

ADAR2-mediated editing of RNA substrates in the nucleolus is inhibited by C/D small nucleolar RNAs

Patrice Vitali,^{1,3} Eugenia Basyuk,² Elodie Le Meur,⁴ Edouard Bertrand,² Françoise Muscatelli,⁴ Jérôme Cavaillé,¹ and Alexander Huttenhofer³

¹Laboratoire de Biologie Moléculaire des Eucaryotes, Centre National de la Recherche Scientifique (CNRS), UMR 5095, Institut Fédératif de Recherche (IFR) 109, 31062 Cedex Toulouse, France

²Institut de Génétique Moléculaire de Montpellier, CNRS, UMR 5535, Université Montpellier II, IFR 122, 34293 Montpellier Cedex 5, France

³Innbruck Biocentre, Division of Genomics and RNomics, Innsbruck Medical University, 6020 Innsbruck, Austria

⁴Institut de Biologie du Développement de Marseille, Campus de Luminy, 13288 Cedex 9, Marseille, France

Posttranscriptional, site-specific adenosine to inosine (A-to-I) base conversions, designated as RNA editing, play significant roles in generating diversity of gene expression. However, little is known about how and in which cellular compartments RNA editing is controlled. Interestingly, the two enzymes that catalyze RNA editing, adenosine deaminases that act on RNA (ADAR) 1 and 2, have recently been demonstrated to dynamically associate with the nucleolus. Moreover, we have identified a brain-specific small RNA, termed MBII-52, which was predicted to function as a nucleolar C/D RNA, thereby

targeting an A-to-I editing site (C-site) within the 5-HT_{2C} serotonin receptor pre-mRNA for 2'-O-methylation. Through the subcellular targeting of minigenes that contain natural editing sites, we show that ADAR2- but not ADAR1-mediated RNA editing occurs in the nucleolus. We also demonstrate that MBII-52 forms a bona fide small nucleolar ribonucleoprotein particle that specifically decreases the efficiency of RNA editing by ADAR2 at the targeted C-site. Our data are consistent with a model in which C/D small nucleolar RNA might play a role in the regulation of RNA editing.

Introduction

Primary RNA transcripts can undergo adenosine to inosine (A-to-I) RNA editing through hydrolytic deamination at the C-6 atom of adenosine (for reviews see Bass, 2002; Schaub and Keller, 2002; and Maas et al., 2003). These posttranscriptional RNA processing events are catalyzed by specific enzymes called adenosine deaminases that act on RNA (ADARs; Keller et al., 1999; Bass, 2002). Three structurally related members of the ADAR family have been characterized: ADAR1 and 2, which are ubiquitously expressed in many tissues with strong expression in the brain (Wagner et al., 1990); and ADAR3/RED2, which is only expressed in neuronal tissues and has not been proven to be an active enzyme (Melcher et al., 1996a; Chen et al., 2000). A-to-I RNA editing occurs within completely or partially double-stranded (ds) RNA structures. Sites of A-to-I editing are generally found within non-coding sequences such as repetitive sequences, introns, and 3' untranslated regions (Morse et al., 2002; Levanon et al., 2004).

However, a few site-specific edited sites have been also detected within the open reading frame of cellular and viral pre-mRNA transcripts. Because the newly introduced inosine residue is decoded as a guanosine by the translation apparatus, A-to-I RNA editing has the potential to diversify the genetic information so that more than one protein is produced from a single gene (Bass, 2002; Schaub and Keller, 2002; Maas et al., 2003; Seeburg and Hartner, 2003).

In the best understood examples of site-specific A-to-I RNA editing in mammals, ionotropic glutamate receptors (GluRs) and serotonin receptors (5-HT_{2C} isoforms), the functional properties of the respective gene products, are modulated (Sommer et al., 1991; Burns et al., 1997; Seeburg and Hartner, 2003). In the case of GluR, RNA editing at the Q/R site of the GluR-B subunit of an α -amino-3-hydroxy-5-methyl-4-isoxazolepropionate receptor (a Gln codon is changed to an Arg codon) decreases the Ca²⁺ permeability of the receptor (Sommer et al., 1991; Seeburg, 1996). Another edited site within GluR-B RNA is the R/G site (an Arg codon is changed to a Gly codon), resulting in an increase in the rate at which the channels recover from desensitization (Lomeli et al., 1994). In the serotonin 5-HT_{2C} receptor pre-mRNA, five closely spaced ed-

Correspondence to Jérôme Cavaillé: cavaille@ibcg.biotoul.fr

Abbreviations used in this paper: ADAR, adenosine deaminases that act on RNA; CB, Cajal body; CMV, cytomegalo virus; dNTP, deoxynucleotide triphosphate; ds, double stranded; GluR, glutamate receptor; PWS, Prader-Willi syndrome; scaRNA, small CB-specific RNA; snoRNA, small nucleolar RNA.

ited adenosines (A–E sites) alter the coding potential of three codons (see Figs. 1 A and 3 A). By decreasing the efficiency of G-protein coupling, the RNA editing of 5-HT_{2C} gives rise to serotonin receptor isoforms with a reduced constitutive activity (Burns et al., 1997; Herrick-Davis et al., 1999; Niswender et al., 1999; Berg et al., 2001).

The mechanism and the spatio-temporal regulation of site-specific RNA editing (Lomeli et al., 1994; Burns et al., 1997; Barbon et al., 2003; Yang et al., 2004) is still poorly understood. Most site-specific RNA editing requires a partial dsRNA structure that involves the exonic sequence encompassing the edited site(s) and a downstream intronic sequence (Higuchi et al., 1993; Burns et al., 1997). Thus, RNA editing and RNA splicing might interfere with each other or might even be coordinated (Higuchi et al., 2000; Wang et al., 2000; Bratt and Ohman, 2003; Flomen et al., 2004). Accordingly, ADAR1 and 2 are found to be associated with the spliceosome in a large nuclear particle, which is consistent with a physical interaction between editing and splicing machineries (Raittskin et al., 2001). Cellular annealing and unwinding activities affecting the overall stability of the dsRNA structure, which is recognized by ADARs, also play key roles to control RNA splicing and/or RNA editing (Reenan et al., 2000; Bratt and Ohman, 2003). Regulation of the intracellular levels of ADARs is expected to affect the overall efficiency of RNA editing (Kawahara et al., 2003; Yang et al., 2003a). In this line, ADAR2 is able to edit its own pre-mRNA to create a new 3' splice site, the utilization of which results in a frameshift that gives rise to a nonfunctional ADAR2 protein. This autonegative feedback loop might regulate the endogenous level of ADAR2 (Rueter et al., 1999). Surprisingly, the treatment of mice with fluoxetine (a serotonin re-uptake inhibitor) affects the RNA editing of 5-HT_{2C} pre-mRNA, suggesting that RNA editing can be regulated in a serotonin-dependent manner (Gurevich et al., 2002).

In vivo, the subnuclear location where A-to-I RNA editing occurs has not been precisely characterized yet. However, because RNA editing reactions require the formation of an RNA duplex formed by base pairing between intronic and exonic sequences, editing has to occur before RNA splicing. Surprisingly, two observations suggest that nucleolar-associated functions might regulate RNA editing to some extent. First, ADAR1 and 2 are dynamically associated with the nucleolus, and, although a functional role of the nucleolus in the editing process is far from being understood, it has been proposed that nucleolar sequestration of the editing enzymes modulates RNA editing (Desterro et al., 2003; Sansam et al., 2003; Yang et al., 2003b; Nie et al., 2004). Also, we have previously identified a brain-specific C/D RNA, designated as MBII-52, that is predicted to target an A-to-I editing site within the 5-HT_{2C} serotonin receptor mRNA for 2'-O-methylation of the ribose of the edited base. In mammals, site-specific 2'-O-methylations of rRNAs (ribosomal RNAs) and U spliceosomal RNAs are directed by C/D RNAs, with most of them accumulating and functioning in the nucleolus (hence their designation as small nucleolar RNAs [snoRNAs]) and a few of them in the Cajal bodies (CBs; hence their designation as small CB-specific

RNAs [scaRNAs]; Kiss, 2002). These C/D RNAs act through the formation of a canonical RNA duplex in which the nucleotide to be 2'-O-methylated in the target RNA is paired to the fifth position upstream from the conserved D (or D') box sequence motif found in all of the C/D small RNA (Cavaillé et al., 1996; Kiss-Laszlo et al., 1996). Importantly, the nucleotide that is predicted to be modified by MBII-52 C/D RNA is precisely the edited C-site within 5-HT_{2C} pre-mRNA. A 2'-O-methylation of the ribose of an adenosine nucleotide has been shown to specifically decrease the deamination rate of the modified adenosine in vitro (Yi-Brunozzi et al., 1999); thus, by its antisense element, MBII-52 is predicted to negatively regulate pre-mRNA editing at the C-site (Cavaillé et al., 2000).

In this study, we designed several experiments to address unresolved questions regarding the potential involvement of the nucleolar compartment in the control of site-specific RNA editing. Thereby, we wanted to specifically address the following points: (1) Are editing enzymes ADAR1 and 2 functional within the nucleolus? (2) Is MBII-52 a bona fide C/D methylation snoRNA guide? And (3) does expression of MBII-52 snoRNA affect RNA editing at the C-site of a 5-HT_{2C} RNA substrate?

Results

ADAR2, but not ADAR1, can edit nucleolar 5-HT_{2C} and GluR-B dsRNA substrates

Several groups have recently reported that endogenous ADAR1 and 2 display a dynamic nucleolar association, with ADAR2 shuttling between the nucleolus and the nucleoplasm and ADAR1 shuttling between the nucleolus, the nucleoplasm, and the cytoplasm (Desterro et al., 2003; Sansam et al., 2003; Yang et al., 2003b; Nie et al., 2004). Although the functional significance of these observations is unknown, it has been proposed that RNA editing might be regulated by the intracellular compartmentalization of these ADARs (Desterro et al., 2003; Sansam et al., 2003). To investigate whether ADARs are active within the nucleolus, we analyzed RNA editing within a 5-HT_{2C} dsRNA substrate (A–E-sites) and two GluR-B dsRNA substrates (Q/R and R/G sites), transcribed either from a cytomegalo virus (CMV) promoter (Pol II) or a ribosomal gene promoter (Pol I; Fig. 1, A and B). We reasoned that the distinct subcellular location expected for Pol II and Pol I transcripts (i.e., in the nucleoplasm and the nucleolus, respectively; Fig. 1 C) allows discrimination between nucleolar and nucleoplasmic RNA editing. Indeed, previous studies have shown that the majority of Pol I transcripts do not leave the nucleolus to a large extent (Cavaillé et al., 1996; Ganot et al., 1999; Jady et al., 2003). Pol I- and Pol II-5-HT_{2C} minigenes were, therefore, transiently transfected into NIH-3T3 (which contains low levels of endogenous editing activities) together with either hADAR1- or hADAR2-expressing vectors. RNA editing efficiency was measured at each edited site by direct sequencing of the RT-PCR products (Fig. 2 A). Consistent with earlier reports (Burns et al., 1997; Higuchi et al., 2000; Hartner et al., 2004), when nucleoplasmic RNA editing was assayed, we observed that RNA editing at the D-site is mainly

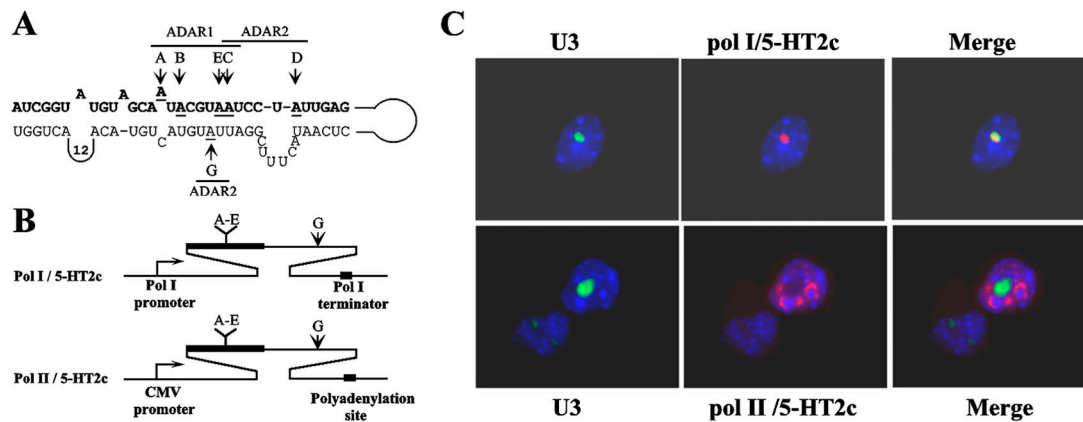


Figure 1. Differential subnuclear locations of Pol I- and Pol II-derived transcripts. (A) RNA stem-loop structure of 5-HT2C formed by exon V (bold characters) and intron 5. Exonic (A–E) and intronic (G; this study) editing sites are underlined, and ADARs specificity is mentioned. 12 indicates the size (in nucleotides) of the bulge. (B) Schematic representation of Pol I/5-HT2C and Pol II/5-HT2C constructs. (C) Pol I/5-HT2C and Pol II/5-HT2C constructs give rise to RNAs that accumulate in the nucleolus and in the nucleoplasm, respectively. In situ hybridization was performed with Cy3-labeled oligonucleotide probes complementary to Pol I and Pol II/5-HT2C RNAs. U3 snoRNA was detected with a Cy5-labeled oligonucleotide probe and was used as a nucleolar marker.

catalyzed by ADAR2, whereas ADAR1 preferentially edits the A- and B-sites; E- and C-sites are catalyzed by both enzymes (Fig. 2 A). In the course of this work, we also detected a novel edited site: the G-site edited by ADAR2 (Fig. 2 A), which maps within an intronic segment that base pairs with the exonic sequence containing the A–E-sites (Fig. 1 A). Intriguingly, this novel edited site was not detected in total RNA that was extracted from mouse brains (unpublished data); thus, its biological relevance is questionable.

Surprisingly, when nucleolar RNA editing was assayed, only adenosines at the D- and G-sites (edited by ADAR2) were found to be significantly converted into inosines ($58.01 \pm 4.6\%$ and $15.3 \pm 3.2\%$, respectively), whereas A- and B-sites (edited by ADAR1) remained fully unedited. Nevertheless, sequencing of the cloned RT-PCR products obtained from the same transfection experiments indicated that $\sim 5\%$ of the C-site was edited by ADAR2 in the nucleolus (unpublished data). Therefore, we reanalyzed RNA editing at the C-site by the more sensitive primer extension method (Burns et al., 1997). As shown in Fig. 2 A (right), nucleolar RNA editing mediated by ADAR2 is readily detected at the C-site ($3.9 \pm 0.63\%$) and also at the E-site, albeit at very low levels.

We also tested whether nucleolar RNA editing can occur at the Q/R and R/G edited sites in GluR-B dsRNA substrates. Again, we found that the Q/R site (only edited by ADAR2 [Higuchi et al., 2000]) as well as the R/G site (edited by ADAR1 and 2 [Maas et al., 1996; Melcher et al., 1996a,b]) are efficiently deaminated by ADAR2 in either Pol II or Pol I/GluR-B transcripts (efficiency of editing at Q/R and R/G sites in the Pol I transcripts is $40.1 \pm 2.65\%$ and $49.85 \pm 13\%$, respectively; Fig. 2 B). In contrast, ADAR1 faithfully edits the R/G site in the Pol II/GluR-B dsRNAs, but not within Pol I transcripts, because it is targeted to the nucleolus (Fig. 2 B). Thus, we conclude that ADAR2 is active within the nucleolar compartment, whereas ADAR1 is not.

Given that Desterro et al. (2003) recently reported that an NH_2 -terminal deletion mutant of ADAR1 (lacking aa 1–442),

termed GFP-ADAR1C-Term, is exclusively targeted to the nucleolus, we tested its ability to be active in RNA editing. Again, although the truncated GFP-ADAR1C-Term protein can edit the A- and B-edited sites as well as the R/G site in a Pol II-transcribed dsRNA substrate, no RNA editing could be detected within the same Pol I-transcribed dsRNA (Fig. 2 C). As controls, we monitored the distribution of the transfected GFP-tagged versions of ADAR2 and ADAR1C-Term. In agreement with an earlier report (Desterro et al., 2003), the two GFP-tagged proteins were mainly detected within the nucleolus (Fig. 2 D). Altogether, these data indicate that although both GFP-ADAR1C-Term and ADAR2-GFP proteins are detected in the nucleolus, only ADAR2 is able to efficiently edit a dsRNA structure within this organelle. It is noteworthy that endogenous levels of ADAR2 in NIH-3T3 do not support an efficient nucleolar RNA editing (Fig. 2 A, right). This is in contrast to the neuroblastoma cell line Neuro2A (unpublished data), suggesting that the intracellular level of endogenous ADAR2 protein might be critical for the nucleolar activity of the enzyme.

MBII-52 exhibits all of the functional properties of a C/D nucleolar methylation RNA guide

In addition to a potential role of the nucleolus in sequestering ADAR1 and 2 activities (Desterro et al., 2003; Sansam et al., 2003) and/or representing one of the nuclear sites of ADAR2-mediated RNA editing (Fig. 2), nucleolar-associated functions might also be required to fine tune RNA editing in a sequence-specific manner. Consistent with that assumption, MBII-52 (a C/D small RNA) displays a long antisense element matching a nucleotide tract within 5-HT2C pre-mRNA. This undergoes both alternative splicing (Canton et al., 1996; Wang et al., 2000) and A-to-I RNA editing (Burns et al., 1997), thus raising the possibility that this small RNA might control 5-HT2C expression (Cavallé et al., 2000; Fig. 3 A). To get better insight into that hypothesis, we first asked whether this C/D RNA belongs to the snoRNA that accumulates within the nucleolus or

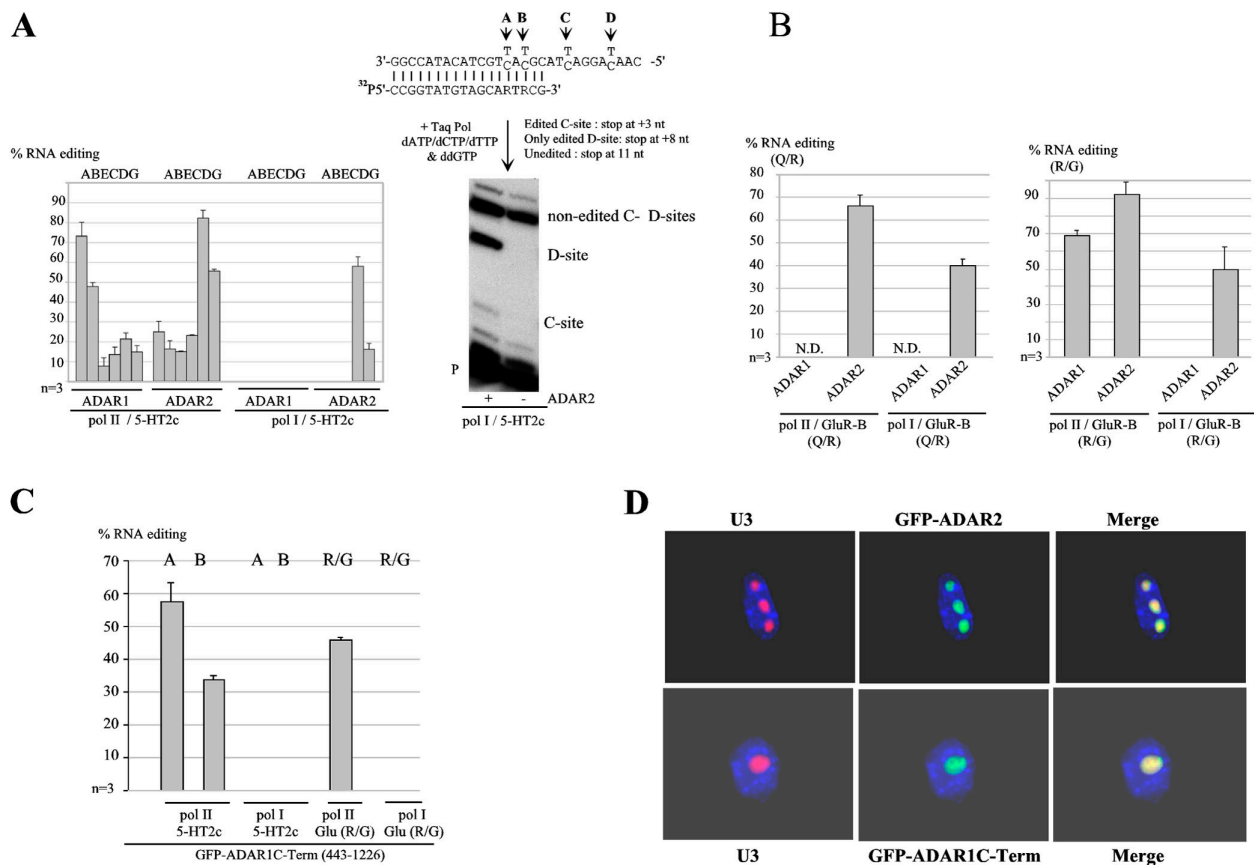


Figure 2. Transfected hADAR2, but not transfected hADAR1 or ADAR1C-Term, is functional in the nucleolus. Histograms show the percentage of RNA editing at each edited adenosine after transient transfection in NIH-3T3 cells with the indicated constructs (A–C). Values represent the mean of three independent experiments ($n = 3$; error bars represent SD). Note: because the Q/R site of GluR-B is specifically edited by ADAR2, the editing efficiency of Pol I and Pol II/GluR-B (Q/R) upon ADAR1 transfection was not determined (ND). (A, right) Primer extension analysis of RNA editing at the C- and D-sites. The nucleotide sequence of the 5' end-labeled (32 P) oligonucleotide sense primer, the antisense strand of the RT-PCR template, and the positions of the editing sites are indicated. P, primer. (D) Subcellular localization of GFP-ADAR2 and GFP-ADAR1C-Term in transiently transfected NIH-3T3. Nucleoli are visualized by a fluorescent oligonucleotide probe (Cy5) directed against U3 snoRNA.

to the scaRNA, a recently identified class of C/D RNAs that are only detected within the CBs (Kiss, 2002). In situ hybridizations with specific Cy3-labeled fluorescent oligonucleotide probes against MBII-52 was performed on adult rat brain sections. As shown in Fig. 3 B (left), MBII-52-specific signals merge perfectly with those of U3 C/D snoRNA, which is known to reside in the nucleolus. In addition, in many cells, this probe also gives rise to a more punctate nucleoplasmic signal outside the nucleolus (Fig. 3 B, right) that has been identified as CBs based on colocalization with coilin, a marker of this nuclear body. Note that MBII-85 is present in the nucleolus of the cell as well (Fig. 3 B). Immunoprecipitation performed from a rat brain total extract also reveals that these two C/D RNAs interact with three common C/D RNA-binding proteins: namely, fibrillarin, NHPX, and NOP58 (Fig. 3 C).

We next examined whether MBII-52 can act as an RNA methylation guide by expressing a predicted, minimal RNA target (Fig. 3 A). A short fragment that was complementary to the antisense elements of MBII-52 was placed under the transcriptional control of a Pol I promoter (Pol I-52 target), which allows specific targeting of the RNA substrate to the nucleolus (Cavaillé et al., 1996; Fig. 1). The plasmid construct was tran-

siently transfected into NIH-3T3 cells in the presence or absence of an MBII-52-expressing vector. The presence of the targeted 2'-O-methylation was then assayed by primer extension analysis, as described previously (Maden, 2001). As shown in Fig. 3 D, a deoxynucleotide triphosphate (dNTP) concentration-dependent pause of reverse transcriptase was mapped at the expected nucleotide only in total RNA that was extracted from NIH-3T3 cotransfected with MBII-52-expressing plasmids. The same vector was also introduced into Neuro2A cells, a neuroblastoma cell line that endogenously expresses MBII-52, albeit at low levels (unpublished data); again, the same dNTP concentration-dependent pause pattern was observed. We conclude that the endogenously expressed MBII-52, as well as MBII-52 expressed from a transfected plasmid, can direct a sequence-specific 2'-O-methylation into an RNA target. Hence, MBII-52 can be considered as a bona fide methylation guide C/D snoRNA.

Partial coexpression of MBII-52 and 5-HT2C serotonin receptor mRNA in the adult mouse brain

A prerequisite for the in vivo role of MBII-52 in the editing of 5-HT2C receptor pre-mRNA is to show the coexpression of

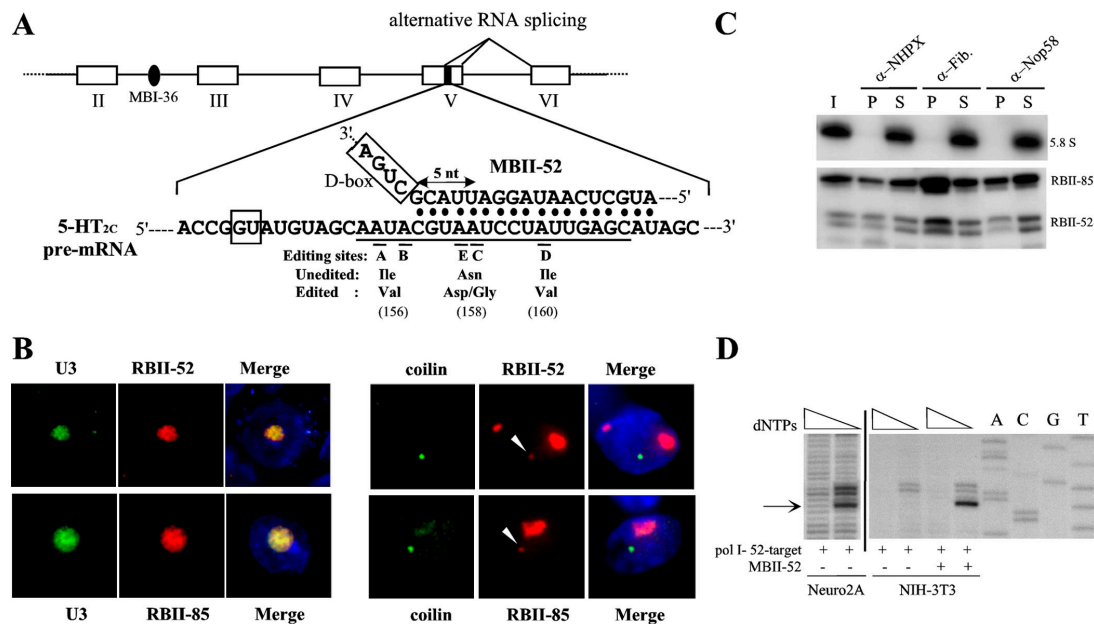


Figure 3. MBII-52 is a bona fide C/D box snoRNA. (A, top) Structure of the mouse serotonin receptor 5-HT_{2C} gene. The exon V segment, including the alternative 5' splice site and the edited sites (A–E-sites), are indicated by a black box, whereas the MBI-36 H/ACA box RNA gene that is encoded within the second intron is shown by a black ellipse (not drawn to scale). (bottom) Predicted base pairing between MBII-52 and 5-HT_{2C}–pre-mRNA. The edited C-site predicted to be targeted by MBII-52 is located at the fifth nucleotide upstream from the D-box. The alternative 5' splicing site is boxed. The editing sites A–E are underlined, and the corresponding amino acid changes in the receptor encoded by the edited and unedited mRNAs are indicated. (B) MBII-52 and MBII-85 can be detected both in the nucleolus and in CBs. (left) In situ hybridization from adult rat brain sections by using specific fluorescent oligonucleotide probes against RBII-52 (Cy3), RBII-85 (Cy3), and U3 C/D snoRNA as a nucleolar marker (Cy5). (right) Same probes were used, except that coilin was used as a CBs marker (immunodetection with anticoinlin antibodies). White arrowheads indicate the position of CBs. (C) MBII-52 and MBII-85 C/D RNA associate with NHPX, fibrillarin, and NOP58. Immunoprecipitation was performed from whole rat brain extracts as previously described (Cavaillé et al., 2001) with anti-NHPX R86, anti-NOP58, and antifibrillarin 72B9. RBII-52 and RBII-85 were detected by Northern blot hybridization with specific ³²P-labeled oligonucleotide probes. 5.8 S rRNA was used as a negative control. I, input (1/10); P, pellet; S, supernatant. (D) Endogenous as well as transfected MBII-52 C/D RNA can direct a sequence-specific 2'-O-ribose methylation only on an RNA target to the nucleolus. NIH-3T3 and Neuro2A cells were transiently transfected as indicated, and the ribose 2'-O-methylated nucleotide was mapped by the primer extension assay method (Maden, 2001). The arrow denotes the position of the dNTP concentration–dependent pause of reverse transcriptase.

both transcripts in the same brain regions. Thus, we performed in situ hybridization on adjacent sections of adult mouse brains with probes specific for MBII-52 and 5-HT_{2C} receptor pre-mRNA to compare their expression patterns. To reveal trace levels of serotonin 5-HT_{2C} expression (which could not have been detected with a 5-HT_{2C} receptor antisense riboprobe), we also used a probe for the H/ACA snoRNA MBI-36. This small RNA is specifically encoded within intron 2 of 5-HT_{2C} pre-mRNA (Fig. 3 A) and accumulates as a stable product concentrated in the nucleus; thus, it was easily detected. MBII-52 is expressed only in neurons of a broad variety of adult mouse brain structures—including the dentate gyrus; CA1, CA2, and CA3 in the hippocampus, granular cell layer, mitral cell layer, and glomerular cell layer in the olfactory bulb; or Purkinje cells in the cerebellum (unpublished data)—but is never expressed in the choroids plexus, whereas the 5-HT_{2C} receptor and MBI-36 are expressed within this structure (Fig. 4, A–C). This is consistent with earlier reports (Molineaux et al., 1989; Cavaillé et al., 2000). However, among some structures where MBII-52 is expressed, we also detected an expression of 5-HT_{2C} and MBI-36 (Fig. 4, D–L). Indeed, in the subthalamic nucleus (Fig. 4, D–F) and the piriform cortex (Fig. 4, G–I), MBII-52, 5-HT_{2C} receptor, and MBI-36 display relevant, overlapping expression patterns. In some other brain structures, such as the

medial and lateral parts of the geniculate nuclei, MBII-52, 5-HT_{2C} receptor, and MBI-36, expression patterns partially overlap with a more restricted expression of 5-HT_{2C} receptor (Fig. 4, J–L). These results are consistent with the ability of MBII-52 to play a role in the processing of 5-HT_{2C} pre-mRNA in the neurons of some adult brain structures.

Nucleolar, but not nucleoplasmic, RNA editing can be specifically inhibited by a targeted C/D snoRNA

Although it is an attractive model, the *in vivo* involvement of MBII-52 in the posttranscriptional regulation of 5-HT_{2C} pre-mRNA expression is difficult to formally prove because: (1) no classical methodology is sensitive enough to reliably identify 2'-O-methylated nucleotides in low-abundance RNA; (2) the transient nature of the *in vivo* interaction between C/D RNA and its RNA target hampers the use of classical biochemical approaches; and (3) no easily tractable cell lines express MBII-52 and 5-HT_{2C} genes at levels comparable with those found in brain tissues (unpublished data). We next asked whether the expression of C/D snoRNAs bearing an antisense element that is designed to target an adenosine to be edited can interfere with its deamination level, as proposed by the model that MBII-52 snoRNA targets 5-HT_{2C} pre-mRNA. To test this possibility,

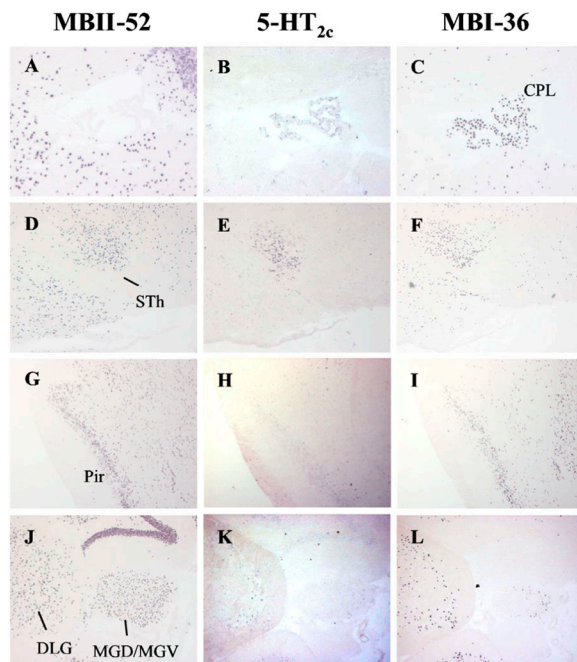


Figure 4. Partial coexpression of MBII-52 and 5-HT2C receptor transcripts in adult mouse brains. In situ hybridization on sagittal and adjacent sections of adult mouse brains confirms that 5-HT2C (B) and MBI-36 (C) are highly expressed in choroids plexus, whereas MBII-52 (A) is not, and also reveals coexpression of MBII-52 (D, G, and J) and 5-HT2C receptor transcripts (E, H, and K) in other discrete regions of adult brains. The snoRNA MBI-36 (F, I, and L), which is present within intron 2 of the 5HT-2C pre-mRNA. MBII-52, 5-HT2C receptor, and MBI-36 are coexpressed in the subthalamic nucleus (D–F), in the piriform cortex (G–I), and in the medial and lateral parts of the geniculate nuclei (J–L). CPL, choroid plexus; STh, subthalamic nucleus; Pir, piriform cortex; DLG, dorsal lateral geniculate nuclei; MGD, medial geniculate nucleus, dorsal part; MGV, medial geniculate nucleus, ventral part.

we first cotransfected either the Pol II/ or Pol I/5-HT2C vector in the presence or absence of an MBII-52 snoRNA-expressing vector into the NIH-3T3 cell line. In addition, cells were cotransfected with a plasmid expressing ADAR2 (Fig. 5 A). Although the expression of MBII-52 does not significantly affect RNA editing at the D- and C-sites in Pol II/5-HT2C RNA, we observed a very substantial decrease at the C-site ($51.3 \pm 1.3\%$) in the Pol I/5-HT2C transcript. Editing at the D-site was unchanged. We also showed that an artificial snoRNA (termed α -D snoRNA) carrying an antisense element directed against the D-site (Fig. 5 A, top) can specifically affect deamination levels at this site, but only when its target was transcribed within the nucleolus (a decrease of $37.8 \pm 2\%$). Finally, the targeting of other artificial C/D snoRNAs (Fig. 5 B, top) against the Q/R or R/G sites within GluR-B dsRNA substrates also resulted in a significant decrease in the nucleolar editing ($49 \pm 5.5\%$ and $56 \pm 6.2\%$, respectively), whereas nucleoplasmic editing was unaffected (Fig. 5 B). In all of these experiments, all transfected C/D snoRNAs were only detected within the nucleolus and were found to be expressed at the same levels as judged by Northern blot analysis (unpublished data). From these data, we conclude that MBII-52 as well as artificial C/D snoRNAs have the potential to inhibit RNA editing within the nucleolar compartment.

Discussion

In recent years, it has become evident that gene expression in eukaryotes is linked to spatial nuclear compartmentalization (Carmo-Fonseca, 2002; Chubb and Bickmore, 2003). One of the best known examples is the nucleolus, which plays a key role in ribosome biogenesis as well as in a broad range of cellular processes, RNA-processing steps, and nuclear functions (Olson et al., 2002). The nucleolus is a nonmembrane-bound nuclear structure, and although its organization is not completely understood, recent studies lead to the view that there is a constant flux of molecules between the nucleolus and its surrounding nucleoplasmic environment (Phair and Misteli, 2000; Andersen et al., 2005). In this line, ADARs, the enzymes that catalyze A-to-I RNA editing, display a complex subcellular compartmentalization, with ADAR2 being localized in the nucleolus as well as in the nucleoplasm, whereas ADAR1 locates throughout the nucleolus, nucleoplasm, and cytoplasm (Desterro et al., 2003; Sansam et al., 2003; Yang et al., 2003b; Nie et al., 2004). Photobleaching experiments indicate that ADARs are constantly and rapidly shuttling in and out of the nucleolus (Desterro et al., 2003; Sansam et al., 2003). Furthermore, upon the transfection of a Pol II transcript–containing edited site, both ADAR1 and 2 are excluded from the nucleoli, and this translocation step is correlated with an increased RNA editing in the nucleoplasm (Desterro et al., 2003; Sansam et al., 2003). Overall, these data show that the nucleolar association of ADAR enzymes is transient, and the nucleolar environment might merely represent a storage compartment rather than a functional site of RNA editing. However, the RNA editing activities of nucleolar-localized ADAR enzymes have not been determined until now. Through a specific targeting strategy of dsRNA substrates to the nucleolus (Figs. 1 and 2), we showed that ADAR2- but not ADAR1-mediated RNA editing can occur in the nucleolus, raising the possibility that endogenous cellular and/or viral transcripts might be edited within this subnuclear compartment.

The inability of ADAR1 to perform nucleolar RNA editing is unexpected and deserves several comments. Because human ADAR1 and 2 localize in the same subnucleolar compartment (Desterro et al., 2003), a subtle differential intranucleolar distribution between these two enzymes is unlikely to account for differences in their nucleolar editing activities. ADAR1 exhibits unique features compared with other ADAR enzymes, including two Z-DNA-binding protein domains and an additional (third) dsRNA-binding domain. Thus, ADAR1-mediated RNA editing might require strict coupling with the nucleoplasmic environment and/or with the transcribing Pol II apparatus. In this line, ADAR1 in *Xenopus laevis* associates with transcriptionally active lampbrush chromosomes, suggesting that RNA editing by ADAR1 might occur cotranscriptionally (Doyle and Jantsch, 2003). We also cannot rule out the possibility that ADAR1 is posttransductionally modified in the nucleolus, thus rendering it inactive (J. Desterro, personal communication). Finally, the nucleolar form of ADAR1 might be linked to other functions that are not directly related to RNA editing.

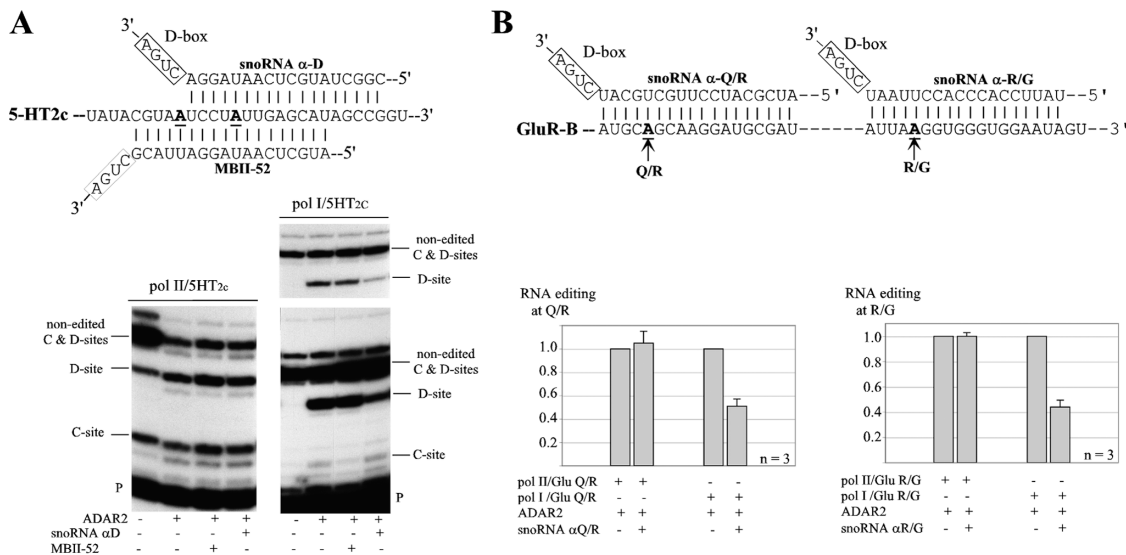


Figure 5. Specific inhibition of ADAR2-mediated nucleolar RNA editing by MBII-52 or artificial C/D snoRNAs. (A and B, top) Canonical base pairing models between the transfected C/D snoRNAs and their RNA targets. Nucleotides that are potentially targeted by these snoRNAs are underlined and indicated in bold. (A, bottom) Primer extension analysis of RNA editing performed on total RNA extracted from NIH-3T3 cells transfected with the plasmids indicated below. Experiments ($n = 3$) were performed as described in Fig. 2 A, and only a representative autoradiogram is shown (in the case of Pol I transcript, two different time exposures of the same gel are shown for the D-site). Quantification data are indicated in the text. It is noteworthy that in the primer extension assay, RNA species edited at both C- and D-sites cannot be detected, and, thus, deamination efficiency at the D-site was determined by direct sequencing of RT-PCR products as described in Fig. 2 A. P, primer. (B, bottom) Histograms showing percentage of RNA editing at the Q/R and R/G sites were determined by the direct sequencing of RT-PCR products obtained from NIH-3T3 that was transiently transfected by the plasmids as indicated below.

Another objective of this study was to investigate the functional relevance of the putative MBII-52/5-HT2C interaction, specifically its capacity to interfere with RNA editing. By localization and associated protein-binding partners, MBII-52 is a bona fide methylation guide C/D snoRNA (Fig. 3) that displays a partially overlapping expression pattern with its putative RNA target in some regions of the adult mouse brain (Fig. 4). Remarkably, our study shows that MBII-52 RNA constructs and artificial C/D snoRNAs reduce ADAR2-mediated nucleolar editing of an RNA substrate in a sequence-specific manner (Fig. 5), whereas nucleoplasmic RNA editing remains unaffected. Artificial C/D snoRNAs have been previously reported to target 2'-O-methylations at ectopic positions in rRNAs or in artificial RNAs (Cavaillé et al., 1996; Kiss-Laszlo et al., 1996; Liu and Fournier, 2004), but to our knowledge our study represents the first demonstration that expression of a cognate C/D snoRNA inhibits a specific RNA processing event in mammalian cells. Together, our results are consistent with a model in which MBII-52 has the potential to inhibit RNA editing in vivo.

We are making the assumption that endogenously expressed 5-HT2C pre-mRNA might be targeted by MBII-52, resulting in decreased RNA editing at the cognate C-site of pre-mRNA. This interaction might eventually fine tune the G-protein coupling of the encoded serotonin 5-HT2C receptor. Indeed, the edited C-site plays (together with the E-site) a pivotal role in regulating serotonergic signal transduction (Burns et al., 1997; Niswender et al., 1999; Wang et al., 2000; Gurevich et al., 2002). Our model implies an at least transient localization of 5-HT2C pre-mRNA in the nucleolus. How this would be achieved is not addressed in this study. It is noteworthy,

however, that pre-mRNA splicing can sometimes be uncoupled from cotranscriptional events and can occur away from the transcription sites (Lopez and Seraphin, 2000). In addition, 5-HT2C pre-mRNA hosts an H/ACA snoRNA within intron 2 (Fig. 3 A), which could promote the targeting of intron-containing pre-mRNA to the nucleolus (a mechanism previously observed in plants; Shaw et al., 1998). In vivo interaction between MBII-52 and its putative RNA target is likely to be complex and could involve distinct cellular regulatory pathways, according to the brain regions in which both transcripts are expressed (Fig. 4). Thus, at this point, we cannot rule out the alternative possibility (e.g., that under some circumstances not mimicked by our experimental transfection assay) that MBII-52 C/D small nucleolar ribonucleoprotein particles might have the ability to function outside of the nucleolus.

The human homologues of MBII-52 genes are encoded at the imprinted 15q11-q13 chromosomal region. This locus is associated with the Prader-Willi syndrome (PWS), which is a neurobehavioral disorder characterized by a complex phenotype, including hyperphagia and behavioral problems with obsessive compulsive disorder (Nicholls and Knepper, 2001). Although the lack of expression of HBII-52 genes is not sufficient to result in PWS (Runte et al., 2005), it is noteworthy that a knockout mice model for 5-HT2C genes leads to epilepsy, to eating disorders as a result of abnormal control over feeding behavior (Tecott et al., 1995), and to compulsive behavior symptoms (Chou-Green et al., 2003) that are described in PWS patients. Hence, it is tempting to speculate that defects of HBII-52 expression, which are presumably associated with the lack of expression of additional paternally expressed genes, might contribute to some aspects of the PWS disease.

Materials and methods

Unless otherwise noted, all techniques for cloning and manipulating nucleic acids were performed according to standard protocols.

Oligonucleotides

Modified and unmodified oligonucleotides were synthesized by Y. de Préval (Laboratoire de Biologie Moléculaire des Eucaryotes, Toulouse, France) on a PerSeptive Biosystems Expedite apparatus or at the MWG Biotech AG.

Plasmid constructs

5-HT2C and GluR-B dsRNA substrates were PCR amplified from rat and mouse genomic DNA, respectively, and were cloned into pW, a ribosomal minigene (Cavaillé et al., 1996), to generate Pol I/5-HT2C, Pol I/GluR-B (Q/R), and Pol I/GluR-B (R/G) vectors. The same PCR products were also inserted under the control of a CMV promoter into pcDNA 3.1 (Invitrogen) to generate Pol II/5-HT2C, Pol II/GluR-B (Q/R), and Pol II/GluR-B (R/G). Minigene Pol I-52 target was derived from pW by inserting a synthetic 20-bp DNA sequence of 5HT2C (Fig. 3 A, underlined sequence). pCMV snoRNA α -D, pCMV snoRNA α -Q/R, and pCMV snoRNA α -R/G were all derived from pCMV-MBII-52 vectors (Cavaillé et al., 2000). The mammalian expression vector constructs of hADAR1 (pCS2-FLIS6-hADAR1) and hADAR2 (pCS2-FLIS6-hADAR2a) were gifts from W. Keller (Biozentrum, Basel, Switzerland), and expression vector GFP-ADAR2 and GFP-ADAR1C-Term were provided by M. Carmo-Fonseca (Institute of Molecular Medicine, Lisboa, Portugal).

Cell cultures and transfections

The murine NIH-3T3 and Neuro2A cells were cultured as monolayers in DME (GIBCO BRL) supplemented with 10% FBS (GIBCO BRL) at 37°C and 10% CO₂. DNA for transfection assays was purified using a plasmid maxiprep kit (QIAGEN). Subconfluent cells (~80% of confluency) were transiently transfected (DAC-30 reagent; Eurogentec) with 500 ng dsRNA substrates expressing plasmids and 4 μ g C/D snoRNA-expressing vectors or 4 μ g of empty pcDNA 3.1 (+) for negative controls.

Detection of ribose-methylated nucleotides

2'-O-ribose methylation was analyzed by reverse transcription by using low dNTP concentrations as described in Maden (2001), except that the lowest concentration was 0.01 mM dNTPs.

Analysis of RNA editing

48 h after transfection, total RNA was extracted using the TRIzol reagent (Invitrogen) according to the manufacturer's protocol. Direct sequencing of RT-PCR products was performed using the BigDye terminator cycle sequencing reaction kit (PE Corp.) and was analyzed on a sequencer (model ABI Prism 377; PerkinElmer) using the Lasergene sequence analysis program package. Editing efficiency was quantified by measuring the height of peaks A (no edited site) and G (edited site) in order to determine the G/A + G peak ratio. This ratio was then reported to 100 to determine the percentage of editing at each site. All transfections were independently repeated three times to evaluate the SD. Primer extension assay was also performed as described in Burns et al. (1997) in the presence of 0.2 mM dATP, dCTP, and dTTP and 0.8 mM ddGTP and Taq polymerase ($n = 50$ cycles). Reactions were analyzed by gel electrophoresis on a 15% polyacrylamide gel, and intensities of the bands were quantified by PhosphorImager (model FLA-3000; Fujifilm). RNA editing levels at the C-sites were determined by calculating the ratio of band intensity for each site to the sum of the three band intensities.

In situ hybridization

FISH on fixed cells or rat brain sections was performed as described in Verheggen et al. (2002) by using oligonucleotide-specific probes for Pol I/5HT2C RNA, Pol II/5HT2C RNA, snoRNA MBII-52 and snoRNA MBII-85 (labeled with Fluorolink Cy3; Amersham Biosciences) and the U3-specific modified oligonucleotide with Fluorolink Cy5 (Amersham Biosciences). Nuclear DNA was stained by DAPI. Colorimetric in situ hybridization was performed on adult brains of C57BL/6 mice purchased from Iffa Credo as described in Tiveron et al. (1996). 5-HT2C receptor riboprobe (225 bp) hybridized to the coding sequence of 5-HT2C receptor mRNA (nt 1060–1284 from GenBank/EMBL/DBJ under accession no. NM_008312). MBI-36 and MBII-52 riboprobes were synthesized from pUC18 plasmids, and an 88-bp-long fragment of MBI-36 and a 77-bp-long fragment of MBII-52 were cloned, respectively. Probes were labeled with digoxigenin and were revealed with an antidigoxigenin antibody conjugated with AP.

All results were observable through either a microscope (model Axiophot; Carl Zeiss Microimaging, Inc.) with plan neofluar Ph1 10 \times /0.30 objective lenses or a microscope (model DMRA; Leica) with Leica 100 \times plan Apo 1.4. The Axiophot microscope was equipped with a camera (model Dxm1200; Nikon) and Nikon ACT1 acquisition software, whereas the DMRA microscope was equipped with a camera (model CoolSNAPfx; Roper Scientific) and Metamorph acquisition software.

Immunoprecipitation, immunostaining, and antibodies

C/D small nucleolar ribonucleoprotein particle immunoprecipitations from rat brain extracts have been performed as described in Cavaillé et al., (2001) with monoclonal antifibrillar 72B9 (a gift of M. Pollard, W.M. Keck Autoimmune Disease Center, La Jolla, CA), rabbit anti-NHPX R86 (a gift of L.B. Chen, Harvard University, Boston, MA), and anti-NOP58 (a gift of R. Luhrmann, Max-Planck-Institute of Biophysical Chemistry, Göttingen, Germany). Immunostaining on rat brain sections have been carried out as described in Cavaillé et al. (2001) with a polyclonal anticoinlin antibody (diluted 1:100; a gift of A. Lamond, Wellcome Trust Biocentre, Dundee, UK).

We thank Dr. J.-P. Bachelier and Pr. G. Canal for critical reading of the manuscript. We also thank N. Joseph for her excellent technical assistance.

Work in the laboratory of J. Cavaillé was supported by the Ministère de l'éducation Nationale, de la Recherche et de la Technologie (Action Concertée Incitative 2003: Biologie cellulaire moléculaire et structurale), and work in the laboratory of A. Huttenhofer was supported by grants from the Deutsche Forschungsgemeinschaft (HU-467/5-1 and 467/5-2) and a grant from the Österreichische Fonds für die Förderung der wissenschaftlichen Forschung (171370). This project (Prader-Willi Syndrome) was also funded by grants from the European Union. The authors declare that they have no competing financial interests.

Submitted: 22 November 2004

Accepted: 14 April 2005

References

- Andersen, J.S., Y.W. Lam, A.K. Leung, S.E. Ong, C.E. Lyon, A.I. Lamond, and M. Mann. 2005. Nucleolar proteome dynamics. *Nature*. 433:77–83.
- Barbon, A., I. Vallini, L. La Via, E. Marchina, and S. Barlati. 2003. Glutamate receptor RNA editing: a molecular analysis of GluR2, GluR5 and GluR6 in human brain tissues and in NT2 cells following in vitro neural differentiation. *Brain Res. Mol. Brain Res.* 117:168–178.
- Bass, B.L. 2002. RNA editing by adenosine deaminases that act on RNA. *Annu. Rev. Biochem.* 71:817–846.
- Berg, K.A., J.D. Cropper, C.M. Niswender, E. Sanders-Bush, R.B. Emeson, and W.P. Clarke. 2001. RNA-editing of the 5-HT(2C) receptor alters agonist-receptor-effector coupling specificity. *Br. J. Pharmacol.* 134:386–392.
- Bratt, E., and M. Ohman. 2003. Coordination of editing and splicing of glutamate receptor pre-mRNA. *RNA*. 9:309–318.
- Burns, C.M., H. Chu, S.M. Rueter, L.K. Hutchinson, H. Canton, E. Sanders-Bush, and R.B. Emeson. 1997. Regulation of serotonin-2C receptor G-protein coupling by RNA editing. *Nature*. 387:303–308.
- Canton, H., R.B. Emeson, E.L. Barker, J.R. Backstrom, J.T. Lu, M.S. Chang, and E. Sanders-Bush. 1996. Identification, molecular cloning, and distribution of a short variant of the 5-hydroxytryptamine2C receptor produced by alternative splicing. *Mol. Pharmacol.* 50:799–807.
- Carmo-Fonseca, M. 2002. The contribution of nuclear compartmentalization to gene regulation. *Cell*. 108:513–521.
- Cavaillé, J., M. Nicoloso, and J.P. Bachelier. 1996. Targeted ribose methylation of RNA in vivo directed by tailored antisense RNA guides. *Nature*. 383:732–735.
- Cavaillé, J., K. Buiting, M. Kiefmann, M. Lalande, C.I. Brannan, B. Horsthemke, J.P. Bachelier, J. Brosius, and A. Huttenhofer. 2000. Identification of brain-specific and imprinted small nucleolar RNA genes exhibiting an unusual genomic organization. *Proc. Natl. Acad. Sci. USA*. 97:14311–14316.
- Cavaillé, J., P. Vitali, E. Basyuk, A. Huttenhofer, and J.P. Bachelier. 2001. A novel brain-specific box C/D small nucleolar RNA processed from tandemly repeated introns of a noncoding RNA gene in rats. *J. Biol. Chem.* 276:26374–26383.
- Chen, C.X., D.S. Cho, Q. Wang, F. Lai, K.C. Carter, and K. Nishikura. 2000. A third member of the RNA-specific adenosine deaminase gene family, ADAR3, contains both single- and double-stranded RNA binding domains. *RNA*. 6:755–767.
- Chou-Green, J.M., T.D. Holscher, M.F. Dallman, and S.F. Akana. 2003. Compulsive behavior in the 5-HT2C receptor knockout mouse. *Physiol. Behav.* 78:641–649.

- Chubb, J.R., and W.A. Bickmore. 2003. Considering nuclear compartmentalization in the light of nuclear dynamics. *Cell*. 112:403–406.
- Desterro, J.M., L.P. Keegan, M. Lafarga, M.T. Berciano, M. O'Connell, and M. Carmo-Fonseca. 2003. Dynamic association of RNA-editing enzymes with the nucleolus. *J. Cell Sci.* 116:1805–1818.
- Doyle, M., and M.F. Jantsch. 2003. Distinct *in vivo* roles for double-stranded RNA-binding domains of the *Xenopus* RNA-editing enzyme ADAR1 in chromosomal targeting. *J. Cell Biol.* 161:309–319.
- Flomen, R., J. Knight, P. Sham, R. Kerwin, and A. Makoff. 2004. Evidence that RNA editing modulates splice site selection in the 5-HT_{2C} receptor gene. *Nucleic Acids Res.* 32:2113–2122.
- Ganot, P., B.E. Jady, M.L. Bortolin, X. Darzacq, and T. Kiss. 1999. Nucleolar factors direct the 2'-O-ribose methylation and pseudouridylation of U6 spliceosomal RNA. *Mol. Cell Biol.* 19:6906–6917.
- Gurevich, I., H. Tamir, V. Arango, A.J. Dwork, J.J. Mann, and C. Schmauss. 2002. Altered editing of serotonin 2C receptor pre-mRNA in the prefrontal cortex of depressed suicide victims. *Neuron*. 34:349–356.
- Hartner, J.C., C. Schmittwolf, A. Kispert, A.M. Muller, M. Higuchi, and P.H. Seeburg. 2004. Liver disintegration in the mouse embryo caused by deficiency in the RNA-editing enzyme ADAR1. *J. Biol. Chem.* 279:4894–4902.
- Herrick-Davis, K., E. Grinde, and C.M. Niswender. 1999. Serotonin 5-HT_{2C} receptor RNA editing alters receptor basal activity: implications for serotonergic signal transduction. *J. Neurochem.* 73:1711–1717.
- Higuchi, M., F.N. Single, M. Kohler, B. Sommer, R. Sprengel, and P.H. Seeburg. 1993. RNA editing of AMPA receptor subunit GluR-B: a base-paired intron-exon structure determines position and efficiency. *Cell*. 75:1361–1370.
- Higuchi, M., S. Maas, F.N. Single, J. Hartner, A. Rozov, N. Burnashev, D. Feldmeyer, R. Sprengel, and P.H. Seeburg. 2000. Point mutation in an AMPA receptor gene rescues lethality in mice deficient in the RNA-editing enzyme ADAR2. *Nature*. 406:78–81.
- Jady, B.E., X. Darzacq, K.E. Tucker, A.G. Matera, E. Bertrand, and T. Kiss. 2003. Modification of Sm small nuclear RNAs occurs in the nucleoplasmic Cajal body following import from the cytoplasm. *EMBO J.* 22:1878–1888.
- Kawahara, Y., K. Ito, H. Sun, I. Kanazawa, and S. Kwak. 2003. Low editing efficiency of GluR2 mRNA is associated with a low relative abundance of ADAR2 mRNA in white matter of normal human brain. *Eur. J. Neurosci.* 18:23–33.
- Keller, W., J. Wolf, and A. Gerber. 1999. Editing of messenger RNA precursors and of tRNAs by adenosine to inosine conversion. *FEBS Lett.* 452:71–76.
- Kiss, T. 2002. Small nucleolar RNAs: an abundant group of noncoding RNAs with diverse cellular functions. *Cell*. 109:145–148.
- Kiss-Laszlo, Z., Y. Henry, J.P. Bachellerie, M. Caizergues-Ferrer, and T. Kiss. 1996. Site-specific ribose methylation of preribosomal RNA: a novel function for small nucleolar RNAs. *Cell*. 85:1077–1088.
- Levanon, E.Y., E. Eisenberg, R. Yelin, S. Nemzer, M. Hallegger, R. Shemesh, Z.Y. Fligelman, A. Shoshan, S.R. Pollock, D. Szybel, et al. 2004. Systematic identification of abundant A-to-I editing sites in the human transcriptome. *Nat. Biotechnol.* 22:1001–1005.
- Liu, B., and M.J. Fournier. 2004. Interference probing of rRNA with snoRNPs: a novel approach for functional mapping of RNA *in vivo*. *RNA*. 10:1130–1141.
- Lomeli, H., J. Mosbacher, T. Melcher, T. Hoger, J.R. Geiger, T. Kuner, H. Monyer, M. Higuchi, A. Bach, and P.H. Seeburg. 1994. Control of kinetic properties of AMPA receptor channels by nuclear RNA editing. *Science*. 266:1709–1713.
- Lopez, P.J., and B. Seraphin. 2000. Uncoupling yeast intron recognition from transcription with recursive splicing. *EMBO Rep.* 1:334–339.
- Maas, S., T. Melcher, A. Herb, P.H. Seeburg, W. Keller, S. Krause, M. Higuchi, and M.A. O'Connell. 1996. Structural requirements for RNA editing in glutamate receptor pre-mRNAs by recombinant double-stranded RNA adenosine deaminase. *J. Biol. Chem.* 271:12221–12226.
- Maas, S., A. Rich, and K. Nishikura. 2003. A-to-I RNA editing: recent news and residual mysteries. *J. Biol. Chem.* 278:1391–1394.
- Maden, B.E. 2001. Mapping 2'-O-methyl groups in ribosomal RNA. *Methods*. 25:374–382.
- Melcher, T., S. Maas, A. Herb, R. Sprengel, M. Higuchi, and P.H. Seeburg. 1996a. RED2, a brain-specific member of the RNA-specific adenosine deaminase family. *J. Biol. Chem.* 271:31795–31798.
- Melcher, T., S. Maas, A. Herb, R. Sprengel, P.H. Seeburg, and M. Higuchi. 1996b. A mammalian RNA editing enzyme. *Nature*. 379:460–464.
- Molineaux, S.M., T.M. Jessell, R. Axel, and D. Julius. 1989. 5-HT_{1c} receptor is a prominent serotonin receptor subtype in the central nervous system. *Proc. Natl. Acad. Sci. USA*. 86:6793–6797.
- Morse, D.P., P.J. Aruscavage, and B.L. Bass. 2002. RNA hairpins in noncoding regions of human brain and *Caenorhabditis elegans* mRNA are edited by adenosine deaminases that act on RNA. *Proc. Natl. Acad. Sci. USA*. 99:7906–7911.
- Nicholls, R.D., and J.L. Knepper. 2001. Genome organization, function, and imprinting in Prader-Willi and Angelman syndromes. *Annu. Rev. Genomics Hum. Genet.* 2:153–175.
- Nie, Y., Q. Zhao, Y. Su, and J.H. Yang. 2004. Subcellular distribution of ADAR1 isoforms is synergistically determined by three nuclear discrimination signals and a regulatory motif. *J. Biol. Chem.* 279:13249–13255.
- Niswender, C.M., S.C. Copeland, K. Herrick-Davis, R.B. Emeson, and E. Sanders-Bush. 1999. RNA editing of the human serotonin 5-hydroxytryptamine 2C receptor silences constitutive activity. *J. Biol. Chem.* 274:9472–9478.
- Olson, M.O., K. Hingorani, and A. Szebeni. 2002. Conventional and nonconventional roles of the nucleolus. *Int. Rev. Cytol.* 219:199–266.
- Phair, R.D., and T. Misteli. 2000. High mobility of proteins in the mammalian cell nucleus. *Nature*. 404:604–609.
- Raitskin, O., D.S. Cho, J. Sperling, K. Nishikura, and R. Sperling. 2001. RNA editing activity is associated with splicing factors in hnRNP particles: the nuclear pre-mRNA processing machinery. *Proc. Natl. Acad. Sci. USA*. 98:6571–6576.
- Reenan, R.A., C.J. Hanrahan, and G. Barry. 2000. The mle (naps) RNA helicase mutation in *Drosophila* results in a splicing catastrophe of the para Na⁺ channel transcript in a region of RNA editing. *Neuron*. 25:139–149.
- Rueter, S.M., T.R. Dawson, and R.B. Emeson. 1999. Regulation of alternative splicing by RNA editing. *Nature*. 399:75–80.
- Runte, M., R. Varon, D. Horn, B. Horsthemke, and K. Buiting. 2005. Exclusion of the C/D box snoRNA gene cluster HBII-52 from a major role in Prader-Willi syndrome. *Hum. Genet.* 116:228–230.
- Sansam, C.L., K.S. Wells, and R.B. Emeson. 2003. Modulation of RNA editing by functional nucleolar sequestration of ADAR2. *Proc. Natl. Acad. Sci. USA*. 100:14018–14023.
- Schaub, M., and W. Keller. 2002. RNA editing by adenosine deaminases generates RNA and protein diversity. *Biochimie*. 84:791–803.
- Seeburg, P.H. 1996. The role of RNA editing in controlling glutamate receptor channel properties. *J. Neurochem.* 66:1–5.
- Seeburg, P.H., and J. Hartner. 2003. Regulation of ion channel/neurotransmitter receptor function by RNA editing. *Curr. Opin. Neurobiol.* 13:279–283.
- Shaw, P.J., A.F. Beven, D.J. Leader, and J.W. Brown. 1998. Localization and processing from a polycistronic precursor of novel snoRNAs in maize. *J. Cell Sci.* 111:2121–2128.
- Sommer, B., M. Kohler, R. Sprengel, and P.H. Seeburg. 1991. RNA editing in brain controls a determinant of ion flow in glutamate-gated channels. *Cell*. 67:11–19.
- Tecott, L.H., L.M. Sun, S.F. Akana, A.M. Strack, D.H. Lowenstein, M.F. Dallman, and D. Julius. 1995. Eating disorder and epilepsy in mice lacking 5-HT_{2c} serotonin receptors. *Nature*. 374:542–546.
- Tiveron, M.C., M.R. Hirsch, and J.F. Brunet. 1996. The expression pattern of the transcription factor Phox2 delineates synaptic pathways of the autonomic nervous system. *J. Neurosci.* 16:7649–7660.
- Verheggen, C., D.L. Lafontaine, D. Samarsky, J. Mouaikel, J.M. Blanchard, R. Bordonne, and E. Bertrand. 2002. Mammalian and yeast U3 snoRNPs are matured in specific and related nuclear compartments. *EMBO J.* 21:2736–2745.
- Wagner, R.W., C. Yoo, L. Wrabetz, J. Kamholz, J. Buchhalter, N.F. Hassan, K. Khalili, S.U. Kim, B. Perussia, F.A. McMorris, et al. 1990. Double-stranded RNA unwinding and modifying activity is detected ubiquitously in primary tissues and cell lines. *Mol. Cell Biol.* 10:5586–5590.
- Wang, Q., P.J. O'Brien, C.X. Chen, D.S. Cho, J.M. Murray, and K. Nishikura. 2000. Altered G protein-coupling functions of RNA editing isoform and splicing variant serotonin_{2C} receptors. *J. Neurochem.* 74:1290–1300.
- Yang, J.H., X. Luo, Y. Nie, Y. Su, Q. Zhao, K. Kabir, D. Zhang, and R. Rabinovici. 2003a. Widespread inosine-containing mRNA in lymphocytes regulated by ADAR1 in response to inflammation. *Immunology*. 109:15–23.
- Yang, J.H., Y. Nie, Q. Zhao, Y. Su, M. Pypaert, H. Su, and R. Rabinovici. 2003b. Intracellular localization of differentially regulated RNA-specific adenosine deaminase isoforms in inflammation. *J. Biol. Chem.* 278:45833–45842.
- Yang, W., Q. Wang, S.J. Kanes, J.M. Murray, and K. Nishikura. 2004. Altered RNA editing of serotonin 5-HT_{2C} receptor induced by interferon: implications for depression associated with cytokine therapy. *Brain Res. Mol. Brain Res.* 124:70–78.
- Yi-Brunozzi, H.Y., L.M. Easterwood, G.M. Kamilar, and P.A. Beal. 1999. Synthetic substrate analogs for the RNA-editing adenosine deaminase ADAR-2. *Nucleic Acids Res.* 27:2912–2917.