Molecular Therapy
Nucleic Acids
Commentary

Deciphering the NSP12/7/8 complex: Key insights into coronavirus replication and potential therapeutic targets

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Members of the *Coronaviridae*, particularly the betacoronaviruses, pose serious health threats, the latest of which is SARS-CoV-2, responsible for the COVID-19 pandemic.[1,2] RNA virus genome replication and transcription are driven by the RNA-dependent RNA polymerase (RdRp); in coronaviruses, non-structural protein (NSP)12, NSP8, and NSP7 assemble to form the RdRp holoenzyme.[3] This complex forms the replication-transcription complex (RTC) with RNA and other viral proteins.[4] Due to its critical role in the RNA viral life cycle and conservation in the *Coronaviridae* family, the RTC is a major therapeutic target.[5] The RdRp holoenzyme enables RNA replication, with NSP7 and NSP8 being essential cofactors for NSP12.[6,7] Studies on SARS-CoV-1 show contradictory results regarding the role of NSP7 and NSP8 in NSP12 polymerase activity. In contrast, SARS-CoV-2 studies confirm that both cofactors are important for NSP12 activity,[3] although one study reported weak polymerase activity.[8] Thus, it is important to investigate the interaction between NSP7-NSP8 and NSP12 to understand viral multiplication and therapy development. Recently, Singh et al. published a study investigating the molecular mechanisms controlling RdRp complex formation and the specific contributions of NSP12, NSP7, and NSP8 to RNA polymerase activity. This research provides valuable insights into the molecular interactions of NSPs (NSP12, NSP7, and NSP8) in assembling an active polymerase complex, emphasizing their cooperative and inhibitory dynamics.

In this study, Singh et al.[9] successfully produced recombinant NSP12, NSP7, and NSP8 in a bacterial expression system. Interestingly, they reconstituted the functional RdRp holoenzyme *in vitro* by co-incubating NSP12, NSP8, and NSP7 at a 1:2:1 ratio and optimized the concentrations. Furthermore, they have examined the detrimental effects of NSP7 or NSP8 alone on NSP12 polymerase activity and found that NSP12 alone can perform polymerase activity, although at a reduced level compared to the RdRp holoenzyme. However, its activity is completely abolished when added with NSP7 or NSP8. Next, they investigated the impact of NSP7 or NSP8 alone or in combination on template-NSP12 complex formation. Their findings showed that the presence of both NSP cofactors together significantly enhanced RNA binding to NSP12, whereas this was not the case in the complete absence of either of the NSP cofactors. Furthermore, Singh et al. investigated the impact of NSP7 or NSP8 alone, as well as in combination, on NSP12-RNA complex formation and found that when NSP7 or NSP8 alone binds to NSP12 within the NSP12-RNA complex, it displaces the RNA duplex in a time-dependent manner, with NSP8 causing more rapid displacement than NSP7. In contrast, the simultaneous presence of both NSP7 and NSP8 had no such disruptive effect on the bound RNA duplex; instead, they facilitated the formation of the replication complex. Through *in silico* molecular dynamics (MD) simulations, they demonstrated that the lack of RdRp activity in the NSP12/7 and NSP12/8 complexes results from NSP7- and NSP8-induced structural changes in NSP12. These changes cause a severe constriction of the RNA entry/binding tunnel, creating unfavorable conditions for RNA binding. In contrast, the relatively weak polymerase activity of NSP12 alone is attributed to an increased RNA-binding tunnel volume compared to the RdRp holoenzyme, which disrupts optimal template binding.

Overall, the findings by Singh et al.[9] offer new insights into the molecular mechanisms underlying RdRp complex formation and the specific roles of NSP12, NSP7, and NSP8 in RNA polymerase activity. They concluded that while NSP7 and NSP8 work synergistically to enhance NSP12 activity, they act antagonistically when present alone. These findings emphasize the need for further targeted structural studies to validate the results and enhance our understanding of the proposed inhibitory mechanisms of NSP7 and NSP8. In addition, scientists should also work on developing new drugs and compounds that target and inhibit the interactions between NSP7 or NSP8 and NSP12. Such drugs can effectively inhibit coronavirus replication, offering a promising strategy in the fight against this formidable threat.

DECLARATION OF INTERESTS

There are no competing interests to disclose.

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