(Form 1)

Kyoto University	Doctor of Philosophy in Life Sciences	Name	Garcia Bea Clarise Baluyot
Thesis Title	BUD23-TRMT112 mediates the chromosomal tethering of Borna disease virus and catalyzes the internal m^{7}G methylation in viral RNA		

(Thesis Summary)

Borna disease virus 1 (BoDV-1) is a nonsegmented negative-strand RNA virus belonging to the order *Mononegavirales*. Unique among RNA viruses, BoDV-1 replicates in the cell nucleus and establishes a life-long persistent infection by forming the viral replication center, named as viral speckles of transcripts (vSPOTs), which consists of viral ribonucleoproteins (vRNPs). Previous studies revealed that BoDV-1 tethers vRNPs onto the host chromosomes during mitosis to safely maintain its intranuclear persistency. Although the mechanism regarding how BoDV-1 regulates chromosomal tethering of vRNPs has not been unclear, it is considered that some host proteins, such as HMGB1, are involved in this process. Therefore, understanding the host-viral protein interactions may provide a better insight in the nuclear replication strategy of BoDV-1.

In this study, the applicant tried to identify the host proteins interacting with BoDV-1 large (L) protein, a key component of vRNPs encoding viral RNA-dependent RNA polymerase, by using proximity-dependent biotinylation. The applicant found 77 host proteins, and among them, 59 show the nuclear localization. Among the host proteins, the applicant focused on TRMT112, a partner of methyltransferases (MTases), and investigated its putative role in BoDV-1 infection. It was demonstrated that TRMT112 binds with BoDV-1 L at the RNA-dependent RNA polymerase domain, together with BUD23, an 18S rRNA MTase and 40S ribosomal maturation factor. BUD23-TRMT112 was also observed to associate peripherally with the vSPOTs in the nucleus.

The applicant showed that knockdown of BUD23-TRMT112 could not affect the replication, as well as mRNA expression, of BoDV-1 in cultured cells. On the other hand, the knockdown experiments revealed that BUD23-TRMT112 mediate the tethering of BoDV-1 vRNPs to chromosomes and the MTase activity of BUD23 is essential for the tethering of BoDV-1 vRNPs to chromosomes. Furthermore, the applicant showed the possibility that BUD23 can catalyze the internal m^{7}G methylation in BoDV-1 genome RNA.

This study demonstrated, for the first time, BUD23-TRMT112, host factors involved in the RNA methylation, play an important role in BoDV-1 nuclear infection. The findings in this study provide novel insights into the importance of viral genome RNA modification in the RNA virus infection.

(Form 2)

(Thesis Evaluation Summary)

In this thesis, the applicant has attempted to elucidate the mechanism by which BoDV-1 maintains persistent infection in the nucleus by identifying the host factors that interact with the BoDV-1 L polymerase protein. In previous studies, it has been shown that the interaction between the viral phosphoprotein and HMGB1, a host DNA-binding protein, is important for the intranuclear persistence of BoDV-1 and that the knockdown of HMGB1 in BoDV-1-infected cells disrupts the tethering of BoDV-1 vRNPs to chromosomes, resulting in a gradual breakdown of the persistent infection. However, other host factors involved in the BoDV-1 persistent infection have not yet been identified. In this study, the applicant has demonstrated that the BoDV-1 L binds to TRMT112, a cofactor of several host methyltransferases (MTase). This is the first report of identification of host factor that binds to the BoDV-1 L. The applicant also found that BUD23, a MTase and the binding partner of TRMT112, binds the BoDV-1 L through its RNA-dependent RNA polymerase (RdRp) domain. This result supported the hypothesis that the BoDV-1 L, which does not possess the MTase activity, utilizes the host MTase to complete the mRNA cap structure. To confirm this hypothesis, the applicant performed the knockdown analysis. However, the knockdown of BUD23-TRMT112 did not significantly affect the replication of BoDV-1, indicating that the involvement of BUD23-TRMT112 in the BoDV-1 mRNA maturation may be limited. By more detailed examinations of the dynamics of BoDV-1 in the cells lacking BUD23-TRMT112, the applicant found that the BUD23-TRMT112 is involved in the tethering of BoDV-1 vRNPs to host chromosomes. This finding was significant because this is an unexpected finding that the host MTase links to the persistent infection of BoDV-1. Furthermore, the applicant succeeded to show that the genomic RNA of BoDV-1 may be catalyzed the m^7G methylation by BUD23. In summary, this paper is significant to show that host MTase interacts with the RdRp of RNA virus and affects the intracellular dynamics of viral genomic RNA. The applicant has also succeeded in proposing an important hypothesis on the significance of genomic RNA methylation in the maintenance of RNA virus infection. These findings may be useful for the development of RNA virus replication inhibitors.

This thesis substantiates the candidate's extensive and wide knowledge of life sciences, demonstrates expert research capability in the field of virology, and presents new discoveries and concepts that contribute to the profound understanding and further development of the candidate's research field. Moreover, the thesis is written logically and coherently, which satisfies the degree requirement that the thesis shall serve as a valuable document for future reference. On August 10th, 2021, the PhD thesis oral examination was held. Pursuant to this oral examination, the thesis examination committee hereby concludes that the candidate has passed all of the requirements for the degree of Doctor of Philosophy in Life Sciences.

The thesis, thesis summary, and thesis evaluation summary will be published through the Kyoto University Research Information Repository. If the thesis cannot be published on the website immediately after the degree is awarded, due to patent application, journal publication constraints, or other reasons, please indicate the earliest date that the thesis can be published. (Please note, however, based on Article 8 of the Degree Regulations, that the thesis must be published within three months of the date that the degree is awarded.)

Thesis publication date :