OZBIOSCIENCES
The art of delivery systems

Technical Note - mRNAs

Why choose mRNAs over pDNA?

Reporter, Genome Editing, Vaccine mRNAs

Why choose stabilized mRNA over pDNA?

ADVANTAGE #1: It does not require nuclear uptake for being expressed since translation of mRNA occurs into the cytoplasm. Indeed, nuclear delivery (passing through the nuclear membrane) is one of the principal barriers for transfecting slow or non-dividing cells and consequently, mRNA transfection is particularly attractive for such purpose.

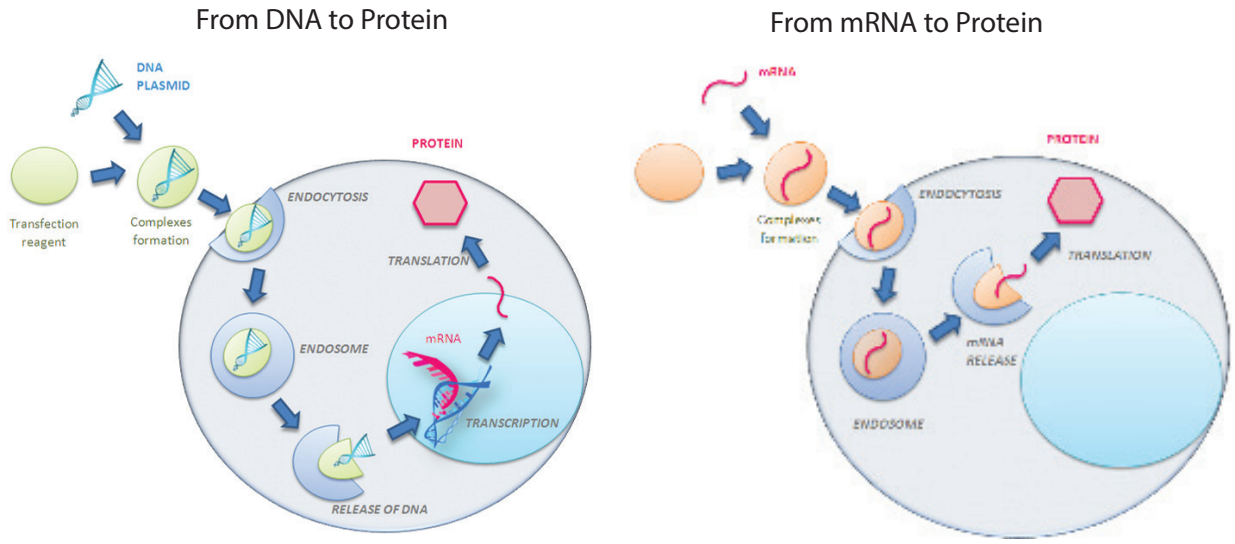


Figure 1: Protein translation process following DNA and mRNA transfection

ADVANTAGE #2: This approach is not integrative. Contrary to pDNA, mRNA cannot lead to genetic insertion causing mutations.

ADVANTAGE #3: Perfect for hard-to-transfect cells. mRNA has several merits over DNA that allows to genetically modify primary and hard-to-transfect cells more easily. Beside the fact that with mRNA there is no risk of integration into the host genome, mRNA transfection is cell cycle-independent, particularly suitable for slow-dividing cells such as endothelial cells or dendritic cells[1].

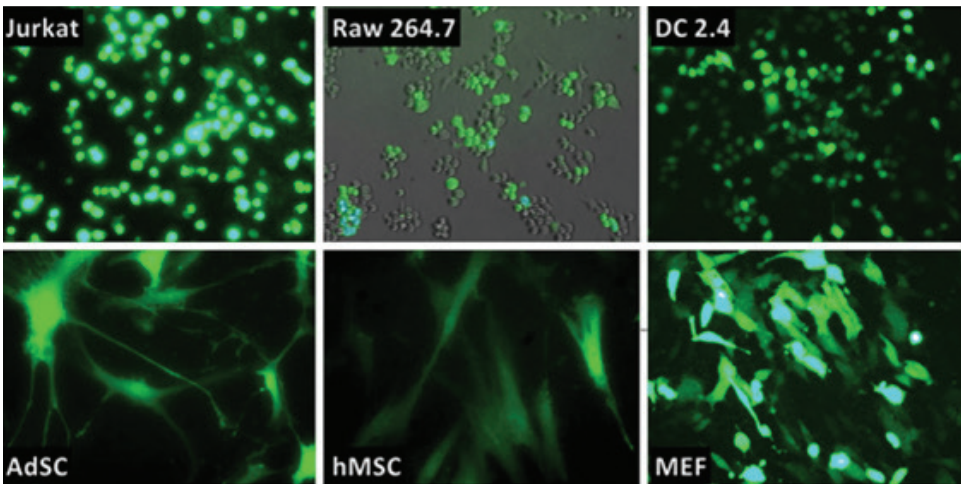


Figure 2: Jurkat T cells, Raw264.7, DC2.4, primary human Adipose Stem Cells, primary human Mesenchymal Stem Cells and Mouse Embryonic Fibroblasts were transfected using RmesFect transfection reagent and mRNA-GFP from OZ Biosciences.

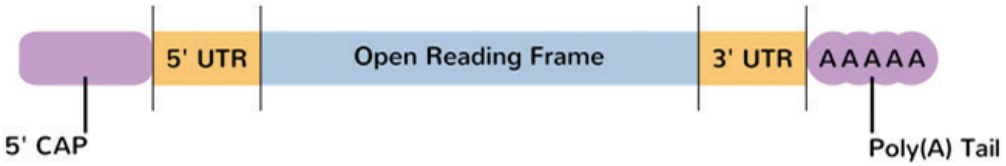
[1]Yamamoto A. et al, Eur J Pharm Biopharm. 2009, Current prospects for mRNA gene delivery.

nstead of plasmid DNA

mRNA complete benefits

- No need for nuclear uptake - protein expression directly in cytoplasm
- Faster protein expression than DNA transfection
- No genomic integration
- Perfect for transfecting slowing or non-dividing cells
- Protein expression in a total promoter-independent manner
- Transient transfection: mRNA based expression of proteins sustains for a limited time

mRNA Structure



5' Cap

This cap structure protects mRNA from degradation and recruits processing and translation factors.
In mammals, the predominant form is a 7-methyl-guanosine (Cap 0) linked via an a 5' to 5' triphosphate bridge to the first transcribed nucleotide which is methylated on the ribose O-2 position (Cap 1).
Other methylations are also observed including first transcribed nucleotide adenosine methylation on position 6 and second transcribed nucleotide ribose O-2 methylation.

5' Untranslated Region (5' UTR)

The 5' UTR is a non-coding region directly upstream from the initiation codon involved in the post-transcriptional regulation of gene expression by modulating mRNA stability, transport, subcellular localization and translation efficiency thus allowing a fine control of the protein product. This region has a high GC content and several secondary structures and comprises the Kozak sequence (GCCGCCRCCAUGG) that plays a major role in the initiation of the translation process.

Open Reading Frame (ORF)

This internal region of eukaryotic mRNA is translated into protein. The ORF begins with a methionine codon (AUG) and ends with a stop codon.

3' Untranslated Region (3'UTR)

The 3' UTR is the part of mRNA that immediately follows the translation termination codon. This region plays a crucial role in gene expression by influencing the localization, stability, export, and translation efficiency of an mRNA. It contains various sequences including microRNA response elements (MREs), AU-rich elements (AREs), and the poly(A) tail.

Poly(A) tail

The poly(A) tail is a long sequence of adenine nucleotides (*0-250 nucleotides with a median length of 50-100 in HeLa and NIH-3T3 cells*)[2] added to the 3' end of the pre-mRNA.
The poly(A) tail contains binding sites for poly(A) binding proteins (PABPs) that play a major role in export from the nucleus, translation, and protection from degradation. Its length is an important determinant of translational efficiency and mRNA stability. This is an important element as its absence or removal often leads to exonuclease-mediated degradation of the mRNA.

[2]Chang H. et al; Mol Cell 2014 - PMID: 24582499A

mRNA Vaccines

Nucleic acid Vaccine combine the positive features of live attenuated vaccines while avoiding many potential safety limitations[1]. These vaccines present several advantages over conventional vaccines such as:

- Mimicking a live infection by expressing antigens *in situ* after immunization and priming both B and T cell responses including cytotoxic T lymphocytes[2].
- Revealing focused immune responses directed toward the selected antigens of interest with no potential reversion to pathogenicity.

mRNA vaccines benefits:

mRNA vaccines present a better safety profile than DNA vaccines: DNA vaccines display a long term expression, a potential risk for genome integration and induction of anti-DNA antibodies[3]. The main advantages of mRNA vaccines come from the intrinsic properties of the mRNA:

- They are produced using cell-free enzymatic transcription.
- The transient expression of mRNA encoded antigen enables a more controlled antigen espression and minimizes the risk of tolerance induction that can be associated with long-term exposure[4].
- There is thus an absence of any additional encoded protein which exclude the possibility of raising undesired immune response or interaction with the host[5]
- Their stabilized design allows higher level of expression *in vivo*[6].
- mRNA serves the dual purpose of expressing the desired antigen as well as acting as an adjuvant.
- mRNA has a superior safety profile compared to inactivated viruses or pathogens.

OZ Biosciences mRNAs for Vaccination/Immunization:

OVA mRNA (Unmodified) ref# MRNA42	Activates innate immune system and production of cytokines in order to engage induced immune response but mRNAs are rapidly degraded.
OVA mRNA (moU) ref# MRNA41	Reduces immune effect compared to unmodified mRNA but lasts longer in the organism due to the stabilization and protection.
Beta-Gal mRNA (moU) ref# MRNA14	Encodes for a protein product of the bacterial LacZ gene.

1. Deering RP et al, Expert Opin Drug Deliv. 2014. Nucleic acid vaccines: prospects for non-viral delivery of mRNA vaccines.
2. Johansson DX et al, PLoS One. 2012. Intradermal electroporation of naked replicon RNA elicits strong immune responses.
3. Pascolo S., Handb Exp Pharmacol. 2008. Vaccination with messenger RNA (mRNA).
4. Pollard C. et al, Trends Mol Med. 2013. Challenges and advances towards the rational design of mRNA vaccines.
5. Schlake T. et al, RNA Biol. 2012. Developing mRNA-vaccine technologies.
6. Kallen KJ. et al, Hum Vaccin Immunother. 2013. A novel, disruptive vaccination technology: self-adjuvanted RNActive(®) vaccines.

Reporter mRNAs

Reporter genes are commonly used in cell biology research. Reporter mRNAs can be used as controls to study transfection and expression in mammalian cells using a variety of assays.
These capped (Cap 1) and polyadenylated mRNAs are optimized for mammalian systems and are composed of unmodified NTPs or are modified (moU replaces U) to reduce immune stimulation.
They mimic fully processed mature mRNAs.

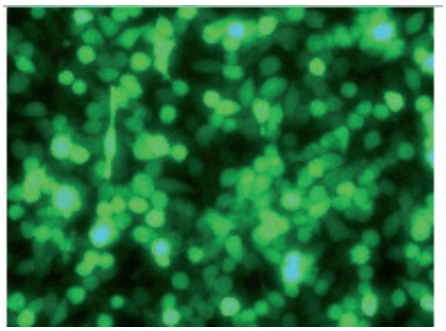


Figure 5: Transfection of GFP mRNA with RmesFect on HeLa cells.

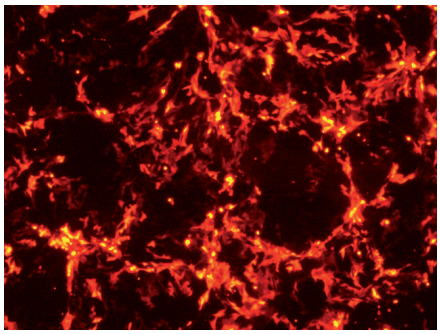


Figure 6: Transfection of mCherry mRNA with RmesFect on SK6 cells.

OZ Biosciences Reporter mRNAs:

GFP mRNA (Unmodified) ref# MRNA15 GFP mRNA (moU) ref# MRNA11	Designed to produce high expression level of Green Fluorescent Protein. It is a commonly used direct detection reporter in mammalian cell culture, yielding bright green fluorescence with an excitation peak at 488 nm and an emission peak at 507 nm.
LUC mRNA (moU) ref# MRNA12	Designed to produce high expression level of FireFly Luciferase. It is commonly used in mammalian cell culture to measure both gene expression and cell viability. FireFly Luciferase emits bioluminescence in the presence of the substrate, luciferin.
mCherry mRNA (moU) ref# MRNA13	Encodes the mCherry fluorescent protein which is derived from DsRed, a protein found in *Discosoma sp.* mCherry is a monomeric fluorophore with a excitation peak at 587 nm and emission at 610 nm. mCherry is photostable and resistant to photobleaching.
Beta-Gal mRNA (moU) ref# MRNA14	Encodes for a protein product of the bacterial LacZ gene. Beta-Gal catalyzes the conversion of Beta-galactosides into monosaccharides. It is a common marker gene used to assess transfection efficiency.

Genome Editing mRNAs

Introduce a variety of genetic alterations (deletion, insertion…) into mammalian cells with Genome Editing techniques.

Genome editing mRNA benefits

Plasmids and viral vectors are traditionally used in genome editing to express the required proteins.
mRNA-based strategy for delivery of CRISPR/Cas9 offers an advantage over pDNA-based approach:

• Cas9: Delivery of DNA/RNA encoding the Cas9 protein is an attractive non-viral method for introduction of the CRISPR/Cas9 machinery into the cell. Unlike gene-based delivery methods, mRNA-based strategies are transient in function, leading to the eventual removal of the nuclease from the cell and circumventing the risks associated with integration into the host genome[1].

• Cre Recombinase: Site specific recombinases are useful tools for manipulation of genomes. However, continued expression of a recombinase in a cell or *in vivo* can result in toxicity and undesired off-target recombination. For this reason, transient expression from mRNA is an ideal method for recombinase expression.

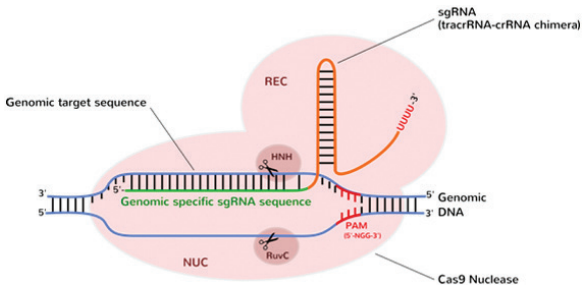


Figure 3: The CRISPR-Cas9 nuclease programmed with sgRNA.

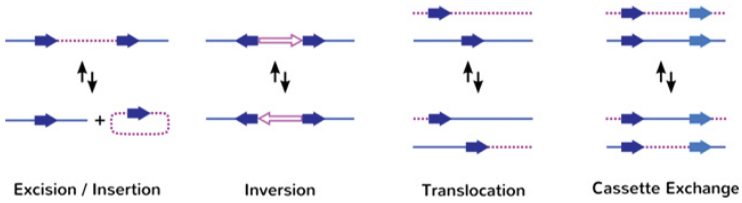


Figure 4: Cre recombinase induces 4 types of site-specific genome modifications. Excision/insertion - Inversion - Translocation - Cassette exchange (DNA is exchanged between two DNA molecules) - Adapted from Nern A. et al, PNAS (108)34; 14198–14203).

OZ Biosciences mRNAs for Genome Editing:

Cas9 endonuclease mRNA (moU) ref# MRNA31	The RNA-guided Cas9 endonuclease is used to induce site-directed double strand breaks in DNA. These breaks can lead to gene inactivation or introduction of heterologous genes, providing efficient tool for Genome Editing[2-5].
CRE Recombinase mRNA (moU) ref# MRNA32	Site-specific DNA recombinases are widely used in cells and organisms to manipulate the structure of genomes and to control gene expression by targeted activation or de-activation. Each recombinase catalyzes 4 types of DNA exchange reactions (figure 4) between short specific target sequences (30-40 nucleotides).

1. Luther DC. et al, Exp. Opin Drug Deliv. 2018;15(9):905–913.
2. Cong L. et al, Science. 2013;339 (6121):819-823.
3. Mali P. et al, Science. 2013;339 (6121):823-826.
4. Jinek M. et al, Science. 2012;337 (6096):816-821.
5. Cho SW. et al, Nat Biotechnol. 2013;31(3):230-232.

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