Minireview

Koala retrovirus: a genome invasion in real time

Jonathan P Stoye

Address: Division of Virology, National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA, UK.
Email: jstoye@nimr.mrc.ac.uk

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Abstract

Koalas are currently undergoing a wave of germline infections by the retrovirus KoRV. Study of this phenomenon not only provides an opportunity for understanding the processes regulating retrovirus endogenization but may also be essential to preventing the extinction of the species.

The genomes of all higher organisms are littered with the remnants of past retroviral infections, some dating back many tens of millions of years. The unique replication cycle of retroviruses, involving the integration of viral genetic information into host-cell DNA as a provirus, allows the formation of a permanent association between the virus and the infected cell. If the infected cell is a germ cell, then that genetic association can persist for many generations, with the provirus forming part of the genome of every cell in progeny.

Until now, we have never had the opportunity of observing or studying such genomic colonization as it takes place. Enter the koala - an Australian icon and a potentially endangered species. A recent paper in *Nature* by Tarlington and colleagues [1] provides evidence that koalas are in the midst of a germline invasion by the koala retrovirus (KoRV). They show that KoRV is present, at variable copy number, in the germline of all koalas found in Queensland, but that animals from some areas of southern Australia lack the provirus. Most notably, KoRV appears completely absent from koalas on Kangaroo Island off the coast of South Australia. This island was stocked with koalas in the early part of the twentieth century and has remained essentially isolated since then; it appears most likely that the small founding population was entirely free of KoRV. Tarlington *et al.* [1] suggest that an ongoing process of infection and endogenization is now occurring, spreading from a focus in northern Australia that quite possibly initiated within the last 100 to 200 years. Studies of the origin, properties and growth of KoRV may provide invaluable insights into the factors influencing retroviral endogenization.

Retroviruses as a fossil record

Inherited proviruses, or endogenous retroviruses (ERVs), are inherited in Mendelian fashion, and thus can provide a 'fossil record' for vertebrate infection by retroviruses [2]. Individual integration events can be distinguished by the cellular sequences flanking the provirus, outside the long terminal repeats (LTRs) that characterize each provirus; for example, a provirus at a common site in two related species implies an insertion event pre-dating the evolutionary split between the species. Studies of primate ERVs indicate an ongoing process of retrovirus acquisition for a period in excess of 30 million years [3]. Analysis of the human genome sequence reveals the presence of between 30 and 40 phylogenetic groups of viruses, ranging in prevalence from 1 copy to more than 1,000. Each group is thought to descend from one cross-species infection, followed by a series of amplification events, most probably including re-infection [4]. Indeed, it appears that proviruses make up a greater fraction of the human genome (6 to 8%) than do protein-coding sequences (1 to 2%) [5]. Only a minute fraction of the inherited proviruses can encode functional retroviruses, as all have suffered mutational decay to an extent related to their period of residence in the genome. Nevertheless, ERVs are associated with a wide range of biological phenomena, including neoplasia. The replication properties of retroviruses and the structures and distribution of proviruses in the germline allow us to infer the likely course of events during a wave of endogenization, but until now the process has not lent itself to experimental study [2]. The ongoing infection of koalas presents an opportunity to remedy that situation.

KoRV was originally described as an endogenous retrovirus based on its ubiquitous presence in all koala samples initially examined [6]. However, unlike most ERVs, KoRV appeared biologically active with ready demonstration of viral particles from cultured koala lymphocytes [6] and significant variation of KoRV copy number [7]. These observations prompted Tarlington *et al.* [1] to investigate the distribution and properties of KoRV in more detail. On the one hand, consistent with the proposition that KoRVs are endogenous, they could show the presence of viral sequences in sperm by fluorescent *in situ* hybridization and demonstrate Mendelian inheritance of specific proviruses in related individuals by Southern hybridization. On the other hand, variation in the KoRV envelope gene sequence was consistent with the propagation of exogenous KoRV. Furthermore, there was considerable variation in the proviral content of unrelated animals, implying that these elements had not been present in the germline for sufficient time to allow genetic fixation.

Studies of koala samples from different geographic locations suggest an on-going process of endogenization spreading from the north of Australia, where all animals contain endogenous KoRV, to the south, where some animals are still virus-free. Setting an accurate time for the start of this epidemic remains a problem; on the basis of the similarity of KoRV to an exogenous virus (one that is not integrated into the germline), called Gibbon Ape Leukemia Virus (GALV), Tarlington *et al.* [1] conclude that it occurred less than 100 years ago. However, this may be an underestimate given the difficulties of determining rates of retrovirus evolution [8]. PCR examination of preserved koala DNA, if any suitable specimens can be identified, might provide a means of addressing this question.

Where did KoRV come from?

Six genera of retroviruses are currently recognized; of these, at least two - deltaviruses and lentiviruses - appear never to have generated ERVs. Although this particular observation may have a fairly trivial explanation, namely the absence of specific receptors for these viruses on germ cells, it does prompt more global questions about the characteristics required for cross-species infection and whether virus evolution either before or after initial colonization is required for successful invasion of the germline. One approach to examining these questions would be to compare the biological properties of the virus that initiates invasion in a species with the one that emerges as a stable ERV, along with any intermediates that can be found. For most ERVs, the progenitor viruses are lost in evolutionary time and cannot be studied, but this approach may be feasible with the KoRVs, due to the relatively recent colonization.

A starting point for the search for the origin of KoRV is its sequence relationship to GALV [6]. Older, pre-genomic studies indicated that GALV in turn is derived from an endogenous retrovirus of the Asian mouse *Mus caroli* or a related species [9]. Using more modern techniques, the hunt is currently under way for one or more viruses from these mice that are closely related to KoRV, and for mammalian vectors that might have allowed the transmission of a virus from mice in Southeast Asia to koalas in Australia. In another paper published recently on the characterization of the koala retrovirus, Oliveira and colleagues [10] describe adaptive changes in the KoRV envelope gene associated with koala infection, highlighting the need for future functional comparisons between the mouse, gibbon, koala and any intermediate retroviruses in order to identify sequences correlated with exogenous and endogenous growth, and to determine whether adaptation to these alternative lifestyles has taken place. For example, one could speculate that selection for low levels of virus replication, perhaps as a result of a weak promoter, would favor virus persistence in the endogenous state but would be incompatible with the exogenous lifestyle.

Benign passenger or pathogen?

An integrated provirus can have five possible fates [2,11]: it can serve as a source for infectious virus; it can evolve to give rise to a viral genome that amplifies itself solely intracellularly; it can decay into junk DNA; it can undergo recombination between the LTRs to leave a solo LTR; or it might contribute a gene that can have a physiological function in the host [12]. These outcomes range from potentially harmful to beneficial to the host. Most replication-competent ERVs identified to date seem relatively nonpathogenic; a species harboring a lethal virus over an extended period of time would presumably be unlikely to survive unless it developed effective countermeasures to prevent virus replication [2,11]. One such measure would be to alter the normal cellular receptor for the virus in such as way as to prevent virus infection but not to affect the normal function of the cellular protein. This phenomenon is known as xenotropism, and explains why some species have multiple genomic copies of replication competent ERVs that can no longer infect cells from those species [13]. Retroviral evolution may also be influenced by the parallel evolution of antiviral factors such as APOBECs (which mutate or lead to the degradation of the products of reverse transcription) or Trim5 and Fv1, intracellular factors that bind to retroviral capsid protein, interfering with post entry events in the viral life cycle [14].

The size of each group of ERVs can vary significantly, presumably reflecting the ease and extent with which viral amplification took place following the initial germ-cell infection. Differential rates of ERV amplification may reflect the properties of the initial provirus. A virus that has undergone a debilitating mutation just before germ-cell infection is unlikely to give rise to many progeny, and studies of

proviruses artificially introduced into the germline by infection of pre-implantation embryos have shown that a surprisingly high percentage of novel proviruses carry such mutations [15]. Alternatively, an initial burst of amplification may be favored by simultaneous expression of endogenous and exogenous viruses. The koalas that have been examined so far have very high levels of circulating KoRV in the blood (viremia) [7], and it is not at all clear whether this results simply from reactivation of recently acquired germline proviruses or whether the animals have also been infected by exogenously transmitted KoRV. It will be important to determine whether germline KoRVs in viremic koalas are still being amplified from generation to generation, and if so, whether such an increase results from amplification of inherited endogenous provirus or from exogenously acquired virus. Similarly, it will be essential to follow the geographic spread of endogenous KoRVs into new locations and ask whether this is due simply to the interbreeding of infected and uninfected animals or whether there is spread of exogenous virus followed by new germline insertions.

The cross-species spread of retroviruses, generating novel ERVs, can be considered a natural evolutionary force. It remains to be seen whether KoRV will belong to the category of benign viruses or whether its presence will compromise the ability of koalas to survive. KoRV appears to be associated with the fatal lymphomas that kill many captive animals [7]. It may also be immunosuppressive, thereby contributing to the chlamydial infections that afflict many koalas [1,16]. The koala already faces the dual threat of shrinking habitat and inbreeding; will KoRV be one burden too many to bear? If so, should we be interfering, perhaps by vaccination, in an attempt to protect it from extinction? If the virus is spreading by exogenous infection followed by new germline insertions, it could be that an appropriate vaccination strategy might stop its spread. Any intervention may well entail laboratory studies, perhaps involving the deliberate infection of koalas. This would appear to be a case where use of some animals in research might be essential to the survival of their species. Hopefully, such studies will simultaneously prove informative about the elements making up a significant fraction of our genomes.

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