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 challenging and 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**).

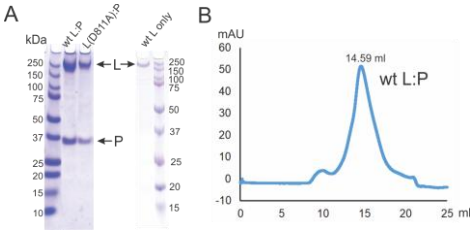

Figure 1: Preparation of the RSV L protein. (A) SDS-PAGE gel shows the expression of full-length wild-type (*wt*) and mutant RSV L:P, and L only in insect cells. **(B)** The size exclusion chromatography shows homogeneity. (*Cao et al. Nat Comm 2020*)

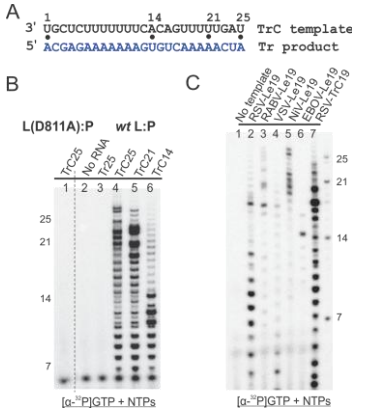

Figure 2: *In vitro* reconstitution of RSV RNA polymerization. (A) the template (TrC) and product (Tr). **(B)** Transcription assay using *wt* and mutant RdRP (L:P) on various RNAs. **(C)** Test RNA templates from different viruses for specificity. (*Cao et al. JVI 2020, Viruses 2022*)

2. In vitro transcription assay. We successfully adapted [1-3] and developed a novel RNA elongation assay to map key promoter features of RSV[4] (**Fig. 2**). Our assay uses 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[5-7] (**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**).

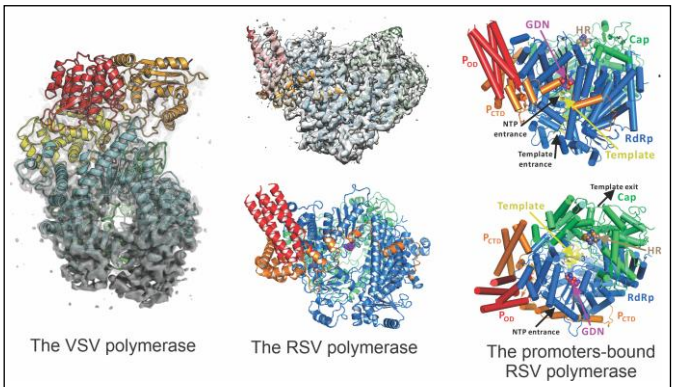

Figure 3: Cryo-EM structures of the NNS RdRPs by the PI. (A) 3.8 Å cryo-EM structure of the VSV RdRP (*Liang et al. Cell, 2015*). **(B)** 3.67 Å cryo-EM structure of the *apo* RSV RdRP (*Cao et al. Nat Comm 2020*). **(C)** 3.40 Å and 3.41 Å cryo-EM structures of the promoters-bound RSV RdRP (*Cao et al. Nature 2024*).

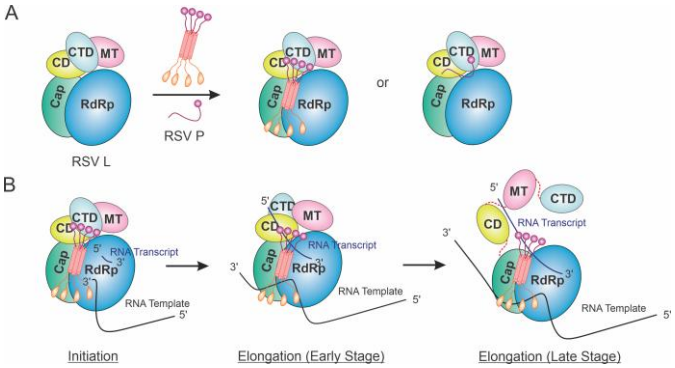

Figure 4: The overview of the proposed work. (A) The RSV RdRP (L:P). The domains of both monomeric L and tetrameric P are shown. P_{frag} is the minimal P fragment used to stabilize the L protein domains in this proposal. **(B) Initiation** and **elongation complexes**. The RNA template and RNA transcript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

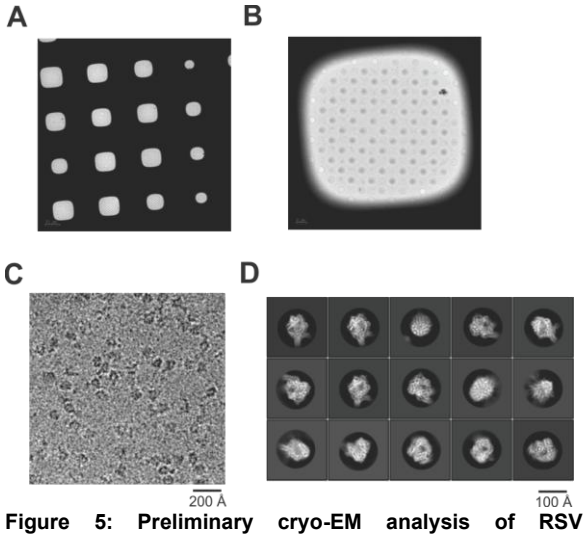

Figure 5: Preliminary cryo-EM analysis of RSV polymerase complex with its RNA (L:P:RNA). (A) The low magnification view of the grid. **(B)** The medium magnification view of a representative grid square. **(C)** The raw image of L:P:RNA complex. **(D)** The class averages show both similar and different 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 (Cao et al., *Nat Comm*, 2020)[6], we determined multiple 2.5-3.5 Å cryo-EM structures of the RSV RdRP in complexes of RNA templates (Cao et al., *Nature*, 2024)[7] and more recently the intermediates involved in the nucleotide addition cycle of the RNA synthesis catalysis (Cao et al., *in preparation*).

REFERENCES:

1. Noton, S.L., Deflube, L.R., Tremaglio, C.Z., and Fearns, R. (2012). The respiratory syncytial virus polymerase has multiple RNA synthesis activities at the promoter. PLoS Pathog *8*, e1002980. 10.1371/journal.ppat.1002980.
2. Morin, B., Rahmeh, A.A., and Whelan, S.P. (2012). Mechanism of RNA synthesis initiation by the vesicular stomatitis virus polymerase. EMBO J *31*, 1320-1329. 10.1038/emboj.2011.483.
3. Morin, B., Liang, B., Gardner, E., Ross, R.A., and Whelan, S.P.J. (2017). An In Vitro RNA Synthesis Assay for Rabies Virus Defines Ribonucleoprotein Interactions Critical for Polymerase Activity. J Virol *91*. 10.1128/JVI.01508-16.
4. Cao, D., Gao, Y., Roesler, C., Rice, S., D'Cunha, P., Zhuang, L., Slack, J., Antonova, A., Romanelli, S., and Liang, B. (2020). In Vitro Primer-Based RNA Elongation and Promoter Fine Mapping of the Respiratory Syncytial Virus. J Virol *95*. 10.1128/JVI.01897-20.
5. Liang, B., Li, Z., Jenni, S., Rahmeh, A.A., Morin, B.M., Grant, T., Grigorieff, N., Harrison, S.C., and Whelan, S.P.J. (2015). Structure of the L Protein of Vesicular Stomatitis Virus from Electron Cryomicroscopy. Cell *162*, 314-327. 10.1016/j.cell.2015.06.018.
6. Cao, D., Gao, Y., Roesler, C., Rice, S., D'Cunha, P., Zhuang, L., Slack, J., Domke, M., Antonova, A., Romanelli, S., et al. (2020). Cryo-EM structure of the respiratory syncytial virus RNA polymerase. Nat Commun *11*, 368. 10.1038/s41467-019-14246-3.
7. Cao, D., Gao, Y., Chen, Z., Gooneratne, I., Roesler, C., Mera, C., D'Cunha, P., Antonova, A., Katta, D., Romanelli, S., et al. (2024). Structures of the promoter-bound respiratory syncytial virus polymerase. Nature *625*, 611-617. 10.1038/s41586-023-06867-y.