

氏 名	HU YIFAN		
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学位論文題目	Study on functional structures of influenza viral polymerase and NP-RNA complex (インフルエンザウイルス RNA ポリメラーゼ及び NP-RNA 複合体の機能構造の研究)		
主 査	筑波大学講師	博士（理学）	小林麻己人
副 査	筑波大学准教授	博士（医学）	齋藤 慎二
副 査	筑波大学准教授	博士（医学）	福田 綾
副 査	筑波大学准教授	博士（農学）	中川 嘉

論文の内容の要旨 Abstract of thesis

（目的 Purpose）

The purpose of this study is to understand the functional structures for viral polymerase and its template nucleoprotein-viral RNA (NP-vRNA) complex. Since the dynamics of viral polymerase along with vRNA synthesis reaction is still unclear, the applicant aimed to determine the conformational change of viral polymerase from initiation state to elongation state with capped mRNA using human influenza A viral polymerase by electron microscopy (EM) structure analysis. In addition, it has been reported that a cellular splicing factor UAP56 provides functional NP-vRNA complex to activate the vRNA synthesis. However, the molecular mechanism of the transfer of NP to vRNA by UAP56 is still unknown. Thus, the applicant aimed to clarify how UAP56 mediates NP-vRNA complex formation through binding with NP.

（対象と方法 Materials and methods）

Human influenza A viral polymerase was purified from virions through column chromatography techniques. Three-dimensional structures of viral polymerase were determined by negative-stained EM and two dimensional hybrid (2D hybrid) analysis using the purified viral polymerase. The recombinant UAP56, wild type NP, and R416 monomeric NP mutant were purified to examine the assembly of functional NP-vRNA complex by gel filtration analysis, atomic force microscopy (AFM) analysis, and electrophoretic mobility shift assay (EMSA).

(結果 Results)

Viral polymerase with high purity and high enzymatic activity was purified from virions. Then, the EM structures of viral polymerases in two different states (transcription initiation state and RNA cleavage state) were determined by 2D hybrid analysis. It was found that the 424-loop was exposed to the outside of viral polymerase at initiation state, and then the 424-loop turned inside to viral polymerase and faced to PA endonuclease domain by the addition of capped RNA, suggesting that the conformational changes may be important for cap cleavage. By deleting 5 amino acid residues of the 424-loop, the cap cleavage activity of the mutant viral polymerase was significantly reduced. UV crosslinking assay showed that the 424-loop recruits RNA chains of capped RNA to PA endonuclease site from non-specific RNA-binding site of viral polymerase. In addition, gel filtration experiments showed that the molecular size of UAP56-NP complex is more than 440 kDa, suggesting that the complex is composed of 6 molecules of NP and 2 molecules of UAP56. By AFM analysis of NP-UAP56, the applicant found a dumbbell-shaped complex which is considered as two trimeric NP connected by UAP56 dimer. The applicant also found that UAP56 stimulates trimeric NP formation from monomeric NP even at physiological salt concentrations. EMSA demonstrated that UAP56 suppresses the loading of excess amount of NP on vRNA.

(考察 Discussion)

The amino acid residues of the 424-loop is highly hydrophilic and contains positively charged residues. The theoretical isoelectric point of the 424-loop is 11.70, indicating that the 424-loop may bind to negatively charged phosphate backbone of capped RNA. In addition, PB2 627-domain and PA endonuclease domain were reported to bind to RNA. Based on the structural information and biochemical experiments, the applicant proposed the hypothetical model as follows: the positively charged 627-domain and the 424-loop bind to the RNA chains of capped RNA and recruit capped RNA to the cap-binding domain. After interaction of capped RNA with PB2, the 424-loop turns inside to face to PA endonuclease domain and functions as a guide for capped RNA, the capped RNA is therefore released from the 627-domain. At the RNA cleavage state, the cap-binding site faces the endonuclease active site across a solvent channel at a distance of about 40-50 Å. Thus, it is possible that PA endonuclease domain cleaves at 10- to 15-nt downstream from cap1 structure.

vRNA binds to NP to form functional NP-vRNA complex as a template, which stimulated by the molecular chaperones for NP. The applicant proposed a more detailed model about this process. In the absence of UAP56, monomeric NP first binds to vRNA, and the additional monomers randomly bind to the monomeric NP on NP-vRNA complex. In contrast, UAP56 facilitates trimeric NP formation from monomeric NP, thereby UAP56 facilitates the transfer of NP to vRNA. Further, UAP56 represses the binding of excess amount of NP to vRNA possibly by transferring trimeric NP.

(結論 Conclusion)

In the RNA cleavage state, the 424-loop in PB2 cap-binding domain of viral polymerase functions as a guide of capped RNA to PA endonuclease domain and it inhibits non-specific binding of capped RNA to other domains. Moreover, vRNA synthesis is enhanced by the formation of functional NP-vRNA complex, because UAP56 stimulates the RNP formation through the assembly of trimeric NP and controls the amount of NP on vRNA for the structural integrity of the NP-vRNA complex for efficient vRNA synthesis.

審査の結果の要旨 Abstract of assessment result

(批評 General Comments)

In this doctoral thesis, Mr. Hu reported the discovery of the 424-loop in influenza viral polymerase PB2 subunit, which functions as a guide of capped RNA to the endonuclease domain of PA subunit. In addition, the applicant demonstrated that the splicing factor UAP56 stimulates trimeric NP formation for the assembly of functional viral ribonucleoprotein complex. This study provides important advances in understanding the structure–function relationships of influenza viral polymerase and NP–RNA complex.

(最終試験の結果 Assessment)

The final examination committee conducted a meeting as a final examination on October 24, 2017. The applicant provided an overview of dissertation, addressed questions and comments raised during Q&A session. All of the committee members reached a final decision that the applicant has passed the final examination.

(結論 Conclusion)

Therefore, the final examination committee approved that the applicant is qualified to be awarded Doctor of Philosophy in Medical Sciences.