Structural Basis of the Respiratory Syncytial Virus Polymerase Complexes

1. Preparation and characterization of the RSV polymerase.

Preparing high-quality and large-quantity of RSV L is the critical first step to understanding RSV catalytic core and has been proven challenging[1]. We have successfully co-expressed and co-purified full-length wild-type (wt) RSV RdRP (L:P) and catalytically inactive L(D811A):P using sf21 insect cells (**Fig. 1**). We will generate a panel of L fragments with one or two functional domains.

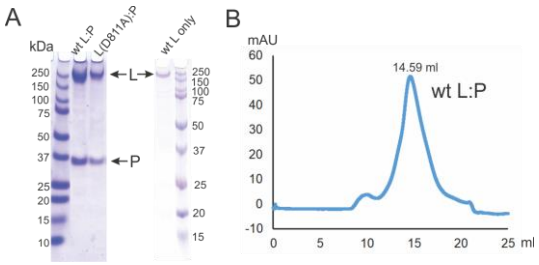

Figure 1: Preparation of the RSV L protein. (A) SDS-PAGE gel shows the expression of full-length wild-type (*wt*) and mutant RSV L:P, and L only in insect cells. **(B)** The size exclusion chromatography shows homogeneity. (*Cao et al. Nat Comm 2020*)

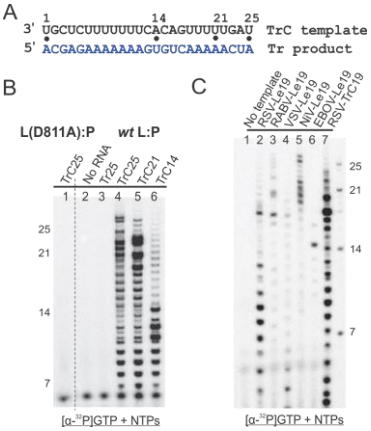

Figure 2: *In vitro* reconstitution of RSV RNA polymerization. (A) the template (TrC) and product (Tr). **(B)** Transcription assay using *wt* and mutant RdRP (L:P) on various RNAs. **(C)** Test RNA templates from different viruses for specificity. (*Cao et al. JVI 2020, Viruses 2022*)

2. In vitro transcription assay.

We successfully adopted [1-3] and developed a novel RNA elongation assay to map key promoter features of RSV[4] (**Fig. 2**). Our assay employs a short RNA template to incorporate [^{32}P]-NTP into the reaction. We will optimize the parameters (i.e., the length and sequence of the template and primers) to identify suitable constructs for in-depth structural analysis.

3. Capture the initiation and elongation of RSV polymerase.

We successfully determined multiple high-resolution structures of the polymerases from NNS RNA viruses using cryo-EM[5,6] (**Fig. 3**). Using the template in the transcription assay and selected NTPs or NTP analogs; we will trap the initiation and elongation stages of the RSV RdRP (**Fig. 4**).

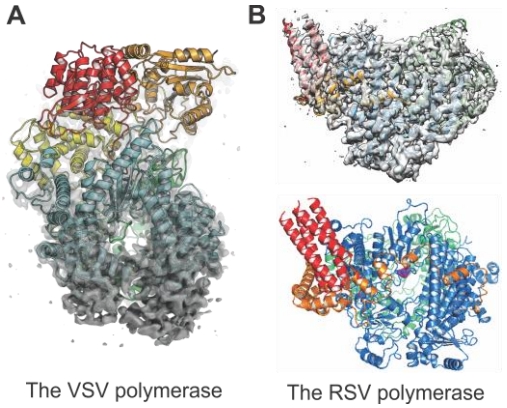

Figure 3: Cryo-EM structures of the polymerases of NNS RNA viruses determined by the PI. (A) 3.8 Å cryo-EM structure of the vesicular stomatitis virus (VSV) polymerase (*Liang et al. Cell, 2015*). **(B)** 3.67 Å cryo-EM structure of the respiratory syncytial virus (RSV) polymerase (*Cao et al. Nat Comm 2020*).

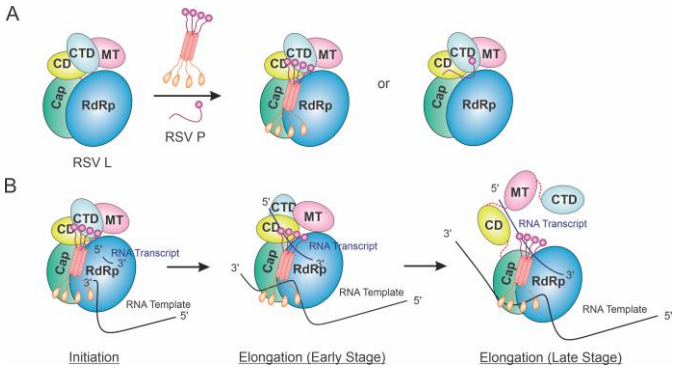

Figure 4: The overview of the proposed work. (A) The RSV RdRP (L:P). The domains of both monomeric L and tetrameric P are shown. P_{frag} is the minimal P fragment used to stabilize the L protein domains in this proposal. **(B) Initiation** and **elongation complexes**. The RNA template and RNA transcript are shown as black or blue lines, respectively.

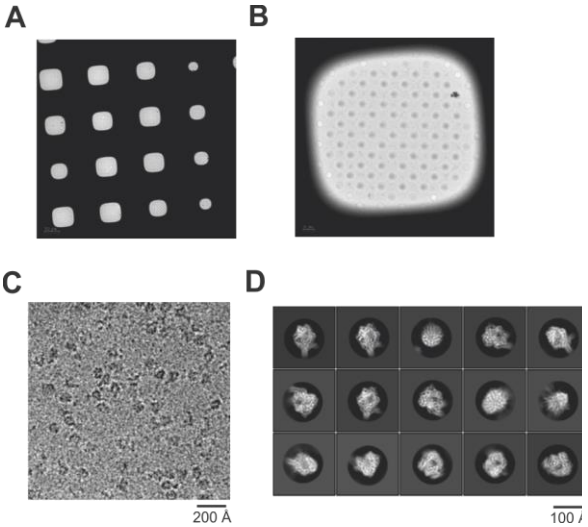

Figure 5: Preliminary cryo-EM analysis of RSV polymerase complex with its RNA (L:P:RNA). (A) The low magnification view of the grid. **(B)** The medium magnification view of a representative grid square. **(C)** The raw image of L:P:RNA complex. **(D)** The class averages show both similar and different views to that of *apo* RSV L:P complex.

Preliminary data suggested that the RSV L:P:RNA complex showed reasonable homogeneity. The initial cryo-screen revealed good ice thickness and gradient in the cryo grids (**Fig. 5A, B**). The preliminary screen showed the particles are readily visible in the grid holes (**Fig. 5C**). A small cryo-EM dataset and the 2D class averages show both similar and different views compared to that of *apo* RSV L:P complex (**Fig. 5D**). In summary, we demonstrated the feasibility of preparing the high-quality RSV L:P:RNA complex. Besides the *apo* RSV RdRP, we determined multiple 2.5-3.5 Å cryo-EM structures of the RSV RdRP in complexes of RNA.

REFERENCES:

1 Noton, S. L., Deflube, L. R., Tremaglio, C. Z. & Fearns, R. The respiratory syncytial virus polymerase has multiple RNA synthesis activities at the promoter. *PLoS Pathog* **8**, e1002980, (2012).

2 Morin, B., Rahmeh, A. A. & Whelan, S. P. Mechanism of RNA synthesis initiation by the vesicular stomatitis virus polymerase. *EMBO J* **31**, 1320-1329, (2012).

3 Morin, B., Liang, B., Gardner, E., Ross, R. A. & Whelan, S. P. J. An In Vitro RNA Synthesis Assay for Rabies Virus Defines Ribonucleoprotein Interactions Critical for Polymerase Activity. *J Virol* **91**, (2017).

4 Cao, D., Gao, Y., Roesler, C., Rice, S., D'Cunha, P., Zhuang, L., . . . Liang, B. In Vitro Primer-Based RNA Elongation and Promoter Fine Mapping of the Respiratory Syncytial Virus. *J Virol* **95**, (2020).

5 Liang, B., Li, Z., Jenni, S., Rahmeh, A. A., Morin, B. M., Grant, T., . . . Whelan, S. P. J. Structure of the L Protein of Vesicular Stomatitis Virus from Electron Cryomicroscopy. *Cell* **162**, 314-327, (2015).

6 Cao, D., Gao, Y., Roesler, C., Rice, S., D'Cunha, P., Zhuang, L., . . . Liang, B. Cryo-EM structure of the respiratory syncytial virus RNA polymerase. *Nat Commun* **11**, 368, (2020).