RNA Silencing as a Natural Antiviral Defense System in Mammals: Where Are We Now?

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Small regulatory noncoding RNAs are increasingly at the forefront of research in molecular biology. They are key elements in a variety of processes together referred to as "RNA silencing." In addition to playing key roles in a large variety of biological processes through the action of microRNAs (miRNAs), RNA silencing was associated very early on with antiviral defense in both plants and insects. However, the question of whether the antiviral arm of RNA silencing was conserved in mammals has been nagging at researchers almost ever since RNA interference was shown to be functional in mammalian systems.[1] Until recently, the consensus was that this was unlikely. Now, in an article recently published in *PLoS Pathogens*, Andrew Fire and collaborators put the current theory to the test.[2] Taking advantage of next-generation sequencing technologies, they cloned and sequenced millions of small RNAs from an impressive number of different cell lines and tissue types infected with different RNA viruses. The massive amount of data thus generated enabled them to identify viral small RNAs (vsRNAs) derived from the viral genomes used in the study; the authors hypothesize that some of these small RNAs indicate that RNA silencing indeed targets these viruses in certain cell types.

Different classes of small RNAs have been characterized, such as miRNAs, small interfering RNAs (siRNAs), and Piwi-interacting RNAs (piRNAs).[3] A large subset of these tiny nucleic acids derives from larger double-stranded RNA (dsRNA) molecules that are usually processed by ribonucleases such as Dicer. After processing of the dsRNA, the end product—typically an RNA 19 to 26 nucleotides long—is assembled into an effector complex, which invariably contains a member of the Piwi/Argonaute family of proteins. Once loaded, this RNA-induced silencing complex (RISC) can regulate the expression of target RNAs through base-pairing with the guide small RNA.[4] Viruses very often produce dsRNA molecules during the course of infection, through either replication intermediates or intramolecular folding. In plants and insects, Dicer or Dicer-like proteins can readily process these viral dsRNAs into viral siRNAs, which can then assemble into a RISC to target the viral genome, resulting in efficient protection against the invading pathogen. As a result, most plant and insect viruses have developed strategies to counter this antiviral response by expressing suppressors of RNA silencing.[5] However, the cloning and sequencing of small RNAs from mammalian cells infected with RNA viruses has failed to identify any siRNAs of viral origin[6]; there was greater evidence that if any small RNAs were to play a role during viral infections in such cells, they would more likely be miRNAs.[7]

Importantly, the experiments described in the study by Fire's group[2] were not performed solely in wild-type host cells but also in mutants of genes essential for RNA silencing (such as Dicer or Ago2) or for classic innate immunity (the interferon (IFN) pathway). All together, these investigators generated small RNA profiles from 41 host systems infected with either flock house virus, dengue virus, vesicular stomatitis virus, poliovirus, West Nile virus, or hepatitis C virus (HCV). Their key findings can be summed up as follows: the overall abundance of vsRNAs is pretty low (about 0.4% on average), but—depending on the virus and the cell line studied—some of the vsRNAs are relatively abundant compared with miRNAs. There is a striking difference in the size distribution of vsRNAs in nematode vs. mammalian samples, with the former showing a strong peak around 21 nucleotides that is missing in the latter. Nevertheless, the strand ratio of cloned vsRNA is between 1:1 and 1:5 (i.e., there are one to five times more vsRNAs originating from the plus strand than from the minus strand of the replication intermediate). Normally, there is an overrepresentation by 100-fold of the plus (genomic) strand compared with the minus strand, so this strand ratio does not fit with what would be expected if vsRNA simply comprised degradation products. In the absence of Dicer, the number of vsRNAs cloned drops, but only by about twofold, which indicates either that some of the vsRNAs are random degradation products or that other unidentified nucleases participate in the process. Conversely, in the absence of IFN α/β, or Ago2, there is an increase in the number of vsRNAs. Finally, as expected, viral infection leads to changes in miRNA profiles. However, the authors do not provide definitive conclusions regarding the latter two findings.

The authors analyzed in greater detail the small RNA profiles of cells infected with HCV. Here they found evidence for hot spots for vsRNA accumulation along the genome, as well as a bias in the nucleotide composition of HCV-derived RNAs. More interestingly, some of the small RNAs can be assembled in duplexes with one to three nucleotides overhanging at the 3′ end, similar to what is known for siRNAs.[8] Finally, HCV small RNAs seem to be enriched in immunoprecipitates of tagged versions of Argonaute proteins.

What is the significance of these findings when it comes to molecular therapy? If indeed viruses can to some extent be targeted by the RNA silencing machinery and give rise to small RNAs, we are forced to ponder whether this could

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pose a problem for the use of viruses as vectors for gene therapy. However, given that most viral vectors are based on retroviruses or small DNA viruses (which are less likely to produce large amounts of dsRNA), this concern is relatively minor. Conversely, one could also speculate about whether any potential therapeutic application can be derived from the findings in this report. The authors hint that these studies could lead to a way of defining potent siRNAs directed against these viruses. This could indeed be the case for the vsRNAs that were found to be associated with Ago2 and hence are more likely to be functional. Otherwise, this study also indicates that by increasing the efficiency of the RNA silencing machinery, an alternative approach to the targeting of viruses might be developed—although this could be problematic, given the other roles of this machinery in infected cells. The results reported by Fire and colleagues[2] do not constitute a formal proof that RNA silencing is indeed naturally directed against viruses in mammals, but they raise the intriguing possibility that this might be the case in certain viruses and cells, and the new findings should fuel this intriguing and important debate.

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