(Form 1)

Kyoto University	Doctor of Philosophy in Life Sciences	Name	Duic Ivana
Thesis Title	Molecular mechanism of viral RNA recognition and MDA5 activation through LGP2		

(Thesis Summary)

RIG-I, MDA5 and LGP2 belong to a family of RNA helicases (collectively termed as RIG-I-Like Receptor, RLR) functioning as a critical sensor for invading and replicating viral RNA. Upon sensing viral double stranded (ds)RNA, RLR triggers antiviral responses and promotes later acquired immunity against viruses. The central helicase domain is conserved in RLR and exhibits ATPase activity upon RNA binding. Genetic deletion analyses revealed that RIG-I and MDA5 sense short (<1,000 bp) and long (>1,000 bp) viral dsRNA, respectively. RIG-I and MDA5 share a N-terminal domain (Caspase Activation and Recruitment Domain, CARD), acting as a molecular switch for signaling. Crystal structure and molecular biological analyses suggest that CARD is masked in inactive state but exposed upon dsRNA detection. Although LGP2 lacks CARD, knockout studies suggest that it participates in the positive regulation by RIG-I and MDA5.

The applicant aimed to delineate the mechanism underlying viral dsRNA sensing by MDA5 and its cooperation with LGP2. MDA5 bound long dsRNA by forming a fiber like polymer along the length of dsRNA by atomic force microscope observation as reported previously by electron microscopic observation. In the presence of LGP2, the fiber formation was highly accelerated. By using molecular tag for LGP2, the applicant revealed that single LGP2 molecule was incorporated in every 5-9 MDA5 molecules in the polymer. Interestingly, the fiber dissociated upon addition of ATP. To analyze fiber formation and its dissociation, the applicant fractionated MDA5 fiber by size exclusion chromatography and revealed that the fiber dissociation was enhanced in the presence of both LGP2 and ATP. Next, to analyze CARD exposure in the fiber and the dissociated MDA5, the samples were subjected to limited trypsin digestion followed by SDS-PAGE analysis. By analyzing the digestion products, it was revealed that CARD of MDA5 was exposed upon dsRNA binding and the exposure was notably enhanced in the presence of LGP2. Furthermore, the dissociated MDA5/LGP2 complex by ATP hydrolysis was isolated and analyzed for CARD exposure. The result strongly suggested that MDA5 kept exposed CARD after dissociation from dsRNA, however CARD of non-activated MDA5 was masked. In summary, LGP2 is MDA5 nucleator, resulting in rapid viral RNA recognition by fiber formation. LGP2 is incorporated into the MDA5-RNA fiber. LGP2 enhances MDA5 conformational changes, promoting the exposure of CARD and dissociation through ATP hydrolysis. The conformational changes are preserved after dissociation from dsRNA and mediate efficient signal transduction in the cytoplasm.

(Form 2)

(Thesis Evaluation Summary)

The applicant aimed to delineate the mechanism underlying viral dsRNA sensing by MDA5 and its cooperation with LGP2. First, the applicant learned AFM technique to visualize the complex of MDA5 and dsRNA at molecular level and confirmed that MDA5 forms fiber-like polymer along dsRNA. It was revealed that LGP2 facilitated the fiber formation and in the presence of ATP, induced dissociation of MDA5 from dsRNA in an ATP hydrolysis-dependent manner. Next, a sophisticated Q-dot technology was developed to localize LGP2 in the fiber and it was revealed that LGP2 was periodically incorporated into the MDA5 fiber. To analyze dissociation of the fiber through ATP hydrolysis, size exclusion chromatography was used to isolate MDA5 fiber and demonstrated that addition of LGP2 and ATP promoted the fiber dissociation. Finally, the applicant determined limited trypsin digestion conditions to analyze conformational change of CARD. Trypsin digestion revealed that CARD was masked in naive MDA5 but MDA5 in the fiber exposes CARD, demonstrating that dsRNA binding confers active conformation of MDA5. Interestingly, even after release from dsRNA through ATP hydrolysis, MDA5 keeps CARD exposed, presumably as oligomers with LGP2. This observation presented a novel sequential signaling model: first, MDA5 conforms active conformation upon viral dsRNA sensing; second, MDA5 was released by ATP hydrolysis and transmit signaling to downstream adaptor molecule by diffusion in the cytoplasm.

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 February 8th, 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 :