A DnaJ Protein, Apobec-1-binding Protein-2, Modulates Apolipoprotein B mRNA Editing*

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Mammalian homologues of DnaJ proteins, also known as Hsp40 proteins, are co-chaperones that complement Hsp70 chaperone function. Using the yeast two-hybrid system, we cloned an apolipoprotein (apo) B mRNA editing complementation protein, called apobec-1-binding protein-2 (ABBP-2), and found that it is a Class II DnaJ homologue. ABBP-2 binds to apobec-1, the mammalian apoB mRNA editase, via its J domain and neighboring G/F domain. It is a ubiquitously expressed protein, and, by transfection analysis of GFP-ABBP-2, we found that the protein is located in both the nucleus and cytosol of transfected cells, with predominance in the nucleus. Down-regulation of ABBP-2 expression in cultured cells inhibits endogenous apobec-1-mediated apoB mRNA editing. Like other Hsp40 proteins, ABBP-2 binds to Hsp70 and has ATPase-stimulating activity. Apobec-1-mediated apoB mRNA editing activity of in vitro tissue extracts requires the presence of Hsp70/ABBP-2. Although exogenously added ATP is not required for editing activity, removal of the endogenous ATP present in these extracts, which disrupts ABBP-2-Hsp70 interaction, completely inhibits editing. ABBP-2 differs from previously described auxiliary proteins (ABBP-1, ACF, and GRY-RBP) in that it does not contain any RNA recognition motifs. Not only is ABBP-2 required for efficient apoB mRNA editing, this newly discovered apobec-1-binding protein may help determine the subcellular distribution and trafficking of apobec-1 via its interaction with the chaperonin Hsp70.

The Hsp70 class of chaperones plays a diverse role in cell physiology (1–3). They participate in different processes, including protein translation, translocation, folding, and the assembly and disassembly of protein complexes. In many cases, the specificity of Hsp70 interaction with their substrates is conferred by the DnaJ class of co-chaperones (4). The binding of Hsp70s to their target polypeptides is ATP hydrolysis-dependent. ATP-bound Hsp70 has a relatively low affinity for its substrate, primarily because of a high off rate, whereas ADP-bound Hsp70 has a high affinity, due to its lower off rate (5). The intrinsic ATPase activity of Hsp70s is extremely weak, and co-chaperones such as DnaJs are essential to Hsp70 action by stimulating ATP hydrolysis. Furthermore, some DnaJ proteins associate directly with substrate polypeptides, which they then transfer to Hsp70 (6–9).

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ing the affinity and specificity of binding of the J domain to its Hsp70 partner (16, 18).

DnaJ proteins belonging to all three classes have been found to mediate many different cellular functions (8, 15, 21–24). A major role of the J domain appears to be the recruitment of Hsp70 to a specific polypeptide substrate; together they mediate functions as diverse as protein translation, translocation, cell cycle regulation, karyogamy, exocytosis, and growth regulation, among others (11).

In this report, we demonstrate that a novel DnaJ protein is involved in eukaryotic mRNA editing. Apolipoprotein B mRNA editing, the sequence-specific deamination of apob-100 to apob-48 mRNA, is mediated by a specific editase, apobec-1, but only in the context of an editosome complex that also contains other auxiliary proteins (25). The DnaJ protein identified, called ABBP-2 (apobec1-binding protein-2), directly interacts with apobec-1. We further showed that ABBP-2 function involves the ATP hydrolysis-dependent recruitment of Hsp70 to the editase, up-regulating apobec-1-mediated apoB mRNA editing, which we show to be ATP-dependent. Down-regulation of the DnaJ protein ABBP-2 inhibits apoB mRNA editing.

**EXPERIMENTAL PROCEDURES**

Two-hybrid Cloning—Five true positives were obtained, by failing to interact with nonspecific diploids, from screening six million Trp Leu colonies according to the mating/screening protocol as described by Harper et al. (26). Two of these clones isolated from a human placenta library were reported by us as ABBP-1 (27) and GR-Y-RBP (28). Another clone that was obtained from a human liver library (CLONTECH, Inc.) contains a partial ABBP-2 cDNA. The 5’ end of human ABBP-2 was obtained by using a CLONTECH 5’ RACE (rapid amplification of cDNA ends) cDNA kit. Mouse ABBP-2 was obtained from a mouse liver Agt11 cDNA library.

Biochemical Methods—Antibodies to glutathione S-transferase (GST)–ABBP-2 fusion protein were raised in New Zealand White rabbits. These polyclonal monospecific antibodies detect a single polypeptide band in Western blots of mouse liver or small intestine S-100 extracts corresponding to the predicted size of ABBP-2. Human tissue RNA blots were purchased from CLONTECH, Inc. Northern hybridization was performed in ExpressHyb hybridization solution (CLONTECH, Inc.) at 68 °C and washed in 2× SSC, 0.05% SDS at room temperature and washed again at 50 °C for 40 min. In vitro binding of GST-ABBP-2 fusion proteins were performed as described previously (27). Briefly, to 1 ml of phosphate-buffered saline, 0.2% Nonidet P-40 solution, 100 μl of GST-ABBP-2 beads were added and incubated with 5 μl of [32P]methionine-labeled apobec-1 obtained by coupled transcription/translation using a Tnt kit (Promega, Inc.) at 4 °C for 1 h with gentle mixing. The beads were washed five times and drained before they were boiled and denatured in SDS loading buffer and analyzed by SDS-PAGE. The truncated GST-ABBP-2 fusion proteins were obtained by polymerase chain reaction subcloning. The GST plasmids, pGEX-4T-1 and pGEX-4T-2, and glutathione-Sephrose 4B beads were obtained from Amersham Pharmacia Biotech.

Transfection of ABBP-2 or ACF Antisense DNA—We used Capture-Tec pHook-3 kits (Invitrogen) and electroporation to transiently transfect antisense DNA of the full-length human ABBP-2 into a stable BNLCL.2 cell line that stably expressed mouse apobec-1 as previously described (29). The plasmid pHook-3 uses the Rous sarcoma virus promoter to express and display the sFv (a single-chain antibody against a specific hapten on the surface of transfected cells). Successfully transfected cells were isolated by the culture from their specific binding to hapten-coated magnetic beads. The expression of antisense-ABBP-2 DNA was under the control of the human early CMV promoter. After 48 h, transfected cells were harvested by using phosphate-buffered saline/3 mM EDTA. We enriched the transfected cells by placing the tubes containing Capture-Tec beads in a magnetic stand (Invitrogen). Bound cells were washed by resuspension in 1 ml of complete minimal essential medium (Life Technologies). Total RNA was isolated by the acid phenol method with an RNA isolation kit (Stratagene). The effect of antisense ABBP-2 RNA on endogenous apoB mRNA editing was assayed by reverse transcription-PCR that specifically amplified apoB mRNA from the total cellular RNA. The gel-purified, amplified products were subjected to primer-extension assay to assess the percentage of edited apoB messages as described previously (30).

A mouse ACF cDNA insert corresponding to the antisense sequence from 57 to 500 of the mouse expressed sequence tag clone (locus AI047471) was PCR-subcloned into pHook3. Transient transfection of the stable BNLCL.2 cell line expressing apobec-1 was performed as described above.

We used the Ecdysone-inducible expression kit (Invitrogen) for mammalian expression to examine the effect of acute down-regulation of the mouse ABBP-2 on editing in an established cell-line. The kit uses a heterodimer of the ecdysone receptor VgEcR derived from Drosophila and modified to contain the VP16 transactivation domain and the retinoid X receptor (RXR) in the presence of ecdysone. The full-length antisense ABBP-2 DNA was subcloned into p Hind-hygromycin plasmid, pTk promoter, and the heterodimeric receptor pVgRXR at 30 °C. The reporter plasmid was transfected into the stable BNLCL.2 cell line expressing apobec-1. The stably transfected colonies were selected by using 125 μg/ml Zeocin (Invitrogen) and 250 μg/ml hygromycin B (Life Technologies). Induction was done by incubating the transfected colonies with 10 μM muristerone A (a synthetic analog of ecdysone) for 48 h. Total RNA was isolated, and PCR editing assays were performed as described above. The samples were separated by 12% Sequagel (National Diagnostics). The radioactive signal on the gels was detected and quantitated using the Cyclone Storage phosphor system (Packard Instrument Co., Inc.).

**RESULTS**

Identification of Apobec1-binding Protein-2 (ABBP-2) as a Class II DnaJ Protein—Using the yeast two-hybrid system and apobec-1 as bait, we have identified a novel apobec-1-interacting protein, which has been named ABBP-2. The sequence of ABBP-2 cDNA predicts a protein of 358 amino acid residues with a calculated molecular mass of 40.5 kDa (Fig. 1A). We also cloned the mouse homologue, which shows a 99% identity to the human sequence.
contains all the conserved residues (tains a DIF tripeptide. The function of the DIF motif (Fig. 1) of E. coli DnaJ J domain deviate from those of the human orthologues, whereas the compact center of the folded helices remains relatively unchanged. The J domain of ABBP-2 is therefore structurally more similar to that of Hdj1 than either one is to DnaJ.

Therefore, structurally ABBP-2 contains a bona fide J domain, a weak G/F region, and a region that contains four cysteines but lacks the zinc finger domain. Both predicted secondary and tertiary structures resemble those of Hdj1. Therefore, it is a Class II DnaJ protein (4, 11, 12).

The J Domain and G/F Region Together Mediate ABBP-2 Binding to Apobec-1.—The two-hybrid clone of human ABBP-2 that we obtained was nearly full-length but was devoid of helix I of the J domain. Next, we mapped the region of ABBP-2 that mediates its binding to apobec-1. GST fusion proteins that contained full-length ABBP-2 and various deletion mutants (Figs. 2, A and B) were used to study their binding to [35S]methionine-labeled apobec-1 synthesized in vitro.

The results of such binding experiments are shown in Fig. 2 (A and C). It is clear that efficient binding to apobec-1 requires the presence of both the J domain and the G/F region. Other studies also showed that the G/F-rich area is critical to specific protein-protein interaction (8, 33). The J domain itself is not sufficient for apobec-1 binding. Deletion mutants missing helix I (mu 1) or the entire J domain (mu 5–9) and mutants missing the G/F region (mu 2, 6–9) do not bind apobec-1 efficiently. These results also indicate that the moderately cysteine-rich domain alone is not sufficient for specific protein-protein interaction. Our results with ABBP-2 are consistent with the tertiary structural requirements for the entire J domain, including helices I to IV together with the G/F-rich region for efficient binding of Hsp40 to unfolded proteins. Thus, the G/F domain of ABBP-2 is also required for ABBP-2’s apobec-1-binding function. These in vitro binding data (Fig. 2, A and B) confirmed the in vivo interaction of the two-hybrid cloned protein, ABBP-2, and apobec-1 inside the yeast nucleus. Their interaction apparently occurs in their native (folded) states in vitro and in vivo.

The ability of ABBP-2, a DnaJ protein, to directly bind to its presumed substrate, apobec-1, in its native state, is not without precedent. Nascent polypeptide chains have been co-immunoprecipitated with antibodies against Hsp40 from a reticulocyte lysate translation, even under conditions where Hsc70 is relatively depleted (6). Although the main function of Hsp40 is to act as a co-chaperone, that is, to provide ATPase activity to stimulate Hsp70 chaperones (12) so that together they bind to and facilitate the folding of proteins, some Hsp40 chaperones additionally have intrinsic ability to bind and fold some misfolded proteins in the absence of Hsp70 (2, 22).

Tissue-specific Expression of ABBP-2.—Northern blot analysis of poly(A)-RNA showed that there are two bands of sizes 1.5 and 2.0 kb that are ubiquitously expressed in all tissues examined (Fig. 3A). Many putative apoB mRNA-editing auxiliary factors, including ABBP-1 and GRY-RBP, are widely distributed in human and/or rodent tissues. A notable exception is ACF, which is essentially not expressed in human small intestine (34), the major tissue of apoB mRNA editing and of apobec-1 expression (29, 35). Evidently in the small intestine, factors other than ACF could be the predominant complemen-

![Fig. 1. Structure of ABBP-2. A, deduced amino acid sequences of human and mouse ABBP-2. The hydrophobic domain and J domain are boxed. The boldface letters represent the preferred amino acids in the J domain. The corresponding GenBank™ access numbers are: Human ABBP-2 (HDJ9) (AB028859) and mouse ABBP-2 (AY054981). B, sequence alignment of the J domain of DnaJ, human Hdj1 (an Hsp40 protein) and ABBP-2. Identical residues are shaded yellow, and conservative substitutions are shaded blue and green. C, tertiary structure of the J domain. The structures of E. coli DnaJ and human Hdj1 were determined by NMR and that of ABBP-2 was predicted by VAST (NCBI) with Hdj1 as template.](http://www.jbc.org/content/46447/6/4467/F1.large.jpg)
Intracellular Localization of ABBP-2—ApoB mRNA editing is an intranuclear event (36). The editing enzyme, apobec-1, as well as the complementation factors, ACF and GRY-RBP, are localized inside the nucleus (37). To investigate the possible intracellular localization of ABBP-2, we expressed GFP-ABBP-2 fusion protein in HepG2 cells by transfection. The tagged protein was found in both the nuclear and cytosolic compartments but with a definite preference for the nucleus (Fig. 3B). The predominantly nuclear localization is reminiscent of the translocation of another DnaJ protein, Hdj1, and Hsp70 from the cytoplasm to the nucleus and nucleolus when mammalian cells are exposed to heat-shock and other types of stress (32, 38). Together they may repair denatured proteins in the nucleus (39). Some members of the Hsp70 family may also assist in the unfolding and folding of proteins in various intracellular compartments as they cross the organelle’s membrane (40). The data presented in Fig. 3B suggest that ABBP-2 may assist Hsp70 to unfold and re-fold apobec-1 during the assembly and disassembly of editosome complexes as they cross the nuclear membrane.

Down-regulation of ABBP-2 Inhibits ApoB mRNA Editing—To qualify as an auxiliary (or complementation) protein, changes in ABBP-2 expression have to be correlated with changes in apoB mRNA editing. We decided to examine this in a mouse liver cell line BNCL-L2. BNCL cells normally express miniscule amounts of apobec-1, and essentially all endogenous apoB mRNA in these cells is unedited. We first produced a stable subline (BNCL-L2) expressing mouse apobec-1 that, like authentic mouse liver, contains ~50% edited apoB mRNA. We transiently transfected BNCL-L2 cells with a mouse ABBP-2 antisense cDNA expression vector cloned in pHook-3. Total RNA was isolated from successfully transfected cells as well as untransfected cells (unbound fraction) as selected by magnetic head-coupled hapten capture of pHook-3-directed antigen expression (see “Experimental Procedures”) and assayed for the extent of endogenous apoB mRNA editing (Fig. 4). We found that transfection with antisense ABBP-2 led to a marked decrease of endogenous ABBP-2 mRNA level (Fig. 4A) and subsequently to a marked decline of apoB mRNA editing in these cells from 54% in the untransfected cells (recovered in the unbound fraction) to 8.3% in the bound fraction, which contains cells expressing the ABBP-2 antisense plasmids (Fig. 4B). We applied the same approach to down-regulate ACF expression in BNCL-L2 cells by ACF antisense expression using the same Capture-Tec pHook-3 system. Indeed, corroborating previous results in in vitro systems (41), we found that antisense down-regulation of ACF in cultured cells reduces editing to 5.5% (Fig. 4B). This is the first demonstration of the complementation activity of both ACF and ABBP-2 expression in apoB mRNA editing inside a living cell system. Interestingly, cotransfection of ABBP-2 and ACF antisense plasmids produced a similar degree of inhibition of editing (down to 7.5%) as transfection with either one alone (Fig. 4B).
To have a more homogeneous population of cells in which we could examine the effect of ABBP-2 antisense expression, we produced a stable cell line containing an inducible antisense construct. We stably transfected an antisense ABBP-2 construct downstream of an inducible ecdysone promoter in the apobec-1-expressing BNLCL.2 cell line. The stably transfected cells were maintained in the absence of inducer. To determine whether inhibition of editing was induced by the antisense expression, we measured the degree of endogenous apoB mRNA editing in three separate stable lines (numbers 1–3, Fig. 4C). We treated these cell lines with a C-mRNA editing in three separate stable lines (expression, we measured the degree of endogenous apoB mRNA expression, we amplified glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (lane 1) and ABBP-2 (lane 2) cDNAs using specific primers by reverse transcription–PCR followed by analysis on a 2% agarose gel. B, endogenous editing of apoB mRNA. Primer extension assays are shown with the unedited product (CAA) and the edited product (TAA) separated on a 12% SequaGel sequencing gel. UbdpAS-ABBP-2 is the Capture-tech-bead-unbound (untransfected) fraction (lane 1) and Bd pAS-ABBP-2, the corresponding bound fraction (lane 2) of BNLCL.2 cells, 48 h after transfection by pHook3 antisense ABBP-2 plasmid; Bd pAS-ACF, the bound fraction transfected with pHook3-antisense ACF DNA (lane 3); Bd pAS-ACF+pAS-ABBP-2, the bound fraction of pHook3-antisense ABBP-2 DNA and pHook3-anti-ACF DNA co-transfected into apobec-1-BNLCL.2 cells (lane 4). C, antisense down-regulation ABBP-2 with an ecdysone-inducible expression system in a stable cell line expressing apobec-1. Permanent apobec-1-expressing BNLCL.2 cells were transfected with buffer (Mock); PIND and pVGRXR (PIND) only; PAS-ABBP-2#1–3, three individual stable cell lines transfected with pIND-antisense ABBP-2 DNA and pVGRXR. Cells were treated with (lanes 7–12) or without (lanes 1–6) muristerone A was carried out for 48 h. D, time course of muristerone A induction. The cell line used was PAS-ABBP-2 #2. , no muristerone A was added (lanes 1 and 2); +, 3 μM muristerone A was added for 1 (lanes 3 and 4), 2 (lanes 5 and 6), or 5 days (lane 7).

Therefore, data generated in mouse liver cell line ABBP-2 antisense expression produced by transient transfection are quite consistent with results obtained by edcsyne-induced antisense expression. They both showed that, under in vivo conditions, antisense down-regulation of ABBP-2 expression leads to inhibition of endogenous apoB mRNA editing. Additionally, we provided the first in vivo evidence that, like ABBP-2, ACF down-regulation in cultured cells also leads to inhibition of apoB mRNA editing, complementing previous results in vitro (41).

**ABBP-2 Functions as an Hsp70 Co-chaperone and Stimulates ATP Hydrolysis**—A major function of mammalian DnaJ homologues is to stimulate ATPase activity of Hsc70, thereby allowing rapid and efficient binding of Hsc70 to unfolded sub-
FIG. 5. Stimulation of ATPase activity and association of ABBP-2, Hsp73/72, and apobec-1 in HepG2 S-100 extracts. A, stimulation of Hsc70 ATPase activity by ABBP-2 as assayed by TLC autoradiograph of reaction products. Equal amounts (1.4 μM) of bovine Hsc70 and human GST-ABBP-2 were incubated with [α-32P]ATP (50 μM) for 5 min (lane 2) and 15 min (lane 3) at 30 °C. Lane 1, no ABBP-2 added. B, time course of ABBP-2-stimulated Hsc70 ATPase activity in vitro. C, immunoprecipitation was performed on S-100 prepared from pFLAG-CMV2-apobec-1 using anti-ABBP-2. Immunoprecipitates were separated on SDS-PAGE, and Western blotting was performed with a monoclonal antibody against ABBP-2. D, immunoprecipitation was performed on S-100 using rabbit polyclonal antibodies against ABBP-2, and protein A beads. The immunoprecipitates were separated on SDS-PAGE, and Western blotting was performed using anti-Hsp70 antibody that recognizes both the inducible (Hsp72) and constitutive (Hsp73) forms of Hsp70. Blots with an antibody against Hsp78 are also shown in the lower panel. Lane 1, no ATP; lane 2, ATP (5 mM) added; lane 3, anti-ABBP-2 antibody omitted. D, immunoprecipitation was performed on HepG2 S-100 using rabbit polyclonal antibodies against ABBP-2, and protein A beads. The immunoprecipitates were separated on SDS-PAGE, and Western blotting was performed as in C. Lane 3, apyrase (50 units/ml) added; lane 2, boiled apyrase added. Anti-Hsp70 monoclonal antibody was used for Western blotting as described in C.

strate proteins, or to nascent polypeptide as they emerge from the ribosome (6). We therefore assayed for ABBP-2 stimulation of ATPase activity of Hsp70. Addition of purified GST-ABBP-2 to Hsc70 (constitutive form) caused a significant stimulation of the Hsc70 ATPase activity (Fig. 5A). The Hsc70-[32P]nucleotide complex was incubated in the absence (Fig. 5A, lane 1) or presence of GST-ABBP-2 for 5 min (lane 2) and for 15 min (lane 3). Hsc70 itself has little ATPase activity (lane 1). The hydrolysis of ATP converted the Hsc70-bound [α-32P]ATP (Fig. 5A, bottom spot) to [α-32P]ADP (top spot) on the thin-layer chromatography paper. The hydrolysis was enhanced upon the addition of ABBP-2, indicating that ABBP-2 has ATPase-stimulating activity. The stimulation by ABBP-2 was 20-fold in 20 min of incubation (Fig. 5B). As shown in Fig. 5 (A and B), there was a time-dependent conversion of [α-32P]ATP to [α-32P]ADP upon addition of ABBP-2; whereas in its absence, ATP hydrolysis was minimal.

Because ABBP-2 has ATP-stimulating activity, we tested whether addition of ATP significantly affected binding of ABBP-2 to apobec-1, the canonical factor required for apob mRNA editing. We prepared an editing-competent S-100 extract from pFLAG-apobec-1-transfected HepG2 cells and immunoprecipitated ABBP-2 with a monospecific antibody to the protein. We then analyzed the immunoprecipitated material by Western blotting using a monoclonal antibody against FLAG. In Fig. 5C, lane S is the Western blot of the total extract, and lanes 1–3 are blots against the immunoprecipitates. We found that the ABBP-2 antibody-immunoprecipitated material also contained FLAG-aposc-1, whether in the absence of exogenously added ATP (lane 1) or in its presence (5 mM, lane 2). Therefore, in editing extracts apobec-1 is detectable in a complex bound to ABBP-2, whether or not ATP is added.

Because many Type II DnaJ proteins have been shown to interact with Hsp70, we used the same approach to determine if antibodies against ABBP-2 would co-immunoprecipitate some of the Hsp proteins from HepG2 S-100 cell extracts. In Fig. 5D, lane S represents Western blots of total S-100 using antisera against Hsp73/72 (upper panel) or Hsp78 (Bip, lower panel). The basal concentration of ATP in the S-100 was 0.3 ± 0.07 (S.D.) mM. At this low ATP concentration, ABBP-2 was associated predominantly with the constitutive Hsp73 (lane 1). Addition of exogenous ATP (5 mM) to the S-100 extract stimulates complex formation between ABBP-2 with Hsp73 as well as with the inducible form Hsp72 (also known as Hsp73 or Hsc70). These data are consistent with our observation that ABBP-2 is localized predominantly inside the nucleus and not in the endoplasmic reticulum and that its partner, either the constitutive Hsc70 (or cognate) or the highly stress-inducible Hsp73, is present within the cytosol and the nucleus.

Finally, we determined if the low concentration (~0.3 mM) of ATP present in the S-100 extracts was necessary for complex formation between ABBP-2 and Hsp72/73. We depleted the ATP pool in these extracts by the addition of an ATPase, apyrase, and examined the association by immunoprecipitation as before. As shown in Fig. 5E (lane 3), depletion of ATP completely eliminated Hsp72/73 from the immunoprecipitate. In contrast, apyrase inactivated by boiling did not have any effect on the association (Fig. 5E, lane 2). Therefore, ATP was necessary for the association of ABBP-2 with Hsp72/73 in the S-100 extract. However, the low concentration of ATP (~0.3 mM) that was present in an S-100 tissue extract was both necessary and sufficient to promote the association.

Apobec-1-mediated ApoB mRNA Editing In Vitro Requires Hsp70/ABBP-2 and Is ATP-dependent—It had been shown previously that exogenous ATP or an ATP-regenerating system
was not required for the apoB mRNA editing activity of S-100 extracts from mammalian small intestine (30). However, the requirement for the ATP present in the extracts themselves was not considered. We tested whether removal of the endogenous ATP by apyrase added exogenously would affect the editing activity of apobec-1-supplemented S-100 from chicken small intestine (C-I S-100), a commonly used editing reaction mixture (25, 42, 43). Chicken apoB mRNA is not edited, and C-I S-100 contains no apobec-1 or editing activity. Apyrase also has no editing activity (Fig. 6A, lane 1). The addition of apobec-1 to the C-I S-100 was required for editing of apoB mRNA substrate in vitro (−75% editing, lanes 2 and 3). The addition of exogenous boiled (inactivated) apyrase did not significantly affect editing (lanes 6 and 7). However, when active apyrase was added to the reaction mixture, it completely inhibited its editing activity (lanes 4 and 5). We further examined whether the apyrase also affected editing in a mammalian S-100 editing extract. We prepared an active S-100 extract from HepG2 cells transfected with apobec-1 (Fig. 6B, lane 2). The addition of active apyrase to the system markedly inhibited its editing activity from 15% (Fig. 6B, lane 2) down to −1.5% (lane 1), whereas the addition of inactive apyrase was without effect (Fig. 6B, lanes 3 and 4). These experiments indicate that basal amounts of ATP are required for editing activity of standard in vitro editing S-100 extracts, whether they are derived from an avian species or a mammalian species.

Because Hsp70 and ABBP-2 are highly expressed cellular proteins, they are not rate-limiting components in the S-100 extracts. Therefore, further increasing the concentration of these proteins by addition of the pure proteins to the extracts had little effect on editing activity (data not shown). We examined the effect of depletion of these proteins from active editing extracts on their editing activity. In Fig. 7A we removed, by immunoprecipitation, individually the putative complementation proteins, ABBP-1, ABBP-2, and GRY-RBP, from active apoB mRNA-editing extracts prepared from apobec-1-expressing HepG2 cells. These extracts had a basal editing activity of −15% (lane 1). The addition of buffer (lane 2) or preimmune serum (lane 6) had little effect on editing. In contrast, removal of ABBP-1 (lane 4), ABBP-2 (lane 3), or GRY-RBP (lane 5) completely blocked editing. Therefore, ABBP-2 is required for editing activity of a standard human tissue-derived editing extract. We next examined the requirement for Hsc70 in the same editing extract (Fig. 7B). Again, the complete extract displayed good basal activity (−12% editing of apoB mRNA substrate, lane 1). Addition of protein A alone in the immunodepletion reaction did not affect editing activity (lane 2), but removal of either ABBP-2 (lane 3) or Hsc70 (lane 4) by immunodepletion completely inactivated the reaction extract. Therefore, the presence of both ABBP-2 and Hsc70 is essential for competent editing in vitro.

**DISCUSSION**

ApoB mRNA editing was the first instance of RNA editing identified in vertebrates (44–46). It is mediated by a multicomponent editosome complex, of which the catalytic subunit was identified as a sequence-specific cytidine deaminase, apobec-1 (25, 46). From the time that apobec-1 was cloned, it was known that, by itself, the enzyme has minimal activity against apoB mRNA as a substrate (25). Optimal editing requires the presence of other protein(s) known as auxiliary or complementation protein(s). In the last several years, three putative auxiliary proteins, ABBP-1 (27), ACF (also known as ASP) (34, 41), and GRY-RBP (28, 37) have been cloned. These proteins share some common properties. They all display binding activity toward apobec-1, which is not surprising because their affinity toward apobec-1 was one of the criteria for their identification. Interestingly, they also share another important common feature. All three proteins contain RNA recognition motifs (RRMs) (28); ABBP-1 contains two copies, and ACF and GRY-RBP, each three copies, of RRM. The fact that these auxiliary factors are RNA-binding proteins suggests that they may be involved in anchoring and presenting the substrate mRNA to apobec-1 within the editosome complex for editing. Although, like other complementation proteins studied to date, ABBP-2 also displays apobec-1-binding activity, it is the only auxiliary protein identified to date that does not contain any known RNA-binding motif. Furthermore, as a DnaJ protein, it is the only auxiliary protein that belongs to a family of co-chaperone proteins.

ABBP-2 is a Class II DnaJ homologue and is a member of the Hsp40 group of co-chaperonins. The J domain of ABBP-2, to-
gether with the neighboring G/F domain, is essential for apo-
bec-1 binding. We showed that, in cultured mammalian cells, 
down-regulation of ABBP-2 inhibits apoB mRNA editing, im-
plicating a direct functional role of the protein in apoB mRNA 
editing. ABBP-2 binds to Hsp70 and displays ATPase activity.
These are unique functions for an auxiliary protein; they pro-
vide a link between apoB-1 and the chaperone protein Hsp70.
Moreover, we showed that low basal levels of ATP are required 
for efficient RNA editing

\[\text{ABBP-2/Hsp70 system modulated by one or more auxiliary proteins that also function}\
\text{as chaperonins.}
\]

Recent evidence suggests that the predomi-

\[\text{cellular machinery that partakes in the assembly and disas-
}\text{sembly of the apoB mRNA editing. ABBP-2 binds to Hsp70 and}
\text{displays ATPase activity.}
\]

In addition to complementing the enzymatic activity of apo-
bec-1, ABBP-2 may be an important determinant of the sub-

cellular localization of apoB-1. GFP-ABBP-2 fusion protein is 
distributed both inside the cytosol as well as the nuclear 
compartments, with a preference toward the latter compart-
ment. Through its interaction with Hsp70, it may be part of the 
cellular machinery that partakes in the assembly and disas-

\[\text{sembly of the apoB mRNA editing. ABBP-2 binds to Hsp70 and}
\text{displays ATPase activity.}
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