International Research Center for Infectious Diseases

Department of Infectious Disease Control
感染制御系

Professor	Yasushi Kawaguchi, D.V.M., Ph.D.	教　授	博士(獣医学)	川　口　　寧
Assistant Professor	Akihisa Kato, Ph.D.	助　教	博士(医学)	加　藤　哲　久
Assistant Professor	Jun Arii, Ph.D.	助　教	博士(獣医学)	有　井　　潤
Assistant Professor	Naoto Koyanagi, Ph.D.	助　教	博士(生命科学)	小　柳　直　人

Our special interest is focused upon searching for effective methods to protect or control viral infection by using accumulated knowledge based on molecular pathogenicity, and developing novel anti-viral drugs and attenuated strains for novel vaccines. The works have been conducted by close collaboration with Division of Molecular Virology, Department of Microbiology and Immunology.

1. Roles of the Interhexamer Contact Site for Hexagonal Lattice Formation of the Herpes Simplex Virus 1 Nuclear Egress Complex in Viral Primary Envelopment and Replication

Jun Arii, Kosuke Takeshima, Yuhei Maruzuru, Naoto Koyanagi, Akihisa Kato, Yasushi Kawaguchi

The scaffolding proteins of several envelope viruses required for virion assembly form high-order lattice structures. However, information on the significance of their lattice formation in infected cells is limited. Herpesviruses acquire envelopes twice during their viral replication. The first envelop acquisition (primary envelopment) is one of the steps in the vesicle-mediated nucleocytoplasmic transport of nascent nucleocapsids, which is unique in biology. HSV-1 NEC, thought to be conserved in all members of the Herpesviridae family, is critical for primary envelopment and was shown to form a hexagonal lattice structure. Here, we investigated the significance of the interhexamer contact site for hexagonal lattice formation of the NEC in HSV-1-infected cells and present evidence suggesting that the lattice formation of the NEC hexamer has an important role in HSV-1 replication by regulating primary envelopment. Our results provide insights into the mechanisms of the envelopment of herpesviruses and other envelope viruses.

During the nuclear export of nascent nucleocapsids of herpes simplex virus 1 (HSV-1), the nucleocapsids acquire a primary envelope by budding through the inner nuclear membrane into the perinuclear space between the inner and outer nuclear membranes. This unique budding process, termed primary envelopment, is initiated by the nuclear egress complex (NEC), composed of the HSV-1 UL31 and UL34 proteins. Earlier biochemical approaches have shown that the NEC has an intrinsic ability to vesiculate membranes through the formation of a hexagonal lattice structure. The significance of intrahexamer interactions of the NEC in the primary envelopment of HSV-1-infected cells has been reported. In contrast, the contribution of lattice formation of the NEC hexamer to primary envelopment in HSV-1-infected cells remains to be elucidated. Therefore, we constructed and characterized a recombinant HSV-1 strain carrying an amino acid substitution in a UL31 residue that is an interhexamer contact site for the lattice formation of the NEC hexamer. This mutation was reported to destabilize the interhexamer interactions of the HSV-1 NEC. Here, we demonstrate that the mutation

causes the aberrant accumulation of nucleocapsids in the nucleus and reduces viral replication in Vero and HeLa cells. Thus, the ability of HSV-1 to form the hexagonal lattice structure of the NEC was linked to an increase in primary envelopment and viral replication. Our results suggest that the lattice formation of the NEC hexamer has an important role in HSV-1 replication by regulating primary envelopment.

2. Identification of the Capsid Binding Site in the Herpes Simplex Virus 1 Nuclear Egress Complex and Its Role in Viral Primary Envelopment and Replication

Kosuke Takeshima, Jun Arii, Yuhei Maruzuru, Naoto Koyanagi, Akihisa Kato and Yasusi Kawaguchi

Binding of HSV-1 NEC to nucleocapsids has been thought to promote nucleocapsid budding at the inner nuclear membrane and subsequent incorporation of nucleocapsids into vesicles during nuclear egress of nucleocapsids. However, data to directly support this hypothesis have not been reported thus far. In this study, we have present data showing that two amino acids in the membrane-distal face of the HSV-1 NEC, which contains the putative capsid binding site based on the solved NEC structure, were in fact required for efficient NEC binding to nucleocapsids and for efficient incorporation of nucleocapsids into vesicles during primary envelopment. This is the first report showing direct linkage between NEC binding to nucleocapsids and an increase in nucleocapsid incorporation into vesicles during herpesvirus primary envelopment.

During nuclear egress of nascent progeny herpesvirus nucleocapsids, the nucleocapsids acquire a primary envelope by budding through the inner nuclear membrane of infected cells into the perinuclear space between the inner and outer nuclear membranes. Herpes simplex virus 1 (HSV-1) UL34 and UL31 proteins form a nuclear egress complex (NEC) and play critical roles in this budding process, designated primary envelopment. To clarify the role of NEC binding to progeny nucleocapsids in HSV-1 primary envelopment, we established an assay system for HSV-1 NEC binding to nucleocapsids and capsid proteins in vitro Using this assay system, we showed that HSV-1 NEC bound to nucleocapsids and to capsid protein UL25 but not to the other capsid proteins tested (i.e., VP5, VP23, and UL17) and that HSV-1 NEC binding of nucleocapsids was mediated by the interaction of NEC with UL25. UL31 residues arginine-281 (R281) and aspartic acid-282 (D282) were required for efficient NEC binding to nucleocapsids and UL25. We also showed that alanine substitution of UL31 R281 and D282 reduced HSV-1 replication, caused aberrant accumulation of capsids in the nucleus, and induced an accumulation of empty vesicles that were similar in size and morphology to primary envelopes in the perinuclear space. These results suggested that NEC binding via UL31 R281 and D282 to nucleocapsids, and probably to UL25 in the nucleocapsids, has an important role in HSV-1 replication by promoting the incorporation of nucleocapsids into vesicles during primary envelopment.

Publications

Takeshima, K., Arii, J., Maruzuru, Y., Koyanagi, N., Kato, A., and Kawaguchi, Y. Identification of the Capsid Binding Site in the Herpes Simplex Virus 1 Nuclear Egress Complex and Its Role in Viral Primary Envelopment and Replication. J. Virol. 93: e01290-19, 2019.

Arii, J., Takeshima, K., Maruzuru, Y., Koyanagi, N., Kato, A., Kawaguchi, Y. Roles of the Interhexamer Contact Site for Hexagonal Lattice Formation of Herpes Simplex Virus 1 Nuclear Egress Complex in Viral Primary Envelopment and Replication. J. Virol. 93: e00498-19, 2019.

Joo, S., Suwanto, A., Sato, A., Nakahashi-Ouchida, R., Mori, H., Uchida, Y., Sato, S., Kurashima, Y., Yuki, Y., Fujihashi, K., Kawaguchi, Y. & Kiyono, H. A role for the CCR5–CCL5 interaction in the preferential migration of HSV-2-specific effector cells to the vaginal mucosa upon nasal immunization. Mucosal Immunol. 12: 1391-1403, 2019.

Watanabe, T., Suzuki, N., Tomonaga, K., Sawa, H., Matsuura, Y., Kawaguchi, Y., Takahashi, H., Nagasaki, K., Kawaoka, Y. Neo-virology: The raison d'etre of viruses. Virus Res. 274: 197751, 2019.