In The Name of God

ANTISENSE THERAPY

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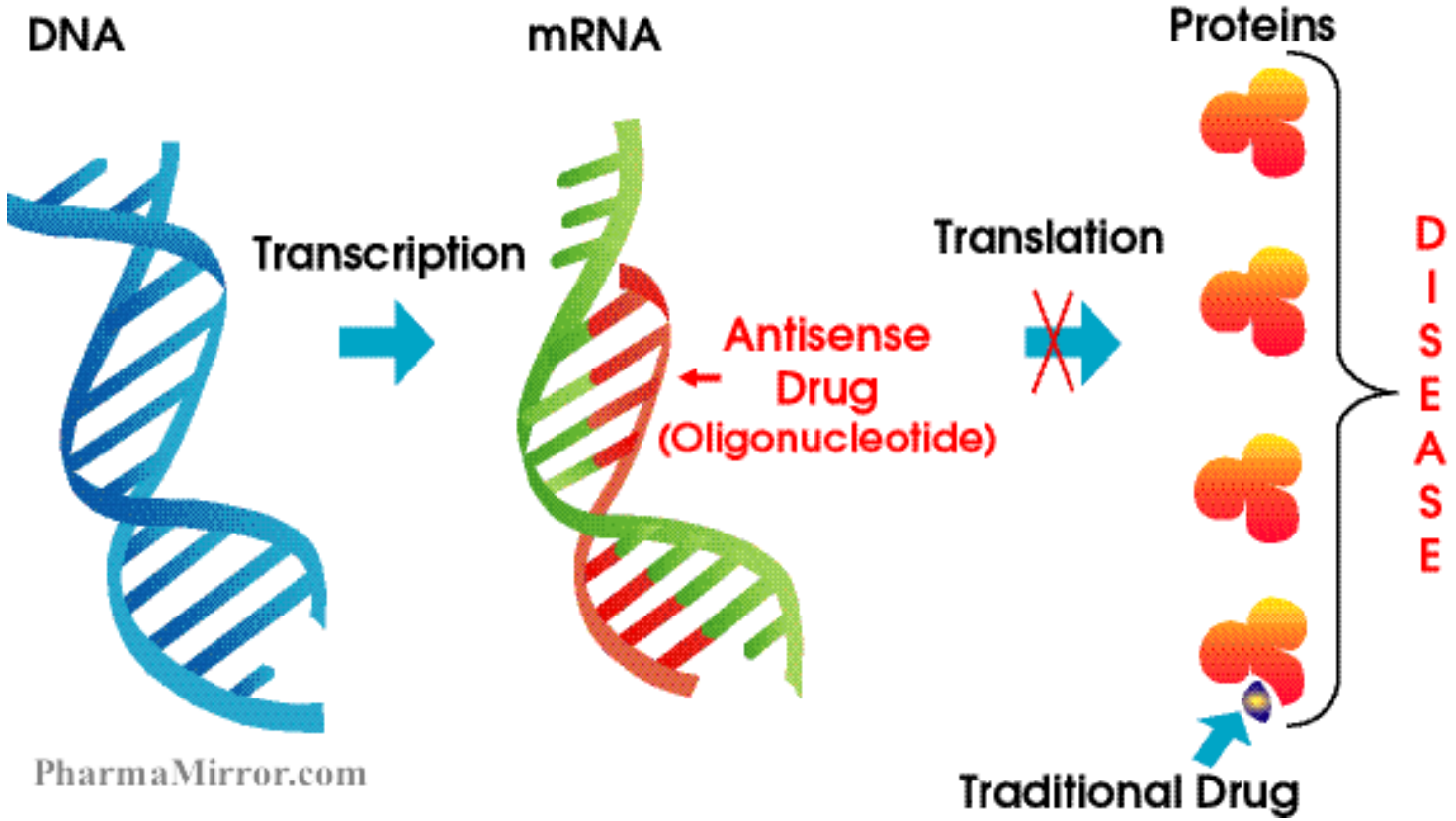
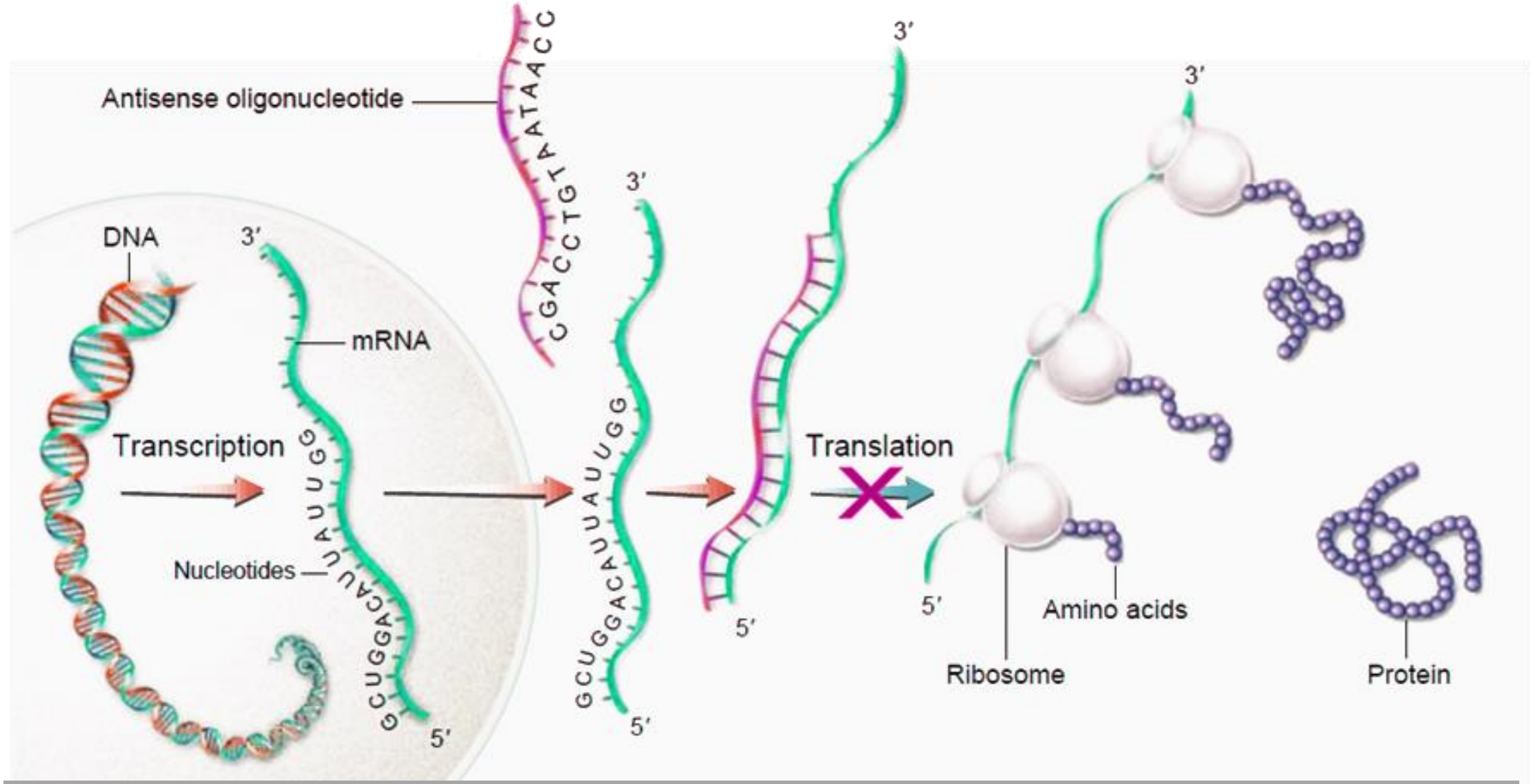
INTRODUCTION

- Antisense oligonucleotide (ASO) therapeutics design drugs that are specific inhibitors of the protein expression.
- Formation of a heteroduplex that inhibits the function of that target RNA



THERAPEUTIC APPLICATION

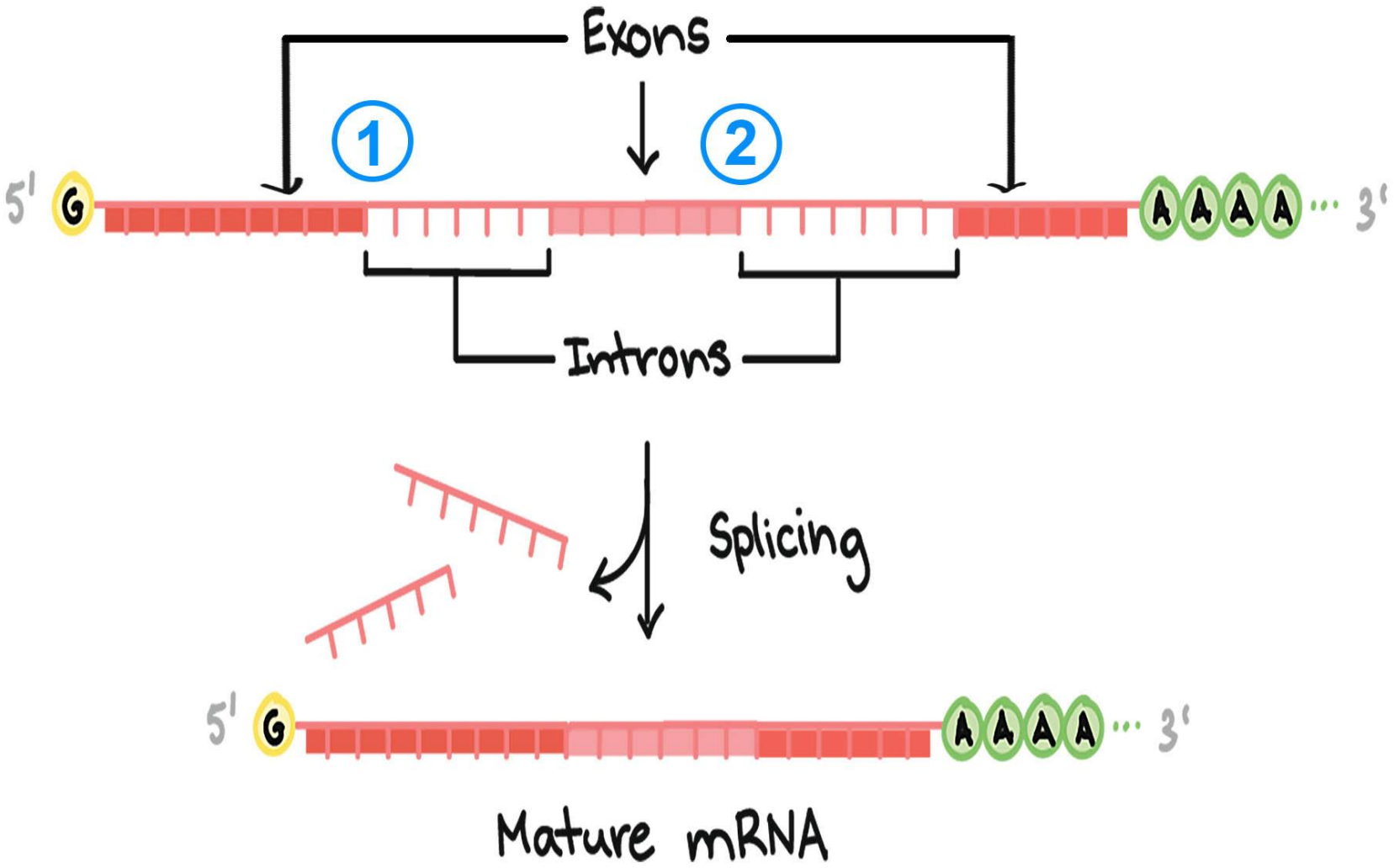
- Diabetes
- ALS
- Parkinson
- Spinal muscle atrophy
- Duchene muscular dystrophy
- Asthma , Arthritis
- Cytomegalovirus retinitis
- Batten disease
- HIV
- Cytomegalovirus retinitis
- Familial hyperchlostrolemia



Antisense Therapy

PROCESS OF PROTEIN EXPRESSION

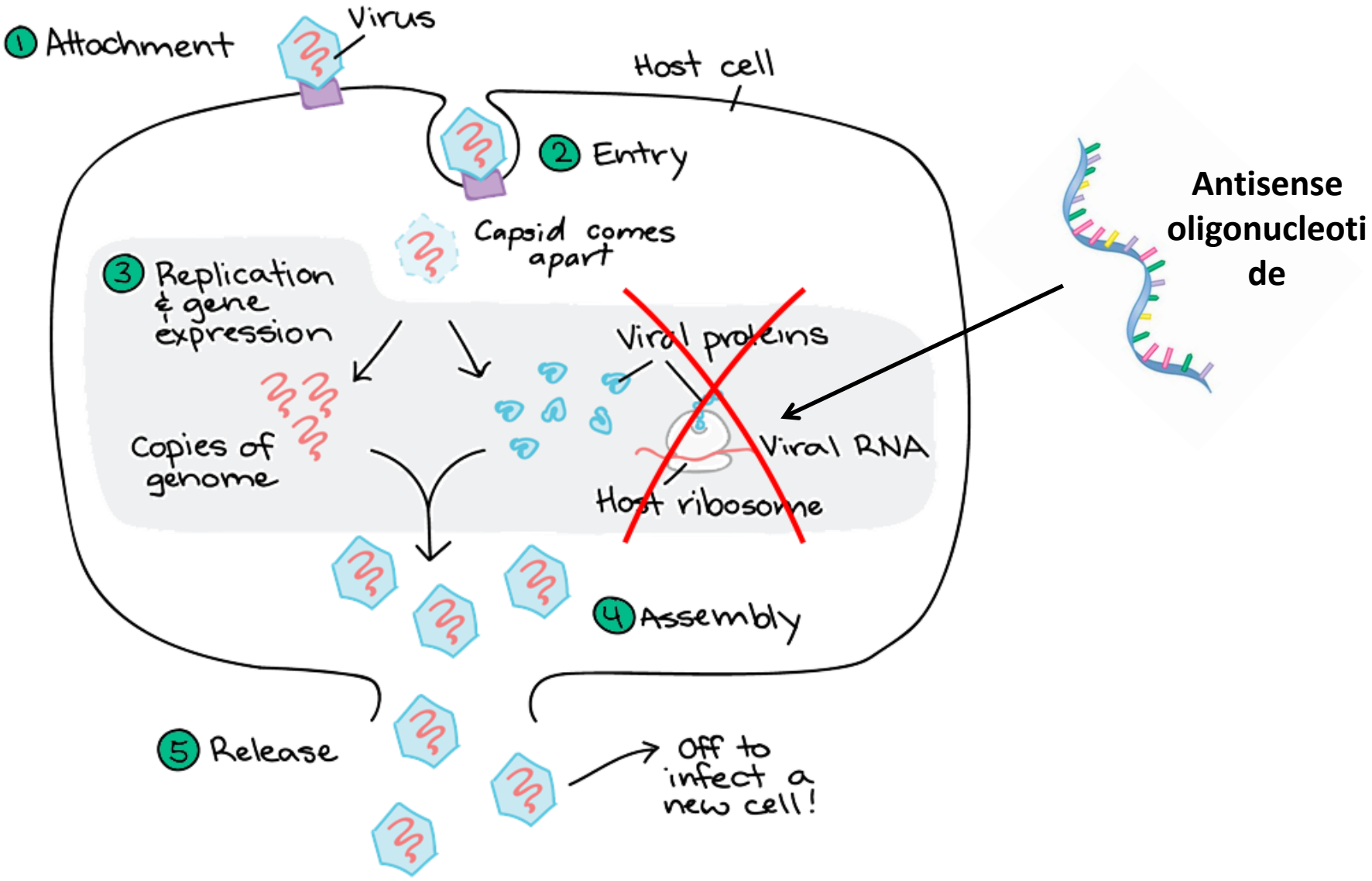
- Splicing
- Intron
- Exon



HISTORY

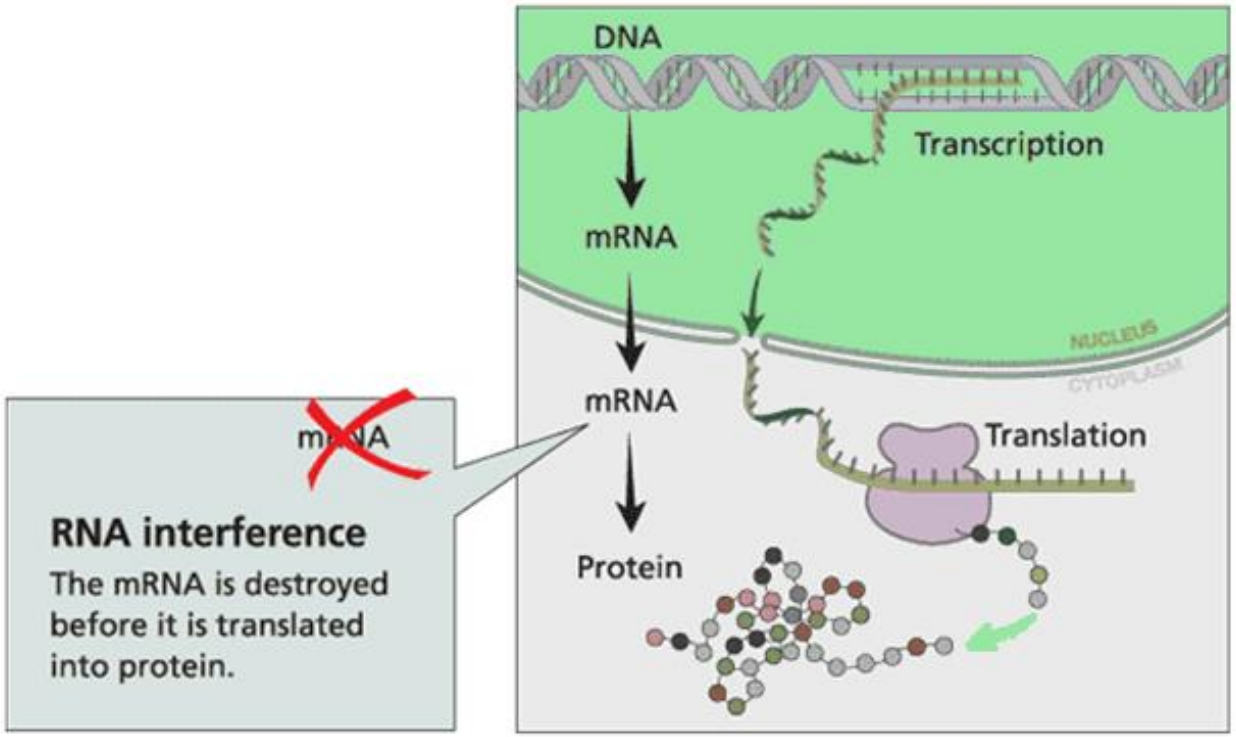
It might also be possible to inhibit the translation of a specific cell protein

- In 1978, Zamecnik and Stephenson; ASO prevented the accumulation of Rous sarcoma virus by inhibiting the translation of proteins.



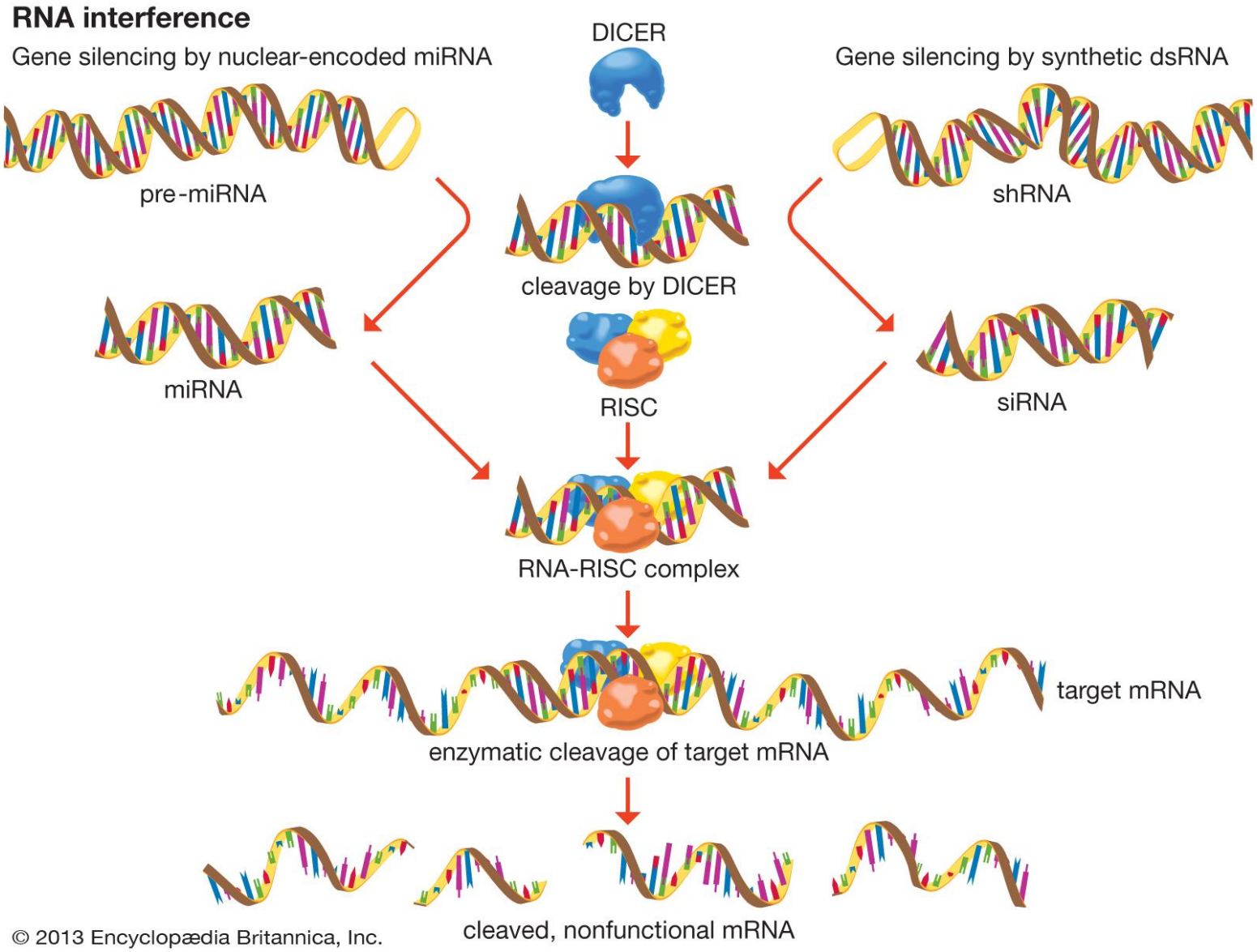
General Diagram of a Virus Lifecycle

HISTORY



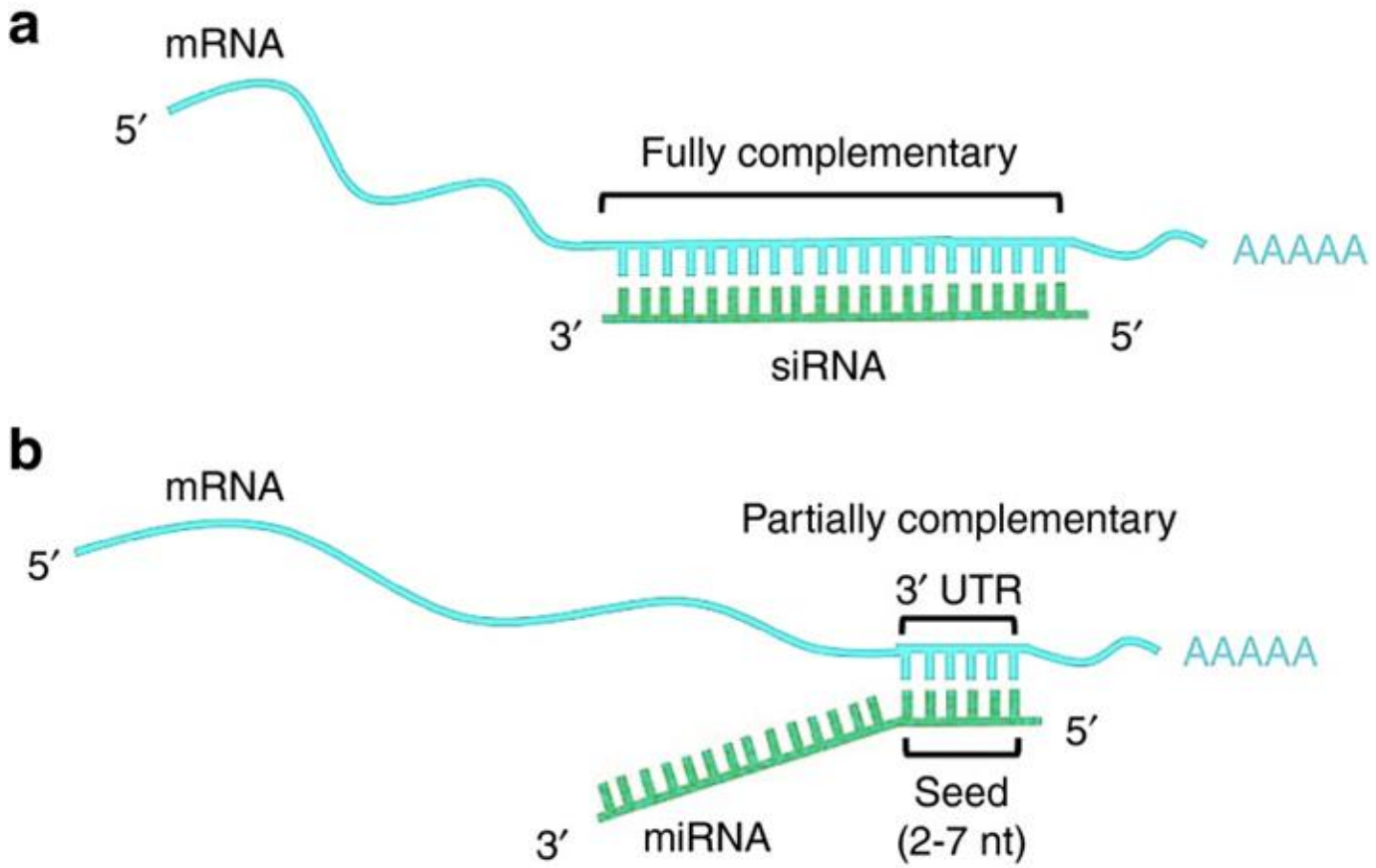
- In 1983, discovery of RNA interference (**RNAi**)
- Posttranslational gene expression regulation

- siRNA
- miRNA



RNA INTERFERENCE

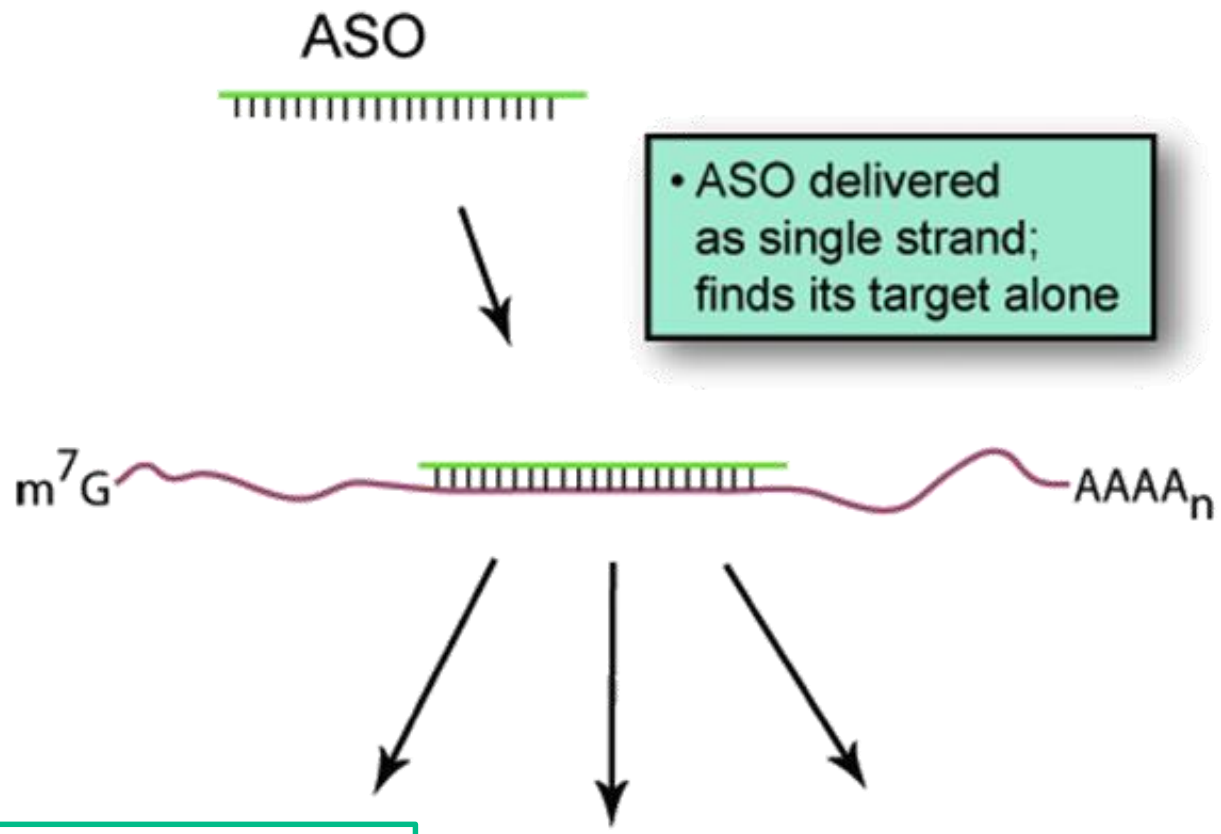
- siRNA vs miRNA



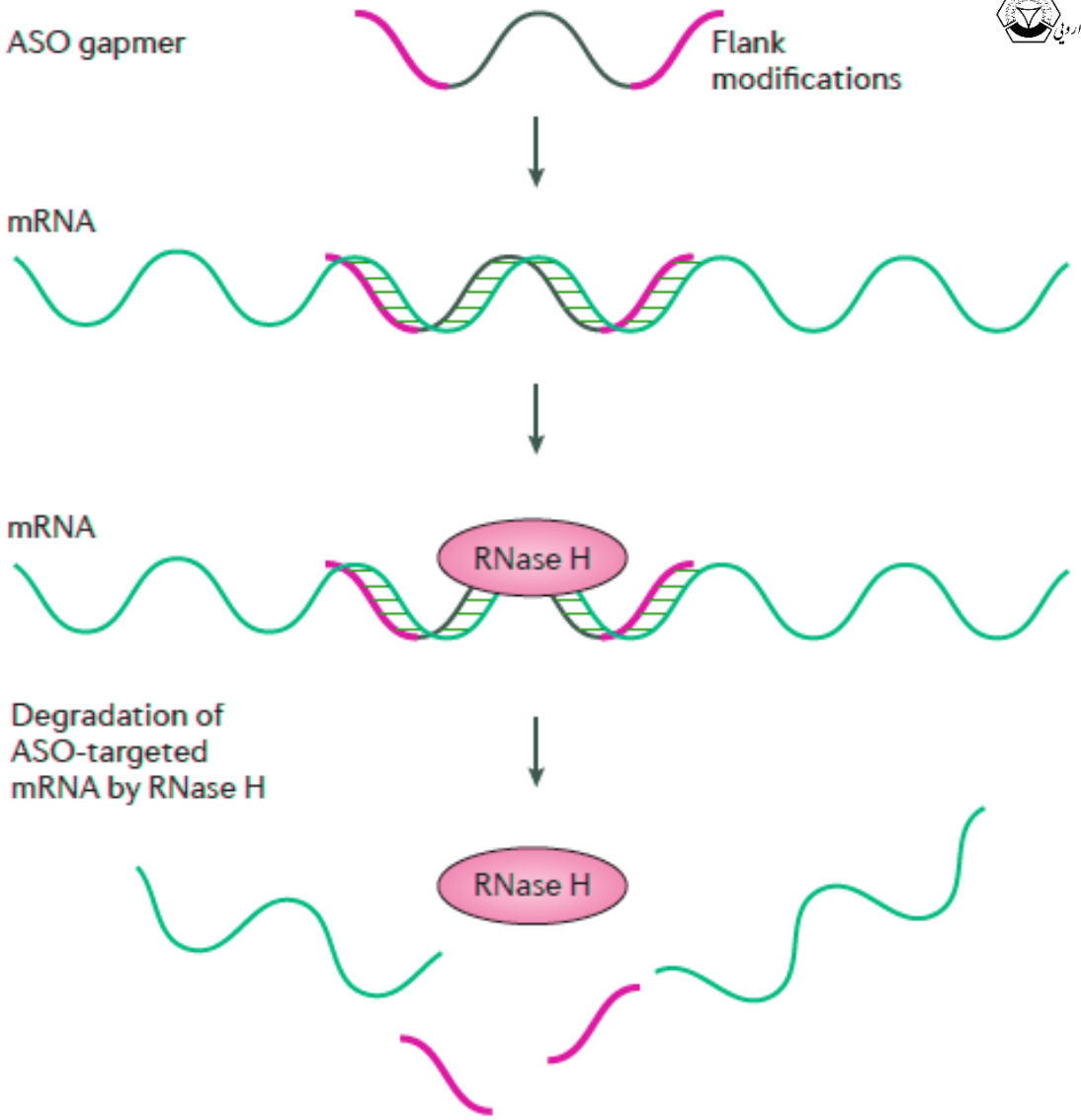
NOTE

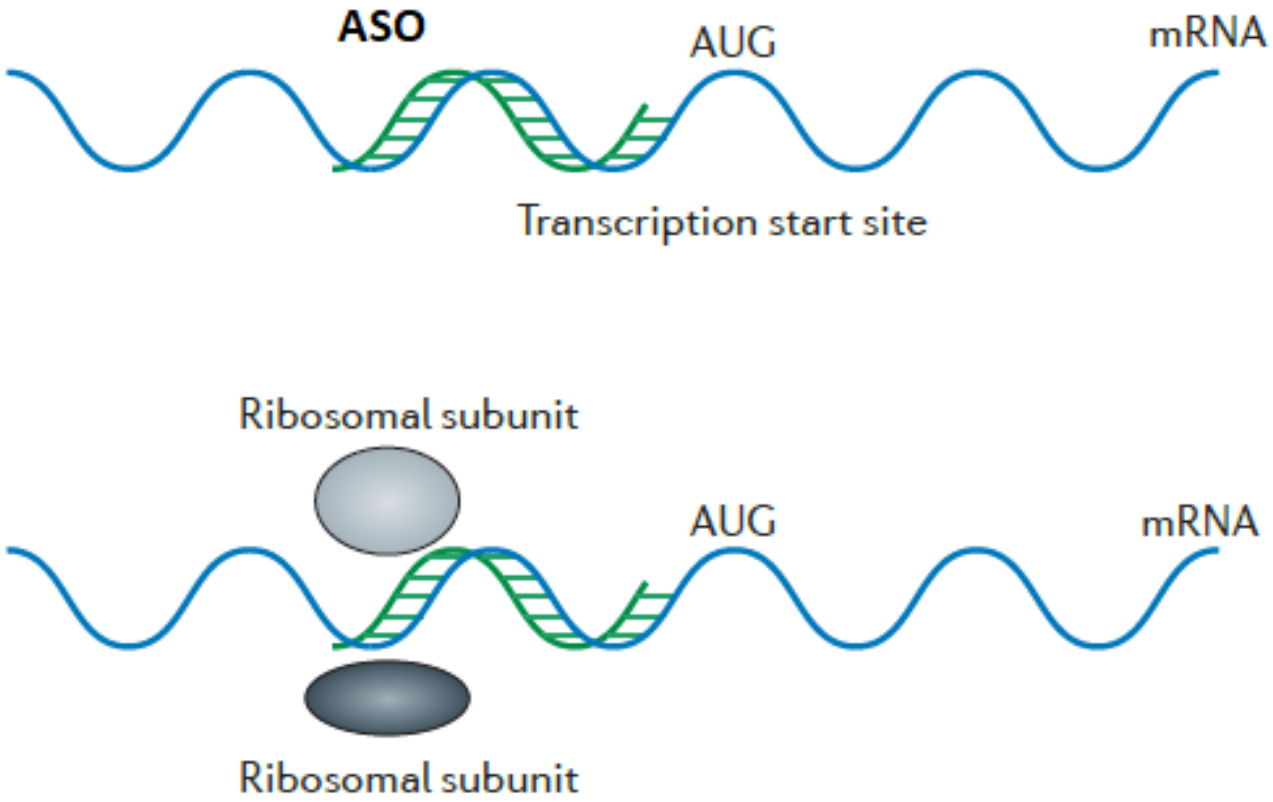
- Although ASOs and siRNA share similarities, they are divergent on some points, and so, choosing ASO or siRNA strategy for gene targeting depends on the target gene.
- ASOs are :
- Single stranded, as opposed to siRNA
- They have lower cost of production
- Eeasier to deliver ASOs *in vivo*
- A simple chemical modification can increase their resistance to nucleases, as opposed to siRNA that need a carrier.

FUNCTIONAL MECHANISMS OF ASOs



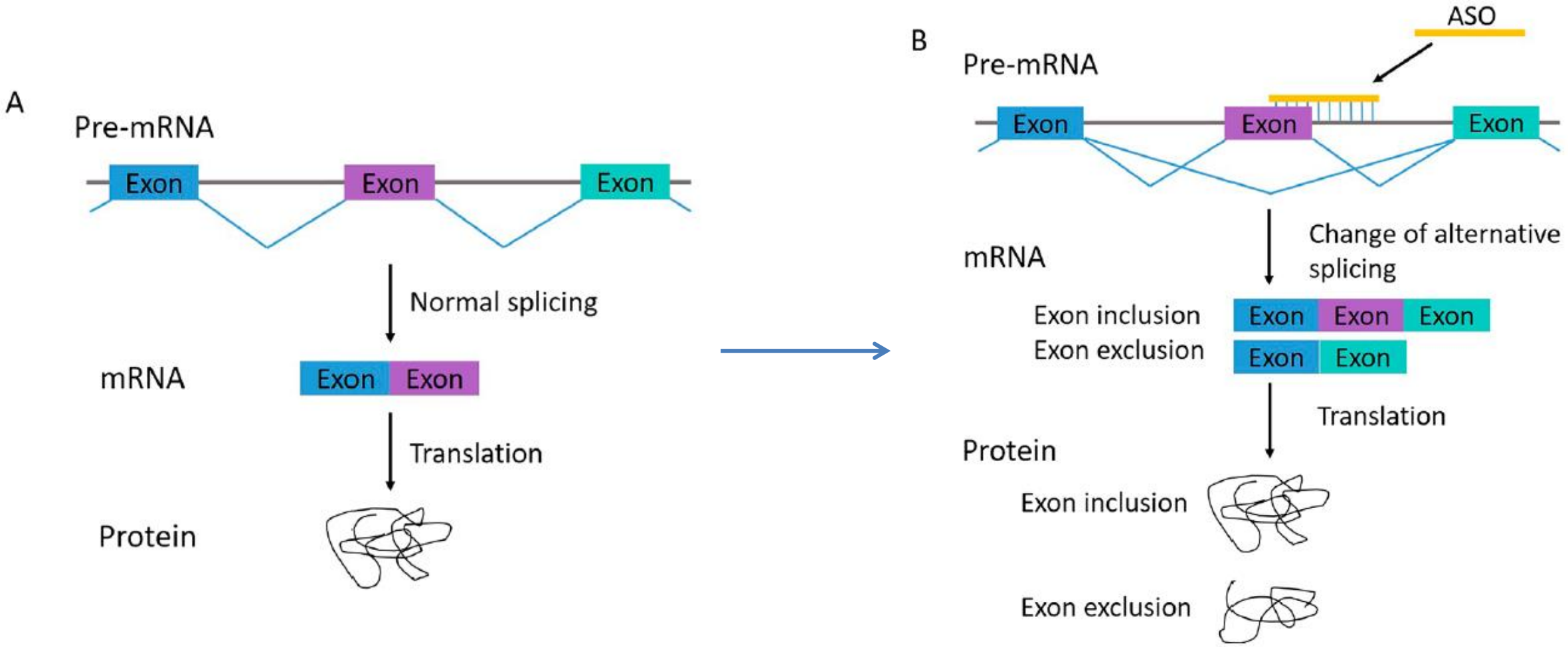
CLEAVAGE BY RNASE H



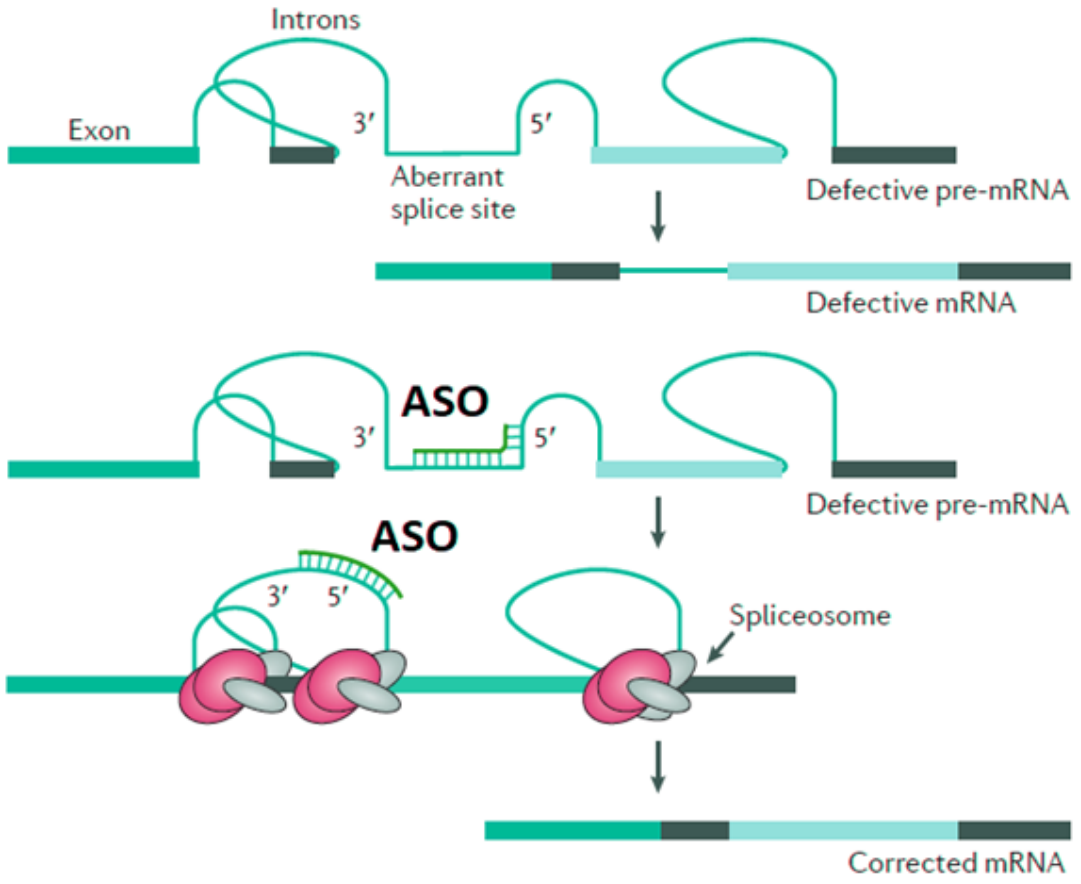


BLOCKING TRANSLATION

MODULATING SPLICING

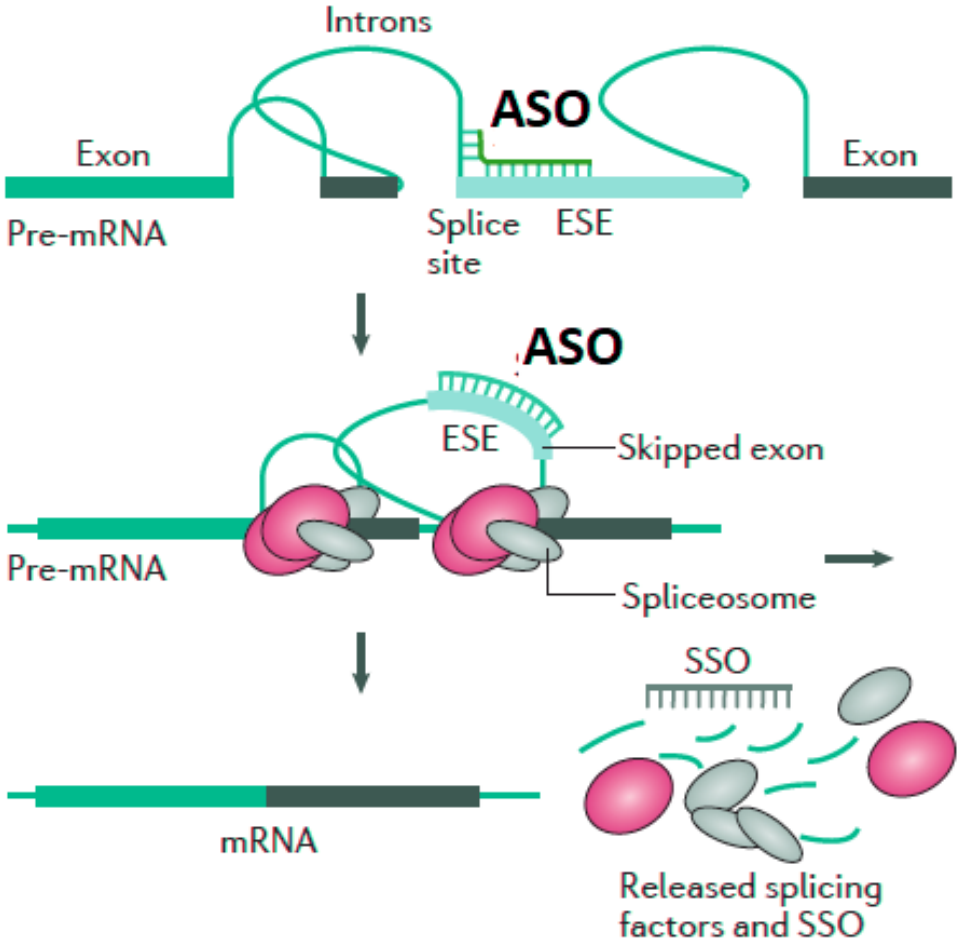


MODULATING SPLICING



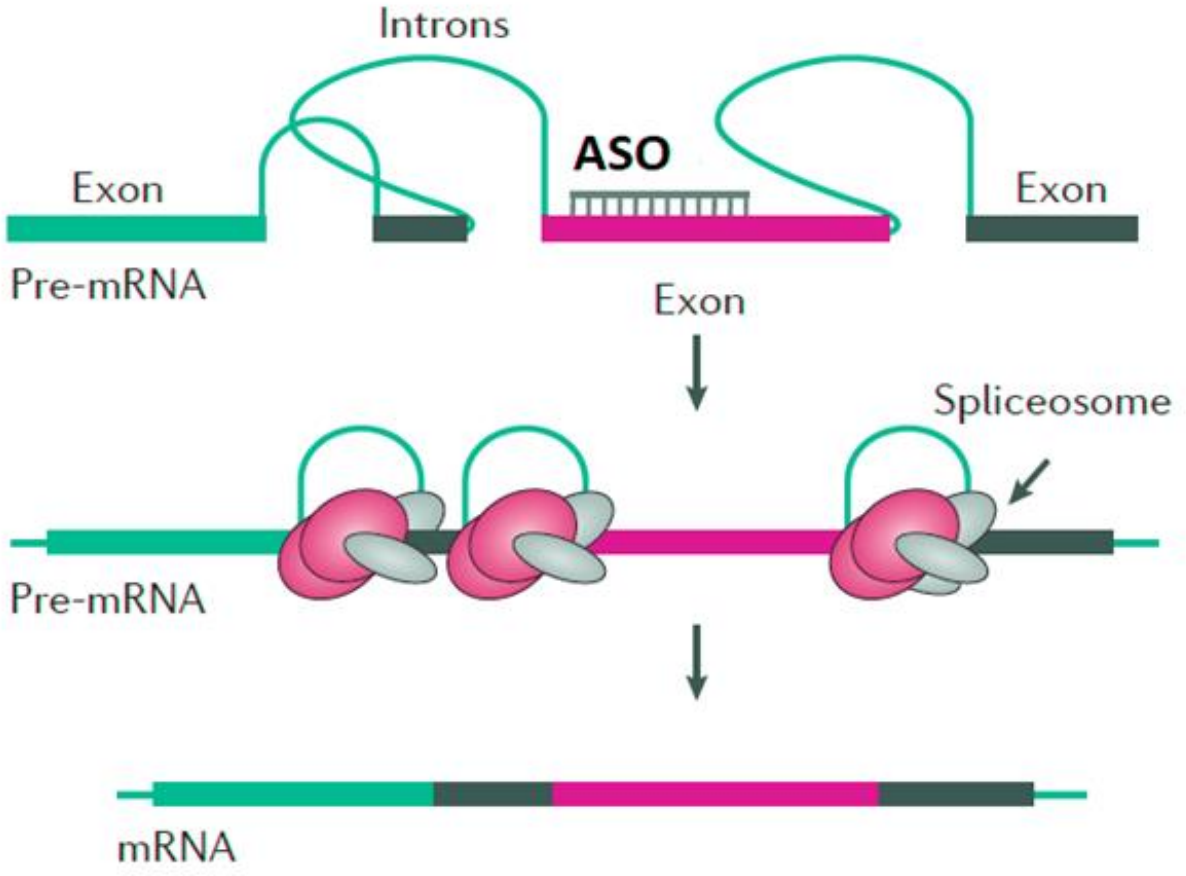
Restoration of correct splicing

MODULATING SPLICING



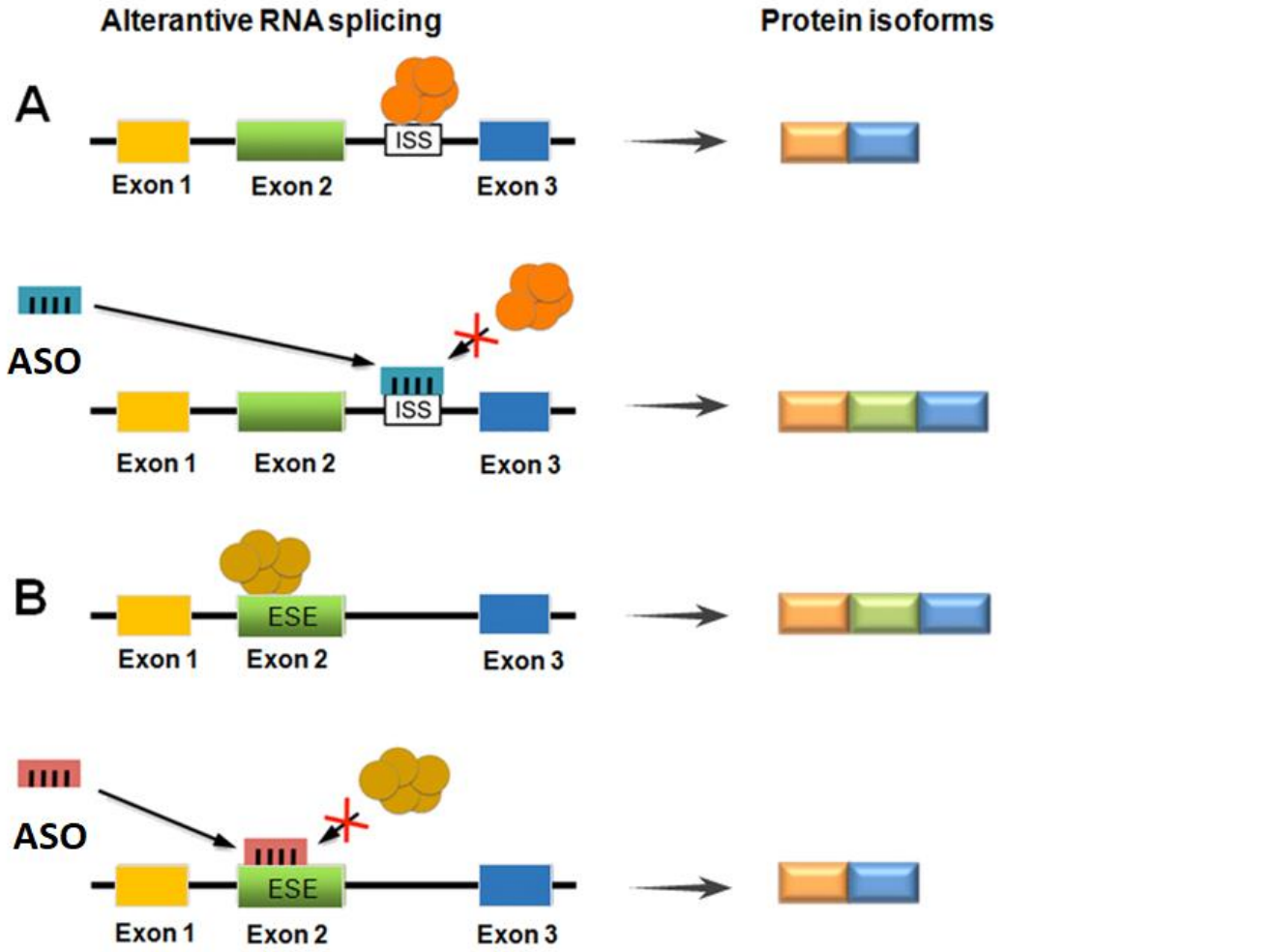
Exon skipping

MODULATING SPLICING



Exon retention

MODULATING SPLICING



Alternative RNA splicing and gene expression regulation

TIMELINE

first antisense drug (fomivirsen) was approved in 1998 to treat CMV eye infections

fourth (nusinersen) in December 2016 For spinal muscular atrophy

1978

Discovery of antisense DNA by Zamecnik and Stephenson in 1978

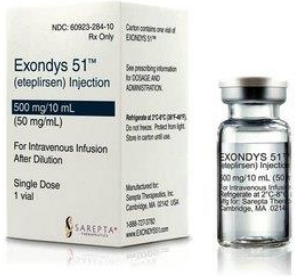
1998

second antisense drug (mipomersen) for familial hypercholesterolemia

2016

third approval (eteplirsen) in August 2016 for Duchenne muscular dystrophy

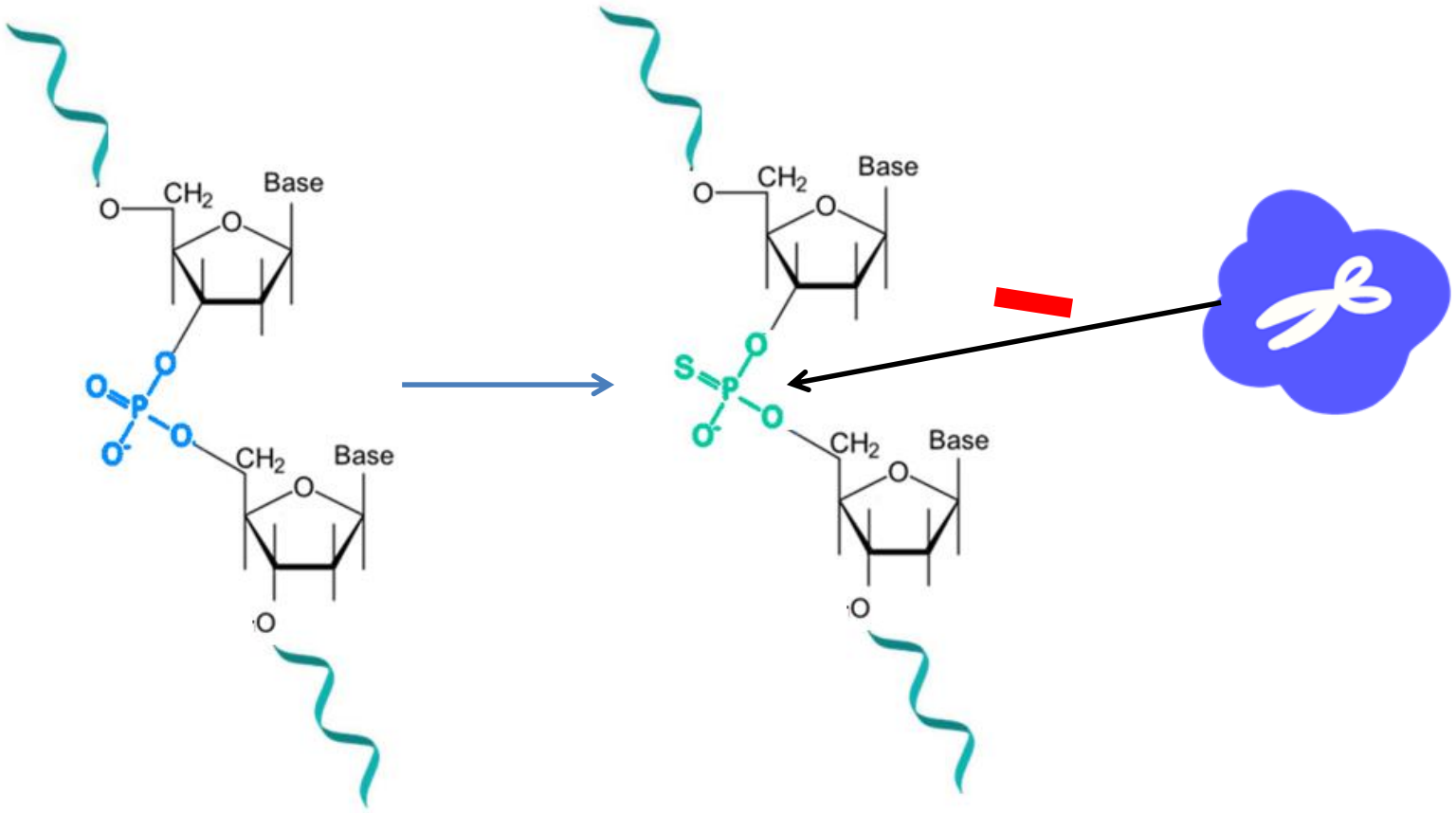
2016



CHALLENGES

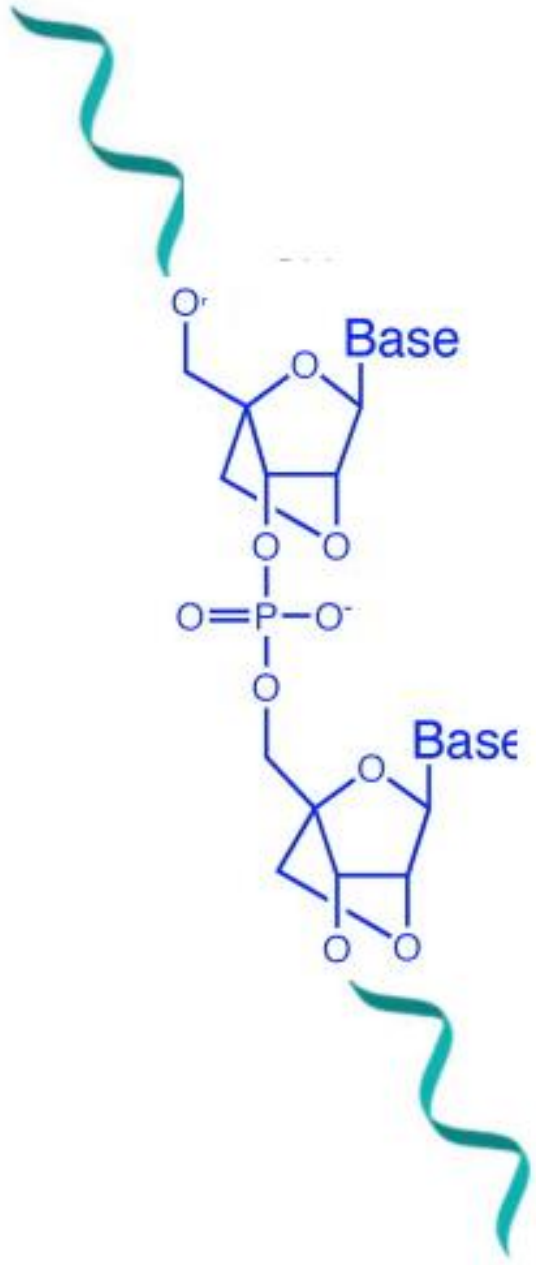
- DNA is rapidly broken down by nucleases inside cells
- Cellular mRNA targets are highly structured and often bound by protein chaperones. The binding affinity of DNA is generally insufficient to overcome this existing structure.
- Foreign nucleic acids can be recognized by the immune system, causing potentially serious side effects.
- Side effects can also arise if the antisense oligonucleotide (ASO) recognizes undesired RNA targets through partial complementarity.
- Unmodified DNA and RNA are not taken up by cells efficiently enough to be used in the clinic.

MEDICINAL CHEMISTRY CAN INCREASE NUCLEASE STABILITY



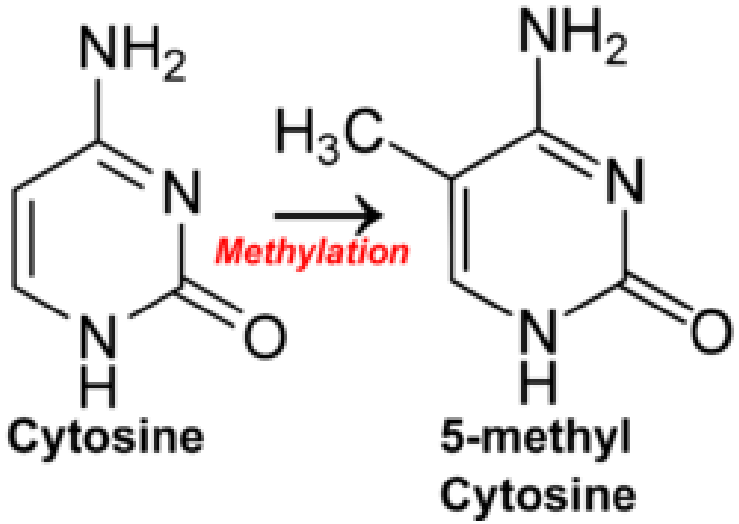
- Unmodified single stranded DNA or RNA oligonucleotide is rapidly digested by serum and cellular nucleases and thus has a half-life too short for clinical activity

MEDICINAL CHEMISTRY CAN INCREASE SPECIFICITY



- locked nucleic acid (LNA)
- **ideal conformation** for base pair binding

MEDICINAL CHEMISTRY CAN REDUCE IMMUNE RESPONSE

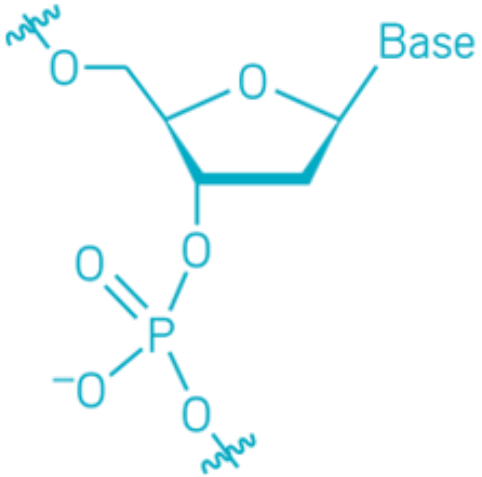


- 5-methylation of cytosine in CpG dinucleotides of DNA can reduce the immune response induced by oligonucleotides.

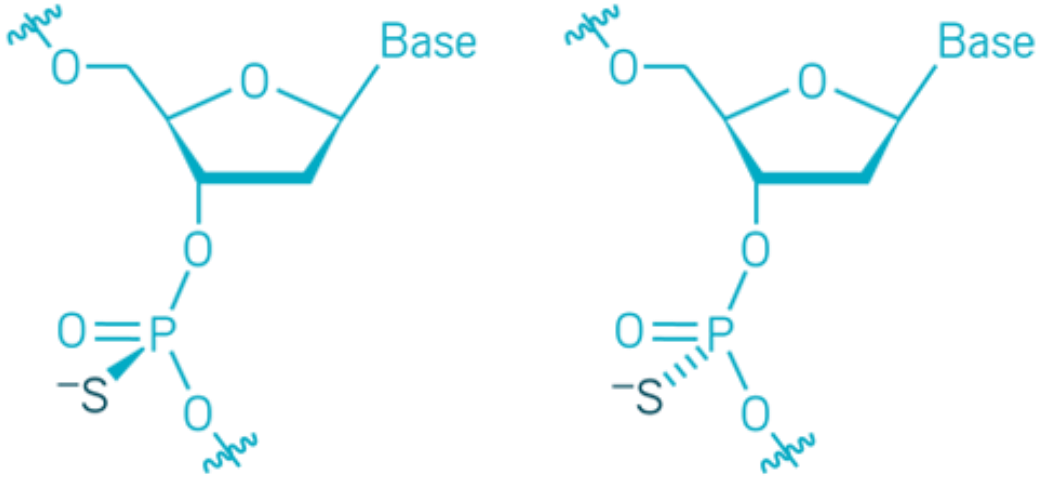
CHEMICAL MODIFICATIONS OF CURRENT IMPORTANCE BY STRUCTURAL CLASS

First generation Antisense oligonucleotides

- Phosphoro-thioate - deoxy-nucleotides
- Better stability to nucleases due to the S conformer
- Can activate RNase H



Phosphodiester bond in DNA

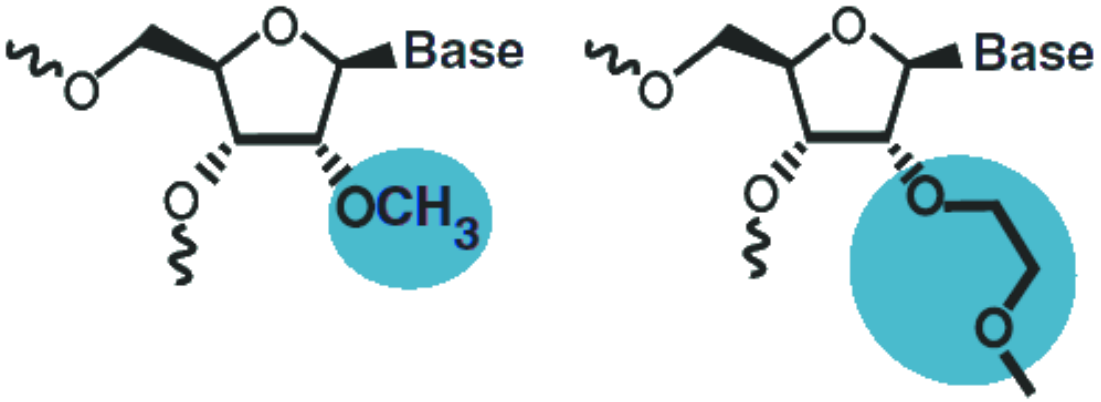


Diastereomers of phosphorothioate bond in DNA
(R isomer on left, S isomer on right)

CHEMICAL MODIFICATIONS OF CURRENT IMPORTANCE BY STRUCTURAL CLASS

Seconed generation Antisense oligonucleotides

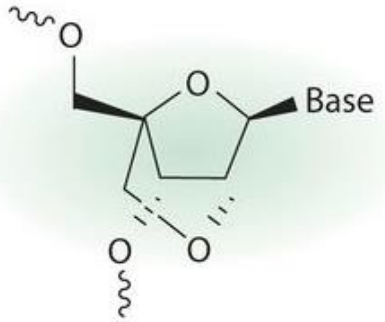
- Resistant to degradation by cellular nucleases
- Target mRNA with higher affinity
- RNase H independent mechanisms
- Modifications at the 2’ position of the ribose
- Mechanism:Translation arrest or splicing modulation



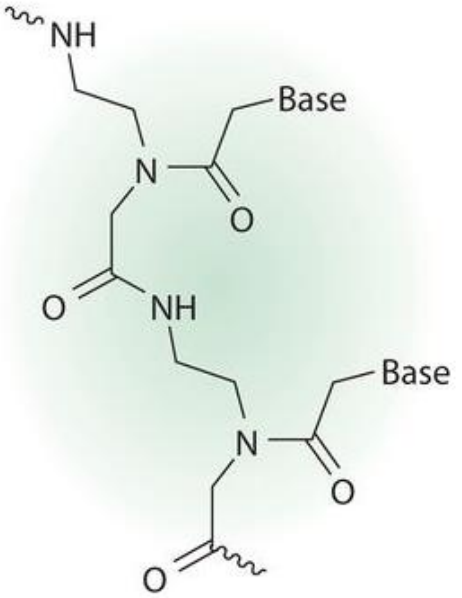
CHEMICAL MODIFICATIONS OF CURRENT IMPORTANCE BY STRUCTURAL CLASS

Third generation Antisense oligonucleotides

- Peptide nucleic acids (PNAs)
- Locked nucleic acid (LNA)



LNA
(locked nucleic acid)



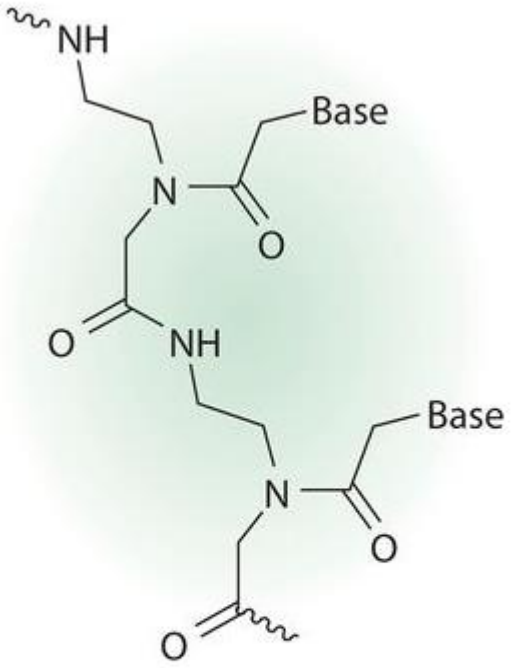
PNA
(Peptide nucleic acid)

CHEMICAL MODIFICATIONS OF CURRENT IMPORTANCE BY STRUCTURAL CLASS

Third generation Antisense oligonucleotides

- Peptide nucleic acids (PNAs)

✓ high-affinity nucleic acid binding can be explained by the lack of electrostatic repulsion because of the absence of negative charges on the PNA



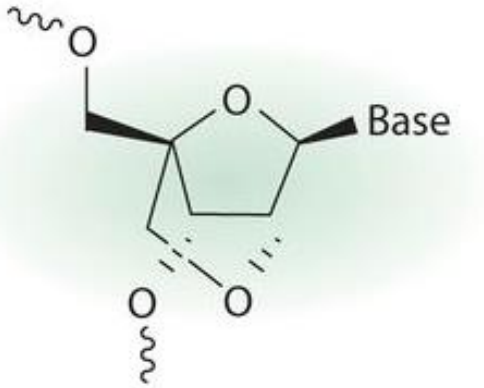
PNA
(Peptide nucleic acid)

CHEMICAL MODIFICATIONS OF CURRENT IMPORTANCE BY STRUCTURAL CLASS

Third generation Antisense oligonucleotides

- Locked nucleic acid (LNA)

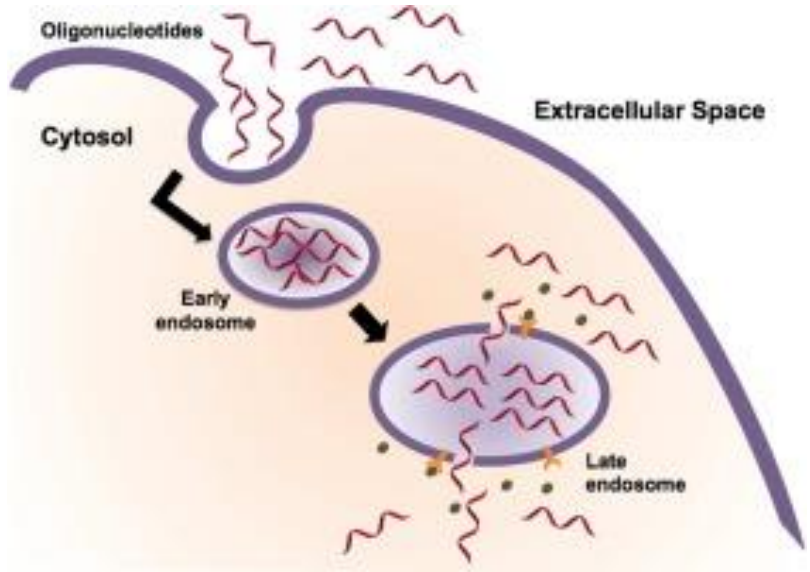
  ✓ locking the ribose ring in the ideal conformation binding.

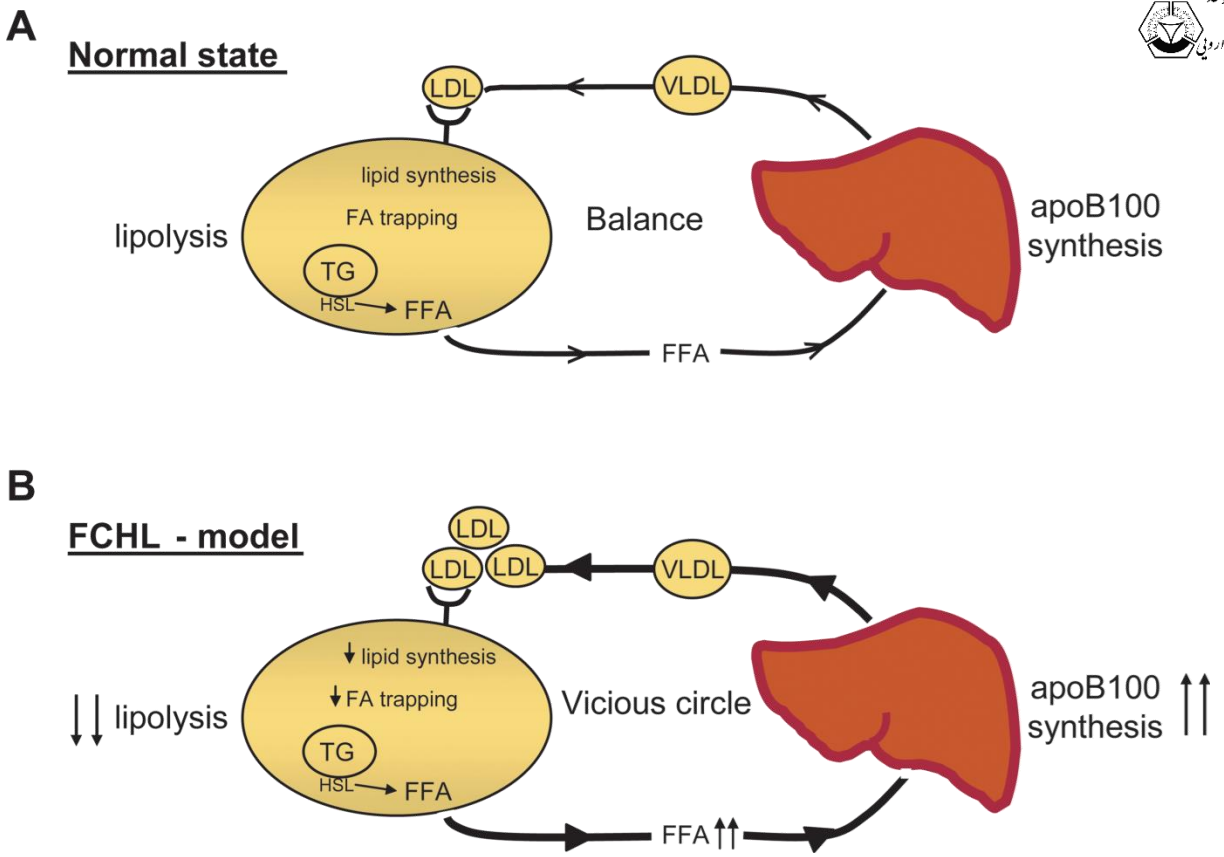


LNA
(locked nucleic acid)

NOTE

- Single-stranded oligonucleotides can be taken up by cell surface receptors and are more readily taken up by cells than are (naked) double-stranded oligonucleotides
- ✓ Single-stranded oligonucleotide are flexible, while duplexes are rigid.
- ✓ Single-stranded oligonucleotides are amphiphilic while duplexes have a surface dominated by heavily hydrated phosphates



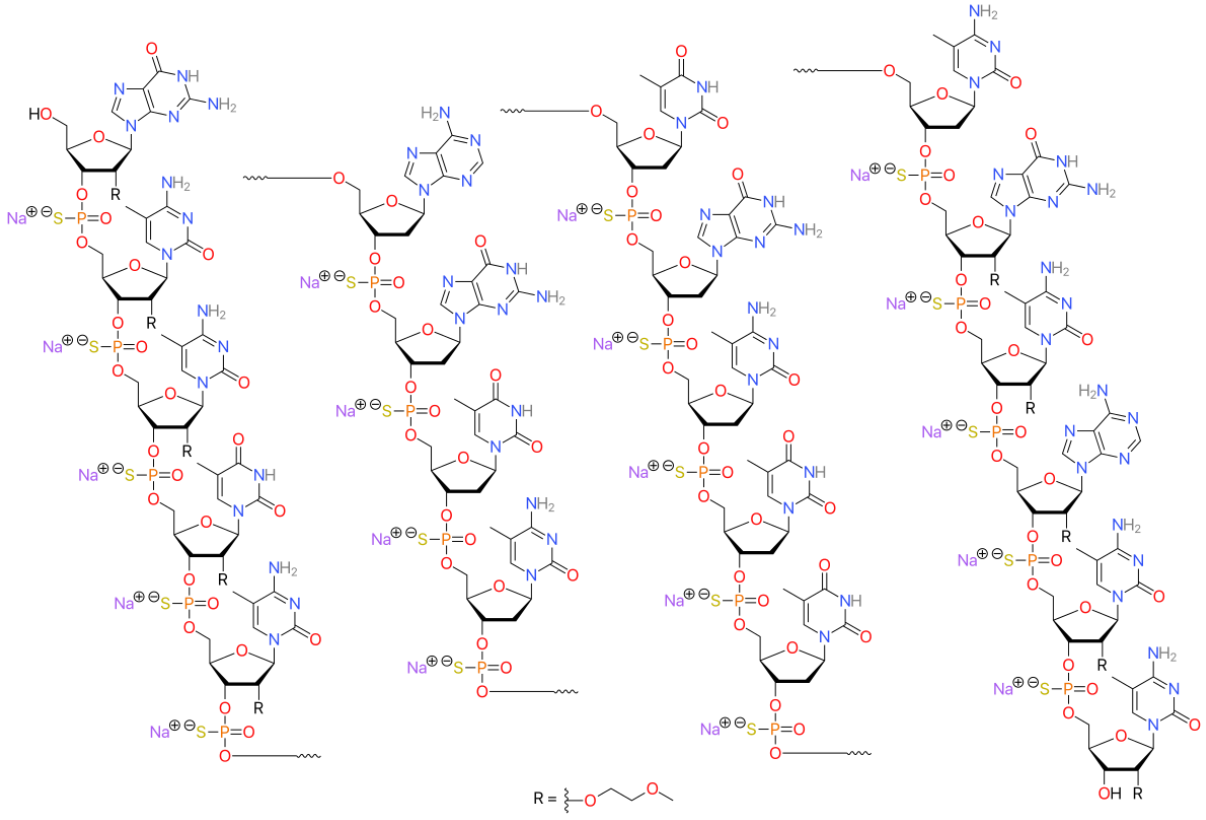


GENETIC DISEASES

Familial Hypercholesterolemia

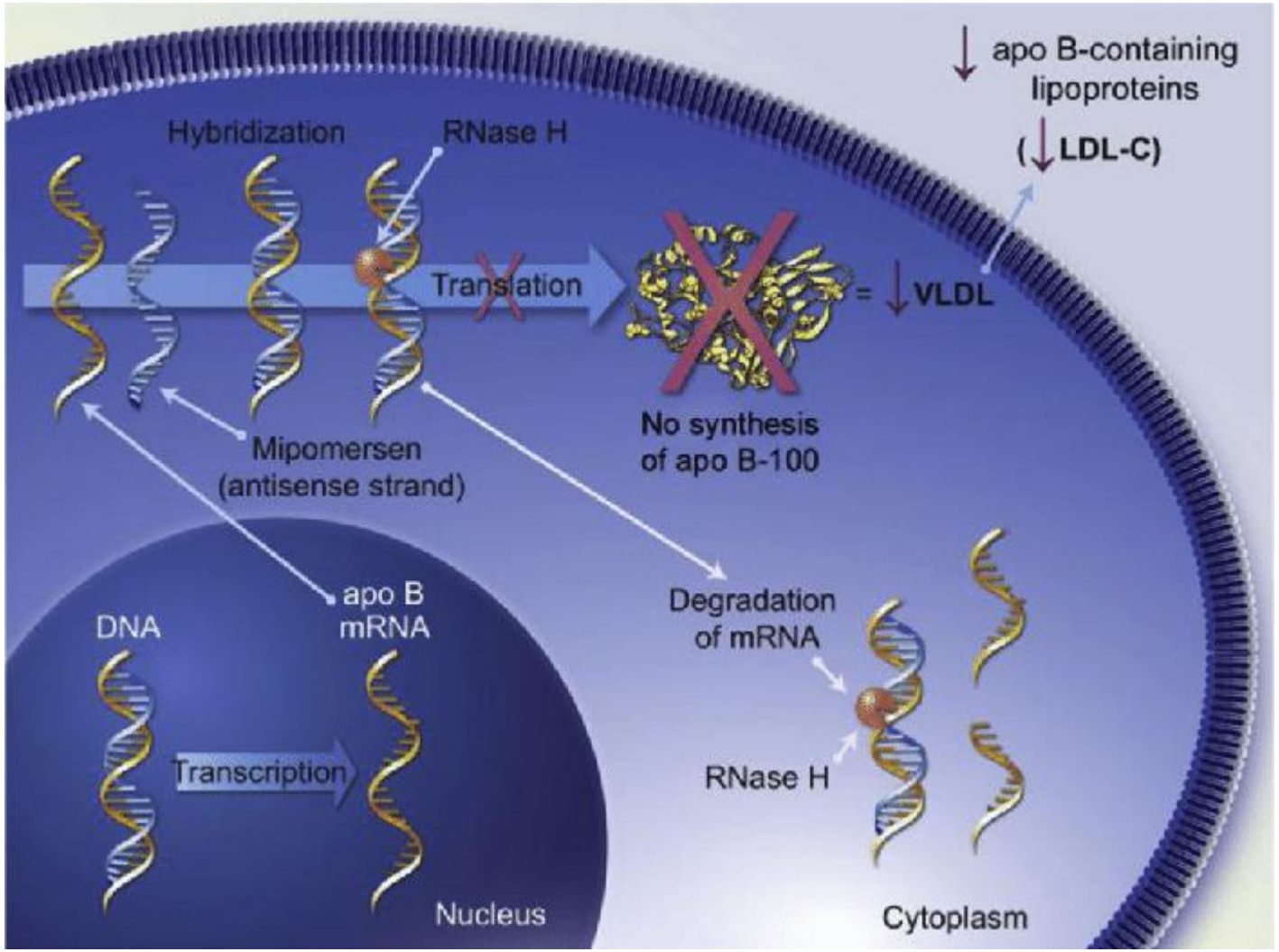
- increased levels of plasma lipoproteins containing apoB100
- Accelerated atherosclerosis and cardiovascular disease (CVD)


NDC 58468-0191-1
KYNAMRO™
(mipomersen sodium) injection
200 mg/mL
Pre-filled syringe contains 200 mg of mipomersen sodium in 1 mL of solution
For subcutaneous injection only
℞ only
1 Single-use syringe containing 1 mL
Discard unused portion



Mipomersen

CLEAVAGE BY RNASE H



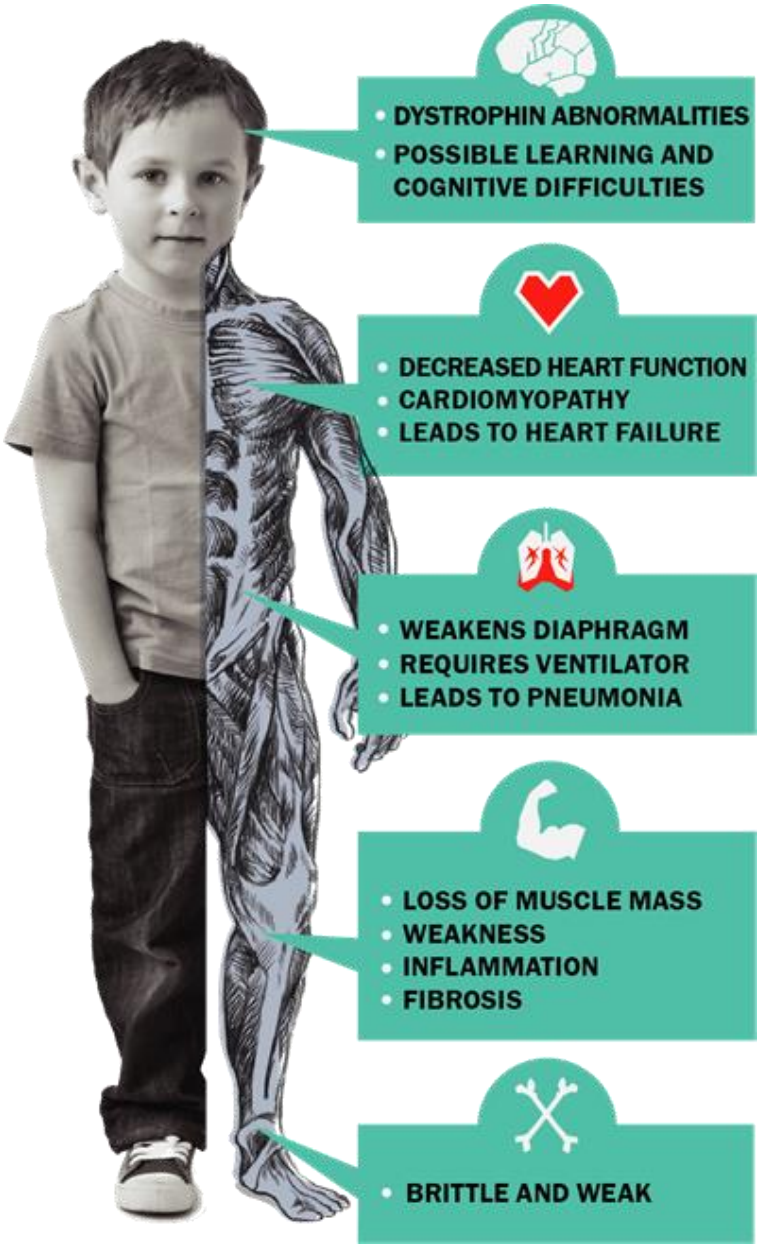
MIPOMERSEN MECHANISM

- APOB100 knockdown by exon skipping

GENETIC DISEASES

Duchenne Muscular Dystrophy (DMD)

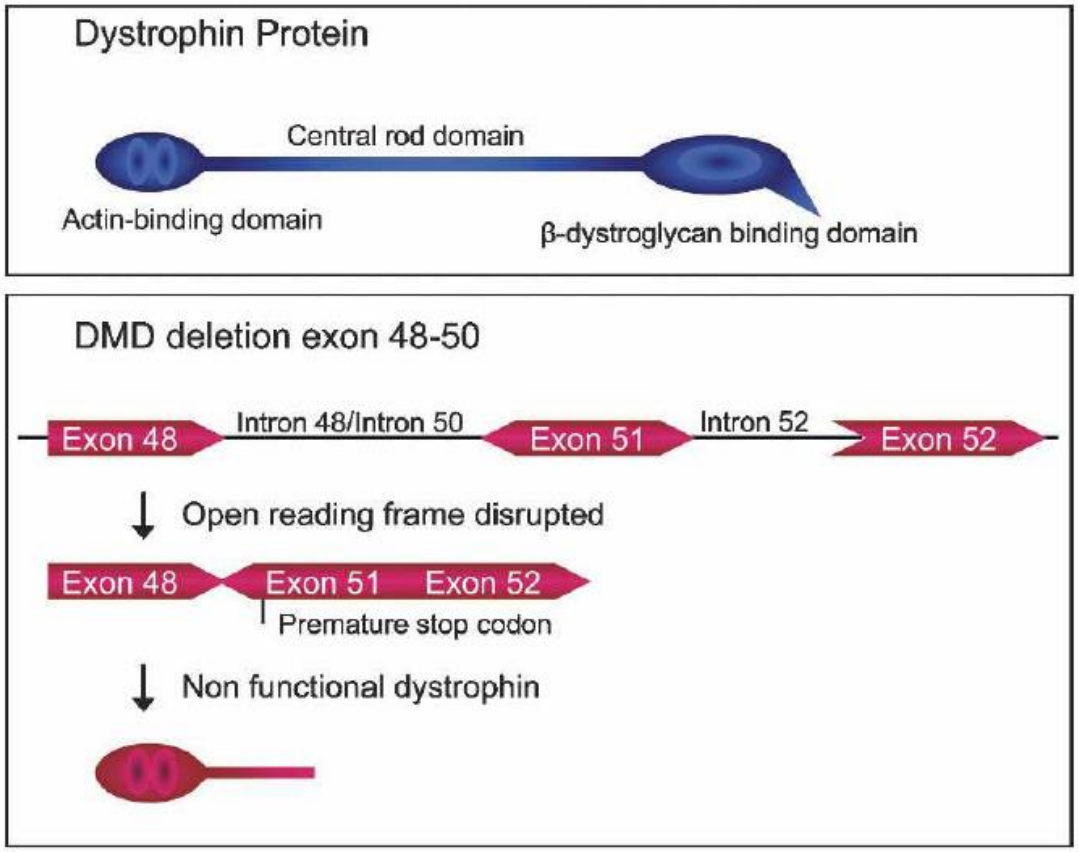
- deletions mutation in dystrophin gene disrupt the reading frame and produce a dysfunctional protein, resulting in musculardystrophy.

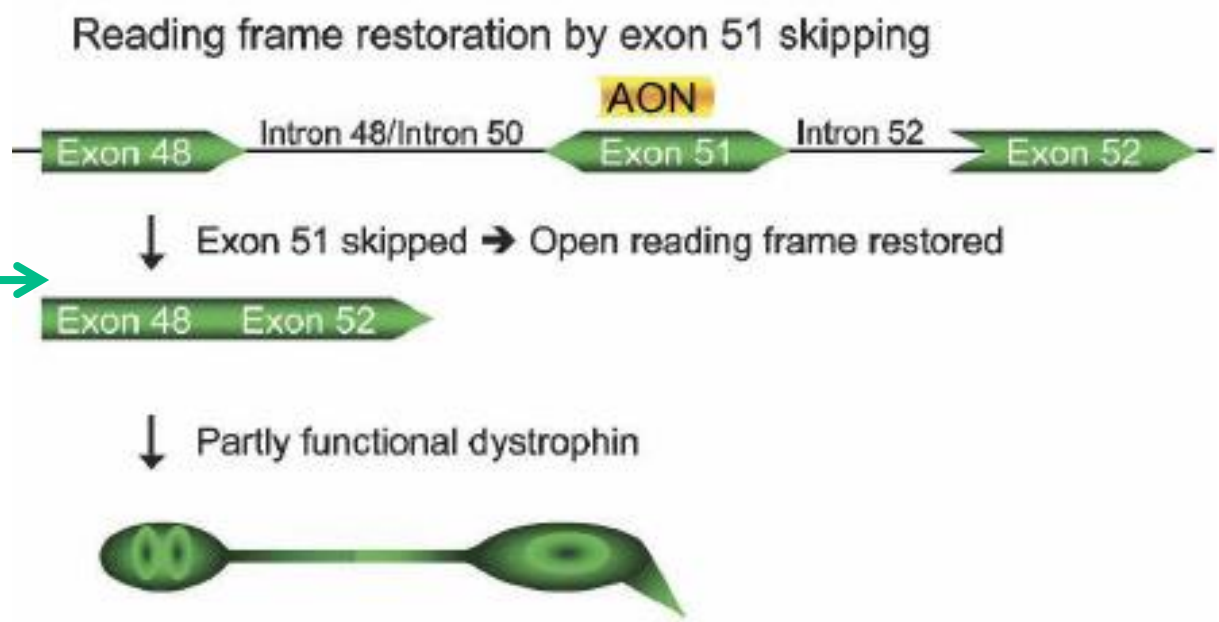


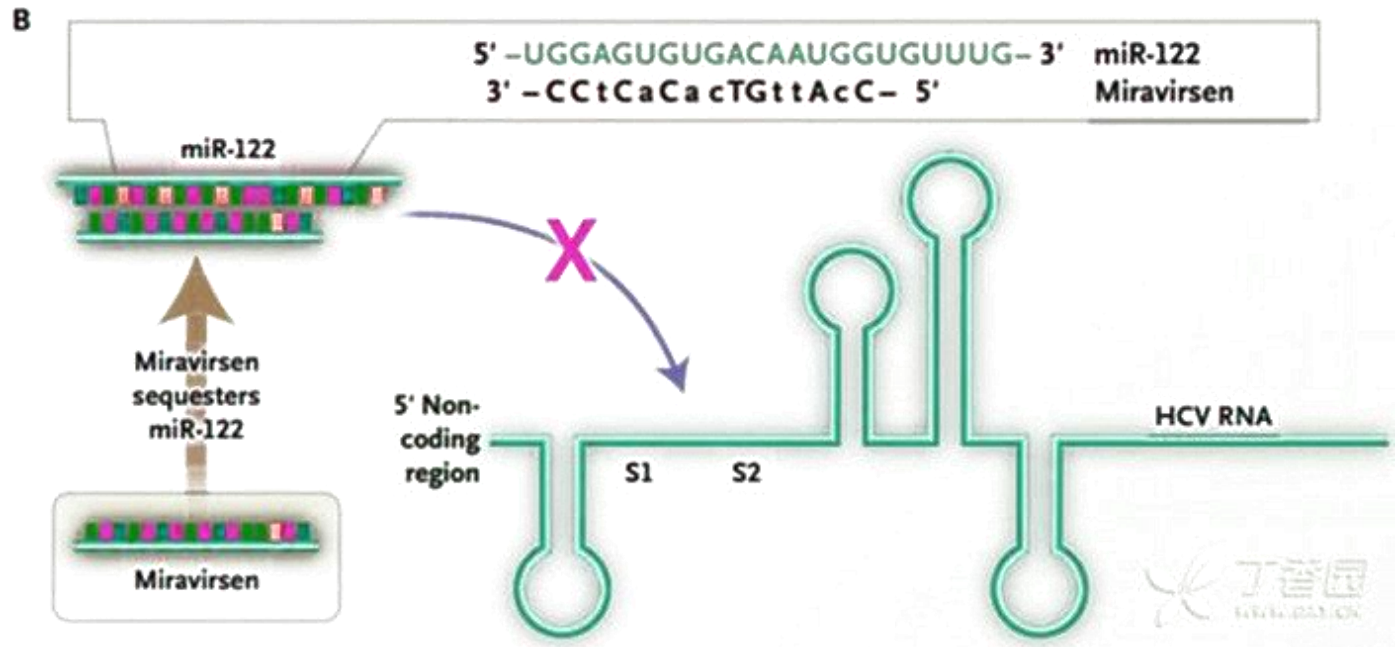
EXON EXCLUSION

Duchenne Muscular Dystrophy

- **Drisapersen** is 2'-*O*-methyl-phosphorothioate
- ASO for Duchenne treatment







Miravirsen, miR-122, and Hepatitis C Virus (HCV)

Miravirsen is a 15-mer LNA-modified ASO

bind to the miR-122 and inhibit its function

miR-122 :Preventing nucleolytic degradation of the HCV genome

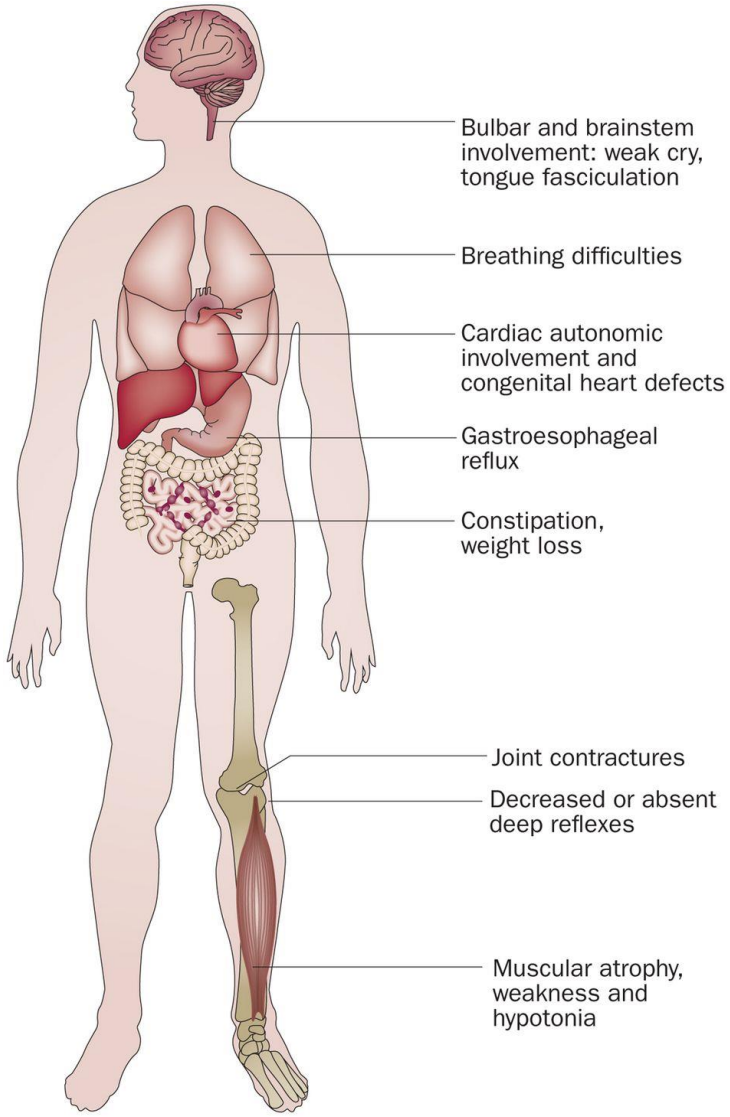
Spinal Muscular Atrophy (SMA)

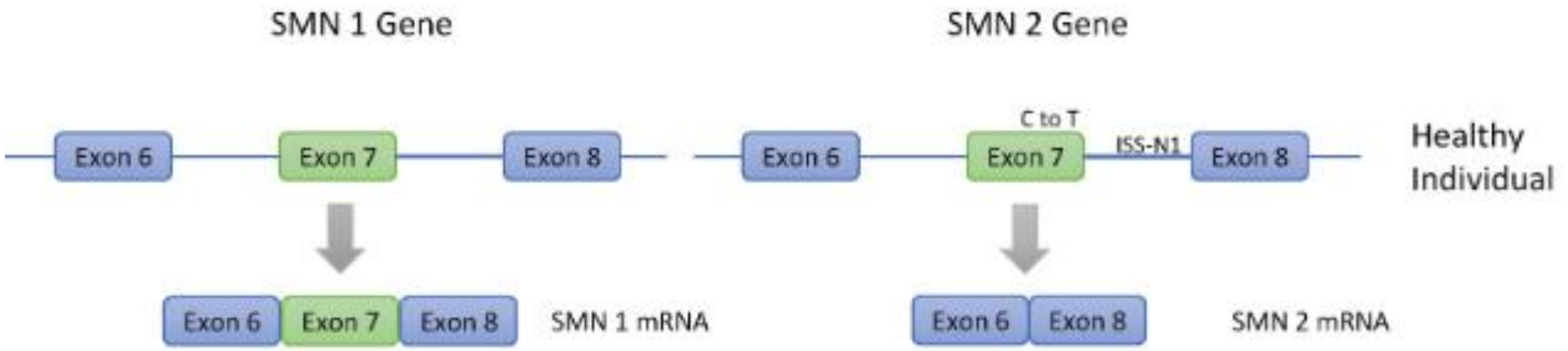
- symptoms of SMA are muscle atrophy and weakness

resulting from motor neuron degeneration in the spinal cord and brain stem

- Paralysis, brain stem defects, and respiratory defects are the primary

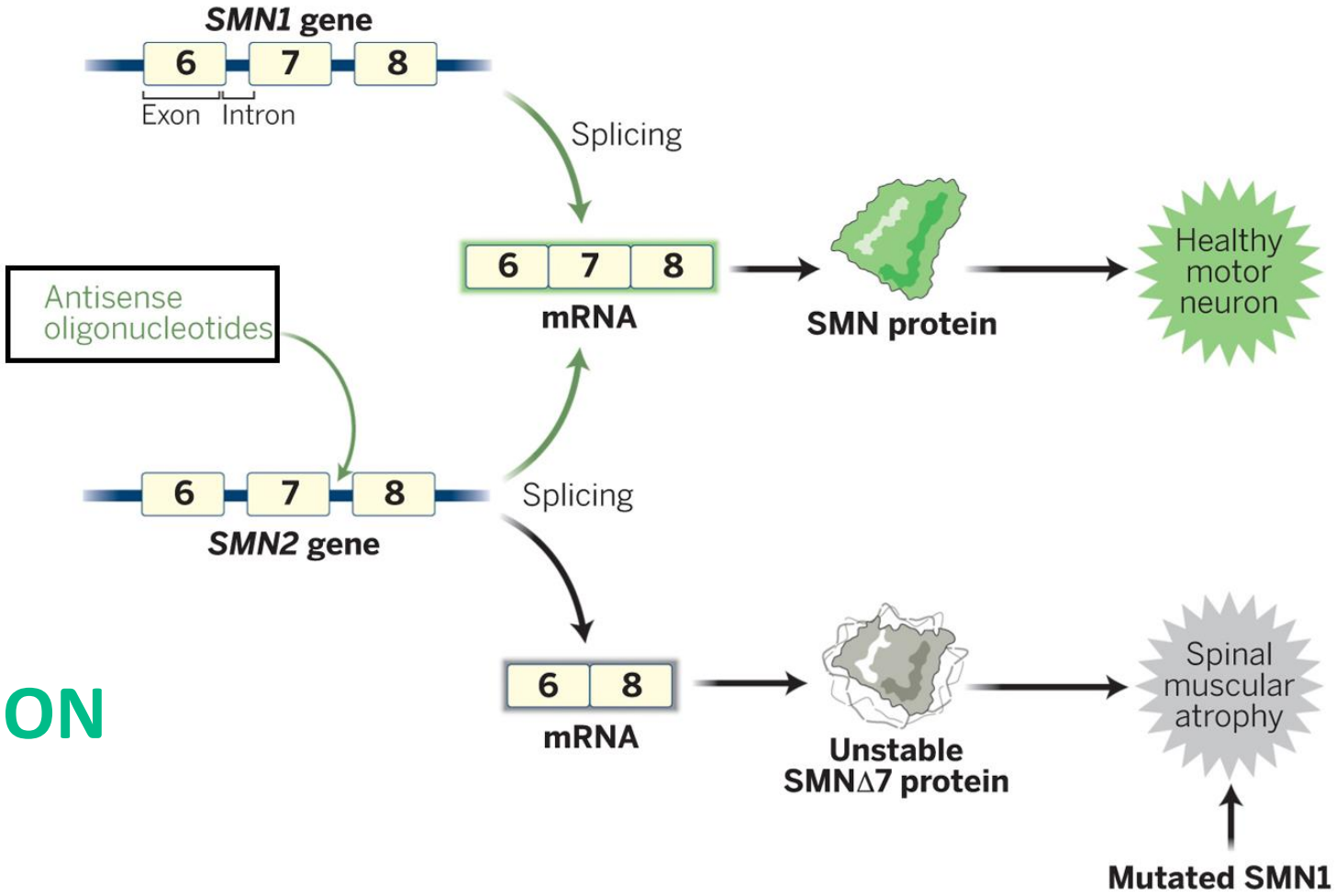
manifestations of this disease and ultimately lead to a shortened life span





Spinal Muscular Atrophy (SMA)

- *SMN1* and *SMN2* genes encode SMN protein
- Exon7: difference between SMN1 and 2
- inclusion of exon 7 in the *SMN2* gene product results in a fully functional SMN protein

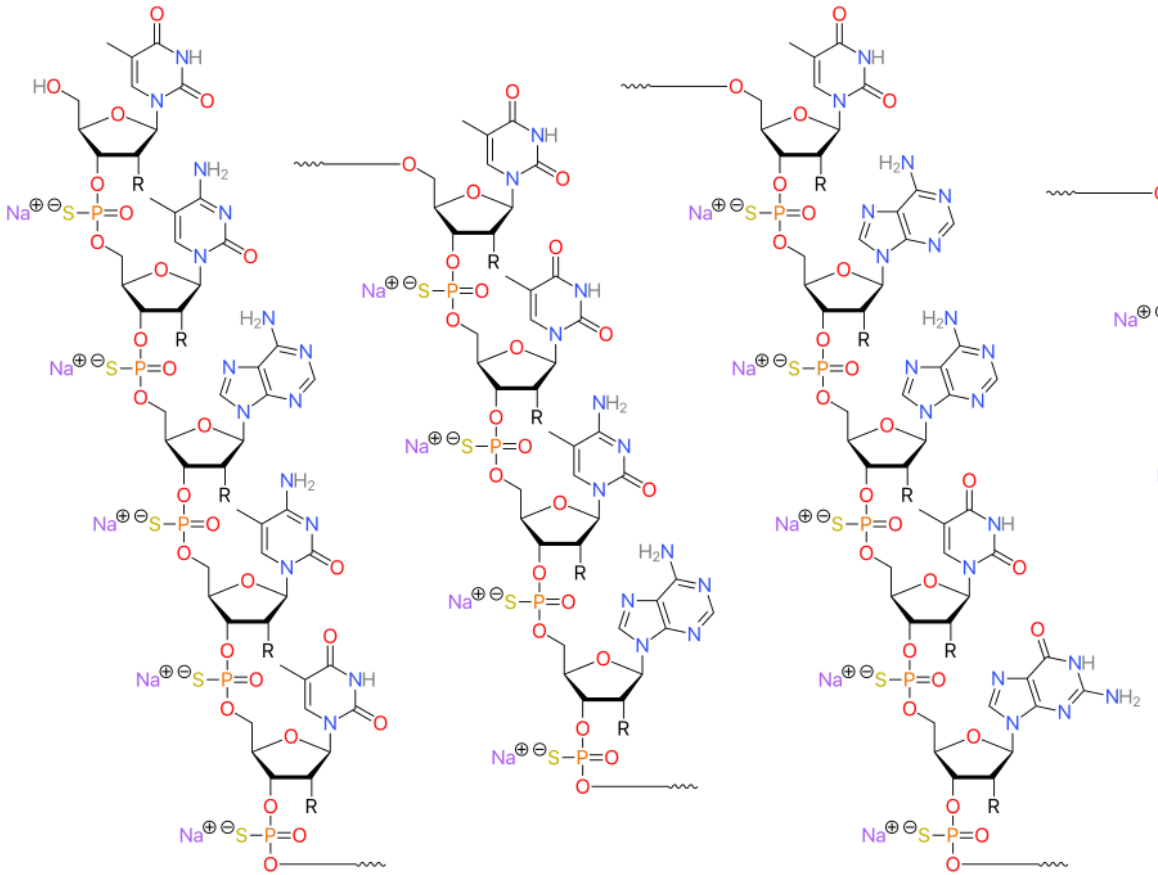


EXON INCLUSION

Nusinersen a 18-mer-modified 2'-*O*-2-methoxyethyl (MOE) phosphorothioate (PS) ASO

- Binds to the specific sequence in the intron downstream of exon 7 on the *SMN2* pre-mRNA
- blocking the exon skipping of exon 7 leading to the production of full-length SMN protein

Nusinersen




Spinraza
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Injection
12 mg/5 mL
(2.4 mg/mL)
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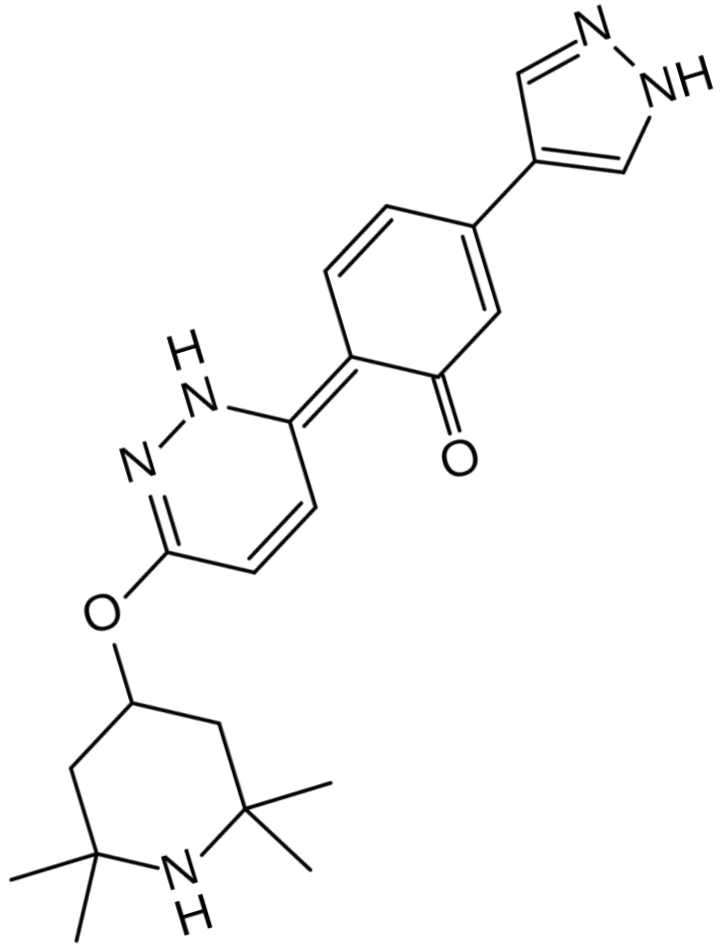
Spinraza™
(nusinersen)
Injection
12 mg /5 mL
(2.4 mg/mL)
Sterile solution for
Intrathecal Use Only
Rx Only
1 Single Dose Vial
Discard Unused Portion

SMALL MOLECULE; MODIFYING SPLICING PATTERN!!!!

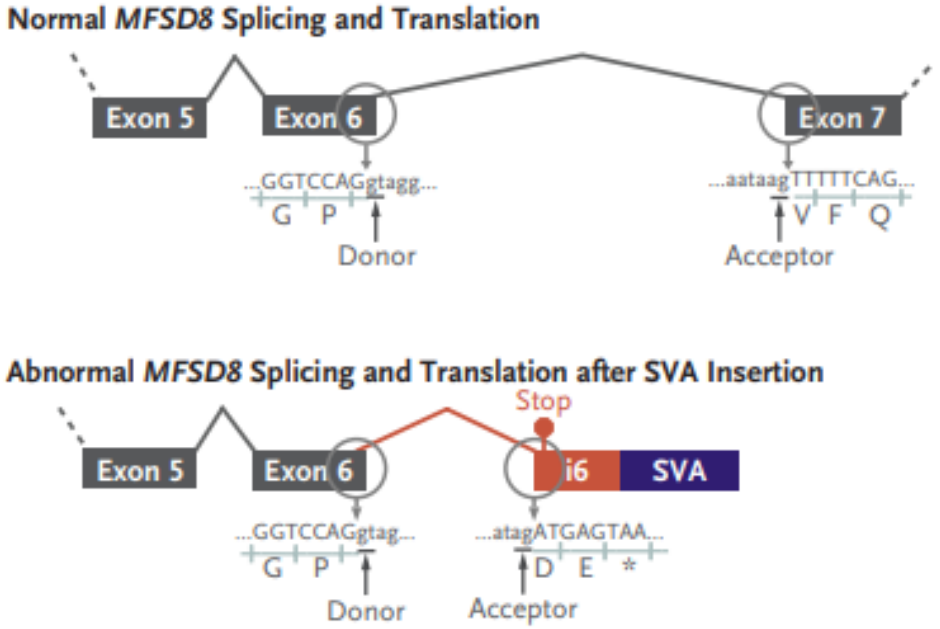
Branaplam

- selective and orally active small molecule experimental drug being developed by Novartis to treat spinal muscular atrophy (SMA).
- pyridazine derivative
- increasing the amount of functional survival of motor neuron protein produced by the *SMN2* gene through modifying its splicing pattern
- As of July 2019, branaplam is in a phase-II clinical trial in children with SMA type 1



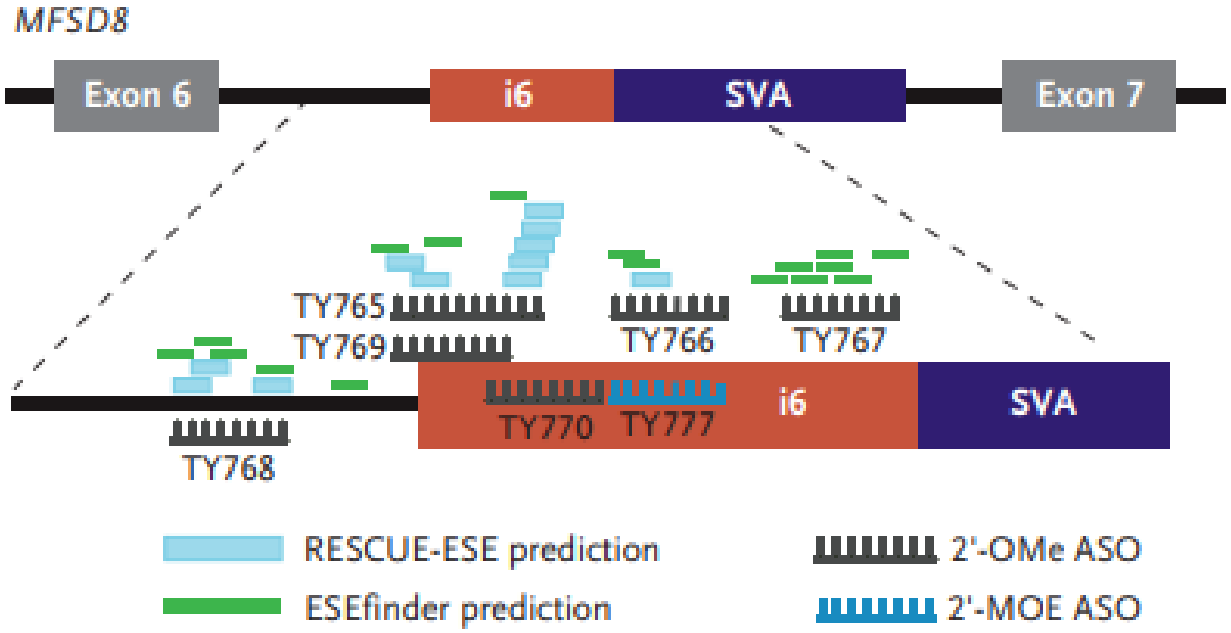
ANTISENSE THERAPY TOWARDS INDIVIDUALIZED MEDICINE

- A 6-year-old girl presented with the insidious onset of blindness, ataxia, seizures, and developmental regression.
- Batten’s disease
- Genetic panel testing for known Batten’s disease genes revealed a single known pathogenic mutation in the gene MFSD8 (also known as CLN7).



- Milasen is a 22-nucleotide antisense oligonucleotide with the same backbone and sugar chemistry modifications (phosphorothioate and 2’-O-methoxyethyl) as nusinersen.
- Milasen itself remains an investigational drug, and it is not suited to the treatment of other patients with Batten’s disease because its design is customized to our patient’s specific mutation.

antisense oligonucleotides to target the i6.SA cryptic splice-acceptor site and nearby splicing enhancers



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