Dimerization of ADAR2 is mediated by the double-stranded RNA binding domain

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ABSTRACT

Members of the family of adenosine deaminases acting on RNA (ADARs) can catalyze the hydrolytic deamination of adenosine to inosine and thereby change the sequence of specific mRNAs with highly double-stranded structures. The ADARs all contain one or more repeats of the double-stranded RNA binding motif (DRBM). By both *in vitro* and *in vivo* assays, we show that the DRBMs of rat ADAR2 are necessary and sufficient for dimerization of the enzyme. Bioluminescence resonance energy transfer (BRET) demonstrates that ADAR2 also exists as dimers in living mammalian cells and that mutation of DRBM1 lowers the dimerization affinity while mutation of DRBM2 does not. Nonetheless, the editing efficiency of the GluR2 Q/R site depends on a functional DRBM2. The ADAR2 DRBMs thus serve differential roles in RNA dimerization and GluR2 Q/R editing, and we propose a model for RNA editing that incorporates the new findings.

Keywords: RNA editing; ADAR; dimerization; DRBM; BRET

INTRODUCTION

RNA editing can specifically change the sequence of an mRNA, and the functional properties of the products from a single gene can in this way be varied depending on, e.g., developmental state or cell type. Thus, editing is a means of increasing the complexity of the proteome. One example of RNA editing is deamination of adenosine to inosine, which is recognized as guanosine by tRNAs, and every metazoan examined expresses adenosine deaminases acting on RNA (ADARs) (Bass 2002).

The initially described ADAR targets were identified serendipitously, and they were mainly transcripts encoding membrane proteins expressed in central nervous systems. Various screening methods have recently extended the list of ADAR substrates substantially, primarily with transcripts affected in untranslated regions (Levanon et al. 2004; Clutterbuck et al. 2005). The best described example of mammalian editing is the receptor of the most abundant excitatory neurotransmitter, glutamate. Different glutamate receptor (GluR) subunit pre-mRNAs are edited at different positions, but the physiologically most important site is the

Q/R site in GluR2, which is edited to ~100% (Sommer et al. 1991). The resultant amino acid change causes significant reduction of the GluR Ca²⁺ permeability (Burnashev et al. 1992), and genetically modified mice have demonstrated that lack of the introduced arginine causes premature death (Higuchi et al. 2000).

The ADARs share a common overall domain structure, where the C-terminal catalytic deaminase domain is preceded by an RNA binding domain with one, two, or three repeats of a double-stranded (ds) RNA binding motif (DRBM) that mediates the interaction with the substrates. The two functional mammalian deaminases ADAR1 and -2 have three and two DRBMs, respectively. The Q/R site is only edited efficiently by ADAR2 (Melcher et al. 1996), but on other substrates, the two enzymes have partly overlapping specificities and they can both deaminate up to half of the adenosines in perfectly dsRNA (Bass 2002).

The DRBM is found in a number of viral, prokaryotic, and eukaryotic proteins with diverse cellular functions, and the proteins generally contain multiple copies of the motif with varying affinities for dsRNA (Chang and Ramos 2005). Several NMR and X-ray crystallographic studies of DRBMs have revealed that the domain adopts an α - β - β - β - α structure with the two α -helices on the same side of a β -sheet (Ryter and Schultz 1998; Ramos et al. 2000). In addition to RNA binding, DRBMs can mediate several

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functions, including protein–protein interactions, activation of transgene expression, and RNA annealing (Chang and Ramos 2005). Furthermore, DRBMs influence the intracellular localization of some proteins, including the ADARs, whose nucleolar localization depends on the DRBMs and, for ADAR1, also the nuclear localization is determined by the DRBMs (Herbert et al. 2002; Scherl et al. 2002; Desterro et al. 2003; Sansam et al. 2003; Andersen et al. 2005).

The current model of the editing process is that mismatches, bulges, and loops in a substrate will restrict how the ADARs can bind (Lehmann and Bass 1999), and the target adenosine is then flipped out for deamination (Hough and Bass 1997). The precise mechanism of how the RNA binding facilitates catalysis is still uncertain, but in vitro studies suggest that formation of a ternary complex with two ADAR2 proteins per substrate is required for activity (Jaikaran et al. 2002), and purifications of ADAR1 and 2 indicate that they both form homodimers (Cho et al. 2003). For the *Drosophila* ADAR, the N terminus and the first DRBM were shown to be necessary for dimerization and catalytic activity (Gallo et al. 2003), while the human ADAR2 does not depend on the N-terminal region for RNA editing (Macbeth et al. 2004), suggesting that the ADAR2 dimerization domain differs from that of the *Drosophila* ADAR.

In the following, we use the term dimerization, although some of the assays do not distinguish between dimers and oligomers. To clarify how two ADAR2 molecules may interact, we have performed yeast two-hybrid and in vitro cross-linking assays, and our analyses show that the dsRNA binding domain is necessary and sufficient for the interaction. Bioluminescence resonance energy transfer (BRET) confirms that ADAR2 forms dimers in living mammalian cells, and to our knowledge, this is the first time that the BRET technique has been successfully applied to a quantitative study of protein interactions in the nucleolus. Both the yeast two-hybrid and the BRET assays show that DRBM1 is more important for the interaction than is DRBM2. To further clarify the functions of the two ADAR2 DRBMs in the biochemical and cellular properties of the protein, we also examined their individual roles in RNA binding and RNA editing. Our results show that in contrast to the *Drosophila* ADAR, rat ADAR2 does not depend on the N terminus for protein dimerization or for GluR2 Q/R editing, and we propose a model for ADAR2 RNA editing whereby binding of DRBM1 to an RNA substrate promotes the RNA binding by DRBM2 as well as protein dimerization and the substrate can be deaminated.

RESULTS

Homodimerization of the ADAR2 RNA binding domain

To confirm that rat ADAR2 is able to dimerize, we made constructs encoding either the Gal4 DNA binding domain

or the Gal4 activation domain fused N-terminally to the full-length rat ADAR2 (Fig. 1A). The plasmids were cotransformed into the yeast strain PJ69, which depends on the addition of adenine for growth unless the two fusion proteins interact. The fusion proteins allowed efficient growth on adenine omission plates, thus confirming that ADAR2 is able to dimerize in the yeast two-hybrid assay (Fig. 1A). As negative controls, we also tested whether the two Gal proteins allowed growth on their own or when only one was fused to full-length ADAR2, but they did not.

To map the region responsible for the interaction, we first tested the ADAR2 RNA binding N terminus (N-term) and the catalytic C terminus (C-term), and we found that the RNA binding domain dimerized as efficiently as the full-length enzyme, while the Gal fusion proteins with the catalytic domain were unable to rescue yeast growth (Fig. 1A). We then studied the two DRBMs individually in the yeast two-hybrid system and found that DRBM1 (R1) allowed robust growth, while DRBM2 (R2) did not (Fig. 1A). We also tested for an interaction between DRBM1 and DRBM2 but found no evidence of this.

Extensive studies of PKR have demonstrated that this protein dimerizes via two N-terminal DRBMs (Thomis and Samuel 1993; Ung et al. 2001). A highly conserved alanine in the first PKR DRBM (the position analogous to A134 in ADAR2) (Fig. 1B) was demonstrated to be critical for the interaction (Zhang et al. 2001), so we tested the effect of changing the ADAR2 DRBM1 alanine to a glutamic acid. In accordance with the PKR studies, A134E abolished the yeast two-hybrid interaction of the ADAR2 DRBM1 (R1 mut) (Fig. 1A).

The constructs N-term R1 mut and FL R1 mut, which contain the wild-type DRBM2, were also unable to rescue yeast growth (Fig. 1A), supporting the lack of DRBM2 dimerization by the isolated domain. To examine if the alanine mutation in one motif might directly influence the dimerization ability of the other motif, we made the similar mutation in DRBM2, A288E, but it had no influence on the ADAR2 interaction in yeast (FL R2 mut), supporting that ADAR2 dimerization depends primarily on a functional DRBM1.

ADAR2 exists as dimers in living mammalian cells

BRET is a newly developed technique that has proven to be a powerful tool for real-time detection of protein–protein interactions within living cells. The technology is based on energy transfer from a luminescent donor to a fluorescent acceptor, and since the transfer is highly dependent on the proximity of donor and acceptor, BRET is ideal for detecting hetero- and homodimerization. It has been especially successfully applied to studies of interaction partners for G-protein–coupled receptors (Gales et al. 2005).

In the mouse neuroblastoma cell line N2A, we transiently coexpressed wild-type ADAR2 fused N-terminally to the

FIGURE 1. The dsRNA binding domain in ADAR2 mediates dimerization. (A) In the schematic representation of the constructs tested, the numbers denote the amino acid positions relative to the N terminus of rat ADAR2. The splice variant of the protein used lacks 10 residues (466–475) in the catalytic domain compared with GenBank file NP_112268. The boxes indicate the functional domains: light gray box, DRBM1; dark gray box, DRBM2; and black box, catalytic domain. A mutation, either A134E or A288E, is indicated by a dot. Two plasmids encoding a given construct fused N-terminally to the Gal activation domain and the Gal transactivating domain, respectively, were cotransformed into the yeast strain PJ69. Interaction was assessed by the ability of transformants to grow on medium without adenine. Robust growth after 4 d is indicated by ++, no detectable growth is indicated by –. (B) An alignment of the seven DRBM sequences from rat ADAR2, human ADAR1, and human PKR. Residues conserved between four or more of the motifs are marked by black boxes. A dot indicates the position of the mutated alanine.

energy donor *Renilla* luciferase and wild-type or mutant forms of ADAR2 N-terminally fused to the energy acceptor EGFP. If the catalytic degradation by the luciferase of Deep Blue C produces an EGFP-signal, the distance between the two proteins must be <100 Å (Xu et al. 1999).

As illustrated in Figure 2B, a strong BRET signal was observed for the two wild-type ADAR2 fusion proteins, which supports that ADAR2 exists as dimers in living cells. We also found that introduction of the alanine mutations into both DRBMs resulted in a BRET signal that was only 15% of the signal observed for wild-type ADAR2 (R1R2 mut*), supporting the yeast results that the dsRNA binding domain is necessary for dimerization. From the yeast experiments, it was also expected that the A288E mutation in DRBM2 should have no effect on dimerization, and this is indeed what we observed (R2 mut). However, with the A134E mutation (R1 mut), we observed an unexpected high value that was 70% of the wild-type signal. To ensure that the reduction was not simply due to an altered N-terminal structure of the R1 mut, which might increase the distance between the N-terminal EGFP and luciferase tags, we also examined the BRET signals from ADAR2 with

tags at the opposite ends. We found that the BRET signal was also reduced by the A134E mutation in this case (data not shown).

To further examine the apparent difference between R1 mut dimerization in the yeast and mammalian systems, we used the more sensitive BRET² assay, where EGFP is replaced by GFP², which has an excitation profile that is more optimal for the Deep Blue C spectrum. In HEK293 cells, a fixed amount of the *Rluc*-ADAR2 plasmid was expressed with varying amounts of plasmids encoding GFP²-tagged proteins, and the BRET² ratio was plotted as a function of the GFP²/*Rluc* expression ratio, which was determined individually in each sample to allow for differences between transfections in expression of the individual constructs.

As expected, the BRET² signal obtained with wild-type ADAR2 increased hyperbolically as a function of the GFP²/*Rluc* expression ratio approaching a maximum when the amount of GFP² was no longer limiting (Fig. 2C), which indicates a specific interaction. The curve for R2 mut was similar to the wild-type curve, while the R1 mut curve was clearly lower.

FIGURE 2. ADAR2 dimerizes in living cells. (A) Schematic representation of the constructs used for the BRET experiments. At the N terminus, the proteins were fused to Rluc, EGFP, or GFP². A mutation is indicated by a dot. (B) BRET was measured in N2A cells 48 h after transfection of 3 μ g Rluc-ADAR2 and 5 μ g of the indicated wild-type or mutated EGFP-ADAR2 fusion protein. The asterisk indicates that the double mutation was also introduced into the Rluc construct, since the protein localizes differently in the nucleus than the wild-type or single mutant proteins (Fig. 5D). The bars show the average and standard deviations of triplicate determinations in a representative experiment. The expression levels of the fusion proteins with mutations relative to those with wild-type ADAR2 are indicated in the two *bottom* rows. (C) HEK293 cells transfected with constant concentrations of Rluc-ADAR2 and varying concentrations of GFP²-ADAR2 (■), GFP²-R1 (▲), GFP²-R2 (▼), or GFP²-nucleolin (◆) were incubated with 5 μ M Deep Blue C, and the light emissions were measured. The BRET² ratios were determined as the ratio of GFP² emitted light to Rluc emitted light corrected for the background signal from Rluc alone. The data shown represent pooled individual readings performed in triplicate from three independent experiments.

The BRET technique has mostly been applied to studies of interactions between membrane associated proteins, and it has, to our knowledge, not previously been used for proteins located in the nucleoli. To confirm that the signals obtained were not simply due to random associations between overexpressed proteins located in a small compartment, we used a nucleolar protein, nucleolin, which is not expected to interact with the ADARs. Electron and fluorescence microscopy have suggested that nucleolin localizes in the dense fibrillar and granular components of the nucleoli (Biggiogera et al. 1990), while ADAR2 localizes to the periphery of the dense fibrillar component (Desterro et al. 2003, 2005). With quantitative confocal microscopy, we observe at least significant colocalization of ADAR2 and nucleolin within the nucleoli (data not shown), and when BRET² signals were measured between Rluc-ADAR2 and GFP² fused to nucleolin, the signal also increased with increased GFP²: Rluc ratio (Fig. 2C), indicating proximity. However, the correlation was linear rather than hyperbolic, which suggests that the signal obtained is due to random collisions of noninteracting

proteins (termed bystander BRET) (Mercier et al. 2002). The R1 mut curve is close to the background, but the steeper initial increase for R1 mut indicates that there is a specific interaction between the energy donor and acceptor; i.e., the second DRBM of ADAR2 is also able to mediate protein dimerization. A comparison of the BRET²₅₀ values, which reflect the affinities of the interaction partners, shows that wild type and R2 mut have similar values, while the BRET²₅₀ value for R1 mut is three times higher (Table 1). R1 mut is thus able to dimerize, but with a lower affinity than the wild-type deaminase, which is consistent with the lack of interaction observed in the yeast system.

DRBM dimerization in vitro

Next, we examined the interaction in vitro by cross-linking experiments to study if the RNA binding domain was able to dimerize directly. A recombinantly expressed fragment of ADAR2 containing the two dsRNA binding domains (d1d2) with a 6xHis-tag was incubated with the chemical cross-linking reagent DMS, which reacts with amine

TABLE 1. Parameters obtained from BRET² saturation curves

	BRET ² max	BRET ² ₅₀
Wild type	278 ± 39	0.63 ± 0.18
R1 mut	248 ± 20	1.94 ± 0.34
R2 mut	335 ± 51	0.87 ± 0.30

The BRET² max is the maximal BRET² obtained for a given pair, and the BRET²₅₀ corresponds to the concentration of acceptor giving 50% of the BRET² max. The results are expressed as the mean ± standard error and were derived from the data presented in Figure 2C.

groups. Prior to the addition of DMS, the protein fragments were incubated with or without poly(rI-rC). Figure 3 shows that even without the addition of dsRNA, d1d2 forms multimers consisting of up to four molecules (lane 2), but increasing amounts of poly(rI-rC) reduce the multimerization to dimerization (lanes 5,6).

RNA binding properties of the DRBMs

Our observation that the two DRBMs have different importance for protein dimerization led us to examine if they also differ in their RNA binding properties. We therefore performed gel mobility shift experiments with both of the DRBMs (d1d2) or either one alone (d1 and d2) by using a 150-nucleotide-labeled RNA probe derived from the sequence of the Q/R hairpin in the rat GluR2 pre-mRNA (Fig. 4). Both d1 (lanes 2–7) and d1d2 (lanes 14–19) formed a complex with the probe that was shifted to higher-order complexes at higher protein concentrations, while d2 alone did not shift the probe at similar concentrations (lanes 8–13). DRBM1 thus appears to be the major contributor to the dsRNA binding of ADAR2.

Effects of DRBM mutations on editing of the GluR2 Q/R site

By deletion and mutation analyses of ADAR1, the importance of the three DRBMs for catalytic activity has been studied (Maas et al. 1996; Liu and Samuel 1999; Liu et al. 1999). For ADAR2, the first DRBM has been shown to be dispensable for editing of a minimal substrate in vitro (Macbeth et al. 2004), but ADAR2 has not been further characterized. We therefore tested the effect of the A134E and A288E mutations on the ability of ADAR2 to edit a minimal substrate derived from the sequence of the Q/R hairpin in the rat GluR2 pre-mRNA. To obtain a broader characterization of the roles of the individual DRBMs in editing, we also tested the effects of deleting or swapping the motifs (Fig. 5A).

N2A-cells were transiently cotransfected with pQR1, from which the substrate is expressed, and a plasmid

encoding either wild-type or mutant ADAR2. After 48 h, total RNA was purified, treated with DNase, and amplified by RT-PCR with pQR1 specific primers. The PCR product was gel purified, and the level of editing at the Q/R site was determined by primer extension (Fig. 5B).

Cotransfection of pQR1 with the EGFP vector resulted in <2% editing of the Q/R site (mock) (Fig. 5C), while the level of editing was 62% when cotransfecting with wild-type ADAR2. Mutation of DRBM1 reduced the ADAR2-mediated editing to 22% (R1 mut) and DRBM1 deletion caused a reduction to 41% (R1 delta), so ADAR2 retains some editing activity even if it lacks a functional DRBM1. In contrast, the editing levels were not significantly above background if DRBM2 was mutated or deleted (R2 mut/R2 delta), and ADAR2 with mutations or deletions of both DRBMs were also unable to induce Q/R site editing (R1R2 mut/R1R2 delta). Replacement of DRBM2 with DRBM1 did not rescue the editing (R1R1), but DRBM1 substituted with DRBM2 gave 34% editing of the Q/R site (R2R2). A functional DRBM2 thus appears to be critical for ADAR2-mediated editing of the GluR2 Q/R site, although DRBM1 is more important for RNA binding and protein dimerization.

Either DRBM can mediate nucleolar localization of ADAR2

The ADAR editing activity can be influenced by the localization of the protein (Poulsen et al. 2001; Sansam

FIGURE 3. The dsRNA binding domain multimerizes in vitro. Chemical cross-linking reactions were performed with 8 μg/mL of the ADAR2 amino acids 69–307 with a C-terminal 6xHis-tag, d1d2. Except in lane 1, DMS was added to a final concentration of 1.6 mM. In lanes 3–6, the protein was preincubated with poly(rI-rC) at concentrations rising 10-fold in each lane from 200 ng/mL (lane 3) to 200 μg/mL (lane 6). Assignments of monomer (mo), dimer (di), trimer (tr), and tetramer (te) were based on a molecular weight standard. The fainter band between the dimer and trimer bands (visible in lanes 2–4) probably represents an alternatively cross-linked dimer with different mobility.

FIGURE 4. Binding of dsRNA by ADAR2 is primarily mediated by DRBM1. Gel mobility assays of a ^{32}P -labeled RNA derived from the Q/R editing site of rat GluR2 with His-tagged d1 (ADAR2 amino acids 69–152), d2 (ADAR2 amino acids 223–307), or d1d2 (ADAR2 amino acids 69–307). Lane 1 contained 20 μL 1.25 nM RNA only, and in lanes 2–19, the indicated proteins were included at the following concentrations: 2.5 nM (lanes 2,8,14), 7.5 nM (lanes 3,9,15), 30 nM (lanes 4,10,16), 120 nM (lanes 5,11,17), 300 nM (lanes 6,12,18), and 1200 nM (lanes 7,13,19).

et al. 2003), and it has previously been shown that the ADAR2 DRBMs are necessary for the preferred nucleolar localization of the protein (Desterro et al. 2003; Sansam et al. 2003). We therefore examined the localization of the proteins tested for editing activity by fluorescence microscopy (Fig. 5D). All of the proteins were exclusively nuclear, but there was a difference in localization within the nucleus: ADAR2 was concentrated in the nucleoli (wild type), while simultaneous mutation or deletion of both of the DRBMs resulted in a homogenous staining of the nucleoplasm (R1R2 mut/R1R2 delta) as previously shown (Sansam et al. 2003). Interestingly, the proteins with at least one functional DRBM (R1 mut/R1 delta/R2 mut/R2 delta/R1R1/R2R2) all localized to the nucleoli, so either of the ADAR2 DRBMs is able to direct the enzyme to the nucleolus.

ADAR2 shuttles rapidly between nucleoli (Sansam et al. 2003), and the shuttling is likely necessary for editing to occur in competition with splicing. We performed photobleach experiments with wild type, R1 mut, and R2 mut to examine if the DRBM mutations influenced the shuttling, but we observed no difference in their shuttling kinetics (data not shown).

DISCUSSION

In this report, we demonstrate that rat ADAR2 can homodimerize in both yeast and mammalian cells. By yeast two-hybrid and BRET assays, we map the site of dimer-

ization to the ADAR2 DRBMs, and in vitro studies show that a recombinantly expressed protein, d1d2, which contains only the two DRBMs and the linker, is efficiently cross-linked (Fig. 3) suggesting that the dsRNA binding domain is both necessary and sufficient for ADAR2 dimerization. DRBM2 is dispensable for the dimerization, but the BRET assay shows that in the absence of DRBM1, DRBM2 is able to mediate an interaction although with lower affinity (Fig. 2C). The DRBMs are also necessary for editing of the GluR2 Q/R site in transfected cells, but the editing activity depends more strongly on a functional DRBM2.

In contrast to our results, Gallo and coworkers (Gallo et al. 2003) have reported that dimerization and editing activity of the *Drosophila* ADAR depend on both the first DRBM and the extreme N terminus. To confirm that the mammalian ADAR2 differs from the otherwise very similar *Drosophila* ADAR in its mode of dimerization, we have examined a truncated version of ADAR2 lacking the 68 N-terminal residues, and we find that the mutant dimerizes in the BRET assay and edits the GluR2 Q/R site efficiently (data not shown). This is supported by a report from Macbeth and coworkers (Macbeth et al. 2004) showing that the region N-terminal to DRBM2 of human ADAR2 is dispensable for catalytic activity in vitro.

We find that DRBM1 is more important for both RNA binding (Fig. 4) and protein dimerization (Figs. 1A, 2C) than is DRBM2, but is RNA binding a prerequisite for the dimerization? Most of the evidence indicates that the answer is yes. First, mobility shift assays have demonstrated consecutive binding of two ADAR2 monomers to an RNA (Ohman et al. 2000; Jaikaran et al. 2002). Second, high concentrations of RNA inhibit editing suggesting that excess of RNA leaves no enzymes free in solution for dimerization (Hough and Bass 1994). In accordance, a study of the change in ADAR2 tryptophan fluorescence with increasing amounts of dsRNA showed that the maximal conformational change in the catalytic domain (which is likely a sign of catalytic activity) occurred when the molar ratio of protein:RNA was 2:1, while emission dropped again at higher RNA concentrations (Yi-Brunozzi et al. 2001). Third, the sedimentation coefficient of *Drosophila* ADAR indicates that the protein is dimeric in the presence of RNA but monomeric in its absence (Gallo et al. 2003). Fourth, ADAR1 interacts with the NF90 proteins (which also contain DRBMs) via dsRNA (Nie et al. 2005).

In opposition, Cho and coworkers (Cho et al. 2003) reported that ADAR dimerization was RNA independent, since RNase treatment did not disrupt the dimers. However, we speculate that the ADARs may have been protecting the RNA from degradation in this assay.

A model for RNA editing by ADAR2

The current model of PKR activation is based on an NMR study showing that PKR's DRBM1 has a considerably

FIGURE 5. (Continued on next page)

higher conformational flexibility than does DRBM2, while only DRBM2 interacts with the kinase domain, suggesting that a flexible DRBM1 can bind dsRNA, promote co-operative binding by DRBM2, and thereby release the kinase domain (Nanduri et al. 2000). The data presented here are consistent with a largely similar model for ADAR2 (Fig. 6) with the main difference that DRBM1 (and not DRBM2) has been shown to have an auto-inhibitory effect on ADAR2 catalysis, which is released upon RNA binding (Macbeth et al. 2004). This auto-inhibition explains why a point mutation in DRBM1 is more severe for the editing activity than deletion of the motif (Fig. 5C).

The BRET assay shows that mutation of DRBM1, but not of DRBM2, reduces ADAR2 dimerization (Fig. 2C), suggesting that DRBM1 mediates the initial interaction with a substrate and thereby facilitates dimerization. However, ADAR2 still partly maintains the ability to dimerize and to edit a substrate in the absence of DRBM1 (Figs. 2C, 5C), and it is thus likely that DRBM2 has some affinity for RNA in the full-length protein, although we could not show this in a gel mobility assay (Fig. 4). Mutating DRBM2 or replacing it with DRBM1 abolished editing (Fig. 5C), so after RNA binding and protein dimerization, a conformational change involving DRBM2 appears to be required for editing to occur. In concord, DRBM2 was recently shown to be important for positioning of ADAR2 on a minimal Q/R substrate, since introduction of a benzyl group at the RNA site expected to be bound by DRBM2 reduced the deamination kinetics by >70% (Stephens et al. 2004). For ADAR1, mutational analyses have also demonstrated that the DRBM closest to the catalytic domain is critical for catalytic activity (Maas et al. 1996; Liu and Samuel 1999; Liu et al. 1999), which might suggest that both deaminases depend on the C-terminal DRBM for activation of the catalytic domain.

We find that ADAR2 was unable to edit the GluR2 Q/R substrate when the DRBMs were deleted (Fig. 5C), and we speculate that the ADARs generally depend on the DRBMs for substrate recognition and activation. However, an ADAR1 lacking all three DRBMs was reported to edit a short hairpin RNA in

transfected cells (Herbert and Rich 2001), and the ADAR2 catalytic domain alone can deaminate dsRNA in vitro (Macbeth et al. 2005). Our data do not support ADAR dimerization in the absence of the DRBMs (Figs. 1A, 2B), suggesting that the deamination observed with the catalytic domains was not the result of protein dimerization, but we cannot rule out that the catalytic domain has an intrinsic ability to dimerize under special conditions, e.g., in the presence of a high concentration of substrate.

In *Drosophila*, ADAR heterodimerization with an inactive mutant down-regulates editing (Gallo et al. 2003), and there are also indications that the two *Caenorhabditis elegans* ADARs, ADR-1 and ADR-2, act as heterodimers

FIGURE 5. DRBM2 is essential for RNA editing by ADAR2. N2A cells were cotransfected with expression plasmids encoding an editing construct, pQR1, encompassing the Q/R site of the rat GluR2 pre-mRNA in a minimal hairpin and the EGFP-ADAR2 fusion constructs schematically represented. A dot indicates a mutation; a line indicates a deletion (A). Forty-eight hours after transfection, RNA was purified, DNase treated, and subjected to RT-PCR. (B) The editing level at the Q/R-site was determined by primer extension. The *lower* band represents unextended primer, P; the *middle* band represents cDNA from unedited transcripts, U; and the *top* band represents cDNA from transcripts edited at the Q/R site, E. In the lane marked *pQR1*, the plasmid encoding the editing substrate was used for the primer extension. (C) The U and E signals were quantitated by PhosphorImaging, and the level of editing was determined as the intensity of E relative to the sum of the E and U intensities. The average and standard deviation of the results from a representative experiment performed in triplicate are shown. (D) Fluorescence microscope images (Zeiss Axiovert200) of living N2A cells were taken 24 h after transfections with the indicated EGFP-ADAR2 constructs.

(Tonkin et al. 2002). Furthermore, ADAR1 interacts with the NF90 proteins that contain DRBMs (Nie et al. 2005). The most interesting perspective of this is that the ADAR activities may generally be regulated by heterodimerization between different ADARs or with other dsRNA binding proteins, which would facilitate a greater variability in regulation of activities and specificities, and it is tempting to speculate that the observed mutual inhibition of ADAR1, -2 and -3 (Chen et al. 2000) may not be due only to steric hindrance, but to the formation of heterodimers with altered specificities. In opposition to this idea, Cho and

coworkers (Cho et al. 2003) found that human ADAR1 and ADAR2 formed stable homodimers after expression in insect cells, but they were unable to detect heterodimerization. The reason may be that the construct used for expression, ADAR2 and the interferon-induced form of ADAR1, encodes proteins with nuclear and cytoplasmic localizations, respectively, while we have indications that the constitutively expressed form of ADAR1, which localizes to the nucleus, can interact with ADAR2 in BRET and yeast two-hybrid experiments (H. Poulsen, unpubl.).

MATERIALS AND METHODS

Plasmid constructs

The open reading frame of the full-length rat ADAR2 gene cloned into the BamHI and EcoRI restriction sites of pcDNA3 (Invitrogen) or pGEX-4T-3 (Amersham) was used for further subcloning. Mutageneses of the DRBMs were achieved by a modified version of the megaprimer method (Ke and Madison 1997) on the pcDNA3 ADAR2.

The yeast two-hybrid constructs were generated by insertion of different fragments from the pGEX-construct into the pGAD and pGBD vectors (James et al. 1996), yielding the Gal4 activation domain and Gal4 DNA binding domain, respectively, fused in frame N-terminally to all of the following ADAR2 regions: full-length (the BamHI-SalI fragment inserted into the same sites), C-term (the MscI-SalI fragment inserted into the SmaI-SalI digested vectors), N-term (the BamHI-TaqI fragment inserted into the BamHI-ClaI digested vectors), R1 (the EcoRI-BsaAI fragment of FL into the EcoRI-SmaI digested vectors), and R2 (the BsaAI-BglII fragment of N-term inserted into the SmaI-BglII digested vectors). The constructs with mutations in the DRBMs were made by

subcloning from the pcDNA3 constructs with mutated versions of ADAR2.

The Rluc-ADAR2 construct for BRET measurements was generated by PCR amplification of the *Renilla* luciferase open reading frame from the pRL-CMV vector (Promega) adding KpnI and BamHI restriction sites by which the fragment was inserted into the pcDNA3 ADAR2 construct. Inserting the BamHI-EcoRI fragment of the pcDNA3 ADAR2 construct into the enhanced green fluorescence protein (EGFP) C1 vector (Clontech) generated the EGFP-ADAR2 fusion construct. The constructs with mutations in the DRBMs were made by subcloning from the relevant pcDNA3 ADAR2 constructs. The GFP²-ADAR2 constructs were

FIGURE 6. A model for RNA editing by ADAR2. In the absence of RNA (*left*), DRBM1 has an auto-inhibitory role. In the presence of a substrate (*middle*), DRBM1 binds the RNA, the auto-inhibition is released, and the protein will be able to dimerize. Upon dimerization and binding of DRBM2 to the substrate (*right*), a conformational change occurs giving an active enzyme.

created by replacing the EGFP sequence of the EGFP-ADAR2 constructs with the GFP² sequence from pGFP²-β-arrestin2 (PerkinElmer). To obtain ADAR2 constructs with C-terminal tags, the open reading frames of *Renilla* luciferase and GFP² were PCR amplified and inserted into the pcDNA3.1+ vector (Invitrogen) with EcoRI/XbaI and NotI/XhoI restriction sites, respectively. ADAR2 wild-type and R1 mut sequences were subsequently inserted into the EcoRI sites after PCR amplification to remove the stop codons. The editing construct pQR1 contains part of the sequence of the rat GluR2 gene with the Q/R site in the pcDNA3 vector (H. Poulsen, unpubl.).

The plasmids for *Escherichia coli* expression were generated by PCR amplification of either or both DRBMs in the pcDNA3 constructs, adding EcoRI and BamHI restriction sites by which the fragments were inserted into the pET H-His vector (Jensen et al. 1997), yielding the d1 (ADAR2 amino acids 69–152), d2 (ADAR2 amino acids 223–307), and d1d2 (ADAR2 amino acids 69–307) with C-terminal 6xHis-tags.

Yeast two-hybrid assays

The yeast strain PJ69 cotransformed with pGAD and pGBD constructs was plated on selective medium lacking leucine and tryptophan. Double transformants were restreaked on selective medium also lacking adenine to test for interaction of the two fusion proteins. Plates were incubated for 4 d at 30°C.

Cell cultures and transfections

The human embryonic kidney cell line HEK293 was cultured at 37°C, 7.5% CO₂ in Dulbecco's modified Eagle medium containing 10% fetal calf serum, and the cells were transfected with the calcium phosphate precipitation method. The mouse neuroblastoma cell line N2A was cultured at 37°C, 7.5% CO₂ in Dulbecco's modified Eagle medium containing 5% fetal calf serum, and Lipofectamine (Invitrogen) was used for transient transfections according to the manufacturer's instructions.

BRET measurements

BRET is based on the catalytic degradation of a *Renilla* luciferase substrate that leads to the emission of light, which can excite the energy acceptor, EGFP or GFP², if the two proteins are in proximity. After excitation, the energy acceptor emits light with a peak at a higher wavelength, and the ratio of light emitted by

acceptor (515 nm) and by donor (400 nm) determines the BRET ratio. Forty-eight hours after transfection, 2×10^5 cells suspended in assay buffer (Dulbecco's PBS containing 0.1 g/L CaCl₂, 0.1 g/L MgCl₂, and 1 g/L L-glucose) were distributed into wells in a 96-well microplate (white Optiplate, PerkinElmer). The *Renilla* luciferase substrate Deep Blue C (Packard Bioscience) was injected to a final concentration of 5 μM, and the signals were detected with a Mithras LB 940 plate reader (Berthold Technologies) that allows the sequential integration of the signals detected in the 400- and 515-nm windows. The background signal from Rluc was determined by coexpressing the Rluc construct with empty pcDNA3 vector, and the BRET ratio generated from this transfection was subtracted from all other BRET ratios.

In the experiment with EGFP constructs, the expression levels of the Rluc fusion proteins were determined with a TopCount NXT (Packard Bioscience) measuring the light emission from 50,000 cells after addition of Deep Blue C to 5 μM. In the experiment with GFP², the Rluc expression levels were determined in the Mithras readings, since there is no emission from the acceptor at 400 nm. The expression levels of the energy acceptor proteins were determined with a NOVostar (BMG Labtechnologies) measuring the fluorescence of 50,000 cells at 520 nm after excitation at 420 nm. Both donor and acceptor values were corrected by subtracting the values obtained with the same number of untransfected cells.

Protein expression

The pET constructs were expressed in the *E. coli* strain BL21(DE3) yielding the proteins d1, d2, and d1d2. Bacterial cultures were induced in early log phase with 1 mM IPTG for 3 h at room temperature. Bacteria recovered by centrifugation were resuspended in buffer A (300 mM NaCl, 5 mM imidazole, 25 mM Hepes at pH 7.6, 2 mM 2-mercaptoethanol, lysozyme, complete EDTA-free protease inhibitor; Roche), lysed by French Press and cleared by centrifugation. The supernatant was incubated with Ni-NTA (Qiagen) for 1 h at 4°C; loaded onto a column; and washed with buffer A, buffer B (300 mM NaCl, 40 mM imidazole, 25 mM Hepes at pH 7.6, 2 mM 2-mercaptoethanol); and buffer C (1 M NaCl, 5 mM imidazole, 25 mM Hepes at pH 7.6, 2 mM 2-mercaptoethanol), and bound proteins were eluted with buffer E (300 mM NaCl, 150 mM imidazole, 25 mM Hepes at pH 7.6, 2 mM 2-mercaptoethanol). Top fractions were dialyzed into storage buffer (300 mM NaCl, 25 mM Hepes at pH 7.6, 1 mM DTT, 1 mM MgCl₂, 0.1 mM EDTA, 10% glycerol).

Protein cross-linking

Two microliters of protein and 2 μL poly(rI-rC) diluted in PBS were mixed and incubated for 20 min on ice; 16 μL 2 mM dimethyl superimide (DMS; Pierce) in 10 mM Hepes (pH 7.9), 100 mM NaCl was added and the samples were incubated for 90 min at room temperature before adding 0.5 M glycine and SDS loading buffer to terminate the reaction. The reactions were separated on 10% SDS-polyacrylamide gels and blotted to an Immobilon-P membrane (Millipore), and Western blotting was performed with an antibody against the 6xHis tag (Roche). Bands were visualized by standard techniques using a secondary antibody and ECL (Amersham).

Gel electrophoresis mobility shift

Five microliters of 5 nM 32 P-labeled RNA (in 10 mM Hepes at pH 7.9, 30 mM KCl) was renatured by incubation at 85°C and slow cooling. Fifteen microliters of protein (in 13 mM Tris-HCl at pH 8.0, 33 mM KCl, 13 mM NaCl, 1.3 mM MgCl₂, 0.7 mM DTT, 13% glycerol, 0.13 g/l BSA) was added, and the reaction was incubated at room temperature for 20 min before direct loading onto a 4% native polyacrylamide gel with 1× TBE. Electrophoresis was performed at room temperature for 2 h at 150 V.

RNA editing

The EGFP-ADAR2 expressing constructs were cotransfected into N2A cells with pQR1. Two days after transfection, RNA was purified with the RNeasy kit (Qiagen) and treated with DNase (Roche), and after purification, Superscript II RT (Invitrogen) was used for reverse transcription (RT) of the RNA with an SP6 primer at 42°C for 40 min followed by 10 min at 45°C. The cDNA was amplified by PCR using a T7 primer and an SP6 primer, and RNA not subjected to the RT reaction was tested for the presence of DNA by similar PCR. A PCR product with a length of 130 bp corresponding to the GluR2 RNA was purified after agarose gel electrophoresis and used to estimate the editing at the Q/R site by 30 cycles with Thermo Sequenase (Amersham) extending the primer 5'-[32 P]CCG AGC TCG GAT CCT TTA-3' (radiolabeled using T4 PNK [New England BioLabs] with [γ - 32 P]ATP [3000 Ci/mmol; Amersham]) in the presence of 333 μ M ddATP and 33 μ M dGTP, dCTP and dTTP. The reactions were analyzed by gel electrophoresis on a 15% polyacrylamide gel, and the intensities of the bands were quantified by PhosphorImager. The editing level at the Q/R site was determined as the signal obtained from extension of the primer by four nucleotides (the Q/R site A deaminated) relative to the sum of the signals from primers extended four and seven nucleotides (the latter corresponding to unedited RNA). As demonstrated by light emission measurements from BRET experiments, the expression levels of the mutant EGFP-ADAR2 constructs were similar to that of wild-type ADAR2.

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REFERENCES

Andersen, J.S., Lam, Y.W., Leung, A.K., Ong, S.E., Lyon, C.E., Lamond, A.I., and Mann, M. 2005. Nucleolar proteome dynamics. *Nature* **433**: 77–83.
 Bass, B.L. 2002. RNA editing by adenosine deaminases that act on RNA. *Annu. Rev. Biochem.* **71**: 817–846.
 Biggiogera, M., Burki, K., Kaufmann, S.H., Shaper, J.H., Gas, N., Amalric, F., and Fakan, S. 1990. Nucleolar distribution of proteins

B23 and nucleolin in mouse preimplantation embryos as visualized by immunoelectron microscopy. *Development* **110**: 1263–1270.
 Burnashev, N., Khodorova, A., Jonas, P., Helm, P.J., Wisden, W., Monyer, H., Seeburg, P.H., and Sakmann, B. 1992. Calcium-permeable AMPA-kainate receptors in fusiform cerebellar glial cells. *Science* **256**: 1566–1570.
 Chang, K.Y. and Ramos, A. 2005. The double-stranded RNA-binding motif: A versatile macromolecular docking platform. *FEBS J.* **272**: 2109–2117.
 Chen, C.X., Cho, D.S., Wang, Q., Lai, F., Carter, K.C., and Nishikura, K. 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.
 Cho, D.S., Yang, W., Lee, J.T., Shiekhata, R., Murray, J.M., and Nishikura, K. 2003. Requirement of dimerization for RNA editing activity of adenosine deaminases acting on RNA. *J. Biol. Chem.* **278**: 17093–17102.
 Clutterbuck, D.R., Leroy, A., O'Connell, M.A., and Semple, C.A. 2005. A bioinformatic screen for novel A-I RNA editing sites reveals recoding editing in BC10. *Bioinformatics* **21**: 2590–2595.
 Desterro, J.M., Keegan, L.P., Lafarga, M., Berciano, M.T., O'Connell, M., and Carmo-Fonseca, M. 2003. Dynamic association of RNA-editing enzymes with the nucleolus. *J. Cell Sci.* **116**: 1805–1818.
 Desterro, J.M., Keegan, L.P., Jaffray, E., Hay, R.T., O'Connell, M.A., and Carmo-Fonseca, M. 2005. SUMO-1 modification alters ADAR1 editing activity. *Mol. Biol. Cell* **16**: 5115–5126.
 Gales, C., Rebois, R.V., Hogue, M., Trieu, P., Breit, A., Hebert, T.E., and Bouvier, M. 2005. Real-time monitoring of receptor and G-protein interactions in living cells. *Nat. Methods* **2**: 177–184.
 Gallo, A., Keegan, L.P., Ring, G.M., and O'Connell, M.A. 2003. An ADAR that edits transcripts encoding ion channel subunits functions as a dimer. *EMBO J.* **22**: 3421–3430.
 Herbert, A. and Rich, A. 2001. The role of binding domains for dsRNA and Z-DNA in the in vivo editing of minimal substrates by ADAR1. *Proc. Natl. Acad. Sci.* **98**: 12132–12137.
 Herbert, A., Wagner, S., and Nickerson, J.A. 2002. Induction of protein translation by ADAR1 within living cell nuclei is not dependent on RNA editing. *Mol. Cell* **10**: 1235–1246.
 Higuchi, M., Maas, S., Single, F.N., Hartner, J., Rozov, A., Burnashev, N., Feldmeyer, D., Sprengel, R., and Seeburg, P.H. 2000. Point mutation in an AMPA receptor gene rescues lethality in mice deficient in the RNA-editing enzyme ADAR2. *Nature* **406**: 78–81.
 Hough, R.F. and Bass, B.L. 1994. Purification of the *Xenopus laevis* double-stranded RNA adenosine deaminase. *J. Biol. Chem.* **269**: 9933–9939.
 ———. 1997. Analysis of *Xenopus* dsRNA adenosine deaminase cDNAs reveals similarities to DNA methyltransferases. *RNA* **3**: 356–370.
 Jaikaran, D.C., Collins, C.H., and MacMillan, A.M. 2002. Adenosine to inosine editing by ADAR2 requires formation of a ternary complex on the GluR-B R/G site. *J. Biol. Chem.* **277**: 37624–37629.
 James, P., Halladay, J., and Craig, E.A. 1996. Genomic libraries and a host strain designed for highly efficient two-hybrid selection in yeast. *Genetics* **144**: 1425–1436.
 Jensen, T.H., Jensen, A., Szilvay, A.M., and Kjems, J. 1997. Probing the structure of HIV-1 Rev by protein footprinting of multiple monoclonal antibody-binding sites. *FEBS Lett.* **414**: 50–54.
 Ke, S.H. and Madison, E.L. 1997. Rapid and efficient site-directed mutagenesis by single-tube “megaprimer” PCR method. *Nucleic Acids Res.* **25**: 3371–3372.
 Lehmann, K.A. and Bass, B.L. 1999. The importance of internal loops within RNA substrates of ADAR1. *J. Mol. Biol.* **291**: 1–13.
 Levanon, E.Y., Eisenberg, E., Yelin, R., Nemzer, S., Hallegger, M., Shemesh, R., Fligelman, Z.Y., Shoshan, A., Pollock, S.R., Szybel, D., et al. 2004. Systematic identification of abundant

- A-to-I editing sites in the human transcriptome. *Nat. Biotechnol.* **22**: 1001–1005.
- Liu, Y. and Samuel, C.E. 1999. Editing of glutamate receptor subunit B pre-mRNA by splice-site variants of interferon-inducible double-stranded RNA-specific adenosine deaminase ADAR1. *J. Biol. Chem.* **274**: 5070–5077.
- Liu, Y., Emeson, R.B., and Samuel, C.E. 1999. Serotonin-2C receptor pre-mRNA editing in rat brain and in vitro by splice site variants of the interferon-inducible double-stranded RNA-specific adenosine deaminase ADAR1. *J. Biol. Chem.* **274**: 18351–18358.
- Maas, S., Melcher, T., Herb, A., Seeburg, P.H., Keller, W., Krause, S., Higuchi, M., and O'Connell, M.A. 1996. Structural requirements for RNA editing in glutamate receptor pre-mRNAs by recombinant double-stranded RNA adenosine deaminase. *J. Biol. Chem.* **271**: 12221–12226.
- Macbeth, M.R., Lingam, A.T., and Bass, B.L. 2004. Evidence for autoinhibition by the N terminus of hADAR2 and activation by dsRNA binding. *RNA* **10**: 1563–1571.
- Macbeth, M.R., Schubert, H.L., Vandemark, A.P., Lingam, A.T., Hill, C.P., and Bass, B.L. 2005. Inositol hexakisphosphate is bound in the ADAR2 core and required for RNA editing. *Science* **309**: 1534–1539.
- Melcher, T., Maas, S., Herb, A., Sprengel, R., Higuchi, M., and Seeburg, P.H. 1996. RED2, a brain-specific member of the RNA-specific adenosine deaminase family. *J. Biol. Chem.* **271**: 31795–31798.
- Mercier, J.F., Salahpour, A., Angers, S., Breit, A., and Bouvier, M. 2002. Quantitative assessment of β 1- and β 2-adrenergic receptor homo- and heterodimerization by bioluminescence resonance energy transfer. *J. Biol. Chem.* **277**: 44925–44931.
- Nanduri, S., Rahman, F., Williams, B.R., and Qin, J. 2000. A dynamically tuned double-stranded RNA binding mechanism for the activation of antiviral kinase PKR. *EMBO J.* **19**: 5567–5574.
- Nie, Y., Ding, L., Kao, P.N., Braun, R., and Yang, J.H. 2005. ADAR1 interacts with NF90 through double-stranded RNA and regulates NF90-mediated gene expression independently of RNA editing. *Mol. Cell. Biol.* **25**: 6956–6963.
- Ohman, M., Kallman, A.M., and Bass, B.L. 2000. In vitro analysis of the binding of ADAR2 to the pre-mRNA encoding the GluR-B R/G site. *RNA* **6**: 687–697.
- Poulsen, H., Nilsson, J., Damgaard, C.K., Egebjerg, J., and Kjems, J. 2001. CRM1 mediates the export of ADAR1 through a nuclear export signal within the Z-DNA binding domain. *Mol. Cell. Biol.* **21**: 7862–7871.
- Ramos, A., Grunert, S., Adams, J., Micklem, D.R., Proctor, M.R., Freund, S., Bycroft, M., St Johnston, D., and Varani, G. 2000. RNA recognition by a Staufen double-stranded RNA-binding domain. *EMBO J.* **19**: 997–1009.
- Ryter, J.M. and Schultz, S.C. 1998. Molecular basis of double-stranded RNA-protein interactions: Structure of a dsRNA-binding domain complexed with dsRNA. *EMBO J.* **17**: 7505–7513.
- Sansam, C.L., Wells, K.S., and Emeson, R.B. 2003. Modulation of RNA editing by functional nucleolar sequestration of ADAR2. *Proc. Natl. Acad. Sci.* **100**: 14018–14023.
- Scherl, A., Coute, Y., Deon, C., Calle, A., Kindbeiter, K., Sanchez, J.C., Greco, A., Hochstrasser, D., and Diaz, J.J. 2002. Functional proteomic analysis of human nucleolus. *Mol. Biol. Cell* **13**: 4100–4109.
- Sommer, B., Kohler, M., Sprengel, R., and Seeburg, P.H. 1991. RNA editing in brain controls a determinant of ion flow in glutamate-gated channels. *Cell* **67**: 11–19.
- Stephens, O.M., Haudenschild, B.L., and Beal, P.A. 2004. The binding selectivity of ADAR2's dsRBMs contributes to RNA-editing selectivity. *Chem. Biol.* **11**: 1239–1250.
- Thomis, D.C. and Samuel, C.E. 1993. Mechanism of interferon action: Evidence for intermolecular autophosphorylation and autoactivation of the interferon-induced, RNA-dependent protein kinase PKR. *J. Virol.* **67**: 7695–7700.
- Tonkin, L.A., Saccomanno, L., Morse, D.P., Brodigan, T., Krause, M., and Bass, B.L. 2002. RNA editing by ADARs is important for normal behavior in *Caenorhabditis elegans*. *EMBO J.* **21**: 6025–6035.
- Ung, T.L., Cao, C., Lu, J., Ozato, K., and Dever, T.E. 2001. Heterologous dimerization domains functionally substitute for the double-stranded RNA binding domains of the kinase PKR. *EMBO J.* **20**: 3728–3737.
- Xu, Y., Piston, D.W., and Johnson, C.H. 1999. A bioluminescence resonance energy transfer (BRET) system: Application to interacting circadian clock proteins. *Proc. Natl. Acad. Sci.* **96**: 151–156.
- Yi-Brunozzi, H.Y., Stephens, O.M., and Beal, P.A. 2001. Conformational changes that occur during an RNA-editing adenosine deamination reaction. *J. Biol. Chem.* **276**: 37827–37833.
- Zhang, F., Romano, P.R., Nagamura-Inoue, T., Tian, B., Dever, T.E., Mathews, M.B., Ozato, K., and Hinnebusch, A.G. 2001. Binding of double-stranded RNA to protein kinase PKR is required for dimerization and promotes critical autophosphorylation events in the activation loop. *J. Biol. Chem.* **276**: 24946–24958.

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