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VIRAL EVOLUTION

Fooling the coronavirus proofreading machinery

> ExoN proofreading protects SARS-CoV from the deleterious effects of mutagens

The polymerase of RNA viruses lacks the proofreading capacity found in the polymerase of DNA viruses, leading to high error rates and low replicative fidelity. Although the high mutation rate of RNA viruses enables them to readily adapt to environmental changes, they also risk 'lethal mutagenesis' when accumulating mutations corrupt essential functions. Here, Smith *et al.* reveal that, in the case of severe acute respiratory syndrome coronavirus (SARS-CoV), an exoribonuclease domain (ExoN) in non-structural protein 14 provides proofreading activity that protects the virus from mutagenesis.

DIGITAL VISION

Previous work in coronaviruses, the largest RNA viruses, had shown that ExoN deletion leads to reduced replicative fidelity and attenuation of virulence. Thus, Smith *et al.* speculated that ExoN is a proofreading enzyme. To test this hypothesis, they infected cells with wild-type ($ExoN^+$) or ExoN-deleted ($ExoN^-$) SARS-CoV in the presence of the mutagenic pyrimidine analogue 5-fluorouracil (5-FU). Indeed, loss of ExoN sensitized the virus to 5-FU, as shown by a 160-fold reduction in viral replication for $ExoN^-$ SARS-CoV compared with $ExoN^+$ SARS-CoV. Furthermore, genome sequencing of viral populations after 5-FU treatment revealed that $ExoN^-$ SARS-CoV harboured 3,648 mutations, whereas $ExoN^+$ SARS-CoV accumulated only 259 mutations. Of these mutations, 3,304 and 197 were U-to-C and A-to-G transitions, respectively, which are characteristic for nucleotide mismatches caused by the incorporation of 5-FU metabolites. Taken together, these results show that ExoN proofreading protects SARS-CoV from the deleterious effects of mutagens, such as 5-FU.

To date, no antiviral treatment exists for coronaviruses; neither ribavirin nor interferon-α, the only two broadly acting antivirals available in the clinic today, have a consistent antiviral effect against SARS-CoV. However, because ribavirin is a mutagenic nucleoside analogue, it might have similar effects to 5-FU in the absence of ExoN. Consistent with this, $ExoN^-$ murine CoV was more sensitive to ribavirin than $ExoN^+$ murine CoV, although further experiments indicated that other mechanisms besides mutagenesis (for example, reduced RNA synthesis) contributed to this ExoN-dependent effect. As the cells used for SARS-CoV infections failed to take up ribavirin, the sensitivity of $ExoN^-$ SARS-CoV to ribavirin remains unclear.

Nevertheless, coronavirus ExoN is the first proofreading protein reported in an RNA virus, and targeting ExoN is a promising strategy to develop new coronavirus inhibitors and sensitizers to existing antivirals.

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ORIGINAL RESEARCH PAPER Smith, E. C. *et al.* Coronaviruses lacking exoribonuclease activity are susceptible to lethal mutagenesis: evidence for proofreading and potential therapeutics. *PLoS Pathog.* **9**, e1003565 (2013)
FURTHER READING Lauring, A. S., Frydman, J. & Andino, R. The role of mutational robustness in RNA virus evolution. *Nature Rev. Microbiol.* **11**, 327–336 (2013)