Understanding Preferences for Double-Stranded RNA Cleavage By SARS-Cov-2 Enzyme Nsp15

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Coronaviruses including SARS-CoV-2 evade detection by the host's immune system using a protein called nsp15; in fact, several studies have demonstrated that nsp15 activity has a direct and significant influence on the severity and duration of COVID-19 infection in animal models. Nsp15 functions to remove evidence of viral replication by cleaving viral double- stranded RNA (dsRNA) that would otherwise accumulate and be recognized by the host's pattern recognition receptors. Nsp15 cleaves RNA with broad specificity, primarily acting at the site of uridine (U) bases without requiring any extended sequence motifs for recognition. Recent structural studies have revealed that though the active site of nsp15 is too small to directly accommodate dsRNA, nsp15 can cleave dsRNA through a base-flipping mechanism, where a single U base flips away from the dsRNA and engages with the nsp15 active site. However, it remains to be seen whether nsp15 has the ability to actively flip U bases out of dsRNA or must passively take advantage of flipped-out U bases that occur through normal conformational flexibility of the dsRNA.

We aim to address this question through two complimentary research directions: 1) characterizing the context-dependence of U base flipping in the absence of nsp15 – i.e., the effect of bases that neighbor a U on the U's tendency to flip, and 2) comparing the cleavage activity of nsp15 on dsRNA substrates that contain paired, mismatched, or unpaired Us in a variety of sequence contexts. To accomplish our first research focus, we are using fluorine NMR, which can distinguish between stacked and flipped states in dsRNA substrates that contain a fluorine-labeled U. By comparing the proportion of stacked to flipped U in different sequence contexts, we can quantitatively describe the influence of neighboring bases on the U's tendency to flip. To accomplish our second research focus, we are using enzymatic assays that track the rate and patterns of dsRNA cleavage by nsp15 for dsRNA substrates that place U bases in a variety of sequence contexts, and using this information to select particularly interesting substrates for structural study by CryoEM. Taken together, these results will allow us to determine how well the rate of cleavage by nsp15 at a particular U aligns with that U's tendency to flip on its own. Characterizing the context-dependence of dsRNA cleavage by nsp15 will shed light on sites in viral RNA that are likely to be targeted by nsp15, which can further our understanding of the role played by nsp15 during COVID- 19 infection.