

mRNA vaccines: Why and how they should be modified

Isidoro Feliciello, Alfredo Procino

Department of Clinical Medicine and Surgery, University of Naples Federico II, School of Medicine, Naples, Italy

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 confirmed.³⁻⁹ In our opinion, these studies should take in consideration that the human genome con-

tains 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

Correspondence: Isidoro Feliciello, Department of Clinical Medicine and Surgery, University of Naples Federico II, School of Medicine, via Sergio Pansini 5, 80130 Napoli, Italy.
Tel.: +39 3495250441.
E-mail: isidoro.feliciello@unina.it

Key words: L1 retrotransposon; mRNA-vaccines; mutation.

Contributions: IF wrote the paper. AP provided critical feedback. All authors read and approved the final manuscript.

Conflict of interest: The authors declare no conflict of interest.

Funding: this work was supported by the Italian Ministry of Education, University and Research (MIUR), fund for Investments on Basic Research (FFABR).

Ethics approval and consent to participate: Not applicable.

Consent for publication: Not applicable.

Availability of data and material: Not applicable.

Received for publication: 3 September 2021.

Revision received: 29 September 2021.

Accepted for publication: 29 September 2021.

©Copyright: the Author(s), 2021
Licensee PAGEPress, Italy
Journal of Biological Research 2021; 94:10072
doi:10.4081/jbr.2021.10072

This article is distributed under the terms of the Creative Commons Attribution Noncommercial License (by-nc 4.0) which permits any noncommercial use, distribution, and reproduction in any medium, provided the original author(s) and source are credited.

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}

References

- Feliciello I, Procinio A. The pulmonary-proteoliposome as a new therapeutic approach for Coronaviruses. *Hum Vaccin Immunother* 2020;16:2373.
- Tombácz I, Weissman D, Pardi N. Vaccination with messenger RNA: a promising alternative to DNA vaccination. *Methods Mol Biol* 2021;2197:13-31.
- Pardi N, Weissman D. Nucleoside modified mRNA vaccines for infectious diseases. *Methods Mol Biol* 2017;1499:109-21.
- Youn H, Chung JK. Modified mRNA as an alternative to plasmid DNA (pDNA) for transcript replacement and vaccination therapy. *Expert Opin Biol Ther* 2015;15:1337-48.
- Funk CD, Laferrière C, Ardakani A. A snapshot of the global race for vaccines targeting SARS-CoV-2 and the COVID-19 pandemic. *Front Pharmacol* 2020;11:937.
- Orlandini von Niessen AG, Poleganov MA, Rechner C, et al. Improving mRNA-based therapeutic gene delivery by expression-augmenting 3' UTRs identified by cellular library screening. *Mol Ther* 2019;27:824-36.
- Wadhwa A, Aljabbari A, Lokras A, et al. Opportunities and challenges in the delivery of mRNA-based vaccines. *Pharmaceutics* 2020;12:102.
- Liu MA. A comparison of plasmid DNA and mRNA as vaccine technologies. *Vaccines (Basel)* 2019;7:37.
- Doerfler W. Adenoviral vector DNA- and SARS-CoV-2 mRNA-based Covid-19 vaccines: possible integration into the human genome - are adenoviral genes expressed in vector-based vaccines? *Virus Res* 2021;302:198466.
- Jones RB, Song H, Xu Y, et al. LINE-1 retrotransposable element DNA accumulates in HIV-1-infected cells. *J Virol* 2013;87:13307-20.
- International Human Genome Sequencing Consortium. Initial sequencing and analysis of the human genome. *Nature* 2001;409:860-921. Erratum in: *Nature* 2001;412:565. Erratum in: *Nature* 2001;411:720.
- Mita P, Wudzinska A, Sun X, et al. LINE-1 protein localization and functional dynamics during the cell cycle. *Elife* 2018;7:e30058.
- Denli AM, Narvaiza I, Kerman BE, T et al. Primate-specific ORF0 contributes to retrotransposon-mediated diversity. *Cell* 2015;163:583-93.
- Baillie JK, Barnett MW, Upton KR, et al. Somatic retrotransposition alters the genetic landscape of the human brain. *Nature* 2011;479:534-7.
- Zhang Y, Li S, Abyzov A, Gerstein MB. Landscape and variation of novel retroduplications in 26 human populations. *PLoS Comput Biol* 2017;13:e1005567.
- Casola C, Betrán E. The genomic impact of gene retrocopies: what have we learned from comparative genomics, population genomics, and transcriptomic analyses? *Genome Biol Evol* 2017;9:1351-73.
- Doucet AJ, Wilusz JE, Miyoshi T et al. A 3' poly(A) tract is required for LINE-1 retrotransposition. *Mol Cell* 2015;60:728-41.
- Naufer MN, Furano AV, Williams MC. Protein-nucleic acid interactions of LINE-1 ORF1p. *Semin Cell Dev Biol* 2019;86:140-9.
- Kazazian HH Jr, Moran JV. Mobile DNA in health and disease. *N Engl J Med* 2017;377:361-70.
- Naufer MN, Furano AV, Williams MC. Protein-nucleic acid interactions of LINE-1 ORF1p. *Semin Cell Dev Biol* 2019;86:140-9.
- Richardson SR, Salvador-Palomeque C, Faulkner GJ. Diversity through duplication: Whole-genome sequencing reveals novel gene retrocopies in the human population. *BioEssays* 2014;36:475-81.
- Chatron N, Cassinari K, Quenez O, et al. Identification of mobile retrocopies during genetic testing: Consequences for routine diagnosis. *Human Mutation* 2019;40:1993-2000.
- Gardner EJ, Prigmore E, Gallone G, et al. Contribution of retrotransposition to developmental disorders. *Nat Commun* 2019;10:4630.
- ICGC Breast Cancer Group, Cooke SL, Shlien A, Marshall J et al. Processed pseudogenes acquired somatically during cancer development. *Nat Commun* 2014;5:3644.
- Scott E, Devine S. The role of somatic L1 retrotransposition in human cancers. *Viruses* 2017;9:131.
- PCAWG Structural Variation Working Group, PCAWG Consortium, Rodriguez-Martin B, Alvarez EG, Baez-Ortega et al. Pan-cancer analysis of whole genomes identifies driver rearrangements promoted by LINE-1 retrotransposition. *Nat Genet* 2020;52:306-19.
- Andries O, Mc Cafferty S, De Smedt SC, et al. N1-methylpseudouridine-incorporated mRNA outperforms pseudouridine-incorporated mRNA by providing enhanced protein expression and reduced immunogenicity in mammalian cell lines and mice. *J Control Release* 2015;217:337-44.
- Parr CJC, Wada S, Kotake K et al. N 1-Methylpseudouridine substitution enhances the performance of synthetic mRNA switches in cells. *Nucleic Acids Research* 2020;48:e35-e35.
- Barragán-Iglesias P, Lou TF, Bhat VD et al. Inhibition of Poly(A)-binding protein with a synthetic RNA mimic reduces pain sensitization in mice. *Nat Commun* 2018;9:10.
- Zhang L, Richards A, Barrasa MI, et al. Reverse-transcribed SARS-CoV-2 RNA can integrate into the genome of cultured human cells and can be expressed in patient-derived tissues. *Proc Natl Acad Sci USA* 2021;118:e2105968118.