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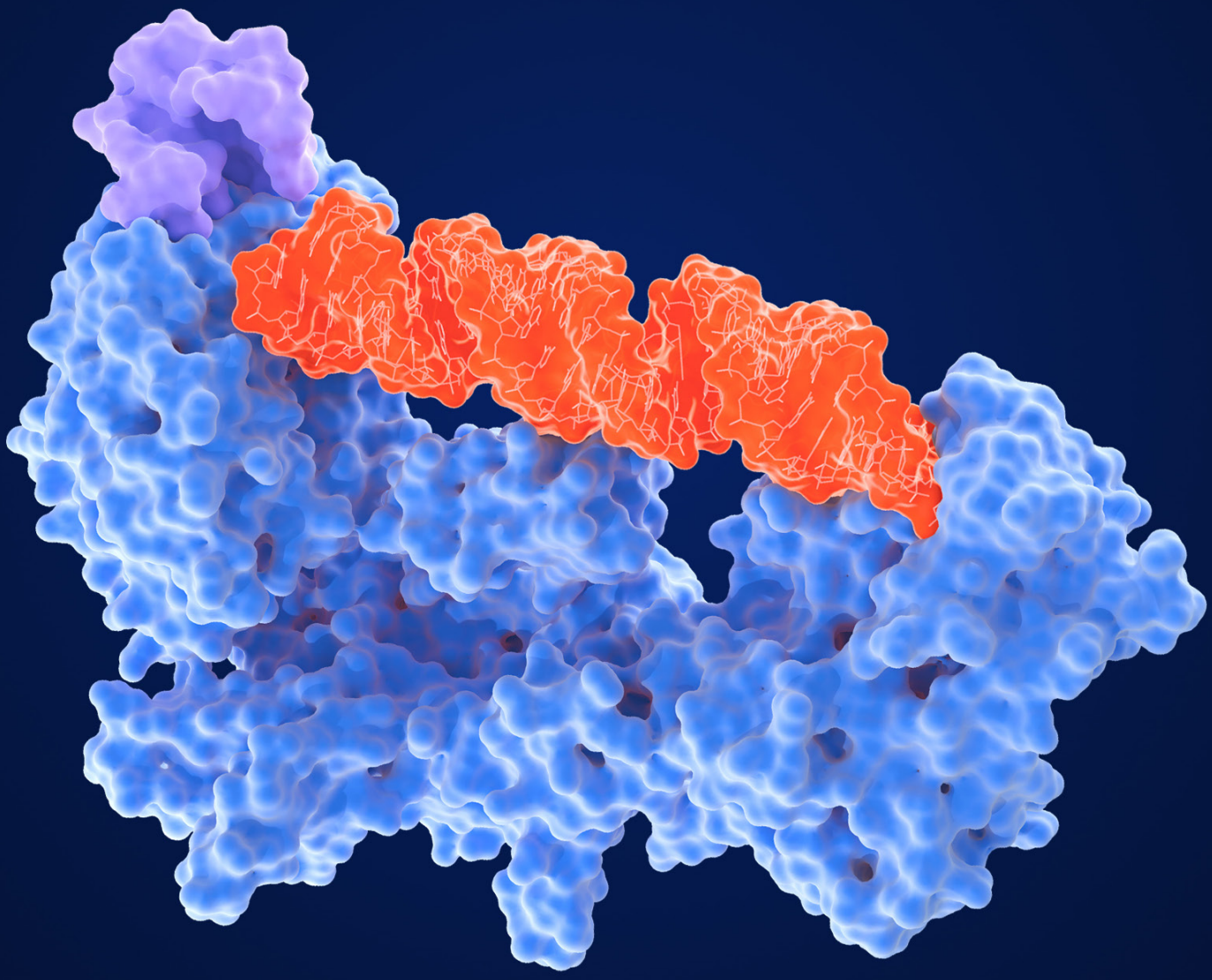
The New RNA World: Evolving RNA from Messenger to Versatile Therapeutic



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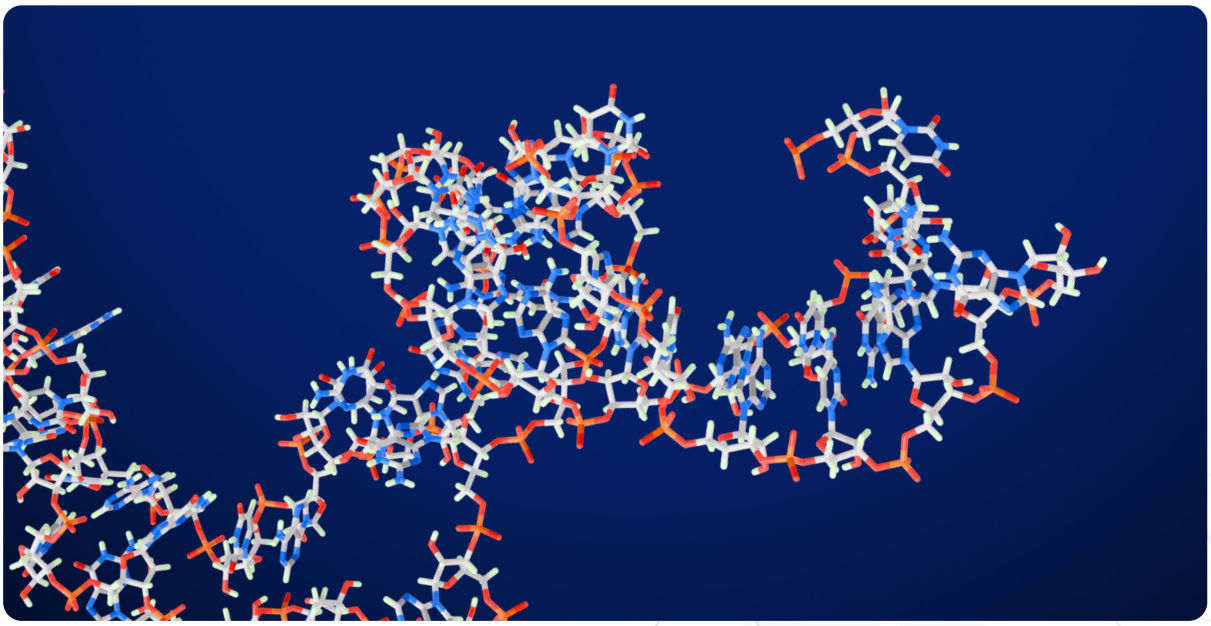
Overview

RNA has evolved from a molecular messenger into a powerful and versatile therapeutic tool. From the early days of antisense technology and RNA interference to the rise of mRNA vaccines and gene-editing guide RNAs, RNA-based modalities are now shaping the future of medicine. New delivery methods, AI-driven design, and expanding sequence libraries are accelerating the path from concept to clinic.

This article will explore:

- The Biological and Therapeutic Origins of RNA
- Expanding Modalities: mRNA, RNAi, ASOs, Aptamers, and gRNAs
- Clinical and Technological Breakthroughs in RNA Therapeutics
- How AI is Supercharging RNA Design, Delivery, and Decision-Making
- A Perspective on Data-Driven Collaboration in the New RNA World



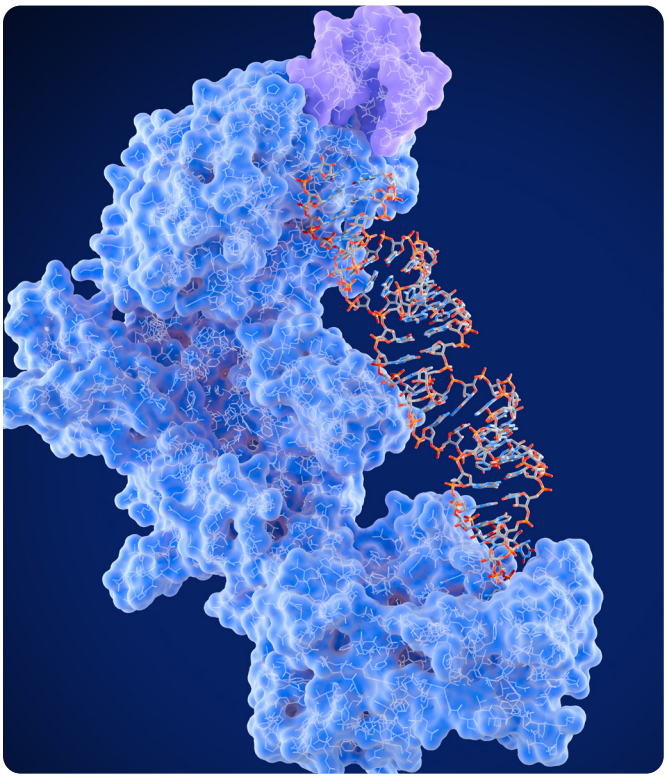
Introduction

RNA is believed to be as old as life itself. It has been hypothesized that the earliest biomolecules were RNA (or something similar) due to RNA's capacity to catalyze reactions as ribozymes, carry information as a nucleic acid, and evolve as a self-replicating molecule.[3-5] While these shared functions have evolved into the more complex system involving DNA, RNA, and protein, the "RNA world" persists today, with RNA not solely as an intermediary, but as a key regulatory system. Almost immediately following its discovery, scientists developed technology to target RNA in research and for therapeutic applications, leading to therapeutic modalities including mRNA, RNA interference (RNAi), antisense oligonucleotides (ASOs), aptamers, and finding a place in genome engineering as guide RNA (gRNA). RNA-based technologies gained prominence for their success fighting the COVID-19 pandemic and have earned a flurry of approvals in recent years, especially for RNAi and ASO-based therapies.[6]

Inserting novel cellular messages with mRNA

Messenger RNA (mRNA) is the best-known type of RNA and the intermediary between DNA and protein and is subject to regulation by other RNAs and proteins.[1,2] mRNA-based therapeutics aim to insert new messages into the mRNA pool to assemble designated proteins. Since the mid-1990s, mRNA has been explored for enzyme replacement therapies and to generate antigens as a potential vaccine modality. The first mRNA vaccine designed for cancer treatment in mice was published in 1995,7 but the chance for mRNA vaccines to prove valuable wouldn't come until more than 2 decades later.

The COVID-19 pandemic was the first real-world test of mRNA vaccines[6,8,9]: vaccines developed by Pfizer and Moderna showcased the rapid development and scalability of mRNA technology.[10]



Unlike traditional vaccines, which inject attenuated viral particles or purified protein antigens, mRNA vaccines instruct cells to produce antigens against which the body can develop an immune response. Compared to traditional vaccines in efficacy, mRNA vaccines have distinct advantages with their ease of development, scale-up, and manufacturing speed.[10] mRNA vaccines are also comparatively easy to update, with mRNA sequence alterations not requiring extensive characterization and testing for stability and immunogenicity compared with protein-based vaccines.

A key advantage of mRNA over protein-based therapeutics is the ability to package numerous mRNA species into a single drug product. Multivalent influenza vaccines have been tested in mice and have shown efficacy in clinical trials,[11,12] and combination influenza-COVID-19 vaccines are currently being tested. Beyond acute disease, the ability to package a cocktail of mRNA-encoding antigens is being explored in bespoke cancer vaccines, where personalization and scalability are not mutually exclusive,[13] and in chronic infections, such as hepatitis B and C.[14,15]

Beyond the Messenger: RNA interference as an additional level of gene expression control

RNA does more than act as the go-between for DNA and protein, with an assortment of species playing non-protein-coding functions as well. RNA interference (RNAi) is a phenomenon first demonstrated in 1998 and was rapidly adopted by researchers as a fast alternative to the laborious and time-consuming process of generating gene-deleted knockout models.[7] The discovery that RNA could base pair with other RNA molecules the way that DNA does led to the discovery of small interfering RNA (siRNA) and microRNA (miRNA), the mediators of RNAi.[7] Both of these RNA species "knock down" gene expression through the RNAi pathway, but differ in their origin and form.

miRNAs are endogenous, short, non-coding RNAs that can regulate multiple targets, while siRNAs are short (about 20 nucleotides long) molecules that have sequence-specific targets and are typically exogenous in origin. While no miRNA therapies have been approved yet, there are over 10 investigational miRNA agents in Phase 1-3 clinical trials in development for wound healing, heart failure, lymphoma, hepatitis, and glioblastoma, among other indications.[8]

siRNA has seen several approvals and a large development pipeline of over 25 investigational drugs in Phase 1-3 trials.[8] Patisiran is an siRNA drug developed to treat hereditary transthyretin-mediated amyloidosis (hATTR) in adults and became the first FDA-approved siRNA drug in 2018.[7,16] This disease is caused by a mutation that causes the accumulation of misfolded transthyretin proteins. Patisiran suppresses the translation of the transthyretin protein to reduce overall protein levels and reduce amyloid deposits. Since this first approval, at least three other siRNA drugs have been approved: givosiran was approved to treat acute hepatic porphyria, lumasiran to treat hyperoxaluria type 1, and inclisiran to treat heterozygous familial hypercholesterolemia.[7,17]

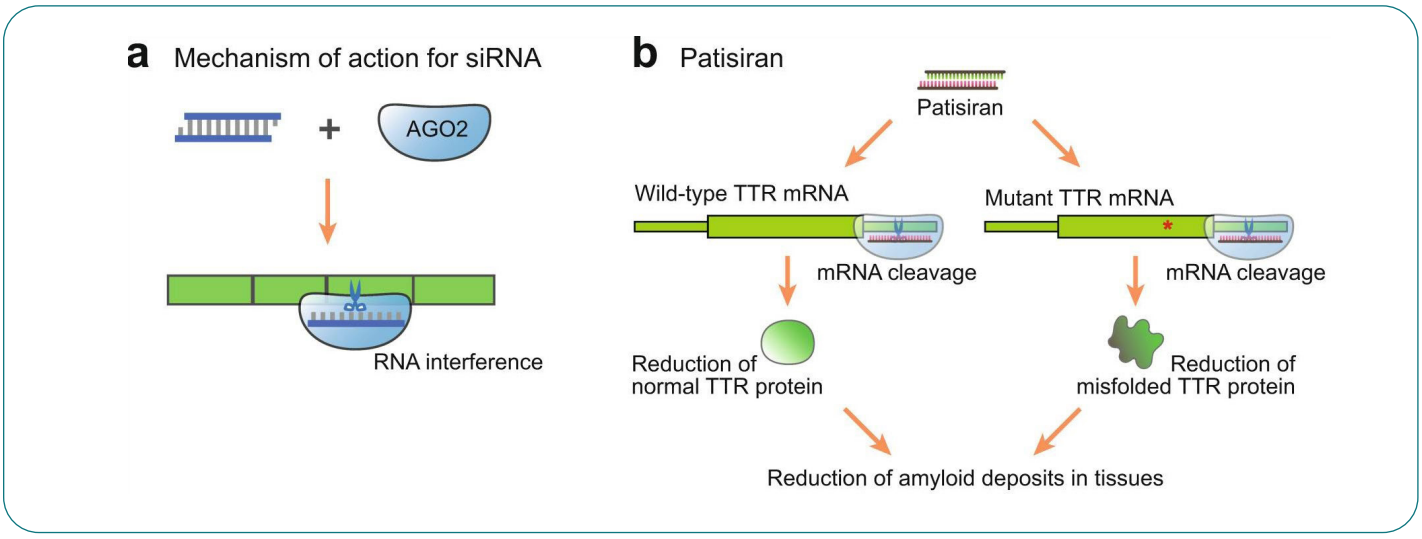


Figure 1. General mechanism for siRNA (left) and for patisiran (right). Source: Kim, Exp Mol Med 2022.[7]

Antisense oligonucleotides to control gene expression

ASOs are DNA or RNA constructs that bind to complementary mRNA targets. ASOs bind to the "sense" strand of mRNA, hence their name. ASOs can be developed to arrest translation, promote mRNA degradation, or modify splicing.[8] The first ASO was approved in 1993 to treat beta thalassemia by reducing the production of the mutated protein.[18]

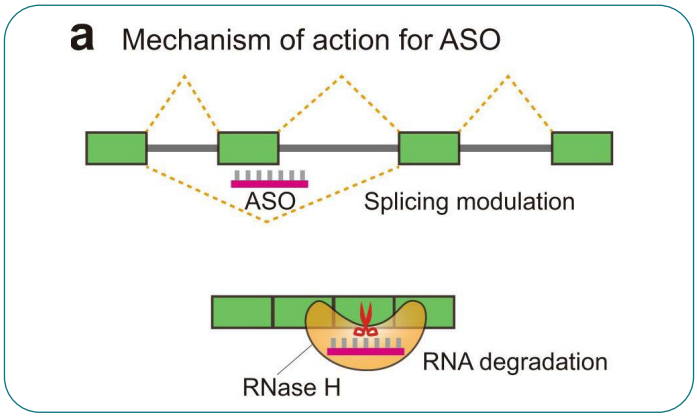


Figure 2. Mechanism of action of ASOs generally involve modulating splicing (top) or inducing RNA degradation by RNase H. Source: Kim, Exp Mol Med 2022.[7]

Spinal muscular atrophy (SMA) is a devastating condition caused by a defect in the survival motor neuron 1 (SMN1) gene.[19] Nusinersen (Spinraza®) is a splicing-modifying ASO that forces the inclusion of exon 7 in the paralog SMN2, allowing SMN protein levels that compensate for the defective SMN1 gene, slowing the rate of atrophy and improving survival.

While nusinersen is a splice-promoting ASO, inotersen is a degradation-promoting ASO for the treatment of hATTR. It is based on the same rationale as the siRNA drug patisiran; however, degradation-promoting ASOs work by recruiting RNase H, while siRNAs work through the RNAi pathway.[8] Both methods share the goal of globally reducing transcript levels to lower protein levels—in this case, reducing transthyretin to minimize the formation of amyloid deposits that lead to the manifestations of hATTR.

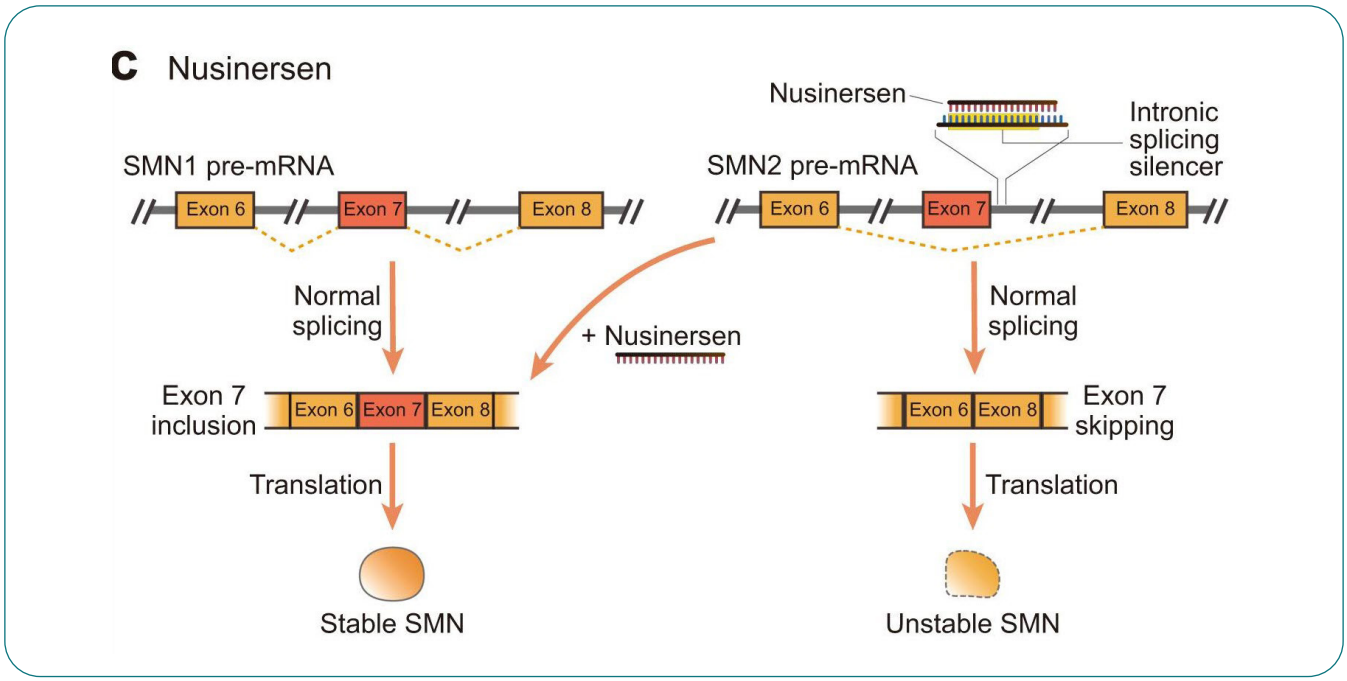


Figure 3. Nusinersen treats SMA as an intronic splicing silencer of the SMN2 mRNA, inducing inclusion of exon 7 into SMN2, allowing it to functionally replace defective SMN1. Source: Kim, Exp Mol Med 2022.[7]

Using RNA as molecular binders: Aptamers

Spinal muscular atrophy (SMA) is a devastating Aptamers are folded RNA species that can bind other molecular entities—particularly proteins—and modify their function. The applications of aptamers range from allowing fluorescent labeling in Situ, such as using aptamers to bind fluorescent proteins to label chromatin sites[20] or creating protein-binding RNA as therapeutic agents.

Unlike RNAi or ASOs that aim to knock down or eliminate a gene, aptamers can act analogously to monoclonal antibodies to bind to target proteins. Aptamers can be delivered to cells similarly to other RNA therapeutic modalities, namely by nanoparticles or engineered viruses. While RNA is generally considered poorer at molecular specificity than proteins, such as antibodies, an advantage to an aptamer is the ability to target intracellular molecules, whereas antibodies are limited to cell-surface targets. The first FDA-approved RNA therapeutic was an aptamer for age-related macular degeneration, though it has since been discontinued due to low market demand.[7] In total, two aptamers have earned approval, and several are in clinical trials for glioblastoma, diabetic nephropathy, and pancreatic cancer.[8]

Guide RNAs in gene therapies and genomic engineering

The discovery of CRISPR has revolutionized the field of gene therapy. This bacterial defense mechanism against viruses was found to use guide RNA (gRNA) to target foreign double-stranded DNA, like a molecular immune system.[21] Unlike gene engineering tools that came before it, the gRNA aspect of CRISPR/Cas9 offered higher-fidelity molecular scissors to target the genome for deletions, insertions, or mutations through homologous recombination and is blurring the lines between RNA and gene therapies.[8]

CRISPR technology made it possible to create knockout animals faster than previous methods and to perform in situ gene deletions in cultured human cells to better study the cellular basis of diseases. However, CRISPR is limited by its tendency to produce off-target DNA breaks. With this limitation, CRISPR is being explored in ex vivo cell therapies, like chimeric antigen receptor (CAR) T cells,[22] where quality can be assured before infusing it into a patient. While no direct-acting CRISPR therapies have been approved, over 25 investigational CRISPR-based therapies are in clinical trials.[7]

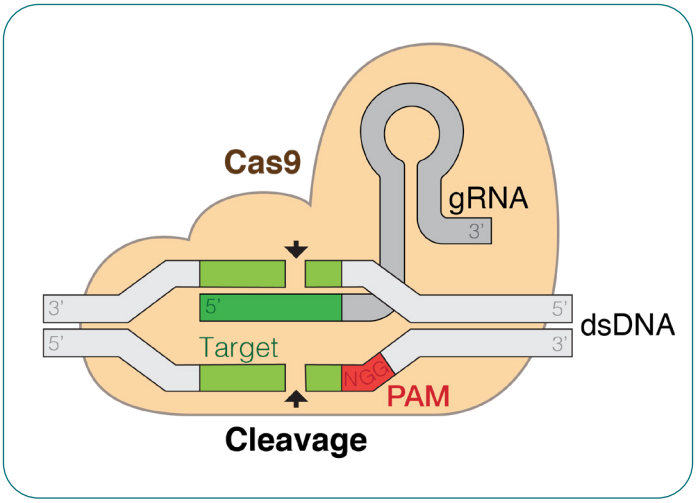
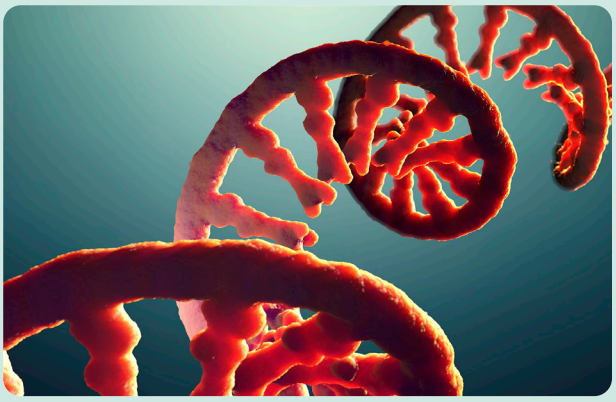


Figure 4. CRISPR-Cas9 mechanism. Complementary base-pairing by the gRNA provides specificity to the Cas9 enzyme, which also requires a specific protospacer-adjacent motif (PAM) in the target. Image by Marius Walter, CC BY-SA 4.0, https://commons.wikimedia.org/w/index.php?curid=103390868

Supercharging RNA therapies with AI

In the 6 decades since RNA was discovered, a myriad of modalities have proven their worth, mainly in upregulating or downregulating key targets. Large language models are expected to accelerate the development of RNA therapeutics—along with many other modalities. Language models using RNA sequences have been published, and as large scale RNA sequencing data becomes more and more common, these models will be able to connect sequence to function. These advances will have dramatic implications for RNA therapy development by streamlining design. Traditional development for ASOs and RNAi are done by creating assorted complementary sequences spanning the length of a target gene, and "walking" the sequence until efficient knockdown is achieved.[8] AI may be able to predict the most likely regions of effect to reduce the labor required to produce candidate molecules.

Recently-unveiled AlphaFold3 capabilities include predicted RNA-biomolecule interactions based on sequence.[23] The advances in RNA binding and docking could be compared to our knowledge of protein-protein interactions, with ever-increasing experimental data driving AI-assisted insights. The ability of AI to identify patterns in large datasets may also work together with research innovation to address one of the RNA therapy modality's biggest challenges: cell-type targeting and effective delivery. Deep Genomics is using AI to predict tissue-specific factors in RNA regulation, expression, and binding interactions of microRNAs and proteins, with one outcome being the design of potential RNA therapeutics.[24] AI is being used to predict higher-order RNA structures to accelerate candidate selection for potential RNA therapies.[25] With an expanding pool of knowledge and sequence data, AI could help identify features and formulations that are more effective for achieving desired therapeutic outcomes.[25]



Revvity Signals Perspective

RNA therapeutics as we know them today wouldn't exist without deep, cross-disciplinary collaboration—from understanding sequence and structural modifications to optimizing delivery and minimizing off-target effects. With RNA's complexity and its overlap with other modalities, clear communication and connected workflows across teams and partners are essential. Revvity's RNA therapeutics solution addresses these challenges across the Design, Make, Test, and Decide (DMTD) process. Scientists can design and visualize complex sequences and conjugates using HELM-powered ChemDraw. Signals Notebook enables seamless data capture and communication across modalities. In the Test and Decide phases, SignalsOne brings assay data together into multiparameter dashboards, helping teams evaluate efficacy, stability, and safety to confidently select or reoptimize candidates. This solution is purpose built for RNA needs, where sequence, chemistry, and biology converge, and supports the fast and iterative decision-making that is critical for advancing high-potential RNA therapies

RNA Back to the Future

RNA is one of the fastest-growing sectors of medical research and development.[6,8] In fact, RNAi and ASOs are poised to top $1 billion in annual revenue by 2030.[6] Achieving cell-type specificity remains a challenge, though recent advances in delivery technologies, like N-acetylgalactosamine (GalNAc) conjugation that enable targeted delivery to the liver, could be the first of many to overcome this.

New modalities are being developed to address current shortcomings in RNA therapies. Self-amplifying RNA, being developed by AstraZeneca and Imperial College London, aims to address challenges in protein yield from mRNA-based therapeutics, increasing potency with smaller doses given.[6]

The discovery of a direct recombination mechanism involving RNA and a transposase enzyme from a radiation-resistant bacteria may be the next leap in genome engineering.[26] While gRNA tells the Cas9 enzyme where to cut in CRISPR, relying on natural recombination processes to repair the DNA break, a new generation of genome engineering is taking shape. Bridge RNAs contain programmable sequences with distinct regions for donor and target sequences to direct recombination. This technology may have improved fidelity compared to CRISPR/Cas9 to minimize off-target recombination that limits in Vivo genome engineering, and the fidelity will be able to direct large-scale genome rearrangements in research to better understand the diseases we seek to treat.

As the repertoire of RNA therapeutics expands, it's worth thinking about the new RNA world in which we find ourselves. The versatility of RNA could be what gave rise to biology from chemistry, and it could be that the new RNA world is one where chemistry drives specific outcomes in biology.

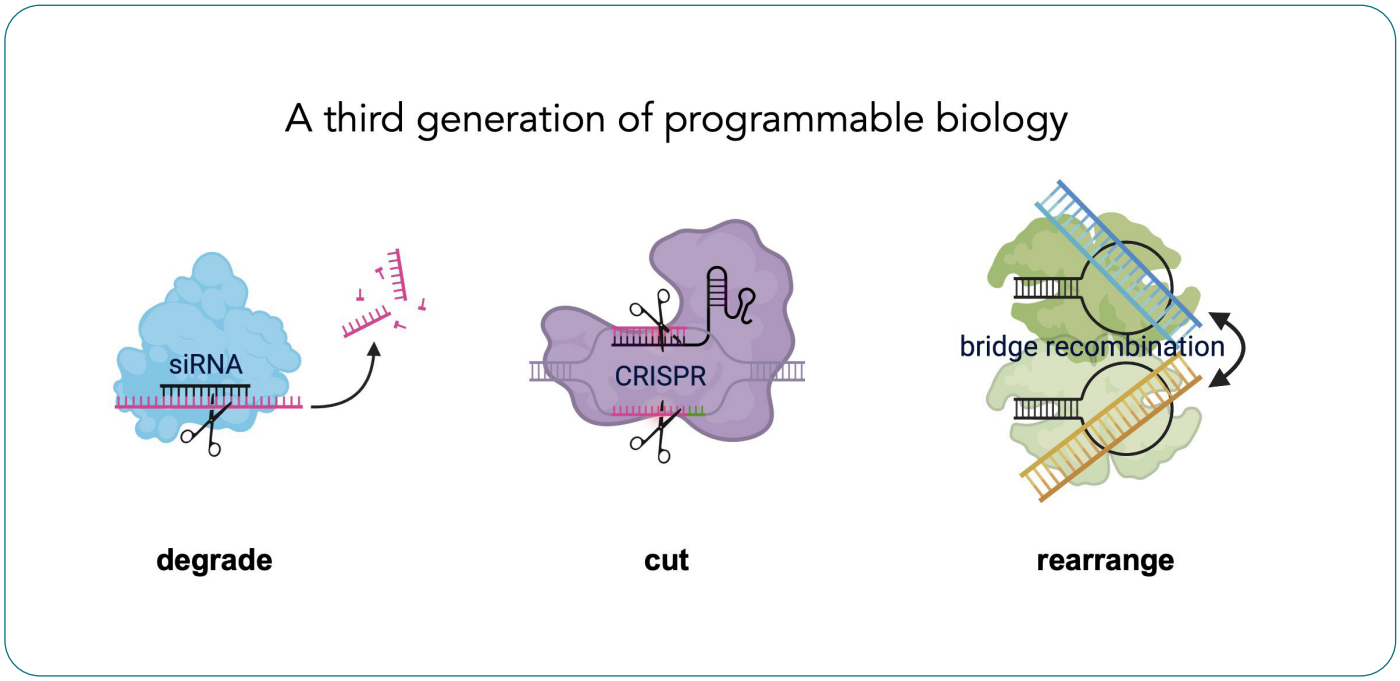


Figure 5. Bridge RNAs as a third generation of programmable biology. Image source: Grinstein 202426

References

1. Brenner S, Jacob F, Meselson M. An unstable intermediate carrying information from genes to ribosomes for protein synthesis. Nature. 1961;190:576-581. doi:10.1038/190576a0
2. Crick FH. On protein synthesis. Symp Soc Exp Biol. 1958;12:138-163.
3. Salditt A, Karr L, Salibi E, Le Vay K, Braun D, Mutschler H. Ribozyme-mediated RNA synthesis and replication in a model Hadean microenvironment. Nat Commun. 2023;14:1495. doi:10.1038/s41467-023-37206-4
4. Papastavrou N, Horning DP, Joyce GF. RNA-catalyzed evolution of catalytic RNA. Proceedings of the National Academy of Sciences. 2024;121(11):e2321592121. doi:10.1073/pnas.2321592121
5. Higgs PG, Lehman N. The RNA World: molecular cooperation at the origins of life. Nat Rev Genet. 2015;16(1):7-17. doi:10.1038/nrg3841
6. Chowdhury S, Chakrabarty A, Malik N. RNA therapeutics as we enter 2025: looking beyond the horizon of first-generation success. Advancing RNA. January 9, 2025. Accessed February 15, 2025. https://www.advancingrna.com/doc/rna-therapeutics-as-we-enter-2025-looking-beyond-the-horizon-of-first-generation-success-0001
7. Kim YK. RNA therapy: rich history, various applications and unlimited future prospects. Exp Mol Med. 2022;54(4):455-465. doi:10.1038/s12276-022-00757-5
8. Jones CH, Androsavich JR, So N, et al. Breaking the mold with RNA—a "RNAissance" of life science. npj Genom Med. 2024;9(1):1-14. doi:10.1038/s41525-023-00387-4
9. Rubio I, Khimani AH, Fraser M. Novel RNA therapeutic modalities. GEN - Genetic Engineering and Biotechnology News. April 1, 2024. Accessed January 21, 2025. https://www.genengnews.com/topics/translational-medicine/novel-rna-therapeutic-modalities/
10. Gote V, Bolla PK, Kommineni N, et al. A comprehensive review of mRNA vaccines. Int J Mol Sci. 2023;24(3):2700. doi:10.3390/ijms24032700
11. Freyn AW, Ramos da Silva J, Rosado VC, et al. A Multi-Targeting, Nucleoside-Modified mRNA Influenza Virus Vaccine Provides Broad Protection in Mice. Mol Ther. 2020;28(7):1569-1584. doi:10.1016/j.ymthe.2020.04.018
12. Moderna R&D Day Highlights Progress and Strategic Priorities. Accessed February 16, 2025. https://investors.modernatx.com/news/news-details/2024/Moderna-RD-Day-Highlights-Progress-and-Strategic-Priorities/default.aspx
13. Sayour EJ, Boczkowski D, Mitchell DA, Nair SK. Cancer mRNA vaccines: clinical advances and future opportunities. Nat Rev Clin Oncol. 2024;21(7):489-500. doi:10.1038/s41571-024-00902-1
14. Zhao H, Shao X, Yu Y, et al. A therapeutic hepatitis B mRNA vaccine with strong immunogenicity and persistent virological suppression. npj Vaccines. 2024;9(1):1-6. doi:10.1038/s41541-024-00813-3
15. Patra T, Meyer K, Haga Y, Reagan EK, Weissman D, Ray R. Hepatitis C virus E1 and modified E2 delivered from an mRNA vaccine induces protective immunity. npj Vaccines. 2023;8(1):1-9. doi:10.1038/s41541-023-00635-9
16. Adams D, Gonzalez-Duarte A, O'Riordan WD, et al. Patisiran, an RNAi therapeutic, for hereditary transthyretin amyloidosis. New England Journal of Medicine. 2018;379(1):11-21. doi:10.1056/NEJMoa1716153
17. Garrelfs SF, Frishberg Y, Hulton SA, et al. Lumasiran, an RNAi Therapeutic for Primary Hyperoxaluria Type 1. New England Journal of Medicine. 2021;384(13):1216-1226. doi:10.1056/NEJMoa2021712
18. Dominski Z, Kole R. Restoration of correct splicing in thalassemic pre-mRNA by antisense oligonucleotides. Proceedings of the National Academy of Sciences. 1993;90(18):8673-8677. doi:10.1073/pnas.90.18.8673
19. Spinal Muscular Atrophy (SMA) - Diseases. Muscular Dystrophy Association. December 18, 2015. Accessed February 17, 2025. https://www.mda.org/disease/spinal-muscular-atrophy
20. Wang S, Su JH, Zhang F, Zhuang X. An RNA-aptamer-based two-color CRISPR labeling system. Sci Rep. 2016;6(1):26857. doi:10.1038/srep26857
21. Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA, Charpentier E. A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. Science. 2012;337(6096):816-821. doi:10.1126/science.1225829
22. Wei W, Chen ZN, Wang K. CRISPR/Cas9: A Powerful Strategy to Improve CAR-T Cell Persistence. Int J Mol Sci. 2023;24(15):12317. doi:10.3390/ijms241512317
23. Abramson J, Adler J, Dunger J, et al. Accurate structure prediction of biomolecular interactions with AlphaFold 3. Nature. Published online May 8, 2024:1-3. doi:10.1038/s41586-024-07487-w
24. Deep Genomics. Deep Genomics Introduces the Most Advanced AI Foundation Model for RNA Disease Mechanisms and Candidate Therapeutics. Deep Genomics. Accessed February 17, 2025. https://www.deepgenomics.com/news/most-advanced-ai-foundation-model-for-rna/www.deepgenomics.com/news/most-advanced-ai-foundation-model-for-rna/
25. Tani H. Recent Advances and Prospects in RNA Drug Development. International Journal of Molecular Sciences. 2024;25(22):12284. doi:10.3390/ijms252212284
26. Grinstein JD. Come together: bridge RNAs close the gap to genome design. GEN - Genetic Engineering and Biotechnology News. June 26, 2024. Accessed February 17, 2025. https://www.genengnews.com/gen-edge/come-together-bridge-rnas-close-the-gap-to-genome-design/

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