Halting coronavirus polymerase

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The nucleotide analogue remdesivir is an investigational drug for the treatment of human coronavirus infection. Remdesivir is a phosphoramidate prodrug and is known to target viral RNA-dependent RNA polymerases. In this issue, Gordon *et al.* identify that remdesivir acts as a delayed RNA chain terminator for MERS-CoV polymerase complexes.

Coronaviruses are important pathogens of many animal species, including humans. Periodic virus jumps from animals into humans have led to viral outbreaks of SARS-CoV in 2002 and MERS-CoV in 2012. The recent emergence of SARS-CoV-2 that began in 2019 has developed into a global health concern where the virus has demonstrated a strong capacity for human-to-human transmission and the ability to cross international borders. Despite the importance of these pathogenic viruses, currently there are no approved antiviral drugs for the treatment of human coronavirus infections. Although several nucleotide analogue drugs targeting viral polymerases have been approved to treat a broad range of RNA viruses, an editing exonuclease in coronaviruses provides a natural resistance to many of these small molecules. However, the drug remdesivir, originally developed for the treatment of Ebola virus (1), shows promise. Remdesivir is a phosphoramidate nucleotide analogue prodrug that is metabolized to a triphosphate form in cells and has been identified as a broad inhibitor of RNA viruses, including filo-, pneumo-, paramxyo-, and coronaviruses (2, 3). Remdesivir has shown effectiveness against a number of coronaviruses, with IC_{50} values of <0.1 μM in human airway epithelial cell models of coronavirus infection. The nucleotide analogue also prevents pathology when given prophylactically and reduces pathology when given therapeutically in animal models of coronavirus infection (3). Remdesivir is currently being trialed as an antiviral therapy to treat SARS-CoV-2 infection. In this issue, Gordon *et al.* characterize the mechanism of remdesivir acting against the MERS-CoV polymerase complex (4).

Upon infecting a host cell, the coronavirus positive-sense RNA genome is translated to produce viral polyproteins. These polyproteins are cleaved by viral proteases to yield 16 nonstructural proteins (nsp)[2] responsible for replication and transcription of the viral genome. These nsp form a multisubunit complex containing many enzymatic activities, including an RNA-dependent polymerase, nsp12 (5). RNA-dependent polymerases are common features of RNA viruses, as the host cell lacks the machinery for the virus to copy its RNA genome. Being distinct from host protein machinery, these viral RNA-dependent polymerases are excellent targets for antiviral drugs.

The RNA-dependent polymerase accepts nucleotides as substrates, and many nucleotide analogues have found utility in broadly inhibiting viral RNA synthesis (6). However, in addition to the nsp12 RNA polymerase, coronaviruses also encode an exonuclease, nsp14, responsible for editing mismatches that occur during viral replication, which also removes many incorporated nucleotide analogues (7). This editing activity makes coronaviruses naturally resistant to several broad-spectrum RNA virus antivirals. One nucleotide inhibitor that has shown efficacy against coronaviruses in the laboratory is remdesivir, an adenosine analogue developed by Gilead Sciences (3) (Gilead Sciences Update on the Company's Ongoing Response to COVID-19, Gilead Sciences, Foster City, CA). Remdesivir is now undergoing phase III clinical trials for the treatment of human coronavirus infections (8). Earlier investigations on the mechanism of remdesivir action using respiratory syncytial virus (RSV) suggested that this antiviral acted as a delayed terminator of RNA chain elongation, but there was no mechanistic understanding of how remdesivir acts against coronaviruses was unknown.

In their new work, Gordon *et al.* determined the mechanism of action of remdesivir against MERS-CoV (4). To accomplish this, the authors used an nsp5 protease-nsp7-nsp8-nsp12 co-expression strategy using the baculovirus expression system to produce a purified complex of viral nsp8 and nsp12 for their *in vitro* measurements of MERS-CoV polymerase activity. Their data show that remdesivir is incorporated into the growing RNA chains, where the viral polymerase surprisingly demonstrated a preference for the analogue over the natural substrate ATP. As found for RSV, remdesivir induces termination of RNA elongation in MERS-CoV polymerase complexes. However, the termination of RNA synthesis did not occur until a further three nucleotides were incorporated into the nascent RNA, leading the authors to propose a mechanism of delayed chain termination similar to that of RSV (6) (Fig. 1). The authors suggest the hypothesis that because chain termination occurs three nucleotides after remdesivir is incorporated, the analogue may be protected from excision by the viral nsp14 exonuclease. Determination of how nucleotide mismatches and incorporated analogues are sensed and edited by nsp14 requires direct testing and further study.

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[2] The abbreviations used are: nsp, nonstructural protein(s); RSV, respiratory syncytial virus.

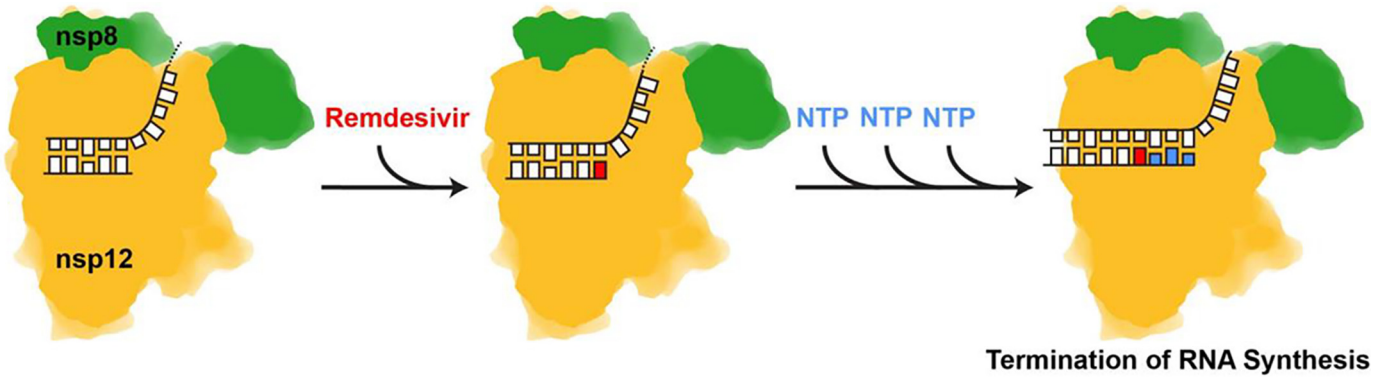

Figure 1. Mechanism of RNA termination by remdesivir. After incorporation of remdesivir by the coronavirus nsp12 RNA polymerase, a further three nucleotides are added to the growing RNA chain before the chain terminates due to the nucleotide analogue.

Knockout of exonuclease activity from related coronaviruses increases the potency of remdesivir, suggesting that the nsp14 exonuclease activity plays some role in limiting the antiviral effect of remdesivir (8).

The results of this study are consistent with the previous findings that remdesivir acts as a chain terminator of RNA virus polymerases. The finding that MERS-CoV polymerase preferentially incorporates remdesivir over ATP suggests a reason for the antiviral's high potency and the difficulty for coronaviruses to evolve resistance (8). Previous work has suggested that a minimal complex for coronavirus nsp12 polymerase activity required both nsp8 and nsp7 co-factors (9). Structural work of the polymerase complex has shown that nsp12 binds nsp8 directly as well as interacting with a nsp7-nsp8 heterodimer, where contacts with nsp12 are mediated primarily by nsp7 (10). Although the authors co-expressed nsp7, the subunit did not co-purify with nsp8 and nsp12. One possible explanation for this may be that an insufficient amount of nsp8 was produced to form nsp7-nsp8 heterodimers. Moreover, the activity of the nsp8-nsp12 complexes indicates that nsp7 was not required for activity in this study, in contrast to previous work. This may be due to the short primer-template pairs used here to assess polymerase activity. As nsp7 has been identified as a processivity factor for the viral RNA synthesis complex, it would be interesting to know the effects of remdesivir as a chain terminator in the presence of this additional viral co-factor. Continued work to provide insights into the mechanisms of antiviral compounds is key to the development of therapies for viral infection. The work by Gordon *et al.* (4) provides key mechanistic insight into the action of remdesivir and makes important hypotheses as to the effectiveness of this nucleotide analogue against the difficult-to-target coronavirus RNA synthesis complex.

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