(Form 1)

Kyoto University	Doctor of Philosophy in Life Sciences	Name	WILAIPORN SAIKURUANG
Thesis Title	Defining the molecular role of RNA helicase DDX3 in antiviral signaling pathways		

(Thesis Summary)

DDX3 is one of the members of DEAD-box RNA helicase family. It is a multifunctional helicase related to RNA metabolism. Previous studies reported that DDX3 accelerates production of an antiviral cytokine, type I interferon (IFN-I), whose expression is induced by viral infection. Viral double-stranded RNA (dsRNA) is sensed by RIG-I-like receptor (also belong to DEAD-box RNA helicase family) and activates the innate immune signaling through several adaptor molecules, followed by activation of the transcription factor IRF-3, which directly induces IFN-I gene expression. Previous studies suggested that DDX3 facilitates recognition of viral dsRNA and enhances the signaling cascade. In this study, the applicant analyzed the underlying mechanism of IFN-I gene expression, enhanced by DDX3. The applicant generated DDX3 knockout cell lines to examine the roles of DDX3 on innate immune signaling, introduced DDX3 mutants into the cells to clarify the critical amino acid residues conferring to the innate immune responses, and performed biochemical analyzes such as immunoblotting and co-immunoprecipitation to reveal the mechanisms of DDX3-mediated enhancement of IFN-I gene expression. These results clearly showed that DDX3 does not influence on recognition of viral dsRNA nor the cytoplasmic signaling cascade, not as previously reported. Importantly, this study revealed that DDX3 participates in the transcriptional activation by interacting with IRF-3 and that DDX3 is a critical component for the transcriptional complex to associate with the IFN-I gene promoter. It is unique that the DEAD-box RNA helicase regulates transcription by forming a protein complex. Thus, this study adds new insight into our understanding of the mechanism of antiviral innate immunity.

(Form 2)

(Thesis Evaluation Summary)

DDX3 is a DEAD-box RNA helicase and plays multiple roles in RNA metabolism. It has been reported that DDX3 plays a positive regulatory role in the production of type I interferon (IFN-I) in virus-infected cells. Several groups reported that DDX3 facilitates viral dsRNA sensing and subsequent signal transduction. Viral dsRNA, either as incoming viral genome or intermediates of viral replication, is sensed by RIG-I-like receptor and activates several adaptor molecules including MAVS, leading to the activation of protein kinases, TBK1 and IKKε, which catalyze activation of the transcription factor IRF-3 by its phosphorylation. It was well established that phosphorylated IRF-3 forms homodimer and translocates into the nucleus to form a complex with p300 and/or CBP (holocomplex). The holocomplex exhibits binding activity to IRF binding motifs (core sequence: AAGTGA) in the IFN-I promoter. The applicant first intended to verify previous studies. The effect of DDX3 in IFN-I induction was examined using wild-type and DDX3 KO cell lines. DDX3 KO cells produced diminished levels of IFN-I, confirming that DDX3 is critical in the IFN-I induction. However, IRF-3 activation was independent of DDX3 as judged by phosphorylation and dimer formation, and nuclear translocation. The applicant finally delineated that the last step to the IFN gene transcription: the formation of transcription factor complex with IRF motif-binding activity, was dependent on DDX3. Further, the ATP binding motif of DDX3 was essential for the IFN-I induction, suggesting ATP hydrolysis by DDX3 is involved in the transcriptional activation. These findings are novel and further advance our understanding of the molecular events leading to the IFN-I induction upon viral infection.

This thesis substantiates the candidate's extensive and wide knowledge of life sciences, demonstrates expert research capability in the field of immunology, 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 April 4th, 2022, 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.

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Thesis publication date :