Mechanism of RNA capping of the SARS-CoV-2 genome

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Abstract

Nidovirus RdRp-Associated Nucleotidyltransferase (NiRAN) domain of the SARS-CoV-2 nsp12 is an essential enzyme catalyzing the first two steps of 5'-end capping of the RNA genome. It covalently bonds nsp9 to the 5'-pppA-end of the genome (RNAylation). Subsequently, nsp9 is delinked from the RNA by GDP-polyribonucleotidyltransferase reaction (deRNAylation), forming capped GpppA-RNA. Additionally, NiRAN NMPylates nsp9 using NTP and deNMPylates nsp9 to form GpppN. We demonstrate that GpppN is a substrate for NMPylation, but not its methylated form. Structure-guided mutagenesis reveals that the NiRAN-bound NTP's orientation during NMPylation is similar to that seen in the evolutionarily related pseudokinase Selenoprotein-O, while during RNAylation the RNA's 5'-pppA-end is oriented perpendicular. nsp12-Asp711 contributes to the exclusive selection of 5'-adenine-containing RNA. nsp13 NTPase flips NiRAN's preference for NMPylation over RNAylation, while RdRp's substrate engagement diminishes RNAylation. Thus, the study unravels mechanistic details of NiRAN's substrate binding and orchestration of its multiple activities, with implications to therapeutics.