Therapeutic Modalities: Antisense Oligonucleotides

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Rare Bootcamp
Ultragenyx Pharmaceutical
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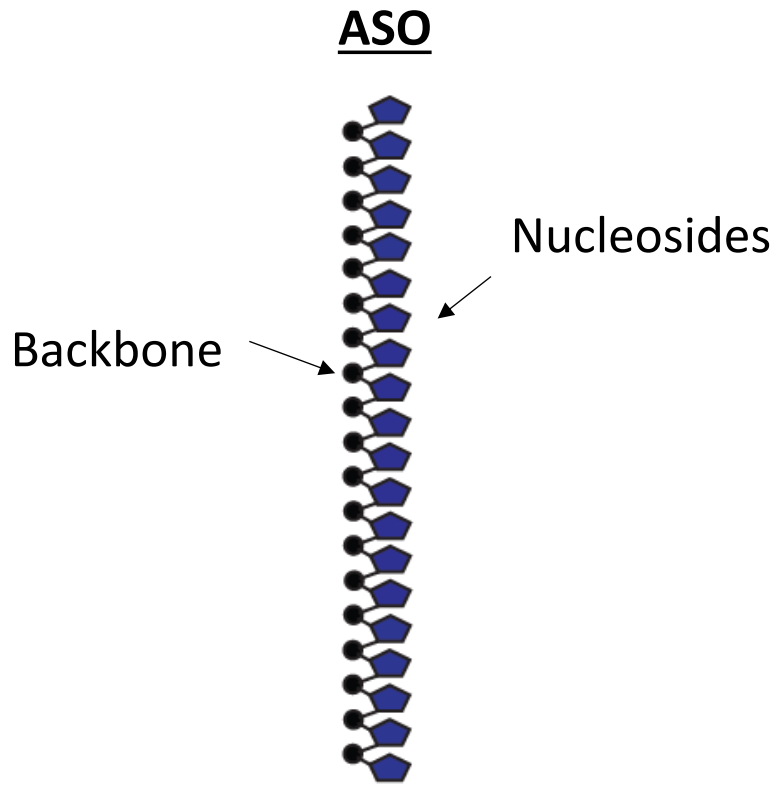
DINDOT LABORATORY

Outline

- Overview of Antisense Oligonucleotides (ASOs)
- ASO Design and Mechanisms of Action
- ASO Pharmacokinetics: The Basics
- ASO Nuances and Challenges
- ASOs vs siRNAs
- Conclusions and Future

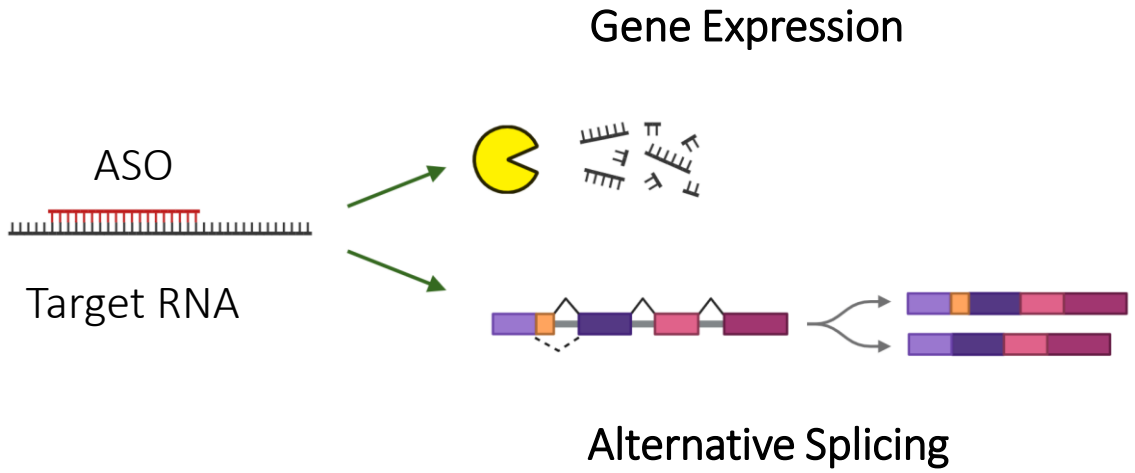
ASO Overview

- Single-stranded oligonucleotide
  - Comprised of ribonucleosides and/or deoxyribonucleosides
  - 14-22 nucleotides long
- Chemically modified to protect the molecule from nucleases and enhance its pharmacological properties.
  - Synthesized on machine
  - FDA considers an ASO a drug (not biologic)



ASO Overview

- Binds to a target RNA via Watson-Crick base pairing
  - Highly specific
- Function
  - Downregulates or upregulates the expression of a target gene
  - Alters the splicing of a target gene to generate different RNA or protein isoforms



ASOs have been around for decades

1978 - ASO approach proposed

1989 - ASO Medicinal Chemistry

1990 – Optimal ASO length identified

1996 – 2’MOE Chemistry

1998 – LNA chemistry

1998 – Formiversen Approved

2001 – IT, ID, and aerosol dosing

2011 – SMA Clinical Trials

2013 – Mipomersen Approved

2016 – Nusinersen Approved

2016 – Eteplirsen Approved

2018 – Inotersen Approved

2019 – Valenosorsen Approved

2019 – FDA allows N-of-1 (Milasen)

2019 – Golodirsen Approved

2020 – Angelman Syndrome Clinical Trials (GeneTx/Ultragenyx, Roche, Ionis)

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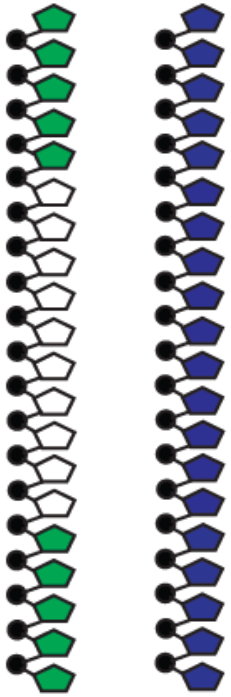
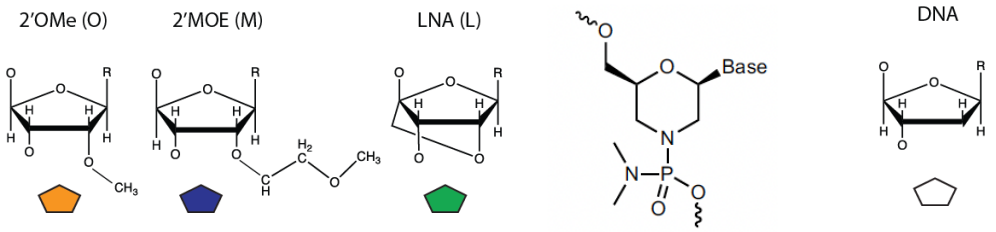
ASOs are chemically modified versions of RNA/DNA

ASOs



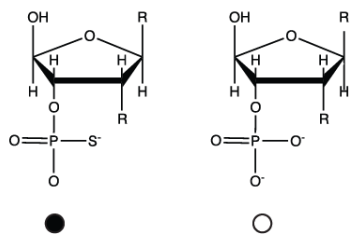
Nucleosides

Ribonucleosides | Morpholino | Deoxyribonucleoside



Backbone linkages

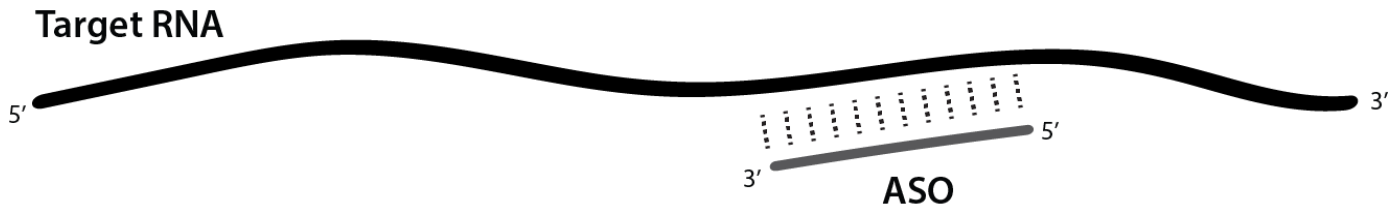
Phosphorothioate (PS) | Phosphodiester (PO)



Chemical Modifications

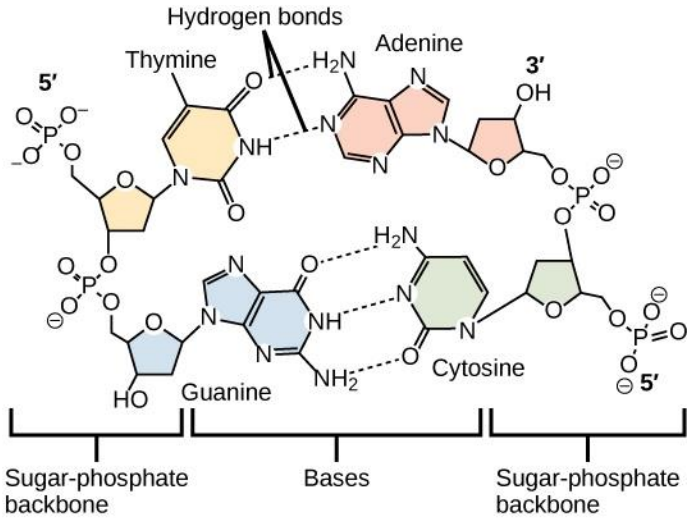
- Determine the mechanism of action
- Increase stability
- Enhance pharmacological properties

ASOs are specific to a target RNA via Watson-Crick base pairing



Bioinformatic analyses, likely replaced by artificial intelligence

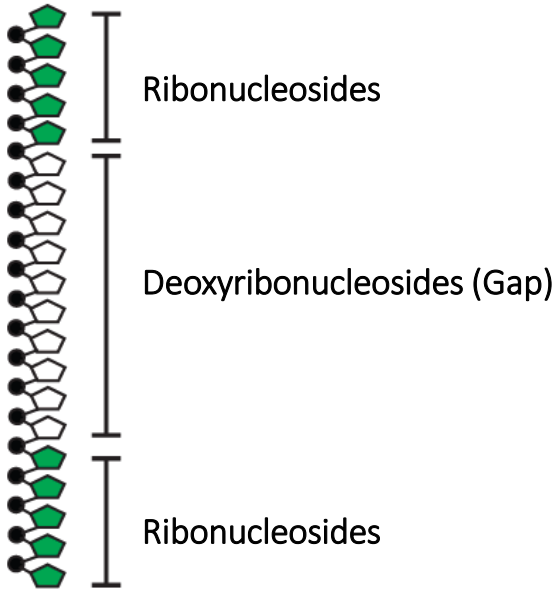
Watson-Crick Base Pairing



Adenine : Thymine/Uracil
Guanine : Cytosine

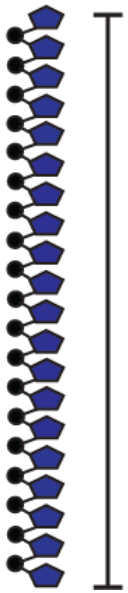
The chemical structure of an ASO determines its mechanism of action

Gapmer ASO



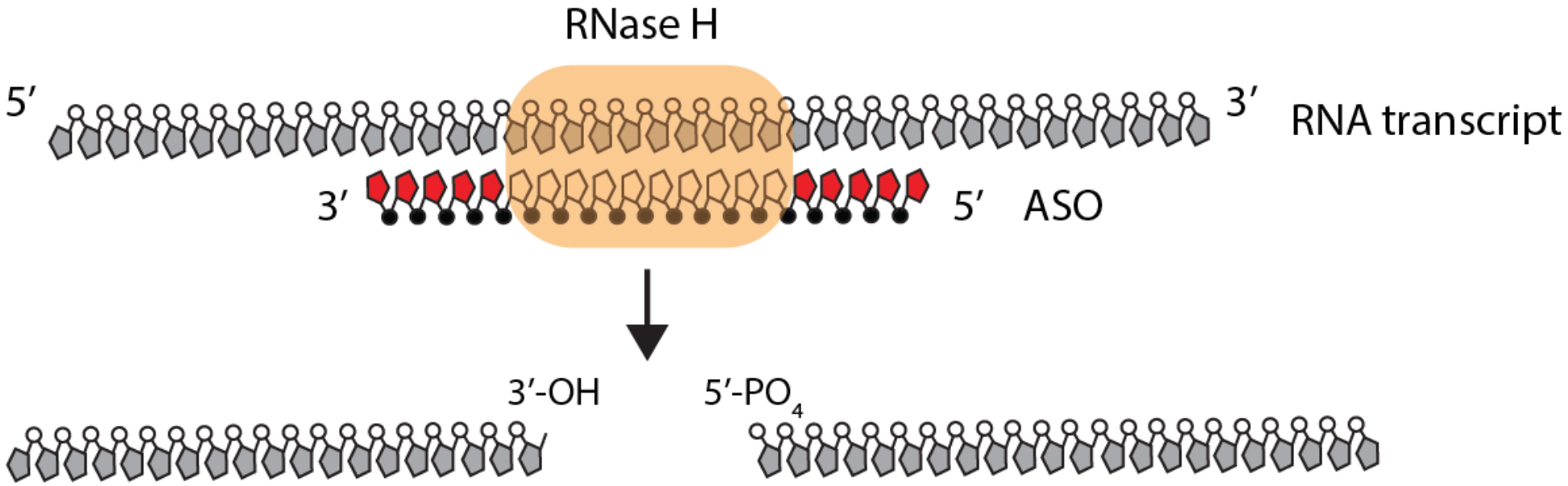
Induces degradation of target RNA

Steric Hindrance ASO

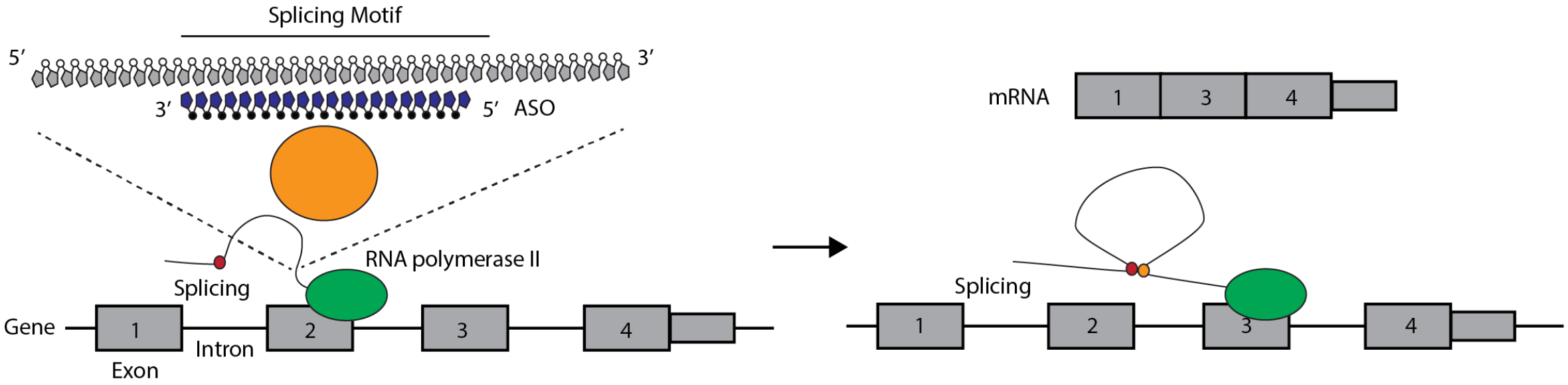


Blocks the binding of proteins or RNAs

Gapmer ASOs induce the degradation of a target RNA

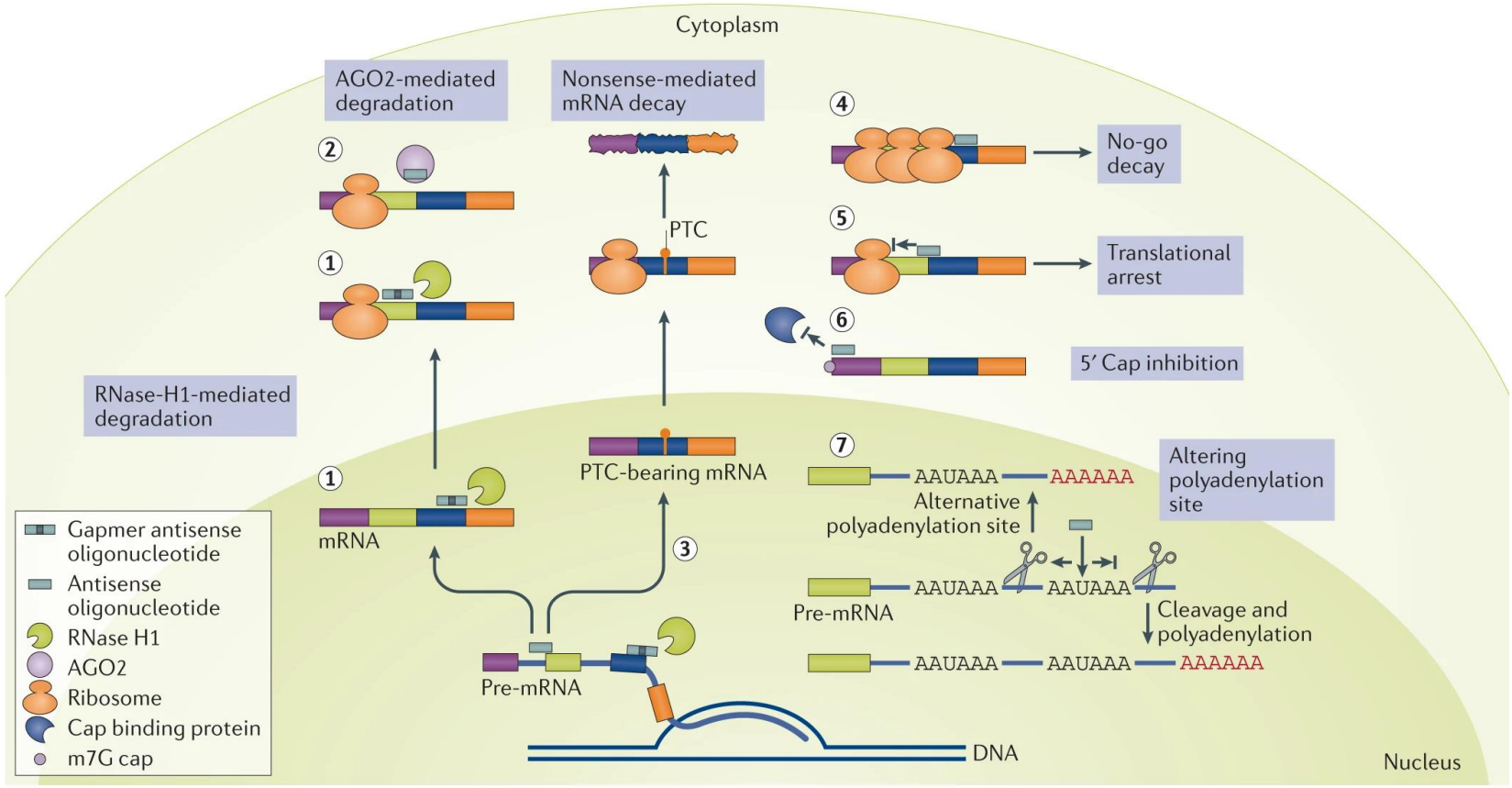


Steric Hindrance ASOs inhibit RNA-Binding Proteins and RNAs



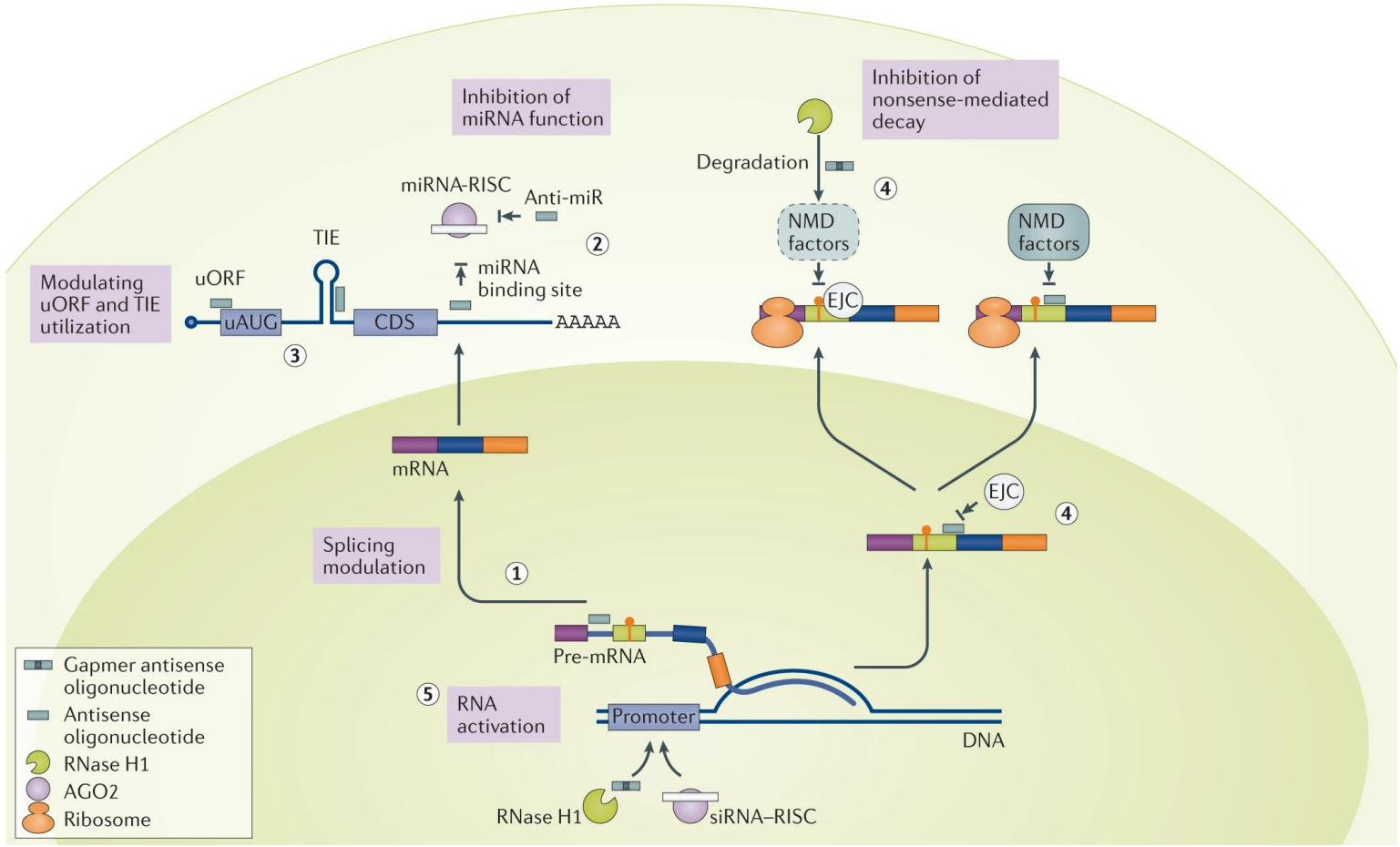
The mechanism of action of an ASO can vary in many ways

ASO-Mediated Repression of Gene Expression



Crooke ST, Baker BF, Crooke RM, Liang XH. Antisense technology: an overview and prospectus. Nat Rev Drug Discov. 2021 Jun;20(6):427-453. doi: 10.1038/s41573-021-00162-z. Epub 2021 Mar 24. PMID: 33762737.

ASO-Mediated Upregulation of Gene Expression



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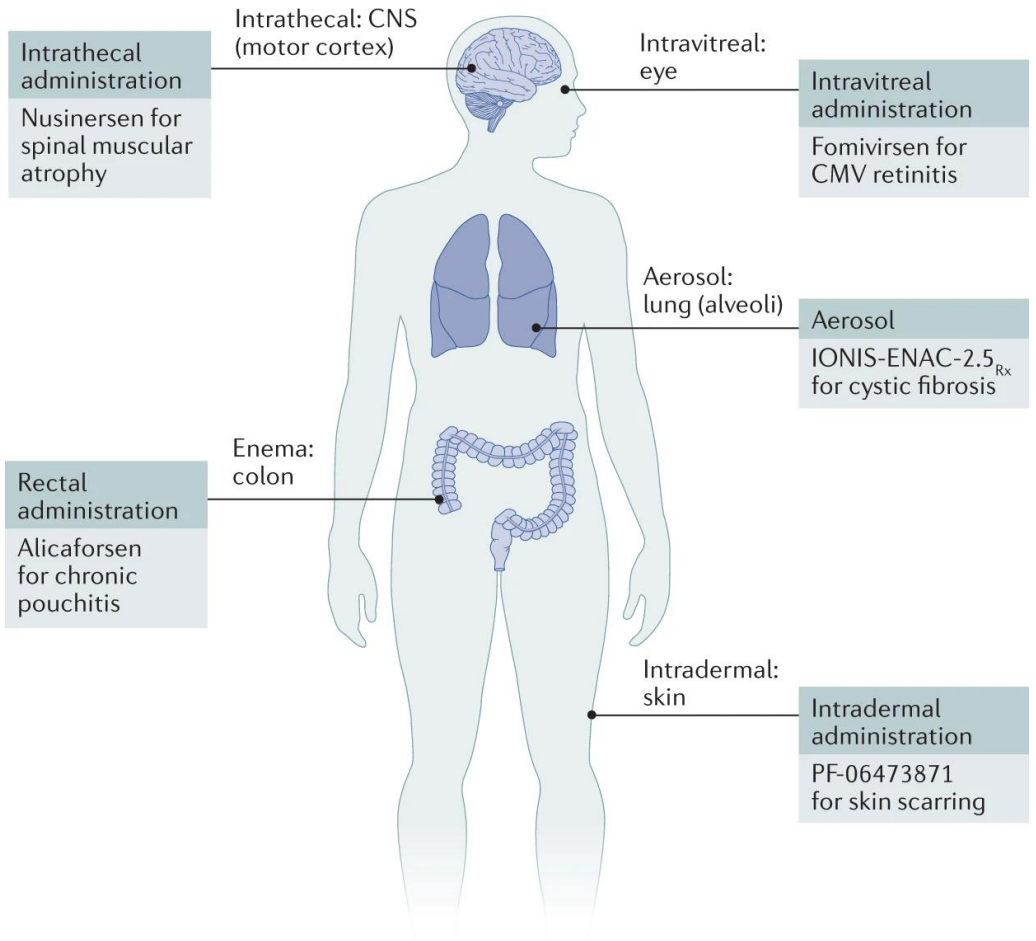
ASO Pharmacokinetics: The Basics

- ASOs are water soluble and do not require a lipofection agent to enter the cell.
  - gymnosis = naked delivery
- ASOs are taken up by all cell types via the endocytic pathway.
- IV administered ASOs distribute throughout the body, following the flow of blood.
  - ASOs do not cross the blood-brain barrier.
- CNS-targeted ASOs are delivered directly to the cerebral spinal fluid.
- ASOs can be conjugated for targeted organ delivery (GalNac [liver], Transferrin receptor - brain).
- ASOs have a relatively long half-life (weeks – months [chemistry dependent]).

ASOs can be administered by different routes



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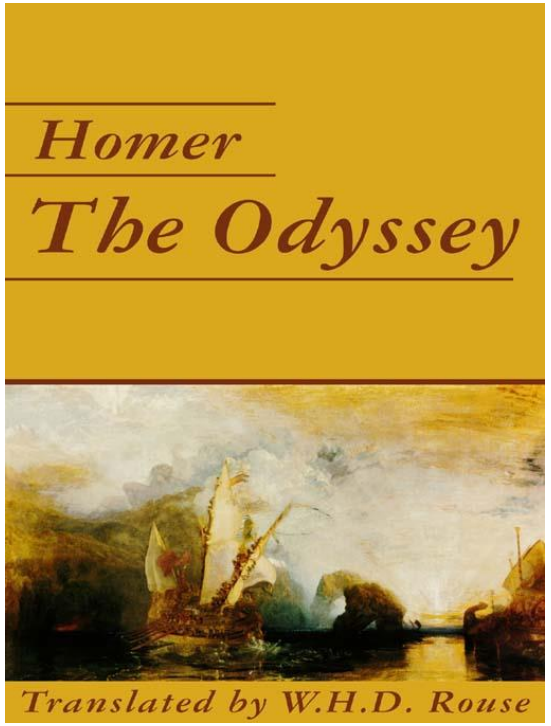
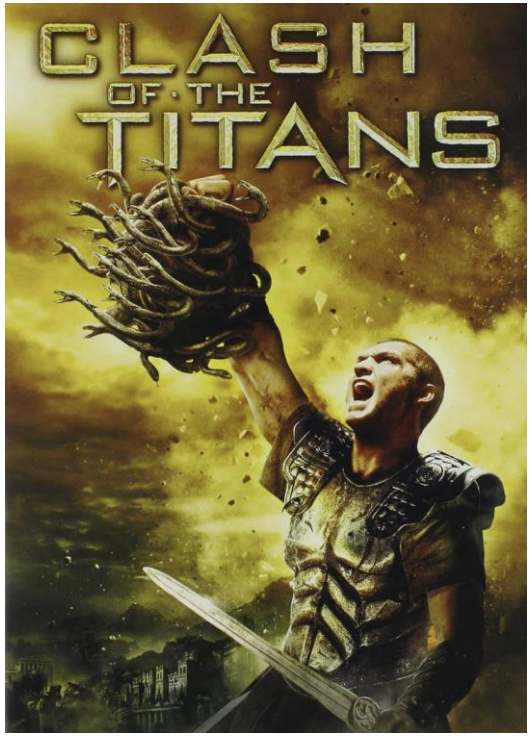
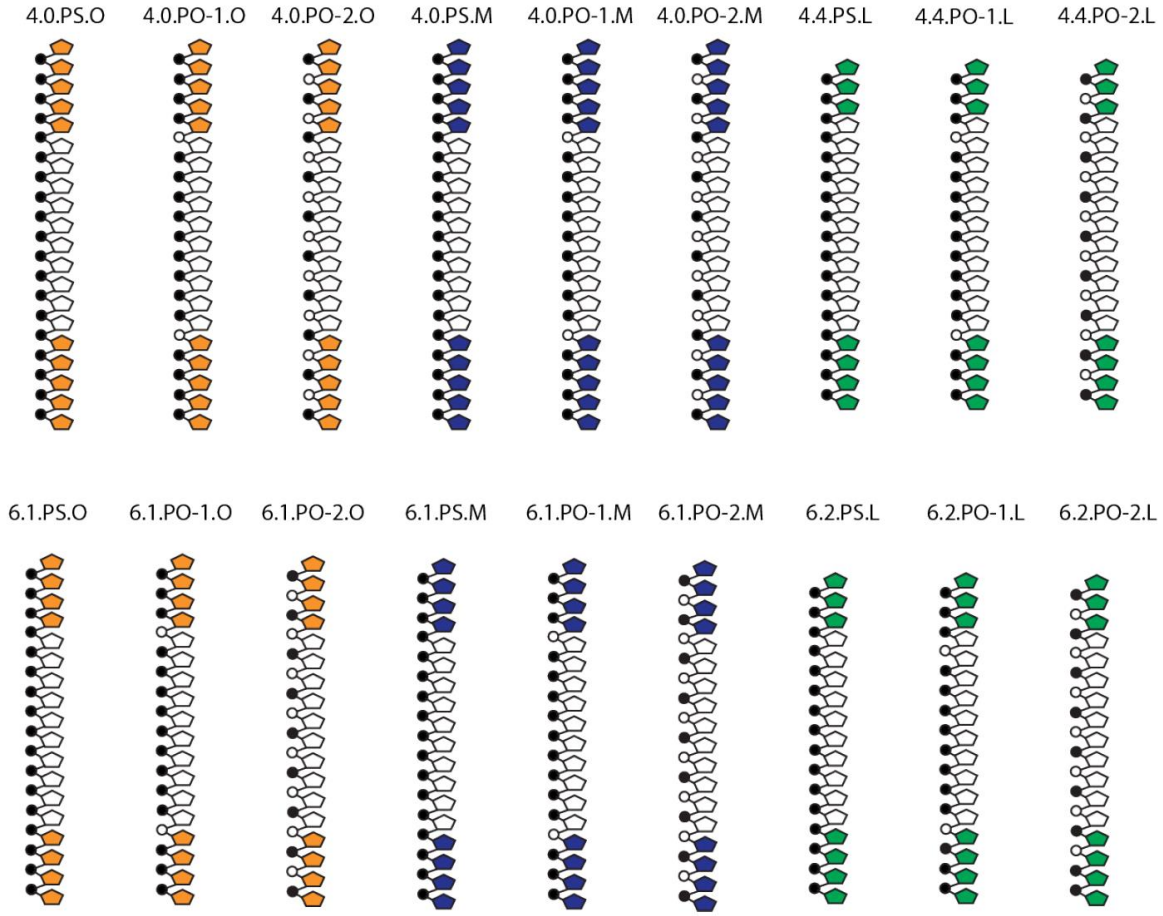
Developing an ASO is a journey

The pharmacological properties of an ASO are dependent on many factors and largely unknown.

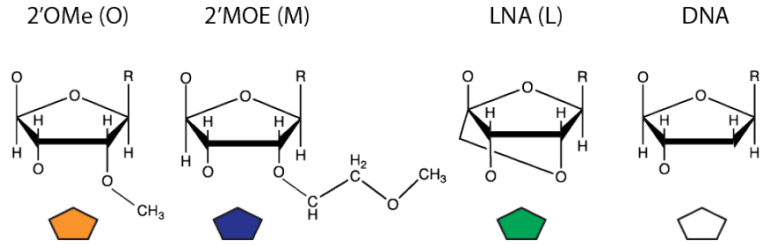




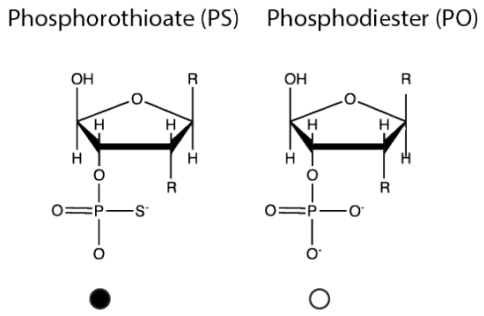
Designing ASOs is complicated by an exponential number of sequence and chemistry combinations



Nucleosides

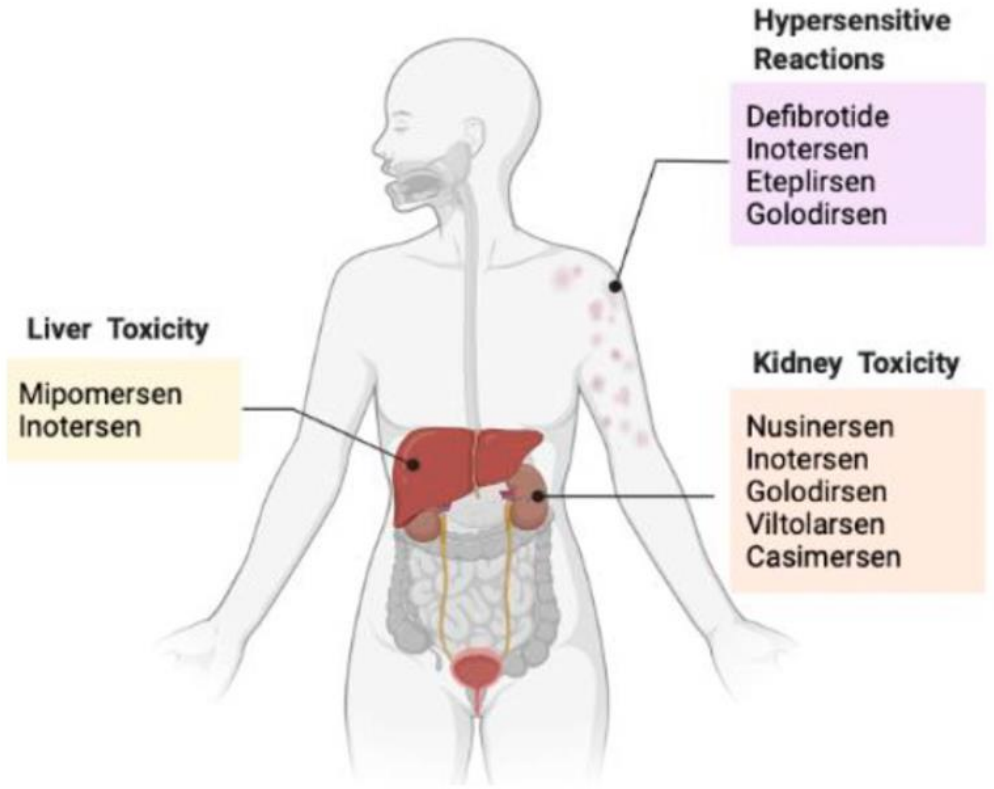


Backbone Linkages



Dindot SV, et al. An ASO therapy for Angelman syndrome that targets an evolutionarily conserved region at the start of the *UBE3A-AS* transcript. Sci Transl Med. 2023 Mar 22;15(688):eabf4077. doi: 10.1126/scitranslmed.abf4077. Epub 2023 Mar 22. PMID: 36947593.

ASOs can be toxic



Alhamadani F, et al. Adverse Drug Reactions and Toxicity of the Food and Drug Administration-Approved Antisense Oligonucleotide Drugs. Drug Metab Dispos. 2022 Jun;50(6):879-887. doi: 10.1124/dmd.121.000418.

Minor changes to ASOs can have massive effects

In Vivo Evaluation of Candidate Allele-specific Mutant Huntingtin Gene Silencing Antisense Oligonucleotides

Amber L Southwell[1], Niels H Skotte[1], Holly B Kordasiewicz[2], Michael E Østergaard[2], Andrew T Watt[2], Jeffrey B Carroll[3], Crystal N Doty[1], Erika B Villanueva[1], Eugenia Petoukhov[1], Kuljeet Vaid[1], Yuanyun Xie[1], Susan M Freier[2], Eric E Swayze[2], Punit P Seth[2], Clarence Frank Bennett[2] and Michael R Hayden[1]

Based on all the ASOs tested:

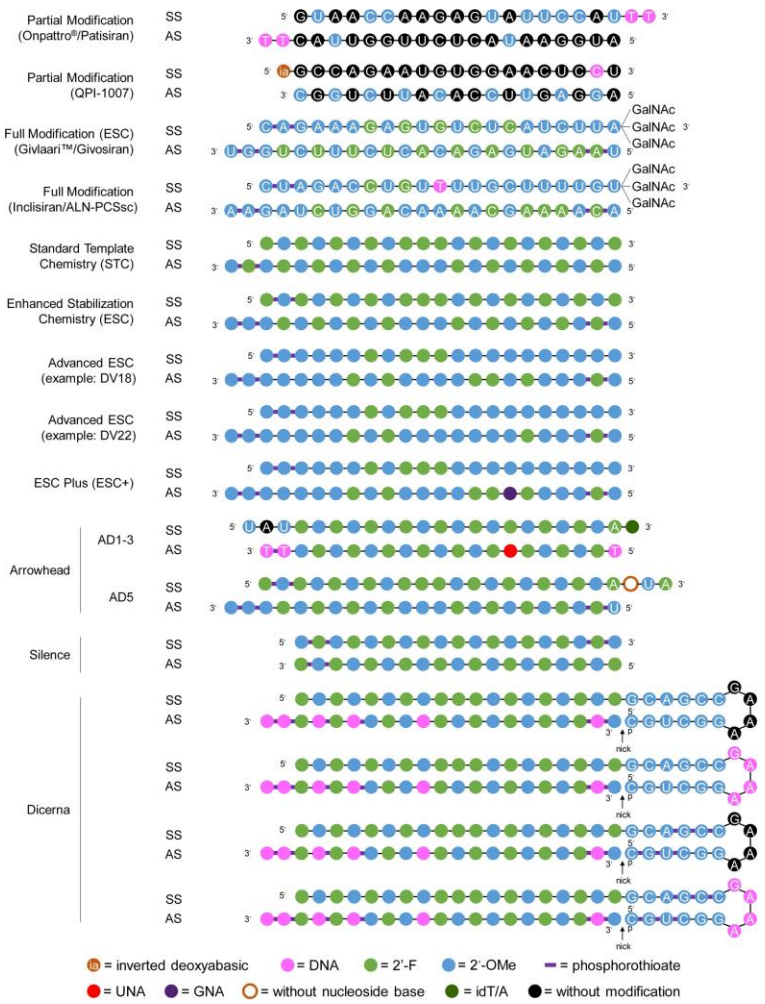
"we were unable to define the governing principles of ASO design…"

"recommend evaluation of multiple molecules to identify optimal ASO candidate drugs."

ASOs vs siRNAs

Small Interfering RNAs (siRNAs)

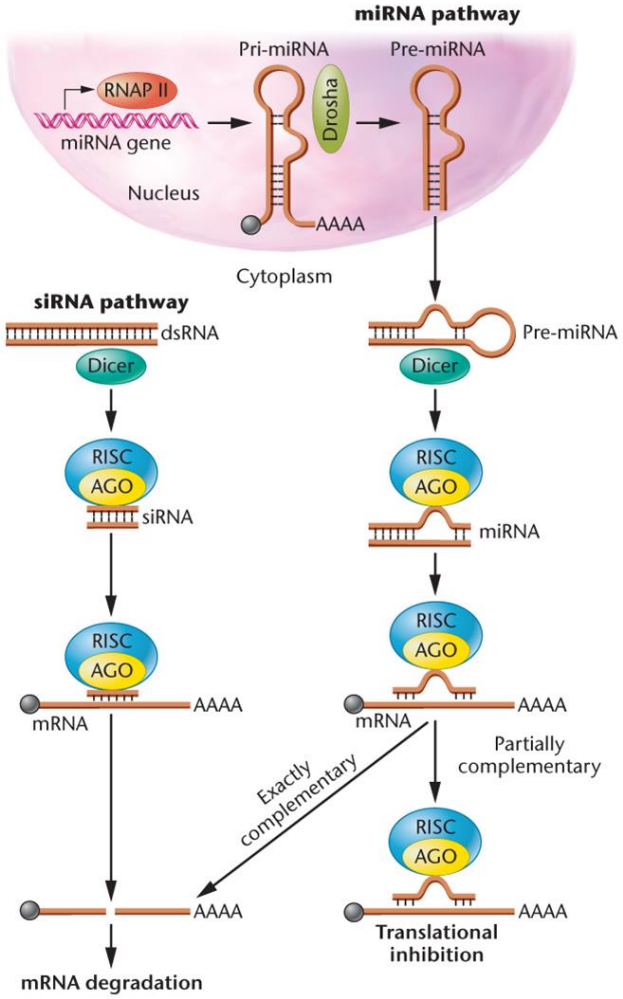
- Double-stranded oligonucleotide, comprised of ribonucleosides
- 20-31 nucleotides long
  - typically 20-22 nucleotides
- Synthetic version of microRNAs
- Function
  - Downregulates gene expression
    - Degradation of mRNA
    - Regulation of translation



siRNA-Meditated Repression of Gene Expression

- Require transfection agent or conjugate (e.g., lipid, GalNac) to enter the cell

- Primarily function in the cytoplasm and not the nucleus



Conclusions

- ASOs and siRNAs are powerful modalities for developing disease-modifying therapies.
  - Every gene and approach is different.

- The ASO research and clinical enterprise is expanding.

- The landscape of ASO therapies is rapidly evolving, with the number of both approved and developing ASO therapies increasing at an exponential rate.

The Future

- The principles governing the pharmacological and toxicological properties of ASOs are unclear.
  - Need for better bioinformatics/algorithms/artificial intelligence to design ASOs

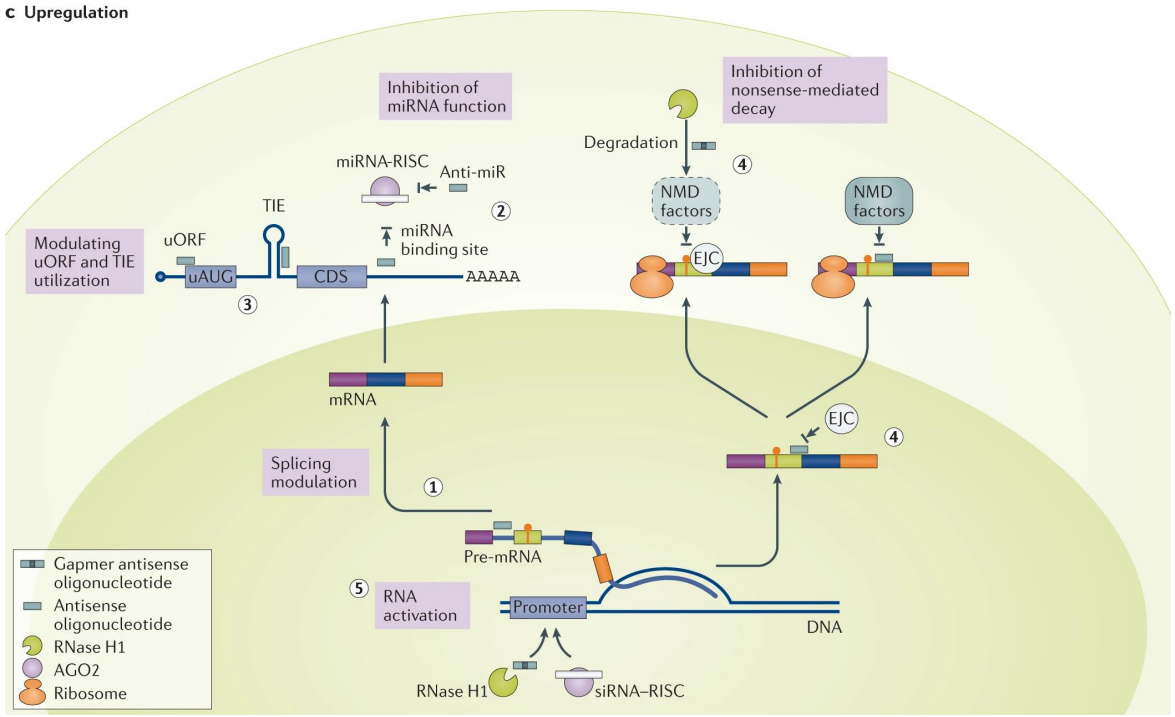
- Delivery is the biggest challenge for ASOs and siRNAs.
  - The field is developing conjugates for targeted delivery of ASOs/siRNAs to organs and cells.

- New nucleic acid therapies will undoubtedly be developed.
  - Longer half-life, more potent, less toxic

Thank You!

Appendix

ASOs: Upregulation



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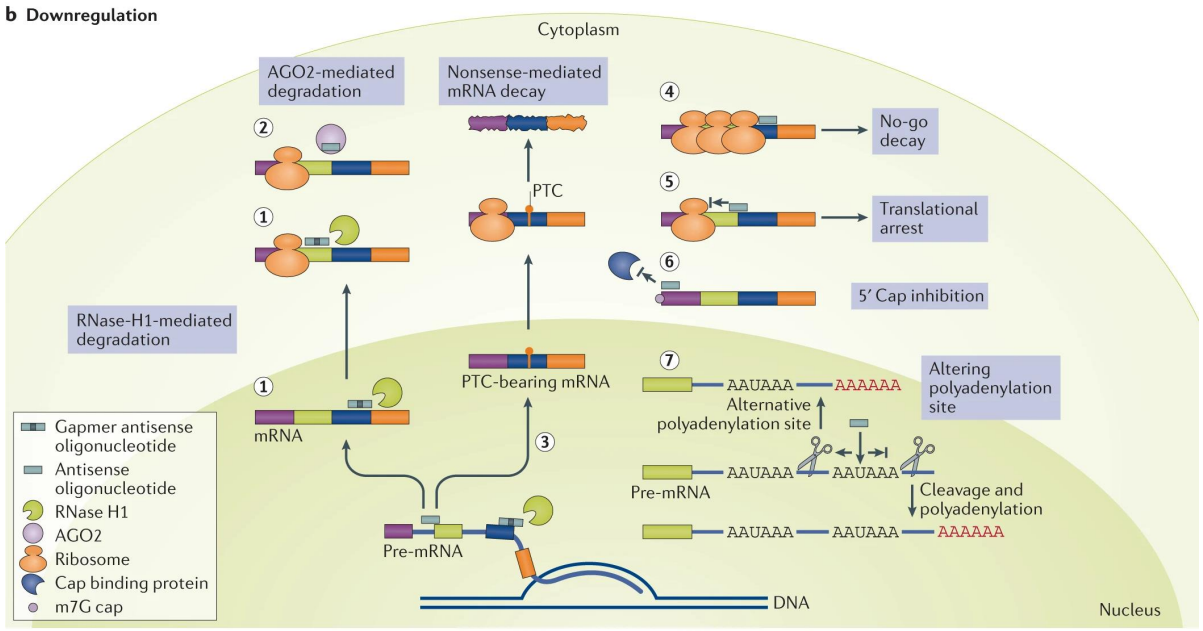
- Steric hindrance ASOs
  - trigger alternative splicing of pre-mRNAs, leading to mRNAs without PTCs, thereby increasing the stability and levels of mRNAs induce cleavage of RNA by RNase H1
  - inhibit miRNA function can increase expression of the miRNA target genes
  - enhance translation by inhibiting translation suppression elements, such as upstream open reading frames (uORFs) and translation inhibitory elements (TIEs) within the 5′ untranslated region (UTR)
- Gapmer ASOs
  - inhibit NMD
  - target promoter regions to enhance transcription

ASOs: Downregulation



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- Gapmer ASOs
  - induce cleavage of RNA by RNase H1
    - cytoplasm = reduces mRNA level
    - nucleus = terminate transcription
  - induce AGO2-mediated RNA degradation, similar to siRNAs
  - cleave 5’-cap and 3’-polyA tails
- Steric hindrance ASOs
  - modulate splicing, generating mRNAs with premature termination codons, leading to nonsense-mediated decay
  - block ribosome scanning and arrest translation
  - bind to the 5’-end of a mRNA inhibiting the binding of translation initiation factors