Conserved Proofreading by SARS-CoV-2 ExoN as a Broad-Spectrum Antiviral Target

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The emergence of novel RNA viruses with pandemic potential (MERS, SARS, and SARS-CoV-2) highlights the need to elucidate the molecular mechanisms underlying viral replication and transcription. At the core of these processes is the replication-transcription complex (RTC), which relies on key enzymes including the nonstructural proteins nsp10, nsp12, nsp14, each serving a specific purpose. The activities of these enzymes allow CoVs to sustain their unusually large genomes—approximately 30 kb—significantly larger than those of most RNA viruses, which typically have genomes under 15 kb. Of the proteins in the RTC, the cofactor nsp10 increases the activity level of nsp14 while nsp12 serves as the polymerase. Although those two enzymes serve important functions, it is suggested the most crucial is nsp14, a bifunctional enzyme with an N-terminal exonuclease proofreading domain (ExoN) and a C-terminal N7-methyltransferate (N7-MTase). The bifunctionality of nsp14 allows the virus to evolve against antivirals while maintaining crucial genome sequences. To investigate the catalytic mechanism of CoV-ExoN proofreading, we integrated comparative sequence and structural analyses with an extensive review of the literature. Our preliminary analysis suggests that the ExoN domain employs a two-metal-ion (2M)–assisted mechanism that resembles, though is not identical to, the conserved catalytic strategies seen in enzymatic processing of DNA/RNA. Ongoing Molecular Dynamics (MD) and Quantum Mechanics/Molecular Mechanics (QM/MM) simulations will offer detailed insights into the dynamics and the mechanistic aspects of this process. Furthermore, we performed multiple sequence alignments, phylogenetic reconstructions, and coevolution studies, which identified conserved regions on ExoN that are critical for interactions with RTC enzymes (including nsp12 and nsp10), and pinpointed residues that remain conserved across multiple CoVs. The conserved structure of the nsp14-ExoN/nsp10 complex, coupled with its functional significance, highlights its potential as a compelling target for inhibition, thereby supporting CoV proofreading as a promising avenue for the design of novel antiviral therapies.