MicroRNA expression by an oncogenic retrovirus

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Although >250 viral microRNAs (miRNAs) are expressed by a range of nuclear DNA viruses, efforts to identify miRNAs expressed by RNA viruses have so far been in vain (1, 2). In PNAS, Kincaid et al. (3) report the identification of five miRNAs encoded by the delta retrovirus bovine leukemia virus (BLV) that are expressed in BLV-transformed B cells. Interestingly, BLV uses RNA polymerase III (pol III) to directly transcribe the pre-miRNA hairpins that give rise to these viral miRNAs, thereby preventing the cleavage of viral genomic RNA and mRNA species as a result of pre-miRNA excision. Moreover, one of the BLV miRNAs, miR-B4, shares the same seed sequence as cellular miR-29 and therefore can down-regulate many of the same mRNA targets. As miR-29 overexpression can induce chronic lymphocytic leukemia (CLL) in transgenic mice (4), and BLV induces CLL-like B-cell neoplasms in infected cattle and sheep (5), it appears likely that these viral miRNAs play an important role in BLV pathogenesis.

Although the first miRNA was identified 18 y ago, it was only in 2001, with the development of technologies that allowed the efficient cDNA cloning and sequencing of small RNA species, that it became apparent that all multicellular eukaryotes encode numerous members of this class of small regulatory RNAs. Shortly after the identification of the first human miRNAs, the first virally encoded miRNAs were reported in the human herpesvirus Epstein–Barr virus (EBV) (6). Although this initial discovery suggested that viruses in general might use miRNAs to down-regulate cellular factors that inhibit viral replication, the subsequent analysis of a wide range of RNA viruses failed to identify any viral miRNAs (1, 2). However, virally encoded miRNAs are expressed by many members of the herpesvirus family of nuclear DNA viruses and are also found in a small number of other nuclear DNA viruses, particularly polymaviruses.

Consideration of the canonical miRNA biogenesis pathway suggested at least two reasons why RNA viruses might not encode any miRNA species. The vast majority of both cellular and viral miRNAs are initially transcribed by pol II as long capped, polyadenylated transcripts called pri-miRNAs, in which the miRNA forms part of one arm of an ~80-nt stem-loop structure (Fig. 1). This stem loop is cleaved by the nuclear RNase III enzyme Drosha, excising the ~65-nt pre-miRNA hairpin. The pre-miRNA is then exported to the cytoplasm where the mature miRNA is released after cleavage by a second RNase III enzyme, called Dicer. Because the large majority of RNA viruses replicate exclusively in the cytoplasm (the major exceptions to this generalization are retroviruses and orthomyxoviruses), it seemed possible that one reason for the lack of RNA virus miRNAs might be the nuclear localization of the first step in miRNA biogenesis, mediated by Drosha (1, 2). However, analysis of the retroviruses HIV type 1 (HIV-1) and human T-cell leukemia virus type 1 (HTLV-1), as well as the orthomyxovirus influenza virus, also failed to reveal any viral miRNA species (7–9). A perhaps more compelling hypothesis for the lack of RNA virus miRNAs suggests that excision of a pre-miRNA from an RNA virus genome, antigenome, or mRNA would result in the endonucleolytic cleavage and degradation of that viral RNA, which would likely inhibit virus replication (1, 2). However, one can also conceive of strategies that RNA viruses might evolve to express viral miRNAs while preserving the integrity of longer viral RNA species. In particular, a murine herpesvirus, mouse γ-herpesvirus 68 (MHV68), has been shown to use pol III, not pol II, to generate short pri-miRNAs that are then processed into miRNAs by mechanisms that do not involve Drosha (10). In MHV68, the viral pri-miRNAs consist of short, fusion transcripts containing a 5′ tRNA-like molecule linked to a 3′ pre-RNA hairpin containing the viral miRNA (Fig. 1). After transcription by pol III, directed by a promoter located within the tRNA, the pri-miRNA is released by cleavage by cellular tRNase Z, which normally defines the 3′ end of cellular tRNAs, to yield a nonfunctional viral tRNA and a viral pre-miRNA that is then exported to the cytoplasm and processed by Dicer as normal (10).

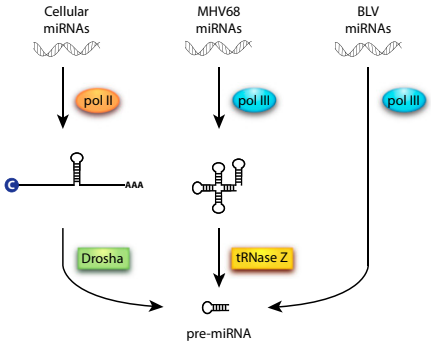

Fig. 1. Almost all cellular miRNAs are initially transcribed by pol II to generate a capped, polyadenylated pri-miRNA that is then cleaved by nuclear Drosha to liberate the pre-miRNA intermediate (*Left*). In MHV68, the pri-miRNA is initially transcribed by pol III and the 3′ pre-miRNA hairpin is then released by cleavage with tRNase Z (*Center*). Finally, in the case of BLV, the pre-miRNA hairpin is directly transcribed by pol III and does not require any RNA processing step (*Right*).

Kincaid et al. (3) hypothesize that retroviruses, RNA viruses that replicate through a nuclear DNA intermediate, might also use pol III to generate short pri-miRNA species that are processed into mature miRNAs without Drosha cleavage, thus sparing the retroviral genomic RNAs from nuclear degradation. Using a custom computer program, they therefore search all known retroviral genomes for the sequence motifs characteristic of pol III promoter elements and identify two candidate pol III promoters in BLV. Subsequent deep sequencing of small RNAs isolated from a BLV-transformed tumor cell line in fact identify five viral miRNA species, all of which are then shown to be transcribed by pol III and initially expressed as ~55-nt hairpin RNAs that are structurally similar to the pre-miRNA intermediate generated by Drosha cleavage (Fig. 1). Importantly, however, insertion of these miRNA genes into the context of an mRNA does not cause that mRNA to be cleaved by Drosha, most probably because the stem found in cellular pri-miRNA stem loops has to be ~32 bp in length to be recognized by Drosha, whereas the stem present in the BLV pre-miRNAs is only ~22 bp long (11).

Analysis of the sequence of the BLV miRNAs shows that one miRNA, miR-B4, shares an identical seed sequence with a known pro-oncogenic cellular miRNA, miR-29. The 7-nt miRNA seed sequence, extending from position 2 to 7 or 8 from the miRNA 5′ end, is the major determinant of mRNA target recognition in miRNAs (12) and, indeed, the authors are

Author contributions: B.R.C. wrote the paper.

The author declares no conflict of interest.

See companion article on page 3077.

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able to show that miR-B4 and miR-29 could down-regulate a shared set of mRNA targets (3). This result is reminiscent of another pro-oncogenic cellular miRNA, miR-155, which is mimicked by viral miRNAs encoded by two oncogenic herpesviruses, human Kaposi's sarcoma-associated herpesvirus (KSHV) and the avian herpesvirus Marek's disease virus (MDV) (13–15). Overexpression of miR-155 is associated with the development of B-cell lymphomas (16). KSHV infection is also associated with B-cell lymphomas, whereas MDV infection largely induces T-cell lymphomas (13–15). Importantly, an MDV mutant lacking the viral miR-155 analog grows normally in culture, yet is no longer capable of causing lymphomas in experimentally infected chickens (15). Similarly, miR-29 overexpression is causatively linked to CLL in humans and mice (4), and BLV causes B-cell tumors that closely resemble CLL (5). By analogy to MDV and miR-155, it would certainly be very interesting to see if a BLV mutant lacking miR-B4 loses the ability to induce B-cell tumors in infected ruminants.

In conclusion, this paper is therefore of interest in at least two ways. First, this is a unique report of miRNAs encoded by an RNA virus and therefore proves that this action is indeed possible. However, the pol III-dependent mechanism used by BLV to express viral miRNAs, although avoiding the Drosha-mediated cleavage of BLV genomic RNA, is one that can apply only to RNA viruses that replicate through a DNA intermediate, i.e., retroviruses. Moreover, this action may be quite unusual as not only HIV-1, a lentivirus, but also another delta retrovirus, HTLV-1, have been reported to lack viral miRNAs (7, 8). Nevertheless, this work should prompt new efforts to analyze a wider range of retrovirus species for the expression of miRNAs of viral origin. Second, this work represents the second example, after MDV, of a transforming virus that not only expresses an analog of a pro-oncogenic host miRNA, but also causes tumors closely analogous to those seen upon overexpression of that host miRNA. There are an increasing number of viral miRNAs that contain seed sequences identical to known cellular miRNAs. For example, the EBV miRNAs miR-BART1 and miR-BART3, which have seed sequences either identical to miR-B4 and miR-29 (miR-BART1) or offset by one nucleotide from the miR-B4 and miR-29 seed (miR-BART3), have recently been shown to share a number of mRNA targets with miR-29 (17). EBV is also an established transforming virus in human B cells, so the importance of viral mimics of cellular miRNAs in viral tumorigenesis in general, and of viral analogs of miR-29 in B-cell lymphoma in particular, certainly merits additional investigation.

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ACKNOWLEDGMENTS. Work in my laboratory on viral microRNA function and viral small RNAs is funded by National Institutes of Health Grants R01-AI067968, R01-DA030086, and R21-AI088327.

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