

Enhancing mRNA results through strategic RNA design and quality control



INTERVIEW

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In the last few years, mRNA has moved to the fore in the vaccine and cell and gene therapy fields, and as a result, its successful production for these downstream applications has never been more crucial. In this podcast episode, **Róisín McGuigan**, Editor, *Nucleic Acid Insights*, speaks to **Ryan Lahr**, Senior R&D Scientist, CELLSCRIPT™, about design, production, and quality control of synthesized mRNA for translation in cells.

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Q Can you tell me a bit about yourself and the current work that you do?

RL I work as part of the product development team at CELLSCRIPT™. Our company develops high-quality, easy-to-use technologies for downstream applications where mRNA is translated in cells, which includes the areas of cell and gene therapy

research and mRNA vaccine development. We provide technologies for all aspects of mRNA synthesis, including the use of modified nucleosides in mRNA. Additionally, we have recently launched benchtop PAGE-based quality control kits for assessing mRNA capping efficiency and tail length.

Q Can you frame the key challenges and considerations when optimizing mRNA design for different research applications in the vaccine and cell and gene therapy fields?

RL Robust protein expression of mRNA is essential for successful applications in vaccine development and cell and gene therapy research. The structure of an mRNA molecule consists of a 5' cap, an RNA transcript, and a 3'-poly(A) tail. The transcript contains the coding region to be translated into a protein by ribosomes in cells. The 5' cap interacts with cellular translation machinery for recruitment of the mRNA to ribosomes for translation, whereas the 3'-poly(A) tail is involved in protecting the transcript from exonuclease digestion in cells. All three regions must be carefully considered when designing your transcript for successful translation in cells.

There are several design elements known to improve stability and longevity of mRNA in cells while also reducing innate immunogenicity, which is the body's first line of defense against foreign substances. Researchers should consider incorporating these design elements into their mRNA to address their specific application needs. One critically important consideration is minimization of immunogenic response by cells. A well-documented method for reducing immunogenicity is incorporation of modified nucleosides like pseudouridine and N1-methyl-pseudouridine into mRNA transcripts.

Another important consideration concerns the 5' cap structure. Complete capping of the 5' end of all mRNAs in your sample, as well as modifications to the 5' cap structure, are important for both the recruitment of cellular machinery for translation and *in vivo* stability of your mRNA. Additionally, 3'-poly(A) tail length should be taken into account to ensure the mRNA transcript survives exonuclease digestion in cells. Synthesized mRNA with poly(A) tails shorter than 150 to 200 A's—the typical tail length found in nature—may experience digestion that negatively affects stability of the transcript.

Q Can you expand on the impact design decisions around nucleoside composition, cap structure, and length of poly(A) tail have on both the quality and functionality of mRNA?

RL Methylation of the 5' cap (Cap-0) results in a structure called Cap-1 that helps pattern recognition sensors in the cell to mark the mRNA as 'self' versus 'non-self'. As mentioned, the poly(A) tail protects mRNA from exonuclease degradation on the 3' end. However, it is also required for mRNA expression, as the binding of proteins to the poly(A) tail stimulate ribosome recruitment for translation.

Poly(A)-tail length shortens over time in the cell through a process known as deadenylation. Once a poly(A) tail reaches a minimum length, the mRNA is decapped and then degraded from the 5' end. In nature, the rate of deadenylation plays an important role in

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gene regulation as the length of the poly(A) tail determines the half-life of the mRNA. Having longer tails will increase the half-life of your mRNA transcript and therefore increase the total quantity of protein expressed. With all this in mind, it is recommended to start with tails of at least 150–200 A’s.

Q How can the risk of innate immunity development best be addressed?

RL Cells have evolved mechanisms to quickly recognize and respond to foreign or ‘non-self’ RNA as a defense against viral infection. These mechanisms are part of what is referred to as innate immunity and include specialized protein receptors called pattern recognition sensors that constantly search RNA for landmarks that denote ‘self’ versus ‘non-self’ RNA. The design challenge for researchers is to introduce foreign RNA for protein expression in cells that also has incorporated features that pattern recognition sensors will confirm as ‘self’.

Incorporation of modified nucleosides, like pseudouridine and N1-methyl-pseudouridine, has been shown to reduce cellular innate immune responses. Use of modified nucleosides in mRNA has been particularly beneficial for therapeutic applications.

Another modification that also helps to mark the mRNA as ‘self’ is the presence of a Cap-1, which is a cap methylation modification only found in higher eukaryotes. ‘Non-self’ RNAs are either quickly degraded in the cell or elicit apoptosis. Because of this, Cap-1 capped mRNA is expressed at higher levels in cells when compared to Cap-0 capped or uncapped RNA.

Because double-stranded RNA (dsRNA) is present during the replication cycles of most eukaryotic viral infections, cells have evolved pattern recognition sensors to detect the presence of dsRNA in the cell and then elicit an innate immune response. Unfortunately, dsRNA is also produced as an unwanted byproduct during *in vitro* transcription and, thus, all mRNA preps contain some percentage of dsRNA. Removal of this byproduct is essential for reducing immunogenicity and thus ensuring increased expression of the mRNA.

Q What advice would you share with developers looking to optimize mRNA design and synthesis for their own specific application?

RL There are many resources that discuss optimization of mRNA design. I’d recommend checking out CELLSCRIPT’s website, where you can find answers to frequently asked questions and additional tips and tricks for design. You can also find a variety of kits, enzymes, and assays for both mRNA synthesis and QC, as well as can connect directly with an mRNA expert to discuss your research needs in a one-on-one format.

Finally, don't forget quality control testing of your mRNA—confirming that your mRNA is of sufficient quality for your application is a step that shouldn't be overlooked.

Q What are the key considerations—and the common pitfalls—when developing a quality control strategy for synthesized transcripts? What analytical tools and techniques are available to assess quality?

RL The current USP guidelines recommend that both capping and tailing analysis be performed via LC-MS or HPLC. However, there is a high capital cost associated with this equipment both from the initial investment for the equipment to specialized training and ongoing maintenance that these systems require. The high-cost barrier for this instrumentation often means that researchers will use a third party to perform their testing.

Outsourcing quality control testing can have a lot of downsides, including large sample amount requirements, the possibility that samples can be lost or damaged during shipment, long lead times (often weeks) to get results, and the extensive communication needed with the testing facility. Additionally, third-party outsourcing can prove to be very expensive.

As a new entry into the mRNA QC market, CELLSRIPT™ has recently introduced our EZ-QC™ mRNA QC Assay Kits. The EZ-QC kits are fluorescence-based PAGE assays that analyze 5'-capping efficiency and 3'-poly(A)-tail length. Assays are performed on the benchtop, using common lab equipment, and can be completed in a single day.

Q Finally, what advice would you give to new researchers interested in using mRNA for translation in cells?

RL Take your time when designing your mRNA and focus on the downstream application. For example, how concerned are you about immunogenicity? Would using modified nucleosides be beneficial for your application? How much dsRNA contamination can your application tolerate? Also, just like with any area of science, expect that there might be a lot of trial and error for new scientists.

BIOGRAPHY

Ryan Lahr received his BA from the University of Northern Iowa, Cedar Falls, IA, USA and has since built a career of over 15 years in the biotech industry, where he has developed products for Hologic, Illumina, and now CELLSRIPT™ as a Senior R&D Scientist.

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AUTHORSHIP & CONFLICT OF INTEREST

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