

# Mutations in RNAi Rescue Aberrant Chemotaxis of ADAR Mutants

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Adenosine deaminases that act on RNA (ADARs) are RNA editing enzymes that convert adenosine (A) to inosine (I) within double-stranded RNA (dsRNA) [reviewed in (1)]. ADARs are found in metazoa where they are highly expressed in neuronal tissues. *Drosophila melanogaster* and *Caenorhabditis elegans* strains lacking all ADAR activity are viable but exhibit behavioral defects (2, 3), whereas mice lacking ADARs die embryonically or shortly after birth (4, 5).

ADARs target messenger RNAs (mRNAs) in coding regions, as well as in noncoding sequences such as 5' and 3' untranslated regions (UTRs). A to I changes in coding sequences often alters codon meaning. For example, A to I editing in mammalian glutamate receptor B mRNA changes a glutamine (Q) codon to an arginine (R) codon at a position critical for determining ion permeability of the glutamate-gated channel; at least some of the defects of mice lacking ADARs derive from the absence of Q-R site editing (5).

The purpose of inosines within noncoding regions of an mRNA is unclear. This type of editing has been observed in mRNA of *C. elegans* and human brain and may be more common than editing within codons (6, 7). The double-stranded structures that mediate editing in UTRs are remarkable in their length and stability, sometimes consisting of several hundred nearly contiguous base pairs (6, 7). Given this, the question arises as to why these structures do not trigger RNA interference (RNAi). One possibility, raised by recent results (8), is that RNA editing prohibits dsRNA from triggering RNAi. A to I changes would destroy the complementarity between a dsRNA and mRNA that is required for RNAi. Further, because ADARs convert AU basepairs to IU mismatches, the UTR structures would become more single-stranded and less suited for binding dsRNA binding proteins such as Dicer. According to this scenario, the phenotypes of ADAR mutants would result because mRNAs that were normally "protected" by editing would be degraded by the RNAi pathway.

To explore this idea, we crossed *C. elegans* strains lacking ADARs with RNAi defective strains. The RNAi defective strains we chose contain mutations in the *rde-1* or *rde-4* gene, and are incapable of eliciting an

RNAi response but otherwise appear normal (9–11). Previously, we observed that deletions in each or both of the *C. elegans* *adr* genes (*adr-1* and *adr-2*) produce animals with defects in chemotaxis (2), a measure of the efficacy of a worm's olfactory system. As shown (Fig. 1A), wild-type animals exposed to chemicals that smell like food move toward the chemoattractant in a concentration-

dependent manner. The chemotaxis index reflects the number of animals reaching the chemoattractant, and *adr-1(gv6);adr-2(gv42)* double mutants exhibit significantly lower chemotaxis indices. We observed that two *rde-1* alleles and an *rde-4* allele rescued the chemotaxis defects of the *adr-1(gv6);adr-2(gv42)* animals. Animals containing only a mutation in the *rde-1* or *rde-4* gene showed normal chemotaxis behavior (Fig. 1B). Rescue was specific for the chemotaxis defects caused by mutations in the *adr* genes, because crossing *rde-1* alleles into other chemotaxis-deficient strains did not lead to rescue (Fig. 1C).

Our results have several implications. First, they suggest that, in addition to changing codon meaning, ADARs play a role in regulating whether a dsRNA enters the RNAi pathway. This function could be quite far-reaching: not only could ADARs regulate silencing triggered by intramolecular structures in mRNA, but also that caused by spurious antisense transcription throughout the genome (12). Finally, we speculate that dsRNA-mediated gene silencing might play a special role in the nervous system.

## References and Notes

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## Supporting Online Material

www.sciencemag.org/cgi/content/full/302/5651/1725/DC1

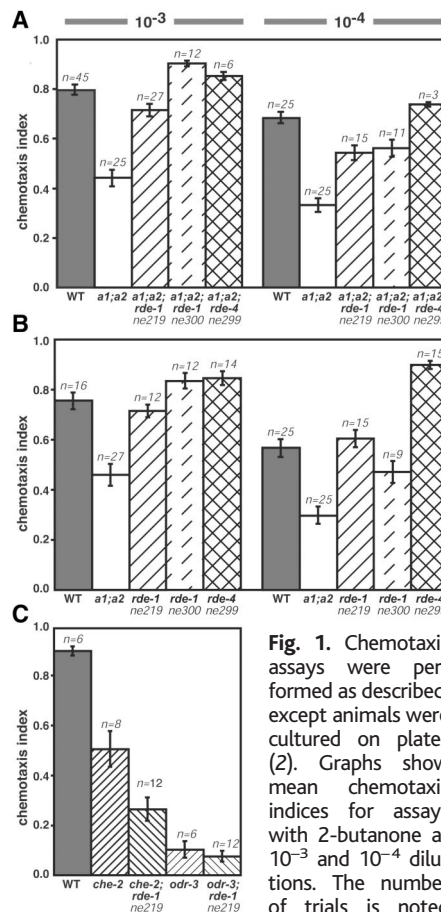
SOM Text

Fig. S1

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**Fig. 1. Chemotaxis assays** were performed as described, except animals were cultured on plates (2). Graphs show mean chemotaxis indices for assays with 2-butanone at  $10^{-3}$  and  $10^{-4}$  dilutions. The number of trials is noted above error bars (SEM). Wild-type (WT) and *adr-1(gv6);adr-2(gv42)* double-mutants (*a1;a2*) were compared with (A) two *rde-1* rescue strains and an *rde-4* rescue strain or (B) with *rde-1* and *rde-4* strains. Values for rescue strains were significantly different ( $P \leq 0.0001$ ) from *a1;a2* strains, but not from WT. Similar results were observed with diacetyl (fig. S1). (C) Defects of *che-2(e1033)* and *odr-3(n2150)* were not rescued by *rde-1* alleles. *P* values are from a Student's *t* test (two-tailed, unequal variance).