Modified RNA building blocks yield unique signatures during incorporation by polymerases

TU Delft researchers have, in collaboration with their colleagues at the University of Erlangen, the University of Minnesota, and Penn State University, revealed that poliovirus RNA-dependent RNA polymerase, a key protein found in this and other RNA viruses, pauses in distinct ways when incorporating different nucleotide analogue inhibitors. These findings are important because such inhibitors are used to target viral activity. The research results were published on October 24 in the open access scientific journal *Cell Reports.*

RNA viruses and their RNA-dependent RNA polymerases

RNA viruses represent a threat to global public health, as outbreaks of West Nile virus, severe acute respiratory syndrome coronavirus, chikungunya virus, Middle East respiratory syndrome coronavirus, and currently Zika virus, attest to. It remains not only challenging to treat or prevent infections once the outbreak is underway, but also to predict the next outbreak. Antiviral therapeutics with broad-spectrum activity would greatly improve our readiness for the inevitable outbreaks of the future. Such therapeutics are few, at best. All RNA viruses encode a highly conserved RNA-dependent RNA polymerase (RdRp) that produces both viral mRNA and progeny genomes. This conservation makes the viral RdRp a very attractive target for developing broad-spectrum antiviral therapeutics. Indeed, ribavirin, a nucleoside analogue, is one of the few antiviral therapeutics with broad-spectrum activity. Thus, knowledge of the manner in which RNA-dependent RNA polymerase incorporates nucleotide analogues is not only important for understanding its mechanics, but ultimately impacts our ability to inhibit viral activity.

Following nucleotide incorporation by individual RNA-dependent RNA polymerases in real time

For their research, the scientists primarily employed an instrument called the magnetic tweezers that is capable of measuring on individually tethered RNA molecules. This allowed the researchers to follow the process of RNA synthesis by the poliovirus RNA-dependent RNA polymerase in real time, with a resolution of a few RNA bases. Because the magnetic tweezer instrument was used in a highly parallelized mode, the activity of hundreds of RNA-dependent RNA polymerases could be processed simultaneously, allowing the researchers to gather very large datasets. These datasets, which reported on the time spent by RNA-dependent RNA polymerase in synthesizing many consecutive short segments of RNA, could then be fitted to probabilistic models of polymerase pausing.

Different analogues yield distinct signatures, also within the cell

By repeating their measurements for different nucleotide analogues, the researchers could determine that pauses of a certain duration could be associated with the incorporation of a specific type of nucleotide analogue by the RNA-dependent RNA polymerase. They could show that nucleotide analogues ribavirin and inosine are incorporated as errors, inducing accompanying pausing, but that these do not diminish the overall extent of RNA synthesis, limiting their efficacy. They also showed that chain

terminating nucleotide analogues did diminish the overall extent of RNA synthesis, but that sometimes this inhibition could be overcome, presumably through their excision. Finally, these researchers showed that the pyrazine-carboxamide nucleotide T-1106 could, surprisingly, induce backtracking by the polymerase, a mechanism that had been previously unsuspected. This manifested itself as inhibition in bulk experiments and showed a consistent response in cells.

These findings are of great importance for our understanding of the way that different nucleotide analogues impact viruses. This study was performed on the RNA-dependent RNA polymerase from poliovirus, but future studies will determine whether RNA-dependent RNA polymerase from other viruses can be influenced in similar fashion.

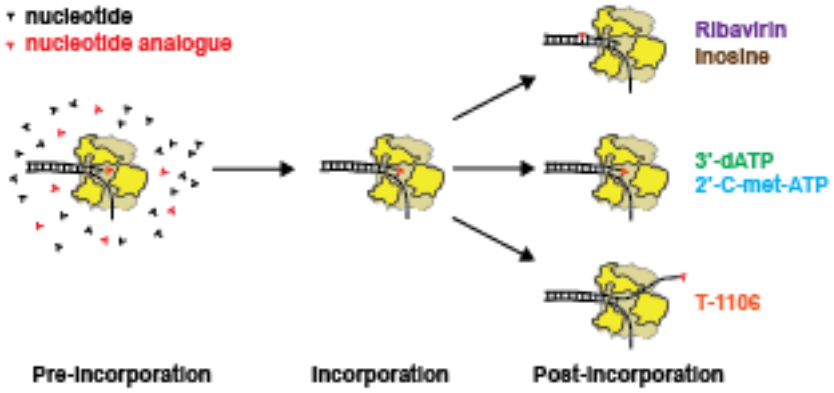

Figure 1. Summary of the different signatures exhibited by nucleotide analogues upon their incorporation by poliovirus RNA-dependent RNA polymerase. Nucleotide analogues ribavirin and inosine are incorporated as errors, inducing accompanying pausing. Chain terminators 3'-dATP and 2'-C-met-ATP reduce the overall extent of RNA synthesis, as expected, but can be excised in a few percent of cases, allowing the polymerase to recover. The pyrazine-carboxamide nucleotide T-1106 could, surprisingly, induce polymerase backtracking, a mechanism that had been previously unsuspected.

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David Dulin, Jamie J. Arnold, Theo van Laar, Hyung-Suk Oh, Cheri Lee, Daniel A. Harki, Martin Depken, Craig E. Cameron, and Nynke H. Dekker,
*"Signatures of nucleotide analogue incorporation by an RNA-dependent RNA polymerase revealed by using high-throughput magnetic tweezers", **Cell Reports*** **(2017),** http://www.cell.com/cell-reports/fulltext/S2211-1247(17)31422-5