

C to U RNA editing mediated by APOBEC1 requires RNA-binding protein RBM47

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Abstract

Cytidine (C) to Uridine (U) RNA editing is a post-transcriptional modification that is accomplished by the deaminase APOBEC1 and its partnership with the RNA-binding protein A1CF. We identify and characterise here a novel RNA-binding protein, RBM47, that interacts with APOBEC1 and A1CF and is expressed in tissues where C to U RNA editing occurs. RBM47 can substitute for A1CF and is necessary and sufficient for APOBEC1-mediated editing *in vitro*. Editing is further impaired in *Rbm47*-deficient mutant mice. These findings suggest that RBM47 and APOBEC1 constitute the basic machinery for C to U RNA editing.

Keywords APOBEC1; Apolipoprotein b; intestine; RBM47; RNA editing

Subject Category RNA Biology

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Introduction

Cytidine (C) to Uridine (U) RNA editing is a mechanism of post-transcriptional regulation of gene activity. It is achieved by the multi-protein 27S editosome [1,2]. Two proteins are known to constitute the holoenzyme of the editosome based on *in vitro* experiments: the deaminase *Apolipoprotein-b*-mRNA-editing-enzyme-1 (APOBEC1) and a cofactor that is believed to be the RNA-binding protein (RBP) APOBEC1-Complementation-Factor (A1CF; also known as ACF) [3–8].

While other transcripts that are subjected to this modification have been identified [9], editing of the *Apolipoprotein-b* (*Apob*) messenger (m)RNA is the most widely studied example of C to U RNA editing [8]. It results in the production of two protein isoforms, APOB100 and APOB48 that are both involved in the regulation of lipid metabolism [10,11]. APOB100 is produced in the liver, while APOB48 is synthesised in the small intestine as a result of C to U editing at position 6666 of *APOB* mRNA that creates a premature UAA stop codon [8,10].

We identified a novel RBP, RNA-Binding-Motif-protein-47 (RBM47), in a screen for genes that are preferentially expressed in

the foregut endoderm of embryonic day (E) 8.5 mouse embryos [12]. Here, we show that RBM47 interacts with APOBEC1 and A1CF but can also substitute for A1CF in the holoenzyme of the editosome to act with APOBEC1 in editing *Apob* transcripts *in vitro*. The disruption of RNA editing in *Rbm47*-deficient mice showed that RBM47 is indispensable for *Apob* editing *in vivo*. RBM47 also promotes C to U editing of four other transcripts tested in our study. Taken together, our results demonstrate that RBM47 is a novel RBP that is essential for C to U RNA editing.

Results

RBM47 is a RNA-binding protein

Rbm47 encodes a 64 kDa protein that contains three RNA recognition motifs (RRM) (Fig 1A). RBM47 proteins are found in multiple vertebrate species. Mouse and human RBM47 are 94.3% identical (Supplementary Fig S1A). To examine the expression pattern and the function of RBM47, we used an anti-RBM47 antibody that recognises purified HIS₆-RBM47 (Supplementary Fig S2) and RBM47 in lysate of 3T3 cells transfected with a *Rbm47* expression vector (Supplementary Fig S3A). Consistent with the known expression of *Rbm47* in the endoderm of the mouse embryo [12], RBM47 was detected in Caco-2 cells, which are human epithelial colorectal adenocarcinoma cells (Supplementary Fig S3B). RBM47 can be immunoprecipitated from Caco-2 cells (Supplementary Fig S3C), and RNA bound to RBM47 was detected in a cross-link immunoprecipitation (CLIP) assay [13] (Fig 1B). Similarly, RNA was detected by CLIP using an antibody that recognises two known RBPs, hnRNP1 and C2 (Fig 1B). No RNA was detected without cross-linking or by using an antibody that does not recognise any RBP. These findings suggest that RBM47 is a RBP.

The functional attributes of a RBP are reflected by its subcellular localisation. In Caco-2 cells, RBM47 was found in the nucleus. Weaker immunofluorescence was observed in the cytoplasm (Fig 1C). A similar subcellular distribution was observed for a RBM47-GFP fusion protein expressed in Caco-2 cells and in 3T3 embryonic fibroblasts (Fig 1D). These results were corroborated by

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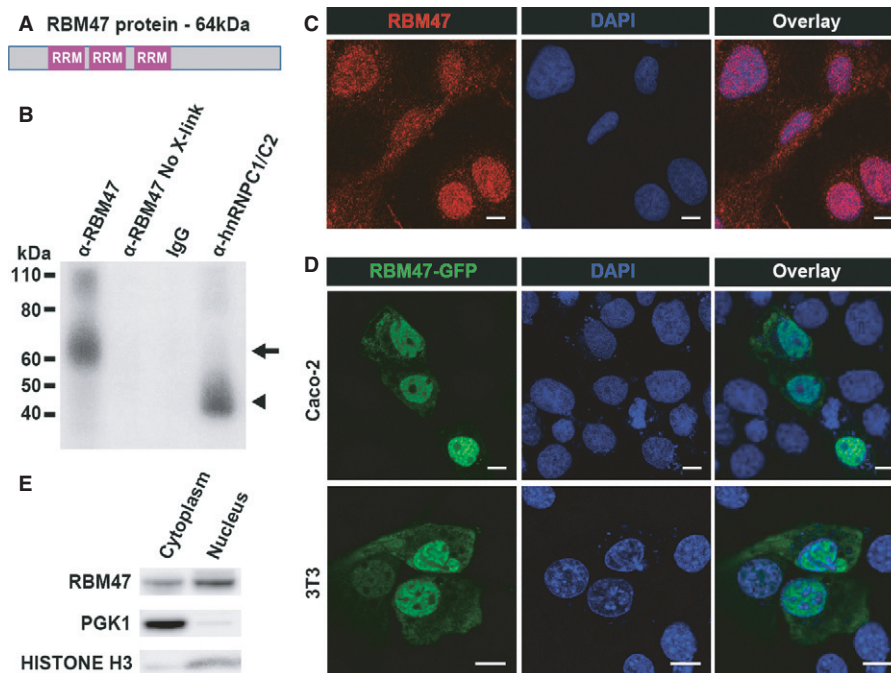


Figure 1. RBM47 is a RNA-binding protein that is more abundant in the nucleus.

- A RBM47 protein characteristics. RRM, RNA recognition motif.
- B RBM47-RNA complexes (arrow) or hnRNPC1/C2-RNA complexes (arrowhead) detected in CLIP assays performed with the antibodies indicated. IgG stands for non-specific antibody. No X-link indicates an IP experiment done without cross-linking.
- C RBM47 (immunostaining) and nuclei (DAPI staining) visualised by confocal microscopy in Caco-2 cells. Scale bars: 10 μ m.
- D RBM47-GFP and nuclei (DAPI staining) visualised by confocal microscopy in Caco-2 and 3T3 cells transfected with a pCMV-*Rbm47*-GFP expression vector. Scale bars: 10 μ m.
- E Western blot analysis of RBM47, PGK1 and Histone H3 in the cytoplasmic and the nuclear protein fractions isolated from Caco-2 cells, with the same amount of protein samples loaded on the gel.

the detection of more RBM47 in the Histone H3-containing nuclear protein fraction than the PGK-containing cytoplasmic fraction of the Caco-2 cells (Fig 1E).

RBM47 co-localises and interacts with APOBEC1 and A1CF

Sequence analysis showed that RBM47 is closely related to A1CF (Supplementary Fig S1B). They are 47.9% identical, and both have three RRM. The sequences of the RRM are 74.9% identical. In view of the role of A1CF as cofactor of APOBEC1 for C to U RNA editing, we investigated whether RBM47 may have a similar function. C to U RNA editing takes place in the epithelial cells of the small intestine [8]. *Rbm47* expression was detected with *Apobec1* and *A1cf* in human small intestine and epithelial cells isolated from mouse small intestine (Fig 2A and B). *Apobec1*-FLAG, -V5 or *A1cf*-V5 expression vector was co-transfected with *Rbm47*-GFP or -HA expression vector in Caco-2 cells. Confocal immunofluorescence microscopy revealed the co-localisation of RBM47-GFP with APOBEC1-FLAG and A1CF-V5 (Fig 2C and D). Immunoprecipitation followed by Western blot analysis further showed that RBM47-HA was specifically and reciprocally co-immunoprecipitated with APOBEC1-V5 and A1CF-V5 (Fig 2E and F). Two RBM47 mutant isoforms of RBM47 were generated: the 3RRM-RBM47 variant form containing only the three RRM and the Δ RRM-RBM47 variant which lacked the RRM (Fig 2G). In co-immunoprecipitation

experiments, none of the isoforms showed an interaction with APOBEC1-V5 (Fig 2H and I), while Δ RRM-RBM47-HA (Fig 2J), but not 3RRM-RBM47-HA (Fig 2K), was immunoprecipitated with A1CF-V5. To confirm these results, we also performed a two-hybrid assay. AH109 yeast strain grows on SD/-Leu/-Trp media when co-transfected with pGADT7 and pGBT7 plasmids, but only grows on SD/-Leu/-Trp/-Ade/-His media if both proteins produced by the plasmids interact. Using this method, we confirmed that RBM47 interacts with APOBEC1 and A1CF and that Δ RRM-RBM47 interacts with A1CF (Fig 2L). In this case, we also showed an interaction with Δ RRM-RBM47 and APOBEC1 that appeared weaker and may explain why it was not detected by co-immunoprecipitation. Similar to the co-immunoprecipitation experiments, we did not observe any interaction of 3RRM-RBM47 with APOBEC1 or A1CF (Fig 2L). These results suggest that RBM47 interacts with APOBEC1 and A1CF using a domain that is not the RRM. Finally, we investigated the interaction between the endogenous proteins in epithelial cells isolated from the small intestine. Unfortunately, none of the commercially available anti-APOBEC1 antibodies that we have tested recognised endogenous APOBEC1. However, one anti-A1CF antibody recognised A1CF. Using this antibody and the anti-RBM47 antibody, we demonstrated an interaction between RBM47 and A1CF (Fig 2M). Collectively, these results show that RBM47 may be a component of the editosome complex.

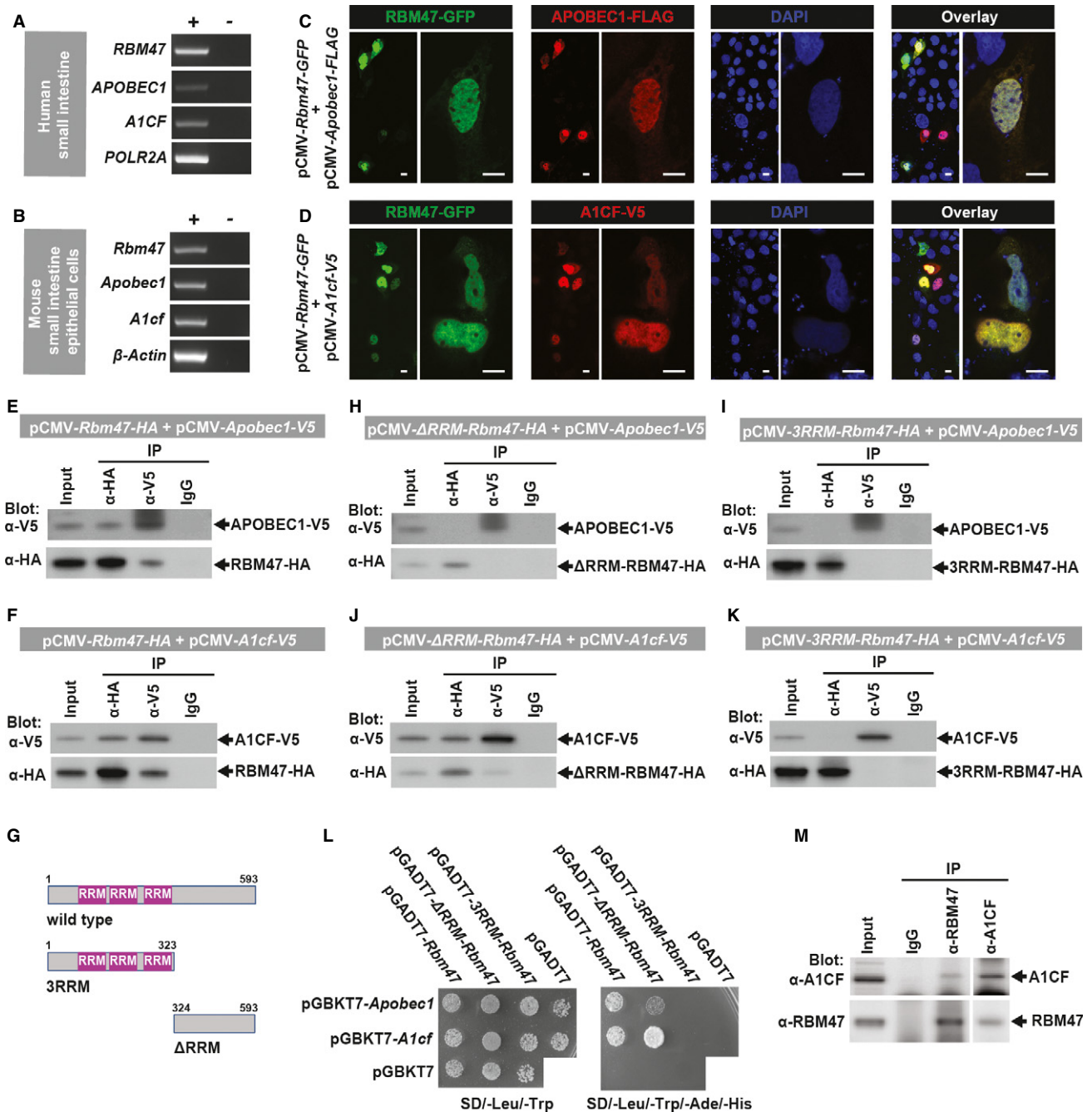


Figure 2. RBM47 co-localises and interacts with APOBEC1 and A1CF.

A, B Detection of *RBM47*, *APOBEC1*, *A1CF* and *POLR2A* expression in human small intestine tissue by RT-PCR (A). Detection of *Rbm47*, *Apobec1*, *A1cf* and β -Actin expression in epithelial cells isolated from mouse small intestine by RT-PCR (B). The PCR was performed with (+) or without (-) cDNA.

C, D RBM47-GFP, APOBEC1-FLAG (immunostaining; C), A1CF-V5 (immunostaining; D) and nuclei (DAPI staining) visualised by confocal microscopy in Caco-2 cells transfected with the expression vectors indicated. Scale bars: 10 μ m.

E, F Detection of RBM47-HA (Blot: α -HA) and APOBEC1-V5 (E; Blot: α -V5) or A1CF-V5 (F; Blot: α -V5) after IP of lysates of transfected Caco-2 cells. Expression vectors used for transfection and antibodies used for IP are indicated. IgG stands for non-specific antibody.

G Characteristics of RBM47 mutant isoforms. RRM, RNA recognition motif.

H-K Detection of Δ RRM-RBM47-HA (H, J; Blot: α -HA) or 3RRM-RBM47-HA (I, K; Blot: α -HA) and APOBEC1-V5 (H, I; Blot: α -V5) or A1CF-V5 (J, K; Blot: α -V5) after IP of lysates of transfected Caco-2 cells. Expression vectors used for transfection and antibodies used for IP are indicated. IgG stands for non-specific antibody.

L Two-hybrid assay where the growth of the AH109 yeast tester strain on synthetic dropout media -leucine -tryptophan (SD/-Leu/-Trp) or synthetic dropout media -leucine -tryptophan -adenine -histidine (SD/-Leu/-Trp/-Ade/-His) was assessed after co-transfection with the expression vectors indicated.

M Detection of RBM47 and A1CF after IP of lysates of epithelial cells isolated from mouse small intestine. Antibodies used for IP are indicated. IgG stands for non-specific antibody. Note: the three first lanes (Input, IP IgG and IP α -RBM47) and the last lane (IP α -A1CF) are from the same blot.

RBM47 mediates C to U editing

In view of *Apob* RNA being the most studied RNA to be C to U edited, we tested whether RBM47 could bind to *Apob* RNA. By RNA Immunoprecipitation (RIP)-Polymerase Chain Reaction (PCR), we showed that *Apob* RNA was specifically immunoprecipitated with RBM47 from Caco2 cell extracts (Fig 3A).

We then tested whether RBM47 may play a role in the editing of *Apob* RNA. We expressed a fragment of *Apob* that contained the editing site C 6666 together with APOBEC1, RBM47 and A1CF in 3T3 cells that normally do not express *Apob* or the C to U editing factors. A primer extension assay was performed to detect both unedited and edited *Apob* RNA isoforms (Fig 3B) [2]. As predicted, the presence of *Apob* RNA alone or together with either APOBEC1 or A1CF did not elicit editing, whereas *Apob* RNA was edited in the presence of both APOBEC1 and A1CF (Fig 3C). RBM47 alone did not produce any significant editing of *Apob* (Fig 3C). However, when RBM47 was co-expressed with APOBEC1, *Apob* RNA was edited (Fig 3C). This suggests that RBM47 can mediate C to U editing activity of APOBEC1. When combined with A1CF, no significant enhancement of the editing elicited by RBM47 and APOBEC1 was observed (Fig 3C). Furthermore, no to very little editing activity was detected when 3RRM-RBM47 or ΔRRM-RBM47 mutants (Fig 2G) were expressed in combination with APOBEC1 (Fig 3C). However, we cannot exclude that the lack of editing by ΔRRM-RBM47 may be due to inefficient expression compared to RBM47 or 3RRM-RBM47

(Fig 3C, HA Blot). Nevertheless, our results suggest that both parts of RBM47 are required for editing as RBM47 needs to bind both APOBEC1 and *Apob* RNA to elicit C to U editing.

To confirm that RBM47 together with APOBEC1 is sufficient to mediate editing, we tested their activity in an *in vitro* cell-free editing assay [14,15] using purified recombinant proteins (Supplementary Fig S2) and a fragment of *Apob* RNA containing C 6666. No editing was observed when *Apob* RNA was present alone or with only one of the three proteins. *Apob* RNA editing was detected in the presence of APOBEC1 with A1CF or APOBEC1 with RBM47 (Fig 3D). As in the 3T3 cells, editing elicited by RBM47 and APOBEC1 was not enhanced by additional A1CF (Fig 3D). These results demonstrate that RBM47, in combination with APOBEC1, is sufficient to mediate the C to U editing of *Apob* RNA and can act in lieu of A1CF.

RBM47 is essential for C to U RNA editing

Rbm47 expression was detected in the foregut and the yolk sac of E8.5 mouse embryos (Fig 4A), consistent with our previous microarray results [12]. At E9.5, *Rbm47* was expressed in the pharyngeal pouches, the gut and the organ rudiments of the liver and the pancreas (Fig 4B). *Rbm47* was weakly expressed in the anterior forebrain, the otic vesicle and the dorsal midline. In adult mice, RBM47 protein was present in endoderm derivatives such as the small intestine, the liver, the pancreas and the lung (Fig 4C and

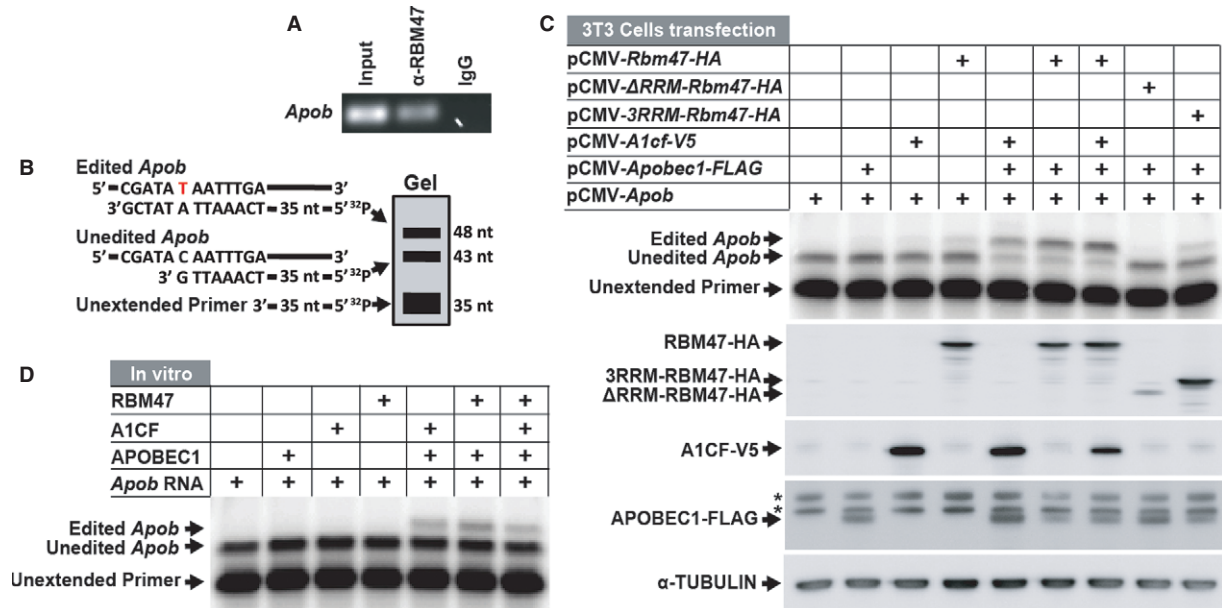


Figure 3. RBM47 mediates C to U RNA editing.

- A *Apob* RNA detected by RT-PCR after RIP experiment performed on Caco-2 cells with antibodies indicated. IgG stands for non-specific antibody.
- B Schematic representation of the primer extension assay. A 35-nucleotide (nt) antisense primer radiolabelled at the 5' end with ³²P anneals 8 nt downstream of the editing site of *Apob* cDNA. It is extended until the addition of a ddGTP resulting in the extension of the primer to 43 nt for unedited *Apob* and to 48 nt for edited *Apob*. The extended primers as well as the excess of unextended primer are separated and visualised on acrylamide-urea gel.
- C Detection of edited and unedited *Apob* RNA by primer extension assay of *Apob* RNA extracted from 3T3 cells that were transfected with the expression vectors indicated (+). Gels below show the result of Western blot (with anti-HA, anti-V5, anti-FLAG and anti-α-tubulin) of the proteins produced in the transfected 3T3 cells. * mark non-specific bands.
- D Detection of edited and unedited *Apob* RNA by primer extension assay of *Apob* RNA synthesised *in vitro* and incubated by itself or with the recombinant proteins indicated (+).

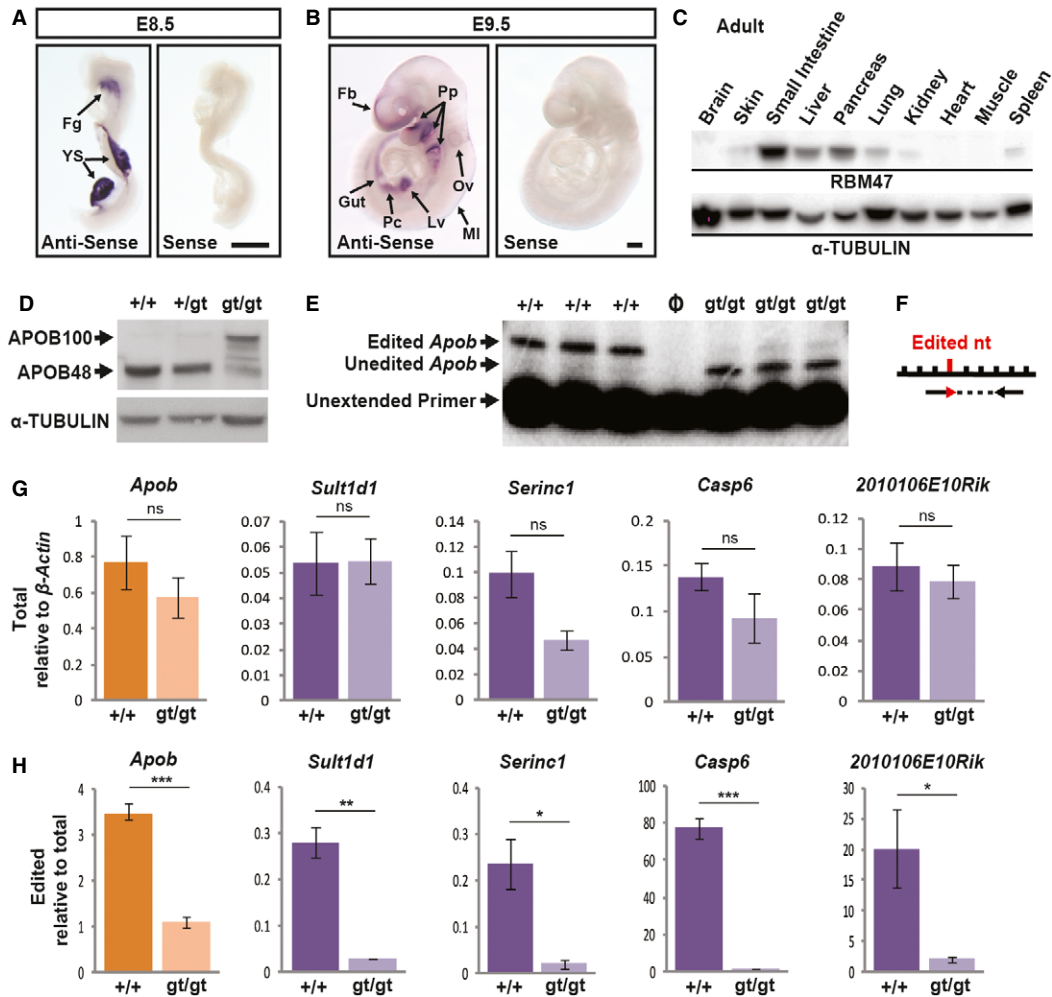


Figure 4. RBM47 is essential for C to U RNA editing in mouse.

A, B *Rbm47* expression visualised by *in situ* hybridisation with antisense probe in E8.5 (A) and E9.5 (B) mouse embryos. *In situ* hybridisation controls performed with a sense probe are also shown. Side view; Anterior to the left. Fb, Forebrain; Fg, Foregut; Lv, Liver; MI, Midline; Ov, Otic vesicle; Pc, Pancreas; Pp, Pharyngeal pouches; YS, Yolk Sac. Scale bars: 200 μ m.

C Detection of RBM47 and α -tubulin by Western blot of protein extracts from organs of adult mouse.

D Visualisation of APOB isoforms and α -tubulin by Western blot of protein extracts from small intestine of adult *Rbm47*^{+/+}, *Rbm47*^{+/^{gt}} and *Rbm47*^{gt/^{gt}} mice.

E Detection of edited and unedited *Apob* RNA by primer extension assay on RNA extracted from small intestine of adult *Rbm47*^{+/+} and *Rbm47*^{gt/^{gt}} mice. Φ : Assay performed without RNA.

F RT-PCR strategy to detect edited RNA isoform. The last nucleotide at the 3' end (red triangle) of one of the two primers for real-time RT-PCR is complementary to the edited nucleotide (red bar) of the edited molecule.

G, H Real-time RT-PCR quantitation of *Apob*, *Sult1d1*, *Serinc1*, *Casp6* and *2010106E10Rik* expression relative to β -Actin expression (G) and edited RNA isoform relative to total expression of both edited and unedited isoforms (H) in the small intestine of adult *Rbm47*^{+/+} and *Rbm47*^{gt/^{gt}} mice. *N* = 3 mice for each genotype. The data represent the mean \pm standard errors of triplicate real-time RT-PCR analysis for each mouse. *P*-value < 0.05 (*), < 0.01 (**), < 0.001 (***) and > 0.05 (no significant; ns) by *t*-test.

Supplementary Fig S3D). It was also detected in other organs such as the kidney, the spleen and the skin, but not in the brain, the heart or the skeletal muscle. Of particular interest, RBM47 protein was detected in the small intestine which in human is the only tissue displaying active C to U RNA editing [8].

To elucidate the function of RBM47 in RNA editing in the gut tissues, we generated a mutant mouse harbouring a gene-trap (gt) mutant allele that expressed β geo instead of *Rbm47* (Supplementary Fig S4). β geo expression recapitulated the endogenous *Rbm47* expression pattern (Supplementary Fig S4B–E). *Rbm47*^{+/^{gt}} mutant mice were healthy and fertile. *Rbm47*^{gt/^{gt}} mutants were functionally

null, showing no detectable *Rbm47* mRNA or RBM47 protein (Supplementary Fig S4F–H). The majority of *Rbm47*^{gt/^{gt}} mice died before birth (Supplementary Fig S4I). Some survived beyond weaning (Supplementary Fig S4J) and showed no *Rbm47* activity (Supplementary Fig S4H). The small intestine of these mice was used for the analysis of the editing activity of RBM47 *in vivo*.

As expected, APOB48 (encoded by edited *Apob*) was the main isoform detected in the small intestine of wild-type mice (Fig 4D). The same was true for *Rbm47*^{+/^{gt}} mice suggesting that one functional *Rbm47* allele is sufficient for RNA editing. In *Rbm47*^{gt/^{gt}} mutants, APOB100 (encoded by the unedited mRNA) was the

predominant isoform though some APOB48 could still be detected (Fig 4D). While the total amount of *ApoB* RNA was not changed (Fig 4G), editing was highly reduced in *Rbm47^{gt/gt}* mutant tissues compared to wild-type mice where nearly all *ApoB* RNA was edited (Fig 4E). These results highlight a key role of RBM47 in C to U editing of *ApoB* RNA in the mouse.

We investigated further whether RBM47 had a wider editing role on other potential RNA targets [9]. Four of these targets, *Sult1d1*, *Serinc1*, *Casp6* and *2010106E10Rik*, were chosen for analysis on the basis that they are expressed and edited in the small intestine [9]. C to U editing of these targets was analysed by a real-time PCR strategy (Fig 4F). The efficacy of this strategy was verified by the detection of the edited version of *ApoB* RNA that replicated the results of the primer extension assay, and the observation of a reduced amount of edited *ApoB* RNA in *Rbm47^{gt/gt}* mutants (Fig 4H). Using this technique, we showed that, while their level of gene expression was not changed (Fig 4G), the C to U RNA editing of *Sult1d1*, *Serinc1*, *Casp6* and *2010106E10Rik* was significantly reduced in the *Rbm47^{gt/gt}* mutants (Fig 4H). This finding points to a critical role of RBM47 in C to U RNA editing of not only *ApoB*, but other RNA molecules that are targets of this post-transcriptional modification.

Discussion

Among the proteins of the editosome [1,2], only APOBEC1 has been shown to be indispensable for C to U RNA editing *in vivo*. C to U RNA editing is absent in *ApoBec1^{-/-}* mutant mice [9,16–18]. Nevertheless, it is reputed that the holoenzyme of the editosome is composed of APOBEC1 and A1CF [8]. This is based on the observation that A1CF can interact with APOBEC1 and act as its cofactor *in vitro* [5–7]. However, editing is enhanced in *A1cf^{+/-}* mutant mice (*A1cf^{-/-}* mice die early during gestation and thus are not available for studying the editing activity) which is inconsistent with A1CF requirement for editing *in vitro* [19]. Here we demonstrated that RBM47 is a RBP that interacts with APOBEC1. We also showed that RBM47 interacts with A1CF. However, the role of A1CF in the editosome *in vivo* and the functional consequences of its interaction with RBM47 are unclear. On the other hand, we demonstrated that RBM47 can substitute for A1CF as the cofactor of APOBEC1. We also showed that, in agreement with its function *in vitro*, RBM47 is

essential for editing in mouse tissues. RBM47 is the first cofactor of APOBEC1 shown to be necessary for C to U RNA editing *in vivo*. Moreover, we revealed that RBM47 is essential for the editing of other transcripts, suggesting that RBM47 may have a universal function in the C to U RNA editing machinery. RBM47 is important for the production of APOB48. Rosenberg *et al* [9] suggested that C to U editing may also play a role in the stabilisation, the polyadenylation, the translation or the localisation of *Sult1d1*, *Serinc1*, *Casp6* and *2010106E10Rik* mRNA. RBM47 may therefore contribute to these processes. In the light of these results, we propose that RBM47 is the universal cofactor of APOBEC1 and that together they constitute the holoenzyme of the C to U editosome (Fig 5).

Some *Rbm47^{gt/gt}* mice die during embryogenesis. The *ApoBec1^{-/-}* mutant mice are normal and viable despite the absence of C to U editing [9,16–18]. This suggests that the prenatal lethality of the *Rbm47^{gt/gt}* mice is not due to the absence of C to U editing mediated by APOBEC1. *A1cf^{-/-}* mutant mice also die before birth which may indicate a potential functional relationship between RBM47 and A1CF during embryogenesis [19]. However *A1cf^{-/-}* embryos die before implantation, while *Rbm47^{gt/gt}* die after. A recent study has shown that the inactivation of *Rbm47* orthologue with morpholino in zebrafish leads to head truncation [20], a phenotype not found in *Rbm47^{gt/gt}* mouse embryos. Therefore, *Rbm47* is likely to have other roles during mouse development that are independent from C to U RNA editing and still to be discovered.

Materials and Methods

Primary antibodies

The anti-RBM47 antibody was generated by Mimotopes according to standard protocols using peptide whose sequence VYGGYAGYIP-QAFPAA is specific to RBM47 and is located at the carboxy-terminal end of the protein (Supplementary Fig S1). It is a rabbit affinity-purified polyclonal antibody. Other primary antibodies are specific for: GFP (Invitrogen, A11122), hnRNPC1/C2 (Santa Cruz, sc-15386), PGK1 (Abcam, ab38007), Histone H3 (Cell Signaling Technology, 9715), HA tag (Santa Cruz, sc-805; Abcam, ab9134), FLAG tag (Abcam, ab1257, ab1162), V5 Tag (Abcam, ab9116, ab9137), APOB (APOB48 and APOB100; Abcam, ab20737), α -tubulin

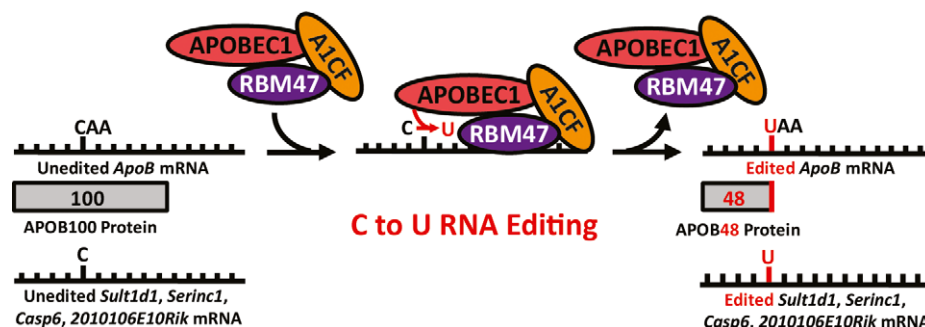


Figure 5. Model of the C to U editosome holoenzyme.

RBM47 constitutes, with APOBEC1, the core component of the editosome. RBM47 can also interact with A1CF. By complexing with both RNA and APOBEC1, RBM47 facilitates APOBEC1-mediated C to U editing of RNA such as those encoded by *ApoB*, *Sult1d1*, *Serinc1*, *Casp6* and *2010106E10Rik*. For *ApoB* RNA, this results in the production of APOB48 instead of APOB100 protein, while in the case of the other transcripts, it may affect their stability, polyadenylation, translation or localisation [9].

(Sigma-Aldrich, T6199), A1CF (Abcam, ab89050) and APOBEC1 (Abcam, ab80493).

Isolation of epithelial cells from the small intestine

Isolation was done according to a protocol described by Rosenberg *et al* [9].

Production and purification of recombinant proteins

RBM47, APOBEC1 and A1CF recombinant proteins were expressed and purified using a protocol adapted from Cohen *et al* [21].

Protein and RNA immunoprecipitation

Protein and RNA were precipitated from Caco-2 or epithelial cells using the anti-RBM47, the anti-HA, the anti-V5 or the anti-A1CF antibodies. Anti-GFP antibody and anti hnRNPC1/C2 antibody were used as negative and positive controls, respectively. For RIP, precipitated RNA was reverse transcribed before PCR. CLIP protocol was adapted from König *et al* [13].

Yeast two-hybrid

Experiments were performed using the GAL4 Matchmaker Two-Hybrid System 3 (Clontech).

Editing experiments and primer extension assay

For the editing experiments in 3T3 cells, the cells were transfected 48 h before RNA extraction. For the editing experiments *in vitro*, *ApoB* RNA was incubated with the recombinant proteins according to a protocol adapted from Blanc *et al* [14] and Mehta and Driscoll [15]. RNA was extracted, purified and reverse transcribed. A 145-bp region of *ApoB* that contains the editing site was amplified by PCR from the cDNA and purified for the primer extension assay. The protocol for the primer extension assay was adapted from Chen *et al* [2].

Rbm47 gene-trap mutant mouse

We obtained the modified *Rbm47*^{+/gt} embryonic stem (ES) cell line (Clone ID #D027B04) from the German Gene-Trap Consortium. *Rbm47*^{+/gt} mutant mouse was generated from the *Rbm47*^{+/gt} ES cells according to standard protocol.

In situ hybridisation, X-Gal staining, histology and immunofluorescence

Whole mount *in situ* hybridisation, X-Gal staining, histology, haematoxylin-eosin staining and immunofluorescence staining were performed as previously described [12].

RT-PCR

RNA was extracted with RNeasy Mini kit (Qiagen) according to manufacturer instructions. RNA from human small intestine was obtained from Clontech. RNA was reverse transcribed using the

SuperScript III First-Strand kit (Invitrogen). Real-time PCR was performed in triplicate from cDNA of each sample.

Plasmids, primers (Supplementary Table S1) as well as other materials and detailed protocols used for experiments are described in the Supplementary Materials and Methods.

Supplementary information for this article is available online: <http://embor.embopress.org>

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Author contributions

NF and PPLT designed research; NF, KT, TR, KB, DL, JBS, MP and VJ performed research; DAFL performed preliminary research; NF, KT, TR and KB analysed data; NF and PPLT wrote the manuscript.

Conflict of interest

The authors declare that they have no conflict of interest.

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