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Co-factor Interactions in *Alpha* and *Betacoronavirus* Core Polymerase Complexes

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Coronaviruses are a large family of animal pathogens potentially causing severe respiratory or enteric disease. Recent history is punctuated by the emergence of pathogenic coronaviruses such as SARS-CoV, MERS-CoV and SARS-CoV-2 that crossed into human circulation from animal reservoirs [1] similar to the zoonoses responsible for deadly influenza virus pandemics [2] and Ebola virus outbreaks [3]. Coronaviruses of the *Alphacoronavirus* genus also precipitate serious pathogenesis in animal livestock including pigs. Several serotypes of transmissible gastroenteritis virus (TGEV) and porcine epidemic diarrhea virus (PEDv) circulate globally causing enteric diseases in weaned pigs and high lethality in neonatal piglets [4-6]. Animal coronaviruses are globally prevalent pathogens that continuously emerge and evolve, thereby making them challenging moving targets for prevention and treatment of infection. Previous efforts prioritizing studies of betacoronaviruses have neglected other coronavirus genera including highly pathogenic animal coronaviruses of the *Alphacoronavirus* genus such as PEDv.

The coronavirus RNA synthesis machinery is multi-subunit complex of non-structural proteins (nsp) derived from the viral polyprotein [7]. At the core of this complex is the viral RNA-dependent RNA, nsp12 polymerase. However, this subunit alone is insufficient for RNA synthesis activity in vitro, requiring both nsp7 and nsp8 co-factors [8]. Structural studies of coronavirus polymerase core complexes have focused exclusively on SARS-CoV and SARS-CoV-2 [9-11]. While this has provided a wealth of information, whether these findings could be extended to other coronaviruses is unknown.

To improve our understanding of PEDv replication and draw parallels across the coronavirus family, we have determined the structure of the PEDv polymerase core complex using single-particle cryo-EM. The structure includes the viral RNA-dependent RNA polymerase nsp12 and required cofactors nsps 7 and 8 and a bound RNA primer-template pair. In comparison to prior SARS-CoV and SARS-CoV-2 complex structures, our structural analysis of the PEDv polymerase complex shows altered interactions of the nsp12 polymerase with nsp7 and nsp8 co-factors particularly around nsp8 binding sites.

We have validated the importance of these altered interactions using mutagenesis and *in vitro* biochemistry. This work additionally demonstrated the *in vitro* RNA synthesis activity of an alphacoronavirus polymerase for the first time. Testing of hypotheses based on the PEDv structure using biochemical methods for both SARS-CoV-2 and PEDv polymerases has allowed us to determine essential functions of the nsp7 and nsp8 co-factors in viral RNA synthesis across the coronavirus family. A more comprehensive understanding of coronavirus replication mechanics across the viral family is critical for our development of both broad acting and disease-specific antivirals, preparing us for a future coronavirus pandemic.

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