Living fossil or evolving virus?

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Although virus-like organisms are thought to have appeared together with the earliest forms of cellular life, their origin remains a mystery and we have little idea of the features of ancient viruses. Conversely, we know a lot about modern viruses, which show great diversity in genome organization and replication machinery, probably due to their antagonistic co-evolution with host defence mechanisms. However, not only is it extremely difficult to follow the details of these evolutionary processes, it is also hard to predict what ancient viruses might have been like, because only retroviruses have left a fossil record of their infection in hosts.

Against this background, the study of bornaviruses is shedding light on the murky past of viral origins and evolution. Bornaviruses are non-segmented, negative-strand RNA viruses that are characterized by their ability to infect nervous system cells without causing cell damage. A mammalian bornavirus, Borna disease virus (BDV), is infectious to a wide variety of host species and causes central nervous system disorders (Ikuta *et al*, 2002). Records dating to the seventeenth century in Germany, for example, detail neurological disorders in horses that we now know are caused by BDV infection.

The most striking feature of bornaviruses is that they establish a persistent infection in host cell nuclei, making them the only animal RNA virus capable of this type of intranuclear parasitism. Another important property of bornaviruses was uncovered recently by an international team of researchers (Horie *et al*, 2010): BDV was shown to integrate a DNA copy of its mRNA into the genetic material of the host in persistently infected cells. Although other non-retroviral RNA viruses are known to produce DNA versions of their genomes during replication (Klenerman *et al*, 1997; Geuking *et al*, 2009), bornaviruses are so far unique in having left endogenous fragments of themselves in the genomes of many mammalian species, including humans (Horie *et al*, 2010). At least four copies of these elements—homologous to the BDV nucleoprotein and thus named endogenous Borna-like nucleoprotein (EBLN)—have been found in the human genome, and their orthologues in the genomes of other primates. A phylogenetic analysis of these fragments has revealed that humans probably first acquired an EBLN fragment at least 40 million years ago. EBLN is therefore regarded as the first endogenous, non-retroviral viral element to be found in a mammalian genome, and it might preserve the features of ancestral bornaviruses.

Given this tantalizing possibility, the discovery of EBLN fragments has given rise to a host of new questions. The first surprise is the extremely long coexistence of bornaviruses and humans over 40 million years, in spite of which, we have not yet overcome their infection. More strangely, despite tens of millions of years spent replicating as exogenous viruses, the sequences of current bornaviruses are similar to those of EBLNs.

A main form of modern bornavirus infection is a chronic, life-long persistence in the host. Such a harmonious coexistence with the host might have enabled the virus to limit the accumulation of genetic variation during evolution. Of course, it is also possible that other events and processes—natural selection, recombination and switching to alternative host species—might also have been crucial in the conservation of bornavirus sequences. Is the modern bornavirus a living fossil that has retained the features of the ancient virus? Solving this puzzle of high sequence conservation is key to exploring the evolution and origin of RNA viruses.

The integration of BDV DNA into host genomes could represent a novel mode of pathogenesis in RNA virus infection. Persistent infection with BDV causes neurological disorders in animals in the absence of inflammation in the brain, which suggests that BDV directly induces the functional disturbance of infected neurons (Gonzalez-Dunia *et al*, 2005). Although the frequency of BDV integration into the host genome appears to be low, the functional disturbance caused by BDV might be a consequence of gene disruption due to the insertion of its DNA. This intriguing hypothesis is under investigation. Notably, some primate EBLNs are known to be expressed and to interact with several cellular proteins (Ewing *et al*, 2007); it will be interesting to determine their cellular function. It is also enticing to consider whether RNA viruses in general have had a role as drivers of genetic innovation or mutation during the evolution of their hosts.

The specific sequence characteristics of EBLNs and integrated BDV DNAs suggests that retrotransposons, such as the long interspersed nucleotide element 1 (L1) family, are involved in the integration of bornavirus sequences. This, in turn, suggests that EBLNs are processed pseudogenes derived from ancient bornaviruses. Yet, if L1s are responsible for bornavirus integration into the host genome, what ensures the specificity of L1s for bornavirus nucleoprotein mRNA without targeting other viral RNAs? This is one of many intriguing topics that remains to be explored; the unknown mechanism that enables bornavirus intranuclear residence could perhaps explain this specificity. The template switching of L1-encoded proteins was proposed recently as a mechanism of chimeric pseudogene formation from non-L1 cellular RNAs (Garcia-Perez *et al*, 2007). On the one hand, it is tempting to speculate that the bornavirus mRNA could be a target of this template-switching mechanism in the nucleus. On the other hand, a better control of bornavirus DNA integration into host genomes will also be necessary to make wide use of a recently established BDV-based vector system.

The study of such an ancient RNA virus will hopefully reinvigorate interest in this broad research area. A better understanding of the evolution of bornaviruses might provide clues as to the origin and/or evolution of RNA viruses in general.

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