mRNA vaccines: Why and how they should be modified

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The COVID-19 pandemic has stimulated the production of different therapeutic approaches for the resolution of coronavirus infections. On one hand, nanobiomolecules have been proposed as bait material for viruses,[1,2] on the other hand unconventional messenger RNA vaccines have been produced like SARS-CoV-2 mRNA vaccines (BioNTech/Pfizer BNT162b2 and Moderna mRNA-1273). A not negligible advantage of these mRNA-based vaccines is the speed with which they can be developed, especially in light of the discovery of new viral genetic variants and the need to adapt the vaccine to the rapid genetic changes of the virus. However, the biology of "retrotransposons" suggests greater caution in their large-scale use. The idea that the mRNAs of vaccines used to stimulate the immune response to SARS-CoV-2 are reluctant to integrate into the cellular genome needs more in-depth studies to be confirmd.[3-9] In our opinion, these studies should take in consideration that the human genome contains L1 retrotransposons, DNA sequences that are autonomously capable of increasing their copy number through a mechanism of retro-transcription, from RNA to DNA, and concomitant insertion of the neo-DNA copy in a different genomic locus than the original. In theory, any mRNA of the cellular cytoplasm could be recognized by the proteins of the molecular machinery of the endogenous L1 retroelements, and could be integrated into the genome in the form of a new copy of DNA. We hope to convince the scientific community that further studies are needed to better understand the mutagenicity of mRNA vaccines and in vitro experiments should be design to elucidate molecular strategies able to limit the effects of L1 on mRNA vaccine. L1 retroelements are DNA elements of approximately 6 kilobases and make up nearly 20% of the human genome. Their copies are replicated in the genome by a mechanism of L1-retrotransposition.[10,11] L1 messenger RNA encodes a few proteins that bind to their own messenger RNA, including ORF1p and ORF2p. The latter is a multifunctional protein with endonuclease and reverse transcriptase enzymatic activities: the most important properties to increase the number of L1 copy in a genome. In the nucleus of cells, the mRNA-L1 is eventually retro-transcribed and integrated into consensus regions of genome, 5'-TTTT / AA-3', rich in Adenine/Thymine.[12] For completeness of information, another L1 protein, ORF0p, should also be mentioned, which would help to improve the efficiency of retrotransposition.[13] The result of the mechanism of L1-retrotransposition is the massive accumulation of mobile elements in all cells of the genomes, from germ cells to somatic cells, including nerve cells, where the phenomenon of retrotransposition is well studied.[14]

In many eukaryotes, cellular mRNAs are endogenously retro-transcribed and reintegrated into their own genome, producing an increase in the number of copies, or rather, retrocopies. This process is extensively studied in primates and mice.[15,16] The mechanism of retrotransposition is mainly based on the binding of the ORF2p protein, encoded by L1, to the poly-A tail of the L1 mRNA. In this case, the interaction is called "*cis"*-association to differentiate it from "*trans"*-association when the L1 protein complex recognizes messenger RNAs that are not of L1 origin.[17] The binding of ORF2p to the poly-A tail of the mRNA plays a crucial role in this process.[18] Hypothetically, proteins encoded by L1, including ORF1p and ORF2p, could interact with any mRNA, including exogenous mRNA that is carried by the vaccine which could be reverse transcribed and integrated into the genome.[19,20] It is estimated that in humans there are several thousand retrocopies that may be at the origin of genes for some human diseases, including tumors.[16,21-26]

Structurally, the messenger RNA of both the BNT162b2 vaccine and the mRNA-1273 vaccine exhibit typical eukaryotic messenger RNA architecture, with some useful modifications to improve its translation and escape the immune system.[27,28] One of these structural elements is the poly-A tail at the 3' end of 110 nucleotides which, as reported above, should make these mRNAs

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excellent targets suitable for L1-governed trans retro-integration.[17] It is urgent and necessary to undertake a specific experimental study that demonstrates the real possibility of vaccine mRNAs being captured by the L1 machinery and being retro-integrated into the genome. These studies should also help to understand how to avoid trans-association between L1 proteins and vaccine mRNAs. In our opinion, genetic modification of the 3'-end of the poly-A should be done to evaluate, in in-vitro experiments, the kinetics of RNA-L1 protein association.[29] Recently, the 3'-end of the SARS-CoV-2 genome, shows to be more frequently integrated into cellular DNA than sequences closer to the 5' end.[30] Interestingly, the same study has showed that mRNAs from the SARS-CoV-2 genome can be back-transcribed by L1 elements and integrated into the genome of cultured human cells especially after viral infection.[10,30]

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