ADBP-1 regulates ADR-2 nuclear localization to control editing substrate selection

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ABSTRACT

Adenosine-to-inosine (A-to-I) RNA editing, catalyzed by ADAR enzymes, is a prevalent and conserved RNA modification. While A-to-I RNA editing is essential in mammals, in *Caenorhabditis elegans*, it is not, making them invaluable for RNA editing research. ADR-2 is the sole A-to-I editing enzyme in *C. elegans*. Although ADAR localization is well-studied in humans, it is not well-established in *C. elegans*. In this study, we examine the cellular and tissue-specific localization of ADR-2. We show that while ADR-2 is present in most cells in the embryo, at later stages, the expression is both tissue- and cell-type-specific. Additionally, ADR-2 is present mainly in the nucleus, adjacent to the chromosomes, at all cell cycle stages. We showed that endogenous ADR-2 nuclear localization depends on ADBP-1 and not ADR-1, a prominent RNA editing regulator in *C. elegans*. In *adbp-1* mutant worms, the mislocalization of ADR-2 leads to decreased editing levels as well as *de-novo* editing, mostly in exons, suggesting that ADR-2 is also functional in the cytoplasm. Besides, ADBP-1 absence affects gene expression. Furthermore, our analysis showed that ADR-2 targets adenosines with different surrounding nucleotides in exons and introns. Our findings indicate that ADR-2 cellular localization is highly regulated and affects its functions.

INTRODUCTION

RNA editing is a common post-transcriptional process essential for RNA function[1]. The most prevalent type of RNA editing is A-to-I RNA editing[2]. In this process, adenosine (A) is deaminated into inosine (I), which is recognized by the splicing and translational machinery as guanosine (G)[3,4]. The enzymes that catalyze this conversion are the Adenosine Deaminases Acting on RNA (ADAR)[5]. This seemingly minor modification has an extensive effect both on the structure and function of the edited RNA. The inosine, a non-canonical nucleotide, signals the cell that the modified RNA is not foreign, preventing undesirable activation of the immune system[6]. RNA editing can also alter proteins' amino acid sequence and generate protein isoforms[7]. Moreover, alteration of editing activity occurs in many neurological disorders and cancer[8–13].

In humans, two catalytically active ADARs are known: ADAR1 and ADAR2; both are essential, expressed in most tissues, and target only double-stranded RNA[4]. ADAR1 has two isoforms capable of shuttling between the nucleus and cytoplasm[14]. ADAR1 p110 is constitutively expressed and mostly nuclear, while ADAR1 p150 is interferon-induced and thus is predominantly expressed during viral infection[14]. The latter tends to accumulate in the cytoplasm owing to a nuclear export signal (NES) found in its unique N-terminal region. A non-classical nuclear localization signal (NLS) overlaps the third double-stranded RNA binding domain (dsRBM) in both isoforms[15–17]. The localization of the different isoforms is thought to be one of the regulatory mechanisms affecting ADAR1 RNA editing activity, which occurs mainly in non-coding regions[15,18,19.] Although ADAR2 is nuclear, it can be sequestered into the nucleolus via an NLS found in its dsRBM domains (also in the N-terminus region of the enzyme)[20], thus resembling the form of regulation seen in ADAR1. ADAR2 edits coding regions that can lead to protein recoding but also non-coding regions[21]. Because RNA editing is important for neuronal function, ADARs are essential in mammals; for example, mice containing a homozygous deletion for ADAR2 die shortly after birth[22]. However, in *Caenorhabditis elegans,* deletion of either or both of its ADAR genes is not lethal[23], making this well-characterized model organism ideal for researching the function of RNA editing[24].

C. elegans has two ADAR genes: ADR-1 and ADR-2[24]. The function of ADR-1 is mainly regulatory[25–28], while ADR-2 is catalytically active and edits mainly non-coding sequences[23,25,29]. mRNA expression of *adr-2* has been shown in the germline, neurons, hypodermis, muscle, and intestine of individual *C. elegans* in the larval stage[30]. However, previous studies have been unsuccessful at determining the expression pattern of the ADR-2 protein by using translational reporters, presumably because *adr-2* is situated in a six-gene operon and there are undefined control areas, or it is possible that overexpression of *adr-2* is lethal [23].

The first indication of regulation of ADR-2 localization came from the finding that ADBP-1 (ADR-2 Binding Protein 1) affected the subcellular localization of a heterologous expressed ADR-2 transgene[31]. ADBP-1 was shown to interact with ADR-2, and in the absence of ADBP-1, four known targets of ADR-2 did not undergo RNA editing[31]. However, whether the function of ADR-2 was compromised because of the different localization was not determined.

To gain more insight into the localization of ADR-2 and, thus, the mechanisms of regulation of its RNA editing activity, we performed immunofluorescence studies and RNA-seq experiments. We

found that ADR-2 is ubiquitously expressed in wild-type embryos and localized with the chromosomes throughout the entire cell cycle. In contrast, except for gonads, ADR-2 is not ubiquitously expressed in adult worm tissues. We also show that in the lack of ADBP-1, ADR-2 appears to be cytoplasmatic. It can still edit mRNA; however, this editing happens in lower levels, unlike when ADR-2 is nuclear localized. Although editing levels decrease, ADR-2 mislocalization causes *de-novo* editing, which looks sporadic. Additionally, we found that ADR-2 targets adenosine with surrounding nucleotides specific to untranslated regions, coding exons and introns in wild-type and *adbp-1* mutant worms. Our results suggest that the localization of ADR-2 is highly regulated and likely affects the cellular transcripts' editing levels and expression.

MATERIAL AND METHODS

Maintenance and handling of *C. elegans* strains

Experiments were performed with the wild-type Bristol strain N2[32], BB21 (*adr-1(tm668)* I; *adr-2(ok735)* III[23]), RB886 (*adr-2(ok735)* III[33]), QD1 (*adbp-1(qj1)* II[31]) and ALM517 *(adr-1(uu49*). ALM517 was obtained but outcrossing 3 times and separating *adr-1(uu49)* from strain BB239 (*adr-1(uu49)* I; *adr-2(uu28)* III)[34]. All *C. elegans* strains were grown at 15°C on NGM agar 5-cm plates and seeded with E. coli OP50 bacteria. For mRNA-seq libraries preparation, strains were grown at 20°C.

DNA and RNA Sanger sequencing

DNA extraction was performed according to a protocol in which worm lysis buffer is applied to a sample of ~5 worms, followed by cycles of cooling and heating temperatures (-80°C for 15 minutes, 60°C for 60 minutes, 95°C for 15 minutes, finally cooling down to 4°C)[35].

To obtain cDNA, extracted RNA was treated with turbo DNase (Ambion). Then, a reverse transcriptase reaction (Quanta - qScript Flex cDNA Kit) was done using oligo-dT. The amplification products were sequenced by Sanger sequencing.

mRNA-seq libraries preparation

Worms were washed with M9 and treated with sodium hyperchlorite. The embryos were either resuspended in EN buffer and frozen in liquid nitrogen, or resuspended in M9 buffer and left overnight in a rotator at 20°C. The hatched and synchronized L1 larvae were placed on NGM media with OP50 until they reached the L4 stage. The L4 larvae were washed with EN buffer and frozen with liquid nitrogen. Frozen pellets were ground to powder with liquid nitrogen chilled mortar and pestle. RNA was extracted by using Direct-zol RNA MiniPrep Plus (ZYMO). The RNA was treated with TURBO DNAse (Ambion). mRNA sequencing libraries were prepared by using Illumina HiSeq 2500.

A-to-I RNA editing detection

The current A-to-I RNA editing detection is based on at least three different biological RNA-seq replicas of the following strains: N2 (wild-type), ADAR mutant worms (BB21), and *adbp-1* mutant worms (QD1), at the embryo and L4 stages.

The quality of RNA-seq reads was estimated by FastQC[36]. Afterward, reads with poor base quality at their edges were trimmed by an in-house script to improve alignment and editing detection. Identical reads were collapsed in all the examined samples by an in-house script. The reads were aligned to *C. elegans* WS220 genome using Bowtie[37] by the following command: bowtie -p 23 -n 3 -e 120 -a --strata --best --sam -m 2 –un. SAM format files, the alignment output files, were processed to BAM files using SAMtools[38]. BAM files of samples representing the same strain and development stage were merged and processed to a pileup format using SAMtools. To detect editing of known editing sites[29] in wild-type and *adbp-1* mutant worms, sites that appeared to undergo editing above 3% in the BB21 mutant were removed from the pileup files to avoid artifacts that might be derived from non-A-to-I-RNA-editing events. An in-house script annotated the remaining sites in the pileup files to known editing sites. To detect if editing exists in the annotated site, we considered nucleotide changes with Phred quality ≥ 25 to increase editing detection precision. An annotated site was considered as an editing site if A-to-G or T-to-C (the revered strand) changes appeared to be ≥ 1%, and no more than 1% of other nucleotides were changed along the nucleotides covering the site. The known A-to-I editing sites analysis included a comparison of editing both on a single site level and at the whole gene editing level by custom scripts. The final lists of known editing sites that appeared in the used samples are presented in the supplemental tables. The results were plotted using the ggplot2 R package.

De-novo A-to-I RNA editing sites search based on the pipeline published by Goldstein et al., 2017[29]. However, this study did consider the Phred quality of the reads. This analysis included different pileup files for each sample and pileup files representing merged data by strain and developmental stage. To identify nucleotide changes in the RNA that are not a result of RNA editing events, we eliminated nucleotide changes derived from the DNA sequence from the pileup files using DNA-seq[29] and single nucleotide polymorphisms dataset[39]. Next, we eliminated each nucleotide change in the ADAR mutant from the pileup files. Next, we defined a site as edited if it meets the following criteria; Nucleotide change was considered if its Phred quality ≥ 25. The most abundant nucleotide change among the site reads appears in at least two expressed reads. The percentage of the most abundant nucleotide change ≥ 5% of the total expressed reads covering the site. The not abundant nucleotide changes appear to be ≤ 1% of the total expressed reads covering the site. The edited site appeared in at least two biological replicas. *De-novo* A-to-I editing sites in the *adbp-1* mutant are editing sites which are not appeared to be edited in wild-type samples both in the known editing site analysis and in *de-novo* A-to-I RNA editing sites search. An in-house script annotated the editing sites in the pileup files to genes.

We used Logomaker, a Python package[40], to create the logos of the nucleotides surrounding editing sites and random adenosines. The logo represents a probability matrix, which means the probability of observing each possible nucleotide at each possible position within a particular sequence type. The following equation calculated the probability: $P_{ic} = \frac{n_{ic}+\lambda}{\sum_{c'} n_{ic'}+C\lambda}$, where P_{ic} represents matrix elements. C is the number of possible characters, and λ is a user-defined pseudo count. A probability logo has heights given by these P_{ic} values.

Gene expression analysis

The RNA-seq data for A-to-I RNA editing site detection were analyzed for gene expression. The reads were aligned to *C. elegans* WS220 transcriptome using Bowtie[37] using a command that allows multiple alignments for isoform-aware alignments: bowtie -p 3 -n 3 -e 120 -a --strata --best --sam -m 10 –un. We pre-filtered the count genes, so only genes with at least ten reads with a coefficient of variation < 1 were analyzed. To test the significance of lncRNA and 3'UTR edited genes to all the expressed genes in all samples, we applied the Welch two-sample t-test between chosen two groups (two-sided). We used DESeq2[41], an R package, to analyze gene counts and identify differentially expressed transcripts. We considered genes as differentially expressed (DE) between the wild-type and mutated strains that adhered to the following criteria: |log2FoldChange| > 1 and padj < 0.05 at the embryo stage and |log2FoldChange| > 2 and padj < 0.05 at the L4 stage. The results are exhibited by volcano plots created by the 'EnhancedVolcano' package in R[42]. We applied enrichment analysis of the DE genes by avoiding the mutated genes in WormBase Enrichment Suite[43,44]. P-values in Venn diagrams were determined by hypergeometric distribution using the Phyper function in R.

Immunostaining

In order to visualize *C. elegans* embryos, adult worms containing embryos were fixed and prepared for immunostaining according to a previously described fixation protocol with methanol-acetone[45]. In order to visualize larvae and adult worms a mix of worms present at different stages was fixated with 1% formaldehyde and permeabilized as described previously[46]. Primary rabbit anti-ADR-2 (IU529)[27] and anti-ADR-1 were used at a 1:50 dilution. Donkey anti-rabbit Secondary Antibody, Alexa Fluor 568 (Life Technologies, #A10042) was used at a 1:200 dilution. Primary mouse anti-MH27 (# MH-27-s) from DSHB (Developmental Studies Hybridoma Bank), The University of Iowa, was used at a 1:300 dilution (gift from Benjamin Podbilewicz's lab). Secondary antibody Alexa Fluor 488 donkey anti-mouse (Life technologies, #A21202) was used at a 1:500 dilution. DAPI (4′,6-diamidino-2-phenylindole, Sigma) was used at a 1:1000- 1:2000 dilution for DNA staining.

Microscope images of embryos and most of the adult worms were obtained with a Spinning Disk Confocal microscope from Nikon with CSU-W1 Confocal Scanner Unit with dual camera from Yokogawa. The objective used for all images was x100 oil (NA = 1.45) CFI PLAN APOCHROMAT. For the embryo images exposure was set at 200 milliseconds for both channels (405nm for DAPI and 561nm for ADR-2 antibody). For the adult and larva images exposure was set at 400 milliseconds for 405nm and 100 milliseconds for both 561nm and 488nm (for MH27 antibody). Laser intensities were set at 35% for 405nm, 22.1% for 568nm and 15% for 488nm. Z-stacks for all images were obtained in a range of 7 µm in the z-axis. The acquisition was performed using the NIS-Elements AR software. Maximal intensity z-projection of the stacks was created using Fiji (imageJ, NIH) for images of the hermaphrodite body and tail belonging to wild-type and *adbp-1*$^{-/-}$strains. The rest of the images are individual slices taken from their corresponding z-stacks (hermaphrodite head for both strains, embryo and cell cycle images).

Images of larvae and adults were also obtained using an inverted microscope (Nikon Ti ECLISPE) with a Confocal Spinning Disk (Yokagawa CSU-X), with an Andor iXon3 camera (DU-897-

CSO-#BV). The objectives used were a 60x oil Plan Apochromat (NA = 1.4) lens or a 40x oil Plan Fluor (NA= 1.3) lens. Some images were enhanced with an additional x1.5 amplification. The laser intensity was set at 7%, exposure at 100 milliseconds and the gain at 300 for both channels (405nm for DAPI and 561nm for ADR-2 antibody). Z-stacks were obtained in a range of 5 µm in the z-axis. The software used was the Molecular Devices Metamorph.

All microscope images were corrected for brightness and contrast together with the controls, merged and stacked to RGB using Fiji (imageJ, NIH).

RESULTS

ADR-2 is localized to chromosomes throughout the cell cycle

In *C. elegans,* most A-to-I RNA editing sites are, as in humans, in non-coding regions, including introns, indicating that most of the editing occurs in the nucleoplasm[29,47–49]. However, ADR-2 localization has not been completely established. To explore the intracellular localization of ADR-2, we conducted immunostaining experiments on wild-type embryos using an antibody against ADR-2[48]. ADR-2 co-localizes with the DNA in the wild-type embryo, indicating the protein is mainly present inside nuclei (Figure 1A). This is in line with previous studies that showed that *adr-2* expression is high at the early stages of development and with the ADR-2 editing function at the embryo stage[29,33,47]. Moreover, our results show that ADR-2 is located at the chromosomes at all cell cycle stages (Figure 1B). Interestingly, ADR-2 presence is not dispersed equally along the chromosomes' length. Previous studies showed that edited dsRNAs are enriched in the autosomal chromosomes' distal arm, practically adjacent to repetitive sequences[34,49,50]. In our immunostainings, ADR-2 seems to be adjacent to specific regions along the chromosomes; however, not only to the chromosomes' distal arm (Figure 1B).

Next, we wanted to explore ADR-2 expression during the later stages of development and in different tissues of adult worms, including germlines. We immunostained adult worms with the same ADR-2 antibody utilizing a technique that preserves the worm structure while allowing permeability (see material and methods). As a staining control, we used the MH27 antibody, which stains the apical borders of epithelial cells. Strikingly, while ADR-2 is expressed in nuclei throughout the entire oogenesis process in wild-type hermaphrodite gonads (Figure 2A) and intestine (Supplemental Figure 1), it does not appear to be expressed in sperm, nor is it ubiquitously expressed in cells in the head, body, and tail of the wild-type adult hermaphrodite (n=20, Figure 2B).

To conclude, ADR-2 is expressed mainly in the nuclei adjacent to the chromosome through the entire cell cycle. This proximity suggests that editing occurs co-transcriptionally when transcription begins or may indicate ADR-2 binding to nascent transcripts. Moreover, while ADR-2 exists in the embryo and gonads, ADR-2 is not ubiquitously expressed in the worm's later developmental stages and the sperm.

ADR-2 nuclear localization is dependent on ADBP-1 but not on ADR-1

Previously, it was shown that the lack of *adbp-1* altered the editing of four substrates and increased cytoplasmic localization of a transgenically expressed ADR-2-green fluorescent protein (GFP) driven

by a hypodermal promoter[31]. We wanted to test further how ADR-2 subcellular localization is established and how localization affects ADR-2 function. Because it was shown that ADR-1 regulates ADR-2 editing activity and target selection[25–27], we wondered whether ADR-1 also affects ADR-2 localization. Using immunostaining, we showed that ADR-1 and ADR-2 are localized in the nuclei (Supplemental Figure 1). Immunostaining of embryos lacking *adr-1* with ADR-2 antibody showed similar localization of ADR-2 as in wild-type worms (Figure 1A). ADR-2 localization during the cell cycle in *adr-1* mutant worms was similar to wild-type worms (Figure 1B). Therefore, we concluded that ADR-1 is not required for the nuclear localization of ADR-2.

To further test the role of ADBP-1 in the localization of ADR-2, we examined the localization of endogenous ADR-2 in the *adbp-1* mutant strain. Immunostaining of *adbp-1* mutant embryos with ADR-2 antibody showed that endogenous ADR-2 is mislocalized, mainly residing in the cytoplasm (Figure 1A). To ensure that ADR-2 mislocalization is not due to mutation in the *adr-2* gene, we sequenced the entire gene using several primers in the *adbp-1* mutant strain (Supplemental Table S1 and Supplemental Figure 2). The sequencing results showed that the *adbp-1* mutant strain has an intact *adr-2* gene with a wild-type sequence. We also verified that the *adr-2* gene is expressed using real-time PCR (Supplemental Figure 2). ADR-2 remains cytoplasmic and is not localized to the chromosomes during all cell cycle stages in *adbp-1* mutant embryos (Figure 1B). Interestingly, when staining ADR-2 in *adbp-1* mutant adult worms, ADR-2 seems to reside more in cytoplasmic speckles in the gonads (Figure 2A) and the head and body of the worm (Supplemental Figure 3). By tracking the expression of *adr-2* and *adbp-1* in wild-type worms, we found that both genes are expressed throughout the entire oogenesis process (Supplemental Figure 4) and are not specific to a particular stage in oocyte development. We concluded that ADBP-1 is required for ADR-2 nuclear localization, while ADR-1, although regulating editing by ADR-2, does not.

RNA editing still occurs in the *adbp-1* mutant; however, it involves mostly coding regions

Ohta et al. observed that RNA editing is attenuated in *adbp-1* mutant worms when testing a handful of transcripts[31]. To examine if RNA editing is globally abolished in *adbp-1* mutant worms, we generated RNA-seq libraries from three biological replicas of *adbp-1* mutant worms at the embryo or L4 developmental stages. The expression data of these libraries were compared to that of libraries generated at the same developmental stages from wild-type worms (N2) and BB21 worms, which lack both *adr-1* and *adr-2* genes (*adr-1*(-/-);*adr-2*(-/-))[29]. All worms were grown under the same conditions, and most of the libraries were generated at the same time. To determine if editing occurs in the *adbp-1* mutant, we globally tested editing sites that were previously identified from transcriptome-wide studies[25,29] for editing in the RNA-seq data. RNA-seq data from RNA editing mutant worms lacking *adr-1* and *adr-2*, were used as a control to estimate editing false-positive sites. We counted the number of edited sites in the wild-type and the *adbp-1* mutants (Figure 3A). As expected, in both embryo and L4 developmental stages, in the *adbp-1* mutant, the number of edited sites is significantly reduced. However, although the number of edited sites in the *adbp-1* mutant was very low compared to wild-type worms, we still observed editing (Figure 3B). Next, we wanted to examine if the distribution of editing

sites in wild-type worms is different than in *adbp-1* mutant worms. In wild-type worms, almost all editing sites at both developmental stages reside in introns, as shown before [29,47–49] (Figure 3C, D). In striking contrast, the profile of editing sites in *adbp-1* mutant worms was very different. Most editing sites in *adbp-1* mutants reside in exons in both developmental stages (Figure 3E, F). This finding aligns with our immunostaining data, which points to mainly cytoplasmic localization of ADR-2 in *adbp-1* mutant worms. As many edited genes possess several editing sites, we next compared the editing levels of each gene between wild-type and *adbp-1* mutants. We found that a significant portion of the genes undergoes editing in both the wild-type and *adbp-1* mutant worms, strains at the same developmental stage (Figure 3G, H, P-value < 5.38e-47 and P-value < 1.14e-21, respectively). However, many genes undergo editing only in one of the strains.

Previously we showed that editing levels are attenuated in *adr-1* mutant worms[25]. In addition, we showed that ADR-1 binds directly edited genes or as part of a complex, as was shown by RIP-seq[25]. Hence, ADR-1 directs ADR-2 to editing sites. We aimed to test if the genes edited in *adbp-1* mutants are also bound by ADR-1 determined by the RIP-seq analysis. Thus, we looked for overlap between edited genes in worms lacking *adbp-1* with ADR-1 binding genes at the embryo and L4 stage. The analysis shows that a third of the genes edited in *adbp-1* mutant worms in the embryo stage and about a third of the genes edited in the L4 stage are bound by ADR-1 (P-value = 5.52e-15 and P-value = 2.61e-09 respectively; Supplemental Figure 5A).

To conclude, our results show that worms lacking *adbp-1* still exhibit non-negligible editing and many of the genes edited in *adbp-1* mutant worms are genes shown to be directly bound by ADR-1. However, the editing profile in *adbp-1* mutants is very different, with most sites residing in exons and not introns, as in wild-type. This profile is most probably determined by the cytoplasmic localization of ADR-2, where ADR-2 encounters spliced transcripts.

ADR-2 cytoplasmic localization creates *de-novo* editing sites

The above editing analysis in *adbp-1* mutant worms focused on detecting editing sites found in transcriptome-wide studies[25,29] (Figure 3). Next, we wondered if the cytoplasmic localization of ADR-2 creates *de-novo* editing sites that are not present in wild-type worms. To check this, we searched for new editing sites in the wild-type and *adbp-1* mutant RNA-seq using a pipeline described in Goldstein et al., 2019, and Light et al., 2021[29,51]. To be more restrictive, we considered nucleotide changes with only a Phred quality score ≥ 25 to increase the precision of nucleotide calling. We defined a *de-novo* editing site in a*dbp-1* mutant worms when it did not appear to be edited in the wild-type new editing search or previous works[25,29].

At the embryo stage, our pipeline detected 32 genes that had undergone editing only in *adbp-1* mutant worms. Each gene has one editing site (Figure 4A**,** Supplemental Table 2). At the L4 stage, 44 genes were found to be edited exclusively in *adbp-1* mutant worms, with only one editing site per gene. (Figure 4B**,** Supplemental Table 2). Most of the editing sites at both developmental stages reside in exons, as was shown in the analysis of known editing sites (Figure 3E, F).

Interestingly, we also detected *de-novo* editing in genes normally edited in wild-type worms at the embryo and L4 stages (Figure 4A, B). At the embryo stage, only half of them reside in exons, and 43% reside in exons at the L4 stage.

We wondered what the ADR-2 targets are in the *adbp-1* mutant worms. We discovered that the unique edited sites of the *adbp-1* mutant belong to highly expressed transcripts (Supplemental Figure 6). In addition, there is no significant overlap between the newly edited genes and ADR-1-bound genes (Supplemental Figure 5B). These findings suggest that the high expression of these genes may increase the probability of ADR-2 targeting them, and ADR-2 probably acts independently of ADR-1 in a random matter.

In summary, we discovered genes edited exclusively in *adbp-1* mutants. These genes, edited mostly in exons, are highly expressed and are not targeted by ADR-1.

ADR-2 has a preferential site selection along the gene

Few works tried to find if ADAR enzymes have preferable nucleotides surrounding their target site[49,52–57]. Although each work done on human ADARs showed slightly different motifs[52–55], they found in common that human ADAR1 and ADAR2 prefer uridine at the 5' of the edited sites and guanosine at its 3'. Previous works showed that in contrast to human ADARs, ADR-2 in *C.elegans* prefers adenosines on both sides of the editing site [49,57]. We found the same pattern by analyzing the nucleotides surrounding the overall editing sites previously found in wild-type worms[25,29] (Figure 4C). *adbp-1* mutant worms have a significant portion of editing in exons compared to wild-type worms (Figure 3C, D and Figure 4A, B), we aimed to understand whether the nearest-neighbor preferences of ADR-2 change in the *adbp-1* mutant. We focused on coding genes and looked at the probability of each nucleotide surrounding the editing site. To understand better the nucleotide preference, we focused on the different parts of the genes. We divided the results into coding regions (exons), introns, and UTRs. We analyzed random adenosines (as a control), editing sites previously found in wild-type worms at all developmental stages[25,29], and the editing sites we found in *adbp-1* mutant worms in this study (Figure 3B, E, F, and Figure 4A, B). The analysis of edited sites in wild-type worms shows that the prevalent nucleotides surrounding them are also adenosines (Figure 4C). In contrast, in *adbp-1* mutant worms, the probability of guanosines being on both sites of the edited site is the highest. Interestingly, we saw that in coding regions and UTRs, guanosines are more prevalent in neighbor editing sites both in wild type and *adbp-1* mutant worms, while random adenosine are surrounded also by adenosines. Overall the nucleotides surrounding editing sites in exons in wild-type and *adbp-1* mutant worms is very similar. Wild-type editing sites in introns show adenosines as neighbors with the highest probability on both sides. However, in *adbp-1* mutant introns, uridine (shown as thymine in Figure 4C) is the most common nucleotide at 5' nearest neighbor position (T>G=C=A). In contrast, the 3' nearest neighbor preferable nucleotide is adenosine. However, we would like to note that the number of editing sites in *adbp-1* mutants that reside in introns is very low, which might skew the preference for a certain nucleotide. This deep look at the neighboring nucleotides of the editing sites in the different regions of the gene can explain the difference in the overall motif between wild-type and *adbp-1* mutant worms. Most editing sites (80%) in wild-type worms reside in introns. In contrast, most editing sites in *adbp-1* mutant worms

(65.5%) reside in exons. Therefore, the overall editing sites motif in each strain is a mirror of the nucleotide preference in the region of the most abundant sites. To conclude, the results show that ADR-2 has different preferences for the targeted adenosines, which depend on their location in the gene.

3'UTR edited genes and lncRNAs expression is altered in *adbp-1* mutant worms

Previously, we showed that 3'UTR edited genes and lncRNAs are downregulated in worms lacking *adr-2* at the embryo stage compared to wild-type worms[25,29]. This downregulation was associated with a lack of editing that allows RNAi to target substrates normally protected by editing[29]. We wanted to test if the mislocalization of ADR-2 causes a similar downregulation. Using the RNA-seq databases we generated, we confirmed the downregulation of 3'UTR edited genes and lncRNA genes in *adr-1* and *adr-2* mutant worms compared to wild-type worms at the embryo stage (Figure 5A, B, P-value = 0.001 and P-value < 2.2e-16, respectively). Comparing the expression of 3'UTR edited genes and lncRNAs in *adbp-1* mutant worms to wild-type worms at the embryo stage, we saw the same trend as in *adr-1* and *adr-2* mutant worms, e.g., downregulation of 3'UTR edited genes and lncRNAs in wild-type embryos (Figure 5C, D, P-value = 9.4e-08 and P-value < 2.2e-16, respectively). Low levels of editing in *adbp-1* mutant worms can explain this finding.

To further study ADBP-1 function, we wanted to explore the effect of lacking ADBP-1 on global gene expression. For this purpose, we analyzed the RNA-seq data of embryo and L4 stages, searching for differentially expressed genes. We defined a gene as differentially expressed (DE) if its expression differed more than two-fold between the two strains, with a P-value after Benjamini-Hochberg correction < 0.05. When comparing wild-type embryos' gene expression to *adr-1;adr-2* mutant embryos, we did not observe many differentially expressed genes in both strains (Figure 5E, Supplemental Table 3). Similarly, comparing wild-type worms to *adbp-1* mutant worms showed only a few DE genes (Figure 5F). Even when comparing *adbp-1* mutant to *adr-1;adr-2* mutant worms, the same trend of only a few DE genes was shown (Supplemental Figure 7E, Supplemental Table 3). Hence, it is unsurprising that functional enrichment analyses of the DE of wild-type versus *adbp-1* mutant genes showed no significant enrichment[43,44]. Although both mutant strains showed downregulation of 3'UTR edited genes and lncRNAs, only a few met DE genes' criteria. Some DE genes found in the differential expression analysis between wild-type to *adr-1;adr-2* mutant worms were found to be DE also between wild-type to *adbp-1* mutant worms: *T11A5.8*, *R07B5.10*, *math-46*, and *F21H12.3*. Interestingly, only *math-46* was found to be edited in wild-type worms. In contrast, the three other genes, and most DE genes in the two comparisons, are neither edited in wild-type worms nor *adbp-1* mutant (Supplemental Table 3). This indicates that they might be affected by upstream processes influenced by impaired editing.

Expression analysis of 3'UTR edited genes, lncRNAs, and DE genes at the L4 stage was not always consistent with the embryo stage results. At the L4 stage, 3'UTR edited genes showed slight upregulation in *adr-1*;*adr-2* mutant worms compared to wild-type worms. However, the P-value was close to 0.05, our significance cutoff (Supplemental Figure 7A, P-value = 0.04594). Previously we reported that 3'UTR edited genes downregulated in *adr-1*;*adr-2* mutant worms compared to wild-type worms[29]. However, variations between the samples might reduce the expression and significance reflected by the P-value. lncRNAs were upregulated in *adr-1*;*adr-2* mutant worms, compared to wild-

type worms (Supplemental Figure 7B, P-value = 1.047e-10), similarly to what we showed before[29]. In contrast to *adr-1*;*adr-2* mutant worms, in *adbp-1* mutant worms, 3'UTR edited genes were downregulated compared to wild-type worms (Supplemental Figure 7C, P-value = 0.0001894). At the same time, lncRNAs were not significantly changed (Supplemental Figure 7D, P-value = 0.119).

DE analysis of all mutant strains compared to wild-type, and *adr-1*;*adr-2* mutant compared to *adbp-1* mutant worms, showed much more DE genes, even when we looked for DE with a greater fold-change than at the embryo stage analysis (|log2FoldChange| > 2 and P-adjusted < 0.05, Supplemental Figure 7F-H). However, these variations can result from different L4 substages of the worms and not changes resulting from the difference between the strains.

Taking together all of our findings, we propose the following model. In wild-type worms, ADBP-1 mediates ADR-2 import to the nucleus. In the nucleus, ADR-1 directs ADR-2 to specific editing sites in dsRNA molecules, primarily introns (Figure 6). In addition, ADR-2 proximity to chromosomes enables co-transcriptional editing or nascent RNA binding. Without ADBP-1, ADR-2 remains in the cytoplasm and edits spliced transcripts. Hence, most editing happens in exons, sporadically in highly expressed dsRNA molecules. ADR-2 prefers adenosines with specific neighbors in both the nucleus and the cytoplasm, changing between exons and introns targets (Figure 4C).

DISCUSSION

In this work, we sought to examine the intracellular and tissue-specific localization of ADR-2 in *C. elegans* and the effect the localization might have on its function. Although the general assumption is that editing occurs mainly in the nucleus[31,47], surprisingly, the exact location of the endogenous ADR-2 protein in *C. elegans* has not been directly shown. Using a specific antibody against ADR-2, we showed that in wild-type worms, ADR-2 resides in nuclei and is adjacent to the chromosomes at all cell cycle stages.

We use *adbp-1* and *adr-1* mutant worms to show that ADBP-1, not ADR-1, facilitates endogenous ADR-2 localization to the nucleus. In the absence of ADBP-1, ADR-2 appears in the cytoplasm. In addition, our bioinformatical analysis showed that although the editing level decreased in the absence of ADBP-1, ADR-2 is still enzymatically active. Worms lacking *adbp-1* still exhibit non-negligible editing, mainly in exons, probably due to its cytoplasmic localization. Many genes undergo editing in both wild-type and *adbp-1* mutant worms.

Moreover, the mislocalization of ADR-2 leads to *de-novo* editing sites that do not exist in wild-type worms. *De-novo* editing appeared in highly expressed genes that were not found to be bound by ADR-1, indicating sporadic editing of ADR-2 in the cytoplasm. Our bioinformatical analysis showed that ADR-2 preferences for nucleotides surrounding targeted adenosines in exons and introns differ.

Looking at RNA expression levels in ADBP-1 mutant embryos, we noticed the downregulation of genes that undergo editing in their 3'UTR normally and lncRNAs. Similar downregulation, previously observed by us in ADAR mutant worms[29], is attributed to the sensitivity of unedited transcripts to RNAi. ADR-2 is highly expressed in all embryo cells, as shown before[29,47]. In contrast, it is not ubiquitously

expressed in the somatic tissues of adults. In wild-type adult hermaphrodites, ADR-2 is expressed mainly in the gonad (Figure 2A), not in sperm (Figure 2B).

ADR-2 nuclear localization proximity to the chromosomes suggests co-transcriptional editing

In the embryo, we showed that ADR-2 is expressed in the nuclei of most cells (Figure 1A), which is consistent with research showing that the expression of *adr-2* mRNA is highest at the early developmental stages[29,47].

Not only does ADR-2 reside in the nuclei in embryos, it also seems to reside near the chromosomes (Figure 1B). This proximity could allude to the importance of ADR-2 being close to the DNA so that the editing process can happen co-transcriptionally when transcription begins or ADR-2 binding to nascent transcripts. In addition, most of the editing sites in *C. elegans* being in introns[47], also suggesting co-transcriptional editing. This finding aligns with other research showing co-transcriptional RNA editing in humans[58,59] and *Drosophila*[60]. The study in humans revealed that A-to-I RNA editing events occur in nascent RNA associated with chromatin before polyadenylation[58,59].

In addition to ADR-2 chromosome proximity, we observed that ADR-2 is localized in specific regions along the chromosomes (Figure 1B). Previous studies in *C. elegans* showed that autosomal chromosomes distal arms are enriched with dsRNA, practically in repetitive sequences[34,49,50]. Although it is difficult to differentiate the exact localization of ADR-2 along the chromosomes in our results, ADR-2 may also be localized at the autosome distal arm and co-transcriptionally edits its targets. The localization of ADR-2 in the absence of ADR-1 is identical to that of the wild-type. This points to two independent stages in localizing ADR-2; in the first, ADR-2 is brought to chromosomes (and possibly to the particular chromosome areas) by regulator/s other than ADR-1, and, at the second stage, ADR-2 is targeted by ADR-1 to its specific editing substrates. This suggests that more regulators of this process remain to be discovered.

ADBP-1 regulates the nuclear localization of ADR-2

In human, both ADAR1 isoforms, 110-kDa, and 150-kDa protein, can shuttle between the nucleus and cytoplasm. However, ADAR1 p150 has a strong nuclear export signal, overlapping its third dsRBM, which leads to its accumulation in the cytoplasm[16,17]. In contrast, human ADAR2 is mainly localized to the nucleolus[2]. Human ADAR2 localization is regulated by binding to pin1[61]. In *C. elegans*, ADBP-1 was shown to regulate the localization of the transgenic ADR-2[31]. In contrast to using a transgenic ADR-2, we aimed to understand how ADBP-1 affects endogenous ADR-2. We confirmed the finding that ADBP-1 regulates the nuclear localization of *C. elegans* ADR-2 (Figures 1, 2). ADR-1 was shown to regulate editing by ADR-2, probably by directing ADR-2 to the editing sites[25–27]. Our results suggest that ADR-1 regulates editing but does not affect ADR-2 localization. It is still unclear whether ADBP-1 affects ADR-2 localization directly or indirectly. To understand more about *C. elegans* ADR-2 shuttling, we tried to predict the existence of nuclear localization signal (NLS) in ADR-2 using NLS prediction tools[62–65]; however, we could not find any NLS in ADR-2. Though, these tools are limited because they mainly cover classical NLSs, not accounting for non-classical ones, as in human ADAR1[15].

Moreover, it is known that small water-soluble molecules weighing less than ~60 kDa can diffuse into the nucleus[66]. As ADR-2 has a molecular weight of 55 kDa, it is possible that NLS is not required for ADR-2 as it can passively diffuse through nuclear pore complexes (NPCs). In such case, in the absence of ADBP-1, ADR-2 can be present in a nucleus but at low levels. Indeed, we found a fraction of intron-residing editing sites to undergo editing in ADBP-1 mutant worms (Figure 3E-F and Figure 4A-B). In addition, when we analyzed if *adbp-1* mutant worms have editing in genes previously identified from transcriptome-wide studies[25,29] (Figure 3), we found that although the small number of edited sites, a significant fraction of these edited genes are bound by ADR-1 (Supplemental Figure 5A).

Interestingly, we could not detect NLS in ADBP-1 as well. ADBP-1 may have a non-classical NLS or serve as an adaptor protein, mediating the active import of ADR-2 to the nucleus (Figure 6). Alternatively, a lack of NLS can suggest that ADR-2 and ADBP-1 may also have a cytoplasmic activity, which can be expressed only in specific cells or tissues. In such a case, the localization of ADR-2 probably regulates its function. In addition, it can suggest that other importin proteins are involved in the shuttling of ADR-2 to the nucleus.

We previously showed that ADR-1 binds dsRNA at editing sites[25]. In addition, ADR-2 has a low affinity to dsRNA, which increases upon its binding to ADR-1[27]. Thus, after ADBP-1 brings ADR-2 to the nucleus, the binding of ADR-2 to ADR-1 brings ADR-2 to its proper RNA targets. The cytoplasmic location of ADR-2 leads to different editing patterns, for several reasons. High cytoplasmic levels of ADR-2 result in editing in the cytoplasm, mainly in exons, including novel sites. On the other hand, low ADR-2 levels in the nucleus result in a lack of editing in introns (See our model Figure 6). In addition, in the cytoplasm, the lack of introns in the transcripts decreases dsRNA structures, which are ADR-2 substrates. Hence, editing levels drop and mainly occur in exons.

adbp-1 mutation downregulates the expression of 3'UTR edited genes and lncRNAs in embryos. This can be explained by the fact that 3'UTR edited genes and lncRNAs fail to undergo editing in the nucleus, like introns. Upon entering the cytoplasm, they do not get edited as well. In the cytoplasm, there is no ADR-1 to bring ADR-2 to its proper targets. Having long unedited dsRNA stretches, 3'UTR edited genes and lncRNAs are successfully targeted by RNAi machinery, leading to the observed downregulation of their levels, similar to what is happening in ADR-2 mutants.

ADR-2 expression pattern in embryo and adult worm is different

In contrast to the embryo, ADR-2 expression is not evident in cells of somatic tissues of the worm's head, body, and tail (Figure 2B). In humans, ADAR proteins, mainly ADAR2, have essential functions in the nervous system[9–12]. In *C. elegans,* neuronal genes were found to undergo editing[29,48] and one of the most prominent phenotypes of worms lacking *adr-2* is chemotaxis defects[23,48]. However, our immunostaining data suggest that ADR-2 is not expressed in all cells in the head and tail of the worm and are possibility neuronal cells.

The ubiquitous presence of ADR-2 in the oocytes indicates that RNA editing is needed for the entire developmental process, which starts with oocyte development and maturation and continues after fertilization into embryonic development. This could be consistent with other studies showing that editing levels are highest during *C. elegans* earlier stages of development (embryo and L1)[29,34,47]. In a striking

difference to a strong expression of ADR-2 in the gonad (Figure 2A, B), we could not detect ADR-2 in sperm. The existence of ADR-2 only in certain tissues and cells indicates a control that occurs not only at the intracellular level but also at the tissue level.

ADR-2 prefers to edit adenosines with different nucleotides surrounding it in different regions of the gene

While the previous works analyzed the nucleotides surrounding the targeted adenosine and looked at the overall editing sites, we focused on nucleotides surrounding edited adenosines in UTRs, coding exons, and introns separately (Figure 4C). By looking at the nucleotides surrounding overall edited adenosine that appears at all genes' parts together, our results align with previous works done in *C. elegans*[25,29]. Surprisingly, when we focused on the different gene parts, we found different nucleotides surrounding the editing site in each gene part. The specific editing motif of each gene part was very similar in the wild-type and the *adbp-1* mutant. This suggests that each gene part's structure and nucleotide usage affect the motif and not ADR-2 cellular localization. We suggest that prediction tools for editing should also consider the location of the editing site along the gene.

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CONFLICT OF INTEREST

The authors declare no competing interests.

REFERENCES

1. Gott, J. M. & Emeson, R. B. Functions and Mechanisms of RNA Editing. *Annu. Rev. Genet.* 499–531 (2000).
2. Erdmann, E. A., Mahapatra, A., Mukherjee, P., Yang, B. & Hundley, H. A. To protect and

modify double-stranded RNA - the critical roles of ADARs in development, immunity and oncogenesis. *Crit. Rev. Biochem. Mol. Biol.* **56**, 54–87 (2021).

3. Walkley, C. R. & Li, J. B. Rewriting the transcriptome: adenosine-to-inosine RNA editing by ADARs. *Genome Biol.* **18**, 205 (2017).
4. Levanon, Y. E. & Eisenberg, E. A-to-I RNA editing — immune protector and transcriptome diversifier. *Nat. Rev. Genet.* **19**, (2018).
5. Bass, B. L. RNA Editing by Adenosine Deaminases That Act on RNA. *Annu. Rev. Biochem.* **71**, 817–846 (2002).
6. Quin, J. *et al.* ADAR RNA Modifications, the Epitranscriptome and Innate Immunity. *Trends Biochem. Sci.* **46**, 758–771 (2021).
7. Higuchi, M. *et al.* RNA Editing of AMPA Receptor Subunit GluR-9: A Base-Paired Intron-Exon Structure Determines Position and Efficiency. *Cell* **75**, 1361–1370 (1993).
8. Paz, N. *et al.* Altered adenosine-to-inosine RNA editing in human cancer. *Genome Biol.* 1586–1595 (2007) doi:10.1101/gr.6493107.1.
9. Kawahara, Y. *et al.* RNA editing and death of motor neurons. *Nature* **427**, 2004 (2004).
10. Jain, M., Jantsch, M. F. & Licht, K. The Editor's I on Disease Development. *Trends Genet.* **35**, 903–913 (2019).
11. Song, B., Shiromoto, Y., Minakuchi, M. & Nishikura, K. The role of RNA editing enzyme ADAR1 in human disease. *Wiley Interdiscip. Rev. RNA* **13**, 1–35 (2022).
12. Yang, Y., Okada, S. & Sakurai, M. Adenosine-to-inosine RNA editing in neurological development and disease. *RNA Biol.* **18**, 999–1013 (2021).
13. Baker, A. R. & Slack, F. J. ADAR1 and its implications in cancer development and treatment. *Trends Genet.* **38**, 821–830 (2022).
14. Patterson, J. B. & Samuel, C. E. Expression and regulation by interferon of a double-stranded-RNA-specific adenosine deaminase from human cells: evidence for two forms of the deaminase. *Mol. Cell. Biol.* **15**, 5376–5388 (1995).
15. Barraud, P., Banerjee, S., Mohamed, W. I., Jantsch, M. F. & Allain, F. H. A bimodular nuclear localization signal assembled via an extended double-stranded RNA-binding domain acts as an RNA-sensing signal for transportin 1. (2014) doi:10.1073/pnas.1323698111.
16. Fritz, J. *et al.* RNA-Regulated Interaction of Transportin-1 and Exportin-5 with the Double-Stranded RNA-Binding Domain Regulates Nucleocytoplasmic Shuttling of ADAR1. *Mol. Cell. Biol.* **29**, 1487–1497 (2009).
17. Poulsen, H., Nilsson, J., Damgaard, C. K., Egebjerg, J. & Kjems, J. CRM1 Mediates the Export of ADAR1 through a Nuclear Export Signal within the Z-DNA Binding Domain. *Mol. Cell. Biol.* **21**, 7862–7871 (2001).
18. Patterson, J. B. & Samuel, C. E. Expression and regulation by interferon of a double-stranded-RNA-specific adenosine deaminase from human cells: evidence for two forms of the deaminase. *Mol. Cell. Biol.* **15**, 5376–5388 (1995).
19. Kleinova, R. *et al.* The ADAR1 editome reveals drivers of editing-specificity for ADAR1-isoforms. *bioRxiv* 2021.11.24.469911 (2021).

20. Desterro, J. M. P. *et al.* Dynamic association of RNA-editing enzymes with the nucleolus. *J. Cell Sci.* **116**, 1805–1818 (2003).
21. Roth, S. H., Levanon, E. Y. & Eisenberg, E. Genome-wide quantification of ADAR adenosine-to-inosine RNA editing activity. *Nat. Methods* **16**, (2019).
22. Higuchi, M. *et al.* Point mutation in an AMPA receptor gene rescues lethality in mice deficient in the RNA-editing enzyme ADAR2. *Nature* **406**, 1998–2001 (2000).
23. Tonkin, L. A. *et al.* RNA editing by ADARs is important for normal behavior in Caenorhabditis elegans. *EMBO J* **21**, 6025–6035 (2002).
24. Arribere, J. A., Kuroyanagi, H. & Hundley, H. A. mRNA editing, processing and quality control in caenorhabditis elegans. *Genetics* **215**, 531–568 (2020).
25. Ganem, N. S. *et al.* Disruption in A-to-I Editing Levels Affects C. elegans Development More Than a Complete Lack of Editing. *Cell Rep.* **27**, 1244-1253.e4 (2019).
26. Washburn, M. C. *et al.* Report The dsRBP and Inactive Editor ADR-1 Utilizes dsRNA Binding to Regulate A-to-I RNA Editing across the C . elegans Transcriptome. *CellReports* **6**, 599–607 (2014).
27. Rajendren, S. *et al.* A protein-protein interaction underlies the molecular basis for substrate recognition by an adenosine-to-inosine RNA-editing enzyme. *Nucleic Acids Res.* (2018) doi:10.1093/nar/gky800.
28. Rajendren, S. *et al.* Profiling neural editomes reveals a molecular mechanism to regulate RNA editing during development. *Genome Res.* **31**, 27–39 (2021).
29. Goldstein, B. *et al.* A-to-I RNA editing promotes developmental stage-specific gene and lncRNA expression. *Genome Res.* **27**, 462–470 (2017).
30. Cao, J. *et al.* Comprehensive single-cell transcriptional profiling of a multicellular organism. *Science (80-.).* **357**, 661–667 (2017).
31. Ohta, H., Fujiwara, M., Ohshima, Y. & Ishihara, T. ADBP-1 Regulates an ADAR RNA-Editing Enzyme to Antagonize RNA-Interference-Mediated Gene Silencing in Caenorhabditis elegans. **796**, 785–796 (2008).
32. Brenner, S. The Genetics of Caenorhabditis Elegans. *Genetics* **4**, 71–94 (1974).
33. Hundley, H. A., Krauchuk, A. A. & Bass, B. L. C. elegans and H. sapiens mRNAs with edited 3′ UTRs are present on polysomes. *Rna* **14**, 2050–2060 (2008).
34. Reich, D. P., Tyc, K. M. & Bass, B. L. C. elegans ADARs antagonize silencing of cellular dsRNAs by the antiviral RNAi pathway. *Genes Dev.* **32**, 271–282 (2018).
35. Williams, B. D., Schrank, B., Huynh, C., Shownkeen, R. & Waterston, R. H. A genetic mapping system in Caenorhabditis elegans based on polymorphic sequence-tagged sites. *Genetics* **131**, 609–624 (1992).
36. Andrews, Simon. FastQC: a quality control tool for high throughput sequence data. 2010. (2017): W29-33.
37. Langmead, B., Trapnell, C., Pop, M. & Salzberg, S. L. Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. *Genome Biol.* **10**, (2009).
38. Li, H. *et al.* The Sequence Alignment/Map format and SAMtools. *Bioinformatics* **25**, 2078–2079

(2009).
39. Harris, T. W. *et al.* WormBase: a comprehensive resource for nematode research. *Nucleic Acids Res.* **38**, D463–D467 (2010).
40. Tareen, A. & Kinney, J. B. Logomaker: Beautiful sequence logos in Python. *Bioinformatics* **36**, 2272–2274 (2020).
41. Love, M. I., Huber, W. & Anders, S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol.* **15**, 1–21 (2014).
42. Blighe K, Rana S, L. M. EnhancedVolcano: Publication-ready volcano plots with enhanced colouring and labeling. (2022).
43. Angeles-Albores, D., Raymond, R. Y., Chan, J. & Sternberg, P. W. Tissue enrichment analysis for C. elegans genomics. *BMC Bioinformatics* **17**, 1–10 (2016).
44. Angeles-Albores, D., Lee, R., Chan, J. & Sternberg, P. Two new functions in the WormBase Enrichment Suite. *microPublication Biol.* 25–2 (2018) doi:10.17912/W25Q2N.
45. Margalit, A., Segura-Totten, M., Gruenbaum, Y. & Wilson, K. L. Barrier-to-autointegration factor is required to segregate and enclose chromosomes within the nuclear envelope and assemble the nuclear lamina. *Proc. Natl. Acad. Sci. U. S. A.* **102**, 3290–3295 (2005).
46. Finney, M. & Ruvkun, G. The unc-86 gene product couples cell lineage and cell identity in C. elegans. *Cell* **63**, 895–905 (1990).
47. Zhao, H. Q. *et al.* Profiling the RNA editomes of wild-type C. elegans and ADAR mutants. *Genome Res* **25**, 66–75 (2015).
48. Deffit, S. N. *et al.* The C. elegans neural editome reveals an ADAR target mRNA required for proper chemotaxis. *Elife* **6**, (2017).
49. Whipple, J. M. *et al.* Genome-wide profiling of the C. elegans dsRNAome. *Rna* **21**, 786–800 (2015).
50. Reich, D. P. & Bass, B. L. Inverted repeat structures are associated with essential and highly expressed genes on C. Elegans autosome distal arms. *Rna* **24**, 1634–1646 (2018).
51. Light, D. *et al.* RESIC: A Tool for Comprehensive Adenosine to Inosine RNA Editing Site Identification and Classification. *Front. Genet.* **12**, 686851 (2021).
52. Eggington, J. M., Greene, T. & Bass, B. L. Predicting sites of ADAR editing in double-stranded RNA. *Nat Commun* **2**, 319 (2011).
53. Lehmann, K. A. & Bass, B. L. Double-stranded RNA adenosine deaminases ADAR1 and ADAR2 have overlapping specificities. *Biochemistry* **39**, 12875–12884 (2000).
54. Polson, A. G. & Bass, B. L. Preferential selection of adenosines for modification by double-stranded RNA adenosine deaminase. *EMBO J.* **13**, 5701–5711 (1994).
55. Riedmann, E. M., Schopoff, S., Hartner, J. C. & Jantsch, M. F. Specificity of ADAR-mediated RNA editing in newly identified targets. *Rna* **14**, 1110–1118 (2008).
56. Morse, D. P. & Bass, B. L. Long RNA hairpins that contain inosine are present in Caenorhabditis elegans poly(A)+ RNA. *Proc Natl Acad Sci U S A* **96**, 6048–6053 (1999).
57. Washburn, M. C. *et al.* The dsRBP and inactive editor, ADR-1, utilizes dsRNA binding to regulate A-to-I RNA editing across the C. elegans transcriptome. *Cell Rep.* **6**, 599–607 (2014).

58. Raitskin, O., Cho, D. S., Sperling, J., Nishikura, K. & Sperling, R. RNA editing activity is associated with splicing factors in lnRNP particles: The nuclear pre-mRNA processing machinery. *Proc. Natl. Acad. Sci. U. S. A.* **98**, 6571–6576 (2001).
59. Hsiao, Y.-H. E. *et al.* RNA editing in nascent RNA affects pre-mRNA splicing. *Genome Res.* **28**, 812–823 (2018).
60. Rodriguez, J., Menet, J. S. & Rosbash, M. Nascent-seq indicates widespread cotranscriptional RNA editing in Drosophila. *Mol Cell* **47**, 27–37 (2012).
61. Marcucci, R. *et al.* Pin1 and WWP2 regulate GluR2 Q/R site RNA editing by ADAR2 with opposing effects. *EMBO J.* **30**, 4211–4222 (2011).
62. Nguyen Ba, A. N., Pogoutse, A., Provart, N. & Moses, A. M. NLStradamus: A simple Hidden Markov Model for nuclear localization signal prediction. *BMC Bioinformatics* **10**, 1–11 (2009).
63. Lin, J. rong & Hu, J. SeqNLS: nuclear localization signal prediction based on frequent pattern mining and linear motif scoring. *PLoS One* **8**, (2013).
64. Bernhofer, M. *et al.* NLSdb-major update for database of nuclear localization signals and nuclear export signals. *Nucleic Acids Res.* **46**, D503–D508 (2018).
65. Kosugi, S., Hasebe, M., Tomita, M. & Yanagawa, H. Systematic identification of cell cycle-dependent yeast nucleocytoplasmic shuttling proteins by prediction of composite motifs. *Proc. Natl. Acad. Sci. U. S. A.* **106**, 10171–10176 (2009).
66. Shimozono, S., Tsutsui, H. & Miyawaki, A. Diffusion of large molecules into assembling nuclei revealed using an optical highlighting technique. *Biophys. J.* **97**, 1288–1294 (2009).

FIGURES

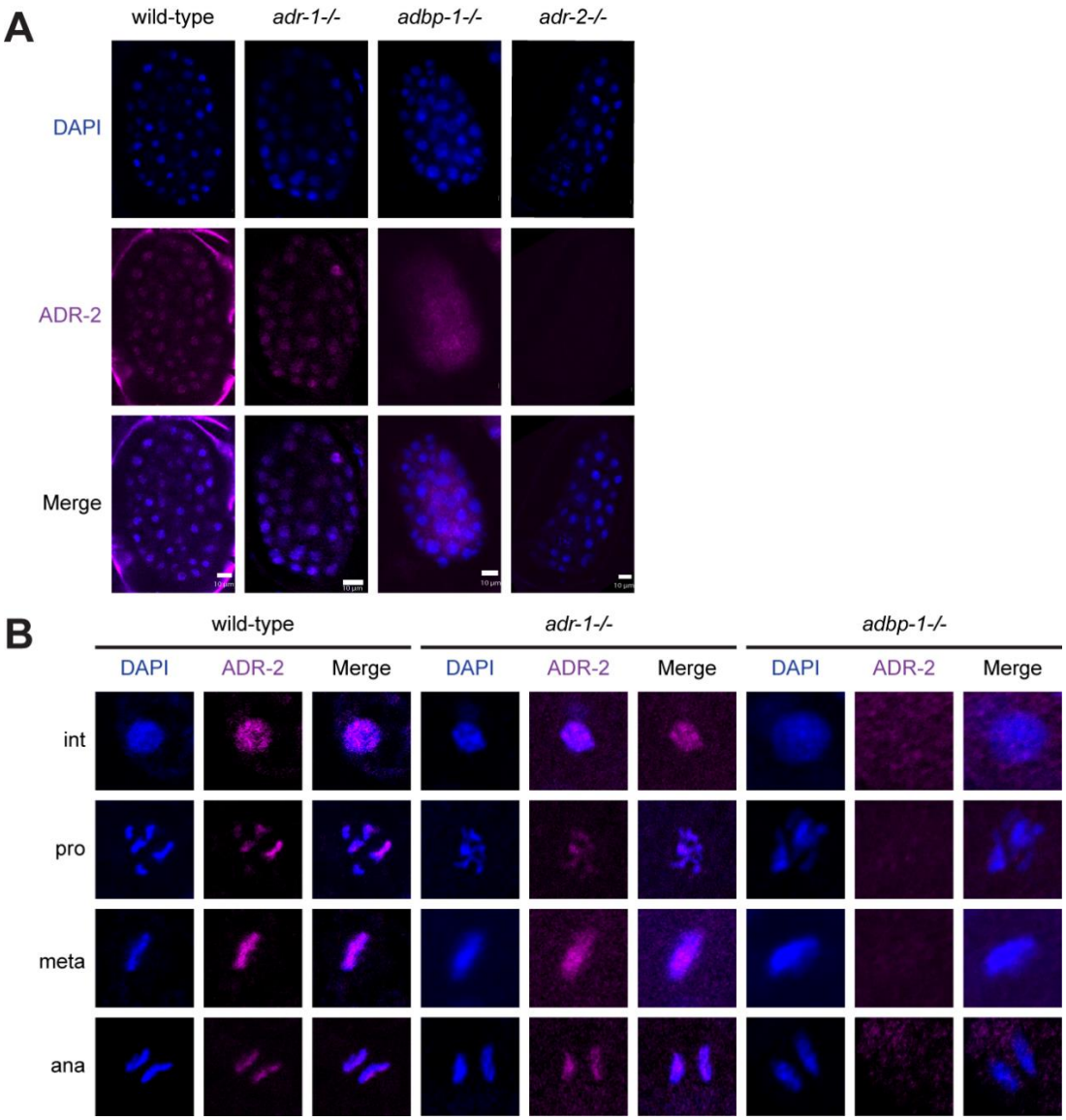

Figure 1. Localization of ADR-2 in embryos at different stages of mitosis. (A) Representative immunofluorescence images of DNA (DAPI in blue) and ADR-2 (magenta) in embryos from wild-type (N2), *adr-1-/-* and *adbp-1-/-* worms, including *adr-2-/-* worms as control strain. Their colocalization is shown as the overlap of both images. (B) Embryos of wild-type (N2), *adr-1-/-* and *adbp-1-/-* strains. A representative nucleus from each stage is shown: Int, interphase; Pro, prophase; Meta, metaphase; and Ana, anaphase. Scale bar, 10 µm.

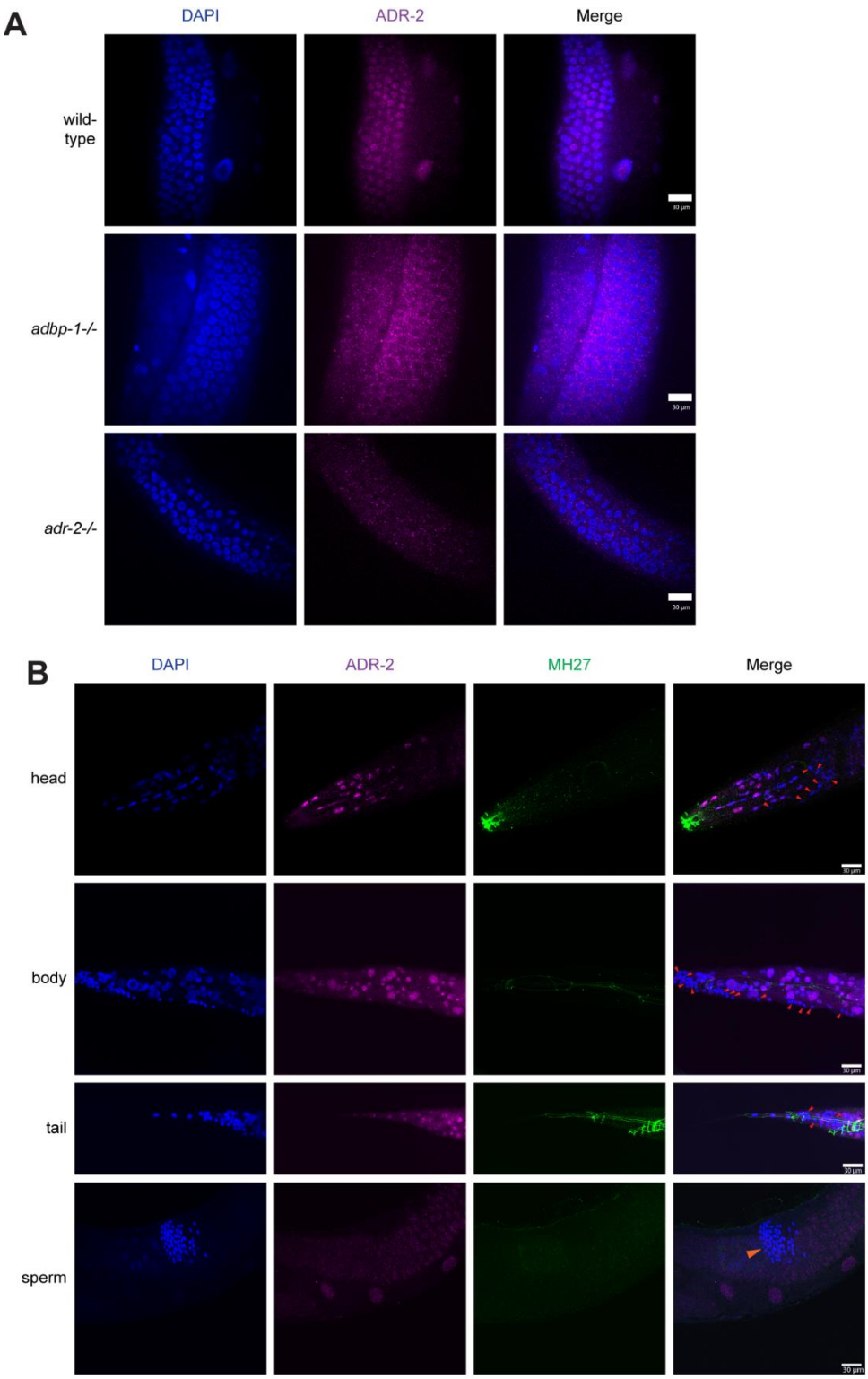

Figure 2. Localization of ADR-2 in the hermaphrodite adult gonads, head, body, tail, and sperm. (A) Representative immunofluorescence images of DNA (blue) and ADR-2 (magenta) from wild-type (N2) (top), *adbp-1* mutant (middle), and *adr-2-/-* control (bottom) strains. Their colocalization is shown as the overlap of both images. Scale bar, 30 µm. (B) Representative immunofluorescence images of DNA (blue), ADR-2 (magenta), and MH27 (green) from the head, body, tail, and sperm of wild-type (N2) strain. Their colocalization is shown as the overlap of the three images. Red arrows indicate where ADR-2 is absent. The orange arrow indicates the location of the sperm. Scale bar, 30 µm.

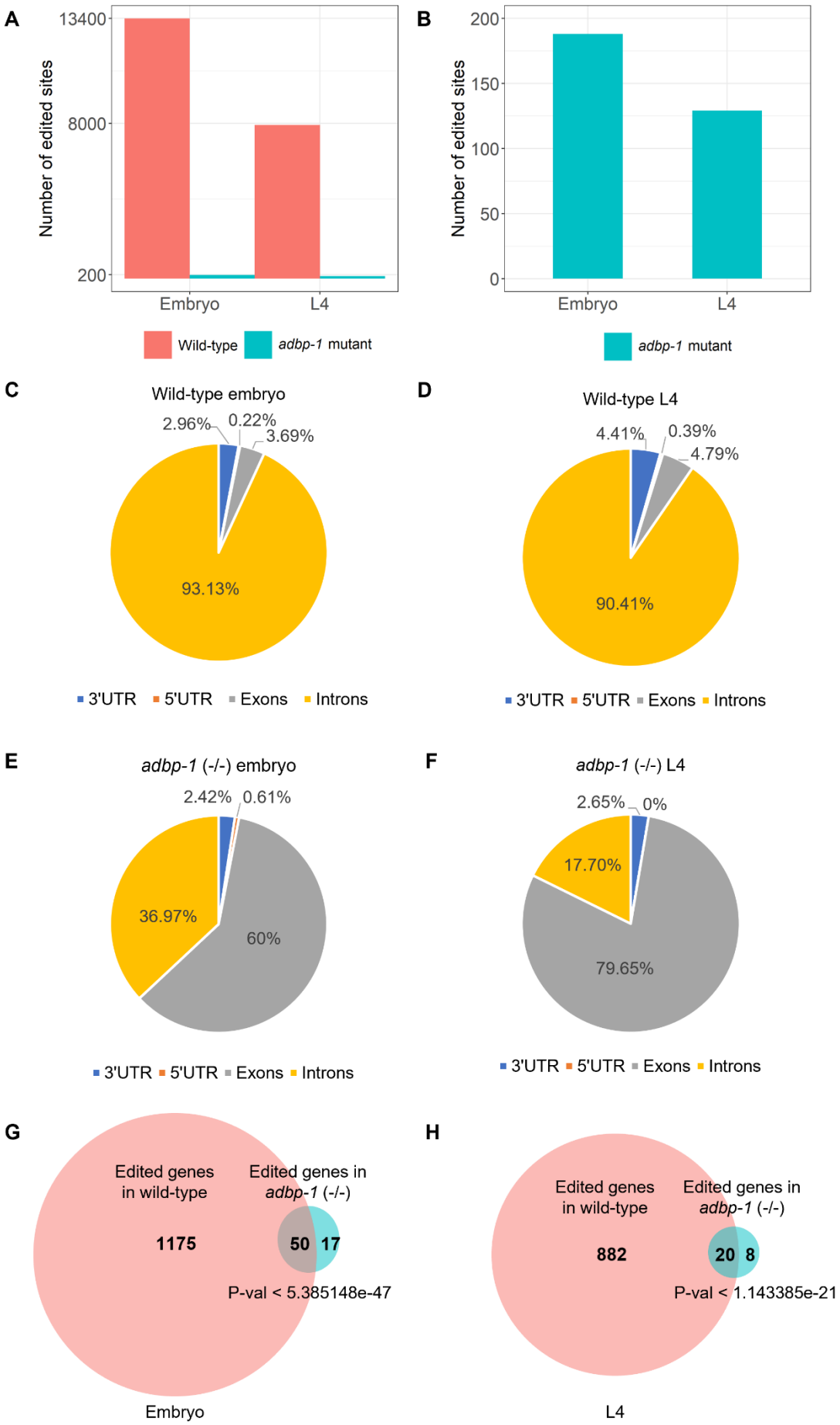
A
13400
8000
200
Number of edited sites
Embryo
L4
Wild-type
adbp-1 mutant
B
200
150
100
50
0
Number of edited sites
Embryo
L4
adbp-1 mutant
C
Wild-type embryo
2.96%
0.22%
3.69%
93.13%
3'UTR
5'UTR
Exons
Introns
D
Wild-type L4
4.41%
0.39%
4.79%
90.41%
3'UTR
5'UTR
Exons
Introns
E
adbp-1 (-/-) embryo
2.42%
0.61%
36.97%
60%
3'UTR
5'UTR
Exons
Introns
F
adbp-1 (-/-) L4
2.65%
0%
17.70%
79.65%
3'UTR
5'UTR
Exons
Introns
G
Edited genes in wild-type
Edited genes in adbp-1 (-/-)
1175
50
17
P-val < 5.385148e-47
Embryo
H
Edited genes in wild-type
Edited genes in adbp-1 (-/-)
882
20
8
P-val < 1.143385e-21
L4

Figure 3. Editing sites in *adbp-1* mutant worms reside primarily in exons. (A) The bar plot represents known edited sites in the wild-type worms compared to the *adbp-1* mutant worms both at the embryo stage and at the L4 larval stage. (B) The bar plot represents known edited sites in *adbp-1* mutant worms, which were not found in wild-type, at the embryo stage, and at the L4 larval stage. (C-F) Pie charts represent the distribution of annotated known edited sites in wild-type worms and the *adbp-1* mutant worms in the embryo and L4 stages. In the wild-type embryo worms, we detected 13398 annotated editing sites (C), and 7908 annotated editing sites at the L4 stage (D). In the *adbp-1* mutant embryo samples, we detected 188 editing sites (E), while in the *adbp-1* mutant L4 worms, we detected 129 editing sites (F). (G-H) Wild-type worms and *adbp-1* mutant worms share significant common edited genes.

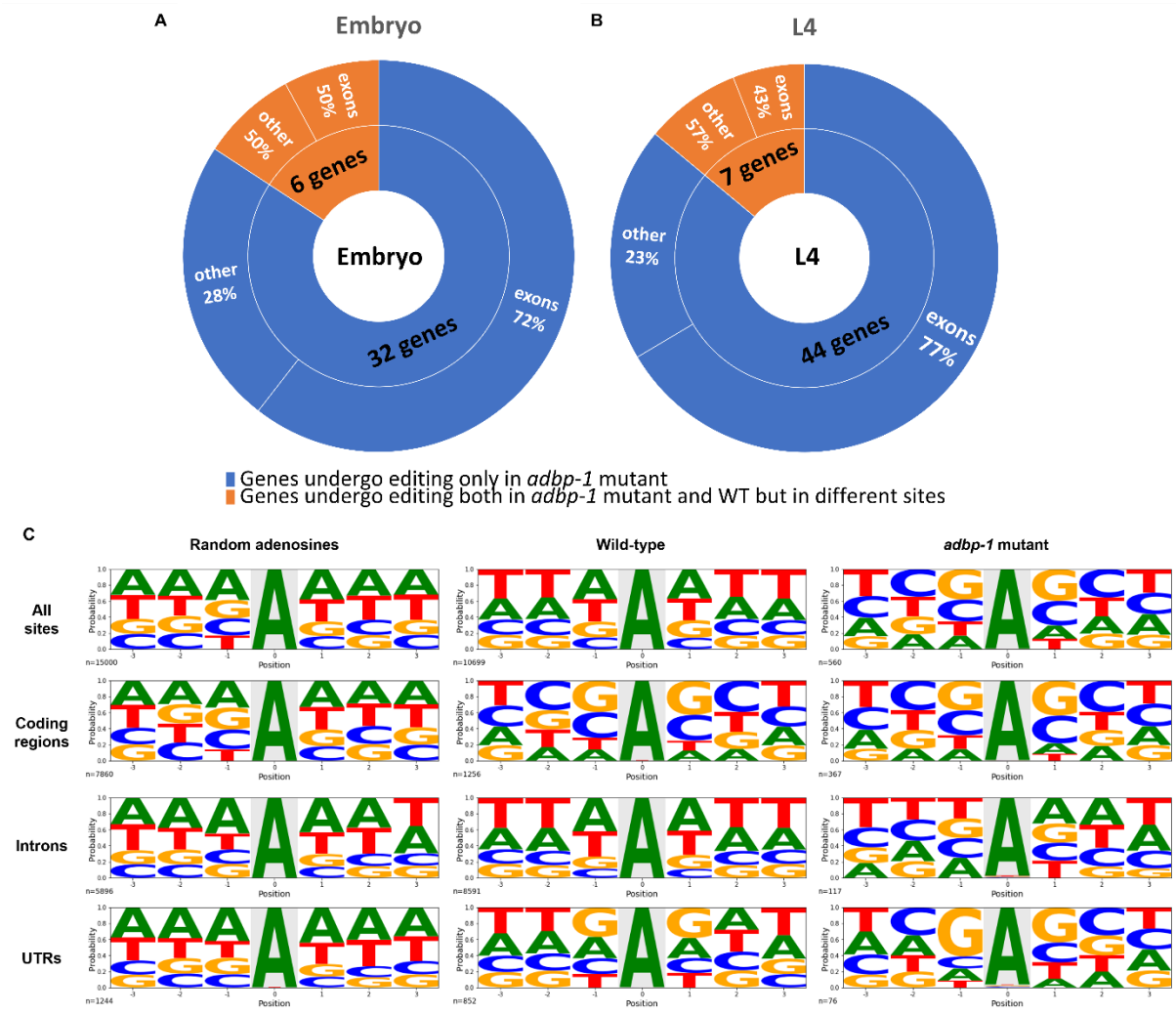

Figure 4. *adbp-1* mutant worms have unique edited sites, which show nucleotide preference to editing in a coding region. The pie charts describe editing sites in genes found by a pipeline searching for new editing sites in *adbp-1* mutant at the embryo (A) and L4 (B) stages. Genes found edited only in *adbp-1* mutant and not in wild-type worms (WT) are represented by the color blue. In contrast, the orange color represents genes in *adbp-1* mutant worms that also undergo editing in wild-type worms; however, the

edited sites in each gene differ between the strains. Editing sites uniquely found in *adbp-1* mutant worms tend to be in exons. (C) Distribution of nucleotides surrounding random adenosine sites and editing sites in wild-type and *adbp-1* mutant worms at coding regions, introns, and UTRs. The x-axis represents the position of the editing site (0) and its surrounding nucleotides. The y-axis represents the probability of finding each nucleotide in each position.

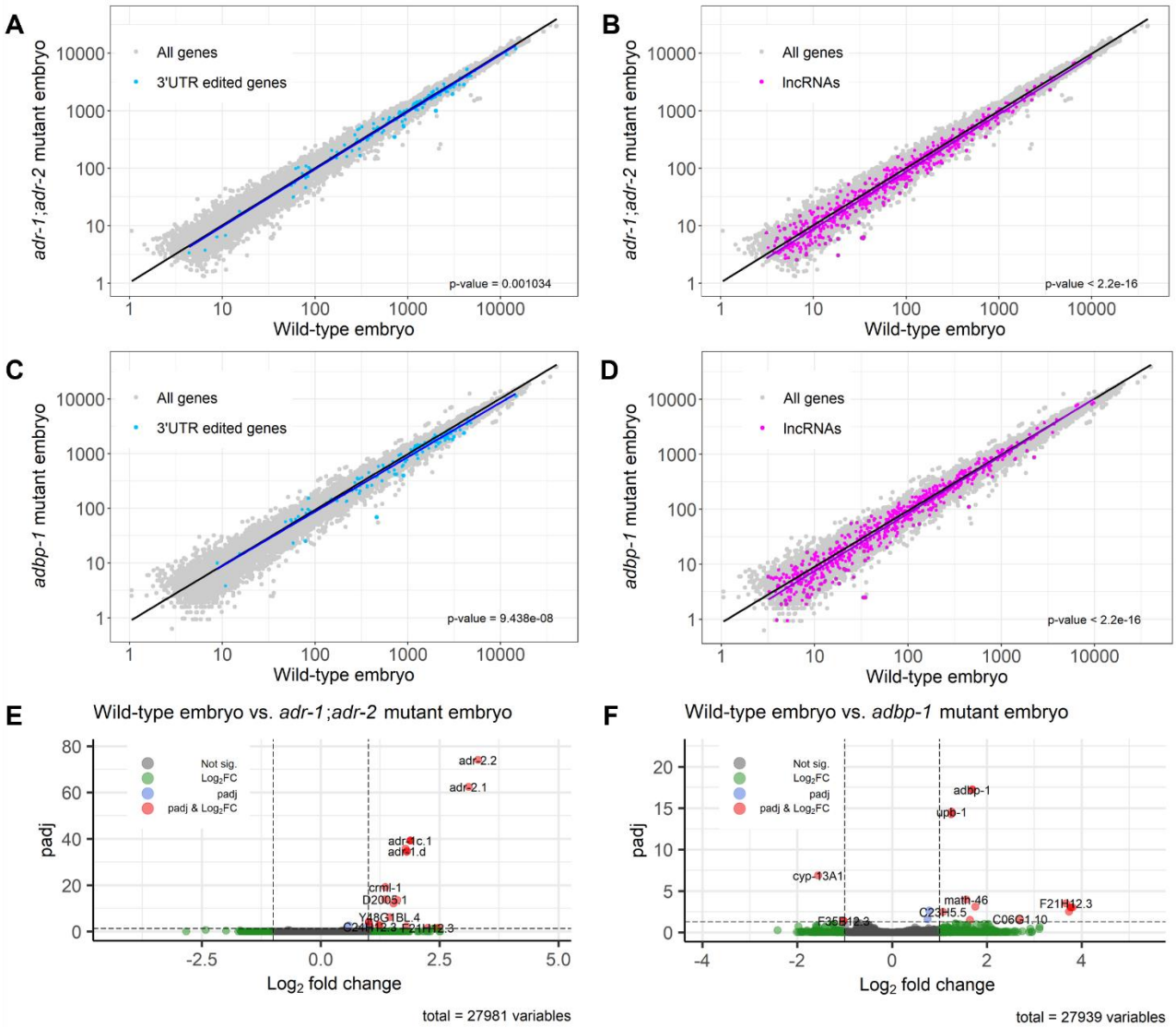

Figure 5. Low editing levels impair edited gene expression at the embryo stage. (A-D) The genes expressed in wild-type worms versus *adbp-1* mutant worms and the genes expressed in wild-type worms versus ADAR mutant worms, both in the embryo stage, are represented in log scale plots. Each dot represents a gene. Grey dots represent all the genes, blue dots represent edited genes at their 3'UTR, and purple dots represent lncRNAs. The black line is a regression line for all genes, the blue line is the regression line for genes edited at their 3'UTR, and the purple line is the regression line for the lncRNAs. (E-F) The volcano plots describe the log2 fold change versus -log10(P-adjusted) between the genes expressed in wild-type worms to *adr-1;adr-2* mutant worms, and wild-type worms to *adbp-1* mutant worms at the embryo. Non-significant genes are colored in grey. Differentially expressed genes,

which adhere to the following criteria: |log2FoldChange| > 1 and P-adjusted < 0.05, are highlighted in red. Genes with only |log2FoldChange| > 1 are colored green, and genes with only P-adjusted < 0.05 are colored blue.

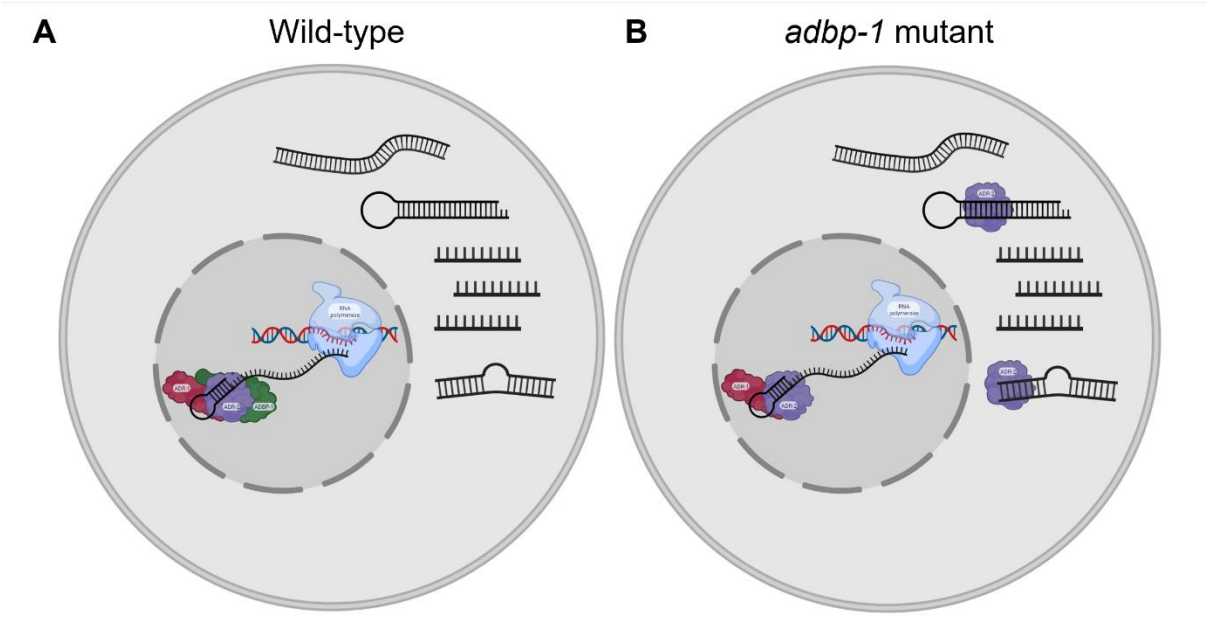

Figure 6. A proposed model of the interaction between ADR-2 and ADBP-1. In wild-type worms, ADBP-1 mediated ADR-2 import to the nucleus. Once ADR-2 is in the nucleus, it is adjacent to the chromosomes, where RNA editing occurs co-transcriptionally, regulated by the binding of ADR-1 to ADR-2. In the *adbp-1* mutant, ADR-2 remains in the cytoplasm and edits sites randomly in exons.