Review of PhD thesis by Helena Farkašová

entitled

Host-virus interactions of mammalian endogenous retroviruses

PhD thesis by Helena Farkašová was prepared in the Laboratory of Viral and Cellular Genetics, Institute of Molecular Genetics of the ASCR under supervision of MUDr. Daniel Elleder, PhD. The thesis contributes to understanding of the assorted diversity of interactions between endogenous retroviruses and their hosts.

The thesis is focused on four endogenous retroviruses: (i) an endogenous Lentivirus of Malayan colugo (*Galeopterus variegatus)*; (ii) an endogenous Deltaretrovirus MINERVa of long-fingered (*Miniopteridae)* bats; (iii) an endogenous Gammaretrovirus of mule deer *Odocoileus hemionus*; and (iv) the presumed Gammaretrovirus of Chinese hamster (*Cricetulus griseus)*. The study of endogenous retroviruses from several groups harbored in animals from only remotely related taxa is unified by general interest in phenomenon of endogenization and its mechanism.

Helena Farkašová performed a computational screen of 104 mammalian genomes available, aimed at detecting unusual cases of endogenous retroviruses, including endogenous lentiviruses and deltaviruses. By this approach, she detected a novel endogenous Lentivirus in the Malayan colugo genome, denoting it ELVgv. ELVgv is by far the oldest known Lentivirus, present in more than three copies in the genome of *G. variegatus*. Interestingly, orthologous provirus sequences were detected in the only other extant dermopteran species *Cynocephalus volans.*

In addition to ELVgv, Helena Farkašová discovered an endogenous Deltaretrovirus called MINERVa in the genomes of Miniopteridae bats. This virus, which contained large internal deletion was found in the host genome only in a single copy. Its affiliation to deltaretroviruses is documented by presence of ORFs in similar position as Tax and Rex ORFs in HTLV and phylogenetic relatedness of *gag*.

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Furthermore, endogenization mechanism was studied on example of cervid endogenous gammaretrovirus (CrERV) from mule deer cells. Thousands of insertionally polymorphic CrERV integration sites suggest that CrERV fits in the category of modern ERVs that entered the host genome after speciation. Helena Farkašová showed that replication competent CrERV was induced by cocultivation with susceptible human cells and concluded that the xenotropic nature of this retrovirus is probably caused by a receptor interference and a later lock in deer cells.

Finally, Helena Farkašová investigated infection block to amphotropic viruses in the Chinese hamster CHOK1 cell by the presence of a secreted inhibitor. Analysis of secreted proteins showed that they originate from gammaretrovirus envelopes related to, but not identical, to FeLV. These experiments, apparently in progress, represent the only unpublished part of the thesis.

The thesis is organized to 1. Hypothesis and Aims, 2. Introduction, 3. Materials and Methods, 4. Results and Discussion, 5. Conclusion, 6. Significance of results and Future prospects, 7. Involvement of the student in the publication, 8. References, and 9 Supplement. The supplement contains 4 papers. In 2 of them, Helena Farkašová is the first author (PNAS, IF=9.4, and Virology, IF=3.2), in 2 others, she is the second author (Retrovirology, IF=5.236, and Mol. Biol. Evol., IF=13.649). The thesis successfully synthesizes heterogeneous subjects to unique study on endogenization of retroviruses.

Helena Farkašová could clarify or discuss during defense of her thesis the following points.

1. Is it possible to define on the basis of presented results the conditions necessary and sufficient for endogenization of retroviruses into the host genome? Could be the exogenous retroviruses circulating at present in the human population endogenized?
2. What is the role of host restriction factors on endogenization?
3. Majority of exogenous retrovirus particles in infected organisms are not replication competent. What is the role of defective retroviruses during endogenization process?
4. How old are the oldest endogenous CrERVs?

5. The statement that “The function of dUTPase in lentiviral genome is still not clearly elucidated” (p. 36) might be modulated, see Priet et al., Curr HIV Res 2006, 4, 32.
6. Also the statement that “2.5.3.6. Viperin was not shown to play a role in fighting retroviral infections” (p. 45) could be less categorical (Nasr, N. et al. Blood 20121, 120, 778; Lim, ES, Retrovirology 2012 9 55; Tang, YD. Et al., J Virol 2014, 88, 12296).
7. Abbreviation pSIVgml should be used rigorously (p. 38, pSIV is sometimes confusing).
8. Figures 3, 5, 7, 8 and 28 are partially illegible.

Minor points

1. In the paragraph 2.6.2.1 “Fighting fire with fire” should be mentioned the old concept of “viral interference“
2. Galactoceramide is among important HIV receptors (p. 41)
3. Several sentences throughout the text start with a digit.
4. “because” instead of becuase (p. 48, 3rd line from the bottom)
5. “Bromodeoxyuridine” instead of Bromodeoxouridine (p. 49, the last line)

In conclusion, the thesis of Mgr. Helena Farkašová perfectly fulfills requirements demanded for the level of dissertation work. Helena Farkašová is the first author of two excellent papers, and the second author of two other high quality papers published in highly impacted journals. I heartily recommend submitted work for defense, and depending on the outcome of defense procedure for approval of doctor degree.

Prague, March 30, 2017 RNDr. Ivan Hirsch, CSc