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Molecular Characterization of Koala Retroviruses Isolated from Koalas *(Phascolarctos cinereus)* Reared in Japanese Zoos

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Abstract. In northern Australia most koalas *(Phascolarctos cinereus)* are infected with the gammaretrovirus known as koala retrovirus (KoRV). KoRV is believed to be currently endogenizing into its host. Koalas were first introduced into three Japanese zoos in 1984 and now about 50 koalas are held in eight zoos. In 2007 KoRV was isolated from koalas reared in Japanese zoos, and, for the first time, an infectious molecular clone termed pKoRV522 was constructed. Using the molecular clone and KoRV isolates, we revealed the budding mechanism of KoRV and genomic diversity of KoRVs isolated from Japanese koalas. We found that KoRV utilizes the multivesicular body-sorting pathway. We also discovered a novel KoRV subgroup, named KoRV-J, which utilizes thiamine transport protein 1 as an entry receptor. The original KoRV, which utilizes Pit-1 as an entry receptor, is now named KoRV-A. In two Queensland koalas examined, the copy numbers of KoRV-J was less than 1 copy per cell and varied in tissues. These data, at least in these two koalas, suggest that KoRV-J is an exogenous retrovirus not an endogenous retrovirus.

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Endogenous retroviruses (ERVs), occupy about 8 to 13 percent of mammalian genomes. Most ERVs are defective due to genomic mutations and deletions. However, some ERVs retain functionality and contribute to host physiological processes, exemplified by the human syncytins in placentation (Feschotte & Gilberet, 2012). In this regard, ERVs are believed to play a role in the evolution of mammals, yet the process of endogenization of retroviruses, resulting in the establishment of ERVs, has not been elucidated. The koala retrovirus (KoRV), found in koalas *(Phascolarctos cinereus)*, is a gammaretrovirus which is believed to be currently endogenizing into its host, thus providing us with a rare opportunity to investigate the mechanisms involved in retrovirus endogenization (Stoye, 2006; Tarlinton *et al*., 2006). Genetically and phylogenetically, KoRV is closely related to gibbon ape leukemia virus (GALV) which is an exogenous gammaretrovirus and induces leukemia/lymphoma in gibbons (Delassus *et al*., 1989). In addition, KoRV shares the viral receptor (Pit-1, a phosphate transporter) with GALV when it infects cells (Oliveira *et al*., 2007).

In addition to benefits provided by ERVs, there are also negative consequences of harboring them in the host genome. Indeed, increased levels of KoRV infection in koalas have been associated with several diseases. For instance, koalas suffer from leukemia and lymphoma with a rate of 3–5% in the wild and an even higher rate of up to 60 % in some captive colonies (Canfield *et al*., 1988; Hanger *et al*., 2000). Tarlinton et al. reported that, using quantitative real-time reverse transcriptase (RT)-PCR, KoRV RNA levels in plasma were significantly increased in koalas suffering from leukemia or lymphoma when compared with