様式（二）

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論文題目　Studies on molecular biological interactions between Akabane virus and host cell
（アカバネウイルスと宿主細胞の分子生物学的相互作用の研究）

Akabane virus (AKAV) is the member of genus Orthobunyavirus, family Bunyaviridae, which is an enveloped tri-segmented negative single strand RNA virus. AKAV can cause reproductive system and nervous system diseases in cattle, sheep, and goat, resulting in significant economic losses in livestock industries. Recently, a large endemic Schmallenberg virus (Akabane-like virus) infection crisis lead to a new concern about etiological importance of this virus group. The new endemic virus suggests relation to climate changes and agricultural developments on the earth. Although bunyaviruses are the largest family of viruses and affect a wide range of hosts (human, animals, and plants), we have not attained detailed knowledge about virus-host cell interactions through virus entry into cells to release from cells. The present study performed molecular biological approaches for this fundamental question on AKAV infection, contributing one to establish control measures against bunyavirus infections including

AKAV .

In Chapter 1, I evaluated AKAV entry mechanisms using chemical inhibitors of different endocytic pathways, and localization assays were performed for a comparative analyses between non-bovine derived cell lines (Vero cells, HmLu-1 cells and BHK cells) and bovine derived cell lines (LB9.K cells and MDBK cells) endocytosis mechanisms during AKAV infection. The results showed that AKAV penetrates into non-bovine derived cell lines with relation to the classical clathrin–dependent endocytic pathway. By contrast, AKAV entry into bovine derived cell lines required lipid-raft and dynamin. However clathrin, caveolae, actin, microtubule and Arf were not required in this entry process. These results suggest that AKAV entry involved in a novel endocytic pathway. In addition, I demonstrated that endosomal low pH environment was required for uncoating process after AKAV uptake into the cell. No difference on AKAV entry mechanism was observed between different pathotyped strains such as OBE-1 (low pathogenicity) and Iriki (high pathogenicity) strains.

In Chapter 2, I showed the role of actin and microtubule of host cell in AKAV entry, internalization trafficking, replication and assembly steps. The LB9.K cells were treated with cytochalasin D (CytD; actin inhibitor), nocodazole (NOC; microtubule inhibitor), and Brefeldin A (BFA; *GTPases* of the ADP-ribosylation factor (Arf) family inhibitor and ER-Golgi transport protein inhibitor) at various time points upon AKAV infection. The results showed that neither actin nor microtubule was required for AKAV entry and trafficking to replication compartment. However, I observed that both microtubule and ER-Golgi transport protein were essential for AKAV replication and assembly. Immunofluorescense antibody (FA) microscopic analyses revealed displaced AKAV N protein from perinuclear region in NOC- and BFA-treated cells,

indicating that AKAV N transport was inhibited. Also, NOC and BFA treatment affected Golgi compartment position as well. These results demonstrated an essential role of microtubule during AKAV replication and assembly in LB9.K cells.

In Chapter 3, I described the binding property between a regulatory factor of mitochondrial transport, trafficking kinesin-binding protein 2 (TRAK2), and AKAV NSm, which is a viral nonstructural protein encoded by M segment, as revealed by yeast two-hybrid screening assay. In this study, I showed interactions between bTRAK2 and NSm by GST-tag and FLAG-tag pull down assays. Mapping of the binding domain showed that the AKAV NSm interacts with C-terminal domain of bTRAK2, giving a new insight into NSm function of orthobunyavirus replication and assembly events. FA analysis revealed co-localization of bTRAK2 and NSm interaction in perinuclear area where AKAV virions are assembled.

In Chapter 4, I described amino acid difference of the AKAV NSs, which is a viral S segment-encoded nonstructural protein, between OBE-1 and Iriki strains leading to different interferon (IFN) antagonism. The previous study has shown that the NSs of low pathogenic OBE-1 strain rather suppressed IFN action more than 80 times as compared with the NSs of high pathogenic Iriki strain. It is unknown that this functional difference between the NSs may relate with AKAV pathogenicity. Amino acid sequence alignment revealed different residues at three positions of the NSs between OBE-1 and Iriki strains (T for OBE-1 and I for Iriki (T/I) at position 73; F/S at position 85, and T/I at position 86). To determine which residues are responsible for alteration of IFN antagonistic properties between two AKAV strains, I first produced a reassortant AKAV virus (named rIIO), in which L and M segments are derived from Iriki strain and S segment are from OBE-1 strain, by our reverse genetic system with T7 RNA

polymerase. I further generated a series of (a total of 6) NSs mutants with single or double amino acid substitutions in the rIIO virus, and compared their growth kinetics, plaque sizes, and IFN-inducible Mx1 RNA expression in LB9.K cells. All of these mutant viruses showed no difference between plaque sizes, but grew more slowly than wild-type (wt-)Iriki virus. qRT-PCR analysis indicated that a mutant virus with amino acid substitution from threonine to isoleucine at position 73 of the NSs stimulated the host Mx1 mRNA level similar to wt-Iriki, indicating that amino acid at this position of the NSs plays a role in different antagonistic properties between OBE-1 and Iriki strains.

In this study, I described the multiple AKAV entry mechanisms and the essential role of microtubule and ER-Golgi transport protein during AKAV replication and assembly, providing to better understand host cellular responses during virus infection and leading to design antiviral drugs targeted for these unique mechanisms in the future. Study of interaction between TRAK2 and NSm might be a first step to enlightenment further study in relation of mitochondria and AKAV infection. Moreover, it is now clear that one amino acid mutation of the NSs leads to show different host interferon response between OBE-1 and Iriki strains. Together, our findings for molecular dissection on AKAV infection might be able to contribute to understand AKAV different pathogenicities between 2 strains as well as the new emerging AKAV-like virus, hopefully leading to establish control measures against bunyavirus infections.