# mRNA vaccines: Why is the biology of retroposition ignored?

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## Abstract

**The major advantage of mRNA vaccines over more conventional approaches is their potential for rapid development and large-scale deployment in pandemic situations. In the current COVID-19 crisis the two mRNA COVID-19 vaccines have been conditionally approved and broadly applied, while others are still in clinical trials. However, there is no previous experience with the use of mRNA vaccines on the large scale in general population. This warrants a careful evaluation of mRNA vaccine safety properties by considering all available knowledge on the mRNA molecular biology and evolution. Here, I discuss the pervasive claim that mRNA-based vaccines cannot alter genomes. Surprisingly, this notion is widely stated in the mRNA vaccine literature, but never supported by referencing any primary scientific papers that would specifically address this question. This discrepancy becomes even more puzzling if one considers previous work on the molecular and evolutionary aspects of retroposition in murine and human populations that clearly documents the frequent integration of mRNA molecules into genomes, including clinical contexts. By performing basic comparisons, I showed that the sequence features of mRNA vaccines meet all known requirements for retroposition by L1 elements — the most abundant autonomously active retrotransposons in the human genome. In contrast, I found an evolutionary bias in the set of known retrocopy generating genes — a pattern that might help in the future development of retroposition-resistant therapeutic mRNAs. I conclude that is unfounded to *a priori* assume that mRNA-based therapeutics do not impact genomes, and that the route to genome integration of vaccine mRNAs via endogenous L1 retroelements is easily conceivable. This implies that we urgently need experimental studies that would rigorously test for the**

**potential retroposition of vaccine mRNAs. At present, the insertional mutagenesis safety of mRNA-based vaccines should be considered unresolved.**

## Introduction

The research and development of mRNA-based therapeutics gained momentum with the onset of the COVID-19 pandemics. Currently the two mRNA vaccines against SARS-CoV-2 (BioNTech/Pfizer BNT162b2 and Moderna mRNA-1273) have been approved for use in general population in many countries (e.g. 1,2), and several others are under development (3–5). It has often been suggested that the main advantage of mRNA-based vaccines, compared to the more conventional approaches, is the possibility of their rapid development and large-scale deployment (6,7), which are both desirable properties in pandemic situations. The statement that vaccine mRNAs do not pose the risk for genome integration (e.g. 6,8–12), and consequently that there is no insertional mutagenesis risk, is another commonly listed advantage of mRNA-based vaccines, especially when contrasted to the safety profile of DNA-based therapeutics (10,12,13). This claim prompted me to look more carefully into the mRNA vaccine literature to find a rationale for it. Surprisingly, I was not able to track down any experimental or theoretical study that specifically addresses the possibility of genome integration of mRNA therapeutics.

This shortage of relevant studies is reflected in numerous reviews (4–6,9,10,14–18), book chapters on the mRNA vaccines (13,19–22) and documents of international organizations (23–25) which often state that mRNA vaccines do not pose the risk for genome integration, but miss to cite any references in support of this idea. Occasionally, some citations are embedded (e.g. 15,22,26,27), but unfortunately, they are circular as they point to the similar unsupported statements (6,10,21,28–30). This signals that the idea of vaccine mRNAs resistance to genome integration behaves like a meme that self-replicates in the literature, and therefore it should not be considered reliable scientific information. Undoubtedly, there is always a possibility that my literature search missed some important work, however other researchers also notice, although without going into details, the shortage of studies that explicitly deal with the possibility of vaccine mRNA genome integration (13,31–34).

Besides the lack of references, the argumentation line for the claim that the genome integration of vaccine mRNA molecules is not possible, or is negligible, is rather limited in the vast majority of papers. Many of them simply state that vaccine mRNA cannot integrate into the

host genome without explaining why this is not possible (3,10,12,19–22,26,30). Others shortly describe that vaccine mRNAs remain in the cytoplasm of the host cells — in contrast to DNA-based vaccines that must enter the nucleus to be effective — and thus do not have the opportunity to change the genome (4,9,18,27,35).

Recently, some papers argue that the relatively short persistence of mRNA makes genome integration of mRNA vaccines improbable (4,13,27). However, some of them also recognize the possibility of genome integration if vaccine mRNA is reverse-transcribed in the host cells (4,13,31). As a possible source of enzymes for reverse transcription and genome integration human endogenous retroviruses (HERVs) and retroviral infections (e.g. HIV) are mentioned, with conclusion that the integration risk is still highly unlikely (4,31). In contrast, some authors are more cautious and suggest that investigation may be needed to clarify whether vaccine mRNA integration can occur (13).

**The biology of retroposition**

Nevertheless, this discussion within the vaccinology field on the vaccine mRNA genome integration risks is rather brief and surprisingly incomplete as it does not consider the accumulated knowledge on the biology of retroposition (36–40). In many eukaryotes the cellular mRNAs of various genes are endogenously reverse-transcribed and reintegrated into the genome yielding their retrocopies (Fig. 1b) (36,38–40). This process of mRNA-mediated gene duplication is highly frequent in therian mammals (41), and is best studied in primates and mice (36–38,40). Of note, the term retrocopy is often interchanged with other related terms like processed pseudogenes, retrotransposed pseudogenes, retropseudogenes, retroposed gene copies, retroCNVs, and retrogenes, as the terminology related to retroposition is not yet fully settled (38,39).

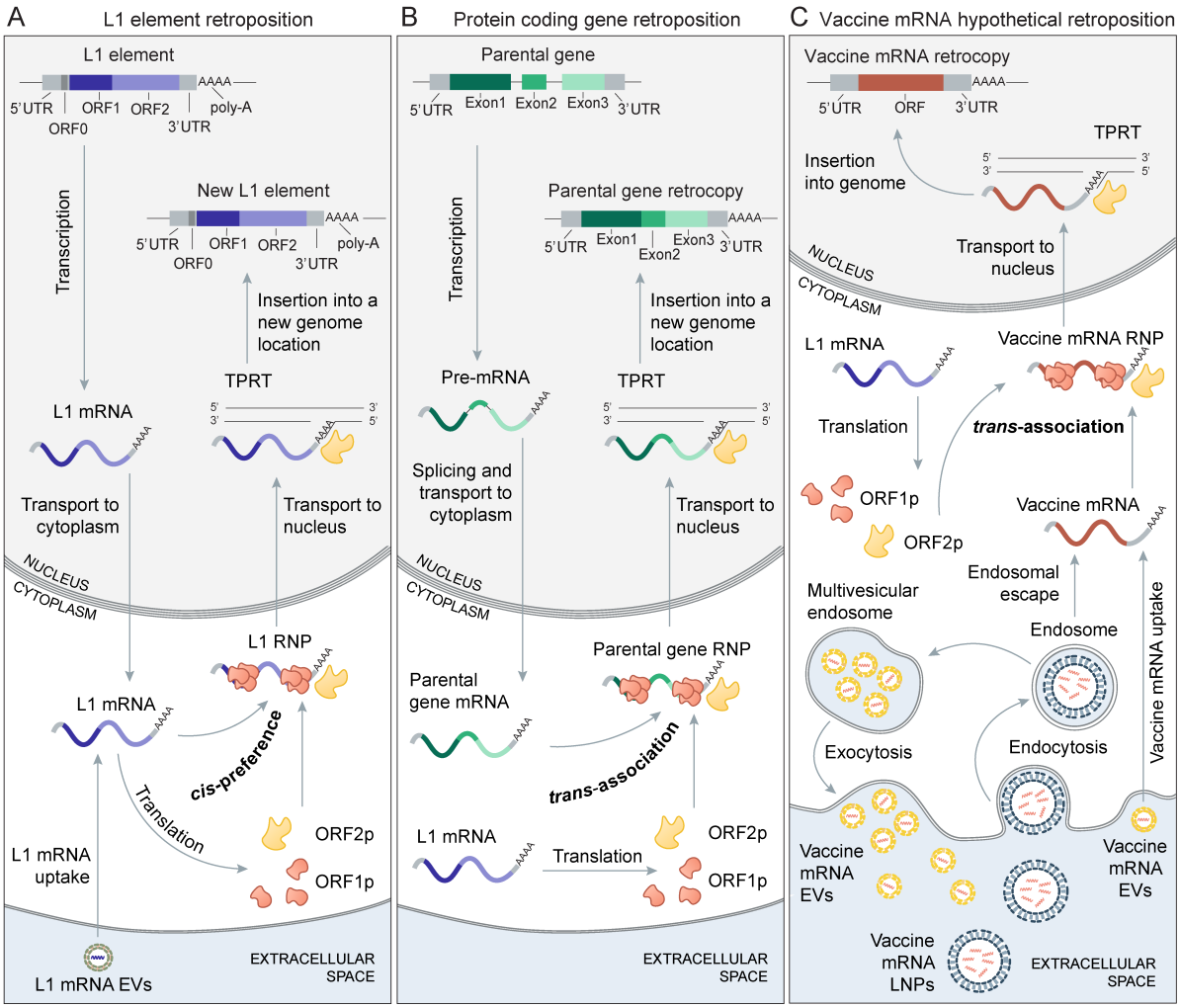


**Figure 1. L1-mediated retroposition. A)** Retroposition cycle of L1 elements. An active L1 element is transcribed in the nucleus and resulting L1 mRNA is transported to the cytoplasm where it undergoes translation (42,43). L1 mRNA codes for ORF1 and ORF2 proteins which preferentially associate with L1 mRNA (*cis*-preference) to form L1 ribonucleoprotein particle (L1 RNP) (42–44). ORF1p is an RNA binding protein with chaperone activity, while ORF2p functions as reverse transcriptase and endonuclease (45,46). By a yet unresolved mechanism L1 RNP, which contains at least L1 mRNA and ORF2p, enters the nucleus. In the nucleus, L1 mRNA is reverse transcribed and integrated into the genome by the process of target-primed reverse transcription (TPRT) (43,45–47). The retroposition mechanism relies on the binding of ORF2p to the L1 mRNA poly-A tail (46,48–50). There is some evidence that the cells could uptake extracellular vesicles (EVs) containing L1 mRNA which can than undergo translation and retroposition (51). **B)** L1-mediated retroposition of protein coding genes. A parental protein coding gene is transcribed in the nucleus. The resulting pre-mRNA is processed and mature parental gene mRNA is then transported to the cytoplasm. L1 proteins (ORF1p and

ORF2p) interact with parental gene mRNA by the process termed *trans*-association to form parental gene ribonucleoprotein particle (parental gene RNP) (36,43,44,47). Similar to L1 RNP, parental gene RNP enters the nucleus where by the TPRT process parental gene mRNA is reverse transcribed and integrated into the genome. The poly-A tail of parental gene mRNA plays the crucial role in this process (36,48–50). **C**) Hypothetical L1-mediated retroposition of vaccine mRNA. Vaccine mRNA formulated in lipid nanoparticles (LNPs) enter the cell by endocytosis (1,2,6,10,52). A fraction of vaccine mRNA enters the cytosol via endosomal escape, the rest of vaccine mRNA undergoes degradation in endosomes (52), or is repackaged in multivesicular endosomes into extracellular vesicles (EVs) and secreted back into the extracellular space (53). The neighboring or distant cells can uptake vaccine mRNA from these EVs (53,54). L1 proteins (ORF1p and ORF2p) interact with vaccine mRNA by the process termed *trans*-association to form vaccine mRNA ribonucleoprotein particle (vaccine mRNA RNP) (36,43,44,47). Like L1 and parental gene RNPs, vaccine mRNA RNP enters the nucleus where by the TPRT process vaccine mRNA is reverse transcribed and integrated into the genome. The poly-A tail of vaccine mRNA plays the crucial role in this process (36,48–50).

Depending on the annotation methodology, the estimated number of retrocopies in the human genome vary, but the figures in most studies revolve around 8,000 (38,39,55,56), and these retrocopies are derived from around 2,500 parental genes (55,57) — i.e. genes whose mRNAs are reverse transcribed and integrated into genome (Fig. 1a,b). These values are similarly high in all screened therian mammals and reflect endogenous retroposition activity during ~200 My of their evolution (41,57). However, the continuous activity of retroposition is also apparent in extant human populations where substantial polymorphism of novel retrocopies is revealed (37,56,58–60). For instance, it was estimated that an individual harbors in average six novel retrocopies which are absent from the human reference genome, and that these retrocopies were derived from the pool of 503 unique parental genes (37). These values indicate a rather high retroposition activity in present human populations.

A recent study in mice suggests that the actual rate of retrocopy generation in extant populations is even higher and possibly similar between humans and mice (40), and hence it is not surprising that retrocopy variation is detected in medical contexts (61,62). However, it is also suggested that due to the use of unoptimized analytical pipelines many retrocopies have often been overlooked in the routine genetic testings (40,61). At present, there are several documented cases of retrocopy emergence related to diseases in animals (47,61,63), and one

case of pathogenic retrocopy in humans (47,61,64,65), but more could be expected to be discovered (40). Actually, it seems that retrocopy variation in human populations might be more phenotypically relevant and population-specific than single nucleotide polymorphisms (37,40), and that the most of newly transposed retrocopies have a deleterious impact (40). All of this suggests that the mutation load coming from the retroposition activity in extant human populations is medically relevant.

Regardless of the initial selective purge (40), retrocopies are the source of novel genes with adaptive significance that contribute to human biology and health (36,39). Previously, retrocopies have been viewed as the unfunctional remnants of evolutionary turnover, termed processed pseudogenes (39), mainly because it was presumed that retrocopies inherently lack transcription-driving elements and thus could not be transcribed (39–41). A similar argument is recently raised in the vaccinology field when the possibility of vaccine mRNA genome integration and its impact on phenotypes is discussed (13). However, after it was realized that the most regions of a mammalian genome are transcribed (66–68), and that retrocopies could easily gain their own regulatory elements (36,38,40,41), it has become apparent that most retrocopies show evidence of transcription (38,40,41).

These transcribed retrocopies are thus the source of evolutionary innovations as they could be further transformed to novel protein coding or RNA retrogenes (36,38,41,69). Approximately several hundred RNA and several hundred protein coding retrogenes are estimated to be active in humans and mice (36,38). For most of them functional significance has yet to be determined, but some are known to be human disease genes (70,71) or to have discernible phenotypes (36,38).

Many of the retrocopies I have discussed so far are vertically transmitted through the germline, but mRNA retroposition also occurs in somatic tissues. Somatic retroposition is substantially less studied, but it is known to be common in cancer tissues (58,72–75), and to occur during early development (64,65). However, the activity of endogenous retroelements that drive retroduplication in humans suggests that mRNA retroposition events should be found in other somatic tissues as well (see below). This indicates that retrocopies continuously reshape the human genome, not only at the population level and deeper evolutionary time scale, but also in somatic tissues during individual development. It is therefore important to consider the

176 endogenous mechanisms of retroposition in humans when the genomic integration probability
177 of mRNA vaccines is evaluated.
178
179 **The mechanisms of retrocopy formation**
180 The mechanism that leads to the formation of retrocopies in human lineage is relatively well
181 studied and predominantly includes long interspersed element-1 (Fig. 1a) (LINE-1 or L1)
182 retrotransposons (36,38,40,44,76), albeit there is some evidence that retroposition through long
183 terminal repeat (LTR) retrotransposons is also possible (38,76). L1 retroelements are around 6
184 kb long, make 17 percent of the human genome and around one hundred of them are active in
185 spreading their copies in the genome by means of retroposition of their own mRNA (Fig. 1a)
186 (42,43,47,77–80). When transcribed L1 produces bicistronic mRNA that codes for two
187 proteins; ORF1p is an RNA binding protein with chaperone activity, while ORF2p functions
188 as reverse transcriptase and endonuclease (42,43,45–47,79,80). Together with a L1 mRNA
189 these proteins assemble in the cytoplasm into a L1 ribonucleoprotein particle (L1 RNP), which
190 can then enter the nucleus (Fig. 1a) (42,43,45–47,79,80).
191
192 In the nucleus, L1 mRNA is eventually reverse transcribed and integrated into the genome at
193 A/T rich consensus target sites by the process termed target-primed reverse transcription
194 (TPRT) (Fig. 1a) (43,45–47). In the antisense direction L1 also codes for ORF0p, a small
195 peptide that localizes in the nucleus and enhances efficiency of retrotransposition (47,81).
196 During the L1 lifecycle diverse host proteins interact with L1 RNPs by promoting or
197 suppressing their retrotransposition (47,82). L1 protein machinery preferentially targets their
198 encoding mRNA (*cis*-preference), but it can also mobilize a variety of other RNAs present in
199 the cell (*trans*-association) including non-autonomous mobile elements (Alu, SVA),
200 splicesomal RNAs and diverse protein coding mRNAs (Fig. 1b) (43,44,47,78,83).
201
202 This relaxed retroposition behavior of L1 elements, which allows mobilization of various
203 mRNAs through *trans*-association, is responsible for the massive accumulation of non-
204 autonomous mobile elements and retrocopies in genomes (Fig. 1b). The question arises how
205 L1 elements achieve such promiscuous performance. The underlying reason for such behavior
206 is linked to the L1 retroposition mechanism that is contingent on ORF2p binding to the poly-
207 A tail during RNP formation in the cytoplasm (Fig. 1) (48,49). Subsequently in the nucleus,
208 genome integration also relies on the poly-A tail which permits flexibility in DNA priming at
209 the target site during the TPRT process (46,50). Given that poly-A tails are unspecific low

complexity sequences that are almost ubiquitously present at the 3' ends of cellular mRNAs (84), this implies that in principle every mRNA could be a target of L1 protein machinery and undergo the TPRT process (Fig. 1c).

However, the complete lack of retroposition specificity would significantly lower the fitness of L1 elements and compromise their parasitic proliferation in the genomes. To avoid this scenario L1 elements managed to preferentially target their own mRNA regardless of the poly-A tail dependence (44,85,86). A popular model that tries to explain the mechanisms of this *cis*-preference envisage that during translation emerging L1 proteins associate immediately at the ribosome to their encoding mRNA (42,45,48,87). Obviously, this or a similar process ensures the balance between parasitic reproduction of L1 elements and the occasional mobilization of diverse mRNAs by *trans*-association via poly-A tracts (Fig. 1).

**L1 elements in germline and soma**

The overall dynamics of L1 retroelements makes them important contributors to genetic variation within and between individuals with implications on the evolution and disease in humans (43,80,88). Interaction between the host genome and L1 elements is multilayered with beneficial and detrimental effects on the host fitness (88–93). For this reason, the host cells evolved various mechanisms to keep in balance their activity (88,91,94–99). Regardless of these host protection mechanisms, a new retroposition event mediated by L1 elements must occur in the germline to be passed to the next generation (92).

The mere presence of numerous vertically inherited L1 elements, non-autonomous mobile elements and retrocopies in human genomes provides a direct evidence that their mobilization repeatedly occurs in the germline (94). It has also been well established that L1 activity contributes to the ongoing germline mutagenesis (100,101). However, the precise dynamics of retroposition during the germline lifecycle is less clear (91,92,102,103). The current data suggest that L1 elements show expression and retroposition activity in testes (91,100,101,104), spermatozoa (105,106), ovaries (100,101), oocytes (107), and early embryos (92,94,100,102,103,108).

Although it was initially thought that L1 elements are mainly active in the germline, accumulated evidence suggests that they also should be considered an endogenous mutagen in somatic tissues (94,95,101,109). L1 elements are expressed in diverse human somatic tissues

including liver, spleen, adrenal glands, lungs, heart and brain (101), lymphoblastoid cell lines (110), platelets, megakaryocytes and T cells (93). Expression and retroposition activity of L1 elements was detected in vascular endothelial cells as well (104,111). However, somatic L1 retroposition have been extensively studied only in the brain, cancer tissues and the gastrointestinal tract (43,73).

During both embryonic and adult neurogenesis L1 retroposition activity generates significant neuronal mosaicism (56,94,112–116) that further increases in neurological disorders (116,117). L1 retroposition occurs in diverse cell types of the central nervous system including glial cells, neuronal progenitor cells, differentiating neurons and mature non-dividing neurons (113,116,118–121). It is speculated that L1-driven somatic mosaicism may alter functional properties of neural cells and that many of them may contain a unique genome (113,121). However, biological and medical significance of this mosaicism is not fully clear (115–117).

L1 elements are also highly expressed in many human cancers, where they function as an endogenous mutagen, and can be responsible for driving mutations in tumorigenesis (79,80). Epithelial cancers seem to be particularly prone to L1 retroposition (43,73). Interestingly, L1 insertions are found in tumor cells as well as normal cells of liver, stomach, colon and esophagus (122–125), suggesting widespread somatic activity of L1 elements in the gastrointestinal tract. In general, somatic L1 retroposition is highly ontogeny dependent and strongly increases with advanced age due to L1 transcriptional derepression (99,126). In addition to endogenous regulation, the activity of L1 elements is sensitive to exogenous signals and could be induced by numerous environmental factors (88,94,95,109,117). Taken together, it is clear that human germinative and many somatic cells have lasting potential for L1-mediated retroposition by *cis*-preference and *trans*-association (Fig. 1).

**Vaccine mRNAs and retroposition**

Evidently, various mRNAs in humans could be reverse transcribed and integrated into genome via L1 retroelements with negative effects on fitness. However, this does not readily imply that this will occur to vaccine mRNAs. A definitive answer will come from experiments and population monitoring, but for now it is helpful to consider their described properties and evaluate them against the L1 retroposition mechanism (Fig. 1). The active substance of BNT162b2 vaccine is a 4,284-nucleotide long synthetic mRNA molecule that contains N1-methylpseudouridine (m1Ψ), a modified nucleoside that substitutes naturally occurring uridine

(1,127,128). This nucleoside modification reduces innate immune response to exogenous mRNA molecules and enhances their translation (6,129–131). Structurally BNT162b2 mRNA consists of a 5' cap analogue, a 5' untranslated region, a codon-optimized SARS-CoV-2 spike protein coding sequence, a 3' untranslated region and a 110-nucleotide poly-A tail (1,52,127,128). These structural elements follow the usual eukaryotic mRNA architecture and help to increase RNA stability and translational efficiency of mRNA vaccines (6,10,28,128). In contrast to BNT162b2, the exact mRNA sequence of mRNA-1273 vaccine seems not to be publicly disclosed (52). However, its general design is similar to BNT162b2 mRNA including the use of m1Ψ instead of uridine, the presence of a 5' cap structure, a 5' untranslated region, a codon-optimized spike protein coding sequence, a 3' untranslated region, and a poly-A tail (2,132).

From the perspective of their sequence arrangement BNT162b2 and mRNA-1273 mRNA synthetic molecules appear to be suitable targets for L1 retroposition in *trans* because they structurally and functionally mimic the architecture of native mRNAs that occur in the cytoplasm of eukaryotic cells (6,10). In this regard, probably the most important sequence feature is their poly-A tail that is known to be required for L1-mediated retroposition (Fig. 1) (49). However, the available information on the vaccine mRNA engineering logic reveals that vaccine mRNAs were not specifically constructed to avoid capture by the L1 retroposition machinery (1,2,6,10,52). In fact, it seems that no study in the mRNA vaccine field considered this possibility (e.g. 4,6,10,13,31). For instance, the poly-A tail of BNT162b2 mRNA contains a 10 nucleotides long linker sequence that is flanked by 30 and 70 nucleotides long adenosine tracts (127). Nevertheless, this poly-A tail modification, which helps in increasing translational efficiency (128,133), is unlikely to affect the retroposition propensity of the vaccine mRNA because only nucleotide changes directly neighboring the 3' end of the poly-A tail are known to have significant impact on the L1 retroposition mechanism (49,50,97). Moreover, non-adenosine nucleotides at the 3' end of the poly-A tail are generally avoided in mRNA therapeutics as they hamper translational efficiency (134). Similarly, the m1Ψ ribonucleoside modification, because of the total number of modified nucleotides per mRNA molecule, is perhaps the most striking artificial feature of the vaccine mRNAs — however, these types of ribonucleoside modifications generally do not prevent reverse transcription (135).

**Parental genes and BNT162b2**

In the comparative context, genes known to actively generate retrocopies (parental genes) in extant populations (Fig. 1b) are the best reference to assess general mRNA sequence trends related to retroposition. However, the collective properties of parental genes have not been extensively analyzed. Some studies report that parental genes are enriched in translation, ribosome, intracellular lumen and cell division related functional categories (37,58,60), and that they have a weak tendency to be highly expressed (37), but a more detailed analysis is still missing. It is helpful then to explore here some basic sequence properties of mRNAs transcribed from parental genes known to actively generate retrocopies in extant populations (37,40), and then to relate this information to the vaccine mRNA sequence that is publicly available (i.e. BNT162b2).

The current estimate of 503 parental genes in humans (37) is lower than in mice where 1663 of them are recovered (40). However, the study in mice which use an improved retrocopy detection pipeline and higher sequencing depths, finds that the number of parental genes has not reached saturation, thus the actual number of parental genes should be expected to be higher, especially in humans (40). Regardless of this inherent incompleteness, the available datasets showed that both mouse and human parental genes have a broad distribution of mRNA lengths (Fig. 2a, b). It is also evident that the mRNAs of parental genes tend to have slightly longer sequences than the average for all protein coding genes (Fig. 2a, b). Under the caveat that I here considered only the longest splicing variant per gene, and that shorter and intronless genes might be overlooked in the retrocopy/parental gene detection pipelines, this result revealed that L1-mediated retroposition in *trans* is modulated to some extent by parental gene mRNA sequence length. In any case, the sequence length of BNT162b2 mRNA falls very close to the average mRNA length of parental genes (Fig. 2a, b), indicating that the sequence length of BNT162b2 mRNA will likely not be an obstacle to retroposition.

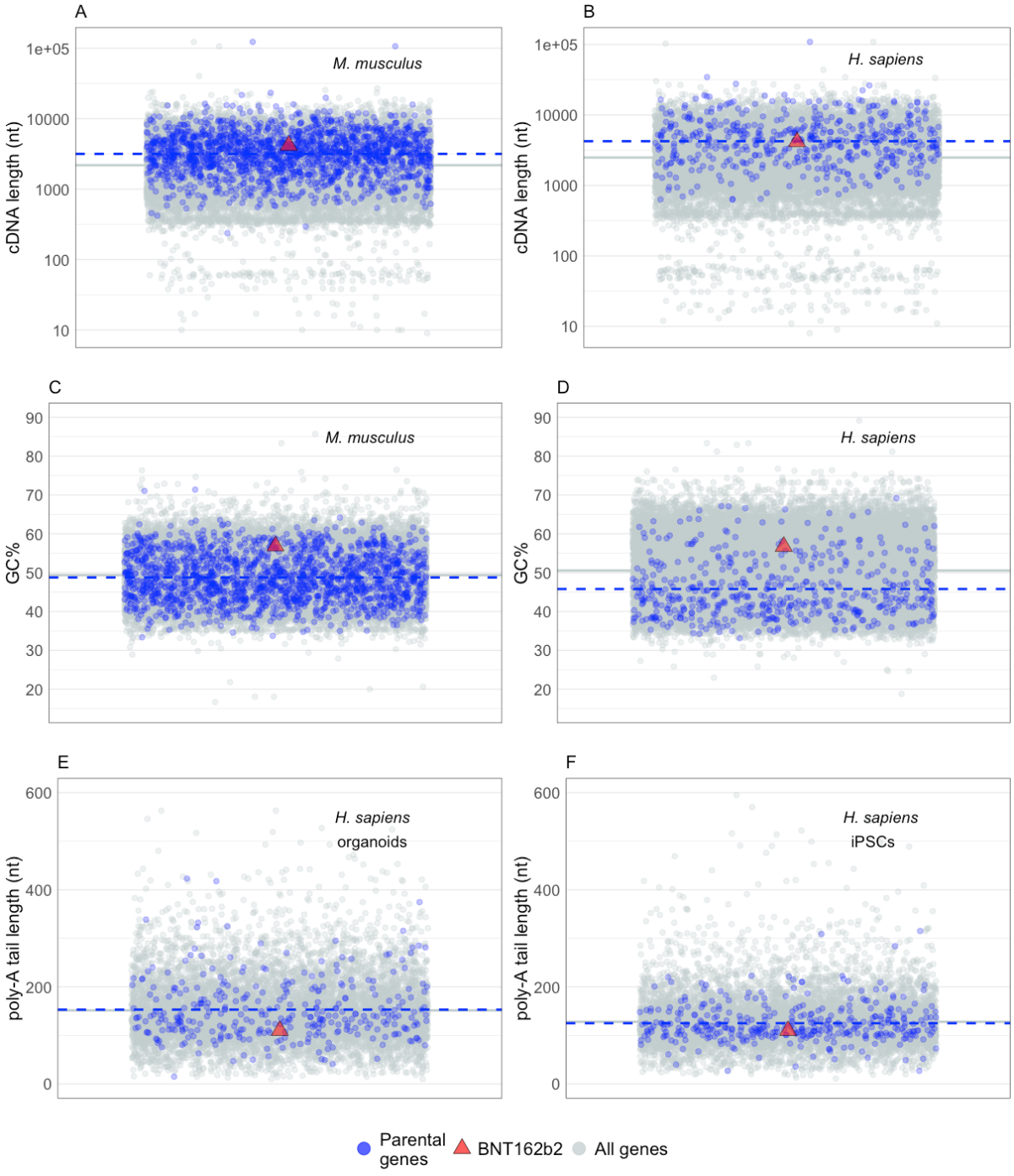


**Figure 2. The basic sequence properties of BNT162b2 mRNA are within the range of parental genes that generate retrocopies.** The jitter plots show parental genes (blue dots) and all genes (gray dots) randomly distributed along x-axis. The red triangle shows BNT162b2 mRNA values. The significance of difference between parental genes average (blue dashed line) and all genes average (gray solid line) are tested by permutation test (two-tailed, $10^6$ permutations). The initial lists contained 503 human (37) and 1,663 mouse parental gene names (40). All mouse and 496 human parental gene names were successfully linked to the sequence data. Poly-A tail lengths were obtained for 7,760 (organoids, replicate 1) and 9,132 (iPSCs,

replicate 1) human genes by averaging multiple estimates per gene (84). **A)** The comparison of cDNA lengths in mice ($p$ = 0; 22,770 all genes, 1,663 parental genes, Ensembl GRCm38.86). **B)** The comparison of cDNA lengths in humans ($p$ = 0; 22,964 all genes, 496 parental genes, Ensemble GRCh38.86) **C)** The comparison of GC content in mice ($p$ = 0.00021; 22,770 all genes, 1,663 parental genes, Ensembl GRCm38.86) **D)** The comparison of GC contents in humans ($p$ = 0, 22,964 all genes, 498 parental genes, Ensemble GRCh38.86) **E)** The comparison of poly-A tail lengths in human iPSCs-derived cerebral organoids ($p$ = 0.69; 7,760 all genes, 330 parental genes, Ensemble GRCh38.84) **F)** The comparison of poly-A tail lengths in human induced pluripotent stem cells (iPSCs) ($p$ = 0.26; 9,132 all genes, 369 parental genes, Ensemble GRCh38.84)

To improve their translation and stability, vaccine mRNAs are frequently sequence and/or codon optimized (1,6,52,136) and this optimization could affect GC content. Hence, to see if the GC content of BNT162b2 mRNA is outside the range of parental genes I explored their GC content in mice and humans. Similar to the mRNA length analysis, GC content of parental genes shows a broad range of values (Fig. 2c, d). In mice, average GC content of parental genes is almost equal to the genome average (Fig. 2c), whereas in humans parental genes tend to have slightly lower average GC content (Fig. 2d). Although the GC content of BNT162b2 mRNA is higher than the average of parental genes, it is well within their range (Fig. 2c, d), thus it is unlikely that peculiarities of BNT162b2 GC content will prevent its retroposition.

The mRNA sequences analyzed so far correspond to bioinformatic cDNA sequences; i.e. coding sequence plus untranslated regions excluding poly-A tail. Commonly, poly-A tails are not considered in genome-based analyses because they are post-transcriptionally added, and it was technically challenging to recover precisely their nucleotide sequence. However, poly-A tails sequencing approaches at the transcriptome scale are continuously improving and recently produced datasets provide an opportunity to get insight into the distribution of their lengths (84). Here I explored poly-A tail lengths estimated using FLAM-seq in human induced pluripotent stem cells (iPSCs) and iPSCs-derived cerebral organoids (84). I found no difference between average poly-A tail lengths of known parental genes and all coding genes (Fig. 1e, f). The distribution range of parental gene poly-A tail lengths is rather broad (Fig. 1e, f), indicating that L1 machinery is mostly insensitive to the variation in poly-A tail lengths. The BNT162b2 poly-A tail with 110 nucleotides is well within the range of these values, so no specific difficulties in retroposition regarding the poly-A tail length are expected. At this point, it is

379 worth mentioning that poly-A tail is present in other mRNA vaccine candidates as well
380 (5,137,138).
381
382 **Parental genes show evolutionary bias**
383 This simple *ad hoc* comparative analysis that covers the length, GC content and poly-A tail
384 length of parental genes that actively produce retrocopies in extant populations (Fig. 2) could
385 be expanded by considering other datasets and sequence traits, or by using more sophisticated
386 analytical approaches. However, its main purpose is to show that effectively any poly-A tail
387 containing mRNA in human cells, including vaccine mRNAs, has some chance to be integrated
388 into the genome by L1 machinery. I hope, this should incite experimental studies that will
389 establish with certainty if some particular mRNA species is retroposition-proof and uncover
390 mechanistic reasons for such behavior (139). On the other hand, we and others previously
391 showed that the computational macroevolutionary analyses of gene sets linked to disease and
392 other phenotypes could bring unexpected insights (140–144) with predictive power that could
393 guide experiments (145–148). This approach could also be applied on the currently available
394 sets of parental genes that actively produce retrocopies, however it appears that this has not
395 been done so far (37,40). To fill this void, at least in part, I made here a pilot macroevolutionary
396 analysis.
397
398 In order to see if the sets of parental genes that actively generate retrocopies in human and
399 mouse (37,40) have some evolutionary bias, I analyzed the phylogenetic origin of their protein
400 sequences using the phylostratigraphic approach (Fig 3). The enrichment profiles on the
401 phylostratigraphic maps show that although protein sequence of parental genes could be traced
402 back to a wide range of phylogenetic levels (phylostrata - ps) they tend to be evolutionary old
403 (Fig 3). I found significant enrichments among genes that are common to all cellular life (ps1,
404 Fig 3), genes that originated in archaea (ps2, Fig3), and among those that emerged at the origin
405 of eukaryotes (ps4, Fig 3). This result suggests that evolutionary ancient genes, for yet
406 unknown reason, tend to have higher retroposition rates in present populations. In addition, this
407 reveals that there is some predictability in the patterns of endogenous mRNA retroposition. In
408 future work this bias could be used as a starting point in search of underlying factors that
409 correlate with the gene age and directly promote or limit mRNA retroposition in mice and
410 humans. Transcription levels, cellular localizations, translation rates, various sequence
411 features, and mRNA regulation and stability are some of the possible factors that could be
412 contrasted between ancient phylostrata enriched with parental genes (ps1, ps2, ps4) and

younger phylostrata that show depletion of them (ps9-ps24). In an ideal case, better understanding of these or other factors could eventually guide experiments and help in the engineering of retroposition-resistant therapeutic mRNAs.

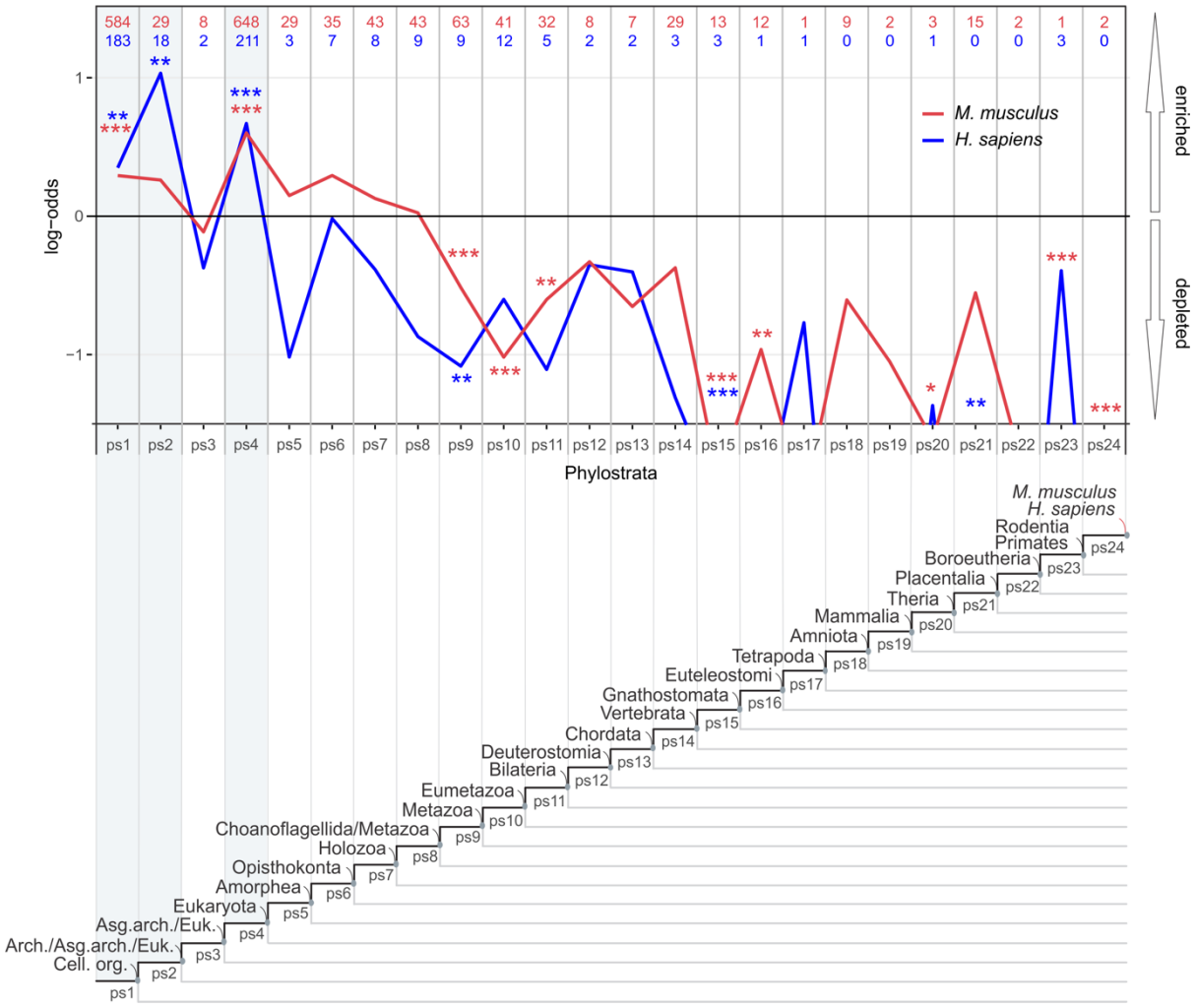


**Figure 3. The parental genes that generate retrocopies in human and mouse populations tend to be evolutionary ancient.** The phylostratigraphic maps of human and mouse protein coding genes are generated using corresponding consensus phylogenies containing 24 internodes (phylostrata - ps). To simplify presentation of the phylostratigraphic results human and mouse phylogenies are overlapped and shown in the lower panel. The two phylogenies differ only in the last two phylostrata (ps23, ps24); i.e. Rodentia-*M. musculus* vs. Primates-*H. sapiens* lineage. Protein sequences of all human (Ensemble GRCh38.86) and mouse genes (Ensembl GRCm38.86) are compared by BLAST against the corresponding custom reference database (e-value 0.001) and mapped on the respective phylogeny using the phylostratigraphic approach (140,142,145,148). The distribution of human (483, blue numbers, (37) and mouse parental genes (1659, red numbers, (40) are shown at the top of upper panel. The log-odds chart

in the upper panel shows deviation from the expected frequency of parental genes in humans (blue line) and mice (red line). Significance of these deviations is tested by the two-way hypergeometric test adjusted for multiple comparisons (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$). The gray shaded phylostrata (ps1 - cellular organisms, ps2 - Archaea/Asgard Archaea/Eukaryota and ps4 - Eukaryota) are enriched for parental genes. Starting with Metazoa (ps9), evolutionary more recent phylostrata show significant depletion in the number of parental genes. This phylostratigraphic pattern is effectively unchanged in the range of e-value cut-offs from 1 to $10^{-20}$, therefore it could be considered fairly robust (148).

**Pharmacology aspects**

Synthetic mRNAs have rather complex pharmacology that is dependent on their nucleotide sequence, formulation and administration route (10,52,149). The likelihood of synthetic mRNA genome integration via L1 elements, beside the nucleotide sequence, depends on its distribution in tissues and organs, and eventually on its concentration and stability in the cell cytosol. The quantity of synthetic mRNA in a single dose is the initial factor that determines the pharmacokinetics and pharmacodynamics of mRNA vaccines (10,149), hence it is helpful to consider declared values for BNT162b2 and mRNA-1273. In a single 30μg BNT162b2 dose (1,150) there are around $1.3 \times 10^{13}$ synthetic mRNA molecules. If we ignore the loss of vaccine mRNAs on the route to the cytosol, and assume their homogenous distribution among roughly $3 \times 10^{12}$ nucleated cells in the human body (151), then every nucleated cell could receive about 26 mRNA copies. This is substantial amount if compared to the expressed human protein coding genes that have on average 25 mRNA copies per cell (152). These values show that the quantity of vaccine mRNA delivered in a single dose of BNT162b2 is large enough to theoretically reprogram the transcriptome of every single human cell that in principle can undergo retroposition. The undisclosed sequence of mRNA-1273 vaccine prevents similar calculation, but under assumption that its sequence length and nucleotide composition is comparable to BNT162b2 (2,5,52), the number of mRNA molecules per nucleated cell are possibly even higher because a single dose of mRNA-1273 vaccine contains 100μg of synthetic mRNA (2,52). This calculation provides the theoretical upper bound of vaccine mRNA cellular uptake, however the lower bound is much more challenging to estimate due to the complex pharmacology of synthetic mRNAs (10) and rather limited data in the literature (1,2,52,150).

After intramuscular inoculation BNT162b2 and mRNA-1273 mRNA molecules should reach the cell cytosol where they are translated to SARS-CoV-2 spike proteins, which eventually elicit the protective immune response (1,2,52,149,153). On this road from the entry site to the cell cytosol some naked and unmodified mRNAs would be mostly degraded by the omnipresent extracellular ribonucleases (5,6,10,154). The remaining mRNAs that eventually enter the cell through endocytosis predominantly end up entrapped in endosomes and degrade over time (10,52,153,154). On top of this, naked mRNAs with unmodified nucleosides are detected in the endosome and cytosol by pattern recognition receptors, which by triggering the interferon signaling and other pathways promote RNA degradation, induce inflammation, and inhibit translation and replication (5,10,52). So even if some external mRNAs reach the cytosol their half-life should be largely compromised. These multiple innate immunity mechanisms against external RNAs show that eukaryotic cells are under strong selective pressure to avoid transcriptome reprograming. By preventing the entry and activity of external mRNAs in the cytosol, these protective mechanisms also largely preclude possible interaction of external mRNAs and endogenous L1 machinery, and consequently lower the chances that some exogenous mRNAs undergo retroposition.

However, mRNA vaccines to be effective must overcome these innate defense mechanisms against exogenous RNAs, reach the cytosol, and have to be efficiently translated by ribosomes (6,10). In the case of BNT162b2 and mRNA-1273 vaccines this is achieved by elaborate sequence optimizations and nucleoside modifications that stabilize synthetic mRNAs and make them largely invisible to innate defense mechanisms (1,2,6,10,52). To further protect them from the harsh extracellular environments, they are formulated in lipid nanoparticles (LNPs) that facilitate their cellular uptake and cytosol entry by endosomal escape (1,2,10,52,149). It is important to note that these remarkable engineering achievements that improve vaccine mRNA cytosol delivery inadvertently increase the chances of vaccine mRNA retroposition (Fig. 1c). This shortcoming stems from the fact that, in principle, any improvement in the vaccine mRNA cytosol delivery increases probability of interaction with the endogenous L1 machinery. Nevertheless, regardless of the increased stability and LNP formulation of vaccine mRNAs, substantial fraction of the initial dose is degraded and will never reach the cytosol (149,153). Unfortunately, accessible information in the public domain on the BNT162b2 and mRNA-1273 does not reveal which percentage of the initial vaccine mRNA dose becomes bioavailable in the cytosol (1,2,149). In any case, any further improvement in the cytosol delivery of vaccine mRNAs, which is a heavily pursued goal in the mRNA vaccinology field

(6,10,149,153,155,156), will concomitantly increase the chances of L1-mediated retroposition (Fig. 1c).

Every mRNA molecule in the cytosol will eventually decay through one of many degradation pathways (157,158). In contrast to exogenous vaccine mRNAs that once degraded are not replaced (6,10,155), the levels of endogenous mRNAs are controlled by the interplay between transcription and decay (157,158). If all other parameters are ignored, this would mean that the probability of L1-mediated retroposition is higher for an endogenous gene with typical levels of expression than for a vaccine mRNA that is transiently present in the cell. However, several additional factors increase the chances of vaccine mRNA retroposition. The number of received doses per individual directly increases the chance of retroposition because it prolongs the time for the encounter of vaccine mRNA with L1 machinery. Currently, BNT162b2 and mRNA-1273 are administered intramuscularly as a series of two doses, three weeks and one month apart respectively (1,2,52). Any eventual increase in the number of required doses would further rise the chances of vaccine mRNA retroposition. This could be a particularly prominent problem if the mRNA vaccines would require long-term recurrent application — like in the case of the current seasonal vaccination program against influenza (159).

Additional property that influences the likelihood of vaccine mRNA genome integration is the stability of vaccine mRNA molecules. The turnover of endogenous mRNA molecules in eukaryotic cells shows great variability, with estimated average half-life of around 7 hours (160). The precise measurements of the vaccine mRNA half-life in cells are not publicly available (1,2), but it is clear that the sequence and codon optimization of vaccine mRNAs increases their functional half-life with an aim to improve their translation efficiency (6,10,27,52,160,161). Undoubtedly, this prolonged functional half-life increases the chances that vaccine mRNAs encounter L1 machinery and eventually retropose into the genome. In addition, it remains unexplored how vaccine mRNAs interact with ribonucleoprotein granules that participate in the regulation of mRNA storage and decay (28,157,162,163) as well as with the cytoplasm residing L1 ribonucleoprotein particles (139).

**Biodistribution profiles**

A biodistribution profile is another important parameter that determines the likelihood of vaccine mRNA genome integration because the activity of L1 elements differs between the cells, tissues and organs (94,95,109). Interestingly, direct biodistribution studies have not been

conducted for the BNT162b2 vaccine (1). However, surrogate studies in mice and rats indicate distribution, in different quantities, from the injection site to most tissues, including liver, adrenal glands, spleen and gonads (1). Direct distribution and pharmacokinetic studies for the mRNA-1273 vaccine were also not conducted, but studies in rats using the same LNPs and a cocktail of mRNAs encoding cytomegalovirus antigens indicate that these mRNAs, with the exception of kidney, could be detected at varying levels in all examined tissues including the injection site muscle, proximal and distal lymph nodes, spleen, eyes, heart, lung, brain and testis (2). Notably, the distribution of mRNA to ovaries is not tested because no female rats were included in this study, as explained in the regulatory documents (2). Obviously, these surrogate biodistribution profiles substantially overlap with organs known to show the activity of L1 elements like liver (122), spleen (101), brain (56,94,112–116), adrenal glands (101), muscles (99,126,164) and gonads (91,100,101,104,107).

If the quantity of vaccine mRNA in a single dose of BNT162b2 or mRNA-1273 is considered, these neither strictly localized nor fully systemic distribution patterns suggest that in some tissues vaccine mRNA likely accumulates in rather high concentrations, with potential to saturate the exogenous mRNA uptake capacity of recipient cells (10,165). To evaluate more precisely the probability of L1 mediated retroposition, it is important to understand which cell types can uptake vaccine mRNA. Dendritic cells and macrophages present at the inoculation site and draining nodes are, according to the regulatory body, the two principal cell types targeted by BNT162b2 and mRNA-1273 vaccines (166). However, the assessment report for the BNT162b2 vaccine states that is unknown whether other cells than professional antigen presenting cells (APCs) may transiently express the vaccine derived spike protein (1). Similarly, the mRNA-1273 vaccine assessment report declares that the delivered vaccine mRNA is mainly expressed by macrophages and dendritic cells (2). This apparently reveals that the mRNA-1273 is expressed in some other cell types as well. It is also indicative that the mechanisms of action that would drive BNT162b2 and mRNA-1273 exclusively/preferentially to dendritic cells and macrophages, if exists, is not explained in these documents (1,2,166).

Although macrophages and dendritic cells, as professional antigen presenting cells (APCs), are specialized in sampling their environment, essentially all nucleated cells are endocytosis competent. The evidence from several studies indicates that the cellular uptake of the mRNA LNPs relies on the apolipoprotein E (ApoE) binding to LNPs and their subsequent endocytosis that is facilitated by low density lipoprotein (LDL) receptors (52,165,167,168). Since ApoE,

LDL and LDL-like receptors are expressed by many cell types throughout the body (169,170) it could be expected that APCs are not the only cell types that internalize mRNA LNPs (52,168). For example, some studies indicate that myocytes, epithelial cells and fibroblast uptake vaccine mRNA and contribute to its expression (52,171–173). These considerations suggest that cell types other than dendritic cells and macrophages most likely internalize BNT162b2 and mRNA-1273 vaccine mRNAs, and that the potential encounter of L1 machinery and vaccine mRNAs may occur in diverse cell types within the broad range of tissues.

Another level of complexity in the transport and uptake of LNP-formulated exogenous mRNA arises with the recent finding that, after endocytosis, LNPs containing mRNA are repackaged in late endosomes and secreted back into extracellular space as extracellular vesicles (EVs) (Fig. 1c) (53). These vaccine mRNA EVs (endo-EVs) protect exogenous mRNA in extracellular fluids during *in vivo* transport to other organs, and deliver intact exogenous mRNA to the cytoplasm of the distant recipient cells (53,54,174–176). Because of their small size vaccine mRNA EVs are less visible than LNPs to innate immunity mechanisms and can pass through the vascular endothelium and the extracellular matrix (53,177). Given that many cell types including dendritic cells (178) and macrophages (179) secrete EVs, the range of cells and tissues that exogenous mRNAs could reach is substantially broadened, if compared to the LNPs route only (Fig. 1c). A recent work shows that L1 mRNAs in cultured cells could also be packaged into EVs, delivered via EVs to recipient cells and retroposed into their genome (Fig. 1a) (51). Together, this suggests that the dynamics of EVs substantially raise the odds for the interaction between active L1 elements and vaccine mRNAs (Fig 1c).

The possibility of vaccine mRNA genome integration in somatic and germline cells (Fig. 1) is not the only adverse effect that should be considered. Theoretically, the vaccine mRNA could also be epigenetically inherited via the sperm RNA cargo (180–183). This could happen if the testis cells of the male germinative lineage uptake LNPs or EVs containing vaccine mRNAs, and if these mRNAs then end up in spermatozoa (181,182,184). Alternatively, during their functional maturation in epididymis, spermatozoa could potentially actively internalize vaccine mRNAs delivered by epididymal EVs (183,184).

**Final remarks**

There are some further points that should be mentioned. Several papers report that infection of human cells by viruses, including SARS-Cov-2, increases activity of their endogenous L1 retroelements (185–188) — consistent with the presumed environmental modulation of L1 activity (109). These findings suggest that, paradoxically, mRNA vaccination during active or after resolved viral infection might increase chances of vaccine mRNA genome integration. The COVID-19 vaccine mRNAs code for SARS-CoV-2 spike protein (52), so it is important to know if there is any evidence that SARS-CoV-2 mRNAs could integrate into the genome. Indeed, a recent study shows that upon infection SARS-CoV-2 subgenomic mRNAs can be reverse-transcribed by L1 elements and integrated into the genome of infected cells (185). Interestingly, fragments of mRNAs closer to the 3' end of the SARS-CoV-2 genome, including spike mRNA, are more frequently integrated into the cell DNA than the sequences closer to the 5' end (185). This integration bias could be related to the differences in the abundance of SARS-CoV-2 subgenomic mRNAs (189) as suggested by the authors (185). However, it could also reflect the nested architecture of subgenomic mRNAs (189) coupled with the mechanism of L1 retroposition that relies on the poly-A tail (49) and is prone to truncate transcripts with increasing distance from the 3' end.

L1 retrotransposon activity is closely linked with replication (45,81,190,191), and is suggested that the retroposition of cellular mRNAs is coupled to cell divisions (37,60). This implies that the risk of vaccine mRNA genome integration might be increased in human proliferating cell populations. The biodistribution profiles of vaccine mRNA are not available for tumors, however increased replication activity coupled with elevated L1 retrotransposition in tumor cells (79) make them a favorable environment for possible vaccine mRNA genome integration. In this regard, it would be very informative to test biodistribution profile of mRNA vaccines in murine tumor models, and to look for eventual somatic retroposition events.

At the first glance, it appears that the application of mRNA vaccines could not alter the primary retroposition rates at the individual and population level. The underlying reason is that vaccine mRNAs are not directly mutagenic and that their route to potential genome integration hinges on the endogenous cellular mechanisms; i.e. the activity of L1 elements that continuously operate on the available mRNA pool. Nevertheless, the possible change in primary retroposition rates should not be immediately dismissed because it cannot be excluded without testing that vaccination with LNPs-formulated mRNAs do not modulate L1 activity. As already explained, it is well established that many exogenous factors modify L1 activity (109),

including viral infections (185–188), so the impact of mRNA vaccination should also be evaluated in this regard.

On the other hand, it is apparent that eventual vaccine mRNA genome integration broadens the spectrum of conceivable sequences that could be retrocopied (Fig. 1). Our cells evolved under mutational pressure that comes from the activity of L1 elements which generate retrocopies of our native genes (37,40). However, the transfection of human cells with exogenous and artificially modified mRNAs, which have potential to be retrocopied into the genome (Fig. 1c), extends the standard mutational sequence space to the realm of transgenic modifications. It is rather clear that any possibility of transgenesis in humans has ethical concerns that should be properly addressed.

The retroposition of a vaccine mRNA molecule is in principle a random event that can occur in any transfected cell that shows the activity of L1 elements (Fig. 1c). The clonal expansion of a new retrocopy largely depends on its phenotypic effects and the pre-existing proliferative capacity of the mutated cell. On one extreme, a vaccine mRNA retrocopy that directly inactivates an essential gene (92,192) would result in cell death that would preclude any further spread of that retrocopy. However, a retrocopy that is moderately deleterious or neutral (141,193), and has emerged in a cell with high proliferative potential, has good odds to be propagated to the large number of descendant cells. In adults, the proliferative capacity of many cells in the soma is considerably limited (193,194), and it further drops with aging (195). This implies that the vaccine mRNA retrocopy mosaicism in the adult soma should be largely restricted to smaller cell clusters or individual cells. Nevertheless, a retroposition event in a progenitor cell, an adult stem cell (196) or a premalignant cell (193) would lead to clonal expansion of the retrocopy in much larger chunks of somatic tissue.

In contrast to relatively confined effects of somatic retroposition, a possible heritable vaccine mRNA retroposition event would have a more far-reaching impact by rendering fully transgenic individuals. The hypothetical vaccine mRNA retrocopy with heritable potential could occur in germinative cells or in the pluripotent cells of early embryos (92). As already discussed above, the documents of regulatory agencies state that the surrogate biodistribution studies report distribution of LNP-formulated mRNA to gonads (1,2), which are known to display activity of L1 elements (91,94,100,101,104–107). On the other hand, vaccine mRNA stored in the sperm RNA cargo could hypothetically reach the pluripotent cells of early

664 embryos, which are the hot-spots of L1 activity (88–90,92,102,103), and undergo retroposition
665 there. This in turn could result in somatic mosaicism where the substantial part of cells in an
666 individual could become transgenic, and if the gonads are also affected, the retrocopy could
667 become heritable (92,108).
668
669 The phenotype of a vaccine mRNA retrocopy will depend, among other factors, on the number
670 and identity of cells that become transgenic, the insertion locus, completeness of the inserted
671 sequence, direction of the insertion, peculiarities of the recipient genome and the expression
672 potential of the retrocopy. Although native mRNAs lack transcription-driving elements it is
673 well established that most of their retrocopies show evidence of transcription (38,40,41), hence
674 it could be expected that a hypothetical vaccine mRNA retrocopy would also have good
675 chances to be expressed. Many expressed retrocopies of native genes tend to have a strong
676 negative impact on fitness and are therefore relatively quickly purged from the population (40).
677 It was suggested that these deleterious effects of expressed retrocopies are often related to the
678 interference with their parental genes (40). Since a hypothetical vaccine mRNA retrocopy does
679 not have a parental gene in the host genome (Fig 1c), effects related to the expression
680 interference between the retrocopy and its parental gene are not possible. However, an
681 expressed retrocopy of vaccine mRNA could interact in unpredictable ways with the host
682 immune system, later viral infections, and vaccine mRNAs received in subsequent
683 administration rounds.
684
685 **Conclusions**
686 Current engineering strategies (136) and declared future directions (136,197) for the
687 improvement of mRNA vaccines do not consider the possibility of vaccine mRNA genome
688 integration via L1 retroelements native to human cells. This is at odds with the knowledge base
689 on the biology of L1-based retroposition and its role in the genetics, development, and
690 evolution of humans. Why this risk is overlooked is even more obscure knowing that mRNA
691 retroposition is a biomedically recognized phenomenon outside vaccinology
692 (42,47,58,61,62,64,65,72,74,75,78). To alleviate these discrepancies between the fields, it
693 would be critical to design and perform experimental studies on animal models that aim to
694 detect the existence of vaccine mRNA retrocopies and estimate their retroposition frequencies.
695 As the retroposition propensity via L1 retroelements is sequence dependent, it would be
696 advisable to independently test every mRNA therapeutic candidate. This information could

then guide further vaccine mRNA refinements in the direction of avoiding active L1 cellular environments (198), or by improving their resilience to the L1 machinery capture (97).

Every technology is a double-edged sword, and mRNA therapeutics are not an exception. In this complex COVID-19 crisis it is essential that all pros-and-cons of control measures, procedures, treatments, prophylaxis and vaccine technologies are continually openly discussed and cautiously evaluated from many angles. An encouraging example in this direction are recently published papers that, in a balanced way, discuss the largely ignored negative aspects of COVID-19 pandemic control measures and practices on the overall human microbiome (199), neonatal microbiome (200) and immunity (201). I hope that the possible interplay between mRNA vaccines and L1 elements presented here will also provoke debate and attract the attention of researchers in a broad range of disciplines.

Whether or not the current vaccine mRNAs could integrate into the genome, and by which frequency, has to be ultimately proven by experiments. However, it remains puzzling why and how the mRNA vaccinology field neglected the retroposition biology of L1 retroelements and its theoretical links to possible vaccine mRNA retroposition, if one considers the volume, visibility and significance of the L1 (42,43,56,78–80,99,112) and retroposition research (36–41,43,44,47,56,62,64,72,75). The mRNA vaccinology field started its development more than 30 years ago (11,31) and L1 retroelements in humans are studied for more than 40 years (202,203) but obviously without any crosstalk between the two fields. This awkward silo effect points that in some occasions the structural drawbacks of contemporary science, despite its amassment, globalization and unprecedented dissemination, are deeper than we are willing to admit. I conclude that the broadly reiterated statement that mRNA-based therapeutics could not impact genomes is an unfounded assumption of unclear origin. This implies that the current mRNA vaccine evaluations, lacking studies that specifically address genome integration, are insufficient to declare their genome integration safety. In this regard, it is important that the exact nucleotide sequences of mRNA vaccines are easily publicly accessible, including product information documents (204,205), to allow unambiguous and independent tracking of possible vaccine mRNA integration in the somatic and germinative genomes of already vaccinated people and their progeny.

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**Literature**

1. European Medicines Agency. Comirnaty assessment report. 2020 Dec. EMA/707383/2020. Available from: https://www.ema.europa.eu/en/documents/assessment-report/comirnaty-epar-public-assessment-report_en.pdf

2. European Medicines Agency. COVID-19 Vaccine Moderna assessment report. 2021 Jan. EMA/15689/2021. Available from: https://www.ema.europa.eu/en/documents/assessment-report/covid-19-vaccine-moderna-epar-public-assessment-report_en.pdf

3. 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 Jun 19; 11:937. Available from: https://www.frontiersin.org/article/10.3389/fphar.2020.00937/full

4. Knezevic I, Liu MA, Peden K, Zhou T, Kang H-N. Development of mRNA Vaccines: Scientific and Regulatory Issues. Vaccines. 2021 Jan 23; 9(2):81. Available from: https://www.mdpi.com/2076-393X/9/2/81

5. Park JW, Lagniton PNP, Liu Y, Xu R-H. mRNA vaccines for COVID-19: what, why and how. Int J Biol Sci. 2021; 17(6):1446–60. Available from: https://www.ijbs.com/v17p1446.htm

6. Pardi N, Hogan MJ, Porter FW, Weissman D. mRNA vaccines — a new era in vaccinology. Nat Rev Drug Discov. 2018 Apr; 17(4):261–79. Available from: http://www.nature.com/articles/nrd.2017.243

7. Jackson NAC, Kester KE, Casimiro D, Gurunathan S, DeRosa F. The promise of mRNA vaccines: a biotech and industrial perspective. npj Vaccines. 2020 Dec; 5(1):11. Available from: http://www.nature.com/articles/s41541-020-0159-8

8. Cannon G, Weissman D. RNA Based Vaccines. DNA and Cell Biology. 2002 Dec; 21(12):953–61. Available from: http://www.liebertpub.com/doi/10.1089/104454902762053882

9. Geall AJ, Mandl CW, Ulmer JB. RNA: The new revolution in nucleic acid vaccines. Seminars in Immunology. 2013 Apr; 25(2):152–9. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1044532313000304

10. Sahin U, Karikó K, Türeci Ö. mRNA-based therapeutics — developing a new class of drugs. Nat Rev Drug Discov. 2014 Oct; 13(10):759–80. Available from: http://www.nature.com/articles/nrd4278

11. Verbeke R, Lentacker I, De Smedt SC, Dewitte H. Three decades of messenger RNA vaccine development. Nano Today. 2019 Oct; 28:100766. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1748013219301483

12. Zhang C, Maruggi G, Shan H, Li J. Advances in mRNA Vaccines for Infectious Diseases. Front Immunol. 2019 Mar 27; 10:594. Available from: https://www.frontiersin.org/article/10.3389/fimmu.2019.00594/full

13. Tombácz I, Weissman D, Pardi N. Vaccination with Messenger RNA: A Promising Alternative to DNA Vaccination. In: Sousa Â, editor. DNA Vaccines. New York, NY: Springer US; 2021. p. 13–31. (Methods in Molecular Biology; vol. 2197). Available from: http://link.springer.com/10.1007/978-1-0716-0872-2_2

14. Kreiter S, Diken M, Selmi A, Türeci Ö, Sahin U. Tumor vaccination using messenger RNA: prospects of a future therapy. Current Opinion in Immunology. 2011 Jun; 23(3):399–406. Available from: https://linkinghub.elsevier.com/retrieve/pii/S095279151100032X

15. Weissman D. mRNA transcript therapy. Expert Review of Vaccines. 2015 Feb; 14(2):265–81. Available from: http://www.tandfonline.com/doi/full/10.1586/14760584.2015.973859

16. Maruggi G, Zhang C, Li J, Ulmer JB, Yu D. mRNA as a Transformative Technology for Vaccine Development to Control Infectious Diseases. Molecular Therapy. 2019 Apr; 27(4):757–72. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1525001619300413

17. Dammes N, Peer D. Paving the Road for RNA Therapeutics. Trends in Pharmacological Sciences. 2020 Oct; 41(10):755–75. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0165614720301851

18. Fuller DH, Berglund P. Amplifying RNA Vaccine Development. Phimister EG, editor. N Engl J Med. 2020 Jun 18; 382(25):2469–71. Available from: http://www.nejm.org/doi/10.1056/NEJMcibr2009737

19. Gerer KF, Hoyer S, Dörrie J, Schaft N. Electroporation of mRNA as Universal Technology Platform to Transfect a Variety of Primary Cells with Antigens and Functional Proteins. In: Kramps T, Elbers K, editors. RNA Vaccines. New York, NY: Springer New York; 2017. p. 165–78. (Methods in Molecular Biology; vol. 1499). Available from: http://link.springer.com/10.1007/978-1-4939-6481-9_10

20. Pardi N, Weissman D. Nucleoside Modified mRNA Vaccines for Infectious Diseases. In: Kramps T, Elbers K, editors. RNA Vaccines. New York, NY: Springer New York; 2017. p. 109–21. (Methods in Molecular Biology; vol. 1499). Available from: http://link.springer.com/10.1007/978-1-4939-6481-9_6

21. Hinz T, Kallen K, Britten CM, Flamion B, Granzer U, Hoos A, et al. The European Regulatory Environment of RNA-Based Vaccines. In: Kramps T, Elbers K, editors.

RNA Vaccines. New York, NY: Springer New York; 2017. p. 203–22. (Methods in Molecular Biology; vol. 1499). Available from: http://link.springer.com/10.1007/978-1-4939-6481-9_13

22. Naik R, Peden K. Regulatory Considerations on the Development of mRNA Vaccines. In Berlin, Heidelberg: Springer Berlin Heidelberg; 2020. (Current Topics in Microbiology and Immunology). Available from: http://link.springer.com/10.1007/82_2020_220

23. World Health Organization. Evaluation of the quality, safety and efficacy of RNA-based prophylactic vaccines for infectious diseases: regulatory considerations. (Draft).. 2020 Dec. Available from: https://www.who.int/docs/default-source/biologicals/ecbs/reg-considerations-on-rna-vaccines_1st-draft_pc_tz_22122020.pdf

24. World Health Organization. Background document on the mRNA vaccine BNT162b2 (Pfizer-BioNTech) against COVID-19. 2021 Jan. Available from: https://www.who.int/publications/i/item/background-document-on-mrna-vaccine-bnt162b2-(pfizer-biontech)-against-covid-19

25. World Health Organization. Background document on the mRNA-1273 vaccine (Moderna) against COVID-19. 2021 Feb. Available from: https://www.who.int/publications/i/item/background-document-on-the-mrna-1273-vaccine-(moderna)-against-covid-19

26. Youn H, Chung J-K. Modified mRNA as an alternative to plasmid DNA (pDNA) for transcript replacement and vaccination therapy. Expert Opinion on Biological Therapy. 2015 Sep 2; 15(9):1337–48. Available from: http://www.tandfonline.com/doi/full/10.1517/14712598.2015.1057563

27. Orlandini von Niessen AG, Poleganov MA, Rechner C, Plaschke A, Kranz LM, Fesser S, et al. Improving mRNA-Based Therapeutic Gene Delivery by Expression-Augmenting 3′ UTRs Identified by Cellular Library Screening. Molecular Therapy. 2019 Apr; 27(4):824–36. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1525001618305951

28. N. Kuhn A, Beißert T, Simon P, Vallazza B, Buck J, P. Davies B, et al. mRNA as a Versatile Tool for Exogenous Protein Expression. CGT. 2012 Sep 1; 12(5):347–61. Available from: http://www.eurekaselect.com/openurl/content.php?genre=article&issn=1566-5232&volume=12&issue=5&spage=347

29. Phua KKL, Leong KW, Nair SK. Transfection efficiency and transgene expression kinetics of mRNA delivered in naked and nanoparticle format. Journal of Controlled Release. 2013 Mar; 166(3):227–33. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0168365913000023

30. Yamamoto A, Kormann M, Rosenecker J, Rudolph C. Current prospects for mRNA gene delivery. European Journal of Pharmaceutics and Biopharmaceutics. 2009 Mar; 71(3):484–9. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0939641108003809

851 31. Liu. A Comparison of Plasmid DNA and mRNA as Vaccine Technologies. Vaccines.
852 2019 Apr 24; 7(2):37. Available from: https://www.mdpi.com/2076-393X/7/2/37

853 32. Cimolai N. Do RNA vaccines obviate the need for genotoxicity studies? Mutagenesis.
854 2020 Dec 31; 35(6):509–10. Available from:
855 https://academic.oup.com/mutage/article/35/6/509/5995048

856 33. Meurens F. Flu RNA Vaccine: A Game Changer? Vaccines. 2020 Dec 14; 8(4):760.
857 Available from: https://www.mdpi.com/2076-393X/8/4/760

858 34. Doerfler W. Adenoviral Vector DNA- and SARS-CoV-2 mRNA-Based Covid-19
859 Vaccines: Possible Integration into the Human Genome - Are Adenoviral Genes
860 Expressed in Vector-based Vaccines? Virus Research. 2021 Sep; 302:198466.
861 Available from: https://linkinghub.elsevier.com/retrieve/pii/S0168170221001738

862 35. Wadhwa A, Aljabbari A, Lokras A, Foged C, Thakur A. Opportunities and Challenges
863 in the Delivery of mRNA-Based Vaccines. Pharmaceutics. 2020 Jan 28; 12(2):102.
864 Available from: https://www.mdpi.com/1999-4923/12/2/102

865 36. Kaessmann H, Vinckenbosch N, Long M. RNA-based gene duplication: mechanistic
866 and evolutionary insights. Nat Rev Genet. 2009 Jan; 10(1):19–31. Available from:
867 http://www.nature.com/articles/nrg2487

868 37. Zhang Y, Li S, Abyzov A, Gerstein MB. Landscape and variation of novel
869 retroduplications in 26 human populations. Iakoucheva LM, editor. PLoS Comput
870 Biol. 2017 Jun 29; 13(6):e1005567. Available from:
871 https://dx.plos.org/10.1371/journal.pcbi.1005567

872 38. Casola C, Betrán E. The Genomic Impact of Gene Retrocopies: What Have We Learned
873 from Comparative Genomics, Population Genomics, and Transcriptomic Analyses?
874 Genome Biology and Evolution. 2017 Jun 1; 9(6):1351–73. Available from:
875 https://academic.oup.com/gbe/article/9/6/1351/3866711

876 39. Cheetham SW, Faulkner GJ, Dinger ME. Overcoming challenges and dogmas to
877 understand the functions of pseudogenes. Nat Rev Genet. 2020 Mar; 21(3):191–201.
878 Available from: http://www.nature.com/articles/s41576-019-0196-1

879 40. Zhang W, Xie C, Ullrich K, Zhang YE, Tautz D. The mutational load in natural
880 populations is significantly affected by high primary rates of retroposition. Proc Natl
881 Acad Sci USA. 2021 Feb 9; 118(6):e2013043118. Available from:
882 http://www.pnas.org/lookup/doi/10.1073/pnas.2013043118

883 41. Carelli FN, Hayakawa T, Go Y, Imai H, Warnefors M, Kaessmann H. The life history
884 of retrocopies illuminates the evolution of new mammalian genes. Genome Res. 2016
885 Mar; 26(3):301–14. Available from:
886 http://genome.cshlp.org/lookup/doi/10.1101/gr.198473.115

887 42. Beck CR, Garcia-Perez JL, Badge RM, Moran JV. LINE-1 Elements in Structural
888 Variation and Disease. Annu Rev Genom Hum Genet. 2011 Sep 22; 12(1):187–215.
889 Available from: http://www.annualreviews.org/doi/10.1146/annurev-genom-082509-
890 141802

43. Kazazian HH, Moran JV. Mobile DNA in Health and Disease. Phimister EG, editor. N Engl J Med. 2017 Jul 27; 377(4):361–70. Available from: http://www.nejm.org/doi/10.1056/NEJMra1510092

44. Esnault C, Maestre J, Heidmann T. Human LINE retrotransposons generate processed pseudogenes. Nat Genet. 2000 Apr; 24(4):363–7. Available from: http://www.nature.com/articles/ng0400_363

45. Mita P, Wudzinska A, Sun X, Andrade J, Nayak S, Kahler DJ, et al. LINE-1 protein localization and functional dynamics during the cell cycle. eLife. 2018 Jan 8; 7:e30058. Available from: https://elifesciences.org/articles/30058

46. Naufer MN, Furano AV, Williams MC. Protein-nucleic acid interactions of LINE-1 ORF1p. Seminars in Cell & Developmental Biology. 2019 Feb; 86:140–9. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1084952117304512

47. Hancks DC, Kazazian HH. Roles for retrotransposon insertions in human disease. Mobile DNA. 2016 Dec; 7(1):9. Available from: https://mobilednajournal.biomedcentral.com/articles/10.1186/s13100-016-0065-9

48. Boeke JD. LINEs and Alus — the polyA connection. Nat Genet. 1997 May; 16(1):6–7. Available from: http://www.nature.com/articles/ng0597-6

49. Doucet AJ, Wilusz JE, Miyoshi T, Liu Y, Moran JV. A 3′ Poly(A) Tract Is Required for LINE-1 Retrotransposition. Molecular Cell. 2015 Dec; 60(5):728–41. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1097276515007790

50. Monot C, Kuciak M, Viollet S, Mir AA, Gabus C, Darlix J-L, et al. The Specificity and Flexibility of L1 Reverse Transcription Priming at Imperfect T-Tracts. Eickbush T, editor. PLoS Genet. 2013 May 9; 9(5):e1003499. Available from: https://dx.plos.org/10.1371/journal.pgen.1003499

51. Kawamura Y, Sanchez Calle A, Yamamoto Y, Sato T-A, Ochiya T. Extracellular vesicles mediate the horizontal transfer of an active LINE-1 retrotransposon. Journal of Extracellular Vesicles. 2019 Dec 1; 8(1):1643214. Available from: https://www.tandfonline.com/doi/full/10.1080/20013078.2019.1643214

52. Verbeke R, Lentacker I, De Smedt SC, Dewitte H. The dawn of mRNA vaccines: The COVID-19 case. Journal of Controlled Release. 2021 May; 333:511–20. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0168365921001541

53. Maugeri M, Nawaz M, Papadimitriou A, Angerfors A, Camponeschi A, Na M, et al. Linkage between endosomal escape of LNP-mRNA and loading into EVs for transport to other cells. Nat Commun. 2019 Dec; 10(1):4333. Available from: http://www.nature.com/articles/s41467-019-12275-6

54. Valadi H, Ekström K, Bossios A, Sjöstrand M, Lee JJ, Lötvall JO. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. Nat Cell Biol. 2007 Jun; 9(6):654–9. Available from: http://www.nature.com/articles/ncb1596

930 55. Pei B, Sisu C, Frankish A, Howald C, Habegger L, Mu X, et al. The GENCODE
931 pseudogene resource. Genome Biol. 2012; 13(9):R51. Available from:
932 http://genomebiology.biomedcentral.com/articles/10.1186/gb-2012-13-9-r51

933 56. Richardson SR, Salvador-Palomeque C, Faulkner GJ. Diversity through duplication:
934 Whole-genome sequencing reveals novel gene retrocopies in the human population.
935 BioEssays. 2014 May; 36(5):475–81. Available from:
936 https://onlinelibrary.wiley.com/doi/10.1002/bies.201300181

937 57. Navarro FCP, Galante PAF. A Genome-Wide Landscape of Retrocopies in Primate
938 Genomes. Genome Biol Evol. 2015 Aug; 7(8):2265–75. Available from:
939 https://academic.oup.com/gbe/article-lookup/doi/10.1093/gbe/evv142

940 58. Ewing AD, Ballinger TJ, Earl D, Broad Institute Genome Sequencing and Analysis
941 Program and Platform, Harris CC, Ding L, et al. Retrotransposition of gene transcripts
942 leads to structural variation in mammalian genomes. Genome Biol. 2013; 14(3):R22.
943 Available from: http://genomebiology.biomedcentral.com/articles/10.1186/gb-2013-
944 14-3-r22

945 59. Schrider DR, Navarro FCP, Galante PAF, Parmigiani RB, Camargo AA, Hahn MW, et
946 al. Gene Copy-Number Polymorphism Caused by Retrotransposition in Humans. Akey
947 JM, editor. PLoS Genet. 2013 Jan 24; 9(1):e1003242. Available from:
948 https://dx.plos.org/10.1371/journal.pgen.1003242

949 60. Abyzov A, Iskow R, Gokcumen O, Radke DW, Balasubramanian S, Pei B, et al.
950 Analysis of variable retroduplications in human populations suggests coupling of
951 retrotransposition to cell division. Genome Research. 2013 Dec 1; 23(12):2042–52.
952 Available from: http://genome.cshlp.org/cgi/doi/10.1101/gr.154625.113

953 61. Chatron N, Cassinari K, Quenez O, Baert-Desurmont S, Bardel C, Buisine M, et al.
954 Identification of mobile retrocopies during genetic testing: Consequences for routine
955 diagnosis. Human Mutation. 2019 Nov; 40(11):1993–2000. Available from:
956 https://onlinelibrary.wiley.com/doi/10.1002/humu.23845

957 62. Gardner EJ, Prigmore E, Gallone G, Danecek P, Samocha KE, Handsaker J, et al.
958 Contribution of retrotransposition to developmental disorders. Nat Commun. 2019
959 Dec; 10(1):4630. Available from: http://www.nature.com/articles/s41467-019-12520-y

960 63. Parker HG, VonHoldt BM, Quignon P, Margulies EH, Shao S, Mosher DS, et al. An
961 Expressed Fgf4 Retrogene Is Associated with Breed-Defining Chondrodysplasia in
962 Domestic Dogs. Science. 2009 Aug 21; 325(5943):995–8. Available from:
963 https://www.sciencemag.org/lookup/doi/10.1126/science.1173275

964 64. de Boer M, van Leeuwen K, Geissler J, Weemaes CM, van den Berg TK, Kuijpers TW,
965 et al. Primary Immunodeficiency Caused by an Exonized Retroposed Gene Copy
966 Inserted in the *CYBB* Gene. Human Mutation. 2014 Apr; 35(4):486–96. Available
967 from: https://onlinelibrary.wiley.com/doi/10.1002/humu.22519

968 65. Kazazian HH. Processed pseudogene insertions in somatic cells. Mobile DNA. 2014
969 Dec; 5(1):20. Available from:
970 https://mobilednajournal.biomedcentral.com/articles/10.1186/1759-8753-5-20

971 66. Djebali S, Davis CA, Merkel A, Dobin A, Lassmann T, Mortazavi A, et al. Landscape
972 of transcription in human cells. Nature. 2012 Sep; 489(7414):101–8. Available from:
973 http://www.nature.com/articles/nature11233

974 67. Hangauer MJ, Vaughn IW, McManus MT. Pervasive Transcription of the Human
975 Genome Produces Thousands of Previously Unidentified Long Intergenic Noncoding
976 RNAs. Rinn JL, editor. PLoS Genet. 2013 Jun 20; 9(6):e1003569. Available from:
977 https://dx.plos.org/10.1371/journal.pgen.1003569

978 68. Neme R, Tautz D. Fast turnover of genome transcription across evolutionary time
979 exposes entire non-coding DNA to de novo gene emergence. eLife. 2016 Feb 2;
980 5:e09977. Available from: https://elifesciences.org/articles/09977

981 69. Tautz D, Domazet-Lošo T. The evolutionary origin of orphan genes. Nat Rev Genet.
982 2011 Oct; 12(10):692–702. Available from: http://www.nature.com/articles/nrg3053

983 70. Rohozinski J, Lamb DJ, Bishop CE. UTP14c Is a Recently Acquired Retrogene
984 Associated with Spermatogenesis and Fertility in Man1. Biology of Reproduction.
985 2006 Apr 1; 74(4):644–51. Available from:
986 https://academic.oup.com/biolreprod/article/74/4/644/2666778/UTP14c-Is-a-Recently-
987 Acquired-Retrogene-Associated

988 71. Ciomborowska J, Rosikiewicz W, Szklarczyk D, Makalowski W, Makalowska I.
989 "Orphan" Retrogenes in the Human Genome. Molecular Biology and Evolution. 2013
990 Feb 1; 30(2):384–96. Available from: https://academic.oup.com/mbe/article-
991 lookup/doi/10.1093/molbev/mss235

992 72. ICGC Breast Cancer Group, Cooke SL, Shlien A, Marshall J, Pipinikas CP,
993 Martincorena I, et al. Processed pseudogenes acquired somatically during cancer
994 development. Nat Commun. 2014 May; 5(1):3644. Available from:
995 http://www.nature.com/articles/ncomms4644

996 73. Scott E, Devine S. The Role of Somatic L1 Retrotransposition in Human Cancers.
997 Viruses. 2017 May 31; 9(6):131. Available from: http://www.mdpi.com/1999-
998 4915/9/6/131

999 74. Bim LV, Navarro FCP, Valente FOF, Lima-Junior JV, Delcelo R, Dias-da-Silva MR, et
1000 al. Retroposed copies of RET gene: a somatically acquired event in medullary thyroid
1001 carcinoma. BMC Med Genomics. 2019 Dec; 12(1):104. Available from:
1002 https://bmcmedgenomics.biomedcentral.com/articles/10.1186/s12920-019-0552-1

1003 75. PCAWG Structural Variation Working Group, PCAWG Consortium, Rodriguez-Martin
1004 B, Alvarez EG, Baez-Ortega A, Zamora J, et al. Pan-cancer analysis of whole genomes
1005 identifies driver rearrangements promoted by LINE-1 retrotransposition. Nat Genet.
1006 2020 Mar; 52(3):306–19. Available from: http://www.nature.com/articles/s41588-019-
1007 0562-0

1008 76. Tan S, Cardoso-Moreira M, Shi W, Zhang D, Huang J, Mao Y, et al. LTR-mediated
1009 retroposition as a mechanism of RNA-based duplication in metazoans. Genome Res.
1010 2016 Dec; 26(12):1663–75. Available from:
1011 http://genome.cshlp.org/lookup/doi/10.1101/gr.204925.116

77. International Human Genome Sequencing Consortium. Initial sequencing and analysis of the human genome. Nature. 2001 Feb 15; 409(6822):860–921. Available from: http://www.nature.com/articles/35057062

78. Payer LM, Burns KH. Transposable elements in human genetic disease. Nat Rev Genet. 2019 Dec; 20(12):760–72. Available from: http://www.nature.com/articles/s41576-019-0165-8

79. Burns KH. Transposable elements in cancer. Nat Rev Cancer. 2017 Jul; 17(7):415–24. Available from: http://www.nature.com/articles/nrc.2017.35

80. Burns KH. Our Conflict with Transposable Elements and Its Implications for Human Disease. Annu Rev Pathol Mech Dis. 2020 Jan 24; 15(1):51–70. Available from: https://www.annualreviews.org/doi/10.1146/annurev-pathmechdis-012419-032633

81. Denli AM, Narvaiza I, Kerman BE, Pena M, Benner C, Marchetto MCN, et al. Primate-Specific ORF0 Contributes to Retrotransposon-Mediated Diversity. Cell. 2015 Oct; 163(3):583–93. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0092867415011873

82. Taylor MS, Altukhov I, Molloy KR, Mita P, Jiang H, Adney EM, et al. Dissection of affinity captured LINE-1 macromolecular complexes. eLife. 2018 Jan 8; 7:e30094. Available from: https://elifesciences.org/articles/30094

83. Moldovan JB, Wang Y, Shuman S, Mills RE, Moran JV. RNA ligation precedes the retrotransposition of U6/LINE-1 chimeric RNA. Proc Natl Acad Sci USA. 2019 Oct 8; 116(41):20612–22. Available from: http://www.pnas.org/lookup/doi/10.1073/pnas.1805404116

84. Legnini I, Alles J, Karaiskos N, Ayoub S, Rajewsky N. FLAM-seq: full-length mRNA sequencing reveals principles of poly(A) tail length control. Nat Methods. 2019 Sep; 16(9):879–86. Available from: http://www.nature.com/articles/s41592-019-0503-y

85. Wei W, Gilbert N, Ooi SL, Lawler JF, Ostertag EM, Kazazian HH, et al. Human L1 Retrotransposition: *cis* Preference versus *trans* Complementation. Mol Cell Biol. 2001 Feb 15; 21(4):1429–39. Available from: https://journals.asm.org/doi/10.1128/MCB.21.4.1429-1439.2001

86. Kulpa DA, Moran JV. Cis-preferential LINE-1 reverse transcriptase activity in ribonucleoprotein particles. Nat Struct Mol Biol. 2006 Jul; 13(7):655–60. Available from: http://www.nature.com/articles/nsmb1107

87. Ahl V, Keller H, Schmidt S, Weichenrieder O. Retrotransposition and Crystal Structure of an Alu RNP in the Ribosome-Stalling Conformation. Molecular Cell. 2015 Dec; 60(5):715–27. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1097276515007704

88. Percharde M, Sultana T, Ramalho-Santos M. What Doesn't Kill You Makes You Stronger: Transposons as Dual Players in Chromatin Regulation and Genomic Variation. BioEssays. 2020 Apr; 42(4):1900232. Available from: https://onlinelibrary.wiley.com/doi/10.1002/bies.201900232

89. Beraldi R, Pittoggi C, Sciamanna I, Mattei E, Spadafora C. Expression of LINE-1 retroposons is essential for murine preimplantation development. Mol Reprod Dev. 2006 Mar; 73(3):279–87. Available from: https://onlinelibrary.wiley.com/doi/10.1002/mrd.20423

90. Jachowicz JW, Bing X, Pontabry J, Bošković A, Rando OJ, Torres-Padilla M-E. LINE-1 activation after fertilization regulates global chromatin accessibility in the early mouse embryo. Nat Genet. 2017 Oct; 49(10):1502–10. Available from: http://www.nature.com/articles/ng.3945

91. Newkirk SJ, Lee S, Grandi FC, Gaysinskaya V, Rosser JM, Vanden Berg N, et al. Intact piRNA pathway prevents L1 mobilization in male meiosis. Proc Natl Acad Sci USA. 2017 Jul 11; 114(28):E5635–44. Available from: http://www.pnas.org/lookup/doi/10.1073/pnas.1701069114

92. Richardson SR, Faulkner GJ. Heritable L1 Retrotransposition Events During Development: Understanding Their Origins: Examination of heritable, endogenous L1 retrotransposition in mice opens up exciting new questions and research directions. BioEssays. 2018 Jun; 40(6):1700189. Available from: https://onlinelibrary.wiley.com/doi/10.1002/bies.201700189

93. Schwertz H, Rowley JW, Schumann GG, Thorack U, Campbell RA, Manne BK, et al. Endogenous LINE-1 (Long Interspersed Nuclear Element-1) Reverse Transcriptase Activity in Platelets Controls Translational Events Through RNA–DNA Hybrids. ATVB. 2018 Apr; 38(4):801–15. Available from: https://www.ahajournals.org/doi/10.1161/ATVBAHA.117.310552

94. Levin HL, Moran JV. Dynamic interactions between transposable elements and their hosts. Nat Rev Genet. 2011 Sep; 12(9):615–27. Available from: http://www.nature.com/articles/nrg3030

95. Goodier JL. Restricting retrotransposons: a review. Mobile DNA. 2016 Dec; 7(1):16. Available from: https://mobilednajournal.biomedcentral.com/articles/10.1186/s13100-016-0070-z

96. Pizarro JG, Cristofari G. Post-Transcriptional Control of LINE-1 Retrotransposition by Cellular Host Factors in Somatic Cells. Front Cell Dev Biol. 2016 Mar 7; 4. Available from: http://journal.frontiersin.org/Article/10.3389/fcell.2016.00014/abstract

97. Warkocki Z, Krawczyk PS, Adamska D, Bijata K, Garcia-Perez JL, Dziembowski A. Uridylation by TUT4/7 Restricts Retrotransposition of Human LINE-1s. Cell. 2018 Sep; 174(6):1537-1548.e29. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0092867418309176

98. Sanchez-Luque FJ, Kempen M-JHC, Gerdes P, Vargas-Landin DB, Richardson SR, Troskie R-L, et al. LINE-1 Evasion of Epigenetic Repression in Humans. Molecular Cell. 2019 Aug; 75(3):590-604.e12. Available from: https://linkinghub.elsevier.com/retrieve/pii/S109727651930396X

99. De Cecco M, Ito T, Petrashen AP, Elias AE, Skvir NJ, Criscione SW, et al. L1 drives IFN in senescent cells and promotes age-associated inflammation. Nature. 2019 Feb; 566(7742):73–8. Available from: http://www.nature.com/articles/s41586-018-0784-9

100. Ostertag EM, DeBerardinis RJ, Goodier JL, Zhang Y, Yang N, Gerton GL, et al. A mouse model of human L1 retrotransposition. Nat Genet. 2002 Dec; 32(4):655–60. Available from: http://www.nature.com/articles/ng1022z

101. Belancio VP, Roy-Engel AM, Pochampally RR, Deininger P. Somatic expression of LINE-1 elements in human tissues. Nucleic Acids Research. 2010 Jul; 38(12):3909–22. Available from: https://academic.oup.com/nar/article-lookup/doi/10.1093/nar/gkq132

102. Kano H, Godoy I, Courtney C, Vetter MR, Gerton GL, Ostertag EM, et al. L1 retrotransposition occurs mainly in embryogenesis and creates somatic mosaicism. Genes & Development. 2009 Jun 1; 23(11):1303–12. Available from: http://genesdev.cshlp.org/cgi/doi/10.1101/gad.1803909

103. Kohlrausch FB, Berteli TS, Wang F, Navarro PA, Keefe DL. Control of LINE-1 Expression Maintains Genome Integrity in Germline and Early Embryo Development. Reprod Sci. 2021 Jan 22; Available from: http://link.springer.com/10.1007/s43032-021-00461-1

104. Ergün S, Buschmann C, Heukeshoven J, Dammann K, Schnieders F, Lauke H, et al. Cell Type-specific Expression of LINE-1 Open Reading Frames 1 and 2 in Fetal and Adult Human Tissues. Journal of Biological Chemistry. 2004 Jun; 279(26):27753–63. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0021925820854416

105. Lazaros L, Kitsou C, Kostoulas C, Bellou S, Hatzi E, Ladias P, et al. Retrotransposon expression and incorporation of cloned human and mouse retroelements in human spermatozoa. Fertility and Sterility. 2017 Mar; 107(3):821–30. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0015028216630996

106. Giordano R, Magnano AR, Zaccagnini G, Pittoggi C, Moscufo N, Lorenzini R, et al. Reverse Transcriptase Activity in Mature Spermatozoa of Mouse. Journal of Cell Biology. 2000 Mar 20; 148(6):1107–14. Available from: https://rupress.org/jcb/article/148/6/1107/44986/Reverse-Transcriptase-Activity-in-Mature

107. Georgiou I, Noutsopoulos D, Dimitriadou E, Markopoulos G, Apergi A, Lazaros L, et al. Retrotransposon RNA expression and evidence for retrotransposition events in human oocytes. Human Molecular Genetics. 2009 Jan 8; 18(7):1221–8. Available from: https://academic.oup.com/hmg/article-lookup/doi/10.1093/hmg/ddp022

108. Richardson SR, Gerdes P, Gerhardt DJ, Sanchez-Luque FJ, Bodea G-O, Muñoz-Lopez M, et al. Heritable L1 retrotransposition in the mouse primordial germline and early embryo. Genome Res. 2017 Aug; 27(8):1395–405. Available from: http://genome.cshlp.org/lookup/doi/10.1101/gr.219022.116

109. Del Re B, Giorgi G. Long INterspersed element-1 mobility as a sensor of environmental stresses. Environ Mol Mutagen. 2020 Apr; 61(4):465–93. Available from: https://onlinelibrary.wiley.com/doi/10.1002/em.22366

110. Rangwala SH, Zhang L, Kazazian HH. Many LINE1 elements contribute to the transcriptome of human somatic cells. Genome Biol. 2009 Sep; 10(9):R100. Available from: https://genomebiology.biomedcentral.com/articles/10.1186/gb-2009-10-9-r100

111. Banaz-Yaşar F, Steffen G, Hauschild J, Bongartz BM, Schumann GG, Ergün S. LINE-1 retrotransposition events affect endothelial proliferation and migration. Histochem Cell Biol. 2010 Dec; 134(6):581–9. Available from: http://link.springer.com/10.1007/s00418-010-0758-y

112. Thomas CA, Paquola ACM, Muotri AR. LINE-1 Retrotransposition in the Nervous System. Annu Rev Cell Dev Biol. 2012 Nov 10; 28(1):555–73. Available from: http://www.annualreviews.org/doi/10.1146/annurev-cellbio-101011-155822

113. Upton KR, Gerhardt DJ, Jesuadian JS, Richardson SR, Sánchez-Luque FJ, Bodea GO, et al. Ubiquitous L1 Mosaicism in Hippocampal Neurons. Cell. 2015 Apr; 161(2):228–39. Available from: https://linkinghub.elsevier.com/retrieve/pii/S009286741500313X

114. Erwin JA, Paquola ACM, Singer T, Gallina I, Novotny M, Quayle C, et al. L1-associated genomic regions are deleted in somatic cells of the healthy human brain. Nat Neurosci. 2016 Dec; 19(12):1583–91. Available from: http://www.nature.com/articles/nn.4388

115. Faulkner GJ, Garcia-Perez JL. L1 Mosaicism in Mammals: Extent, Effects, and Evolution. Trends in Genetics. 2017 Nov; 33(11):802–16. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0168952517301130

116. Terry DM, Devine SE. Aberrantly High Levels of Somatic LINE-1 Expression and Retrotransposition in Human Neurological Disorders. Front Genet. 2020 Jan 8; 10:1244. Available from: https://www.frontiersin.org/article/10.3389/fgene.2019.01244/full

117. Bodea GO, McKelvey EGZ, Faulkner GJ. Retrotransposon-induced mosaicism in the neural genome. Open Biol. 2018 Jul; 8(7):180074. Available from: https://royalsocietypublishing.org/doi/10.1098/rsob.180074

118. Muotri AR, Chu VT, Marchetto MCN, Deng W, Moran JV, Gage FH. Somatic mosaicism in neuronal precursor cells mediated by L1 retrotransposition. Nature. 2005 Jun; 435(7044):903–10. Available from: http://www.nature.com/articles/nature03663

119. Coufal NG, Garcia-Perez JL, Peng GE, Yeo GW, Mu Y, Lovci MT, et al. L1 retrotransposition in human neural progenitor cells. Nature. 2009 Aug; 460(7259):1127–31. Available from: http://www.nature.com/articles/nature08248

120. Baillie JK, Barnett MW, Upton KR, Gerhardt DJ, Richmond TA, De Sapio F, et al. Somatic retrotransposition alters the genetic landscape of the human brain. Nature. 2011 Nov; 479(7374):534–7. Available from: http://www.nature.com/articles/nature10531

121. Macia A, Widmann TJ, Heras SR, Ayllon V, Sanchez L, Benkaddour-Boumzaouad M, et al. Engineered LINE-1 retrotransposition in nondividing human neurons. Genome Res. 2017 Mar; 27(3):335–48. Available from: http://genome.cshlp.org/lookup/doi/10.1101/gr.206805.116

122. Shukla R, Upton KR, Muñoz-Lopez M, Gerhardt DJ, Fisher ME, Nguyen T, et al. Endogenous Retrotransposition Activates Oncogenic Pathways in Hepatocellular Carcinoma. Cell. 2013 Mar; 153(1):101–11. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0092867413002213

123. Ewing AD, Gacita A, Wood LD, Ma F, Xing D, Kim M-S, et al. Widespread somatic L1 retrotransposition occurs early during gastrointestinal cancer evolution. Genome Res. 2015 Oct; 25(10):1536–45. Available from: http://genome.cshlp.org/lookup/doi/10.1101/gr.196238.115

124. Doucet-O'Hare TT, Rodić N, Sharma R, Darbari I, Abril G, Choi JA, et al. LINE-1 expression and retrotransposition in Barrett's esophagus and esophageal carcinoma. Proc Natl Acad Sci USA. 2015 Sep 1; 112(35):E4894–900. Available from: http://www.pnas.org/lookup/doi/10.1073/pnas.1502474112

125. Doucet-O'Hare TT, Sharma R, Rodić N, Anders RA, Burns KH, Kazazian HH. Somatically Acquired LINE-1 Insertions in Normal Esophagus Undergo Clonal Expansion in Esophageal Squamous Cell Carcinoma: HUMAN MUTATION. Human Mutation. 2016 Sep; 37(9):942–54. Available from: https://onlinelibrary.wiley.com/doi/10.1002/humu.23027

126. De Cecco M, Criscione SW, Peterson AL, Neretti N, Sedivy JM, Kreiling JA. Transposable elements become active and mobile in the genomes of aging mammalian somatic tissues. Aging. 2013 Dec 7; 5(12):867–83. Available from: https://www.aging-us.com/lookup/doi/10.18632/aging.100621

127. WHO INN (International Nonproprietary Names Program). Messenger RNA encoding the full-length SARS-CoV-2 spike glycoprotein. 2020 Dec. Report No.: (2020) Entry 11889.

128. Vogel AB, Kanevsky I, Che Y, Swanson KA, Muik A, Vormehr M, et al. BNT162b vaccines protect rhesus macaques from SARS-CoV-2. Nature. 2021 Apr 8; 592(7853):283–9. Available from: http://www.nature.com/articles/s41586-021-03275-y

129. Andries O, Mc Cafferty S, De Smedt SC, Weiss R, Sanders NN, Kitada T. N1-methylpseudouridine-incorporated mRNA outperforms pseudouridine-incorporated mRNA by providing enhanced protein expression and reduced immunogenicity in mammalian cell lines and mice. Journal of Controlled Release. 2015 Nov; 217:337–44. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0168365915300948

130. Svitkin YV, Cheng YM, Chakraborty T, Presnyak V, John M, Sonenberg N. N1-methyl-pseudouridine in mRNA enhances translation through eIF2α-dependent and independent mechanisms by increasing ribosome density. Nucleic Acids Research. 2017 Jun 2; 45(10):6023–36. Available from: https://academic.oup.com/nar/article-lookup/doi/10.1093/nar/gkx135

131. Parr CJC, Wada S, Kotake K, Kameda S, Matsuura S, Sakashita S, et al. N 1-Methylpseudouridine substitution enhances the performance of synthetic mRNA switches in cells. Nucleic Acids Research. 2020 Apr 6; 48(6):e35–e35. Available from: https://academic.oup.com/nar/article/48/6/e35/5742781

132. Corbett KS, Edwards DK, Leist SR, Abiona OM, Boyoglu-Barnum S, Gillespie RA, et al. SARS-CoV-2 mRNA vaccine design enabled by prototype pathogen preparedness. Nature. 2020 Oct 22; 586(7830):567–71. Available from: http://www.nature.com/articles/s41586-020-2622-0

133. Trepotec Z, Geiger J, Plank C, Aneja MK, Rudolph C. Segmented poly(A) tails significantly reduce recombination of plasmid DNA without affecting mRNA translation efficiency or half-life. RNA. 2019 Apr; 25(4):507–18. Available from: http://rnajournal.cshlp.org/lookup/doi/10.1261/rna.069286.118

134. Holtkamp S, Kreiter S, Selmi A, Simon P, Koslowski M, Huber C, et al. Modification of antigen-encoding RNA increases stability, translational efficacy, and T-cell stimulatory capacity of dendritic cells. Blood. 2006 Dec 15; 108(13):4009–17. Available from: https://ashpublications.org/blood/article/108/13/4009/6595/Modification-of-antigenencoding-RNA-increases

135. Potapov V, Fu X, Dai N, Corrêa IR, Tanner NA, Ong JL. Base modifications affecting RNA polymerase and reverse transcriptase fidelity. Nucleic Acids Research. 2018 Jun 20; 46(11):5753–63. Available from: https://academic.oup.com/nar/article/46/11/5753/4994676

136. Linares-Fernández S, Lacroix C, Exposito J-Y, Verrier B. Tailoring mRNA Vaccine to Balance Innate/Adaptive Immune Response. Trends in Molecular Medicine. 2020 Mar; 26(3):311–23. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1471491419302448

137. Zhang N-N, Li X-F, Deng Y-Q, Zhao H, Huang Y-J, Yang G, et al. A Thermostable mRNA Vaccine against COVID-19. Cell. 2020 Sep; 182(5):1271-1283.e16. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0092867420309326

138. Rauch S, Roth N, Schwendt K, Fotin-Mleczek M, Mueller SO, Petsch B. mRNA based SARS-CoV-2 vaccine candidate CVnCoV induces high levels of virus neutralizing antibodies and mediates protection in rodents. Immunology; 2020 Oct. Available from: http://biorxiv.org/lookup/doi/10.1101/2020.10.23.351775

139. Mandal PK, Ewing AD, Hancks DC, Kazazian HH. Enrichment of processed pseudogene transcripts in L1-ribonucleoprotein particles. Human Molecular Genetics. 2013 Sep 15; 22(18):3730–48. Available from: https://academic.oup.com/hmg/article-lookup/doi/10.1093/hmg/ddt225

140. Domazet-Lošo T, Brajković J, Tautz D. A phylostratigraphy approach to uncover the genomic history of major adaptations in metazoan lineages. Trends in Genetics. 2007 Nov; 23(11):533–9. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0168952507002995

141. Domazet-Loso T, Tautz D. An Ancient Evolutionary Origin of Genes Associated with Human Genetic Diseases. Molecular Biology and Evolution. 2008 Aug 5; 25(12):2699–707. Available from: https://academic.oup.com/mbe/article-lookup/doi/10.1093/molbev/msn214

142. Domazet-Lošo T, Tautz D. Phylostratigraphic tracking of cancer genes suggests a link to the emergence of multicellularity in metazoa. BMC Biol. 2010 Dec; 8(1):66. Available from: https://bmcbiol.biomedcentral.com/articles/10.1186/1741-7007-8-66

143. Trigos AS, Pearson RB, Papenfuss AT, Goode DL. Altered interactions between unicellular and multicellular genes drive hallmarks of transformation in a diverse range of solid tumors. Proc Natl Acad Sci USA. 2017 Jun 13; 114(24):6406–11. Available from: http://www.pnas.org/lookup/doi/10.1073/pnas.1617743114

144. Trigos AS, Pearson RB, Papenfuss AT, Goode DL. Somatic mutations in early metazoan genes disrupt regulatory links between unicellular and multicellular genes in cancer. eLife. 2019 Feb 26; 8:e40947. Available from: https://elifesciences.org/articles/40947

145. Domazet-Lošo T, Tautz D. A phylogenetically based transcriptome age index mirrors ontogenetic divergence patterns. Nature. 2010 Dec; 468(7325):815–8. Available from: http://www.nature.com/articles/nature09632

146. Domazet-Lošo T, Klimovich A, Anokhin B, Anton-Erxleben F, Hamm MJ, Lange C, et al. Naturally occurring tumours in the basal metazoan Hydra. Nat Commun. 2014 Sep; 5(1):4222. Available from: http://www.nature.com/articles/ncomms5222

147. Shi L, Derouiche A, Pandit S, Rahimi S, Kalantari A, Futo M, et al. Evolutionary analysis of the Bacillus subtilis genome reveals new genes involved in sporulation. Agashe D, editor. Molecular Biology and Evolution. 2020 Feb 15; msaa035. Available from: https://academic.oup.com/mbe/advance-article/doi/10.1093/molbev/msaa035/5736554

148. Futo M, Opašić L, Koska S, Čorak N, Široki T, Ravikumar V, et al. Embryo-Like Features in Developing *Bacillus subtilis* Biofilms. Perna N, editor. Molecular Biology and Evolution. 2021 Jan 4; 38(1):31–47. Available from: https://academic.oup.com/mbe/article/38/1/31/5900268

149. Buschmann MD, Carrasco MJ, Alishetty S, Paige M, Alameh MG, Weissman D. Nanomaterial Delivery Systems for mRNA Vaccines. Vaccines. 2021 Jan 19; 9(1):65. Available from: https://www.mdpi.com/2076-393X/9/1/65

150. Polack FP, Thomas SJ, Kitchin N, Absalon J, Gurtman A, Lockhart S, et al. Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine. N Engl J Med. 2020 Dec 31; 383(27):2603–15. Available from: http://www.nejm.org/doi/10.1056/NEJMoa2034577

151. Sender R, Fuchs S, Milo R. Revised Estimates for the Number of Human and Bacteria Cells in the Body. PLoS Biol. 2016 Aug 19; 14(8):e1002533. Available from: https://dx.plos.org/10.1371/journal.pbio.1002533

152. Marinov GK, Williams BA, McCue K, Schroth GP, Gertz J, Myers RM, et al. From single-cell to cell-pool transcriptomes: Stochasticity in gene expression and RNA

splicing. Genome Research. 2014 Mar 1; 24(3):496–510. Available from: http://genome.cshlp.org/cgi/doi/10.1101/gr.161034.113

153. Kim J, Eygeris Y, Gupta M, Sahay G. Self-assembled mRNA vaccines. Advanced Drug Delivery Reviews. 2021 Mar; 170:83–112. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0169409X20302933

154. Wu Z, Li T. Nanoparticle-Mediated Cytoplasmic Delivery of Messenger RNA Vaccines: Challenges and Future Perspectives. Pharm Res. 2021 Mar; 38(3):473–8. Available from: http://link.springer.com/10.1007/s11095-021-03015-x

155. Hajj KA, Whitehead KA. Tools for translation: non-viral materials for therapeutic mRNA delivery. Nat Rev Mater. 2017 Oct; 2(10):17056. Available from: http://www.nature.com/articles/natrevmats201756

156. Smith SA, Selby LI, Johnston APR, Such GK. The Endosomal Escape of Nanoparticles: Toward More Efficient Cellular Delivery. Bioconjugate Chem. 2019 Feb 20; 30(2):263–72. Available from: https://pubs.acs.org/doi/10.1021/acs.bioconjchem.8b00732

157. Garneau NL, Wilusz J, Wilusz CJ. The highways and byways of mRNA decay. Nat Rev Mol Cell Biol. 2007 Feb; 8(2):113–26. Available from: http://www.nature.com/articles/nrm2104

158. Houseley J, Tollervey D. The Many Pathways of RNA Degradation. Cell. 2009 Feb; 136(4):763–76. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0092867409000671

159. Wei C-J, Crank MC, Shiver J, Graham BS, Mascola JR, Nabel GJ. Next-generation influenza vaccines: opportunities and challenges. Nat Rev Drug Discov. 2020 Apr; 19(4):239–52. Available from: http://www.nature.com/articles/s41573-019-0056-x

160. Shi K, Liu T, Fu H, Li W, Zheng X. Genome-wide analysis of lncRNA stability in human. Chen S-J, editor. PLoS Comput Biol. 2021 Apr 16; 17(4):e1008918. Available from: https://dx.plos.org/10.1371/journal.pcbi.1008918

161. Mauger DM, Cabral BJ, Presnyak V, Su SV, Reid DW, Goodman B, et al. mRNA structure regulates protein expression through changes in functional half-life. Proc Natl Acad Sci USA. 2019 Nov 26; 116(48):24075–83. Available from: http://www.pnas.org/lookup/doi/10.1073/pnas.1908052116

162. Hubstenberger A, Courel M, Bénard M, Souquere S, Ernoult-Lange M, Chouaib R, et al. P-Body Purification Reveals the Condensation of Repressed mRNA Regulons. Molecular Cell. 2017 Oct; 68(1):144-157.e5. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1097276517306512

163. Corbet GA, Parker R. RNP Granule Formation: Lessons from P-Bodies and Stress Granules. Cold Spring Harb Symp Quant Biol. 2019; 84:203–15. Available from: http://symposium.cshlp.org/lookup/doi/10.1101/sqb.2019.84.040329

164. Roberson PA, Romero MA, Osburn SC, Mumford PW, Vann CG, Fox CD, et al. Skeletal muscle LINE-1 ORF1 mRNA is higher in older humans but decreases with

endurance exercise and is negatively associated with higher physical activity. Journal of Applied Physiology. 2019 Oct 1; 127(4):895–904. Available from: https://www.physiology.org/doi/10.1152/japplphysiol.00352.2019

165. Pardi N, Tuyishime S, Muramatsu H, Kariko K, Mui BL, Tam YK, et al. Expression kinetics of nucleoside-modified mRNA delivered in lipid nanoparticles to mice by various routes. Journal of Controlled Release. 2015 Nov; 217:345–51. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0168365915300535

166. European Medicines Agency. Reply to open letter concerning COVID-19 vaccines. March 23, 2021. EMA/140520/2021. Available from: https://www.ema.europa.eu/en/documents/other/reply-open-letter-concerning-vaccines-covid-19_en.pdf

167. Akinc A, Querbes W, De S, Qin J, Frank-Kamenetsky M, Jayaprakash KN, et al. Targeted Delivery of RNAi Therapeutics With Endogenous and Exogenous Ligand-Based Mechanisms. Molecular Therapy. 2010 Jul; 18(7):1357–64. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1525001616310814

168. Cagigi A, Loré K. Immune Responses Induced by mRNA Vaccination in Mice, Monkeys and Humans. Vaccines. 2021 Jan 18; 9(1):61. Available from: https://www.mdpi.com/2076-393X/9/1/61

169. Hussain MM, Strickland DK, Bakillah A. THE MAMMALIAN LOW-DENSITY LIPOPROTEIN RECEPTOR FAMILY. Annu Rev Nutr. 1999 Jul; 19(1):141–72. Available from: http://www.annualreviews.org/doi/10.1146/annurev.nutr.19.1.141

170. Mahley RW, Rall SC. A POLIPOPROTEIN E: Far More Than a Lipid Transport Protein. Annu Rev Genom Hum Genet. 2000 Sep; 1(1):507–37. Available from: http://www.annualreviews.org/doi/10.1146/annurev.genom.1.1.507

171. Probst J, Weide B, Scheel B, Pichler BJ, Hoerr I, Rammensee H-G, et al. Spontaneous cellular uptake of exogenous messenger RNA in vivo is nucleic acid-specific, saturable and ion dependent. Gene Ther. 2007 Aug; 14(15):1175–80. Available from: http://www.nature.com/articles/3302964

172. Lazzaro S, Giovani C, Mangiavacchi S, Magini D, Maione D, Baudner B, et al. CD8 T-cell priming upon mRNA vaccination is restricted to bone-marrow-derived antigen-presenting cells and may involve antigen transfer from myocytes. Immunology. 2015 Oct; 146(2):312–26. Available from: https://onlinelibrary.wiley.com/doi/10.1111/imm.12505

173. Blakney AK, Deletic P, McKay PF, Bouton CR, Ashford M, Shattock RJ, et al. Effect of complexing lipids on cellular uptake and expression of messenger RNA in human skin explants. Journal of Controlled Release. 2021 Feb; 330:1250–61. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0168365920306830

174. Ratajczak J, Miekus K, Kucia M, Zhang J, Reca R, Dvorak P, et al. Embryonic stem cell-derived microvesicles reprogram hematopoietic progenitors: evidence for horizontal transfer of mRNA and protein delivery. Leukemia. 2006 May; 20(5):847–56. Available from: http://www.nature.com/articles/2404132

175. Skog J, Würdinger T, van Rijn S, Meijer DH, Gainche L, Curry WT, et al. Glioblastoma microvesicles transport RNA and proteins that promote tumour growth and provide diagnostic biomarkers. Nat Cell Biol. 2008 Dec; 10(12):1470–6. Available from: http://www.nature.com/articles/ncb1800

176. Ratajczak MZ, Ratajczak J. Extracellular microvesicles/exosomes: discovery, disbelief, acceptance, and the future? Leukemia. 2020 Dec; 34(12):3126–35. Available from: http://www.nature.com/articles/s41375-020-01041-z

177. van den Boorn JG, Schlee M, Coch C, Hartmann G. SiRNA delivery with exosome nanoparticles. Nat Biotechnol. 2011 Apr; 29(4):325–6. Available from: http://www.nature.com/articles/nbt.1830

178. Kowal J, Tkach M. Dendritic cell extracellular vesicles. In: International Review of Cell and Molecular Biology. Elsevier; 2019. p. 213–49. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1937644819300760

179. Ying W, Riopel M, Bandyopadhyay G, Dong Y, Birmingham A, Seo JB, et al. Adipose Tissue Macrophage-Derived Exosomal miRNAs Can Modulate In Vivo and In Vitro Insulin Sensitivity. Cell. 2017 Oct; 171(2):372-384.e12. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0092867417309935

180. Ostermeier GC, Miller D, Huntriss JD, Diamond MP, Krawetz SA. Delivering spermatozoan RNA to the oocyte. Nature. 2004 May; 429(6988):154–154. Available from: http://www.nature.com/articles/429154a

181. Chen Q, Yan W, Duan E. Epigenetic inheritance of acquired traits through sperm RNAs and sperm RNA modifications. Nat Rev Genet. 2016 Dec; 17(12):733–43. Available from: http://www.nature.com/articles/nrg.2016.106

182. Sun YH, Wang A, Song C, Shankar G, Srivastava RK, Au KF, et al. Single-molecule long-read sequencing reveals a conserved intact long RNA profile in sperm. Nat Commun. 2021 Dec; 12(1):1361. Available from: http://www.nature.com/articles/s41467-021-21524-6

183. Sciamanna I, Serafino A, Shapiro JA, Spadafora C. The active role of spermatozoa in transgenerational inheritance. Proc R Soc B. 2019 Aug 28; 286(1909):20191263. Available from: https://royalsocietypublishing.org/doi/10.1098/rspb.2019.1263

184. Cossetti C, Lugini L, Astrologo L, Saggio I, Fais S, Spadafora C. Soma-to-Germline Transmission of RNA in Mice Xenografted with Human Tumour Cells: Possible Transport by Exosomes. Busson P, editor. PLoS ONE. 2014 Jul 3; 9(7):e101629. Available from: https://dx.plos.org/10.1371/journal.pone.0101629

185. Zhang L, Richards A, Barrasa MI, Hughes SH, Young RA, Jaenisch R. 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 May 25; 118(21):e2105968118. Available from: http://www.pnas.org/lookup/doi/10.1073/pnas.2105968118

186. Jones RB, Song H, Xu Y, Garrison KE, Buzdin AA, Anwar N, et al. LINE-1 Retrotransposable Element DNA Accumulates in HIV-1-Infected Cells. J Virol. 2013

Dec 15; 87(24):13307–20. Available from: https://journals.asm.org/doi/10.1128/JVI.02257-13

187. Macchietto MG, Langlois RA, Shen SS. Virus-induced transposable element expression up-regulation in human and mouse host cells. Life Sci Alliance. 2020 Feb; 3(2):e201900536. Available from: https://www.life-science-alliance.org/lookup/doi/10.26508/lsa.201900536

188. Yin Y, Liu X, He X, Zhou L. Exogenous Coronavirus Interacts With Endogenous Retrotransposon in Human Cells. Front Cell Infect Microbiol. 2021 Feb 25; 11:609160. Available from: https://www.frontiersin.org/articles/10.3389/fcimb.2021.609160/full

189. Kim D, Lee J-Y, Yang J-S, Kim JW, Kim VN, Chang H. The Architecture of SARS-CoV-2 Transcriptome. Cell. 2020 May; 181(4):914-921.e10. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0092867420304062

190. Flasch DA, Macia Á, Sánchez L, Ljungman M, Heras SR, García-Pérez JL, et al. Genome-wide de novo L1 Retrotransposition Connects Endonuclease Activity with Replication. Cell. 2019 May; 177(4):837-851.e28. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0092867419302338

191. Sultana T, van Essen D, Siol O, Bailly-Bechet M, Philippe C, Zine El Aabidine A, et al. The Landscape of L1 Retrotransposons in the Human Genome Is Shaped by Pre-insertion Sequence Biases and Post-insertion Selection. Molecular Cell. 2019 May; 74(3):555-570.e7. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1097276519301479

192. Bartha I, di Iulio J, Venter JC, Telenti A. Human gene essentiality. Nat Rev Genet. 2018 Jan; 19(1):51–62. Available from: http://www.nature.com/articles/nrg.2017.75

193. Zhang L, Vijg J. Somatic Mutagenesis in Mammals and Its Implications for Human Disease and Aging. Annu Rev Genet. 2018 Nov 23; 52(1):397–419. Available from: https://www.annualreviews.org/doi/10.1146/annurev-genet-120417-031501

194. Sender R, Milo R. The distribution of cellular turnover in the human body. Nat Med. 2021 Jan; 27(1):45–8. Available from: http://www.nature.com/articles/s41591-020-01182-9

195. Tomasetti C, Poling J, Roberts NJ, London NR, Pittman ME, Haffner MC, et al. Cell division rates decrease with age, providing a potential explanation for the age-dependent deceleration in cancer incidence. Proc Natl Acad Sci USA. 2019 Oct 8; 116(41):20482–8. Available from: http://www.pnas.org/lookup/doi/10.1073/pnas.1905722116

196. Pucella JN, Upadhaya S, Reizis B. The Source and Dynamics of Adult Hematopoiesis: Insights from Lineage Tracing. Annu Rev Cell Dev Biol. 2020 Oct 6; 36(1):529–50. Available from: https://www.annualreviews.org/doi/10.1146/annurev-cellbio-020520-114601

197. Pardi N, Hogan MJ, Weissman D. Recent advances in mRNA vaccine technology. Current Opinion in Immunology. 2020 Aug; 65:14–20. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0952791520300108

198. Jain R, Frederick JP, Huang EY, Burke KE, Mauger DM, Andrianova EA, et al. MicroRNAs Enable mRNA Therapeutics to Selectively Program Cancer Cells to Self-Destruct. Nucleic Acid Therapeutics. 2018 Oct; 28(5):285–96. Available from: https://www.liebertpub.com/doi/10.1089/nat.2018.0734

199. Finlay BB, Amato KR, Azad M, Blaser MJ, Bosch TCG, Chu H, et al. The hygiene hypothesis, the COVID pandemic, and consequences for the human microbiome. Proc Natl Acad Sci USA. 2021 Feb 9; 118(6):e2010217118. Available from: http://www.pnas.org/lookup/doi/10.1073/pnas.2010217118

200. Romano-Keeler J, Zhang J, Sun J. COVID-19 and the neonatal microbiome: will the pandemic cost infants their microbes? Gut Microbes. 2021 Jan 1; 13(1):1912562. Available from: https://www.tandfonline.com/doi/full/10.1080/19490976.2021.1912562

201. Ghanemi A, Yoshioka M, St-Amand J. Coronavirus Disease 2019 (COVID-19) Crisis: Losing Our Immunity When We Need It the Most. Biology. 2021 Jun 18; 10(6):545. Available from: https://www.mdpi.com/2079-7737/10/6/545

202. Adams JW, Kaufman RE, Kretschmer PJ, Harrison M, Nienhuis AW. A family of long reiterated DNA sequences, one copy of which is next to the human beta globin gene. Nucl Acids Res. 1980; 8(24):6113–28. Available from: https://academic.oup.com/nar/article-lookup/doi/10.1093/nar/8.24.6113

203. Skowronski J, Singer MF. Expression of a cytoplasmic LINE-1 transcript is regulated in a human teratocarcinoma cell line. Proceedings of the National Academy of Sciences. 1985 Sep 1; 82(18):6050–4. Available from: http://www.pnas.org/cgi/doi/10.1073/pnas.82.18.6050

204. European Medicines Agency. Comirnaty: EPAR - Product Information. June 02, 2021. Available from: https://www.ema.europa.eu/en/documents/product-information/comirnaty-epar-product-information_en.pdf

205. European Medicines Agency. Spikevax (previously COVID-19 Vaccine Moderna): EPAR - Product information. June 23, 2021. Available from: https://www.ema.europa.eu/en/documents/product-information/spikevax-previously-covid-19-vaccine-moderna-epar-product-information_en.pdf