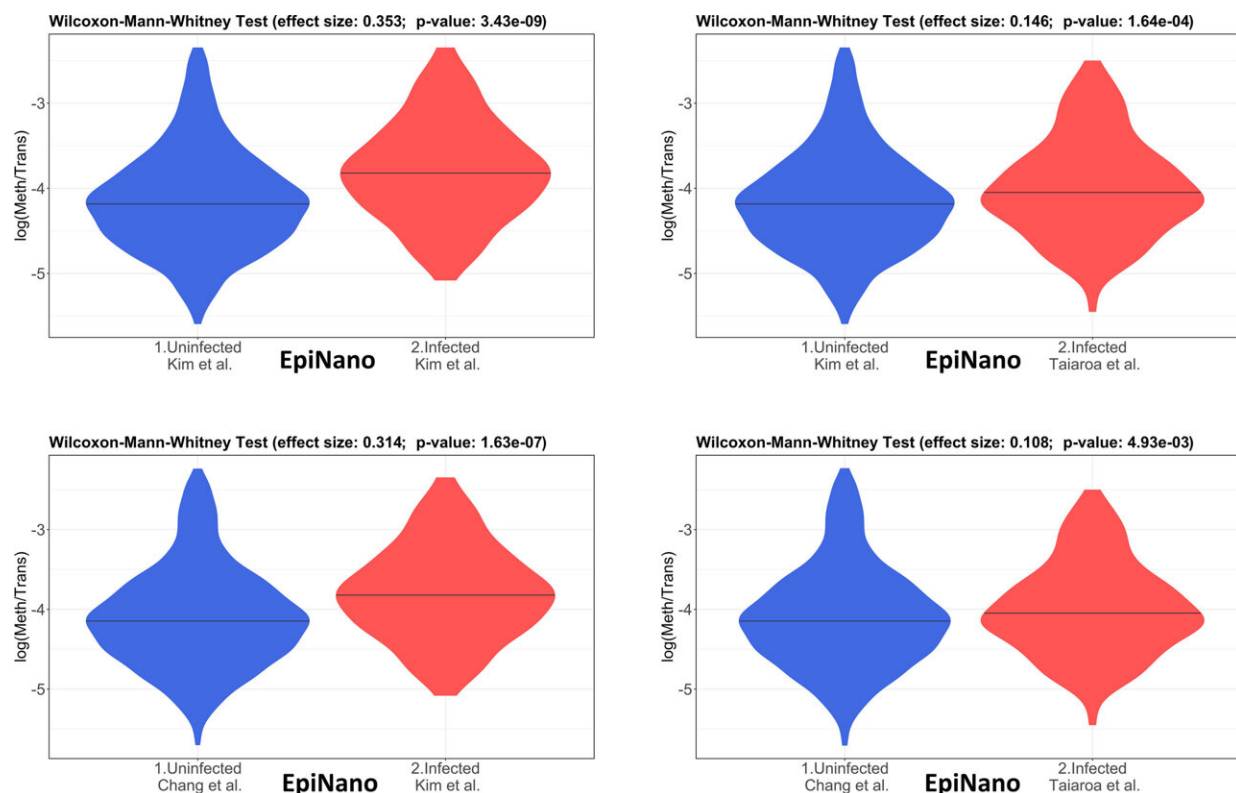


SARS-CoV-2 alters RNA in infected cells, study reveals

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Violin plots of the distributions of differentially methylated transcripts between Uninfected and Infected Vero cell datasets of using program EpiNano. The areas indicate the data distribution of each sample and the horizontal bars in the middle of the areas indicate the Medians. The Effect size and p-values are as obtained by the Wilcoxon-Mann-Whitney test as described in Material and Methods. The abscissas indicate the samples and the ordinates indicate the quantity $S = \log(\text{methylated sites}/\text{transcript})$, which is the logarithm with base 10 of the proportion of methylated sites per transcript, for all transcript types in each dataset. Credit: *Frontiers in Cellular and Infection Microbiology* (2022).

For the first time, scientists at the Federal University of São Paulo (UNIFESP) in Brazil have shown that infection by SARS-CoV-2, the virus that causes COVID-19, changes the functioning of host cell RNA. They arrived at this conclusion by analyzing 13 datasets obtained during four studies of viral, human and animal cell RNA.

The most recent study, reported in an article published in *Frontiers in Cellular and Infection Microbiology*, examined the epitranscriptome of Vero [cells](#) (derived from monkeys) and human Calu-3 cells by direct RNA sequencing. An epitranscriptome is the collection of biochemical modifications of cell RNA, such as methylation.

"Our first important finding in this study was that infection by SARS-CoV-2 increases the level of m6a [N6-methyladenosine], a type of methylation, in host cells compared with non-infected cells," Marcelo Briones, last author of the article, told Agência FAPESP. Briones is a professor at UNIFESP's Medical School (EPM) and a researcher affiliated with its Center for Medical Bioinformatics.

Methylation is a biochemical modification involving the addition of a methyl group to a substrate. It occurs in cells via the action of enzymes capable of transferring part of one molecule to another. This changes the behavior of proteins, enzymes, hormones and genes. The researchers demonstrated changes to infected cell RNA quantitatively by analyzing all the RNAs present in the cells and qualitatively by locating on a map the number of methylations per region in the nucleotides.

The study was a continuation of an earlier genomic analysis published in 2021, where the researchers analyzed the methylation pattern in SARS-

CoV-2.

"Methylation has two functions in viruses. It regulates [protein expression](#), and it defends the virus against the action of interferon, a potent antiviral substance produced by the host organism," Briones said.

In both studies, the researchers analyzed m6a because it is the most common type of RNA nucleotide modification and is involved in several significant processes, such as intracellular location and protein translation. RNA nucleotides contain nitrogenous bases (adenine, guanine, uracil, or cytosine) running along a single strand.

The team also discovered that different strains of the virus displayed variations in the sequences of nitrogenous bases in their nucleotides. "Some strains may be much more methylated than others. If so, they can proliferate better inside host cells," Briones said.

They also found that nucleotide sequences known as m6a DRACH motifs were slightly different in SARS-CoV-2 and in cells. In this acronym, which is frequently used in epigenetics, the letter D stands for adenine, guanine or uracil; R for adenine or guanine; A for the methylated residue; C for cytosine; and H for adenine, cytosine or uracil.

The virus uses cell enzymes for its own methylation, producing evolutionary pressure for adaptation of viral DRACH sequences so that they become more similar to cell sequences. The viral strains that adapt best are able to escape interferon more successfully.

After completing their investigation of how SARS-CoV-2 modifies m6A in host cells, the scientists' next step will be to analyze the stored data in search of a correlation between viral RNA methylation levels and the number of viruses released from each [infected cell](#), known as viral burst size.

"The more methylated the viruses, the more they grow in the cell cytoplasm and the larger the burst size," Briones explained. Under normal conditions, without stimuli, a viral particle replicates a thousand times. "The findings pave the way to novel treatments for COVID-19 and repurposing of known drugs." They also offer elements for a deeper understanding of how [viral strains](#) escape the immune system.

Methodology

The Nanopore direct RNA sequencing method (Oxford Nanopore Technologies) used in the study has several advantages, according to the researchers. One of these is that it dispenses with the modifications required by the conventional method (reverse transcription polymerase chain reaction, or RT-PCR) to read the RNA strand.

To examine a virus using RT-PCR, scientists must first convert its RNA to DNA (reverse transcription). The result is cDNA, where the 'c' stands for complementary. This is because only DNA (which is double-stranded) can be copied. The cDNA is then amplified by being copied hundreds of thousands of times, creating billions of clones so that enough of the target sections of viral DNA are available for analysis, instead of a minuscule amount.

For Briones, researchers may be confused by distortions resulting from the production of viral sequences from cDNA. "Some scientists think nucleotides are switched owing to the presence of epigenetically modified bases. This needs to be investigated in a systematic manner," he said.

The increase in cell methylation was mapped by two m6A detection programs. One of these (m6anet) used a machine learning technique called multiple instance learning (MIL). The other (EpiNano) validated the results using a technique called support vector machine (SVM).

More information: João H. C. Campos et al, The epitranscriptome of Vero cells infected with SARS-CoV-2 assessed by direct RNA sequencing reveals m6A pattern changes and DRACH motif biases in viral and cellular RNAs, *Frontiers in Cellular and Infection Microbiology* (2022). [DOI: 10.3389/fcimb.2022.906578](https://doi.org/10.3389/fcimb.2022.906578)

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