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# Interfering with RNA interference

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## Abstract

Adenine deamination in double-stranded RNA by specific adenine deaminases efficiently blocks RNA interference *in vitro*

# Significance and context

Since it was first described a few years ago, in the nematode *Caenorhabditis elegans*, double-stranded (ds) RNA interference (RNAi) has been the subject of intense study. As a result, we now understand many mechanistic aspects of RNAi and methodologies have been developed for using it to 'knock down' specific gene activity in many organisms. RNAi is not only a fascinating biological phenomenon but also a valuable modern research tool.

In RNAi, long dsRNAs are initially processed by RNase III (the Dicer enzyme in *Drosophila*) to generate small interfering RNAs (siRNAs), 21-23 nucleotides long, that then drive specific nuclease complexes to degrade the cognate mRNAs. This process is thought to comprise part of cellular defenses against viruses and transposable elements. An additional line of defense may be adenine deaminases that act specifically on dsRNA (ADARs). These edit dsRNA by converting adenine (A) to inosine (I). Hyperediting of dsRNA by ADARs would not only generate many missense mutations, thus altering the coding capacity of RNA (I is seen as guanine (G), not A, during translation), but would also significantly affect dsRNA structure by altering proper A-U base pairing to I-U. Such modified dsRNAs could then be targeted for degradation and might constitute poor initiators of RNAi. On the other hand, processing of long dsRNAs to siRNAs by the RNAi machinery would render them poor substrates for deamination by ADARs, as siRNAs are too short to be deaminated. Paradoxically, even though RNAi and ADARs seem to be working towards the same end (cell defense against spurious RNAs) they can potentially antagonize each other. But is this so?

# Key results

Scadden and Smith now present evidence that, at least *in vitro*, ADAR activity can effectively block RNAi. In a *Drosophila* extract that is active for RNAi, they show that long dsRNAs, previously deaminated to about 50% by ADAR, will not trigger RNAi-mediated degradation of cognate mRNAs. Even at lower levels of deamination, RNAi is inhibited in proportion to the extent of hyperediting. To

determine how deamination reduces interference and which step(s) in RNAi is affected, the authors examined the conversion of long, deaminated dsRNA to siRNAs in their *in vitro* system and found that 43% deamination of the input dsRNA is sufficient completely to abolish generation of siRNAs. It appears that hyperedited dsRNAs are poor substrates for RNAse III. With a lower level of editing, siRNAs are generated, albeit less efficiently.

In the case of moderate editing, siRNAs could either originate exclusively from non-edited dsRNA, in which case they would not contain I, or they could contain I if they were the product of processing edited dsRNA. The authors show that when moderately deaminated dsRNA is used, the siRNAs generated do contain I, in amounts proportional to the extent of deamination of the input dsRNA. This indicates that RNase III (Dicer in this case) does not discriminate between edited and non-edited sequences of dsRNA. It is conceivable that I-containing siRNAs might have a much reduced ability to drive specific degradation of cognate mRNAs. This would suggest that ADAR activity impacts on RNAi at two levels: first, reduced processing of deaminated dsRNAs to siRNAs; and second, reduced effectiveness of I-containing siRNAs.

# Conclusions

The authors conclude that antagonism between ADARs and RNAi does exist in their *in vitro* set-up and can be manifested at least at the level of siRNA generation. The same experimental methodology can be used to investigate the effectiveness of deaminated siRNAs in exerting degradation of cognate mRNA. The authors note that for antagonism to occur *in vivo*, ADARs would have to be active in the vicinity of the RNAi machinery. This requires overlapping temporal and spatial expression of ADARs and RNAi machinery components, as well as similar subcellular localization. An interesting peculiarity that might be attributable to antagonism between ADARs and RNAi is the relative ineffectiveness of RNAi in the nervous system and other specialized tissues of *C. elegans*. ADARs are known to be highly expressed in the nervous system and this may, in part, contribute to the limited ability of RNAi to target neuronal genes.

# Reporter's comments

By demonstrating that hyperediting of dsRNA can efficiently antagonize RNAi *in vitro*, Scadden and Smith provoke the interesting question of what, if any, is the *in vivo* role of this clash between processes that presumably serve a similar purpose. To address this question, antagonism needs first to be shown *in vivo*. Ongoing genetic screens in *C. elegans* for mutants that allow efficient RNAi of nervous system genes are expected to shed light on the molecular mechanisms that render this tissue relatively refractory to RNAi. ADAR-related genes may well turn up in these screens. A quicker route might be to directly knock out nematode ADAR-like genes (the closest candidate is the predicted open reading frame T20H4.4), and then test for neuronal susceptibility to RNAi.

# Table of links

*EMBO Reports*

## References

1. Scadden ADJ, Smith CWJ: RNAi is antagonized by A→I hyperediting. EMBO Rep. 2001, 2: 1107-1111. 1469-3178