Trans and *cis* factors affecting A-to-I RNA editing efficiency of a noncoding editing target in *C. elegans*

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ABSTRACT

Adenosine-to-inosine RNA editing by ADARs affects thousands of adenosines in an organism's transcriptome. However, adenosines are not edited at equal levels nor do these editing levels correlate well with ADAR expression levels. Therefore, additional mechanisms are utilized by the cell to dictate the editing efficiency at a given adenosine. To examine *cis*- and *trans*-acting factors that regulate A-to-I editing levels specifically in neural cells, we utilized the model organism *Caenorhabditis elegans*. We demonstrate that a double-stranded RNA (dsRNA) binding protein, ADR-1, inhibits editing in neurons, which is largely masked when examining editing levels from whole animals. Furthermore, expression of ADR-1 and mRNA expression of the editing target can act synergistically to regulate editing efficiency. In addition, we identify a dsRNA region within the *Y75B8A.8* 3′ UTR that acts as a *cis*-regulatory element by enhancing ADR-2 editing efficiency. Together, this work identifies mechanisms that regulate editing efficiency of noncoding A-to-I editing sites, which comprise the largest class of ADAR targets.

Keywords: ADAR; RNA editing; double-stranded RNA; neurons; regulation of RNA editing

INTRODUCTION

Post-transcriptional modification of RNA bases is one mechanism that organisms utilize to diversify the transcripts produced within different cell types from a static genomic sequence (Li and Mason 2014). With over 100 million predicted occurrences in the human transcriptome, one of the most prevalent RNA base modifications is the deamination of adenosine (Bazak et al. 2014). This modification, commonly referred to as RNA editing, is catalyzed by a family of proteins called adenosine deaminases that act on RNA or ADARs (Savva et al. 2012). This type of RNA editing is limited to structured RNA as ADARs only target regions of dsRNA for deamination (Nishikura 2010).

ADARs convert adenosine to inosine, which is a biological mimic of guanosine (Pullirsch and Jantsch 2010). ADAR editing activity has the potential to alter mRNA coding potential, affect alternative splicing, and influence miRNA biogenesis and targeting (Hundley and Bass 2010; Rieder and Reenan 2012; Rosenthal and Seeburg 2012; Wulff and Nishikura 2012). Moreover, the editing levels of individual sites can vary considerably during development and rarely reach 100% editing efficiency demonstrating the dynamic effect of ADARs on the transcriptome (Wahlstedt et al. 2009).

While much recent work has been dedicated to identifying new editing sites, understanding the factors that influence how efficiently an adenosine is deaminated stands out as an area of the RNA editing field with many unanswered questions. ADARs prefer an adenosine, uridine or cytidine 5′ of the target adenosine while guanosine is favored 3′ to the adenosine (Lehmann and Bass 2000; Eggington et al. 2011; Bahn et al. 2012; Bazak et al. 2014). However, editing sites with the same surrounding nucleotide context can exhibit drastically different editing levels, suggesting this is not the only determinant of editing efficiency (Kawahara et al. 2008; Tian et al. 2011; Wheeler et al. 2015). Alternative splicing of ADAR transcripts (Lai et al. 1997; Rueter et al. 1999) and post-transcriptional modification (Desterro et al. 2005) have both been observed to cause decreased ADAR activity. In addition, RNA-binding proteins can both promote and repress editing of specific adenosines (Bhogal et al. 2011; Tariq et al. 2013; Washburn et al. 2014). However, how much these mechanisms interact to determine overall editing efficiency and whether these mechanisms are active in all cell types is poorly understood. Furthermore, it has been suggested that levels of A-to-I editing do not always directly correlate with

ADAR expression levels (Wahlstedt et al. 2009). For example, the editing efficiency of the Filamin A mRNA in mice can be drastically different in various tissues even though these tissues express the same level of ADAR and Filamin A mRNA (Stulić and Jantsch 2013). However, this does not address if altering the expression levels within the same tissue will have an influence on editing efficiency. Increasing the expression of ADAR1 in cell culture has been observed to increase the editing efficiency of *hNeil1* (Daniel et al. 2014). This suggests that altering the ratio of ADARs and the editing target within a singular tissue context can influence editing efficiency; however, this has not yet been examined in vivo. Therefore, understanding the cellular mechanisms that dictate editing efficiency and how these mechanisms are regulated is paramount to understanding ADAR editing activity.

Recently, local dsRNA structures in close proximity, but not encompassing the ADAR editing site, have been observed to influence site-specific editing of coding sequences. In the *Drosophila* editing target *para*, dsRNA elements, including a predicted pseudoknot, created by the intron downstream from exonic editing sites, influence editing efficiency even though these regions lack editing sites (Rieder et al. 2013). Similarly, downstream dsRNA hairpins within the introns of the human *Gabra-3* and *Neil1* transcripts have also been shown to increase editing efficiency of adjacent editing sites in coding regions (Daniel et al. 2012, 2014). While all of these examples involved regulating editing efficiency in coding regions, it is unclear whether this mechanism also extends to editing events in noncoding regions, which harbor the vast majority of editing sites identified to date (Bazak et al. 2014; Washburn et al. 2014; Whipple et al. 2015).

To examine mechanisms that regulate noncoding editing efficiency in vivo, we utilized the model organism *Caenorhabditis elegans*. In *C. elegans*, two ADAR proteins, ADR-1 and ADR-2, are present in the genome (Tonkin et al. 2002). We previously demonstrated that ADR-1 does not edit RNA, but it can function to regulate the extent to which specific adenosines are edited by ADR-2 (Washburn et al. 2014). In addition, we identified a novel *C. elegans* editing target, the mRNA *Y75B8A.8*, and characterized the pattern of editing events within its 3′ UTR (Wheeler et al. 2015). As this 3′ UTR contains two highly edited sites, we utilized this gene to generate a reporter system to examine *cis*- and *trans*-acting factors that affect editing efficiency in vivo. In our prior approaches, we utilized RNA collected from whole animals, which could make examination of editing regulation difficult if distinct regulatory mechanisms are present in different tissues.

Using our reporter system, we demonstrate that the extent of editing of the *Y75B8A.8* 3′ UTR is significantly different in neurons compared to editing levels of *Y75B8A.8* observed from whole animal RNA. We demonstrate that the major determinant in this difference is the presence of ADR-1. In addition, the mRNA expression level of the editing target can act synergistically with ADR-1 expression to alter editing

efficiency. Last, removing an adjacent unedited region of dsRNA significantly decreases ADR-2 editing efficiency, suggesting that this region acts to recruit ADR-2 to the downstream editing sites.

RESULTS AND DISCUSSION

Tissue-specific reporter reveals distinct editing efficiency in neurons

To determine *cis*- and *trans*-acting factors that affect editing efficiency and exclude differences in editing levels due to distinct modes of regulation across tissues, a reporter system to examine editing of the *Y75B8A.8* 3′ UTR exclusively in neurons was developed. Briefly, the dsRNA region of the *Y75B8A.8* 3′ UTR was cloned into a GFP vector under the control of the pan-neuronal *rab3* promoter. The reporter also includes the 3′ UTR of *unc-54*, which is not edited, but promotes transcript stability (Fig. 1A). This neuronal reporter construct together with a control construct expressing RFP, but lacking dsRNA, was introduced into wild-type worms via microinjection. To avoid cross-contamination in analyzing expression and editing of the endogenous and reporter transcripts, specific primers were designed to amplify only the endogenous *Y75B8A.8* 3′ UTR or the neuronal reporter. Compared to the endogenous *Y75B8A.8* 3′ UTR collected from total worm RNA, editing in the neuronal reporter was significantly lower with a 28% reduction in editing at site 227 and a 40% reduction at site 228 (Fig. 1B).

One possibility for the reduced editing of the *Y75B8A.8* 3′ UTR reporter compared to endogenous *Y75B8A.8* mRNA could be that the reporter mRNA expression is significantly lower than the endogenous mRNA expression, thus preventing significant amounts of ADR-2 from binding to the reporter mRNA. Another possibility for the reduced editing of the reporter might be an inability of ADR-2 to compete with neural RNA-binding factors for binding to the reporter mRNA. To examine these possibilities, we sought to increase the mRNA available to ADR-2 by creating additional worm lines that exhibited higher neural mRNA expression. This was accomplished by varying the concentration of the *Y75B8A.8* neuronal reporter DNA in the microinjection mix. For each strain, mRNA expression level of the neuronal reporter and a *rab3*::RFP control, which was maintained at a constant concentration in all of the injection mixes, was determined by qRT-PCR. By comparing the expression of GFP, which is harbored by the neuronal reporter, to the control RFP, the relative mRNA expression of the neuronal reporter constructs was calculated. Using this method, we were able to increase expression of the neuronal reporter by more than 100-fold (Fig. 1C). However, when comparing editing levels at sites 227 and 228 of this highly expressed neuronal reporter to the neuronal reporter that was expressed 100-fold lower, editing levels only differed at site 228 by 5.5% (Fig. 1B). This indicates that differences in mRNA expression between
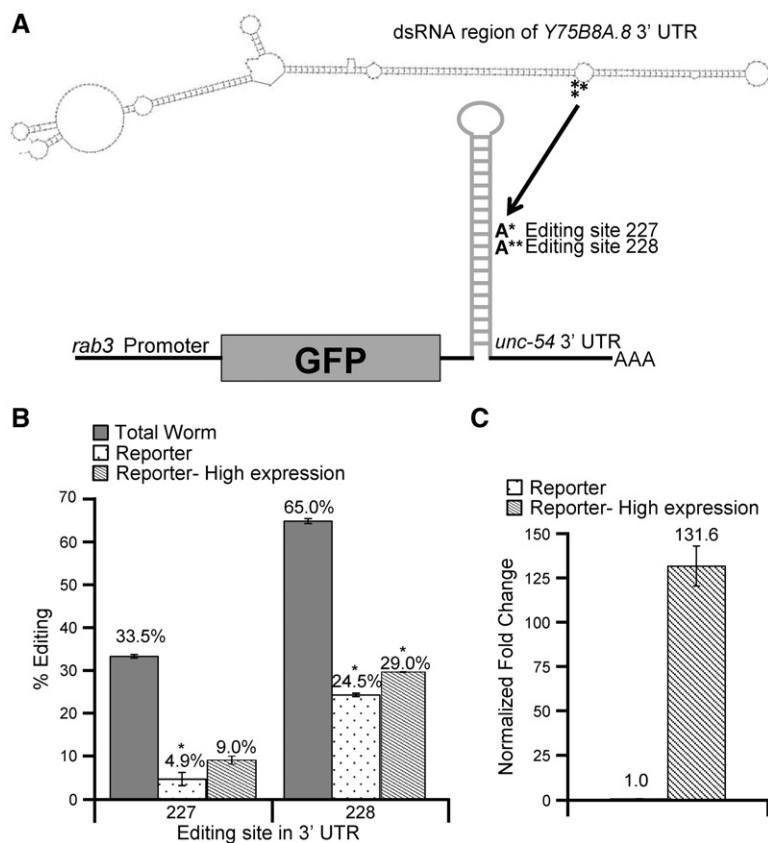
FIGURE 1. *Y75B8A.8* 3′ UTR neuronal editing reporter. (*A*) Schematic of the *Y75B8A.8* 3′ UTR neuronal reporter. The dsRNA region of the *Y75B8A.8* 3′ UTR (322 nt) was cloned into a vector downstream from the GFP coding region and upstream of the *unc-54* 3′ UTR. The pan-neuronal *rab3* promoter drove reporter expression. Secondary structure of *Y75B8A*.8 dsRNA region was generated with mfold (Zuker 2003). Asterisks denote editing sites 227 (*) and 228 (**) in the mfold structure. (*B*, *C*) Error bars represent standard error of the mean (SEM). Significant changes ($P \leq 0.0005$) by Student's *t*-test are marked with an asterisk. (*B*) Editing levels for both the endogenous *Y75B8A.8* 3′ UTR (gray), the *Y75B8A.8* neuronal reporter (black dots), and the highly expressed *Y75B8A.8* 3′ UTR neuronal reporter (hatched) from three biological replicates of staged young-adult wild-type worms. (*C*) Relative expression of the *Y75B8A.8* 3′ UTR neuronal reporter (black dots) or the highly expressed *Y75B8A.8* 3′ UTR neuronal reporter (hatched). Fold change was determined by normalizing to the relative GFP expression of the *Y75B8A.8* 3′ UTR neuronal reporter (black dots).

the endogenous *Y75B8A.8* mRNA and the neuronal reporter are not the sole determinant of the reduced editing levels observed in neurons.

ADR-1 represses editing efficiency of the *Y75B8A.8* 3′ UTR in neurons

To gain insight into the difference in editing levels of *Y75B8A.8* in neurons compared to the whole animal, we next focused on ADR-1. We previously identified ADR-1 as a major regulator of editing in *C. elegans* (Washburn et al. 2014), and ADR-1 is known to be highly expressed in *C. elegans* neurons (Tonkin et al. 2002). To assess if ADR-1 regulates editing levels of the endogenous *Y75B8A.8* 3′ UTR, we examined editing efficiency in *adr-1(-)* null worms. When compared to wild-type, editing levels of the endogenous 3′

UTR are repressed by ADR-1 as editing efficiency increases nearly 6% at site 227 and 14% at site 228 in the absence of ADR-1 expression (Fig. 2). This increased editing level is due to loss of *adr-1* and not other differences in the strain backgrounds, as reintroduction of an ADR-1 transgene restores editing levels of the endogenous *Y75B8A.8* mRNA to wild-type levels (Supplemental Fig. 1). To examine if ADR-1 expression also affects editing efficiency of the *Y75B8A.8* 3′ UTR in neurons, the transgenic animals harboring the *Y75B8A.8* 3′ UTR neuronal reporters were crossed to worms lacking ADR-1 expression to generate animals expressing the *Y75B8A.8* 3′ UTR neuronal reporter in the *adr-1(-)* background. Interestingly, *adr-1(-)* males were unable to successfully mate (data not shown); therefore, *adr-1(-);adr-2(-)* males were utilized for the cross. Consistent with the effects of *adr-1* on endogenous *Y75B8A.8*, editing at both sites 227 and 228 in the neuronal reporter is significantly higher in animals lacking *adr-1* (Fig. 2). However, in neurons, ADR-1 expression has a much greater influence with a 20% increase in editing at site 227 and a nearly 33% increase at site 228. This demonstrates that in the tissue-specific context of neurons, ADR-1 repression of editing efficiency at these sites has a more pronounced effect. This suggests that a portion of endogenous *Y75B8A.8* transcripts is located in cells lacking ADR-1 or with lower expression of ADR-1.

Because ADR-1 represses editing in the *Y75B8A.8* 3′ UTR and ADR-1 is a known dsRNA binding protein, it is possible that ADR-1 binds to the *Y75B8A.8* 3′ UTR in neurons and blocks ADR-2 access to the target adenosines resulting in decreased editing. Additionally, as ADR-1 is known to be expressed approximately 10-fold higher than ADR-2 in the worm (Hundley et al. 2008), this ADR-1 repression of the editing model is consistent with the fact that increasing mRNA expression had a limited influence on editing efficiency in wild-type worms. To examine this, editing levels of the highly expressed neuronal reporters in the *adr-1(-)* background were determined (Fig. 2). In agreement with wild-type worms, mRNA expression does not significantly affect editing at site 227 in the absence of *adr-1*. However, at site 228, editing efficiency increases nearly 14% with increased mRNA expression in the absence of *adr-1*, which is greater than the increase observed in wild-type worms. This suggests
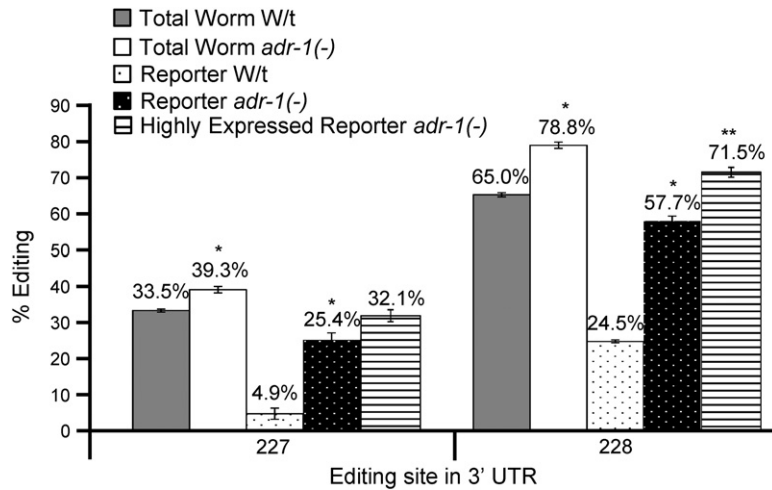
FIGURE 2. ADR-1 has a greater effect on repressing editing efficiency in neurons. (*A*) Editing levels for sites 227 and 228 in the endogenous *Y75B8A.8* 3′ UTR from wild-type worms (gray), endogenous *Y75B8A.8* 3′ UTR from *adr-1(-)* worms (white), the *Y75B8A.8* 3′ UTR neuronal reporter in wild-type worms (black dots), the *Y75B8A.8* 3′ UTR neuronal reporter in *adr-1(-)* worms (white dots), and the highly expressed *Y75B8A.8* 3′ UTR neuronal reporter in *adr-1(-)* worms (horizontal lines) were determined from three biological replicates of staged young-adult worms. Error bars represent SEM. Significant changes ($P \leq 0.005$) between the respective wild-type and *adr-1* editing levels are marked with an asterisk, and significant changes ($P \leq 0.005$) between the highly expressed reporter and the reporter in *adr-1* are marked by two asterisks.

that in the absence of ADR-1, mRNA expression has a greater impact on influencing editing levels at site 228. Furthermore, this demonstrates that while altering mRNA expression alone may have only a slight impact on editing levels, combining high mRNA expression with a loss of ADR-1 expression has an additive effect on increasing editing efficiency.

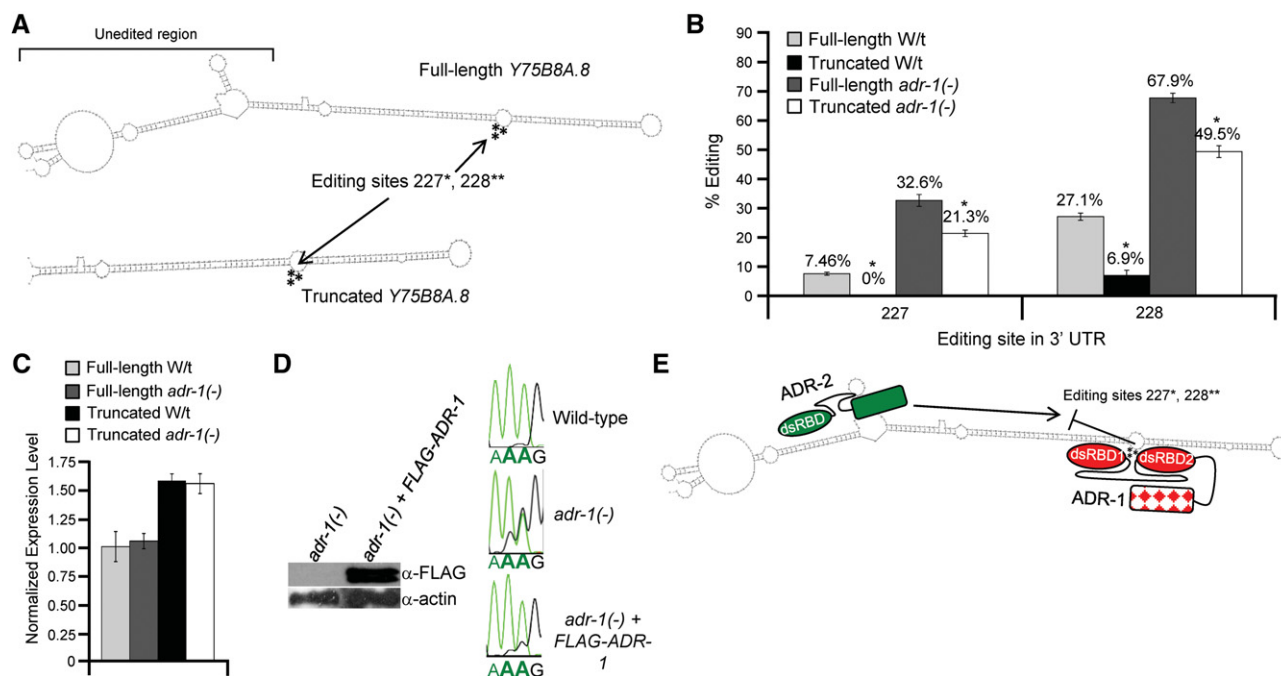## A dsRNA region within the *Y75B8A.8* 3′ UTR enhances editing efficiency

After determining that both mRNA and ADR-1 expression influence editing of the *Y75B8A.8* 3′ UTR neuronal reporter, we wanted to determine the minimal region of the *Y75B8A.8* 3′ UTR required for ADR-1 editing repression. Within the *Y75B8A.8* 3′ UTR, a dsRNA region adjacent to editing sites 227 and 228 contains no detectable editing sites (Wheeler et al. 2015). As ADR-1 likely competes with ADR-2 for access to the dsRNA surrounding the editing sites to repress editing, we speculated this region was not required ADR-1 editing repression. To assess this possibility, we generated a truncated version of the *Y75B8A.8* neuronal reporter (Fig. 3A). Interestingly, the truncated reporter exhibited surprisingly lower editing levels at both editing sites 227 and 228 (Fig. 3B). In wild-type worms, editing at site 227 is undetectable in the truncated reporter and reduced by almost 20% at site 228. Furthermore, greater truncations of the *Y75B8A.8* 3′ UTR did not result in further decreases in editing efficiency (data not shown). This suggests that we serendipitously identified a region of the *Y75B8A.8* that enhances editing efficiency. As the region absent in the truncated reporter does not

contain any editing sites, this reduction in editing efficiency also cannot be attributed to loss of downstream editing sites.

One possibility for the reduction of editing observed in the truncated neuronal reporter could be reduced mRNA expression. However, in comparison to the full-length *Y75B8A.8* neuronal reporter, the truncated reporter has a similar level of expression (Fig. 3C). Another possibility is that the truncation could disrupt the structure of the dsRNA encompassing the editing sites. In this regard, we did not observe any alterations in the predicted mfold structure surrounding the editing sites for the truncated reporter (Fig. 3A; Zuker 2003). If removing this region simply causes enhanced ADR-1 repression of editing, then editing levels of the truncated reporter should return to full-length levels in the absence of ADR-1. To assess this possibility, we examined editing efficiency of the truncated reporter in the absence of ADR-1 (Fig. 3B). Similar to the full-length reporter, editing efficiency of the truncated reporter increases at both sites 227 (over 20% increase) and 228 (over 40% increase) in the absence of ADR-1. This effect on editing can be directly attributed to loss of *adr-1*, as reintroduction of an *adr-1* transgene expressing Flag-tagged ADR-1 (Fig. 3D, left panels) restores editing of the truncated *Y75B8A.8* reporter to wild-type levels (Fig. 3D, right panels). However, despite the increased editing of the truncated reporter in the absence of *adr-1(-)*, editing levels do not return to those observed for the full-length reporter. This suggests that this unedited region acts to increase ADR-2 editing efficiency at sites 227 and 228.

Based on these observations, we propose a model where the unedited dsRNA region serves as a *cis*-acting element to recruit ADR-2 to the *Y75B8A.8* 3′ UTR, thus increasing editing of sites 227 and 228 (Fig. 3E). It is also possible that truncating the *Y75B8A.8* 3′ UTR results in less stable base-pairing of the remaining stem or increases the reporter mRNA export from the nucleus, thus indirectly reducing editing levels. However, as editing of the truncated reporter in the absence of *adr-1* exceeds the editing levels of the full-length reporter in wild-type cells by over 20%, the truncation does not appear to significantly disrupt the dsRNA structure around the editing site. Future experiments to address this model should include tests of whether transferring this sequence to other 3′ UTRs can function to enhance editing and whether this sequence increases editing by ADR-2 in vitro. Furthermore, as fusing the 3′ UTR of *Y75B8A.8* to the GFP reporter has lower editing levels than endogenous *Y75B8A.8*, it is interesting to speculate whether other upstream *cis*-acting elements could also

FIGURE 3. A dsRNA region within the *Y75B8A.8* 3′ UTR enhances editing efficiency. (*A*) Schematic showing the folded secondary structure of the full-length *Y75B8A.8* neuronal reporter and the secondary structure of the truncated *Y75B8A.8* neuronal reporter as predicted by mfold (Zuker 2003). Arrows indicate the location of editing sites 227 and 228 in each construct. The unedited region removed in the truncated reporter is indicated on the full-length reporter. (*B*) Editing levels for sites 227 and 228 in the full-length *Y75B8A.8* reporter from wild-type worms (light gray), the truncated *Y75B8A.8* reporter in wild-type worms (black), the full-length reporter in *adr-1(-)* worms (dark gray), and the truncated *Y75B8A.8* reporter in *adr-1(-)* worms (white) were determined from three biological replicates of staged young-adult worms. Error bars represent SEM. Significant changes between the full-length and truncated reporter in each respective genetic background ($P \leq 0.01$) by Student's *t*-test are marked with an asterisk. (*C*) Relative expression of the full-length *Y75B8A.8* 3′ UTR neuronal reporter in wild-type worms (light gray), the full-length *Y75B8A.8* 3′ UTR neuronal reporter in *adr-1(-)* (dark gray), the truncated *Y75B8A.8* 3′ UTR neuronal reporter in wild-type worms (black), and the truncated *Y75B8A.8* 3′ UTR neuronal reporter in *adr-1(-)* worms (white). Fold change was determined by normalizing to the relative GFP expression of the full-length *Y75B8A.8* 3′ UTR neuronal reporter in wild-type worms (gray). Error bars represent standard error of the mean (SEM). (*D*, *left* panel) Worm lysates of the indicated strains expressing the truncated *Y75B8A.8* reporter were subjected to immunoblotting for the Flag epitope and actin as a loading control. (*Right* panel) Total RNA from the two biological replicates of the indicated strains expressing the truncated *Y75B8A.8* reporter was reverse transcribed, PCR amplified with reporter specific primers and Sanger sequenced. Sequence chromatograms of the PCR products from one biological replicate are shown. The bold A indicates editing sites 227 and 228. (*E*) Proposed model of how the unedited region promotes editing efficiency of ADR-2 and how ADR-1 represses editing in the *Y75B8A.8* 3′ UTR. The unedited region acts to recruit ADR-2 to the *Y75B8A.8* 3′ UTR thereby increasing editing efficiency. If ADR-1 is expressed, it competes with ADR-2 for access to editing sites 227 and 228 resulting in repressing of editing efficiency.

enhance editing of the 3′ UTR. While there are no other predicted double-stranded regions or editing sites present in the endogenous 3′ UTR and lacking in our reporter, editing sites in the first intron of endogenous *Y75B8A.8* mRNA, located 2000 nucleotides upstream of the 3′ UTR, have been previously identified (Zhao et al. 2014). It would be interesting to determine whether this intronic region could influence editing of the downstream 3′ UTR region in endogenous *Y75B8A.8* mRNA. Perhaps by analyzing worm strains that lack factors important for splicing or mutants with reduced speed of splicing, effects of the intron on 3′ UTR editing could be observed.

Conclusions

Using a reporter system to examine factors that affect RNA editing levels in a singular tissue, we have demonstrated three major determinants of editing efficiency for the *Y75B8A.8* 3′ UTR. Foremost, we observed that the dsRBP ADR-1 is the major factor influencing editing efficiency in neurons, which is largely absent when examining RNA from total worms. In neurons, we propose that ADR-1 binds to the 3′ UTR of the *Y75B8A.8* mRNA, which blocks access of ADR-2 to the target adenosine and reduces editing (Fig. 3E). In the future, it will be critical to determine whether this function of ADR-1 is specific to neurons and if it is extends to other transcripts, as ADR-1 is known to be a major regulator of editing in 3′ UTRs. In addition, we observe that altering mRNA expression of the neuronal reporter can slightly influence editing efficiency. However, this influence is increased in the absence of ADR-1 suggesting these two factors have a combinatorial effect on regulating editing efficiency. Finally, we identify a region of the *Y75B8A.8* 3′ UTR that acts to increase ADR-2 editing efficiency. This demonstrates that unedited dsRNA regions of an editing substrate play important roles in regulating editing efficiency of noncoding regions of mRNA.

MATERIALS AND METHODS

Maintenance of worm strains and transgenics

Worm strains were maintained by growth on NGM plates seeded with *Escherichia coli* OP50. Transgenic worm lines were generated by microinjection into the gonads of young-adult worms of the appropriate genetic background. The injection mix used for generating transgenics contained the following: 1, 10, 20, or 40 ng/µL of the transgene of interest, 20 ng/µL of the dominant marker, and 1 kb DNA ladder (NEB) to raise the final concentration of the injection mix to 100 ng/µL. Transgenic strains were maintained by passaging only worms with the dominant marker. The dominant marker used in this study was *rab3::rfp::unc-54* (HH plasmid #22). The transgenes expressing the full-length *Y75B8A.8* 3′ UTR (HH #340) or the truncated version of the *Y75B8A.8* 3′ UTR (HH#344) were injected in a modified version of the *rab3::rfp::unc-54* (#HH21) plasmid, previously published (Hundley et al. 2008). Briefly, the full-length *Y75B8A.8* 3′ UTR and the truncated version were amplified by PCR from N2 genomic DNA with primers designed to add on Sal I and Pst I restriction sites that were introduced into the vector between the stop codon of GFP and the *unc-54* 3′ UTR.

To obtain young adults used in experiments, embryos were isolated from gravid worms grown on 6 cm plates using standard hypochlorite treatment. Young adults were obtained by hatching eggs overnight in M9 buffer, transferring synchronized L1 worms to NGM plates seeded with *Escherichia coli* OP50, and incubating at 20°C for 48–54 h.

The following strains were utilized in this study: Bristol strain N2 (HH#1), BB19 *adr-1(tm668)*, BB21 *adr-1(tm668);adr-2(ok735)* (HH#7), BB21 *adr-1(tm668)* + blmEx1 [3XFLAG-*adr-1* genomic, *rab3::gfp::unc-54*] (HH#76), N2 + blmEx5 [1 ng/µL full-length *Y75B8A.8* 3′ UTR transgene, 20 ng/µL *rab3::rfp::unc-54*] (HH# 179), N2 + blmEx5 (HH #180), BB19 *adr-1(tm668)* + blmEx5 (HH#181), N2 + blmEx6 [10 ng/µL full-length *Y75B8A.8* 3′ UTR transgene, 20 ng/µL *rab3::rfp::unc-54*] (HH#178), N2 + blmEx6 (HH#182), BB19 *adr-1(tm668)* + blmEx6 (HH#183), N2 + blmEx7 [40 ng/µL full-length *Y75B8A.8* 3′ UTR transgene, 20 ng/µL *rab3::rfp::unc-54*] (HH#177), N2 + blmEx7 (HH#184), BB19 *adr-1(tm668)* + blmEx7 (HH#184), N2 + blmEx8 [20 ng/µL truncated *Y75B8A.8* 3′ UTR transgene, 20 ng/µL *rab3::rfp::unc-54*] (HH#168), N2 + blmEx8 (HH#186), BB19 *adr-1(tm668)* + blmEx8 (HH#187).

RNA isolation and editing assays

Total RNA was isolated from young-adult worms using TRIzol (Invitrogen). RNA was further treated with Turbo DNase (Ambion) and then isolated using the RNA Easy Extraction kit (Qiagen). Editing assays were performed using 1 µg of DNase treated RNA, Thermoscript RT (Invitrogen) for reverse transcription and PFX Platinum DNA Polymerase (Invitrogen) for PCR amplification with either endogenous *Y75B8A.8* or transgene-specific primers. PCR products were gel purified and 5 ng of purified DNA was subjected to Sanger sequencing. Editing was quantified by measuring the adenosine and guanosine peak heights in Photoshop. For all editing assays, negative controls were conducted without Thermoscript RT to ensure that all DNA subjected to Sanger sequencing resulted from cDNA amplification.

Transgene quantification

Utilizing DNase treated RNA obtained from young-adult worms, the transgene expression was determined by quantitate real-time PCR. For reverse transcription, 1 µg of total RNA was reverse transcribed with Superscript III (Invitrogen) with random hexamers. Following cDNA synthesis, two microliters of cDNA were analyzed per qRT-PCR in a Mastercycler Realplex[2] instrument (Eppendorf) using the 2X KAPA SYBR FAST qPCR Master Mix (Kapa Biosystems). GFP and RFP were quantified using gene-specific primers. Expression of the neuronal reporter was determined by RT-qPCR for GFP present in the *Y75B8A.8* neuronal reporters. Relative GFP expression levels were determined by correcting with the control *rab3*:RFP.

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