Involvement of a Chaperone Regulator, Bcl2-associated Athanogene-4, in Apolipoprotein B mRNA Editing*

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Apolobec is the catalytic subunit of a multicomponent editosome complex that mediates apolipoprotein B (apoB) mRNA editing. We isolated a novel apolobec-1-interacting protein by yeast two-hybrid cloning and identified the protein as BAG-4. BAG-4, a chaperone-regulating protein, also known as SODD (silenter of death domains), is a member of the BAG family of proteins. In this report, we found that apolobec-1 is localized in the perinuclear compartment in HepG2 cells and rat liver MCR-RH7777 cells. BAG-4 binds to apolobec-1 via its C-terminal region independent of the BAG domain. It is ubiquitously expressed with predominant occurrence in human pancreas, heart, brain, and placenta. Immuno-precipitation experiments confirmed that BAG-4 interacts with Hsc70/Hsp90 in HepG2 cells. BAG-4 tagged with green fluorescent protein (GFP) or FLAG was localized both in cytoplasm of mouse BNCL2 liver cells and human liver HepG2 cells. After nutrient shock, GFP-BAG-4 co-localizes with Hsc70 in the nucleus in HepG2 cells, whereas GFP-BAG-4 mutants lacking the BAG domain remain perinuclear. BAG-4 has no effects on apoB mRNA editing in vitro. However, unlike other apolobec-1 complementation factors studied to date, antisense knockdown of BAG-4 in BNCL2 cells and in MCR-RH7777 cells increases rather than decreases endogenous apoB mRNA editing. Overexpression of BAG-4 in MCR-RH7777 cells also suppresses apoB mRNA editing. ApoB-48 production also increases with antisense BAG-4 expression in MCR-RH7777 cells. We previously showed that apoB mRNA editing is an intranuclear event (Lau, P. P.; Xiong, W. J.; Zhu, H. J.; Chen, S. H., and Chan, L. (1991) J. Biol. Chem. 266, 20550–20554). Thus, BAG-4 overexpression down-regulates apoB mRNA editing by Shutting apolobec-1 from the intranuclear perinuclear compartment to the cytoplasm. We propose that BAG-4 functions as a negative regulator for apolobec-1-mediated apoB mRNA editing through its ability to suppress the Hsp/Hsc70 chaperone activity and thereby editosome formation and, as a consequence, prevents nuclear localization of the apolobec-1 editosome.

Apolobec is the catalytic subunit of an editosome complex that mediates apoB mRNA editing (2). There are three regions

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In addition to these cellular functions, here we identify BAG-4/SODD as a negative regulator of apobec-1 function exerting an inhibitory effect on apoB mRNA editing. We demonstrate how BAG-4 interacts with Hsc70 to modulate apoB mRNA editing.

**EXPERIMENTAL PROCEDURES**

**Two-hybrid Cloning**—Three of the five positive apobec-1-interacting clones that successfully passed the mating assay by failing to interact with nonspecific diploids were reported as two RNA-binding proteins, ABBP-1 (14) and GRY-RBP (15). These two RNA-binding proteins were isolated from a human placenta pGAD cDNA library, when human apobec-1 was used as bait. Subsequently, a DnaJ type of co-chaperone, ABBP-2, was isolated from a human liver library (5). Besides ABBP-2, another clone that was obtained from the human liver library (Clontech) contains a nearly full-length cDNA of a known chaperone regulator, named SODD or BAG-4 (11). Three codons were missing from the 5' end of this BAG-4 cDNA clone, which were recovered by PCR subcloning, and the authenticity of the full-length BAG-4 cDNA was verified by sequencing.

**Biochemical Methods**—Polyclonal antibodies to glutathione S-transferase (GST)-BAG-4 fusion protein and human apobec-1 were raised in New Zealand White rabbits. These BAG-4 antibodies recognized a predominant band on Western blots of mouse S-100 extracts (100,000 g supernatant). Northern blots of human tissues were purchased from Clontech. Hybridization was performed as previously described (15) in ExpressHyb hybridization solution (Clontech). After immobilization to glutathione beads, binding of GST-BAG-4 fusion protein to [35S]methionine-labeled apobec-1 was performed in phosphate-buffered saline, 0.2% Nonidet P-40 solution at 4 °C or a rotary shaker as described previously (5). Truncated GST-BAG-4 cDNAs were constructed into pGEX-4T-2 (Amersham Biosciences) by PCR subcloning, and their sequences were verified. Similarly, *E. coli* expressed truncated GST proteins were purified and immobilized onto the glutathione beads and used for in vitro binding to apobec-1.

**Plasmids and Cell Culture Transfection**—Full-length cDNAs of BAG-4 and human apobec-1 (17) were subcloned in-frame into pFLAG-CMV-2 expression vector (Eastman Kodak Co.). BAG-4 and truncated BAG-4 mutants were cloned into pcDNA3.1/NT-GFP-TOPO (Invitrogen). Antisense DNAs of BAG-4, apobec-1 complementation factor (ACF), and ABBP-2 were subcloned into pHook-3 (Invitrogen). All subclones were verified by direct sequencing. A mouse liver BNLCL.2 cell line that stably expressed mouse apobec-1 and human hepatoma cell line HepG2 cells that stably expressed human apobec-1 (5) were used for transfection. Transient transfection was performed with electroporation or LipofectAMINE. For pHook-3 transfection, the transfected cells were collected by hapten-coated magnetic beads. Besides the single chain antibody against a specific hapten, hemagglutinin A epitope tag and Myc epitope tag were preceded by a signal peptide and co-expressed by the Rous sarcoma virus promoter contained in pHook-3; a CMV promoter was used for expressing the inserted DNA. 12-mm poly-L-lysine coverslips (Becton Dickinson) or collagen-coated cover slips were used for BNLCL.2 cells and HepG2 cells, respectively. After 48 h, cells were fixed by paraformaldehyde, and antibody staining was done at room temperature for 30 min and detected by Rhodamine Red-conjugated secondary rabbit antibody or by MOM fluorescence kit (Vector Laboratories Inc.). In parallel experiments after 48 h, cells were harvested.
and RNA was extracted for reverse transcriptase-PCR amplification for apoB mRNA. Gel-purified fragments of apoB were used for dideoxy-G reverse transcriptase editing assays as described (5).

RESULTS

Structure of BAG-4—Using human apobec-1 cDNA as the bait in a yeast two-hybrid screen, we identified a cloned cDNA for another interacting protein besides ABBP-2 from a human liver library; both clones successfully passed the mating assay. The clone obtained was searched with BLAST and revealed a previously published sequence annotated as SODD or BAG-4. The two-hybrid clone contained all but the first three codons. The predicted protein structure of BAG-4 has a BAG domain in the carboxyl-terminal region (Fig. 1A) but lacks the ubiquitin domain present in BAG-1 or the WW domain present in BAG-3. It contains 458 amino acid residues and has a predicted subunit molecular mass of 49.59 kDa.

The secondary structure of BAG-4 and the BAG-Hsc70 complex is well characterized (18). In the crystal structure of a complex with the ATPase of Hsc70, the BAG domain forms a three-helix bundle, inducing a conformational switch in the ATPase that is incompatible with nucleotide binding in the same fashion as observed in bacterial Hsp70 homologue, DnaK, upon binding of the nucleotide exchange factor GrpE. The tertiary structure of the three helices of the BAG domain as determined by NMR in solution was obtained from a VAST search (Fig. 1A). The second and third helices interact with the ATP-binding pocket of Hsc70/Hsp70. The BAG domain and GrpE of E. coli are structurally unrelated (18). However, they both interact with the same ATPase subdomains of their respective Hsp70 or DnaK.

Tissue Distribution—One criterion for identifying candidate proteins involved in the apoB mRNA editing process is their presence in the tissues actively expressing editing activity. BAG-4 mRNA is present in multiple human tissues including the liver and small intestine as shown on the poly(A) mRNA Northern blots (Fig. 1B). In humans, apoB mRNA editing occurs exclusively in the small intestine, which is also the exclusive tissue for the existence of apobec-1 (17). BAG-4 is a molecular chaperone regulator and participates in various cellular functions, and its universal existence is not unexpected.

BAG-4 Interacts with Apobec-1 at the N Terminus—The full-length human BAG-4 cDNA and different deletion mutants were subcloned into pGEX-4T2. The GST fusion proteins of BAG-4 of different lengths were used for an in vitro binding assay (Fig. 2A) to verify the in vivo interaction of BAG-4 and apobec-1 observed in the two-hybrid screening. Nine mutants of progressive deletion of the C-terminal region and the N-terminal region were used to examine the role of the different parts of BAG-4 that are involved in apobec-1 binding. We found that mutants (mu1–5) lacking the BAG domain still bind to apobec-1 essentially as well as the full-length BAG-4. Only when the P/G-rich region of the N terminus was deleted was there a significant attenuation of apobec-1 binding. The apoBec-1 binding region of ABBP-1 is proline-rich (14). Proline-rich motifs are commonly involved in protein-protein interaction, and proline-rich sequences are commonly found in situations.
requiring the rapid recruitment or interchange of several proteins, such as during initiation of transcription and signal cascades (19). It was postulated that the nonconserved N-terminal regions of BAG proteins target proteins to their right partners. The C-terminal BAG domain is used for Hsc70/Hsp70 binding in the presence of ATP and its co-chaperone, ABBP-2, and subsequently releases Hsc70-bound substrate and ADP.

Apobec-1 Editosome Is Localized in the Perinucleolar Compartment
—Human liver HepG2 cells contain no apobec-1 and are incapable of apob mRNA editing. We introduced apobec-1 into HepG2 cells by transient transfection of pCMV-FLAG-apobec-1 plasmid and examined hundreds of cells. In all cells examined, the presence of apobec-1 was detected by rabbit polyclonal anti-apobec-1 antibodies coupled with Rhodamine Red anti-rabbit IgG second antibodies (Fig. 3A). A, intracellular localization of apobec-1 in HepG2 cells. Human FLAG-tagged apobec-1 was transiently transfected into HepG2 cells. FLAG-apobec-1 was detected by a polyclonal antibody raised against apobec-1 polypeptides (α apobec-1) and by anti-FLAG M5 monoclonal antibodies (α FLAG). Images are merged with the nuclear DAPI staining as indicated (red/blue merge). The red and green images were merged as yellow (right upper panel). All images were merged in the right lower panel. Rat liver MCR-RH7777 cells (McArdle) and apobec-1-transfected mouse liver BNCL.L2 cells (BNCL.L2-apobec-1) were probed with the rabbit polyclonal antibodies (αapobec-1). B, close-up of the merged (yellow) image of a cell indicated by an arrow in A (right upper panel). Note the preferential distribution of apobec-1 in the perinucleolar compartment inside the nucleus (left panel) and a close-up FITC image of (α apobec-1) detection of the nuclei of MCR-RH7777 cells (McArdle), highlighting the extranucleolar distribution of apobec-1. C, intracellular localization of ACF in HepG2 and BNCL.L2 cells (upper panels). Rabbit polyclonal antibodies to ACF (α ACF) were used to detect ACF in HepG2 and BNCL.L2 cells stably expressing mouse apobec-1; rabbit polyclonal antibody against ACF was used to detect ACF (α ACF) in MCR-RH7777 cells. A close-up FITC image of anti-ACF detection in MCR-RH7777 cells is shown in the lower right bottom panel.

Apolipoprotein B mRNA Editing, BAG-4, Chaperone Regulator

Fig. 3. Intracellular localization of apobec-1, ACF, and BAG-4 in HepG2 cells. A, intracellular localization of apobec-1 in HepG2 cells. Human FLAG-tagged apobec-1 was transiently transfected into HepG2 cells. FLAG-apobec-1 was detected by a polyclonal antibody raised against apobec-1 polypeptides (α apobec-1) and by anti-FLAG M5 monoclonal antibodies (α FLAG). Images are merged with the nuclear DAPI staining as indicated (red/blue merge). The red and green images were merged as yellow (right upper panel). All images were merged in the right lower panel. Rat liver MCR-RH7777 cells (McArdle) and apobec-1-transfected mouse liver BNCL.L2 cells (BNCL.L2-apobec-1) were probed with the rabbit polyclonal antibodies (αapobec-1). B, close-up of the merged (yellow) image of a cell indicated by an arrow in A (right upper panel). Note the preferential distribution of apobec-1 in the perinucleolar compartment inside the nucleus (left panel) and a close-up FITC image of (α apobec-1) detection of the nuclei of MCR-RH7777 cells (McArdle), highlighting the extranucleolar distribution of apobec-1. C, intracellular localization of ACF in HepG2 and BNCL.L2 cells (upper panels). Rabbit polyclonal antibodies to ACF (α ACF) were used to detect ACF in HepG2 and BNCL.L2 cells stably expressing mouse apobec-1; rabbit polyclonal antibody against ACF was used to detect ACF (α ACF) in MCR-RH7777 cells. A close-up FITC image of anti-ACF detection in MCR-RH7777 cells is shown in the lower right bottom panel.

polyclonal anti-apobec-1 antibodies coupled with Rhodamine Red anti-rabbit IgG second antibodies (Fig. 3A). The intracellular localization of the protein is identical to that detected by the mouse monoclonal anti-FLAG (M2) antibodies as visualized by fluorescence anti-mouse IgG antibodies. The two images completely overlapped. Thus, by using these two different antibodies, we determined that apobec-1 was predominantly nuclear (Fig. 3A). Apobec-1 was also found similarly located predominantly in the nucleus in (rat liver) MCR-RH7777 cells and apobec-1-transfected (mouse liver) BNCL.L2 cells. This finding is consistent with our earlier observation that apoB mRNA editing is a predominantly intranuclear event (1). Upon close observation of the apobec-1-expressing HepG2 cells, we found that apobec-1 is absent from the nucleoli but is localized exclusively in the nucleoplasm with enrichment in the perinucleolar region (Fig. 3B). A similar observation was obtained in MCR-RH7777 cells (Fig. 3B). The perinucleolar compartment is involved in RNA metabolism including splicing and polyadenylation (20); it is also where other apobec-1 auxiliary factors, such as KSRP, CUG-BP, and heterogeneous nuclear ribo-
nucleoproteins, are located predominantly (20, 21). KSRP is a splicing regulatory protein. CUG-BP is a poly(A)-binding protein. Deletion mutagenesis studies showed that at least three RNA recognition motifs at either the carboxyl or amino end of the polypyrimidine binding proteins are required to target the protein to the perinucleolar compartment, suggesting that RNA binding is needed for such intracellular localization. These RNA-binding proteins were previously found to be involved in apoB mRNA editing (22, 23), and apoB mRNA editing was coincidental with splicing and polyadenylation (1). Besides apobec-1, it is of interest to localize the ACF, the only RNA-binding protein that was reported to enhance apoB RNA editing in vitro (16). A polyclonal rabbit antibody against ACF (a gift from Dr. Donna Driscoll) was used to detect its intracellular localization in HepG2 cells, BNLCL.2 cells, and MCR-RH7777 cells; in contrast to apobec-1, which is predominantly in the nucleus, ACF is localized in both the nuclear and cytoplasmic compartments (Fig. 3C, top panel) in HepG2 cells and BNLCL.2 cells but predominantly nuclear and nonnucleolar (Fig. 3C, bottom panel) in MCR-RH7777 cells. According to observations in other laboratories, ACF intracellular localization varies from cytoplasmic to nuclear in different cell lines and varies according to the antibodies used and whether or not it is tagged with epitopes (24–27, 28). When ACF was tagged with c-Myc epitope in CCL13 (a HeLa cell derivative), ACF was

FIG. 5. Association of BAG-4, Hsc70, Hsp90, and apobec-1 in HepG2 S-100. A, co-immunoprecipitation of BAG-4, Hsc70, and apobec-1. Immunoprecipitation (IP) was performed using anti-FLAG beads on S-100 prepared from apobec-1 stably transfected HepG2 cells, which was also transiently transfected with pFLAG-BAG-4 DNA. The immunoprecipitates were separated on SDS-PAGE and Western blotting was performed using anti-Hsc70 antibody. Reciprocally, a polyclonal antibody against human apobec-1 was used to immunoprecipitate the S-100 extracts followed by GammaBindG beads (bd) pull-down; the control lane has GammaBindG beads only. The Western blot was probed by anti-Hsc70 antibody (α Hsc70) or by a monoclonal antibody to FLAG epitope (M2) (right). B, immunodepletion of Hsp70 and Hsp90 in anti-BAG-4 immunoprecipitate. Reciprocate precipitation was performed using polyclonal antibodies against BAG-4; the Western blots were probed by monoclonal antibodies against Hsp70 (left) or Hsp90 (right). C, BAG-4 stimulates the ATPase activity of Hsc70 in the presence of ABBP-2. TLC autoradiographs of reaction products are indicated. Equal amounts (1 μM) of bovine Hsc70 and GST-ABBP-2 and GST-BAG-4 were incubated with [γ-32P]ATP (50 μM) for 5 min at 30 °C.

FIG. 6. Effect of heat shock on distribution of BAG-4 in HepG2 cells. A, HepG2 cells transiently transfected with GFP-BAG-4 were subjected to heat shock at 43 °C for 75 min and then transferred back to the 37 °C incubator for 2 h. FITC (left, GFP-BAG-4) and the DAPI images (right) are indicated. B, GFP-BAG-4 mutant 2 (mu2) lacking the BAG domain was transfected into HepG2 cells and subjected to heat shock. FITC detection after staining with antibody against Hsp70 and DAPI images is shown. C, BAG-4-mu2-transfected HepG2 cells described as in B were fixed. GFP (FITC) and DAPI images are shown.
predominately localized in the cytoplasm (28). When ACF is
nuclearly localized, the putative nuclear localization signal
motif in ACF may not be responsible for its nucleo-cytoplasmic
trafficking, since the nuclear import of apobec-1 may depend on
protein-protein interaction (26).

Cytoplasmic Localization of BAG-4—We have previously
constructed a HepG2 cell line stably expressing apobec-1 (17). BAG-4 was
not readily detectable with the rabbit polyclonal antibodies in
HepG2 cells, although it was faintly detected in the cytoplasmic
compartment in all of the cells examined. BAG proteins
(BAG-1) are generally not abundant, at least 10-fold less than
HIP (Hsp-interacting protein), so as to allow Hsc70 to fulfill its
expanded spectrum of cellular functions (7). In order to more
clearly ascertain the localization of BAG-4, we transfected
pCMV-FLAG2-BAG-4 cDNA and pcDNA3.1NT-GFP-BAG-4
into this cell line. BAG-4 was detected in the cytoplasmic com-
partment (Fig. 4A), although occasionally we detected GFP-
BAG-4 in both nuclear and cytoplasmic compartments when it
was overly expressed. We also transfected GFP-BAG-4 into a
mouse liver cell line BNLCL2 that stably expressed apobec-1,
producing about 50% apoB mRNA editing (5). GFP-BAG-4 was
localized predominantly in the cytoplasm with some enrich-
mant in the perinuclear region (Fig. 4B). Similarly, BAG-1 and
BAG-1M were mostly located in the cytoplasm but were occa-
sionally found in the nucleus, depending on the cell type and
whether the cells were exposed to stress conditions (13).

Association of BAG-4 with Hsp70 and Hsp90 in HepG2

![Diagram](image.png)

**Fig. 7. Knockdown of BAG-4 by antisense DNA increases editing in vivo. A, down-regulation of BAG-4 increases apoB mRNA editing.**

BNCL2 cells expressing apobec-1 (A) or MCR-RH7777 cells (B) were transiently transfected with pHook-3-antisense-BAG-4 DNA. 48 h after
transfection, cells were harvested using Capture-Tec pHook-3 system. The reverse transcriptase-PCR products of the apoB mRNA isolated from
the bound and unbound cells were purified and subjected to the primer extension assay. Primer extension apoB mRNA editing assays were shown
with the unedited product (CAA) and the edited product (TAA) separated on a 12% SequaGel sequencing gel. A, Mock, pHook3 plasmid; AS
BAG unbd, the unbound (mostly untransfected) fraction of antisense-BAG-4 DNA; AS BAG bd, the corresponding bound (transfected) fraction;
lanes 3–6, AS BAG DNA was transfected into BNCL1-2 cells (stably expressing apobec-1); lane 7, same as in lane 1; lane 8, transfected with
antisense ACF DNA, lane 1, transfected with antisense-ABBP-2 DNA. B, overexpression of antisense or sense BAG-4 DNA in MCR-RH7777 cells.
Lane 1, cells were mock-transfected; lane 2, AS BAG4 bd, bound fraction of cells transfected with antisense BAG-4 DNA; lane 3, mock-transfected
MCR-RH7777 cells; lane 4, SBAG4 unbd, unbound fraction of BAG-4 (sense DNA)-transfected cells; lane 5, SBAG4 bd, bound fraction of
BAG-4-transfected cells. C, endogenous production of apoB-100 and apoB-48 in MCR-RH7777 cells. Lanes 1–3, cells that were mock-transfected;
lanes 4–6, cells transfected with antisense BAG-4 DNA. 48 h after transfection, [35S]methionine-labeled cells were subjected to immunoprecipi-
tation by polyclonal antibodies against apoB. The precipitates were separated on 6% SDS-PAGE. D, apoB-1-100 was prepared from HepG2 cells
transiently transfected with pFLAG-CMV2-apobec-1. In vitro editing was performed with apobec-1-HepG2-S-100 lysate; lane 1, no GST-BAG-4
added; lanes 2–4, 1, 2, and 3 μg/ml GST-BAG-4 was added.
Contrary to these previous examples, we found that antisense to the immunoprecipitate (Fig. 5) noprecipitation using anti-FLAG monoclonal antibody beads. We transfected HepG2 cells with FLAG-BAG-4 and prepared S-100 extracts from them; we then performed an immunoprecipitation using anti-FLAG monoclonal antibody beads. Indeed, we detected Hsc70 with an Hsc70-specific antibody in the immunoprecipitate (Fig. 5A). Conversely, an antibody to apobec-1 also pulled down BAG-4 (Fig. 5A). Similarly, Hsp70 was immunoprecipitated with a polyclonal antibody against BAG-4 (Fig. 5B). In the same immunoprecipitate, Hsp90 was also detected with a monoclonal antibody against Hsp90 (Fig. 5B). Thus, these pull-down experiments suggest that BAG-4 associates with Hsp70 and Hsp90 as well as with apobec-1 inside HepG2 cells.

BAG-1 regulates Hsc70 in a manner opposite to that of HIP (7), which stabilizes the ADP-bound state of Hsc70 and inhibits ATPase activity of Hsc70. BAG proteins are known to stimulate Hsc70 ATPase activity in the presence of a J-protein. Hence, we investigated the effect of BAG-4 acting as a nucleotide exchange factor. In Fig. 5C, we showed that ABBP-2 stimulates Hsc70 ATPase activity and that the addition of BAG-4 enhances the effect of ABBP-2.

BAG-4 Is an Hsp70 Co-chaperone—Previously, we showed that the Hsc70/ABBP-2 pair was involved in modulating apoB mRNA editing in vivo (5). ABBP-2 is a co-chaperone DnaJ protein. The orthodox members of DnaJ subfamily Dj2 and Dj3 have J, G/F, and zinc finger domains and are farnesylated, whereas Dj1 (Hsp40/Hdj-1) and ABBP-2 are noncanonical members that lack the zinc finger domain and the prenylation motif. The BAG-1 protein stimulates refolding of denatured proteins of Hsc70/Dj2 and Hsc70/Dj3 pairs but is not effective with Hsc70/Dj1. BAG-1, BAG-2, and BAG-3 were also reported to suppress chaperone activity of Hsc70 (7, 29). It was further shown that Hsc70/Dj2 and Hsc70/Dj3 do not translocate into the nucleus and remain cytoplasmic and perinuclear after heat shock, whereas the Hsc70/Dj1 pair translocates inside the nucleus as it accumulates in the nucleolus (30). The fact that apobec-1 interacts with both ABBP-2 and BAG-4 has prompted us to investigate whether BAG-4 is a co-chaperone of the Hsc70/ABBP-2 pair. After heat shock, BAG-4 enters the nucleus in HepG2 cells (Fig. 6A). Since the BAG domain is required to suppress Hsc70 chaperone activity, we transfected BAG-4 mutant (mu2) that lacks a functional BAG domain with helix 2 partially deleted and helix 3 totally deleted. The transfected cells were then subjected to heat shock. As expected, BAG-4 mu2 remained perinuclear and cytoplasmic after heat shock (Fig. 6C). In the same mu2-transfected HepG2 cells, Hsc70 was still able to enter the nucleus and nucleolus (Fig. 6B). Thus, BAG-4 is a co-chaperone of Hsc70 and is totally dependent on its BAG domain for its interaction with Hsc70 so that it can move into the nucleus with the latter after heat shock.

Down-regulation of BAG-4 Increases apoB mRNA Editing in Vivo—One criterion to qualify a candidate protein to be an auxiliary factor is that alteration in its expression changes apoB mRNA editing. In all cases studied with one exception to date, down-regulation of the auxiliary factors inhibits apoB mRNA editing in vivo. This was true for ABBP-1 (14), ABBP-2 (5), and ACF (5). In one case (GRY-RBP), there was no effect. Contrary to these previous examples, we found that antisense down-regulation of BAG-4 enhances editing in BNCL2-apobec-1 expressing cells. In Fig. 7A, the bound fraction in the Capture-Tec phook-3 system represents the enriched fraction of antisense BAG-4 DNA-transfected cells; in these cells, editing increased from 50% (mock control in Fig. 7A, lanes 1 and 2) to almost completion, 98% (Fig. 7A, lanes 3–6). This observation indicates that BAG-4 is an inhibitor of apoB mRNA editing in vivo. This is consistent with the hypothesis that BAG-4 inhibits the assembly of apobec-1-editosome in the presence of the Hsc70/ABBP-2 pair. In contrast and consistent with previous observations, we found that antisense DNAs to ACF and ABBP-2 knocked down the endogenous apoB mRNA editing from 55% to 2.5 and 5%, respectively, in BNCL2-apobec-1 expressing cells (Fig. 7A, lanes 7–9). We also tested the effects of antisense BAG-4 cDNA expression in MCA-RH7777 rat hepatoma cells that naturally expressed apobec-1 and displayed about 45% apoB mRNA editing. In these cells, expression of antisense BAG-4 cDNA by transfection increased endogenous apoB mRNA editing from 45 to 96% (Fig. 7B, lanes 1 and 2). On the other hand, overexpression of sense BAG-4 DNA suppressed editing from 40% down to −5% (Fig. 7B, lanes 3–5). To further verify this in vivo effect and the downstream effect of BAG-4 down-regulation, we labeled the cellular proteins in MCR-RH7777 cells with [35S]methionine. SDS-PAGE analysis of the [35S]-labeled apoB immunoprecipitate showed an increase in the amount of [35S]apoB-48 relative to [35S]apoB-100 (from 44 ± 12.8% (S.D.) to 76 ± 13.5%, p = 0.0500) in extracts isolated from the antisense BAG-4 DNA-transfected cells compared to control.

\textsuperscript{a} P. P. Lau and L. Chan, unpublished data.

Fig. 8. Shuttling of apobec-1 to the cytoplasm by BAG-4 overexpression. A. MCR-RH7777 cells were transiently transfected with sense pHook3-BAG-4 DNA; transfected cells harvested with Capture-tech beads were replated and stained with polyclonal antibodies against apobec-1; the FITC (a apobec-1) images (top) and merged DAPI images (bottom) are shown. B, similarly, apobec-1-expressing BNCL2 cells were transfected with pFLAG-BAG-4. 48 h after transfection, the cells were stained with polyclonal antibodies against apobec-1 (Rhadmine Red) and monoclonal antibodies against FLAG (M5) (FITC), sequentially. Rhadmine Red apobec-1 images (a apobec-1) and merged DAPI images are shown. FITC images of FLAG-Bag-4 were detected by anti-FLAG antibodies (a FLAG, right corner panel).
pared with those from mock-transfected cells (Fig. 7C). This observation confirms that down-regulation of BAG-4 increases endogenous editing. Interestingly, when we co-incubated purified BAG-4 with apobec-1-expressing S-100 extract from HepG2 cells, we observed no effect on in vitro editing (Fig. 7D). The data suggest that the effect of BAG-4 on editing in vivo is indirect and probably involves the targeting of apobec-1 and other required proteins to the right partner and/or cellular compartment for editing to occur through regulation of Hsp70 or other chaperones.

Shuttling of Apobec-1 to the Cytoplasm by BAG-4 Overexpression—We found strong evidence for the role of BAG-4 in apobec-1 trafficking (Fig. 8). When BAG-4 was overexpressed, apobec-1 was shuttled to the cytoplasmic compartment in MCR-RH7777 cells (Fig. 8A). Apobec-1 was predominantly localized in the nucleus and changed dramatically to the cytoplasmic compartment after transfection by pHook-3-BAG-4 sense DNA. Similarly, overexpression of BAG-4 in BNLCL.2-apobec-1-expressing cells facilitated apobec-1 export from the nucleus to the cytoplasm (Fig. 8B). Apobec-1 was localized in the nucleus in the extranucleolar compartment in BNLCL2-apobec-1 cells and was exported drastically to the cytoplasm after transfection with pCMV2-FLAG-BAG-4 sense DNA. The appearance of speckles in the cytoplasm may represent the apobec-1 aggregates in cells with the chaperone regulator overly expressed.

DISCUSSION

To date, by two-hybrid cloning using human apobec-1 as bait, our laboratory has identified two RNA-binding proteins, ABBP-1 and GRY-RBP (14, 15), and two co-chaperones, ABBP-2 (5) and Bag-4. Other RNA-binding proteins, such as ACF (ASP), KSRP (16, 22), and CUGBP2 (23) were cloned or identified by other laboratories as auxiliary factors associated with apobec-1 and shown to play a role in editing. In this study, we have further localized apobec-1 in the perinucleolar compartment (Fig. 3B). We note that heterogeneous nuclear ribonucleoprotein, KSRP, and CUGBP are also preferentially localized in this compartment (20). The perinucleolar compartment is a highly dynamic structure; for example, fluorescence recovery after photobleaching analysis revealed a rapid turnover of the polypyrimidine tract-binding protein within this compartment (21). There is evidence indicating that the perinucleolar compartment is actively involved in RNA metabolism (20). Deletion mutagenesis analysis showed that at least three RNA recognition motifs are required for the polypyrimidine tract-binding protein to be targeted to the perinucleolar compartment (31). The identified auxiliary proteins of the apobec-1-editosome, including ABBP-1, GRY-BP, KSRP, and ACF, each contain two or three RNA recognition motifs. Many of them were identified by UV cross-linking to the AU-rich apoB mRNA. Taken together, we speculate that regulated apoB mRNA editing also occurs in the perinucleolar compartment.

Here we further showed that BAG-4 regulates apobec-1-mediated editing through its interaction with Hsc70 and Hsp90. This finding is consistent with our earlier identification of ABBP-2, a DnaJ co-chaperone protein that works with Hsc70, and their involvement in regulating apoB mRNA editing through their interaction with apobec-1. ABBP-2 acts as a positive regulator by stimulating chaperone activity of Hsc70.
and therefore is required for apobec-1 editosome assembly in the presence of HIP by causing a slow ADP release (Fig. 9A).

In Fig. 9A, we depict the possible role of the various cochaperone proteins involved in apobec-1 editosome assembly and transport. In the presence of ATP, Hsc70 and DnaJ (ABBP-2) mediate the assembly of the apobec-1 editosome before it is imported into the nucleus. In the presence of Hsp90 and HOP (heat-shock organizing protein), Hsp90 may assist in the import of apobec-1 into the nucleus. BAG-4 acts as a chaperone regulator by binding to the ATPase domain of Hsc70 N-terminal region, dissociating the Hsp90-editosome complex. This is analogous to the previous findings that BAG-1 regulates p53 stabilization and subcellular localization (8) and that Hsp90 may assist p53 import into the nucleus by exposing its nuclear localization signal (32). Overexpression of BAG-4 would facilitate both the stabilization and cytoplasmic sequestration of apobec-1 aggregates (Fig. 8B). BAG domain proteins stimulate the ATPase activity of Hsc70 in an Hsp40/DnaJ-dependent manner (Fig. 5C) and in this way induce the release of substrates from Hsc70. Efficient interaction of Hsc70 with a polypeptide depends on the conversion of bound ATP to ADP (7). Only the ADP-bound form confers stable substrate binding (33). By accelerating substrate release, the half-life of the Hsp70-substrate complex is decreased (7). Therefore, BAG proteins suppress Hsc70 chaperone refolding activity. Indeed, it was shown in a comparative study (including human BAG-1, BAG-2, BAG-3, BAG-4, and BAG-5) that BAG proteins, which were shown in a comparative study (including human BAG-1, BAG-2, BAG-3, BAG-4, and BAG-5) that BAG proteins, which work as a negative regulator of the Hsc70/ABBP-2 pair in suppressing Hsp90 chaperone system modulates apoB mRNA editing by utilizing a positive regulator, the J-protein ABBP-2 (5), and a negative regulator BAG-4 (this report) to control apobec-1 editosome assembly and its subcellular localization.

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REFERENCES

1. Lau, P. P., Xiong, W. J., Zhu, H. J., Chen, S. H., and Chan, L. (1991) J. Biol. Chem. 266, 20550–20554
2. Chan, L., Chang, B. H., Liao, W., Oka, K., and Lau, P. P. (2000) Recent Prog. Horm. Res. 55
3. Yang, Y., Sowden, M. P., and Smith, H. C. (2001) Exp. Cell Res. 267, 153–164
4. Hartl, F. U., and Hayer-Hartl, M. (2002) Science 295, 1852–1858
5. Lau, P. P., Villanueva, H., Kobayashi, K., Nakamura, M., Chang, B. H., and Chan, L. (2001) J. Biol. Chem. 276, 46445–46452
6. Takayama, S., Sato, T., Krajewski, S., Kocz, K., Irie, S., Millan, J. A., and Reed, J. C. (1995) Cell 80, 279–284
7. Heffold, J., and Jentsch, S. (1997) EMBO J. 16, 6209–6216
8. King, F. W., Wawrzynow, A., Heffold, J., and Zylicz, M. (2001) EMBO J. 20, 6297–6305
9. Takayama, S., Xie, A., and Reed, J. C. (1999) J. Biol. Chem. 274, 781–786
10. Antoku, K., Maser, R. S., Scully, W. J., Jr., Delach, S. M., and Johnson, D. E. (2001) Biochem. Biophys. Res. Commun. 286, 1003–1010
11. Jiang, Y., Wornicz, J. D., Liu, W., and Goeddel, D. V. (1999) Science 283, 543–546
12. Liao, Q., Ozawa, F., Friess, H., Zimmermann, A., Takayama, S., Reed, J. C., Kleeff, J., and Bechler, M. W. (2001) FEBS Lett. 503, 151–157
13. Doung, H., Vrailas, A., and Kohn, E. C. (2002) Cancer Lett. 188, 25–32
14. Lau, P. P., Zhu, H. J., Nakamura, M., and Chan, L. (1997) J. Biol. Chem. 272, 1452–1455
15. Lau, P. P., Chang, B. H., and Chan, L. (2001) Biochem. Biophys. Res. Commun. 282, 977–983
16. Mehta, A., Kinter, M. T., Sherman, N. E., and Driscoll, D. M. (2000) Mol. Cell. Biol. 20, 1846–1854
17. Lau, P. P., Zhu, H. J., Baldini, A., Charansangavee, C., and Chan, L. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 8522–8526
18. Sondermann, H., Scheufler, C., Schneider, C., Hohfeld, J., Hartl, F. U., and Moarefi, I. (2001) Science 291, 1553–1557
19. Kay, B. K., Williamson, M. P., and Suddo, M. (2000) FASEB J. 14, 231–241
20. Huang, S. (2000) J. Struct. Biol. 129, 233–240
21. Huang, S., Deirick, T. J., Ellisman, M. H., and Spector, D. L. (1998) J. Cell Biol. 143, 35–47
22. Lellek, H., Kirsten, R., Diehl, I., Apostol, F., Buck, F., and Greve, J. (2000) J. Biol. Chem. 275, 19848–19856
23. Anant, S., Henderson, J. O., Mok, S. H., Navaratnam, N., Kennedy, S., Min, J., and Davidson, N. O. (2001) J. Biol. Chem. 276, 47338–47351
24. Yang, Y., Sowden, M. P., and Smith, H. C. (2000) J. Biol. Chem. 275, 22663–22669
25. Blanc, V., Henderson, J. O., Andant, S., Kennedy, S., Jarmuz, A., Scott, J., and Davidson, N. O. (2001) J. Biol. Chem. 276, 46386–46393
26. Sowden, M. P., Ballatori, N., Jensen, K. L., Reed, L. H., and Smith, H. C. (2002) J. Biol. Chem. 277, 19617–19625
27. Sowden, M. P., Ballatori, N., Jensen, K. L., Reed, L. H., and Smith, H. C. (2002) J. Biol. Chem. 277, 19617–19625
28. Chester, A., Somasekaram, A., Trinima, M., Jarmuz, A., Gisbourne, J., Kohn, E. C. (2002) Cancer Lett. 188, 25–32
29. Bimston, D., Song, J., Winchester, D., Takayama, S., Reed, J. C., and Moriarto, R. I. (1998) EMBO J. 17, 6871–6878
30. Terada, K., and Mori, M. (2000) J. Biol. Chem. 275, 24728–24734
31. Kimkins, A., and Jackson, R. J. (1998) RNA 4, 826–838
32. Zylicz, M., King, F. W., and Wawrzynow, A. (2001) EMBO J. 20, 4634–4638
33. Minami, Y., Hohfeld, J., Ohtsuka, K., and Hartl, F. U. (1996) J. Biol. Chem. 271, 19617–19624
34. Demad, J., Londers, J., Hohfeld, J., and Zmib, Z. (1998) Mol. Cell. Biol. 18, 2023–2028