Structure and function of the respiratory syncytial virus polymerase

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Respiratory syncytial virus (RSV) is a nonsegmented negative-sense (NNS) RNA virus and shares a similar RNA synthesis strategy with other members of NNS RNA viruses, such as measles, rabies virus, and Ebola virus. RSV RNA synthesis is catalyzed by a multifunctional RNA-dependent RNA polymerase (RdRP), which is composed of a 250 kDa large (L) protein that catalyzes three distinct enzymatic functions (nucleotide polymerization, cap addition, and cap methylation) and an essential coenzyme tetrameric phosphoprotein (P). How RSV L and P coordinate these activities is poorly understood. Here, we present a 3.67 Å cryo-EM structure of the RSV polymerase (L:P) complex. The structure reveals that the RNA dependent RNA polymerase (RdRp) and capping (Cap) domains of L interact with the oligomerization domain (POD) and C-terminal domain (PCTD) of a tetramer of P. The density of the methyltransferase (MT) domain of L and the N-terminal domain of P (PNTD) is missing. The structure we obtained is likely to be at an elongation-compatible stage. Besides, we demonstrated that the RSV polymerase could carry out both de novo and primer-based RNA synthesis. We defined the minimal length of the RNA template for in vitro de novo RNA synthesis using the purified RSV polymerase as 8 nucleotides (nt), shorter than previously reported. We showed that the RSV polymerase catalyzed primer-dependent RNA elongation with different lengths of primers on both short (10-nt) and long (25-nt) RNA templates. We compared the sequence specificity of different viral promoters and identified positions 3, 5, and 8 of the promoter sequence as essential to the in vitro RSV polymerase activity, consistent with the results previously mapped with the in vivo minigenome assay. Overall, these findings agree well with those of previous studies, provide enriched insights into the interrelationship, the inhibitors, and the evolutionary implications of the RSV polymerase and extend our understanding of the mechanism of RSV RNA synthesis.