[1]Weill Institute for Cell and Molecular Biology and Department of Molecular Biology and Genetics, Cornell University, Ithaca, NY 14853, USA

*Correspondence:
ym253@cornell.edu

https://doi.org/10.1016/j.tim.2019.09.001

References

1. Qiu, J. *et al.* (2016) Ubiquitination independent of E1 and E2 enzymes by bacterial effectors. *Nature* 533, 120–124
2. Bhogaraju, S. *et al.* (2016) Phosphoribosylation of ubiquitin promotes serine ubiquitination and impairs conventional ubiquitination. *Cell* 167, 1636–1649.e13
3. Kotewicz, K.M. *et al.* (2017) A single *Legionella* effector catalyzes a multistep ubiquitination pathway to rearrange tubular endoplasmic reticulum for replication. *Cell Host Microbe* 21, 169–181
4. Jeong, K.C. *et al.* (2015) Spatiotemporal regulation of a *Legionella* pneumophila T4SS substrate by the metaeffector SidJ. *PLoS Pathog.* 11, e1004695
5. Wan, M. *et al.* (2019) Deubiquitination of phosphoribosyl-ubiquitin conjugates by PDE domain-containing *Legionella* effectors. *bioRxiv*. Published online August 23, 2019. https://doi.org/10.1101/745331.
6. Havey, J.C. and Roy, C.R. (2015) Toxicity and SidJ-mediated suppression of toxicity require distinct regions in the SidE family of *Legionella pneumophila* effectors. *Infect. Immun.* 83, 3506–3514
7. Black, M.H. *et al.* (2019) Bacterial pseudokinase catalyzes protein polyglutamylation to inhibit the SidE-family ubiquitin ligases. *Science* 364, 787–792
8. Bhogaraju, S. *et al.* (2019) Inhibition of bacterial ubiquitin ligases by SidJ-calmodulin catalysed glutamylation. *Nature* 572, 382–386
9. Gan, N. *et al.* (2019) Regulation of phosphoribosyl ubiquitination by a calmodulin-dependent glutamylase. *Nature* 572, 387–391
10. Sulpizio, A. *et al.* (2019) Protein polyglutamylation catalyzed by the bacterial Calmodulin-dependent pseudokinase SidJ. *bioRxiv*. Published online August 20, 2019. https://doi.org/10.1101/738567.

Spotlight

The Respiratory Syncytial Virus Polymerase: A Multitasking Machine

Rachel Fearns[1,*]

Respiratory syncytial virus (RSV) inflicts a significant toll on human life. An essential element of the virus is its polymerase, a complex capable of transcribing and replicating the viral genome. In an exciting new advance, Gilman *et al.* resolve the structure of this polymerase, providing valuable mechanistic insight into its activities.

RSV infects almost everyone worldwide by the age of two. It is the major cause of respiratory disease in infants, and it afflicts the elderly and immunocompromised [1]. As yet, there is no effective vaccine or antiviral therapy. The RSV polymerase is an essential component of the virus. With three enzymatic domains, it is a 'target-rich' complex for antiviral drug development, and has been subjected to mutations to generate live-attenuated vaccine candidates [2,3]. It is also a fascinating biological machine, capable of performing multiple activities to transcribe and replicate the RSV genome. Despite the importance of the RSV polymerase for translational research and basic biology, until now, its 3D structure remained elusive.

When RSV infects a cell, the viral genome is released from the virus particle into the cell cytoplasm. It is here that the genome undergoes two different processes: transcription to produce mRNAs required for viral protein synthesis, and replication to produce new viral genomes for packaging into virions. The RSV polymerase is capable of performing all enzymatic activities required for viral transcription and genome replication (reviewed in [2]). The genome is a single strand of negative-sense RNA coated along its length with multiple copies of the viral nucleoprotein. It contains ten genes, which are each transcribed into capped and polyadenylated mRNAs (Figure 1A). To transcribe the genome, the polymerase initiates at a promoter at the 3′ end of the genome and then moves towards the 5′ end, transiently displacing the nucleoprotein as it progresses. The polymerase is able to generate subgenomic mRNAs by responding to *cis*-acting signals that flank each gene. It initiates RNA synthesis at the beginning of a gene and cotranscriptionally adds a guanosine cap to the RNA 5′ end (Figure 1B), which it then methylates to produce a cap 1 structure. The polymerase then elongates the RNA until it reaches a gene end signal, where it adds a poly A sequence by repeatedly slipping on a uridine tract (Figure 1C). Following polyadenylation, the mRNA is released and the polymerase scans the genome to locate the start of the next gene and reinitiate RNA synthesis, transcribing the remaining genes. During replication, the nascent RNA is neither capped nor polyadenylated, but instead becomes associated with a growing chain of nucleoprotein as it is synthesized and is elongated to the end of the template. The replicative intermediate acts as a template for further rounds of genome synthesis. Remarkably, the core polymerase capable of all these activities consists of a complex of just two proteins, the large polymerase subunit (L) and phosphoprotein (P). The L subunit contains the RNA-dependent RNA polymerization, capping, and methyltransferase domains. These domains have properties that distinguish them from cellular enzymes. For example, it is likely that capping occurs by an RNA:GDP polyribonucleotidyltransferase rather than guanylyltransferase activity [4]. The P protein is required for efficient expression of L, and for contacting other viral proteins involved in RNA synthesis, such as nucleoprotein and a transcription elongation factor, M2-1 [5,6].

Now in a breakthrough achievement, Gilman and coworkers present the

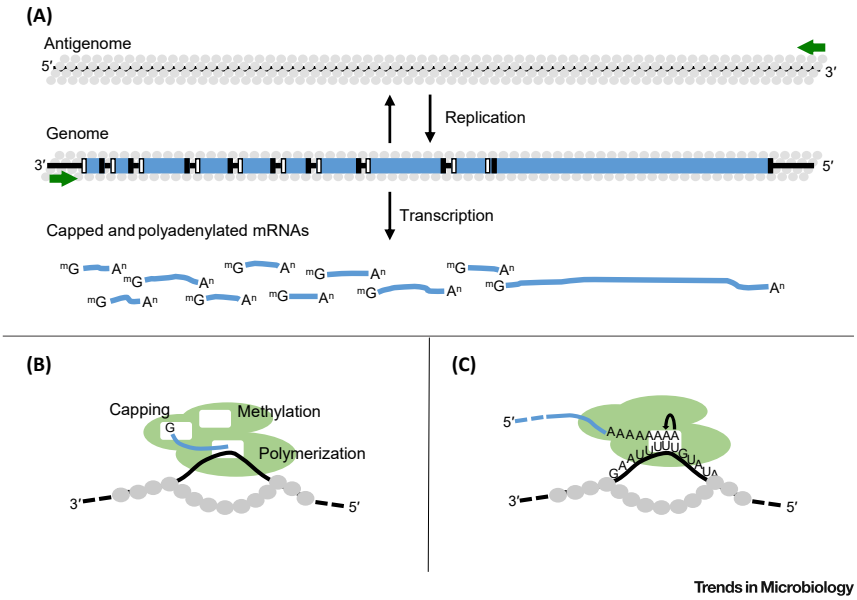

Figure 1. Schematic Diagrams Illustrating Events in Respiratory Syncytial Virus (RSV) Transcription and Replication

(A) The RSV genome and the transcription and replication products [2]. Each gene is depicted with a blue rectangle and is flanked with conserved elements that direct the polymerase during transcription (white and black boxes). The genome and replicative intermediate antigenome are coated with nucleoprotein, as indicated with the gray circles. The promoters are shown as green arrows. (B) Events following initiation at the start of the gene. The three domains of the L protein are shown in green, with active sites shown as white boxes. The RNA template is shown as a black line, with gray circles representing nucleoprotein, and the newly synthesized mRNA as a blue line. It is thought that the 5′ end of the nascent mRNA is channeled from the RNA polymerization domain to the capping domain, where it is modified with a guanosine cap. Presumably, it then transfers to the methyltransferase domain to allow the cap to be methylated. (C) The likely mechanism of polyadenylation. At the end of each gene is a signal containing a U-tract. It is thought that repeated slippage of the transcript, relative to the U-tract in the template, leads to addition of the poly A sequence. Diagrams are not to scale.

structure of the RSV L-P complex [7]. This exciting advance builds on a number of enabling discoveries. Recombinant RSV L-P was first expressed and isolated in 2012, and was found to have properties similar to those of polymerase in infected cells, establishing its validity for structure–function studies [8]. In 2015, the 3D structure of the L protein of a related virus, vesicular stomatitis virus (VSV), was described [9]. This, together with biochemical studies, identified the three enzymatic domains within L and a connector domain, providing the first glimpse of how the different elements within L associate together. The RSV L-P structure provides specific insights into RSV and expands our understanding of how the polymerase functions. At a 3.2 Å resolution there is sufficient information to infer the binding sites of small-molecule inhibitors and to rationalize mechanisms underlying resistance to them. It also affords a mechanistic understanding of an attenuating mutation in a vaccine candidate virus. Of particular significance, the relationship between L and P can be clearly observed. P forms a tetramer, which associates with a large surface area of L, giving it the potential to influence multiple activities. Each P monomer has an extended unstructured arm containing residues necessary for interacting with nucleoprotein and M2-1. Thus, we can begin to speculate how L-P might connect with the other key players of transcription/replication complexes. Comparison of the VSV and RSV polymerase structures also provides an insight into different structural states that the polymerase adopts. The VSV L structure appears to reflect the polymerase in the preinitiation state, in which a loop containing a priming residue [10], an amino acid capable of forming base-stacking interactions with the initiating nucleoside triphosphates (NTPs), protrudes into the active site of the polymerization domain. In contrast, the RSV L-P structure appears to be in an elongation mode, with this loop flipped away from the polymerization active site and more integrated with the capping domain. The capping domain is organized slightly differently, perhaps reflecting an active conformation, and whereas the connector and methyltransferase domains were resolved in the VSV L structure, they could not be resolved in that of RSV L-P. This might reflect flexibility to allow the methyltransferase proximity to the capping domain during RNA synthesis. Thus, comparison of the VSV L and RSV L-P structures provides some understanding of the conformational changes that the polymerase undergoes as it navigates between different activities.

This RSV polymerase structure will significantly advance drug development efforts and has the potential to allow for rational design of attenuated virus vaccines to help combat this important human pathogen. It also provides insight into the intricate interplay of moving parts required for the multiple different events in viral RNA synthesis. Many questions remain, but resolution of this structure represents a leap forward in the field that will allow

studies into this extraordinary protein complex to flourish.

Acknowledgments

The author wishes to thank Drs John Connor and Mohsan Saeed and members of the Fearns group for constructive comments on the manuscript. Research in the author's laboratory is funded by the National Institute of Allergy and Infectious Diseases and National Heart, Lung, and Blood Institute of the National Institutes of Health under award numbers R01AI113321, R01AI133486, R33HL137081, and by sponsored research agreements with Janssen Sciences Ireland UC, and Enanta Pharmaceuticals, Inc. The funders had no role in preparation of the manuscript or decision to publish.

[1]Department of Microbiology, National Emerging Infectious Diseases Laboratories, Boston University School of Medicine, 620 Albany Street, Boston, MA 02118, USA

*Correspondence:
rfearns@bu.edu

https://doi.org/10.1016/j.tim.2019.10.001

References

1. Walsh, E.E. (2017) Respiratory syncytial virus infection: an illness for all ages. *Clin. Chest Med.* 38, 29–36
2. Fearns, R. and Plemper, R.K. (2017) Polymerases of paramyxoviruses and pneumoviruses. *Virus Res.* 234, 87–102
3. Mazur, N.I. *et al.* (2018) The respiratory syncytial virus vaccine landscape: lessons from the graveyard and promising candidates. *Lancet Infect. Dis.* 18, e295–e311
4. Ogino, T. and Banerjee, A.K. (2007) Unconventional mechanism of mRNA capping by the RNA-dependent RNA polymerase of vesicular stomatitis virus. *Mol. Cell* 25, 85–97
5. Pereira, N. *et al.* (2017) New insights into structural disorder in human respiratory syncytial virus phosphoprotein and implications for binding of protein partners. *J. Biol. Chem.* 292, 2120–2131
6. Selvaraj, M. *et al.* (2018) The structure of the human respiratory syncytial virus M2-1 protein bound to the interaction domain of the phosphoprotein P defines the orientation of the complex. *mBio* 9, e01554-18
7. Gilman, M.S.A. *et al.* (2019) Structure of the respiratory syncytial virus polymerase complex. *Cell* 179, 193–204.e14
8. Noton, S.L. *et al.* (2012) The respiratory syncytial virus polymerase has multiple RNA synthesis activities at the promoter. *PLoS Pathog.* 8, e1002980
9. Liang, B. *et al.* (2015) Structure of the L protein of vesicular stomatitis virus from electron cryomicroscopy. *Cell* 162, 314–327
10. Ogino, M. *et al.* (2019) A dual-functional priming-capping loop of rhabdoviral RNA polymerases directs terminal *de novo* initiation and capping intermediate formation. *Nucleic Acids Res.* 47, 299–309