# Gene Vaccines: Can they change your DNA?

Compiled by: Researcher Stophe

## Introduction

- Is your Government lying to you?
- Do Gene Vaccines alter your DNA?
- What are the risks?
- How is this possible?

Fact-Checking

## COVID-19 vaccines are not gene therapy and a Forbes article doesn't say they are

The column attacks anti-vaccine activists and states that the vaccines do not alter DNA.

https://www.poynter.org/fact-checking/2021/covid-19-vaccines-are-notgene-therapy-and-a-forbes-article-doesnt-say-they-are/ https://www.health.gov.au/initiatives-and-programs/covid-19-

vaccines/is-it-true/is-it-true-can-covid-19-vaccines-alter-my-dna



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## Is it true? Can COVID-19 vaccines alter my DNA?

No, COVID-19 vaccines do not alter your DNA. Find out more below.

#### Can COVID-19 vaccines alter my DNA?

No, COVID-19 vaccines do not alter your DNA.

The Pfizer/BioNTech COVID-19 vaccine uses a fragment of messenger RNA (mRNA) to instruct your body to make an immune response against COVID-19.

There is a crucial difference between mRNA and DNA.

DNA, which makes up our genetic code, is larger, double stranded and very long. The mRNA is a single stranded copy of a small part of the DNA, which is often released to send instructions to other parts of the cell.

DNA is stored in the protected centre of our cells – the nucleus. The mRNA is broken down quickly by the body. It never enters the nucleus, and cannot affect or combine with our DNA in any way to change our genetic code.

Instead, COVID-19 mRNA vaccines teach the cell how to make a protein that triggers an immune response specific to COVID-19. The vaccines work with the body's natural defences to develop immunity to disease.

## Outline

- What is a Gene Vaccine?
- How do they work?
- Viral and Bacterial Vectors
- DNA Vaccines
- Designer Gene Vaccines
- Vector for HIV vaccines?
- Who funded this study?
- Self Replicating Genetic Vaccines?
- DNA Alteration?
- References

## What is a Gene Vaccine

- Gene vaccines are a new approach to immunization and immunotherapy in which, rather than a live or inactivated organism (or a subunit thereof), one or more genes that encode proteins of the pathogen are delivered.
- Gene vaccines make use of advances in immunology and molecular biology to more specifically tailor immune responses (cellular or humoral, or both) against selected antigens.

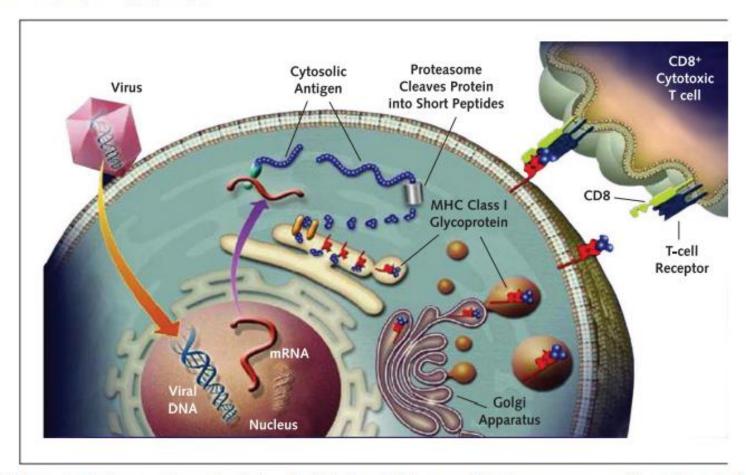


## What is a Gene Vaccine

- Gene vaccines provide a means to generate specific cellular responses while still generating antibodies, if desired.
- In addition, by delivering only the genes that encode the particular proteins against which a protective or therapeutic immune response is desired, the potential limitations and risks of certain other approaches can be avoided.

# How they work

Figure 1. Activation of cytolytic T lymphocytes.



Newly synthesized viral protein in the cytoplasm of an infected cell is degraded into peptides that are transported into the endoplasmic reticulum and then to the Golgi apparatus. Peptides bind to newly synthesized major histocompatibility complex class I molecules; the complex is then expressed on the surface of the cell where, in the presence of appropriate accessory molecules, binding to the T-cell receptor of CD8<sup>+</sup> cells can occur. This binding results in activation of the cytolytic T lymphocyte. Adapted from McDonnell and Askari (2), with permission from the Massachusetts Medical Society.

# Viral and Bacterial Vectors

• Genes encoding a protein antigen from the pathogen (that is, from a different virus or a tumor antigen) are then put in the virus particle. The virus acts as a transporter, in the **manner of a Trojan horse**, to deliver the gene encoding the antigen.

#### VIRAL AND BACTERIAL VECTORS

Viruses have highly evolved structures that enable them to bind to cells and deliver genes into the cells they infect. It may be feasible to use live virus vaccines to induce cytolytic T lymphocytes and thus treat a disease in which cellular immunity is critical. However, in some diseases (such as AIDS), concerns about the safety of a live attenuated vaccine are too great to make this a practical approach (6). In addition, a stronger immune response and an improved safety profile may be obtained by using a vaccine that delivers only the genes that encode the desired antigens (that is, the specific antigen against which the immune response important for protection is directed) rather than all the genes for a given pathogen. Therefore, efforts have been made to use harmless viruses or ones that have been rendered incapable of reproducing for delivery of genes derived from a different pathogen. In other words, a vector is made in which all the genes or key genes of a virus are removed or the virus is nonpathogenic or nonreplicative in humans. Genes encoding a protein antigen from the pathogen (that is, from a different virus or a tumor antigen) are then put in the virus particle. The virus acts as a transporter, in the manner of a Trojan horse, to deliver the gene encoding the antigen.

The viral vector is itself antigenic, and certain viruses cause inflammatory responses. These responses may benefit

## Viral and Bacterial Vectors

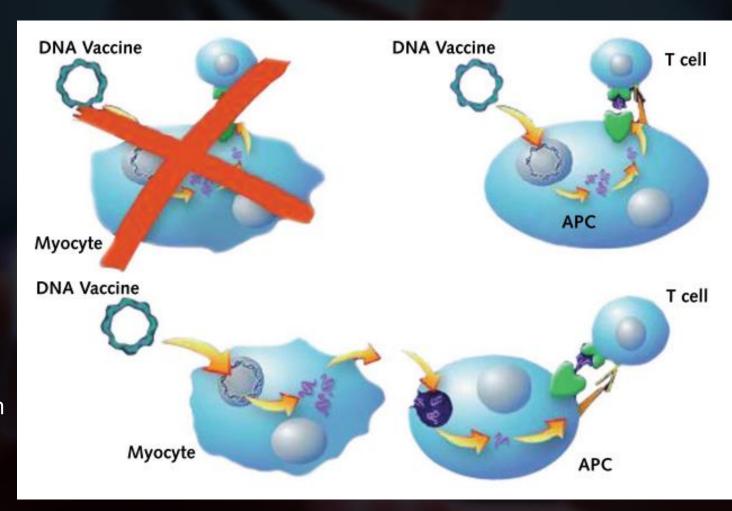
- Preexisting immunity due to previous infection or vaccination may limit the potency of the vaccine by clearing the viral vector before it can infect cells to deliver its payload of antigen genes.
- Similarly, if the vector is reused for a booster immunization, the immune response against the virus itself may eliminate too many of the vector particles before they can deliver the genes.

the desired immune response, depending on their type, location, and extent, but they might also be drawbacks. Preexisting immunity due to previous infection (for example, adenovirus) or vaccination (for example, smallpox) may limit the potency of the vaccine by clearing the viral vector before it can infect cells to deliver its payload of antigen genes. Similarly, if the vector is reused for a booster immunization, the immune response against the virus itself may eliminate too many of the vector particles before they can deliver the genes. Much effort has been directed to making vectors from other strains of viruses, vectors that are not as immunogenic, and vectors that do not induce inflammatory responses.

## **DNA Vaccines**

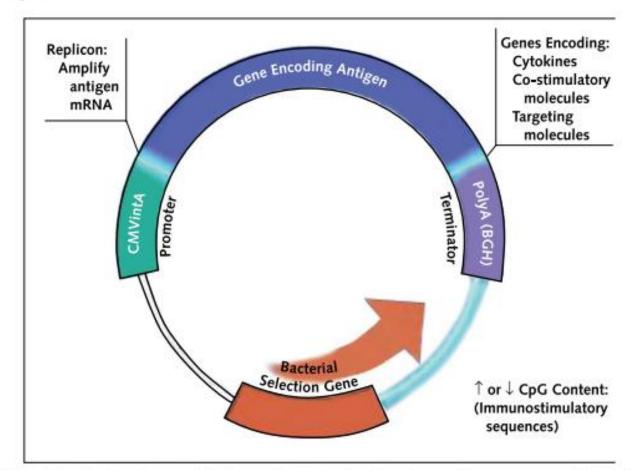
Figure 3. Mechanism of antigen presentation for generation of cytolytic T cells after DNA vaccination.

• Studies in bone marrow chimeric mice demonstrated that after immunization with plasmid DNA encoding an antigen, with plasmid DNA encoding an antigen, cytolytic T lymphocytes were activated by antigen-presenting cells (APC) that either had been directly transfected by the DNA or had received antigen via cross-priming, in which a non—antigen-presenting cell initially produces the protein encoded by the DNA vaccine, then transfers the antigen in some form to a professional antigen-presenting cell for generation of MHC class I restricted cytolytic T cells. Production of the protein antigen in non—antigen-presenting cells, such as myocytes, cannot result in direct stimulation of cytolytic T cells (3).



## Designer Gene Vaccines

Figure 4. Designer gene vaccines.



Sites within a DNA plasmid can be altered to potentially increase the potency of or alter the type of immune responses induced by the DNA vaccine. BGH = bovine growth hormone; CMVintA = cytomegalovirus promoter with the intron A sequence; mRNA = messenger RNA.

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- BGH = bovine growth hormone;
- CMVintA = cytomegalovirus promoter with the intron A sequence
- mRNA = messenger RNA.

## Vector for HIV Vaccines?

- Published in 2003
- Pushing for HIV Gene Vaccines Development?
- Sounds familiar?
  - Refer to Dr. David Martin's Interview with Dr. Reiner Fuellmich
- NIAID involvement?
- Dr. Fauci?
- How deep does this rabbit hole go?
- Who funded this study?

#### SUMMARY

In conclusion, recent developments in immunology and molecular biology have permitted development of DNA vaccines, which have wide-ranging applications (Table 2). Vaccines for such diseases as HIV infection, malaria, and tuberculosis are being developed by using plasmid DNA or viral or bacterial vectors to deliver the genes encoding antigens from pathogens to the host. As with the live attenuated virus vaccines that have been made for decades, antigenic proteins will be made in situ by the host, engendering cellular and humoral immune responses. But unlike live attenuated vaccines, gene-based vaccines are being designed to deliver only the genes encoding the antigens for the vaccine. The vectors themselves cannot replicate or revert to pathogenicity because they are rings of DNA (plasmid) or are viral or bacterial vectors designed at a molecular level to carry only the desired genetic se-

These DNA and other gene-based vaccines should enable generation of specific types of immune responses (cytolytic T cell, antibodies, and the desired type of T-helper cell). The ability to generate cellular as well as humoral responses may be crucial in making effective vaccines against diseases caused by viruses (such as HIV), intracellular bacteria (such as tuberculosis), and parasites (such as malaria), as well as cancer. Similarly, the ability to generate certain forms of an immunogen, such as a protein with a particular structure that can be formed only by mammalian cells in situ, may be a critical feature of gene vaccines. It is thought that gene vaccines will be amenable to production and distribution on a global scale to provide them to the groups most in need of prevention.

## Who Funded this Study?

Gene Vaccines | REVIEW

From Chiron Corporation, Emeryville, California; and Transgene, Strasbourg, France.

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- In the 1990s Genetic vaccine research begins
- DNA vaccines
  - Plasmid-based
  - Replicase-based
- RNA vaccines
- Self replicating genetic vaccines are theoretically very attractive prompting further research

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Vaccine. 1999 Dec 10; 18(9-10): 765-777.

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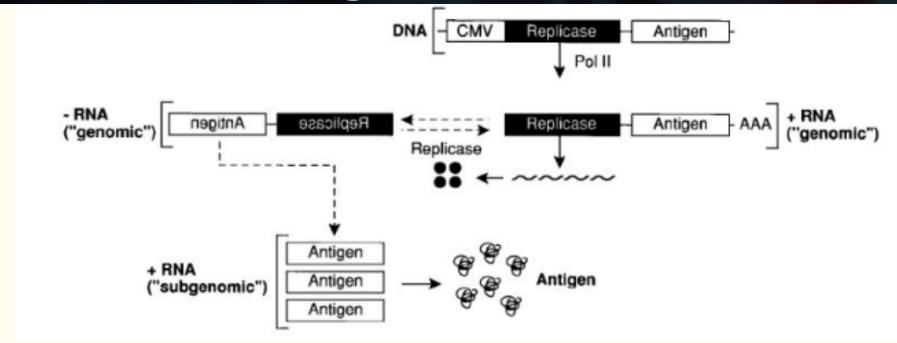
#### DNA and RNA-based vaccines: principles, progress and prospects

Wolfgang W. Leitner, Han Ying, and Nicholas P. Restifo\*

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9. Conclusion Go to: ☑

Ideally, a vaccine should be: safe, highly immunogenic, non-integrating, easy to manipulate, genetically stable and inexpensive to produce. In addition to these features, a therapeutic vaccine must not be compromised by pre-existing immunity of the patient against the vaccine vehicle. While 'conventional' DNA vaccines are frequently hampered by low efficacy, replicase-based vaccines may significantly improve efficacy. 'self-replicating' genetic vaccines may be effective in the fight against diseases that have so far successfully resisted conventional vaccination strategies using recombinant proteins, viruses or bacteria.



## Fig. 3 Self-replicating genetic vaccines

The first product of the self-replicating RNA is a four-subunit-replicase which uses the (+) strand RNA as a template to make (-) strand RNA and more copies of full length (+) strand 'genomic' RNA and (+) strand 'subgenomic' mRNA for the encoded antigen. Due to the high number of RNA-copies, the main product of the transfected cells becomes the encoded antigen. The host cell eventually undergoes apoptosis.

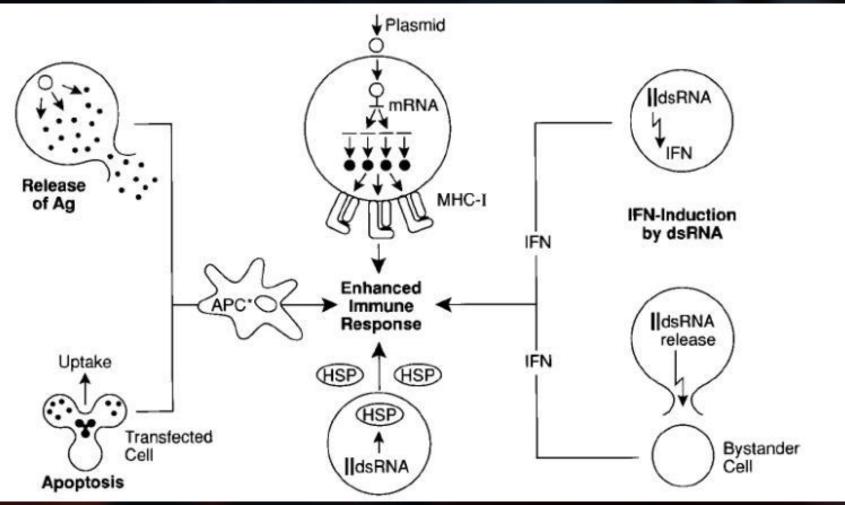


Fig. 4 Potential factors
contributing to the high
immunogenicity of selfreplicating genetic vaccines

(Starting in the upper centre and moving clockwise): Accumulation of antigen in the transfected cell can result in highly efficient MHC-I-loading. A number of 'danger signals' may be generated such as interferon production and interferon release from infected cells resulting from the presence of dsRNA. Interferon may also be produced by bystander cells in response to dsRNA released from dead and lysed transfected cells. Heat shock proteins (HSP) have also been shown to be produced in response to the presence of dsRNA in the cells. Ingestion of antigen-loaded apoptotic cells by APCs can also result in the elicitation of powerful immune responses. Finally, the local release of large amounts of antigen at the site of injection by transfected cells may be fed into resident APC.

#### 6. Enhancing the efficacy of genetic vaccines



A large number of approaches have been used in an attempt to improve the often poor efficacy of DNA vaccines. Because the efficacy of DNA vaccines in many systems has not been satisfactory, the most simple and unexpectedly effective strategy is increasing the intervals between immunizations and, thereby, the 'rest-period' of the immune system [63,65]. In addition, many elements of the plasmid can be optimized for use of the vector as a DNA vaccine [66]. Based on the idea that more antigen is better, most DNA vaccines use strong viral promoters and are geared towards maximum expression. Other sequences that can be optimized in a plasmid include introns, enhancers and poly-adenylation signals [67-69].

### Simple and unexpectedly effective?

• So lets consider this simple possibility, the body is trying to heal itself but they continue to <u>inject toxin producing substances</u>, and these "geniuses" are surprised when they get better results from less frequent vaccination.

Replicase-based DNA or RNA induce apoptotic death of the host cell in vitro just as alphaviral infection induces apoptosis in host cell.

These apoptotic cells may be picked up by dendritic cells for presentation to the immune system.

<u>Transfection</u> with self-replicating genetic vaccines may also cause the production of <u>heat shock</u> proteins in transfected or <u>bystander cells</u>.

Replicase-based DNA or RNA induce apoptotic death of the host cell in vitro just as alphaviral infection induces apoptosis in host cells [116,122]. These apoptotic cells may be picked up by dendritic cells for presentation to the immune system [123]. Transfection with self-replicating genetic vaccines may also cause the production of heat shock proteins in transfected or bystander cells [124]. The activity of the viral replicase may provide a powerful adjuvant-effect because of the requisite production of double stranded RNA (dsRNA) intermediates (Fig. 5). dsRNA itself is a potent inducer of the interferons and virus-derived dsRNA can function as a strong adjuvant for cellular and humoral immune responses [125]. Several molecules are known to bind to and can be activated by dsRNA. The best characterized are 2'-5' oligoadeny-late (2-5A) synthetase and protein kinase-RNA activated (PKR). The 2-5A system contributes to the antiviral effect of the interferons through the synthesis of 2-5A and its activation of RNAse, which degrades both viral and cellular RNA. PKR-expression both induces and is induced by the interferons. PKR is then activated by dsRNA to phosphorylate its substrates, including eIF2. This results in the inhibition of translation, further diminishing viral replication. The cellular death observed in response to dsRNA is likely to be mediated by both the 2-5A system-induced RNAse as well as some substrates of PKR [126,127]. INF-γ potentiates the apoptotic effects of dsRNA [128].

The activity of the viral replicase may provide a <u>powerful adjuvant-effect</u> because of the requisite production of double stranded RNA (dsRNA) intermediates

It is well known that RNA can be "reverse transcribed" into DNA. Residing in our cells are enzymes called "reverse transcriptases". These enzymes convert RNA into DNA. Multiple sources for this class of enzymes exist within our cells. These reverse transcriptases are normally made by other viruses termed "retroviruses". HIV is a retrovirus and so is Hepatitis B, but there are many other retroviruses that fall in this category. In addition to these external viruses, there are viruses that are hard-wired into our genomic DNA called endogenous retroviruses (ERVs). These ERVs harbor instructions to produce reverse transcriptase. In addition to ERVs, there are mobile genetic elements residing in our DNA called LTR-retrotransposons that also encode for reverse transcriptase enzymes. To top it all off, reverse transcriptase is naturally used by our cells to extend the telomeres at the end of chromosomes (Corrigan, 2020).

- The Pfizer/BioNTech (mRNA)
- Moderna (mRNA)
- Oxford/AstraZeneca (DNA, or viral vector)
- Johnson & Johnson (DNA, or viral vector)

These endogenous reverse transcriptase enzymes can essentially take single-stranded RNA and convert it into double-stranded DNA. This DNA can then be integrated into the DNA in the nucleus through an enzyme termed DNA integrase.

https://sciencewithdrdoug.com/2020/11/27/will-an-rna-vaccine-permanently-alter-my-dna/

"SARS-CoV-2 RNA reverse-transcribed and integrated into the human genome." bioRxiv (2020).

#### Commentary:

https://rightsfreedoms.wordpress.com/2021/08/13/mit-harvard-study-suggests-mrna-vaccine-might-permanently-alter-dna-after-all/

#### Extract from article about the study

The Pfizer/BioNTech and Moderna "vaccines" are RNA injections, these transfect their RNA into the cytoplasm of our cells. Through a process called reverse-transcription, injected RNA integrates into our DNA. The Oxford/AstraZeneca and Johnson & Johnson "vaccines" are DNA, or viral vector, injections, which transfect DNA into the nucleus of our cells. Both types of Covid injections can, and probably do, permanently alter our DNA. Because injected DNA or RNA enters the nucleus of our cells, and are treated as our own DNA, they come with a risk of damaging our own DNA, causing mutations, including, potentially, cancer. And, if our cells become permanent Spike Protein producing factories, this could lead to serious autoimmune problems.

https://www.drrobertyoung.com/post/scientific-study-finds-mrna-alters-your-dna-they-told-you-it-was-impossible-they-lied

 The following study was conducted to investigate whether genetically modified RNA could become hardwired into YOUR **DNA.** Although it did not specifically investigate whether genetically modified RNA from animal and/or human sources present in COVID injections could alter YOUR DNA, the data can be used to make that conclusion. The CoV - 19 contains genetically modified RNA that encodes the SARS-CoV-2 spike protein ("Spike Protein") and, like the viral RNA used in the study, it can, and probably does, become hardwired into our **DNA** (Young, 2021).

### <u>Cold Spring Harbor</u> <u>Laboratory</u>

 This interesting study confirms that your DNA can be changed by reversetranscription, this information must be shared since it has profound implications





bioRxiv posts many COVID19-related papers. A reminder: they have not been formally peer-reviewed and should not guide health-related behavior or be reported in the press as conclusive.

New Results

A Follow this preprint

#### SARS-CoV-2 RNA reverse-transcribed and integrated into the human genome

Liguo Zhang, Alexsia Richards, Andrew Khalil, Emile Wogram, Haiting Ma, Richard A. Young, Rudolf Jaenisch doi: https://doi.org/10.1101/2020.12.12.422516

This article is a preprint and has not been certified by peer review [what does this mean?].



### **Abstract**

One possibility is that <u>SARS-CoV-2 RNAs could be reverse-transcribed and integrated into the human genome</u>, and transcription of the integrated DNA copies could be responsible for positive PCR tests.

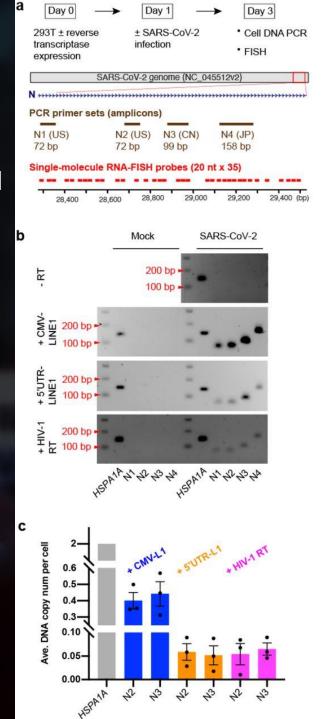
SARS-CoV-2 RNA can be reverse-transcribed and integrated into the human genome in cells overexpressing a reverse transcriptase

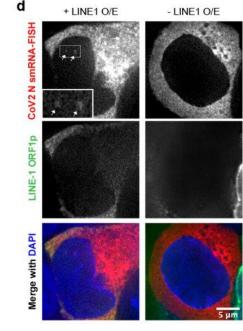
To provide experimental evidence for reverse-transcription and integration of SARS-CoV-2 RNA, we overexpressed human LINE-1 or HIV-1 reverse transcriptase (RT) in HEK293T cells and infected the transduced cells with SARS-CoV-2. The cells were tested 2 days after infection for viral sequences by PCR or fluorescence in situ hybridization (FISH) (Fig. 2a). Considering that the N RNA is the most abundant SARS-CoV-2 sub-genomic RNA31 and is most likely to be retro-integrated (Fig. 1d-e), we chose four N - targeting PCR primer sets that are used in COVID-19 tests (primer source from WHO32, Fig. 2a). PCR amplification of purified cellular DNA showed positive gel-bands in cells with human LINE-1 or HIV-1 RT overexpression (Fig. 2b) but not in non-transfected or non-infected cells. To test whether the DNA copies of N sequences were integrated into the cellular genome, we gel-purified cell genomic DNA (gDNA, >23 kb, Fig. S2a) and qPCR confirmed N sequences in gDNA of cells with expression of all three types of RT (Fig. 2c). Cells with strong expression of LINE-1 driven by a CMV promoter showed ~8-fold higher signals of N sequence detection suggesting a higher copy-number of integrated N sequences than in cells expressing LINE-1 driven by its natural promoter (5'UTR) or HIV-1 RT (Fig. 2c). We were able to clone full-length N DNA from gDNA of cells overexpressing CMV-LINE-1 and confirmed its sequence by Sanger sequencing (Fig. S2b). We did not detect the full-length N sequence from gDNA of cells transfected with 5'UTR-LINE-1 or HIV-1 RT, which may be due to lower expression of RT in these cells (Fig. S2b). We further confirmed that

Figure 2.SARS-CoV-2 RNA can be reverse-transcribed and integrated into the host genome in cells with reverse transcriptase expression.

- a) Experimental workflow (top), PCR primer sets (shown as amplicons, brown) and single-molecule RNA-FISH probes (red) to detect reverse-transcription and integration of SARS-CoV-2 nucleocapsid (N) sequence (middle, blue).
- b) PCR detection of SARS-CoV-2 N sequences in cellular DNA purified from mock (left) or SARS-CoV-2 (right) infected **HEK293T** cells without or with transfection of human LINE-1 (CMV-LINE1 or 5'UTR-LINE1) or HIV-1 RT. *HSPA1A*: human *HSPA1A* gene as control; N1 N4: SARS-CoV-2 N sequences as shown in a).
- c) qPCR detection and copy-number estimation of SARS-CoV-2 N sequences on gel-purified HEK293T genomic DNA. *HSPA1A*: human *HSPA1A* gene as a reference; N2, N3: SARS-CoV-2 N sequences as shown in a). Three biological replicates; mean ± standard error of the mean (s.e.m.).

https://www.biorxiv.org/content/10.1101/2020.12.12.422516v1.full





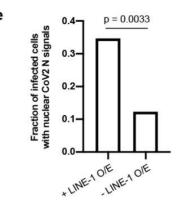
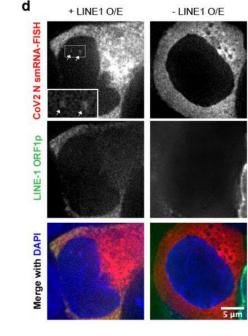
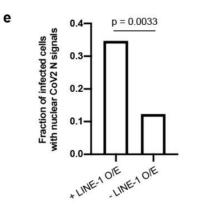


Figure 2.SARS-CoV-2 RNA can be reverse-transcribed and integrated into the host genome in cells with reverse transcriptase expression.

- d) Single-molecule RNA-FISH (red) targeting SARS-CoV-2 N sequence using probes shown in a) plus LINE-1 ORF1 protein immuno-staining (green) and merged channels with DAPI (blue) in SARS-CoV-2 infected HEK293T cells with (left) or without (right) transfected LINE-1. Insets: 2.5x enlargement of region in white-box to show nuclear signals of SARS-CoV-2 N sequence (white arrows). Images were single z-slices from 3D optical sections (0.2-µm z-steps).
- **e)** Fraction of HEK293T cells infected by SARS-CoV-2 (indicated by cytoplasmic FISH signals) showing nuclear FISH signals of N sequence with (+ LINE-1 O/E, n = 75) or without (-LINE-1 O/E, n = 57) LINE-1 overexpression (indicated by LINE-1 ORF1 protein immuno-staining). Combination of two independent cell samples; Chi-Square Test of Homogeneity.

\* FISH SARS-CoV-2





The researchers were puzzled by the fact that there is a respectable number of people who are **testing positive for COVID-19 by PCR long** after the infection was gone. It was also shown that these people were not re-infected.

Through their experiments, they did not find full-length viral RNA integrated into genomic DNA; rather, they found smaller segments of the viral DNA, mostly representing the nucleocapsid (N) protein of the virus, although other viral segments were found integrated into human DNA at a lower frequency.

Quoting from their paper:

"In support of this hypothesis, we found chimeric transcripts consisting of viral fused to cellular sequences in published data sets of SARS-CoV-2 infected cultured cells and primary cells of patients, consistent with the transcription of viral sequences integrated into the genome. To experimentally corroborate the possibility of viral retro-integration, we describe evidence that SARS-CoV-2 RNAs can be reverse transcribed in human cells by reverse transcriptase (RT) from LINE-1 elements or by HIV-1 RT, and that these DNA sequences can be integrated into the cell genome and subsequently be transcribed. Human endogenous LINE-1 expression was induced upon SARS-CoV-2 infection or by cytokine exposure in cultured cells, suggesting a molecular mechanism for SARS-CoV-2 retro-integration in patients. This novel feature of SARS-CoV-2 infection may explain why patients can continue to produce viral RNA after recovery and suggests a new aspect of RNA virus replication."

Why did these researchers bother to investigate whether viral RNA could become hardwired into our genomic DNA? It turns out their motive had nothing to do with mRNA vaccines.

# In this paper, they demonstrate that:

- Segments of SARS-CoV-2 Viral RNA can become integrated into human genomic DNA.
- This newly acquired viral sequence is not silent, meaning that these genetically modified regions of genomic DNA are transcriptionally active (DNA is being converted back into RNA).

Quoting from their paper:

"In support of this hypothesis, we found chimeric transcripts consisting of viral fused to cellular sequences in published data sets of SARS-CoV-2 infected cultured cells and primary cells of patients, consistent with the transcription of viral sequences integrated into the genome. To experimentally corroborate the possibility of viral retro-integration, we describe evidence that SARS-CoV-2 RNAs can be reverse transcribed in human cells by reverse transcriptase (RT) from LINE-1 elements or by HIV-1 RT, and that these DNA sequences can be integrated into the cell genome and subsequently be transcribed. Human endogenous LINE-1 expression was induced upon SARS-CoV-2 infection or by cytokine exposure in cultured cells, suggesting a molecular mechanism for SARS-CoV-2 retro-integration in patients. This novel feature of SARS-CoV-2 infection may explain why patients can continue to produce viral RNA after recovery and suggests a new aspect of RNA virus replication."

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## In this paper, they demonstrate that:

• Segments of SARS-CoV-2 viral RNA retro-integrated into human genomic DNA in cell culture. This retro-integration into genomic DNA of COVID-19 patients is also implied indirectly from the detection of chimeric RNA transcripts in cells derived from COVID-19 patients. Although their RNAseq data suggests that genomic alteration is taking place in COVID-19 patients, to prove this point conclusively, PCR, DNA sequencing, or Southern Blot should be carried out on purified genomic DNA of COVID-19 patients to prove this point conclusively. This is a gap that needs to be closed in the research. The in vitro data in human cell lines, however, is air tight.

## In this paper, they demonstrate that:

- This viral retro-integration of RNA into DNA can be induced by endogenous LINE-1 retrotransposons, which produce an active reverse transcriptase (RT) that converts RNA into DNA. (All humans have multiple copies of LINE-1 retrotransposons residing in their genome.). The frequency of retro-integration of viral RNA into DNA is positively correlated with LINE-1 expression levels in the cell.
- These LINE-1 retrotransposons can be activated by viral infection with SARS-CoV-2, or cytokine exposure to cells, and this increases the probability of retro-integration.

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