Advances in RNA Therapeutics: Mechanistic Basis and Therapeutic Strategies

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***Abstract*—The term "non-druggable" refers to a protein that cannot be targeted pharmacologically; recently, significant efforts have been made to convert these proteins into targets that are reachable or "druggable." Pharmacologically targeting these difficult proteins has emerged as a major challenge in modern drug development, necessitating the innovation and development of new technologies. The idea of using RNA-targeting therapeutics as a platform to reach unreachable targets is very appealing. Antisense oligonucleotides, nucleic acids or aptamers, RNA interference therapeutics, microRNA, and synthetic RNA are examples of RNA-targeting therapeutics. The growing understanding of RNA functions and their crucial roles in diseases promotes the application of various RNAs to selectively function on hitherto "undruggable" proteins, transcripts, and genes, thus potentially broadening the therapeutic targets. Several RNA-based medications have been approved for clinical use, while others are still under investigation or in preclinical trials. Various techniques have been explored to promote RNA intracellular trafficking and metabolic stability, despite significant challenges in developing RNA-based therapeutics. In this review, the mechanisms of action, challenges, solutions, and clinical application of RNA-based therapeutics have been comprehensively summarized.**

***Index Terms*—Drug development, RNA-targeting, medications, metabolic stability.**

I. INTRODUCTION

RNA-targeted therapeutics are treatments that directly interact with RNA molecules to alter gene expression or correct genetic abnormalities. These therapies can degrade, modify, or mimic RNA to treat various diseases, including genetic disorders, cancers, and viral infections.

RNA Therapies: Optimizing Drug Delivery for Treating the Untreatable:

RNA-based therapies should improve the lives of many people affected by difficult-to-treat diseases, provided novel drug delivery methods are fully explored and optimized. Young-Kook Kim at Chonnam National University Medical School in Hwasun, Korea, reviewed the current status of RNA therapies, particularly given recent successes in developing messenger RNA vaccines for COVID-19. RNA therapeutics work by manipulating the expression and activity of specific target molecules, providing the means to treat diseases that do not respond to conventional drug types. Such therapies can be tweaked to cover a wide range of different forms of RNA and protein, and could open the way to personalized medicines and treatments for rare diseases. However, RNA-based drugs are larger molecules than other therapeutics, making targeted delivery within the body more difficult. Kim suggests that ensuring effective RNA drug delivery should be a paramount focus for future research[1].

RNA plays a central role in numerous biological processes, extending beyond its traditional function as a messenger in protein synthesis. RNA molecules, including messenger RNAs (mRNAs) and non-coding RNAs (ncRNAs), are involved in gene regulation, chromatin remodeling, cellular stress responses, and disease progression. Recent research has highlighted RNA as a promising but underexplored therapeutic target, particularly for diseases such as cancer, neurodegenerative disorders, and viral infections. Unlike proteins, which have well-defined binding pockets that facilitate small-molecule drug development, RNA exhibits high structural flexibility and dynamic conformations, making it challenging to develop selective RNA-binding small molecules. Nevertheless, the increasing understanding of RNA structure and function has spurred the development of novel RNA-targeting small molecules, opening new avenues in drug discovery[2].

To date, only approximately 15% of human proteins,

out of around 20,000 human proteins, are deemed druggable.6 Data suggest that the Food and Drug Administration (FDA) has approved only about 700 small-molecule drugs targeting human proteins, underscoring the vast potential for the development of novel drugs independent of traditionally "druggable targets." Since 2018, nearly 30% of FDA-approved drugs have been biologics,8,9 with a subset based on nucleic acid, including antisense oligonucleotides (ASOs), aptamers, messenger RNAs (mRNAs), siRNAs, and miRNAs.10,11,12,13,14,15 This marks the emergence of a new era in RNA-based therapeutics[3]. The consecutive FDA approvals of siRNA drugs, Patisiran and Givosiran, have translated the promise of siRNA into clinical reality.16 With numerous siRNA and mRNA-based therapeutics in the pipeline, the clinical translation of RNA therapeutics has transitioned from mere "hype" to a tangible "hope".

HISTORY:

A Timeline Overview:

RNA-targeted therapeutics have evolved significantly over the last few decades, driven by advances in molecular biology, chemistry, and biotechnology. Here's a brief history:

1970s–1980s: Foundation and Discovery

- 1977: Discovery of introns and RNA splicing laid the groundwork for RNA manipulation.
- 1978: Introduction of synthetic oligonucleotides for gene expression studies.
- Late 1980s: Development of antisense oligonucleotides (ASOs) for gene silencing.
- 1990s: Early Clinical Exploration
- 1998: Fomivirsen (Vitravene) became
- 2016: FDA approved Nusinersen (Spinraza), an ASO for spinal muscular atrophy – a landmark in RNA therapeutics.
- 2018: Approval of Patisiran (Onpattro), the first siRNA drug for hereditary transthyretin-mediated amyloidosis.
- 2020s: RNA Therapeutics Revolution
- 2020–2021: Approval of mRNA vaccines (Pfizer-BioNTech and Moderna) for COVID-19 – global recognition of RNA-based medicines.
- Expansion of RNA therapeutics into cancer, rare genetic diseases, and metabolic disorders.
- Exploration of self-amplifying RNA, CRISPR-based RNA targeting, and circular RNA.
- Present and Future Trends
- Growing use of artificial intelligence for RNA drug design.
- Delivery innovations (e.g., lipid nanoparticles, exosomes).
- Increasing clinical trials and pipeline drugs targeting RNA in neurodegenerative, infectious, and cardiovascular diseases.

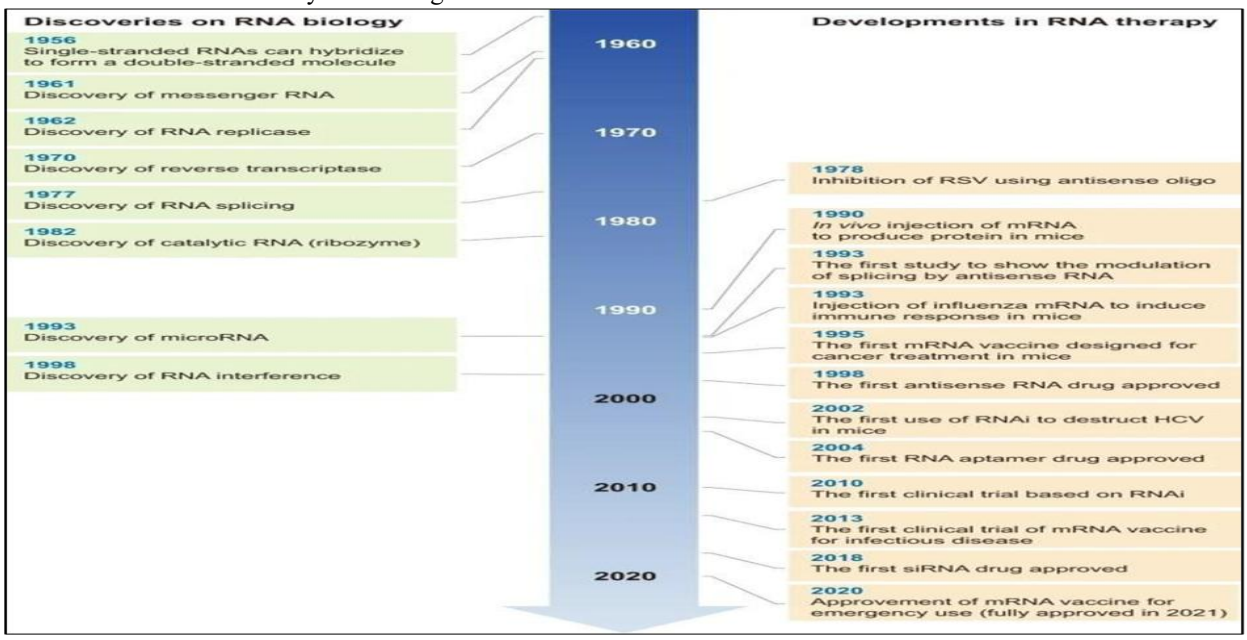

Figure No. 1: The historical timeline of important discoveries in RNA biology and key developments in RNA therapy[4]

II. MECHANISM OF ACTION

One informative way to think of oligonucleotides designed to bind to their cognate sequences in RNA is that they are agents that alter the complex intermediary metabolism of mRNAs; this begins with transcription of a pre-mRNA and includes RNA processing, transport, and utilization by translation, followed by degradation. That these agents are designed to alter the intermediary metabolism of mRNAs implies that the rates of steps in mRNA intermediary metabolism and the rates of drug action are important in defining the ultimate pharmacological effects observed. This has proven to be true (see below).

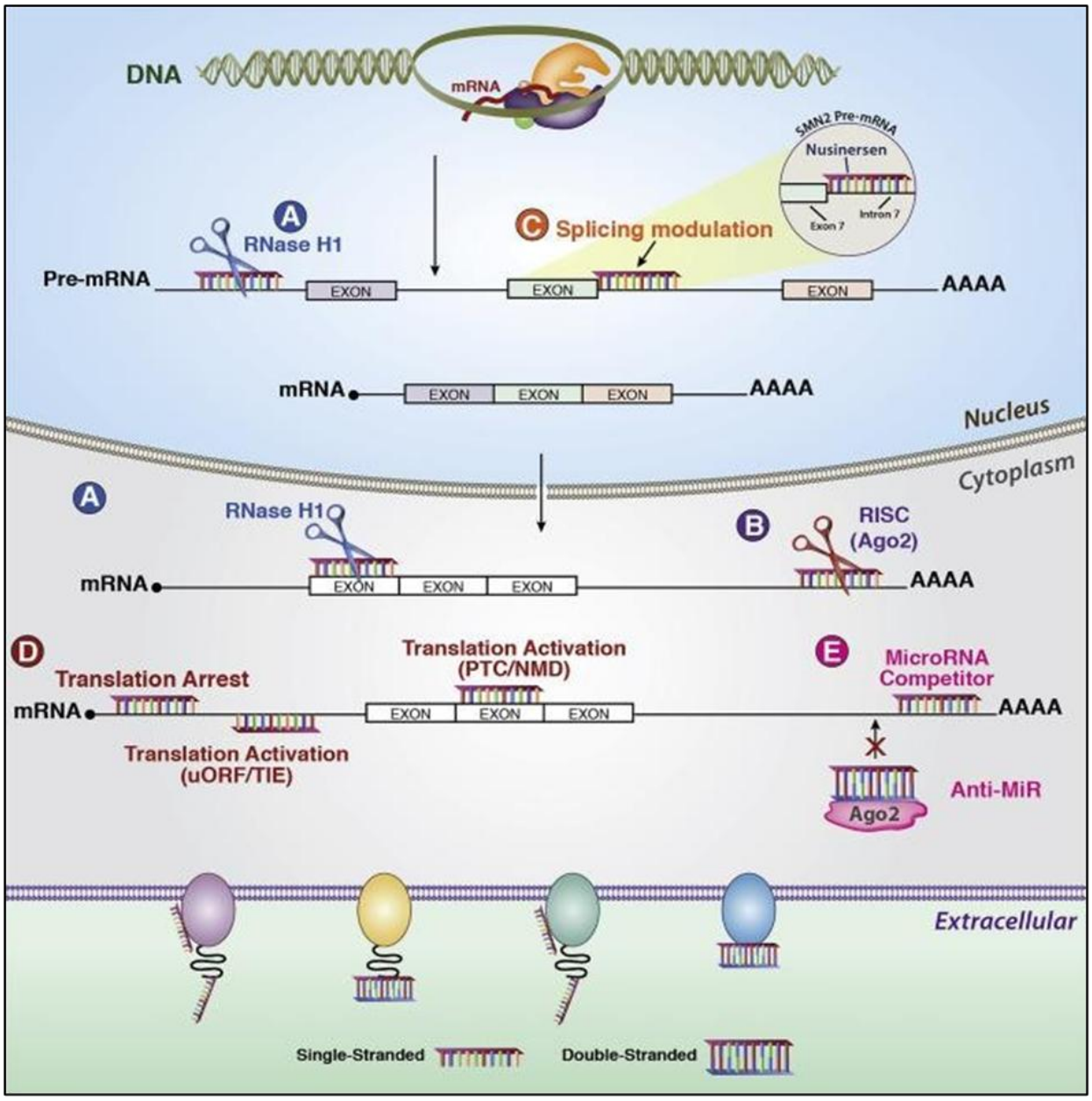

Figure No. 2: Antisense Mechanisms Commonly Used to Modulate Gene Expression

III. TYPES OF RNA THERAPIES

1. ANTISENSE OLIGONUCLEOTIDES:

RNA manipulation is crucial in gene therapy, as it regulates gene expression. Synthetic compounds that bind to RNA can modulate protein production. ASOs are single-stranded synthetic nucleic acids, ranging from 16 to 21 nucleotides, that can identify specific RNA sequences through complementary base pairing[5].

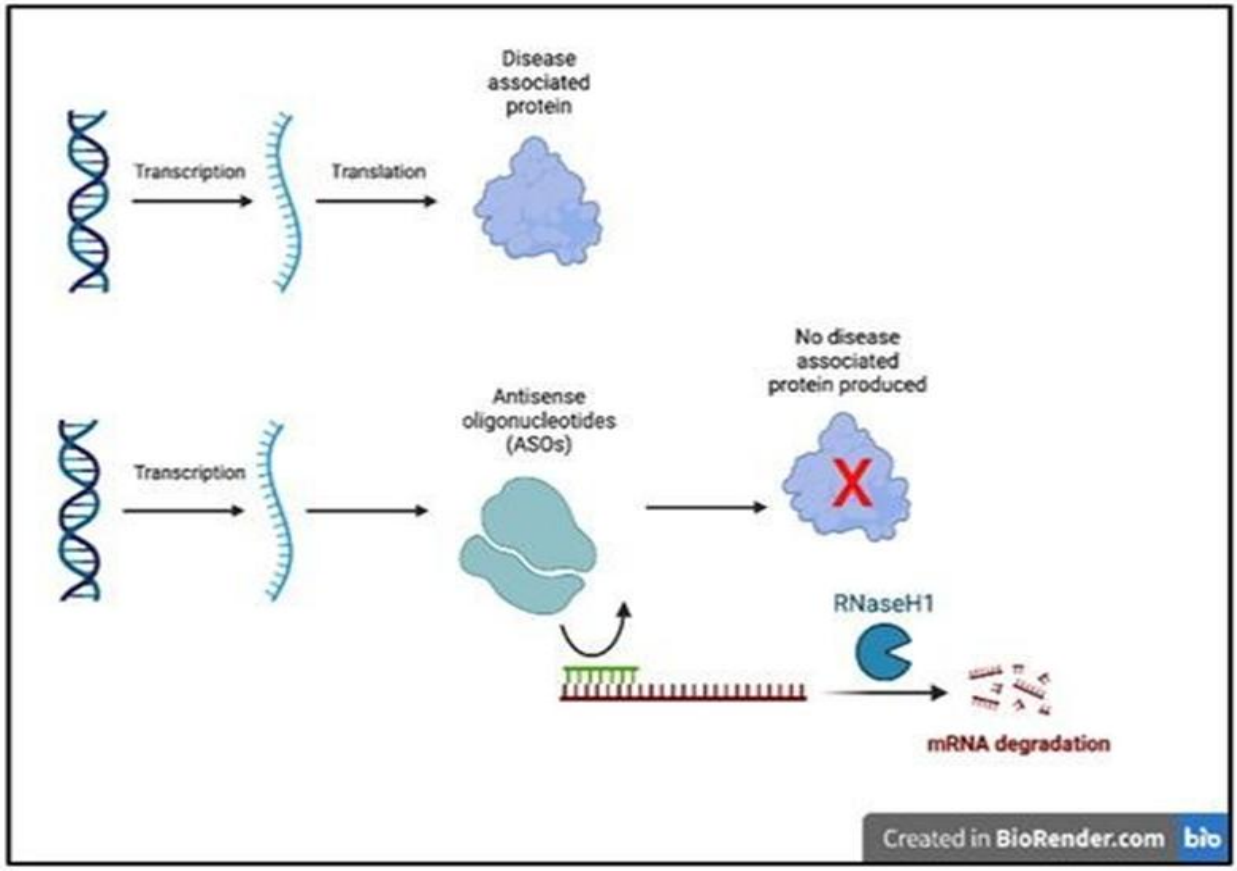

Figure No. 3: MOA of Antisense Oligonucleotide[6]

The central dogma of molecular biology states that DNA encodes RNA, which is then translated into proteins. In recent years, the use of compounds that are able to bind messenger RNAs (mRNAs) has gained increasing interest as inhibition of protein expression can be helpful for controlling the course of inflammatory and neoplastic diseases. The two major therapeutic approaches in this field are the antisense oligonucleotides (ASOs) that inhibit mRNA translation and the oligonucleotides, which function via the RNA interference (RNAi) pathway[7].

Uses:

A. Delivery to the nervous system:

In drug discovery, the aim is to find a substance which is potent, selective, and preferably bioavailable that needs to reach its target at sufficient concentrations[8].

B. Antisense oligonucleotides as a potential treatment for brain deficits observed in myotonic dystrophy type 1. (Ait Benichou, S., Jauvin, D., De Serres-Bérard, T. et al. Antisense oligonucleotides as a potential treatment for brain deficits observed in myotonic dystrophy type 1[9].

2. SMALL INTERFERRING RNA (siRNA):

Small interfering RNA (siRNA), sometimes known as short interfering RNA or silencing RNA, is a class of double-stranded non-coding RNA molecules, typically 20–24 base pairs in length, similar to microRNA (miRNA), and operating within the RNA interference (RNAi) pathway. It interferes with the expression of specific genes with complementary nucleotide sequences by degrading messenger RNA (mRNA) after transcription, preventing translation. It was discovered in 1998 by Andrew Fire at the Carnegie Institution for Science in Washington, D.C. and Craig Mello at the University of Massachusetts in Worcester.

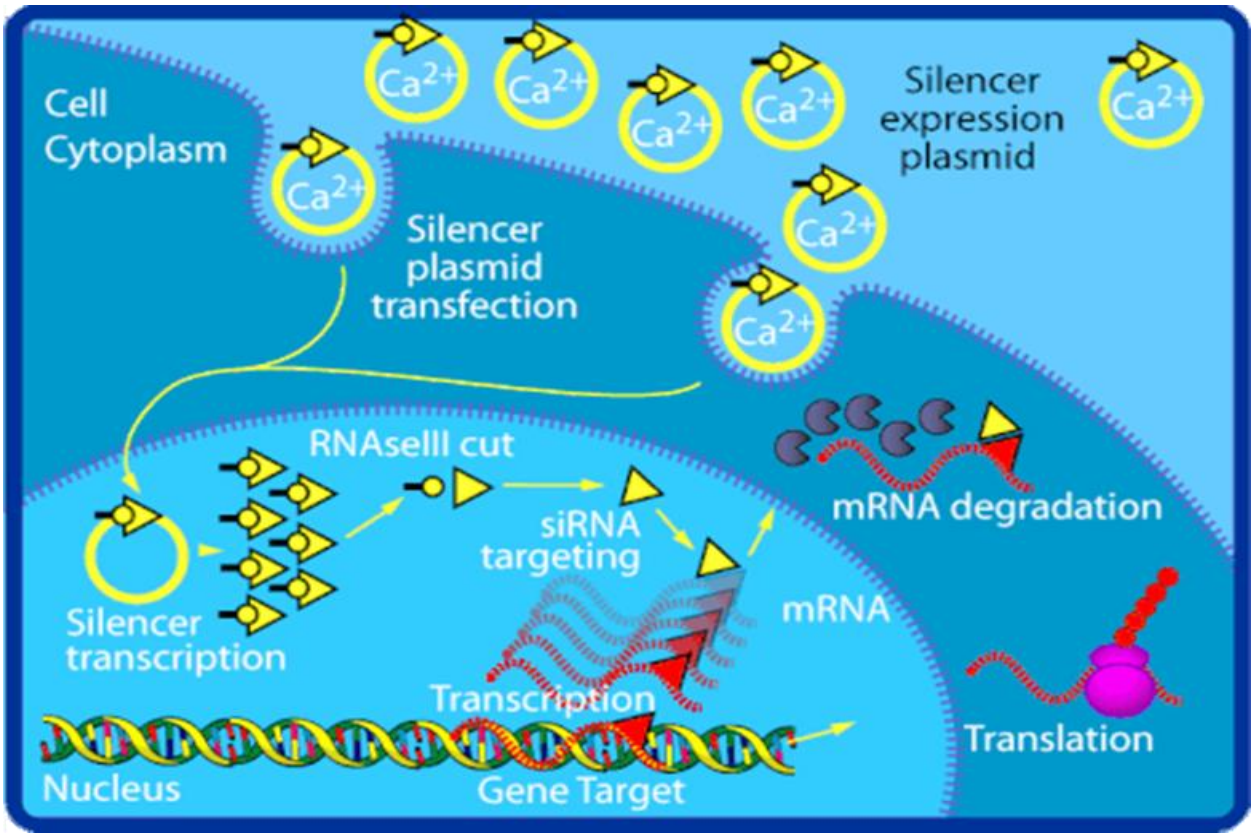

Figure No. 4: Mediating RNA interference in cultured mammalian cells[10]

Uses:

A. siRNAs silence genes at the post-transcriptional level. They cleave mRNA molecules with a sequence complementary to the siRNA molecule and thereby stop the translation process or gene expression.

B. In the past 2 years, extraordinary developments in RNA interference (RNAi)-based methodologies have seen small interfering RNAs (siRNA) become the method of choice for researchers wishing to target specific genes for silencing[11].

3. MICRORNA (miRNA) MODULATORS:

MicroRNAs (miRs) are small (~18–25 nucleotides), noncoding RNAs that regulate gene expression posttranscriptional. Mature miR bind to the 3' untranslated region (UTR) end of target mRNA and either induce mRNA degradation or repress translation. A single miRNA can bind either through perfect or partial complement base pairing to hundreds of sites across the transcriptome thus having the ability to control many different genes. The various biological processes controlled include cell division, proliferation, and death; therefore, miRs can play a role in numerous diseases[12].

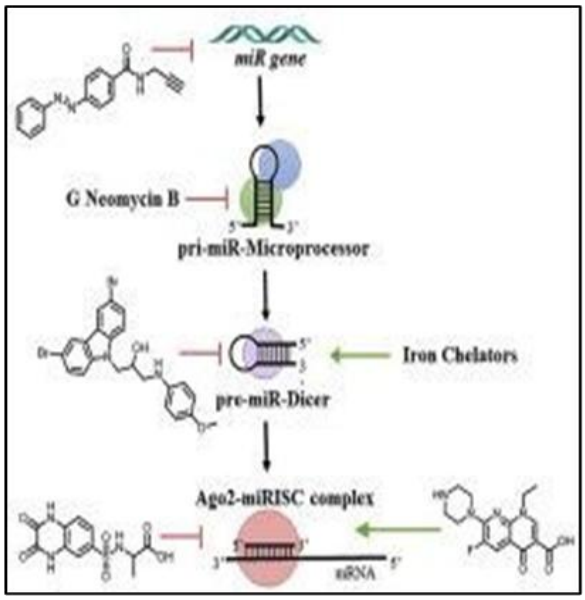

Figure No. 5: MicroRNA Modulation

Uses:

A. MiRNAs are a class of small, naturally occurring RNA molecules that play critical roles in modulating numerous biological pathways by regulating gene

expression[13].

B. There is growing evidence demonstrating frequent dysregulation of microRNAs in cancer cells, which is associated with tumor initiation, development, migration, invasion, resisting cell death, and drug[14].

4. RNA APTAMERS:

RNA Aptamers are defined as RNA oligonucleotides that bind to a specific target with high affinity and specificity, similarly to how an antibody binds to an antigen. Isolation of aptamers from randomized pools of RNA by using a method called Systematic Evolution of Ligands by Exponential enrichment (SELEX) was first developed by Gold and Turek, and by Ellington and Szostak.These RNA molecules were termed as "aptamers," with etymology stemming from the Greek word aptus, which means "to fit" . To date, various aptamers have been successfully selected against different targets and have begun to show promise as diagnostic, prognostic and therapeutic tools in a wide-range of applications including the treatment for human diseases such as cancer, viral infection and macular degeneration[15].

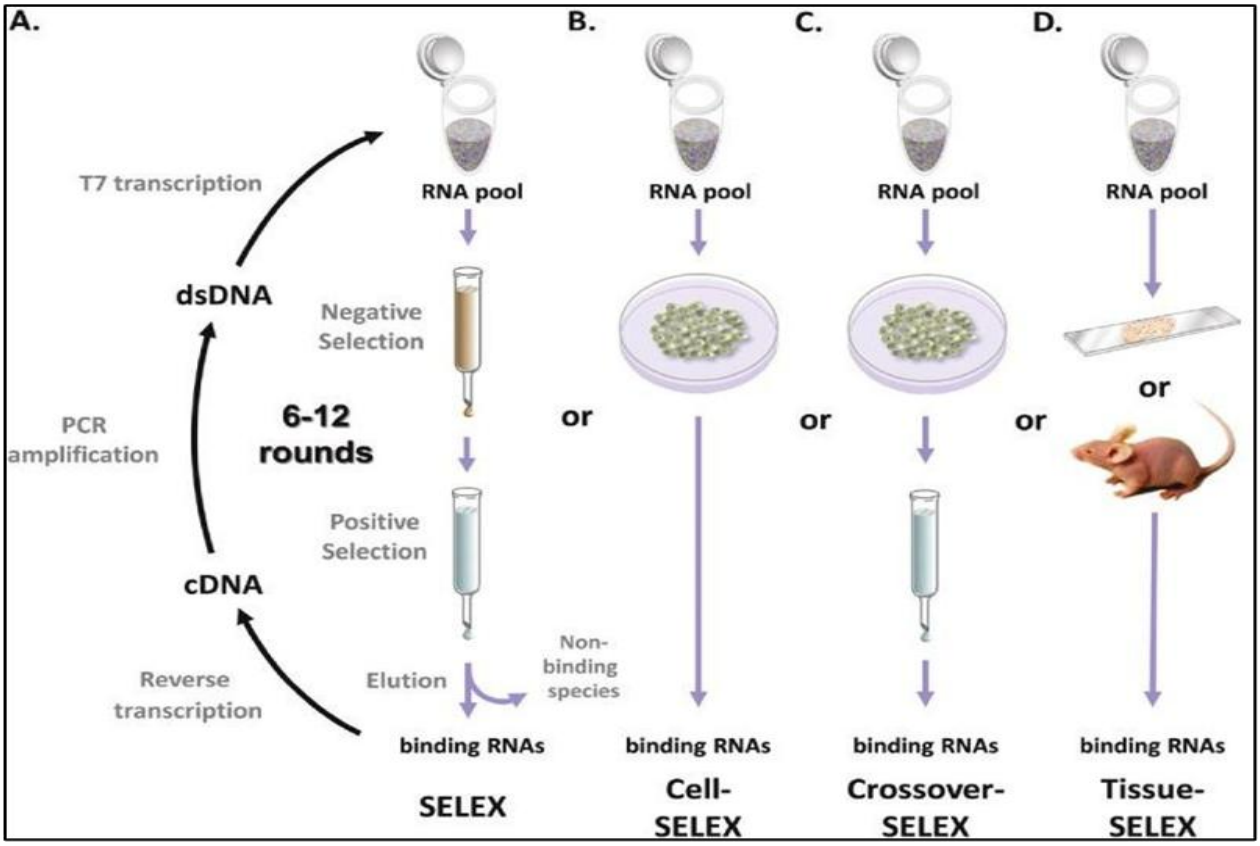

Figure No. 6: RNA Aptamers

Uses:

A. Aptamers are used in biological lab research and medical tests. If multiple aptamers are combined into a single assay, they can measure large numbers of different proteins in a sample[16].

B. Aptamers are single-stranded oligonucleotides that fold into defined architectures and bind to targets such as proteins[17].

5. mRNA THERAPEUTICS:

Messenger RNA (mRNA) is a type of single-stranded ribonucleic acid that is transcribed from a strand of DNA, which carries the coding information for protein synthesis to be further transcribed and processed into functional proteins.1 In vitro transcription (IVT) mRNA was successfully transcribed and expressed in mouse skeletal muscle cells, which establishes the feasibility of mRNA therapy.2 mRNA-based therapeutics were proposed when mRNA could be successfully transfected and produce an immune response in a dose-dependent manner by direct injection into mice to express therapeutic proteins.3 An mRNA-based approach can theoretically produce any protein/peptide via the protein synthesis machine processed in the

transfected cell in vitro or in vivo.4 Unlike DNA-based drugs, mRNA transcripts have a relatively high transfection efficiency and low toxicity because they do not need to enter the nucleus to be functional. Importantly, mRNA has no potential risk of accidental infection or opportunistic insertional mutagenesis. In addition, mRNA has broad potential for treating diseases requiring protein expression and higher therapeutic efficacy due to its continuous translation into encoded proteins/peptides to trigger long-lasting expression compared to transient traditional protein/peptide drugs. Apparently, these advantages of mRNA over DNA or protein/peptide enable the rapid entry of mRNA-based technology and products into various branches of the biomedical fields, which will benefit all aspects of human life, especially millions of patients suffering from incurable diseases[18].

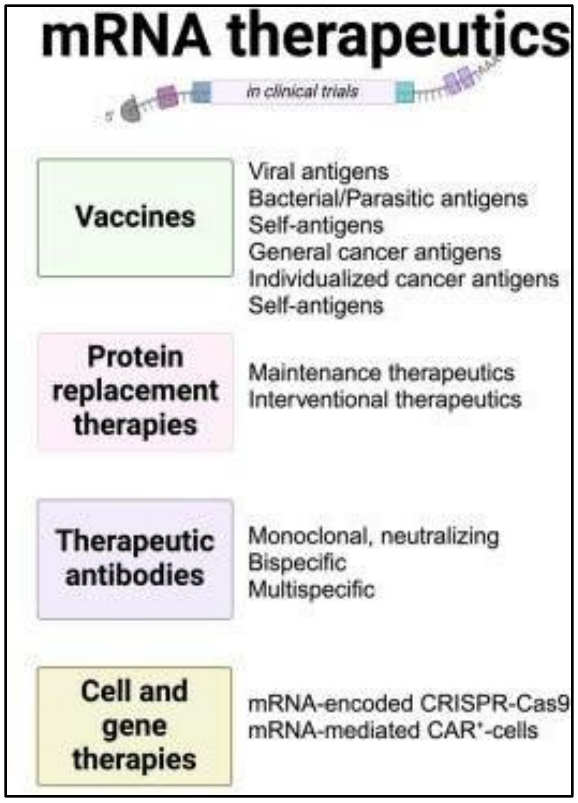

Figure No. 7: An overview of the SELEX procedures[19]

Uses:

mRNA is a versatile tool that can be used for therapeutic protein replacement, gene editing, cell therapy, vaccines, and more.

III. CONCLUSION

RNA-targeted therapeutics have emerged as a transformative class of medicines that expand the possibilities of modern drug discovery and personalized healthcare. From their early conceptual development to the recent clinical successes of RNA-based vaccines and therapies, this field has demonstrated remarkable versatility in targeting previously “undruggable” pathways. By exploiting diverse mechanisms of action including gene silencing, transcript modification, and protein translation control RNA therapies offer precise, adaptable, and highly innovative treatment strategies. The historical progression of RNA therapeutics underscores the importance of technological advances in RNA chemistry, delivery platforms, and stability optimization, which have collectively addressed earlier challenges of safety, specificity, and efficacy. Current modalities such as small interfering RNAs (siRNAs), antisense oligonucleotides (ASOs), aptamers, and mRNA-based platforms highlight the diversity of therapeutic options, each with unique clinical applications across infectious diseases, oncology, genetic disorders, and rare diseases. Looking forward, RNA therapeutics hold the potential to reshape the future of medicine by complementing or even surpassing conventional small molecules and biologics. Continued research into delivery systems, off-target minimization, and long-term safety will be critical to fully realize their potential. As our understanding of RNA biology deepens, RNA-targeted therapies are poised to stand at the forefront of next-generation therapeutics, offering hope for conditions once considered untreatable.

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