Special seminar by Dr. Yuki Takamatsu
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Speaker: Dr. Yuki Takamatsu
Institut für Virologie, Philipps Universität Marburg, Germany

Revealing the molecular mechanisms of Ebola virus replication - 1) Nucleocapsid transport machinery, 2) Regulation of VP30 phosphorylation

Abstract
The filoviruses Marburg virus (MARV) and Ebola virus (EBOV) cause severe hemorrhagic fever with high case-fatality rates in humans and nonhuman primates. No approved specific therapy is available and therefore further understanding of the filovirus life cycle is essential for the development of novel therapeutic options. Here, introducing two research topics focusing on the different stages of EBOV life cycle.
EBOV particles contain the non-segmented negative-sense RNA genome and seven viral proteins: NP, VP35, VP40, GP (glycoprotein), VP30, VP24, and the RNA-dependent RNA polymerase (L). Necessary for EBOV transcription and replication are the nucleocapsid proteins NP, VP30, VP35, and L. VP24 is an additional factor required for nucleocapsid (NC) assembly. A layer of the matrix protein VP40 connects the NC with the viral membrane in which GP is inserted.

1) Nucleocapsid transport machinery
Recent live-cell imaging studies of MARV- and EBOV-infected cells revealed a long-distance actin-dependent transport of NCs from viral inclusions to the plasma membrane. In order to identify the essential viral proteins for the intracellular NC transport, we have constructed a set of fluorescently tagged viral proteins (e.g., VP30-GFP, VP35-GFP, VP24-TagRFP) and developed novel systems to visualize transport of NC-like structures (NCLSs) in the background of filovirus-specific virus-like particle systems. We detected that transport of NCLSs was dependent on the polymerization of actin and proceeded with similar speed as NCs in EBOV- or MARV-infected cells. Moreover, using this system, we identified the viral factors essential for the transport of NCLSs. The newly developed non-infectious live-cell imaging system will further contribute to our understanding of molecular interactions between NCs and cellular proteins, and the development of anti-viral drugs.

2) Regulation of VP30 phosphorylation
The phosphorylation of viral proteins is an important regulatory mechanism in certain RNA viruses. EBOV VP30 is phosphorylated predominantly at six N-proximal serine residues (S29-S31, S42, S44, and S46), and is an essential transcriptional factor. Switching of phosphorylation status of VP30 alters polymerase complexes functions between transcription and replication. Nonphosphorylated VP30 promotes genome transcription, whereas phosphorylated VP30 promotes genome replication. Although phosphatases which dephosphorylate VP30 are identified as PP1 and PP2A, the kinases which phosphorylate VP30 has remained elusive for years. To understand a whole story of EBOV transcription/replication, we have focused on kinases and identified the “kinase X” which phosphorylates VP30. Notably, our findings uncover the key player for reversible VP30 phosphorylation, implying new therapeutic approach which focuses on VP30 phosphorylation.