# The Naturally Occurring $m^1A$ RNA Modification Can Be Efficiently Incorporated into RNA by SARS-CoV-2 RdRp

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**Abstract**: When it is in the template RNA, the naturally occurring $m^1A$ epitranscriptomic RNA modification was recently reported to be able to stop the RNA polymerization reaction catalyzed by the RNA dependent RNA polymerase (RdRp) of SARS-CoV-2. In this report, we report that $m^1A$ via its triphosphate form ($m^1ATP$) can be incorporated into RNA by the same RdRp. These two findings point a new direction for antiviral drug development based on $m^1A$ for combatting COVID-19. More broadly, it is possible that the large pool of epigenetic RNA as well as DNA modifications could serve as a treasury for drug discovery aimed at combating various infectious and other diseases.

## Introduction

Infectious diseases caused by RNA viruses such as HCoV 229E, HCoV OC43, HCV and HIV are notably annoying or difficult to treat. The recent COVID-19 pandemic caused by SARS-CoV-2 once again demonstrated this notion.[1-3] For all infectious diseases, one common feature is that different people show different symptoms, which range from asymptomatic to death. This observation has led to the invention of vaccines.[4-6] During the pandemic, driven by the curiosity to find additional causes for individualized symptoms of infectious diseases beyond those described in the literature such as induced immunity,[7] we studied the effects of epitranscriptomic RNA modifications on the catalytic activity of SARS-CoV-2 RNA dependent RNA polymerase (RdRp).[8-9] We reasoned that the epitranscriptomic systems of different individuals are not identical, and therefore they can modify the viral RNA genome differently. The difference in the modifications may affect viral life cycles by inhibiting or activating the RdRp, and as a result may contribute to the difference of clinical outcomes of COVID-19. Among other observations during the studies, we found that the $m^1A$ modification in an RNA template is capable to stop the RNA polymerization reaction catalyzed by SARS-CoV-2 RdRp.[8] With this finding, we were curious to know if $m^1A$ could be incorporated into RNA by the same RdRp via its triphosphate ($m^1ATP$). If the answer were positive, prodrugs based on $m^1ATP$ and other modified nucleoside triphosphates could be developed to fight SARS-CoV-2 and other RNA viruses.

## Results and Discussion

To test if $m^1A$ can be incorporated into RNA via $m^1ATP$ by SARS-CoV-2 RdRp, the 20-mer primer RNA (**1**), which has a FAM at its 5'-end, and the 30-mer template RNA (**2**) were synthesized. The preformed primer-template duplex (**1**-**2**) was subjected to various RNA polymerization conditions catalyzed by RdRp. The results were analyzed using denatured polyacrylamide gel electrophoresis (PAGE, Figure 1). Lane 1 is a control, and only the preformed primer-template duplex (**1**-**2**) was loaded. The two bands indicate that the duplex can be denatured and the gel can separate the primer and template. Lane 2 is also a control. The RNA polymerization reaction was carried out in the presence of all the four natural NTPs (ATP, CTP, GTP and UTP), and no $m^1ATP$ was added. As expected, the primer was all converted to the 30-mer RNA **3**, where X is A. From this lane, we can also see that the 30-mer extended primer **3**, which has a FAM, can be well separated from the 30-mer template **2**, which does not have a FAM. Lane 3 is the result of $m^1A$ incorporation. As can be seen, when the RNA polymerization reaction was conducted in the presence of $m^1ATP$, CTP, GTP and UTP, even though there was no ATP, the reaction proceeded smoothly. This indicates that $m^1A$ can be incorporated into RNA via $m^1ATP$ by SARS-CoV-2 RdRp.

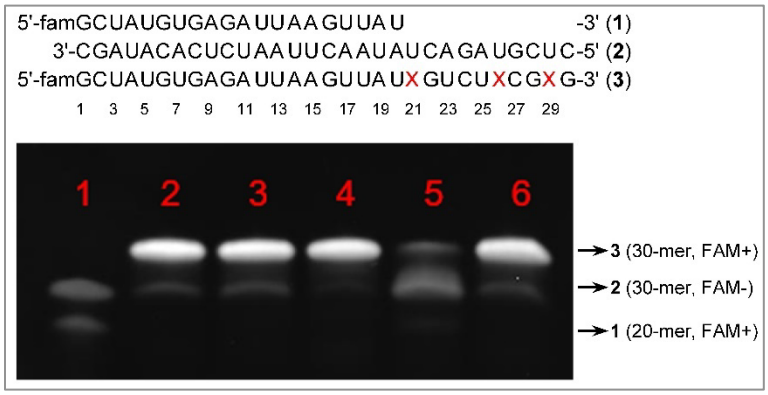


**Figure 1.** Image of denatured PAGE for the analysis of SARS-CoV-2 RdRp catalyzed RNA polymerization reactions. X in **3** is the nucleoside A or $m^1A$ depending on the NTP used. The mixture of RdRp, primer-template duplex **1**-**2** and NTPs was incubated at 37 °C for 2 h. The reaction products were analyzed with PAGE. The image was taken after staining with GelRed. Lane 1: RNA duplex only. Lane 2: ATP, CTP, GTP and UTP, no $m^1ATP$. Lane 3: $m^1ATP$, CTP, GTP and UTP, no ATP. Result indicates that $m^1A$ can be incorporated into RNA via $m^1ATP$. Lane 4: $m^1ATP$ only for 15 min, then ATP, CTP, GTP and UTP. Results indicate $m^1ATP$ does not inhibit RdRp. Lane 5: CTP, GTP, UTP, no ATP, no $m^1ATP$. Results indicate that ATP and $m^1ATP$ in other lanes were needed to reach 30-mer. Results also indicate that one or more of the nucleotides in CTP, GTP or UTP can be incorporated into RNA across U in the template although with low efficiency as indicated by close to complete disappearance of primer, appearance of partially extended primer and small amount of fully extended primer. The latter may also be partially extended 28-mer. The results are consistent with the low fidelity of RdRp. Lane 6: ATP, $m^1ATP$, CTP, GTP and UTP. Overall, the results indicate that $m^1A$ can be incorporated into RNA by SARS-CoV-2 RdRp via $m^1ATP$.

To see if the RNA polymerization reaction could still proceed in the absence of both $m^1ATP$ and ATP, the reaction was conducted in the presence of only CTP, GTP and UTP (lane 5). As can be seen, the reaction did not go well. Only a small portion of the primer was extended to the 30-mer **3**, which might also be the 28-mer RNA resulted from stopping the reaction at the last U (position 29) in **2** because there is a slight chance that the gel could not resolve 28-mer and 30-mer. The majority of the primer was only partially extended, and the reaction might have stopped at any of the three U positions in the single-stranded region of the primer-template duplex. Careful examination of the gel image can also reveal that a small portion of the primer remained unreacted, but this may not be easy to see in Figure 1 as the band is very light. The results in this lane (lane 5) indicate that either $m^1ATP$ or ATP is needed for the reaction to pass the positions

occupied by U in the single stranded region of template **2** at the efficiency observed in experiments related to lanes 2 and 3. This further confirms that $m^1A$ can be incorporated into RNA via $m^1ATP$ by SARS-CoV-2 RdRp. The close to disappearance of primer and the appearance of smear in the region between **1** and **3** in lane 5 is consistent with the low fidelity of the RdRp as reported in the literature.[10-11] Experiments related to lanes 4 and 6 were designed and conducted before we knew that $m^1ATP$ could be an effective substrate of the RdRp. Indeed, we thought that the chance for the RdRp to incorporate $m^1A$ into RNA via $m^1ATP$ were low considering that $m^1A$ in template RNA inhibited the RdRp. In case that were the case, the experiments related to lanes 4 and 6 would determine if $m^1ATP$ could inhibit the incorporation of ATP by RdRp. Now that $m^1A$ can be incorporated, these experiments are irrelevant.

From the above experiments and the data we published earlier regarding inhibition of SARS-CoV-2 RdRp by $m^1A$ in RNA template,[8] it is clear that $m^1ATP$ based prodrugs may be investigated for the treatment of COVID-19. The reason is that once $m^1ATP$ is formed from a prodrug in SARS-CoV-2 infected cells, the viral RdRp could incorporate $m^1A$ into its RNA genome. Once $m^1A$ is in the RNA genome, replication of genome by the viral RdRp would be stopped, and the viral life cycle would be interrupted. It is noted that for this drugging mechanism to function, there is no need for all A in the viral RNA genome to become $m^1A$. Perhaps, only a few or even only one A in a key position is needed to be replaced by $m^1A$ to stop the functioning of the entire viral genome.

Besides $m^1A$, there are over 300 epitranscriptomic RNA modifications.[12-14] Among them, we have found that $m^3C$ in RNA template does not inhibit SARS-CoV-2 RdRp,[8] which was surprising considering that $m^1A$ inhibits the RdRp and both $m^1A$ and $m^3C$ disrupt canonical base pairing. However, even though $m^3C$ does not inhibit RdRp, it is unlikely that the nucleotide incorporated across it is precisely G due to G-C base pair disruption by the modification. Therefore, if $m^3CTP$ could be an effective substrate of RdRp, $m^3CTP$ based prodrugs, which would function through random mutagenesis like favipiravir,[15-16] could also be studied. Besides $m^1A$ and $m^3C$, many of the other epitranscriptomic modifications could be studied for similar purposes.[17]

In addition to SARS-CoV-2, other RNA viruses such as HCoV 229E, HCoV OC43, HCV and HIV[18-19] could also be considered to be combatted using prodrugs based on $m^1A$ and other epitranscriptomic RNA modifications in a similar manner. Of course, the concept can also go beyond RNA viruses and extend to DNA viruses and even non-infectious diseases. Indeed, the large pool of epitranscriptomic RNA modifications and their variations could become a treasury for drug discovery. Importantly, drugs based on these modifications could likely have less adverse effect for reasons such as lower chance to generate toxic metabolites.[20]

## Conclusion

In conclusion, we found that $m^1A$ can be incorporated into RNA via $m^1ATP$ by SARS-CoV-2 RdRp. Earlier, we reported that $m^1A$ in RNA template could inhibit the same RdRp.[8] From these two discoveries, opportunities appear that $m^1A$ based prodrugs could be developed to combat SARS-CoV-2. Using a similar approach, the development of prodrugs based on other epitranscriptomic RNA modifications to fight SARS-CoV-2 and other viruses could be considered. Further investigation of the possibility of using $m^1A$ based prodrugs to combat COVID-19 under more biologically relevant conditions as well as using prodrugs based on other RNA modifications for treating COVID-19 and other infectious diseases are being pursued.

## Supporting Information

Experimental details for SARS-CoV-2 RdRp catalyzed RNA polymerization reaction.

## Conflicts of Interest

Michigan Tech has interest in the IP related to this report.

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