Study on functional structures of influenza viral polymerase and NP-RNA complex

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論　文　概　要

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Purpose: The purpose of this study is to understand the functional structures for viral polymerase and its template nucleoprotein-vrial RNA (NP-vRNA) complex. Although the crystal structures of viral polymerase and NP were determined, the dynamics of viral polymerase along with vRNA synthesis reaction is still unclear. Thus, I first aimed to determine the conformational change of viral polymerase from initiation state to elongation state with capped mRNA using human influenza A virus 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, I aimed to clarify how UAP56 mediates NP-vRNA complex formation through binding with NP.

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).

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, I found a dumbbell-shaped complex which is considered as two trimeric NP connected by UAP56 dimer. I 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.

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, we propose the hypothetical model as follows; the positively charged 627-domain and the 424-loop binds 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, therefore, the capped RNA is 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. We propose 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.

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 formation of functional NP-vRNA complex, this is due to that UAP56 stimulates the RNP formation through the assembly of trimeric NP and controls the amount of NP on vRNA for the

structural integrity of NP-vRNA complex for efficient vRNA synthesis.