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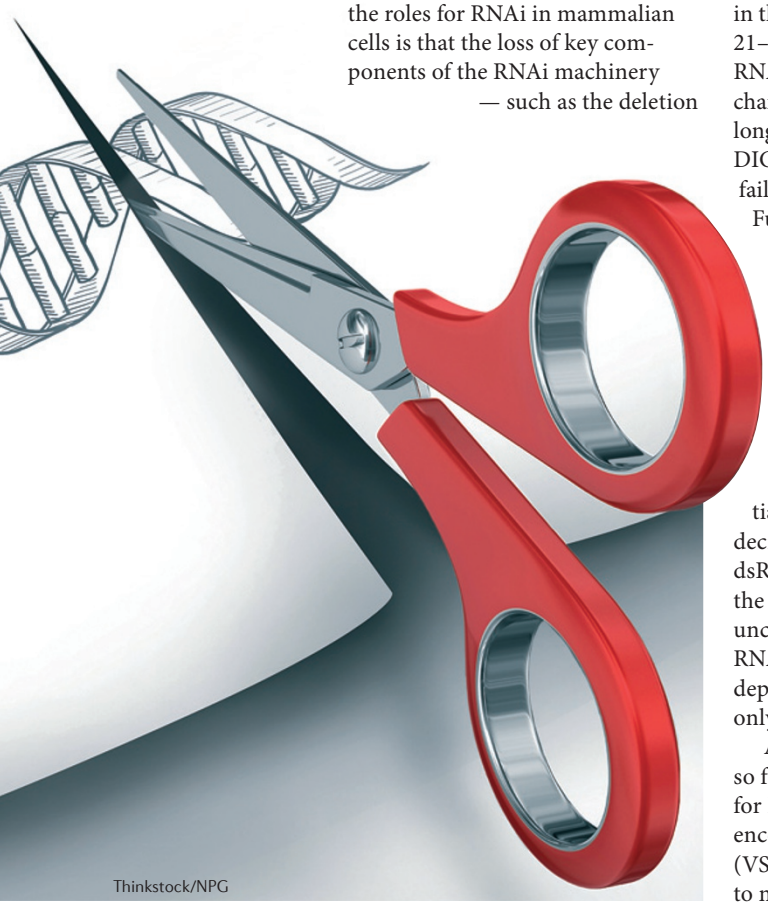
SMALL RNAS

Antiviral RNAi in mammals

> viral accumulation was restored in mESCs lacking all four AGO proteins

The cleavage of viral double-stranded RNAs (dsRNAs) by host RNA interference (RNAi) machinery has been shown to be an important antiviral mechanism in various species, including in plants and insects. However, in mammals, it was unclear whether RNAi had a similar role or whether alternative antiviral mechanisms, such as the interferon response, predominate. Two new studies now provide strong support for an antiviral role for RNAi in mammals.

One challenge of characterizing the roles for RNAi in mammalian cells is that the loss of key components of the RNAi machinery — such as the deletion of *Dicer1* or of all four Argonaute (AGO) genes — is generally incompatible with cell and organism viability.



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Maillard *et al.* took advantage of mouse embryonic stem cells (mESCs), which can survive these manipulations. The authors infected wild-type mESCs with encephalomyocarditis virus (EMCV); this virus has a single-stranded RNA (ssRNA) genome but produces long dsRNAs as part of its infection cycle. High-throughput RNA sequencing revealed that such infection resulted in the production of virus-derived 21–23-nucleotide small interfering RNAs (siRNAs), which are characteristic of the cleavage of long dsRNAs by mammalian DICER1. Indeed, *Dicer1*$^{-/-}$ mESCs failed to produce these siRNAs. Furthermore, biochemical analyses showed that these viral-derived siRNAs became bound by AGO2. Overall, these findings provide support that viral RNAs are processed by mammalian RNAi pathways as a putative antiviral mechanism.

Interestingly, Maillard *et al.* also showed that the differentiation of mESCs substantially decreases the processing of viral dsRNA into siRNAs. Although the reasons for this are currently unclear, it suggests that mammalian RNAi might function as a context-dependent antiviral mechanism only in some cell types.

A further hindrance to efforts so far to identify the antiviral roles for RNAi is that viruses frequently encode viral suppressor of RNAi (VSR) proteins, which are likely to mask antiviral roles for RNAi. Although no VSR is known for EMCV, Maillard *et al.* and, in a separate study, Li *et al.* studied host responses to Nodamura virus (NoV); this virus encodes the dsRNA-binding B2 protein, which functions as a VSR. Similarly to EMCV, NoV has a ssRNA genome and produces long dsRNA during its life cycle.

Both groups used wild-type and B2-deficient NoV strains to determine whether the absence of the VSR protein could unmask an active role for RNAi in controlling viral infections. Maillard *et al.* infected mESCs *in vitro*, whereas Li *et al.* infected hamster fibroblasts *in vitro* and suckling mice *in vivo*. Both groups observed that only in the absence of the VSR were virus-derived siRNAs produced and infections effectively controlled. Further supporting a role for RNAi in this unmasked antiviral response, Li *et al.* found no evidence of alternative RNAi-independent antiviral pathways (such as the interferon response) from gene expression analyses in infected mice, and Maillard *et al.* crucially showed that viral accumulation was restored in mESCs lacking all four AGO proteins.

It will be interesting to assess the relative importance of RNAi and other antiviral responses in different contexts through different stages of mammalian development.

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ORIGINAL RESEARCH PAPERS Maillard, P. V. *et al.* Antiviral RNA interference in mammalian cells. *Science* **342**, 235–238 (2013) | Li, Y. *et al.* RNA interference functions as an antiviral immunity mechanism in mammals. *Science* **342**, 231–234 (2013)