دانشگاه علوم پزشکی و خدمات بهداشتی درمانی کرمانشاه

Antisense Drugs

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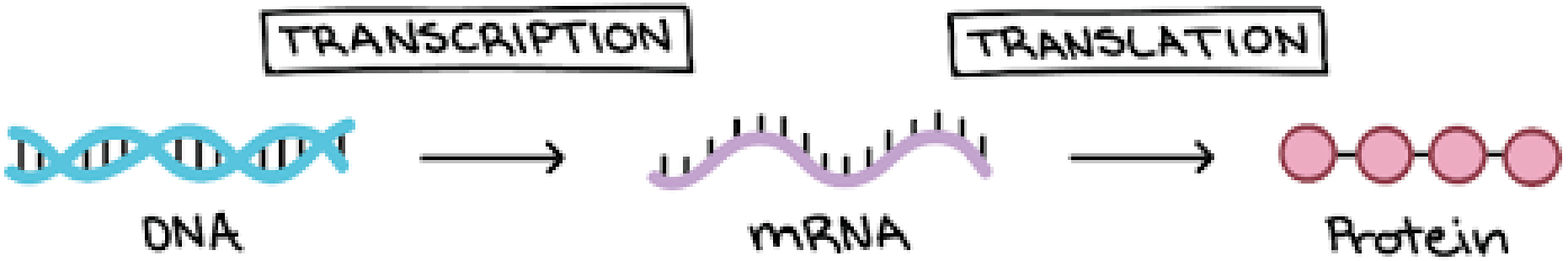
Basic Science:

- Genes contain the information necessary to produce proteins.
- Protein production occurs in two phases called transcription and translation.
- In the transcription phase, the DNA strand is used as a template for manufacturing an mRNA molecule.

- mRNA is responsible for communicating the genetic message in the DNA to the cell so that protein production can take place.

- In the translation phase, the mRNA travels to the ribosome, and carry out protein synthesis.



Overview:

- Currently, a total of ~4,000 genetic disorders are known.
- The mutated genes produce proteins that cannot function properly, leading to the occurance of the diseases.
- Examples: chronic inflammatory disease such as allergies, asthma, chronic inflammatory bowel disease, rheumatoid arthritis, and other autoimmune disorders. metabolic and cardiovascular diseases (CVDs), eye diseases, some neurologic disorders, and also infectious diseases, particularly in the field of viral infections.

How to stop genetic disorder using DNA drugs?

- Design a short DNA sequence that matches the sequence of mRNA that is transcribed from the mutated gene (which causes diseases).

- The DNA drug binds to the mRNA

- The mRNA cannot be translated to protein

- Because no disease-causing protein, disease is cured

What are the ways?

🧬The available possible ways to achieve this include the use of :

- Oligonucleotides
- Ribozyme
- RNAi
- Newer techniques like LNA and CeNA

🧬The Collective use of these techniques is called Antisense Technology

Antisense Technology

Antisense technologies are a suite of techniques that, together, form a very powerful weapon for studying:

🧬Gene function (functional genomics)

🧬For discovering new and more specific treatments of diseases in

- Humans
- Animals
- Plants

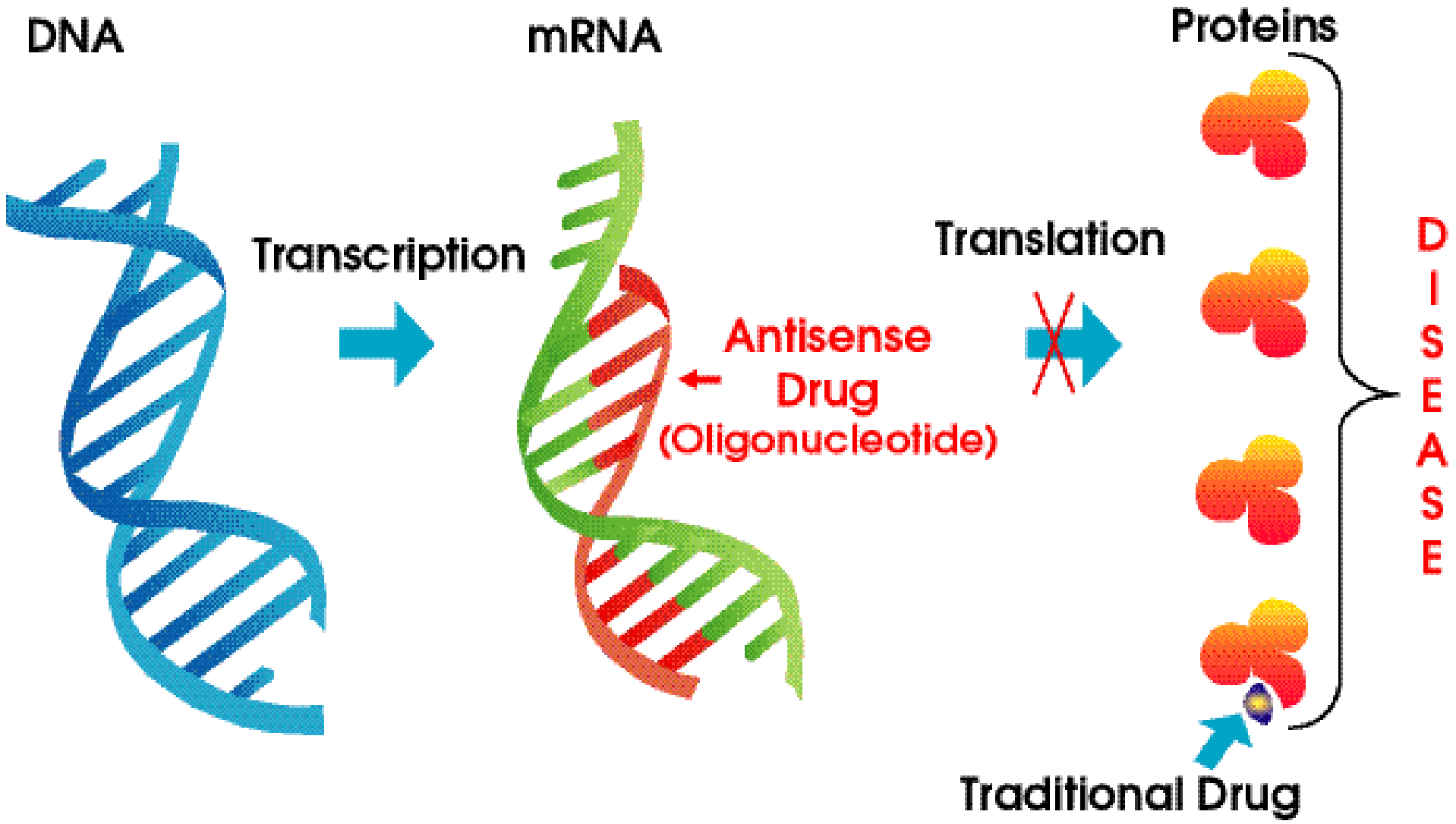
Antisense Technology

Antisense technology interrupts the translation phase of the protein production process by

- Preventing the mRNA instructions from reaching the ribosome.
- Inhibiting the protein systhesis.
- Antisense drugs are short, chemically modified complementary nucleotide chains that hybridize to a specific complementary area of mRNA.

What are Antisense oligonucleotides?

- Antisense Oligonucleotides are unmodified or chemically modified ssDNA, RNA or their analogs.
- They are 13-30 nucleotides long and are specifically designed to hybridize to the corresponding mRNA by Watson-Crick binding.
- In this technique Short segments of single stranded DNA called Oligodeoxynucleotides are introduced in to the cell.
- These Oligonucleotides are complementary to the mRNA, and physically bind to it.


DNA
mRNA
Proteins
Transcription
Translation
Antisense
Drug
(Oligonucleotide)
DISEASE
Traditional Drug

- The antisense effect of a oligonucleotide sequence was first demonstrated in 1970s by Zamecnik and Stephenson, in Rous sarcoma virus.
- When these oligonucleotides combined with target mRNA, a DNA/RNA hybrid is formed, which is degraded by the enzyme RNase H.
- In this technique Short segments of single stranded DNA called Oligodeoxynucleotides are introduced in to the cell.
- These Oligonucleotides are complementary to the mRNA, and physically bind to it.

Despite the simplicity of the idea behind the Antisense oligonucleotides, several problems have to be overcome for successful application:

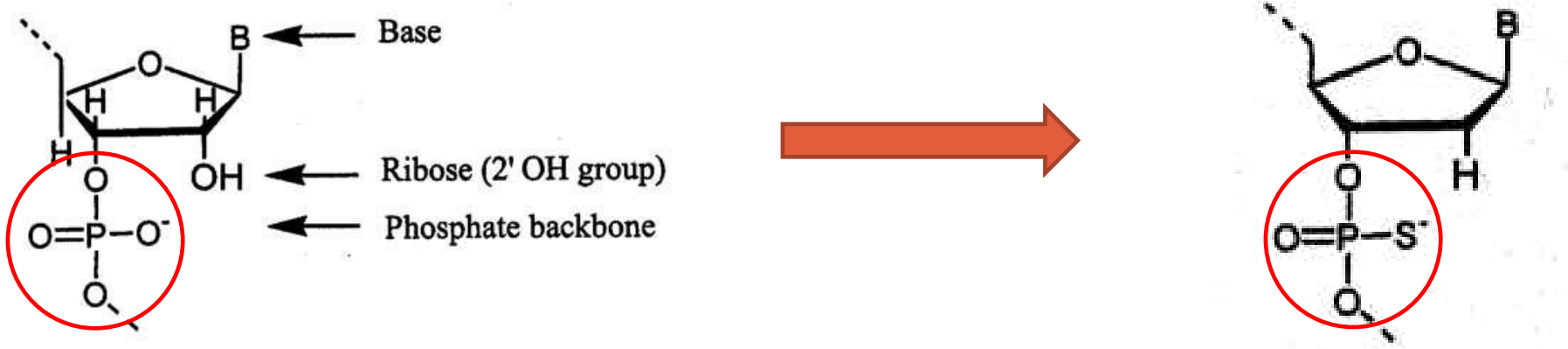
- Accessible sites of the target RNA for oligonucleotide binding have to be identified.
- Antisense agents have to be protected against nuclease enzyme attack.
- Cellular uptake and correct intracellular localization.
- It is therefore necessary to chemically modify antisense oligonucleotides to make them stable in cells.
- Modification of the phosphodiester backbone is likely to inhibit nuclease action and several phosphodiester backbone analogues have been developed with this goal in mind.

On the basis of mechanism of action, two classes of antisense oligonucleotide can be discerned:

- The RNase H - dependent oligonucleotides, which induce the degradation of mRNA
- The steric-blocker oligonucleotides, which physically prevent or inhibit the progression of splicing or the translational machinery.

First generation Antisense oligonucleotides:

- First synthesized by Eckstein and colleagues in 1960s.
- Phosphoro-thioate -deoxy-nucleotides are the first generation oligonucleotides and have a sulfur atom replacing the non-bridging oxygen of the sugar phosphate backbone. It preserves the overall charge and can also activate RNaseH for the degradation of mRNA.



Fomivirsen (brand name Vitravene):

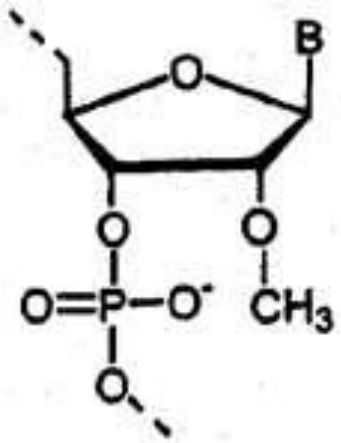
- Is an antisense antiviral drug that was used in the treatment of cytomegalovirus retinitis (CMV) in immunocompromised patients, including those with AIDS.
- 21 nucleotides with phosphorothioate (PS DNA) linkages (which are resistant to degradation by nucleases) and has the sequence:
- 5'-GCG TTT GCT CTT CTT CTT GCG-3'

- Cannot cross the lipid bilayer because of their charge and polarity.
- Once in the circulation they can be taken up by many cell types and not just the cell targeted leading to potential side-effects.
- Observed side effects seen in clinical studies performed on humans include:

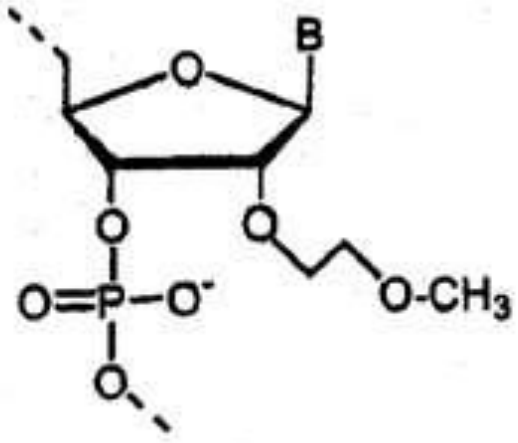
- Thrombocytopenia
- Fatigue
- Fever
- Rashes
- Leukopenia

Second generation Antisense oligonucleotides:

- Second generation Antisense oligonucleotides containing nucleotides with alkyl modifications at the 2′ position of the ribose.
- 2′ -O-methyl and 2′-O-methoxy-ethyl RNA are the most important member of this class



2′ -O-methyl



2′-O-methoxy-ethyl

- These “second-generation” oligonucleotides are resistant to degradation by cellular nucleases and hybridize specifically to their target mRNA with higher affinity than the phosphodiester or phosphorothioate.

- However, such antisense effects result from Rnase H independent mechanisms.

Characterstics of second generation Antisense oligonucleotides:

- Mechanisms of action for the 2' modified oligonucleotides do not rely on RNase H activation but on translation arrest by blocking 80S ribosome complex formation as well as with splicing interference.
- They were developed to try and avoid the toxicity associated with the first generation AS-ONs.
- Show high binding affinity to target mRNA.
- Best stability to nucleases.
- Less toxic than first generation AS-ON.
- Higher lipophilicity compared to first generation ASOns.

Mipomersen (brand name Kynamro):

- Is used to treat homozygous **familial hypercholesterolemia** and is administered by subcutaneous injection.
- Mipomersen binds to the messenger RNA coding for apolipoprotein B-100 (ApoB-100)
- 5’—G*—mC*—mC*—mU*—mC*—dA—dG—dT—dmC—dT—dG—dmC—dT—dT—dmC—G*—mC*—A*—mC*—mC*—3’

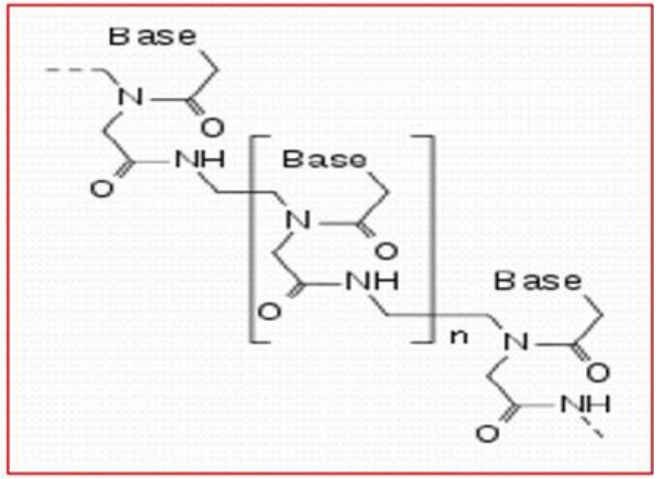
* = 2’-O-(2-methoxyethyl)
m = 5-methyl
d = 2’-deoxy

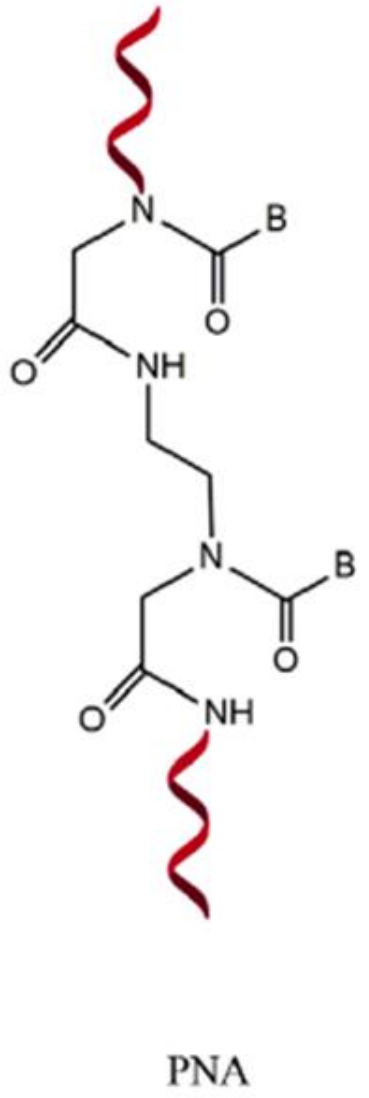
Third generation Antisense oligonucleotides:

- Newest and most promising.
- Enhance binding affinity and biostability.
- Peptide nucleic acids (PNAs)
- Locked nucleic acid (LNA)
- Tricyclo-DNA (tcDNA)
- Cyclohexene nucleic acids (CeNA)

Peptide nucleic acids (PNA):

- In PNAs the deoxyribose phosphate backbone is replaced by polyamide linkages.
- The property of high-affinity nucleic acid binding can be explained by the lack of electrostatic repulsion because of the absence of negative charges on the PNA oligomers.
- The antisense mechanism of PNAs depends on steric hindrance.

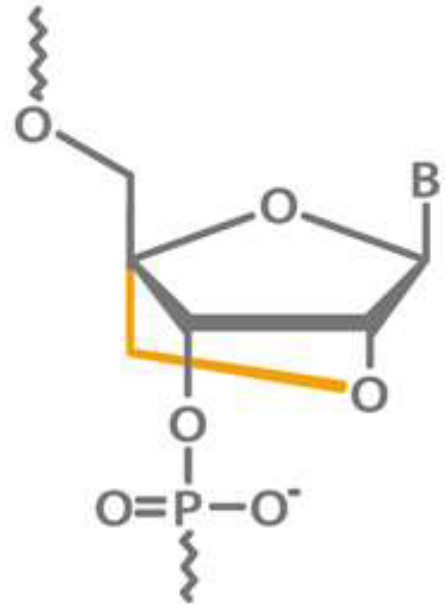




PNA

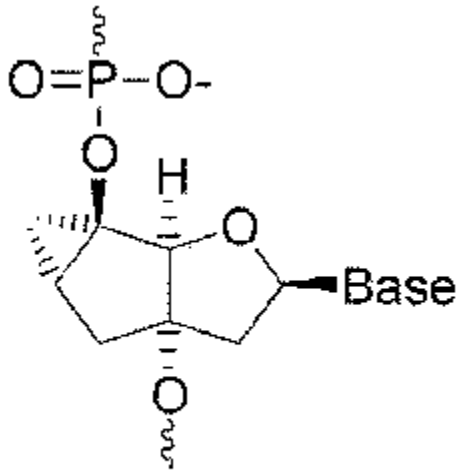
Locked nucleic acid (LNA) :

- The ribose ring is connected by a methylene bridge (orange) between the 2'-O and 4'-C atoms thus “locking” the ribose ring in the ideal conformation for Watson-Crick binding.
- Thus the Pairing with a complementary nucleotide strand is more rapid and increases the stability of the resulting duplex.
- LNA based hepatitis C drug called Miravirsen, targeting miR-122, is in Final testing as of late 2010.
- LNA oligonucleotides exhibit unprecedented thermal stability when hybridized to a complementary DNA or RNA strand.

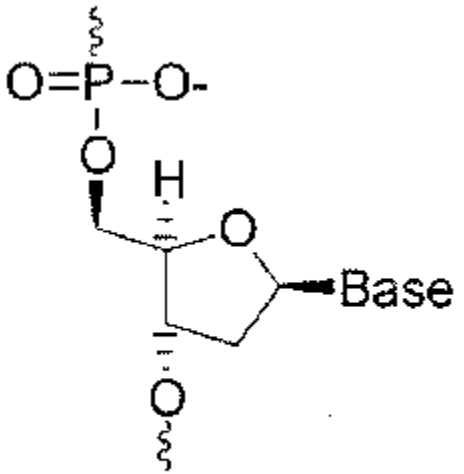


Tricyclo-DNA (tcDNA):

- Chemically, tc-DNA deviates from natural DNA by three additional C-atoms between C(5') and C(3').



tricyclo-DNA

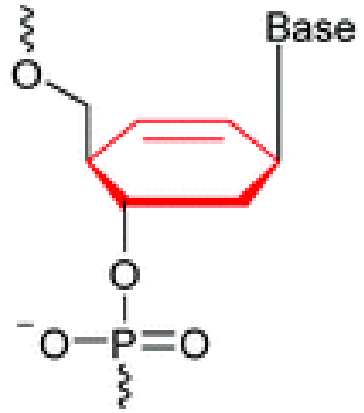


DNA

Cyclohexene nucleic acids (CeNA):

- The replacement of the furanose moiety of DNA by a cyclohexene ring gives Cyclohexene nucleic acids or CeNA.

- CeNA is stable against degradation in serum and a CeNA/RNA hybrid is able to activate RNase H, resulting in cleavage of the RNA strand.



CeNA

These chemical modifications change the properties of natural oligodeoxynucleotides in the following way:

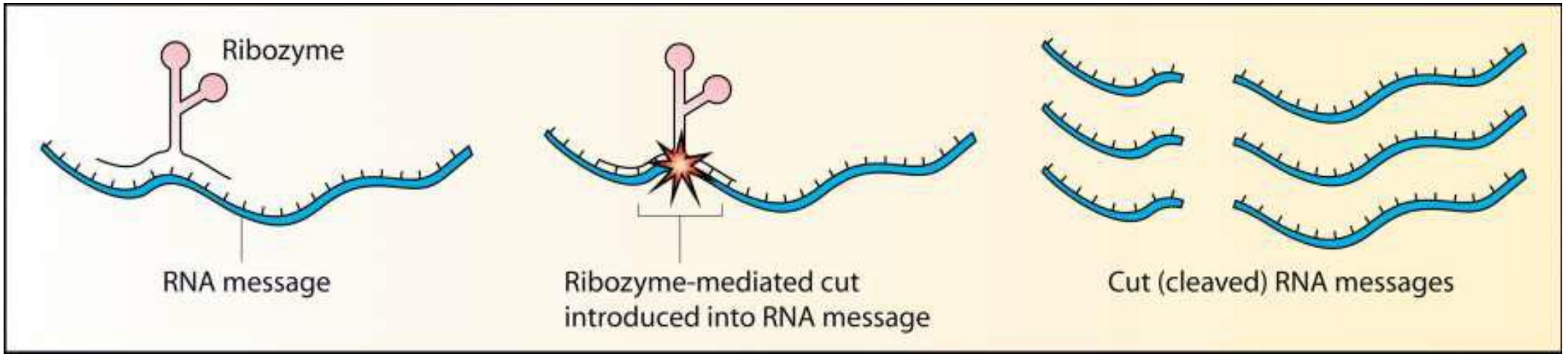
- Increased RNA affinity.
- Increased hydrophobicity.
- Increased stability towards nucleolytic degradation.
- Inability to elicit RNaseH activity.

Application of Antisense Oligonucleotides:

- Lung cancer
- Colorectal carcinoma
- Pancreatic carcinoma
- Malignant melanoma
- Diabetes
- Amyotrophic lateral sclerosis (ALS)
- Duchenne muscular dystrophy
- Asthma
- Arthritis

Ribozymes:

- Ribozymes are RNA molecules that have catalytic activity.
- Ribozyme Bind to the target RNA moiety and inactivate it by cleaving the phosphodiester backbone at a specific cutting site.



Ribozymes in clinical trials:

- Angiozyme : VEGF receptor and angiogenesis inhibitors - treatment of kidney cancer.
- Herzyme : Anti-Human Epidermal growth factor Receptor type 2 (HER2)- treatment of breast and ovarian cancer
- Heptazyme : Reduces Serum HCV RNA Levels In Chronic Hepatitis C Patients

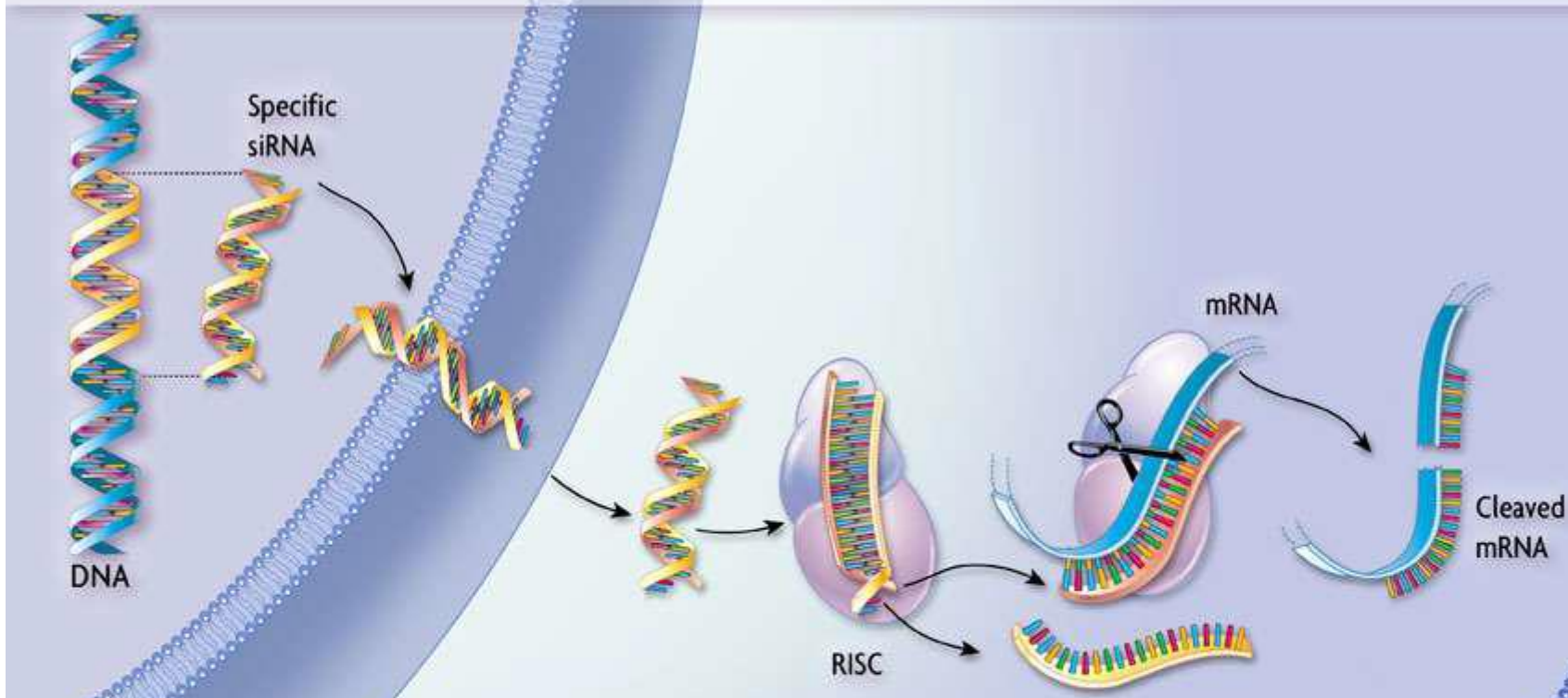
RNA interference (RNAi):

- RNAi is an antisense mechanism that involves using small interfering RNA, or siRNA, to target a mRNA sequence. With siRNA, the cell utilizes a protein complex called RNA-induced silencing complex (RISC) to destroy the mRNA, thereby preventing the production of a disease-causing protein.

Applications of RNAi:

- Cancer
- HIV
- Cardiovascular and Cerebrovascular Diseases
- Neurodegenerative Disorders

The RNAi Natural Process



A Small interfering RNA (siRNA), a 21-25 base pai RNA strand, is targeted to a specific gene.

B Within cells, siRNA unwinds and is incorporated into RISC, a stable protein-RNA complex.

C siRNA is directed to a targeted messenger RNA (mRNA) that is known to be involved in a disease pathway.

D The mRNA undergoes degradation, thereby interrupting the protein synthesis of the targeted gene.

Conclusion:

- Antisense oligonucleotides show a great potential as a molecular biology investigative tool as well as highly selective therapeutic agents.
- They can produce non-specific effects such as hematologic disturbances or activation of immune system components.
- They have limited uptake due to their polarity and specific delivery to target tissues is difficult to obtain.
- 2nd generation antisense oligonucleotides show promise as an alternative to 1st as they can function at other levels than RNase H.
- Drug delivery systems may be the key in making antisense oligonucleotides better therapeutic agents, as they can produce enhanced uptake, they protect from degradation or prevent non-specific effects

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Thank you