Imaging the assembly process of the Human Endogenous Retrovirus K(HML-2)

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Keywords: Human Endogenous Retrovirus (HERV), group-specific antigen (Gag), live-cell imaging, electron microscopy, correlative microscopy

About 42 % of the human genome consists of retroelements. A large number of these elements are human endogenous retroviruses (HERV). Most of them were once infectious exogenous retroviruses that become endogenous. Endogenization of a retrovirus starts with an infection of a germ line cell followed by the characteristic integration of the viral genome into a chromosome. Such an integrated virus (provirus) is then present in each cell of the offspring and is transmitted from generation to generation. Some of the endogenous retroviruses were co-opted by the host and play important roles in physiologic process, but pathogenic implications, such as tumor induction, have also been reported.

During the course of the evolution most, if not all, known proviruses have suffered from inactivating mutations. However, the youngest and best preserved HERV-K family is still able to express functional proteins and form viral particles. Recently, infectious HERV-K(HML-2) viruses were reconstituted, showing its ability to infect present day mammalian cells. One of the best preserved elements is HERV-K113 on chromosome 19p13.11 which has open reading frames for all viral proteins and forms particles [1, 2].

In order to study the assembly release and maturation of HERV-K113 we have applied various microscopic methods including live-cell imaging, transmission-electron microscopy and scanning-electron microscopy.

We were able to label viral particles by fusing a fluorescent protein to the viral protein Gag. This protein encodes a structural polyprotein which is cleaved following the release of the virus from the cell. The labeling of the Gag protein was also used to investigate its intracellular transport (Fig. 1A and B). Labeled Gag protein moves relatively rapid and accumulates at mobile or immobile patches (Fig. 1B). Applying correlative fluorescence- and scanning-electron microscopy we could demonstrate that these patches most probably correspond to the budding sites of virus-like particles. Transmission-electron microscopy revealed that the labeled Gag protein assembled into tubular structures (Fig. 2A and B). However, the formation of retrovirus-like particles was induced by using a defined ratio of labeled und unlabeled Gag proteins (Fig. 2C and D).

References:

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[2] N. Beimforde, K. Hanke, I. Ammar, R. Kurth, N. Bannert. Virology. 2008 Feb 5;371(1): 216-25.

DOI: 10.3217/978-3-85125-062-6-366,

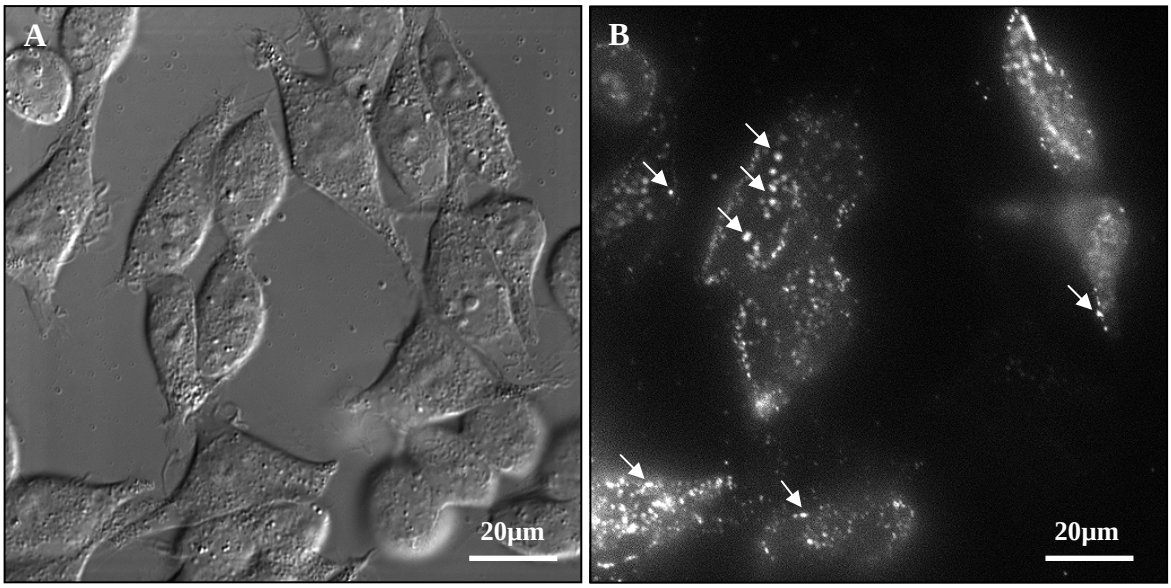

Figure 1. A Image of cultured cells expressing labeled and unlabeled Gag protein at a ratio of 1:10. **B** The fusion protein shows a bright and stable fluorescence which appears diffuse or accumulates (arrows) at the cytoplasm of the cells. (A/B: live-cell imaging)

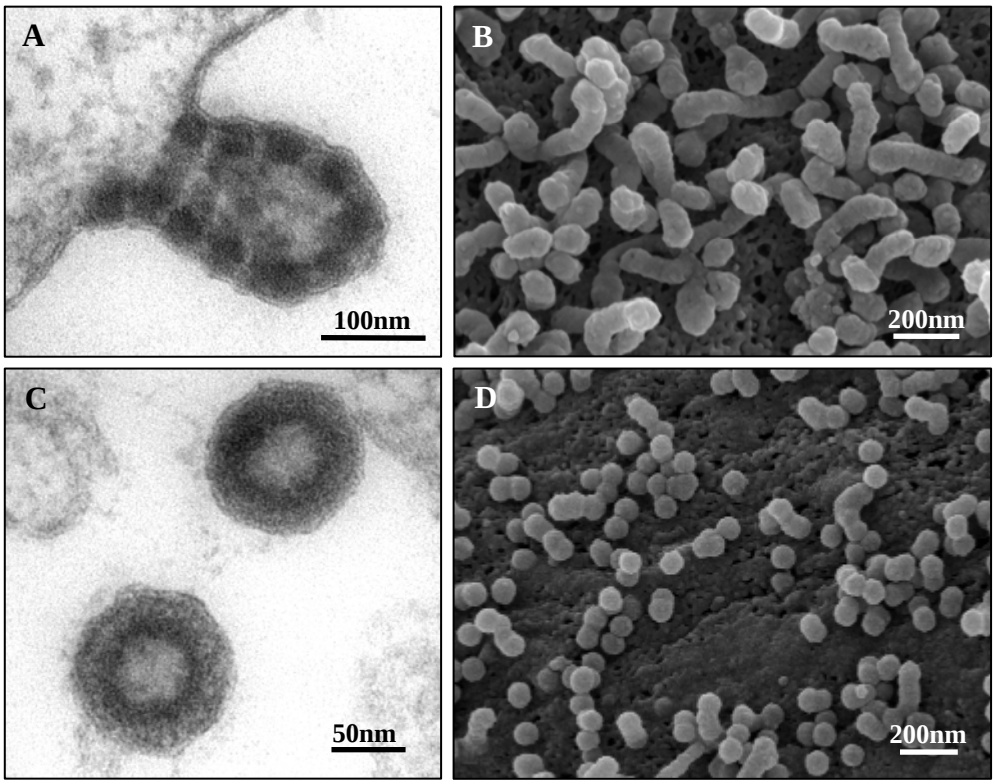

Figure 2. Assembly of Gag proteins. **A** and **B**: Only labeled Gag protein assembled into tubular structures. **C** and **D**: A 1:10 ratio of labeled to unlabeled Gag proteins leads to the formation of virus-like particles at the cell surface. (A/C: transmission-electron microscopy, B/D: scanning-electron microscopy)

M.A. Pabst, G. Zellnig (Eds.): MC2009, Vol. 2: Life Sciences,
DOI: 10.3217/978-3-85125-062-6-366, © Verlag der TU Graz 2009