Partial Characterization of the Auxiliary Factors Involved in Apolipoprotein B mRNA Editing through APOBEC-1 Affinity Chromatography

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APOBEC-1-catalyzed apolipoprotein B (apoB) mRNA editing requires auxiliary factors, but the number and functions of these factors are unknown. We have partially purified the editing activity from extracts of a McArdle cell line overexpressing His6-hemagglutinin-tagged, rat APOBEC-1 using metal-chelating affinity chromatography. The 1,200-fold purification achieved by this approach was partially dependent on exogenously added RNA containing a mooring sequence for editosome assembly. Affinity-purified editing activity could be separated by 300 mM NaCl extraction into two fractions, a salt-resistant fraction (editing fraction 1; EF1) and a salt-soluble fraction (EF2). Neither EF1 nor EF2 alone could edit apoB RNA, but when added together they reconstituted full editing activity. Previously identified candidate auxiliary factors including the p66/p44 apoB RNA binding proteins and the presumptive editosome assembly factor p240 were all present in the affinity-purified editing complex. Moreover, virtually all of p66, p240, and APOBEC-1 were present in EF1, whereas p44 was quantitatively recovered in EF2. This is the first demonstration that p66 and p44 can bind to apoB RNA independently of one another. In addition, 100- and 55-kDa apoB RNA cross-linking proteins have been identified in the APOBEC-1 affinity-purified material. RNA competition studies demonstrated that p100, p66, and p55 bound selectively to apoB RNA, whereas p44 had general RNA cross-linking characteristics. The data underscore the multiplicity of auxiliary factors potentially involved in apoB RNA editing and suggest an editosome far more complicated than may have been previously appreciated.

ApoB mRNA undergoes a post-transcriptional editing process involving a site-specific deamination of cytidine at nucleotide 6666, converting a CAA glutamine codon (2156) to a UAA translation stop codon (1, 2). Translation of edited and unedited variants of apoB mRNA generates two isoforms of apoB proteins: apoB100 and apoB48, whose physiological functions differ markedly in lipoprotein secretion and uptake by peripheral cells. A tripartite RNA sequence motif consisting of a mooring sequence, a spacer region, and a regulatory element is required for site-specific RNA editing (Refs. 3–6; reviewed in Ref. 7). The mooring sequence is also necessary to support assembly of an editosome, a multiprotein complex required for apoB mRNA editing (8, 9).

APOBEC-1 (apoB mRNA-editing catalytic subunit 1) is a cytidine deaminase and the catalytic subunit of the editosome (10). It exists as a homodimer (11, 12), and its catalytic domain shares substantial peptide sequence homology with a variety of cytidine and adenosine deaminases (Refs. 13 and 14; reviewed in Ref. 15). APOBEC-1 has a low affinity and nonselective RNA cross-linking activity and cannot edit apoB RNA in the absence of other editosomal proteins (10–17). Auxiliary activities that complement APOBEC-1 in apoB mRNA editing have been found in diverse tissues and cell types (10, 11, 18–20) and appear to play important but uncharacterized roles in regulating editing during tissue development as well as in response to nutritional stress and hormone stimulation (19–21).

A variety of experimental approaches have been undertaken to characterize the auxiliary proteins. 66- and 44-kDa (p66/p44) apoB RNA binding proteins have been identified by UV cross-linking experiments, and these two proteins cofractionated with the editosome in sedimentation and native gel shift analyses (9, 22–24). A 240-kDa protein (p240) has been identified by Western blot using monoclonal antibodies (mAbs) raised against the in vitro assembled 27 S editosome (25). Immunoprecipitation of McArdle 7777 rat hepatoma cell extracts with anti-p240 mAb yielded p240 along with additional proteins, together referred to as AUX240. APOBEC-1 was not detected in AUX240, nor did AUX240 have autonomous editing activity. Extracts depleted of AUX240 had impaired editosome assembly and editing activity that could be restored by the addition of AUX240 in a concentration-dependent manner (25). Moreover, editing activity in untreated extracts was stimulated by the addition of AUX240.

An APOBEC-1-interacting protein designated ABBP-1 (APOBEC-1-binding protein-1) was cloned recently using the yeast two-hybrid system (26). It is an alternatively spliced variant of previously cloned heterogeneous nuclear ribonucleoprotein A/B. A role for this protein in apoB mRNA editing has been suggested by the findings that ABBP-1 could bind to APOBEC-1 as well as apoB RNA and down-regulation of ABBP-1 in editing competent cells by transfection with an antisense ABBP-1 cDNA construct impaired editing efficiency.
ABBP-1 and APOBEC-1 are, however, not sufficient to edit, suggesting that other auxiliary proteins must be involved.

Affinity columns generated by immobilizing E. coli-expressed 6-histidine-tagged APOBEC-1 (His$_6$-APOBEC-1) on metal-chelating resins have been exploited to isolate auxiliary factors (27, 28). Proteins adsorbed in this manner complement the recombinant APOBEC-1 in editing activity. The molecular masses of masses of proteins obtained by APOBEC-1 affinity chromatography are complex and overlap those observed in AUB240 (25, 27).

In this study, we demonstrated several novel characteristics of partially purified edistomes through a variation of APOBEC-1 affinity chromatography, based entirely on a homologous system of mammalian expressed proteins. Affinity-purified edistomes were dissociated into two fractions by 300 mM salt extraction that could reconstitute fully functional editing complexes when recombined but that alone were insufficient in complementing APOBEC-1. The 300 mM salt-resistant fraction (EF1) contained auxiliary factors associated with APOBEC-1 including p66, p240, and a 100-kDa apoB RNA cross-linking protein (p100). Proteins released from edistomes by 300 mM salt included p44 and a 55-kDa apoB RNA cross-linking protein (p55). The data show for the first time that p66 and p44 are capable of binding apoB RNA independent of one another and that in the context of purified edistomes, p100, p66, and p55 bind apoB RNA selectively, whereas p44 is a general RNA cross-linking protein. Finally, the data suggest that the mooring sequence may play a critical role in recruiting auxiliary factor(s) for edistome assembly.

EXPERIMENTAL PROCEDURES

Plasmid Construction—apo-1 cDNA was amplified from plasmid pPOEXapo-1 (27) by PCR using Pfu DNA polymerase (Stratagene, CA) and primers Y5' (GGGGCGCATATCGTGGGTCGCGACGAG) and Y3' (GCTTAGAAGCTTCTCAGCCGTGT) and subcloned in pcDNA3 (Invitrogen, CA) at the EcoRI/XhoI sites. Sequence encoding His$_6$ and the influenza virus hemagglutinin (HA) tag (YPYDVPDYA) was inserted 5' in frame of apo-1 to produce His$_6$-HA-APOBEC-1 fusion protein.

ApoB RNA (nucleotides 6413–6860) and a 475-nucleotide rat Wilms' tumor suppressor I (WT-1) gene transcript (nucleotides 620–1094) were transcribed in vitro as described previously (8). The region of WT-1 RNA chosen as a control RNA is devoid of UGAU mooring sequence homologous to the 3' UTR of apoB RNA.

Expression of APOBEC-1 in E. coli—His$_6$-HA-APOBEC-1 was expressed in E. coli, purified by nickel-nitrotriacetic acid metal affinity resin (Qiagen, Inc., CA) as described previously (27) and eluted in 1× editing buffer (10 mM Heps, pH 7.9, 10% glycerol, 50 mM KCl, 50 mM EDTA, 0.25 mM dithiothreitol).

Cell Culture—McArdele RH7777 cell was obtained from ATCC (Rockville, MD) and maintained in Dulbecco's modified Eagle's medium containing 10% horse serum and 10% fetal bovine serum. Cells were transfected by the calcium phosphate precipitation method as described before (29). Stable cell lines transfected with apo-1 cDNA were obtained by limiting dilution under 500 μg/ml G418 (Life Technologies, Inc.) selection 48 h after transfection.

Preparation of Cellular Extracts—Fifty 150-mm dishes of 80% confluent cells were scrapped into cold 5× buffer A (50 mM Tris-Cl, pH 8.0, 5 mM MgCl$_2$, 2 mM EGTA, 150 mM NaCl, 0.25 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride (Sigma), 5 μg/ml aprotinin (Sigma), and 5 μg/ml leupeptin (Boehringer Mannheim)) and harvested by centrifugation (800 × g, 3 min), washed once in 5× buffer A minus proteinase inhibitors, and resuspended in 10 packed cell volumes of 1× buffer A.

The cells were allowed to swell for 30 min on ice and were then collected by centrifugation (3,500 × g, 10 min). The packed cells were resuspended in 1× buffer A in 2–4 original packed cell volumes and sheared by three passes each through 18-, 20-, and 22-gauge needles. Sheared cells were brought to 250 mM NaCl and incubated for 10 min on ice, and the cellular debris was cleared by centrifugation (100,000 × g, 20 min).

The 15% (NH$_4$)$_2$SO$_4$ fraction was prepared by adding crystalline ammonium sulfate to a final concentration of 15% (w/v) to the cell extract while stirring on ice. Control experiments determined that virtually all of the editing activity in the extract was recovered in the 15% (NH$_4$)$_2$SO$_4$ fraction. The precipitate was collected (5,000 × g, 15 min) and dialyzed against buffer B (50 mM Tris HCl, pH 7.5, 50 mM NaCl, 10% glycerol, 5 mM β-mercaptoethanol).

Affinity Chromatography—Two volumes of 15% (NH$_4$)$_2$SO$_4$ fractioned extracts preincubated with either in vitro transcribed apoB or WT-1 RNA (10 fmol/μg extract protein) at 30°C for 30 min were mixed with 1 bed volume of TALON* (CLONTECH) metal affinity resin (equilibrated with buffer B containing 0.1% Nonidet P-40 (v/v)) and tumbled for 1 h at 4°C. After the incubation, the resin was allowed to sediment by gravity and resuspended in 1 bed volume of buffer C (20 mM Tris-HCl, pH 7.5, 50 mM NaCl, 5 mM imidazole) and spun through 10 volumes of 1× sucrose in buffer B followed by washing with 30 volumes of buffer C.

Salt extracts were obtained by incubating the resin-bound edistomes, prepared as described above, with 1 bed volume of buffer C containing the indicated concentrations of NaCl at room temperature for 15 min. Elutes were dialyzed against 1 liter of 1× editing buffer for 2 h. The resin was then washed with 50 volumes of buffer C minus imidazole before aliquots were taken for the editing assay or SDS-PAGE.

Editing Assay—Endogenous editing activity was determined as described previously (29). Total cellular RNA was isolated with TriReagent (Molecular Research Center, OH) and amplified by reverse transcriptase-based PCR prior to the poisoned primer extension assay. In vitro apoB RNA editing activity in cell extracts was determined under conditions described previously (4, 27). Forty μl of charged TALON* resin (either after affinity chromatography or subsequent to salt extraction) and 40 fmol of RNA were assayed in 100-μl reactions for editing activity. Editing efficiency was evaluated by poisoned primer extension analysis (4, 8). The primer extension products were resolved on 10% denaturing polyacrylamide gel and quantified by laser densitometric scanning (PhosphorImager model 425E, Molecular Dynamics).

Ultraviolet Light Cross-linking—Editing reactions were transferred to quartz cuvettes (0.5-cm width) on ice and cross-linked with 254-nm wavelength UV light for 5 min as described previously (9). Samples were subsequently digested with 20 units each of RNase A (Sigma) and RNase T1 (Boehringer Mannheim, IN) for 30 min at 37°C and precipitated with 5 volumes of acetone.

SDS-PAGE and Western Blot Analysis—All samples were resuspended in an appropriate volume of sample buffer (125 mM Tris-HCl, pH 6.8, 4% SDS, 20% glycerol, 20 mM dithiothreitol, 0.0005% bromophenol blue) and resolved by 10.5% SDS-PAGE or 4–15% gradient SDS-PAGE (Bio-Rad). Gels were stained with Coomassie Blue or silver (Bio-Rad) or transferred to nitrocellulose membrane (Schleicher & Schuell) and probed with anti-HA mAb (Babco, Richmond, CA) or anti-AUX240 mAb (26) and finally with peroxidase-conjugated goat anti-mouse IgG (Zymed Laboratories Inc.). Immunoreactive proteins were detected with the ECL* kit (Amersham Life Sciences Inc.).

Protein Concentration Determination—Affinity-purified proteins were detected from TALON* resin with 3 bed volumes of 500 mM EDTA according to the manufacturer's protocols (CLONTECH). These proteins and those obtained from NaCl extraction of affinity-purified material were dialyzed against 2 liters of distilled water, quick frozen in dry ice-ethanol bath, and lyophilized to the desired volume. Aliquots were assayd for protein concentration with the BioRad assay system.

RESULTS

Establishment of McArdele Cell Lines Overexpressing APOBEC-1—Previous data from our laboratory (27) and that of Driscoll (28) demonstrated that the auxiliary factors required for apoB RNA editing could be adsorbed from McArdele cell extracts using affinity columns immobilized with E. coli expressed recombinant His$_6$-APOBEC-1. However, these studies have shown that the potential situation that E. coli-expressed APOBEC-1 may not contain the appropriate post-translation modifications required for optimal interactions with the auxiliary factors. We took a novel experimental approach, which was to use endogenously expressed APOBEC-1 as an affinity ligand to copurify the auxiliary factors from an entirely homologous mammalian system. To achieve this we established McArdele cell lines...
overexpressing His<sub>6</sub>-HA-APOBEC-1. One of these cell lines (McAPOBEC) demonstrated a high expression level of His<sub>6</sub>-HA-APOBEC-1 (Fig. 1A). Consistent with previous findings (23, 29), overexpression of APOBEC-1 in McAPOBEC cells stimulated editing of the endogenously expressed apoB mRNA. Editing activity in McArdle and McAPOBEC cells was evaluated by primer extension on reverse transcriptase-based PCR products as described under "Experimental Procedures." Primer extension products were visualized by autoradiography and quantified by PhosphorImager analysis. The positions of primer and the extension products generated from unedited (CAA) and edited (UAA) RNAs are indicated.

To evaluate this possibility, the relative amount of APOBEC-1 in each of the salt-resistant and salt-soluble fractions was evaluated by Western blot. As shown in Fig. 4D, the data demonstrated quantitative retention of APOBEC-1 in the salt-resistant fractions over the entire range of NaCl concentrations evaluated. Only trace amounts of APOBEC-1 were detected in the salt-soluble fractions (particularly at 300 and 500 mM NaCl), apparent only after a substantially longer autoradiographic exposure of the blots (see Fig. 4E, Fig. 4E legend, and Fig. 7B).

The dissociation of auxiliary factors by high salt buffer was further evaluated by reconstitution assays in which salt-soluble fractions obtained by a single-step extraction of affinity-purified editosomes were reconstituted with the 300 mM NaCl-soluble fraction and the affinity-purified editing complexes immobilized on TALON<sup>TM</sup> resin were prepared and assayed for in vitro editing activity as described under "Experimental Procedures." The editing efficiency of each fraction containing 240, 79, and 0.4 μg of protein, respectively, was indicated. The positions of the primer and the extension products generated from unedited (CAA) and edited (UAA) RNAs are indicated. The additional primer extension product above the UAA band corresponds to promiscuous editing of C<sup>5m</sup>U (Ref. 29).

Auxiliary Proteins of the C → U Editosome

**Fig. 1. Overexpression of APOBEC-1 in McArdle cells stimulates editing of endogenous apoB mRNA.** A, expression of His<sub>6</sub>-HA-APOBEC-1 in McAPOBEC cell lines. Lysate of 5 × 10<sup>8</sup> of either parental McArdle (McA) or McAPOBEC cells was resolved on 10.5% SDS-PAGE, Western blotted with anti-HA mAb, and visualized by the ECL. The arrowhead indicates the position of the His<sub>6</sub>-HA-APOBEC-1. B, overexpression of His<sub>6</sub>-HA-APOBEC-1 in McAPOBEC cells stimulated editing of endogenously expressed apoB mRNA. Editing activity in McArdle and McAPOBEC cells was evaluated by primer extension on reverse transcriptase-based PCR products as described under "Experimental Procedures." Primer extension products were visualized by autoradiography and quantified by PhosphorImager analysis. The positions of primer and the extension products generated from unedited (CAA) and edited (UAA) RNAs are indicated.

**Fig. 2. Partial purification of editing activity from McAPOBEC cell extracts by TALON<sup>TM</sup> metal-chelating affinity chromatography.** Forty-μl aliquots from whole McAPOBEC cell extracts, a 15% (NH₄)₂SO₄ fraction, and the affinity-purified editing complexes immobilized on TALON<sup>TM</sup> resin were prepared and assayed for in vitro editing activity as described under "Experimental Procedures." The editing efficiency of each fraction containing 240, 79, and 0.4 μg of protein, respectively, was indicated. The positions of the primer and the extension products generated from unedited (CAA) and edited (UAA) RNAs are indicated. The additional primer extension product above the UAA band corresponds to promiscuous editing of C<sup>5m</sup>U (Ref. 29).
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TABLE I
Purification of editing activity

| Protein                  | Editing activity | Specific activity | Purification |
|--------------------------|------------------|-------------------|--------------|
|                          | µg               | units/µg          | fold         |
| Crude extracts           | 78,000           | 3,249             |              |
| 15% (NH₄)₂SO₄            | 11.820 (15) a,b   | 2,369 (90.5) b    | 0.040        |
| Affinity-purified        | 31 (0.040) b     | 1,474 (45.4) b    | 48           |
|                          |                  |                   | 1189         |

a Units of editing activity were defined as fmols of edited RNA produced per 3 h.
b Percentage yield.

FIG. 3. ApoB RNA facilitates editosome assembly. The 15% (NH₄)₂SO₄ fractionated McAPOBEC extracts were incubated with the indicated amounts of either apoB or control WT-1 RNA to enable the assembly of editosomes as described under “Experimental Procedures.” Assembly reactions were chromatographed on TALON™ column, and the materials recovered on the resin were subjected to primer extension analysis of affinity-purified editing activity using apoB RNA during the assembly reaction. B, primer extension analysis of affinity-purified editing activity using WT-1 RNA during the assembly reaction. C, graphic representation of the quantification of the data in A and B.

We have achieved an approximately 1,200-fold partial purification of editing activity by APOBEC-1 affinity chromatography and characterized the purified editing complex by salt fractionation. Our data demonstrated that the editosome can be operationally divided into two fractions, a 300 mM NaCl-resistant fraction designated as EF1 and a 300 mM NaCl-soluble fraction designated as EF2. Although virtually all of APOBEC-1 was retained in EF1, it was not capable of autonomous editing. On the other hand, EF2 was not sufficient as a source of auxiliary factors, since it did not support apoB RNA editing in the presence of recombinant APOBEC-1. Editing activity could be imparted to EF1 through the addition of EF2 in a concentration-dependent manner. The data argued against the possibility that the loss of editing activity in EF1 was due to denaturation of APOBEC-1 by salt extraction. Rather, the data clearly demonstrated both EF1 and EF2 contained auxiliary factors necessary for complementing APOBEC-1 to edit.

DISCUSSION

Auxiliary Proteins of the C → U Editosome

Fractions were prepared as described under “Experimental Procedures,” and protein concentrations were quantified with the Bio-Rad reagent using bovine serum albumin for a standard curve. Editing assays were conducted and quantified as described under “Experimental Procedures.”

Protein Composition of Affinity-purified Editing Activity— Despite the approximate 1,200-fold purification, the affinity-purified fraction was remarkably complex in protein composition, showing only a marginal differential yield in proteins with molecular masses in the range of 260–180, 160–100, 66–45, and 38–20 kDa (Fig. 7A). Interestingly, most of these proteins were high salt-resistant. The salt-soluble fraction was very low in protein yield and apparent only following silver staining. It was also complex in protein composition, although a 55-kDa protein and a 45-kDa protein dominated the profile. Western blot revealed the presence of APOBEC-1 in all fractions except the salt-soluble fraction (Fig. 7B), consistent with the data in Fig. 4B.

In addition to the bulk protein composition, the occurrence of specific proteins that have been implicated as auxiliary factors in the fractionation profile was evaluated. UV cross-linking analysis demonstrated high levels of p66 and p44 apoB RNA cross-linking proteins in the affinity-purified fraction (Fig. 8). A low cross-linking activity corresponding to where APOBEC-1 migrated was observed, and in addition, cross-linking of apoB RNA to a 55-kDa protein and a 100-kDa protein was apparent. Furthermore, these RNA cross-linking proteins had distinct distributions during salt fractionation. As shown in Fig. 8, EF1 was enriched in p66, p100, and APOBEC-1, whereas EF2 contained the bulk of p44 and p55. The separation of p66/p44 by salt extraction demonstrated for the first time that these proteins were capable of independent cross-linking to apoB RNA.

Cross-linking with all five proteins could be competed with a 70-fold excess of cold apoB RNA (Fig. 9A), but only p100, p66, p55, and APOBEC-1 resisted competition by a 50-fold excess of cold control WT-1 RNA (Fig. 9B). In contrast, p44 showed significant levels of cross-linking to WT-1 RNA, which could be competed by excess cold apoB RNA (Fig. 9B), suggesting that p44 may have general RNA cross-linking properties and may not be selective for the apoB RNA editing site as previously proposed (9, 22–24).

Finally, we evaluated the occurrence and distribution of p240 during fractionation by Western blotting the fractions with anti-p240 mAb. The data demonstrated that p240 was present in the affinity-purified editing complex, and its association with APOBEC-1 was salt-resistant (Fig. 10).
We have found that preincubation with a mooring sequence containing apoB RNA enhanced the recovery of affinity-purified editing activity but did not affect the amount of APOBEC-1 retained on the TALON™ metal-chelating columns (data not shown). These data corroborate with our previous findings that the mooring sequence plays a pivotal role in editosome assembly (9) and suggest that some or all of the interactions of auxiliary factors with APOBEC-1 are mooring sequence-dependent.

Our data differed from those of Mehta et al. (28) who reported that all of the auxiliary factors sufficient to support APOBEC-1 in editing were eluted with 0.4 M KCl from APOBEC-1 affinity resin adsorbed with baboon kidney extracts. There are many differences in the experimental design between the present study and that of Mehta et al. that may...
account for these disparate observations. As mentioned earlier, the role of post-translational modifications in APOBEC-1 interactions with auxiliary factors has not been evaluated. This raises the question of whether auxiliary factor interactions with \( E. coli \)-expressed APOBEC-1 and mammalian expressed APOBEC-1 are the same at the level of salt sensitivity despite their functional consequence. Second, both approaches yielded functional editing activity, but editosome assembly in our study took place in the presence of apoB RNA substrate. In this regard, the salt-resistant fraction of auxiliary factors seen in our study may reflect interactions arising in editosomes that are engaged with apoB RNA, whereas the conditions of Mehta et al. (28) may reflect the organization of editosomes prior to their interaction with RNA substrate. Third, it may not be valid to assume that identical proteins (or the same number of proteins) are involved just because both tissue extracts can serve as a source of auxiliary factors. Finally, the trivial explanation that the differences in observed salt sensitivity was due to the use of KCl versus NaCl or 400 mM versus 300 mM has not been formally ruled out.

Our data demonstrated that the two presumptive apoB RNA-binding proteins p66/p44 were both present in the affinity-purified editosomes, suggesting that both proteins were affiliated with APOBEC-1. The association of p66 with APOBEC-1 was resistant to 300 mM salt extraction, whereas under the same conditions p44 was separated from APOBEC-1. Cross-linking of p66 to apoB RNA has been shown to be mooring sequence-selective (9, 24). Taken together with kinetic data demonstrating that p66 cross-linking to apoB RNA preceded the onset of editing activity, it is tempting to speculate that p66’s function is to bind the mooring sequence and in some way guide APOBEC-1 dimers (11, 12) to the correct cytidine for editing.

In contrast to previous results with crude extracts (9, 23, 24), RNA competition analyses demonstrated that p44 in the affinity-purified editosomes had general RNA cross-linking activity and was not selective for the mooring sequence. We currently cannot explain why purification of the editosomes affected p44's RNA binding specificity. This does not rule out a role for p44 in the editing reaction, since APOBEC-1 also has general RNA-protein interactions (12, 17). Furthermore, both low and high affinity RNA-protein interactions are known to be involved in several forms of RNA processing (30). Whether p44 is required for apoB mRNA editing remains to be determined.

We have found two additional RNA cross-linking proteins of 100 and 55 kDa in the affinity-purified editing fraction. In previous studies, a 55-kDa RNA cross-linking protein was only occasionally observed in crude extracts, and the significance of its occurrence relative to apoB RNA editing was uncertain (9, 24). Our data demonstrated that although the interactions between p55 and APOBEC-1 or other components were salt-sensitive, it bound to apoB RNA selectively. p100 is a novel RNA cross-linking protein and, like p55, demonstrated selective interactions with apoB RNA. In contrast to p55, p100’s association with APOBEC-1 was resistant to 300 mM salt extraction. The data suggest that p100 may also be involved in the editing process. Failure to detect p100 in past studies may have been due to use of crude extract fractions, particularly if p100 is in low abundance.

Finally, we have demonstrated that p240 is a component of affinity-purified editosomes. Previous data suggested that p240 itself or in complex with other proteins as AUX240 mediated protein-protein interactions necessary for the assembly of fully functional editosomes (26). The selective distribution of p240 as a salt-resistant component of the editosome supports this possibility. The yield of p240 in affinity-purified edito-
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Fig. 9. Selectivity of protein-RNA interactions. The selectivity of UV cross-linking activity is evaluated by competition assays. A, UV cross-linking experiments were conducted as described in the Fig. 7 legend, except using a 70-fold excess of cold apoB RNA as competitor. B, UV cross-linking experiments were performed either on labeled apoB RNA substrate competed with 50-fold excess of cold WT-1 RNA (lanes 1 and 2) or on labeled WT-1 RNA substrate competed with 70-fold excess of cold apoB RNA (lanes 3 and 4).

Fig. 10. The occurrence and distribution of the p240 antigen in each fraction during purification. The indicated fractions prepared as described in the Fig. 7 legend were resolved by 4–15% gradient SDS-PAGE, Western blotted with an anti-p240 mAb, and developed by ECL.

In conclusion, we have used mammalian expressed APOBEC-1 as an affinity ligand to purify auxiliary proteins from rat hepatoma cell extracts. This approach has extended our understanding of the auxiliary factor requirement in five ways. 1) Assembly of functional editosomes by the auxiliary factor(s) and APOBEC-1 is sequence-dependent. 2) These functional complexes can be subdivided into two fractions, EF1 and EF2, based on salt solubility. Neither EF1 nor EF2 is sufficient to support editing activity but establishes full editing function when reconstituted. 3) The affinity-isolated editing activity contains the presumptive editosome assembly factor, p240, and four RNA cross-linking proteins, p100, p66, p55, and p44. 4) p100, p66, and p55 are apoB-selective, RNA cross-linking proteins, whereas p44 has general RNA cross-linking capacity. 5) p66 and p44, previously characterized as simultaneously binding to apoB RNA, are shown here to have independent RNA cross-linking capabilities. These data constitute the strongest support to date for the multiprotein composition of the editosome involved in apoB mRNA editing.

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