TECHNOLOGY ADVANCES NEEDED TO DRIVE FUTURE mRNA GROWTH

Both strong growth and further evolution are projected for the mRNA market, with the latter needed to maximize the former. Applications for mRNA are expanding beyond vaccines to therapeutics that treat large populations to personalized therapies tailored to individual patients and to technologies that support CRISPR-Cas9 mediated gene editing. Increasing experience and knowledge will improve contract development and manufacturing services, which will in turn contribute to the ongoing maturation of the sector. In addition, improvements to existing manufacturing approaches, as well as alternatives to plasmid DNA, *in vitro* transcription, and lipid nanoparticle delivery, could have a real impact. Furthermore, considerable interest is building in the development of continuous manufacturing strategies and novel solutions for the storage and shipment of formulated mRNA products, which will be critical to facilitating global access to mRNA vaccines and therapies.

Evolving Application Landscape

Following the rapid and public development of the COVID-19 mRNA vaccines, development efforts in the field of mRNA remain dominated by vaccine candidates that target infectious diseases. However, significant effort has been undertaken in other areas, including cancer treatments, some of which are intended as personalized medicines tailored to the tumor profiles of individual patients. Potential therapeutic applications are being explored, including protein and gene replacement for a range of genetic diseases. Research on gene editing for the treatment and/or cure of human genetic disorders leveraging mRNA for the delivery of CRISPR components is also advancing.

Every Construct is Different

The broadening range of applications will require further expansion of knowledge and the understanding of the role of mRNA in disease and disease treatment. There are many types of RNA, and each of them behaves differently. Similarly, each mRNA construct comprises a different sequence with unique folding patterns and three-dimensional dynamics and hence exhibits unique properties and behaviors. The length of the mRNA, its structure, and whether it is meant to act as a vaccine or therapeutic all have an impact on the selection of a manufacturing strategy. As the sector matures, new problems that require new technological solutions will continue to arise. Production processes therefore need to be highly flexible and adaptable, as do analytical methods.

Limited CDMO Support

That need for flexibility creates challenges for contract development and manufacturing organizations (CDMOs). It also exacerbates a basic issue in the emerging mRNA field: few CDMOs have extensive experience in mRNA manufacturing, particularly at larger scales and under GMP conditions. Those that do largely gained that experience supporting COVID-19 vaccine development and manufacturing efforts that typically involved only one aspect of the overall complex, multi-step process.

Both large and small CDMOs are taking steps to gain expertise in mRNA manufacturing, most again focusing on either drug product or drug substance. A few are seeking to support both drug substance and drug product production, while fewer still are establishing end-to-end services from cell line development for pDNA manufacture through fill/finish of formulated mRNA–LNP products.

Opportunities for Process Optimization

While leaders in the mRNA field have been working for decades on processes for the production of mRNA therapeutics and vaccines, that work has largely been done at lab scale. Consequently, there remain many opportunities — and a real need — for further improvement of every step if these products are to be efficiently and

cost-effectively produced under GMP conditions at large scale for clinical and commercial applications.

Improved mRNA designs and LNP formulations are needed that ensure sufficient bioactivity without causing reactogenicity or undesired immunogenicity. Better enzymes are needed for the *in vitro* transcription (IVT) reaction. Current 5´ capping processes are inefficient and extremely costly. Reduction of by-product formation, particularly double-stranded RNA (dsRNA), is also crucial, as downstream purification to remove dsRNA and other impurities is currently quite challenging and often accompanied by higher costs and product losses.

Seeking Scalable Platform Processes

Another hurdle that CDMOs looking to support mRNA developers must overcome is the broad range of scales at which production capabilities will be needed, given the ever-widening array of vaccine and therapeutic applications. To date, manufacturing up to a few hundred liters has been sufficient to support the commercial needs for COVID-19 vaccines. Some mRNA candidates under development, however, are personalized medicines requiring minute amounts, while others target prevalent diseases and require larger doses, leading to the need for manufacturing at much larger scales.

Flexibility in scale must be accompanied by flexibility in platform design to support the needs of different mRNA sequences, as outlined above. In addition, some solutions that are optimal at small scales are not suitable for large scales, so scaling may involve the need for entirely different process trains leveraging different equipment and production strategies. Other challenges to scaling include limited availability of GMP-grade pDNA and the specialty enzymes required for mRNA production and managing the instability of mRNA, including its sensitivity to shear, temperature, and RNase degradation.

Non-Plasmid DNA Options

One approach to overcoming the limited capacity for pDNA production is to use an alternative source of DNA as the template for IVT. Doggybone DNA (dbDNA™), engineered plasmids constructed using DNA "bricks," antibiotic-free maintenance systems, and synthetic DNA produced via enzymatic processes are all examples of novel approaches under exploration.

The latter is of particular interest, as it requires a smaller manufacturing footprint, affords high yields, involves simpler downstream purification operations, can be scaled up or out, and does not require linearization of the product, and longer poly(A) tails can be incorporated for enhanced stability and translational capacity. Initial concerns about reading errors have largely been overcome through the use of engineered enzymes with proofreading capabilities.

Avoiding the IVT Reaction

Some efforts to improve mRNA production focus on the development of alternatives to IVT. One approach involves extending the use of automated, solid-phase synthesis leveraging phosphoramidite (modified nucleotide) chemistry for the production of short RNA molecules of <100 nucleotides to longer mRNA sequences by ligating the smaller units together. However, the high cost of the required ingredients needs to be addressed to make this approach amenable to large-scale production.

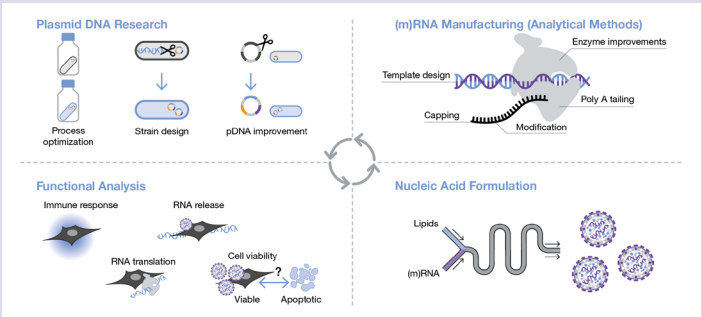
Another potential option involves bacterial fermentation of mRNA. However, there is much work to be done to make this approach practical. With current processes, there is no control over addition of the 5´ cap and poly(A) tail, and yields tend to be low owing to product losses during downstream processing.

RNA Innovation at Wacker Biotech

Given the rapid expansion of the mRNA market and the constant introduction of new and innovative mRNA modalities with wide-ranging applications, it is essential for any CDMO supporting mRNA vaccine and therapeutic developers to continuously invest in R&D and innovation in all aspects of pDNA, mRNA drug substance, and final mRNA drug product design and manufacture.

WACKER's corporate R&D team collaborates closely with universities and research institutions to develop new production platforms and technologies. Efforts are focused on the development of new proprietary bacterial strains and plasmid designs; novel RNA synthesis pathways, including those for mRNA, saRNA, and circRNA; novel lipids for new LNP compositions; and advanced, fit-for-purpose analytical methods for determination of capping efficiency, poly(A) tail length, encapsulation efficiency, dsRNA levels, and the cellular function of LNPs and RNAs.

Know-how gained over decades of optimizing manufacturing processes for recombinant proteins and antibody fragments, vaccines, live biotherapeutic products (LBPs), and pDNA is leveraged in these projects, as is Wacker Biotech's two decades of GMP production experience.



Four teams in WACKER's corporate R&D are working on innovations in pDNA, mRNA and LNP

These R&D efforts are complemented by extensive investments in facilities for mRNA R&D and production. Three-digit € million has been invested in a new facility for mRNA production at Wacker Biotech's production site in Halle, Germany. A new biotech R&D center is also being constructed, which includes facilities for development of processes for the production of a variety of nucleic acids. Wacker intends to invest over €80 million annually in expanding the growth of its biotechnology business over the next few years.

LNP Alternatives in the Longer Term

The predominant solution today for enhancing mRNA stability and facilitating its *in vivo* delivery is to encapsulate the mRNA drug substance in LNPs. However, it can be expected that, for each different mRNA application, some delivery vehicles may be more optimal than others. LNPs have proven fairly effective for vaccines, and they may also be appropriate for mRNA therapeutics targeting the liver.

For products targeting other tissues, however, there is need for improvement on existing LNP technology. That may include the use of novel cationic/ionizable lipids that enable controlled targeting to different tissues, like selective organ targeting (SORT) LNPs. It also may involve new forms of delivery vehicles, such as polymeric solutions or solutions that combine both polymers and lipids, or potentially viral vectors, such as adeno-associated viruses (AAVs). Virus-like particles (VLPs) and extracellular vesicles (EVs) are also under investigation.

Each option has advantages and disadvantages, ranging from payload capacity to product stability and specificity to manufacturability. The optimum vehicle for a given program must be selected on the basis of the specific mRNA sequence and target application. It is exciting that so many new, potentially effective solutions are under development that may enhance delivery and increase safety, potency, and cost-effectiveness.

Addressing Stability for Storage and Transport

The instability of mRNA molecules creates challenges not only during manufacturing but also during storage and distribution. The level of instability is determined by the length of the mRNA sequence, its secondary/tertiary structure alone and when encapsulated in the LNP, and the composition of the final formulation (e.g., the lipids used in the LNP and any other excipients included).

A bigger-picture approach involves producing mRNA products in miniaturized, automated manufacturing systems that can be located near patients, such as within hospitals, thereby eliminating the need for storage and shipment. Another approach involves lyophilization of the drug substance at a central manufacturing site, with final formulation taking place at the point of care by combining the drug substance with a stabilized delivery system, such as LPNs or other suitable carriers. This decentralized method can reduce the complexity and costs associated with cold-chain logistics and ensure that the mRNA drugs remain stable until they are ready for patient administration. Furthermore, this would also allow for customization of doses or formulations based on individual patient needs, providing a more personalized treatment option. In tandem with these strategies, ongoing research into more robust stabilization techniques and materials could further extend the shelf life and reduce the dependency on stringent storage conditions. Ultimately, the goal is to create a flexible, efficient, and patient-centric distribution model that ensures that mRNA therapies are available and effective for all who need them.

Role for Continuous Manufacturing

In a continuous process, recycling and reuse of expensive enzymes and other ingredients would reduce sourcing challenges and costs, while continuous removal of the mRNA drug substance would reduce the complexity of the downstream purification process. As an added benefit, operations only suitable for lab scale could be leveraged in a microfluidic

Germany Pandemic Preparedness

In Germany, Wacker Biotech has entered into a collaboration with the German government to create capacity for the "National Pandemic Preparedness Program." A facility is being constructed at the Halle, Germany, site that will contain capacity earmarked to support a rapid mRNA manufacturing response in the event that novel vaccines are required to alleviate a future pandemic. The plant comprises four independent process lines, each being highly flexible in the sense of production volume and process design. In this, pDNA supply, mRNA drug substance, and LNP formulation are covered by the multipurpose character of the new facility.

Wacker Biotech's mRNA Competence Center in Halle, Germany (expected to be operational by April 2024)

The building and qualification in the context of the "National Pandemic Preparedness Program" is executed under an accelerated schedule and is expected to be operational by April 2024. In this, Wacker Biotech's extensive experience within the CDMO business has been the key factor to successfully fulfill this challenging project. In addition, the new plant will broaden Wacker Biotech's capabilities as a full CDMO in the mRNA field by offering services and capacities to customers from early clinical to commercial projects.

Wacker Biotech feels honored to be selected as a partner by the German government in this ethical and highly beneficial program. This confirms Wacker Biotech's global reputation as a skilled and reliable player in the CDMO business. As a matter of fact, Wacker Biotech's customers will benefit from this new motivation and commitment!

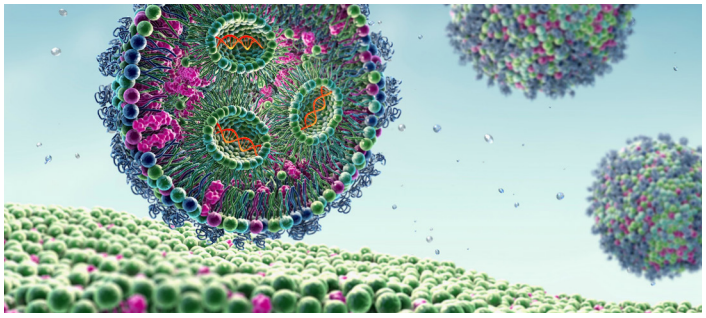
device running in continuous mode, eliminating scalability issues. Greater automation, meanwhile, can provide greater process control while reducing the risk of operator errors, contamination, and product degradation.

There is sufficient interest in continuous mRNA production that the U.S. Food and Drug Administration (FDA)'s Center for Biologics Evaluation and Research is funding a program led by the Massachusetts Institute of Technology (and also including Penn State University and Rensselaer Polytechnic Institute) to design the world's first fully integrated, continuous mRNA manufacturing platform. A few companies are also developing continuous mRNA–LNP manufacturing processes.

Potential of other mRNA Species

Messenger RNA is just one of several RNA molecules with critical roles in human biochemistry. Several RNA species have the potential to serve as novel drugs. They include antisense RNA (RNAi), antisense oligonucleotides (ASOs), small interfering RNA (siRNA), micro-RNA, and aptamers. More recently, self-amplifying RNA (saRNA), also termed replicons and circular RNAs (circRNAs), gained focus.

saRNAs have a structure similar to mRNA, including a 5´ cap and 3´ poly(A) tail, but are much larger because they also encode four additional proteins that allow them to replicate once administered. This ability enables reduced dosages and therefore the need for production of much smaller quantities of drug substance and drug product. Candidates to date have leveraged LNPs.



Lipid nanoparticles (LNPs)

Circular RNAs (circRNAs) are another type of RNA attracting significant interest. These RNA molecules, which are found in most known species, are covalently closed, with the 3´ and 5´ ends connected to one another. This structure allows circRNA to be resistant to exonuclease-mediated degradation and thus exhibit greater stability. While a full understanding of the roles that circRNA molecules play is lacking, there is evidence suggesting that some code for proteins, while others may act as gene regulators.

Conclusion

The mRNA field is poised for strong growth and continued evolution, expanding its applications from vaccines to personalized therapies and gene-editing technologies. The broadening range of mRNA applications presents exciting opportunities and challenges, including the need for flexible manufacturing processes, improved bioactivity, and advanced delivery solutions, as well as an ongoing focus on process optimization and scalability. Alternative approaches to traditional plasmid DNA and IVT are being explored, offering potential advantages in terms of manufacturing efficiency and cost-effectiveness. The future of mRNA also involves addressing stability concerns for storage and transport, including innovations such as lyophilization and miniaturized manufacturing systems for bedside patient care. Furthermore, the exploration of other mRNA species, such as saRNAs and circRNAs, opens new avenues for therapeutic development, as the recent approval of an saRNA based vaccine shows. In this rapidly evolving landscape, ongoing research and innovation are essential to drive future mRNA growth and maximize its potential for improving healthcare, and Wacker Biotech's commitment positions the company at the forefront of this emerging field. This proactive approach ensures that Wacker Biotech remains a trusted and reliable partner, contributing to the advancement of mRNA-based therapies and the fight against future pandemics.

About the Author

Dr. Richter studied Biotechnology at the TU Braunschweig and the University of Waterloo, and received his PhD from the MPI for Terrestrial Microbiology in Marburg. While there, he focused on characterization of CRISPR-Cas systems, specifically the maturation of CRISPR RNAs. Dr. Richter completed his postdoctoral studies at the laboratories of Emmanuelle Charpentier, moving to the Helmholtz-Centre for Infection Biology and later the Max-Planck-Institute for Infection Biology.

At WACKER, Dr. Richter is responsible for business development and technology scouting for innovative approaches, specifically anything related to nucleic acids. He has been a key part of growing WACKER's nucleic acid research from scratch, including plasmid DNA and mRNA manufacturing, as well as formulation and early analysis of the functionality.

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