（２）論文内容要旨ファイルデータ

論 文 の 内 容 の 要 旨

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論文題目：Studies on the molecular biological interactions between bovine viral diarrhea virus nonstructural protein 4A and host cell
（牛ウイルス性下痢ウイルス非構造蛋白質 4A の宿主細胞における分子生物学的相互作用の研究）

Bovine viral diarrhea virus (BVDV) is an economically important, major reproductive pathogen of cattle. Due to the action of the virus in cell culture, a non-cytopathic (ncp) and cytopathic (cp) biotypes can be distinguished. *In vivo*, the two virus biotypes interact to cause the highly fatal mucosal disease in animals that are persistently infected with ncp BVDV. *In vitro*, ncp BVDV has no immediate effect on infected cells, while cp BVDV induces vaculation and cell death. This cell death is mediated by induction of apoptosis. The mechanisms that induce cp BVDV-mediated apoptosis are yet to be clarified. ncp BVDV encodes an active block to type I　interferon (IFN) induction with more than one mechanism to bring about this block. The ability of ncp BVDV to block IFN is critical to establish persistent infection. BVDV is regarded a surrogate model of its relative hepatitis C virus as they share similarities in virological and molecular properties.

BVDV NS4A is a 64-residue protein of about 7-kD similar in size, composition, and hydropathic properties to the NS4A protein of the hepaciviruses. It acts as an essential cofactor of the NS3 serine protease and has a role in viral replication as a part of the viral

replicase complex along with the other nonstructural proteins. Although HCV NS4A has been shown to interact with some host cell signaling pathways, nothing is known about BVDV NS4A interactions with host cell factors. In this thesis the author attempted to study molecular interactions between host-cell and BVDV NS4A.

In order to identify possible interacting partners with NS4A, the author performed a yeast two-hybrid screening and identified two RNA helicase proteins to be interacting partners of NS4A, retinoic acid inducible gene I (RIG-I) and melanoma differentiation associated gene 5 (MDA5). RIG-I and MDA5 are important proteins as they serve as cytoplasmic receptors for viral RNA leading to induction of type I interferons (IFN-α/β). The binding between the two helicases and NS4A was confirmed *in vitro* and *in vivo* in the context of infection with both BVDV biotypes, cp and ncp. The author showed the significance of NS4A interaction with MDA5 and RIG-I. The NS4A binding domains on MDA5 and RIG-I were identified to be helicase domain and C-terminal domain (CTD). Helicase and CTD of MDA5 and RIG-I are responsible of dsRNA binding. Upon binding with dsRNA, MDA5 and RIG-I go through conformational change to release their N-terminal caspase activation and recruitment domains (CARDs) initiating a signaling cascade which leads to IFN induction. Many viruses have been reported to interact with MDA5 and/or RIG-I in order to suppress IFN production. Here, gene reporter assay showed that NS4A has inhibitory effect on MDA5- and RIG-I-mediated activation of IFN-β promoter through its N-terminal domain. This inhibition suggests a role for NS4A in viral strategies to evade IFN response.

"RNA editing" is a biological process in which dsRNA undergoes adenosine (A) to inosine (I) editing through enzymes called adenosine deaminase act on RNA (ADARs). It has been suggested that ADARs play a role in antiviral pathways against various viruses. The author identified ADAR to be binding partner of NS4A and this binding was observed *in vitro* and *in vivo*. According to data from yeast two-hybrid screening, the NS4A binding domain on ADAR seems to be dsRNA binding motif which binds dsRNA to catalyze its hyper-editing. There is possibility that NS4A interferes with ADAR ability to bind dsRNA in order to prevent the action of this protein then prevents elimination of the virus. The ADAR binding domain on NS4A was identified as N-terminal domain.

Collectively, the data in this thesis suggest an important role of NS4A, beside its role in viral replication as a member of the replicase complex, as a player in the game between the virus and the host-cell machinery. The nature of the cellular proteins identified as binding partners of NS4A suggests that NS4A may have affinity to proteins with dsRNA binding capacity which may raise a new feature of NS4A. Being the interacting domain, N-terminal domain of NS4A seems to be important in NS4A interactions with RNA healicases and ADAR. Being the first evidence for BVDV NS4A interaction with some cellular proteins, these results may give new insights into studying the mechanisms by which BVDV can evade host immune system and establish persistent infections in its host.