CPQ Medicine (2023) 14:6
Commentary

The Matryoshka Code of COVID-19 mRNA Vaccines: Overlapping Viral Sequences?

Adonis Sfera[1*], Sabine Hazan[1], Dan O. Sfera[1] & Zisis Kozlakidis[3]

[1]*Patton State Hospital, Hospital in San Bernardino, California*

[2]*University of California, Riverside, United States*

[3]*International Agency for Research On Cancer (IARC), France*

[*]**Correspondence to:** Dr. Adonis Sfera, *et al*. Patton State Hospital, Hospital in San Bernardino, California & University of California, Riverside, United States.

Copyright

Received: 13 April 2023

Published: 18 May 2023

Keywords: *COVID-19; SARS-CoV-2*

We read with great interest the paper by Beaudoin CA *et al*. "Are There Hidden Genes in DNA/RNA Vaccines?", reporting overlapping sequences between the SARS-CoV-2 spike (S) glycoprotein and two viral genes [1]. If translated, the undesired proteins may cause rare, untoward effects, including those recorded in Vaccine Adverse Event Reporting System (VAERS).

These findings are in line with our own research and that of others however, aside from overlapping genes (OLGs), the S protein also contains overlapping molecular structures and signals (heptad repeats, simple sequence repeats, calcium calmodulin kinase II, and prion-like domains) that can lead to VAERS-recorded pathology [2-6].

Overlapping genetic and structural information are important to viruses, as they maximize the number of translated proteins derived from the same genetic information [7]. This compact arrangement also allows for the emergence of mutations without major genetic restructuring [8]. Furthermore, there is evidence that such structures also regulate gene expression in many viruses [9], including coronaviruses [10,11] (Fig. 1).

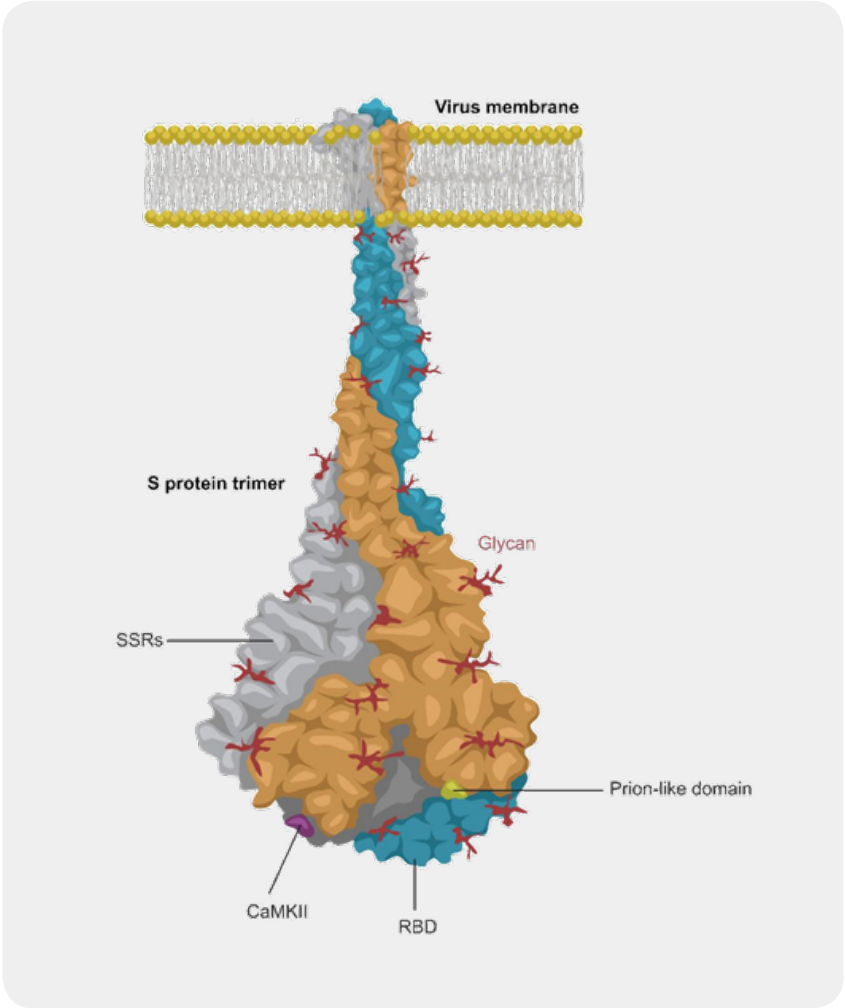

Figure 1: *The overlapping molecular structures and signals in the S protein of SARS-CoV-2 virus. Glycosylation is a viral strategy for successfully exploiting host translational machinery. Vaccination with SARS-CoV-2 S protein lacking glycan shields elicits enhanced responses therefore, glycosylation may have been altered in mRNA vaccines. RBD (receptor binding domain), CaMKII (calcium calmodulin kinase II), SSR(simple sequence repeats).*

Taking the above viral-derived complication into account, messenger RNA (mRNA) vaccines encode the full-length S protein that when expressed on the surface of cells, prompts the generation of neutralizing antibodies [12]. Thus, both OLGs and molecular systems may be translated too, contributing to vaccine complications and potential adverse effects.

Messenger RNA Vaccines, known Modifications

To elicit the generation of neutralizing antibodies, exogenously administered mRNA must be heavily engineered to avoid hydrolysis by the extracellular RNAases and detection by cytosolic immune sensors [13,14]. Placing the nucleic acid backbone into lipid nanoparticles (LNPs), hides it from RNAases, while

codon optimization, replacing uridine with N1-methylpseudouridine (m1Ψ), renders the vaccine undetectable to sensors [15,16]. Other adjustments were made in the untranslated regions (UTRs) and polyadenylated (polyA) tail to protect and stabilize the vaccine [14,16,17]. Another known change, addition of two proline residues, maintain the S antigen in prefusion conformation to augment immune system exposure [18]. Moreover, aside from m1Ψ, codon optimization includes increased the CG content and possibly G-quadruplex structures to enhance translation [6].

Potential Unknown Changes

Aside from the reported changes, the mRNA encoded S antigen may have been engineered further to increase efficacy and translation.

Sense Codon Reassignment?

Pfizer/BioNTech has published the mRNA vaccine sequences, allowing scientists and clinicians to compare codons with the wild type S protein. However, the translated peptides remain proprietary therefore, at this time, it is not possible to rule out sense codon reassignment or introduction of unnatural proteins [19]. This is important as genetic code expansion and incorporation of immunogenic noncanonical amino acids, patented in 2018 (WO2019193416A1), were evaluated for utilization in genetic vaccines [20]. Some unnatural amino acids, especially homoarginine, was associated with heart disease and sudden death therefore, these artificial building blocks may in rare occasions directly contribute to VAERS-recorded events [21,22].

Sugar Coating or Not?

It is unknown at this time whether the S protein glycans were altered to increase the efficacy of the mRNA therapeutics. However, vaccine-elicited neutralizing antibodies exhibit a distinct glycosylation pattern than post-infection antibodies, indicating possible manipulation [23-25]. This is significant as glycosylation plays a major role in cardiovascular and endothelial homeostasis, providing a potential link to VAERS-recorded events [26,27] (Fig. 1).

The S Antigen Molecular Systems

Several biomolecular systems are present in the S protein of SARS-CoV-2 that when translated may trigger secondary pathways linked to vaccine adverse effects. These systems include simple sequence repeats, heptad repeats, calcium calmodulin kinase II, and prion-like domains [3-6]. Translation of these molecular structures may lead to new viral variants, pathological cell-cell fusion, and defective proteostasis. These potential links, derived from the viral legacy of overlapping genetic and structural information will be briefly presented below:

-Simple Sequence Repeats (SSRs)

Also called microsatellites, SSRs are present in the genomes of many viruses, including SARS-CoV-2, accounting for a number of the new, emerging variants [28,29]. Trinucleotide and hexanucleotide repeats

are the most common SSRs that, aside from their role in viral genomes, contribute to skeletal muscle pathology and neurodegeneration, possibly explaining vaccine-induced neuropsychiatric and neuromuscular symptoms [30,31]. Interestingly, SSRs are influenced by the CG content of nucleic acids which is elevated in COVID-19 mRNA vaccines, linking these therapeutics to the emergence of new variants [32,33].

The DNA mismatch repair factor, MSH3, previously associated with trinucleotide repeats, was also found to function as a sensor for G-quadruplexes therefore, opposing codon optimization [34,35]. This is interesting as a novel study found a proprietary, Moderna-owned, reverse MSH3 sequence that matches the SARS-CoV-2 furin cleavage site, suggesting an OLG [36]. Indeed, to protect the optimized CG content and G-quadruplexes, MSH3 may need to be attenuated or inhibited, explaining the reason this reverse sequence could have been patented (US-9587003-B2).

-Heptad Repeats

There are two heptad repeats in the S protein of SARS-CoV-2 that assemble into a six-helix bundle to execute membrane fusion [37]. Translation of these structures likely accounts for vaccine-induced pathological cell-cell fusion, that could result in rare post-vaccination events, such as giant cell myocarditis [38,39].

-Calcium Calmodulin Kinase II

Cell-cell fusion can also be promoted by calcium calmodulin kinase II (CaMKII), a system detected in the S antigen of the SARS-CoV-2 virus [4]. CaMKII may promote post-vaccination pathological syncytia, probably accounting for VAERS-reported multinucleated giant cells thyroiditis or myocarditis [4,40].

-Prion-like Domains

The receptor-binding domain (RBD) of the SARS-CoV-2 virus contains a prion motif that could be translated, leading to pathology [5]. Indeed, post-vaccination Creutzfeldt-Jakob disease (CJD) was reported by two separate studies, indicating that the prion motif may get translated [41,42].

In Summary

OLG and overlapping molecular structures are common occurrence in viruses and contribute to a number of biological processes. However, such overlapping information may also be translated with the vaccine mRNA, thus inadvertently increasing the odds of pathology. An mRNA vaccine expressing only the RBD may lower the susceptibility for adverse effects.

Disclaimer

Where authors are identified as personnel of the International Agency for Research on Cancer/WHO, the authors alone are responsible for the views expressed in this article and they do not necessarily represent the decisions, policy or views of the International Agency for Research on Cancer/WHO.

Bibliography

1. Beaudoin, C. A., Bartas, M., Volná, A., Pečinka, P. & Blundell, T. L. (2022). Are There Hidden Genes in DNA/RNA Vaccines? *Front Immunol., 13,* 801915.

2. Osorio, C., Sfera, A., Anton, J. J., Thomas, K. G., Andronescu, C. V., Li, E., Yahia, R. W., Avalos, A. G., Kozlakidis, Z. (2022). Virus-Induced Membrane Fusion in Neurodegenerative Disorar. *Front Cell Infect Microbiol., 12,* 845580.

3. Garushyants, S. K., Rogozin, I. B. & Koonin, E. V. (2021). Template switching and duplications in SARS-CoV-2 genomes give rise to insertion variants that merit monitoring. *Commun Biol., 4*(1), b1343.

4. Wenzhong, L. & Hualan, L. (2021). COVID-19: the CaMKII-like system of S protein drives membrane fusion and induces syncytial multinucleated giant cells. *Immunol Res., 69*(6), 496-519.

5. Tetz, G. & Tetz, V. (2022). Prion-like Domains in Spike Protein of SARS-CoV-2 Differ across Its Variants and Enable Changes in Affinity to ACE2. *Microorganisms, 10*(2), 280.

6. Xia, X. (2021). Detailed Dissection and Critical Evaluation of the Pfizer/BioNTech and Moderna mRNA Vaccines. *Vaccines (Basel)., 9*(7), 734.

7. Schlub, T. E. & Holmes, E. C. (2020). Properties and abundance of overlapping genes in viruses. *Virus Evolution, 6*(1), veaa009.

8. Cassan, E., *et al*. (2016). Concomitant Emergence of the Antisense Protein Gene of HIV-1 and of the Pandemic. *Proceedings of the National Academy of Sciences of the United States of America,* 11537-11642.

9. Han, L. L., *et al*. (2017). Genetic and Functional Diversity of Ubiquitous DNA Viruses in Selected Chinese Agricultural Soils. *Scientific Reports, 7,* 45142.

10. Okura, T., Shirato, K., Kakizaki, M., Sugimoto, S., Matsuyama, S., Tanaka, T., *et al*. (2022). Hydrophobic Alpha-Helical Short Peptides in Overlapping Reading Frames of the Coronavirus Genome. *Pathogens, 11*(8), 877.

11. Firth, A. E. (2020). A putative new SARS-CoV protein, 3c, encoded in an ORF overlapping ORF3a. *The Journal of General Virology, 101*(10), 1085.

12. Turner, J. S., O'Halloran, J. A., Kalaidina, E., Kim, W., Schmitz, A. J., Zhou, J. Q., *et al*. (2021). SARS-CoV-2 mRNA vaccines induce persistent human germinal centre responses. *Nature, 596*(7870), 109-113.

13. Andries, O., Mc Cafferty, S., De Smedt, S. C., Weiss, R., Sanders, N. N. & Kitada, T. N. (2015). (1)-methylpseudouridine-incorporated mRNA outperforms pseudouridine-incorporated mRNA by providing enhanced protein expression and reduced immunogenicity in mammalian cell lines and mice. *J Control Release., 217,* 337-344.

14. Schoenmaker, L., Witzigmann, D., Kulkarni, J. A., *et al.* (2021). mRNAlipid nanoparticle COVID-19 vaccines: Structure an stability. *Int J Pharm., 601,* 120586.

15. Nance, K. D. & Meier, J. L. (2021). Modifications in an Emergency: The Role of N1-Methylpseudouridine in COVID-19 Vaccines. *ACS Cent Sci., 7*(5), 748-756.

16. Gebre, M. S., Rauch, S., Roth, N., *et al*. (2022). Optimization of non-coding regions for a non-modified mRNA COVID-19 vaccine. *Nature, 601*(7893), 410-414.

17. Jeeva, S., Kim, K. H., Shin, C. H., Wang, B. Z. & Kang, S. M. (2021). An Update on mRNA-Based Viral Vaccines. *Vaccines (Basel)., 9*(9), 965.

18. Riley, T. P., Chou, H. T., Hu, R., Bzymek, K. P., Correia, A. R., Partin, A. C., *et al*. (2021). Enhancing the Prefusion Conformational Stability of SARS-CoV-2 Spike Protein Through Structure-Guided Design. *Front Immunol., 12,* 660198.

19. Theoharides, T. C. (2022). Could SARS-CoV-2 Spike Protein Be Responsible for Long-COVID Syndrome? *Mol Neurobiol., 59*(3), 1850-1861.

20. Fok, J. A. & Mayer, C. (2020). Genetic-Code-Expansion Strategies for Vaccine Development. *Chembiochem., 21*(23), 3291-3300.

21. März, W., Meinitzer, A., Drechsler, C., Pilz, S., Krane, V., Kleber, M. E., *et al.* (2010). Homoarginine, cardiovascular risk, and mortality. *Circulation, 122*(10), 967-975.

22. Drechsler, C., Meinitzer, A., Pilz, S., Krane, V., Tomaschitz, A., Ritz, E., *et al.* (2011). Homoarginine, heart failure, and sudden cardiac death in haemodialysis patients. *Eur J Heart Fail., 13*(8), 852-859.

23. Grant, O. C., Montgomery, D., Ito, K., *et al*. (2020). Analysis of the SARS-CoV-2 spike protein glycan shield reveals implications for immune recognition. *Sci Rep., 10*(1), 14991.

24. Zhao, X., Chen, H. & Wang, H. (2021). Glycans of SARS-CoV-2 Spike Protein in Virus Infection and Antibody Production. *Front Mol Biosci., 8,* 629873.

25. Farkash, I., Feferman, T., Cohen-Saban, N., Avraham, Y., Morgenstern, D., Mayuni, G., *et al*. (2021). Anti-SARS-CoV-2 antibodies elicited by COVID-19 mRNA vaccine exhibit a unique glycosylation pattern. *Cell Rep., 37*(11), 110114.

26. Franzka, P., Krüger, L., Schurig, M. K., Olecka, M., Hoffmann, S., Blanchard, V. & Hübner, C. A. (2021). Altered Glycosylation in the Aging Heart. *Front Mol Biosci., 8,* 673044.

27. Chacko, B. K., Scott, D. W., Chandler, R. T. & Patel, R. P. (2011). Endothelial surface N-glycans mediate monocyte adhesion and are targets for anti-inflammatory effects of peroxisome proliferator-activated receptor γ ligands. *J Biol Chem., 286*(44), 38738-38747.

28. Siddiqe, R. & Ghosh, A. (2021). Genome-wide in silico identification and characterization of Simple Sequence Repeats in diverse completed SARS-CoV-2 genomes. *Gene Rep., 23,* 101020.

29. Naghibzadeh, M., Savari, H., Savadi, A., Saadati, N. & Mehrazin, E. (2020). Developing an ultra-efficient microsatellite discoverer to find structural differences between SARS-CoV-1 and COVID-19. *Inform Med Unlocked, 19,* 100356.

30. Lieberman, A. P., Shakkottai, V. G. & Albin, R. L. (2019). Polyglutamine Repeats in Neurodegenerative Diseases. *Annu Rev Pathol., 14,* 1-27.

31. Yum, K., Wang, E. T. & Kalsotra, A. (2017). Myotonic dystrophy: disease repeat range, penetrance, age of onset, and relationship between repeat size and phenotypes. *Curr Opin Genet Dev., 44,* 30-37.

32. Qi, W. H., Yan, C. C., Li, W. J., Jiang, X. M., Li, G. Z., Zhang, X. Y., *et al.* (2016). Distinct patterns of simple sequence repeats and GC distribution in intragenic and intergenic regions of primate genomes. *Aging (Albany NY)., 8*(11), 2635-2654.

33. Ouyang, Q., Zhao, X., Feng, H., Tian, Y., Li, D., Li, M. & Tan, Z. (2012). High GC content of simple sequence repeats in Herpes simplex virus type 1 genome. *Gene, 499*(1), 37-40.

34. Flower, M., Lomeikaite, V., Ciosi, M., Cumming, S., Morales, F., Lo, K., *et al.* (2019). MSH3 modifies somatic instability and disease severity in Huntington's and myotonic dystrophy type 1. *Brain, 142*(7), 1876-1886.

35. Fleming, A. M. & Burrows, C. J. (2020). Interplay of Guanine Oxidation and G-Quadruplex Folding in Gene Promoters. *J. Am. Chem. Soc., 142*(3), 1115-1136.

36. Ambati, B. K., Varshney, A., Lundstrom, K., Palú, G., Uhal, B. D., Uversky, V. N., *et al.* (2022). MSH3 Homology and Potential Recombination Link to SARS-CoV-2 Furin Cleavage Site. *Front. Virol., 2*.

37. Hu, B., Guo, H., Zhou, P., *et al.* (2021). Characteristics of SARS-CoV-2 and COVID-19. *Nat Rev Microbiol., 19*(3), 141-154.

38. Sung, K., McCain, J., King, K. R., Hong, K., Aisagbonhi, O., Adler, E. D. & Urey, M. A. (2022). Biopsy-Proven Giant Cell Myocarditis Following the COVID-19 Vaccine. *Circ Heart Fail., 15*(4), e009321.

39. Kang, D. H., Na, J. Y., Yang, J. H., Moon, S. H., Kim, S. H., Jung, J. J., *et al*. (2022). Fulminant Giant Cell Myocarditis following Heterologous Vaccination of ChAdOx1 nCoV-19 and Pfizer-BioNTech COVID-19. *Medicina (Kaunas), 58*(3), 449.

40. Bornemann, C., Woyk, K. & Bouter, C. (2021). Case Report: Two Cases of Subacute Thyroiditis Following SARS-CoV-2 Vaccination. *Front Med (Lausanne)., 8,* 737142.

41. Creutzfeldt-Jakob Disease After the COVID-19 Vaccination. *Turk J Intensive Care*.

42. Jean claude Perez, claire Moret-Chalmin & RIP Luc Montagnier (2022). Towards the emergence of a new form of the neurodegenerative Creutzfeldt-Jakob disease: Twenty six cases of CJD declared a few days after a COVID-19 "vaccine" Jab (Version V4). Zenodo.