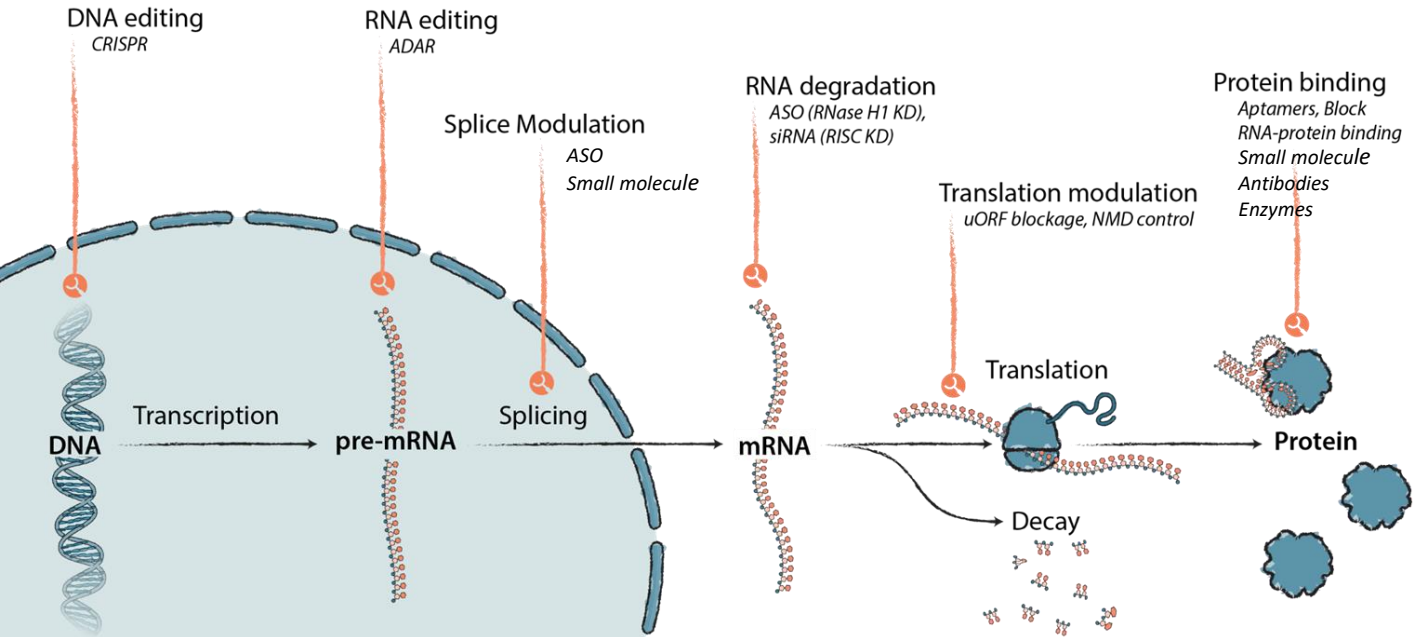
Modalities Overview

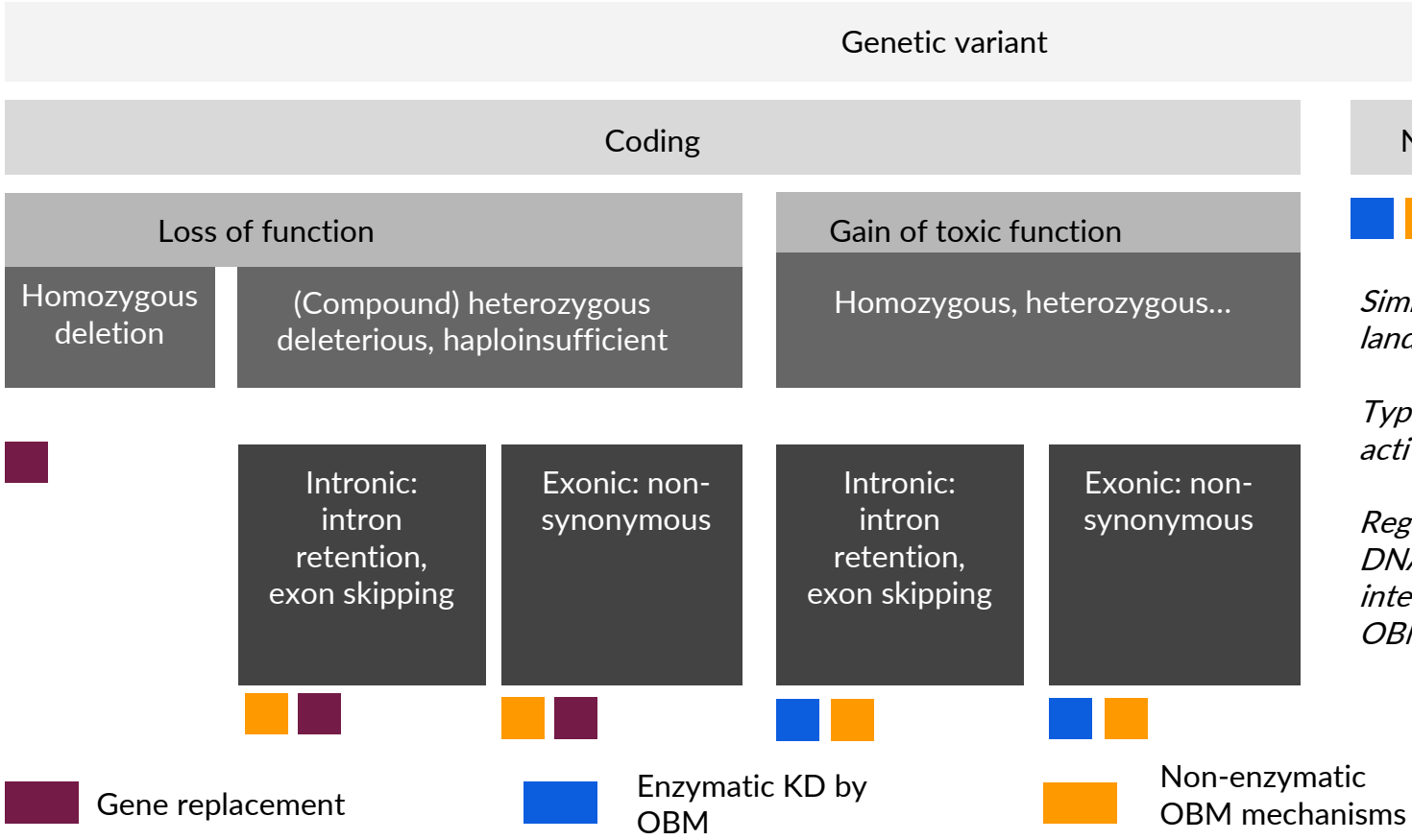
Rare disease is often genetic ∴ interacting/correcting affected downstream processes, machines and structural elements of the cell offer possible treatments



Key consideration of different modalities is where to intervene to correct the genetic processes in the most efficient way

Different pathogenic variants within the same rare disease may be amenable to different modalities

Genetic defects directly addressable with OBMs



Similar nuances in mutational landscape

Typically require OBM activity in the nucleus

Regulatory modulation at DNA / RNA structure, interaction etc. possible with OBMs

ASOs

(and a bit about other oligonucleotide based medicines)

April 27, 2023

Chris Hart, Ph.D.
Co-Founder / CEO
Creyon Bio, Inc.

Outline

- What are ASOs, how do they relate to other Oligonucleotide based medicines (OBMs)
- How do they work
- When ASOs (or other OBMs) may be appropriate for developing a treatment, or for basic research
- Overview of developing ASO medicines
- Questions

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- **What are ASOs, how do they relate to other Oligonucleotide based medicines (OBMs)**
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What are OBMs: Chemically synthesized, short, chemically modified, nucleotide polymers.

OBMs:

- Typically 15-22 nt, almost always <100 nt
- DNA/RNA-*like* molecules that are synthetically created (e.g., not created in cells or through enzymology)
- Chemically modified to impart better pharmacological properties (e.g., make them good drugs)

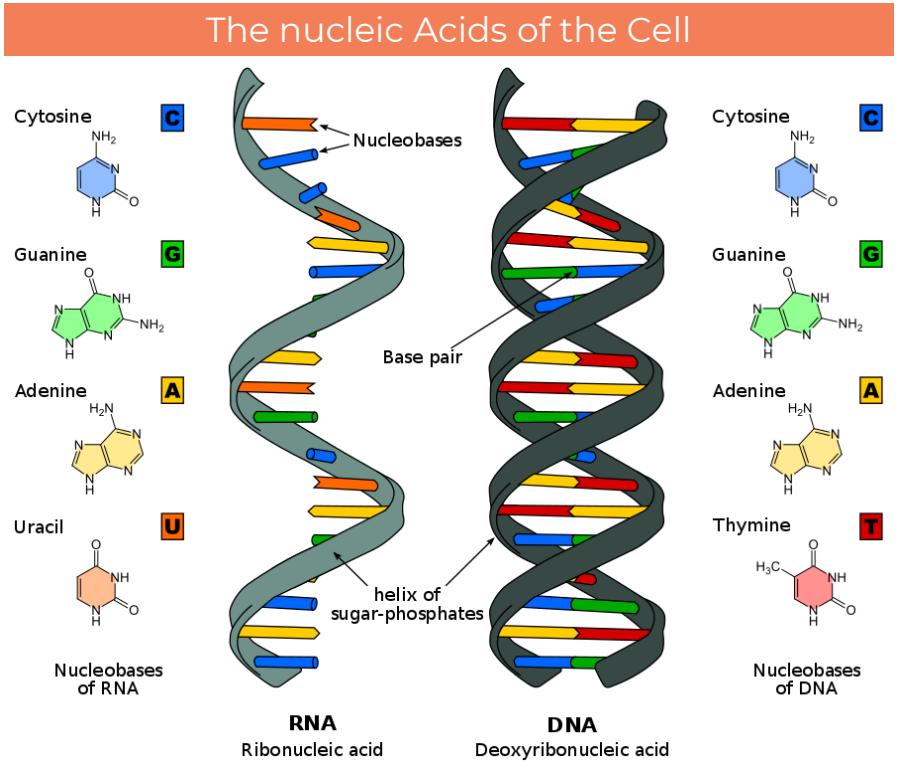
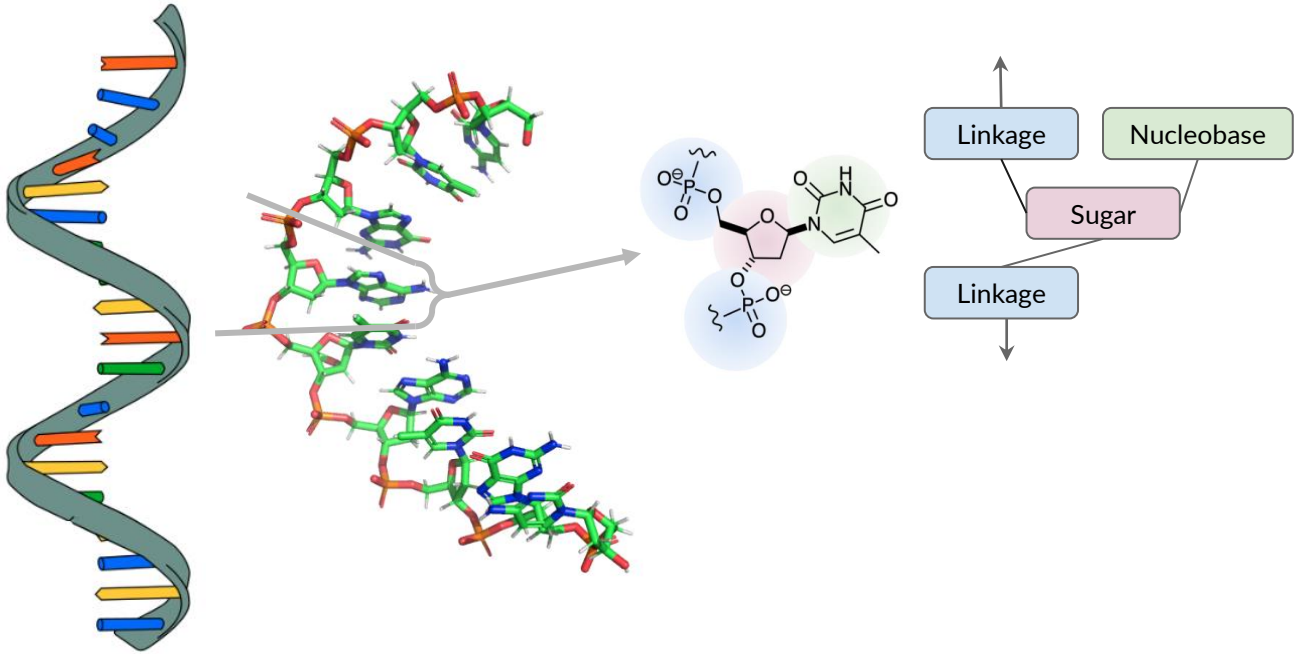
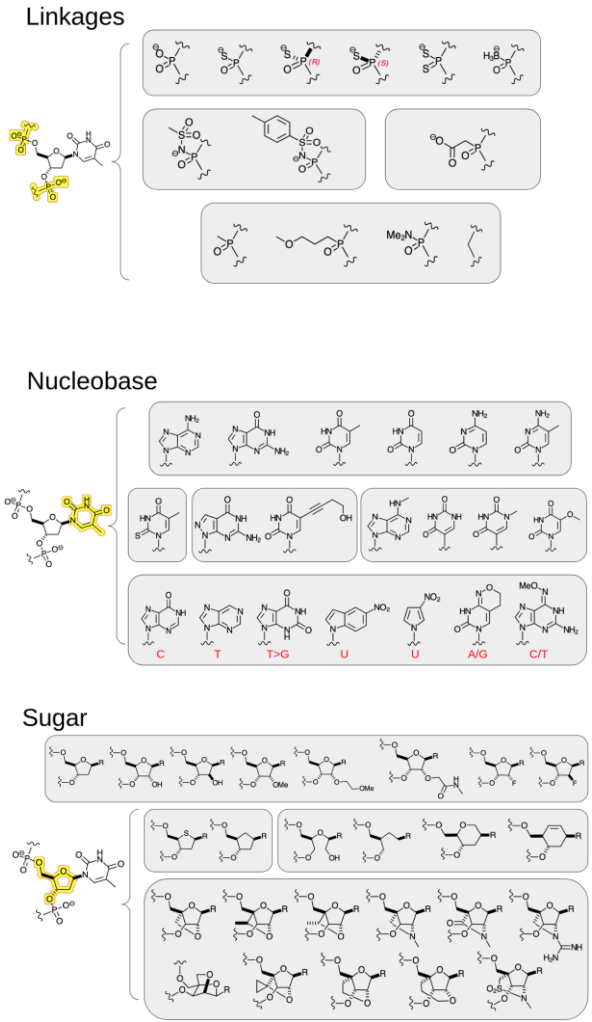


Image source: wikipedia

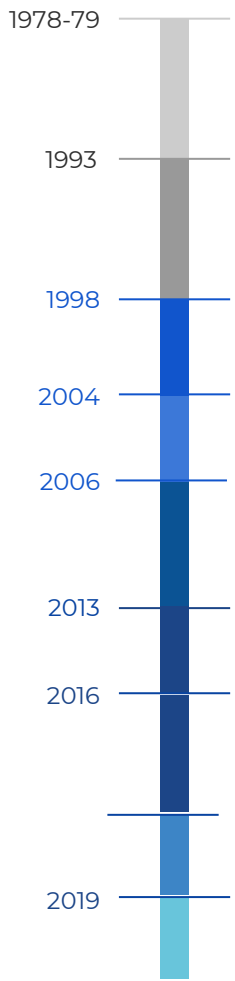
OBM design requires optimization of both sequence and chemistry





Oligonucleotide Based Medicines (OBMs) have been researched for decades

- There is a rich understanding of the mechanism-of-action and molecular biology/pharmacology of OBMs
- Multiple types of OBMs have been approved - ASOs, siRNAs, aptamers
- Many pharma and biotech companies are actively advancing ASOs and siRNAs for rare and common diseases



1978-79 — DNA oligonucleotide and RNase H

Stephenson and Zamecnik showed 13-mer DNA oligonucleotide could inhibit Rous sarcoma virus in cell culture. Donnis-Keller *et al.* described RNase H site specific cleavage

1993 — Splice switching antisense oligonucleotides

Dominski *et al.* reports splice switching application of ASOs in beta-thalassemia

1998 — FDA approval of first antiviral ASO

FDA approves (Fomivirsen) Vitravene for the treatment of cytomegalovirus retinitis in AIDS patients

2004 — FDA approval of aptamer drug

Macugen (Pegatanib) approved for the treatment of angiogenesis in patients with age related macular degeneration

2006 — Nobel Prize awarded for RNAi discovery

Nobel Prize in Physiology or Medicine awarded to Andrew Fire and Craig Mello for their discovery that RNA triggers suppression of gene activity in a homology-dependent manner

2013 — FDA approval of RNase H1 engaging ASO drugs

Mipomersen (Kynamro) approved for treatment of familial hypercholesterolemia

2016 — FDA approves splice switching ASO drugs

FDA accelerated approval of Eteplirsen (EXONDYS 51) and Nusinersen (SPINRAZA) for muscular dystrophy and SMA respectively

FDA approval of siRNA drug

Onpattro (patisiran) approved for the treatment of transthyretin-mediated amyloidosis in adult patients

2019 — FDA allows N-of-1 ASO drug

Development of N-of-1 custom splice-modulatory ASO for Mila Makovec led by Tim Yu, FDA approved under an expanded-access investigational clinical protocol

Different types of OBMs use different chemistries tuned to optimize different molecular mechanisms; have different pharmacology

Antisense Oligonucleotides (ASOs)

- Single stranded (14-21 nt)
- Can be absorbed into cells with or without targeting
- Work through multiple molecular mechanisms:
  - Splice altering
  - RNAseH1 Knock Down
  - Can target nuclear and non-nuclear RNAs
  - Other steric blocking

Short Interfering Ribonucleic Acids (siRNAs)

- Double Stranded (20-25 nt)
- Requires delivery formulation or conjugation
- Works by loading into RISC and directing it to degrade specific mRNAs

Guides, Aptamers, and more

- Typically longer (30-80nt)
- Variable cellular uptake
- Can use different chemistries
- Work through multiple molecular mechanisms:
  - aptamers - Ab like binding
  - recruit endogenous enzymes (e.g., ADAR)
  - recruit exogenous enzymes (e.g., CRISPR)

Oligonucleotide Based Medicines (OBMs) can do many things

AGCCGCAAAG
TCGGCGTTTC

Precise Watson-Crick-Franklin Hybridization Defined Target Recognition

Control Gene Expression

siRNA (RISC KD)

ASO (RNaseH1 KD)

Splice Modulation

uORF blockage

NMD control

...

Guides for Editing Enzymes

ADAR

CRISPR

...

General Steric Blocking

Viral Packaging

Block RNA binding

Repeat expansion

Influence RNA/DNA structures

...

Structurally Complex Macromolecules

Aptamers

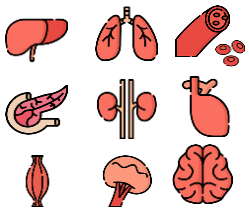
Diverse molecular targeting moieties

Favorable Pharmacology

Multiple routes of administration

Active for weeks to months

Broad Tissue Access



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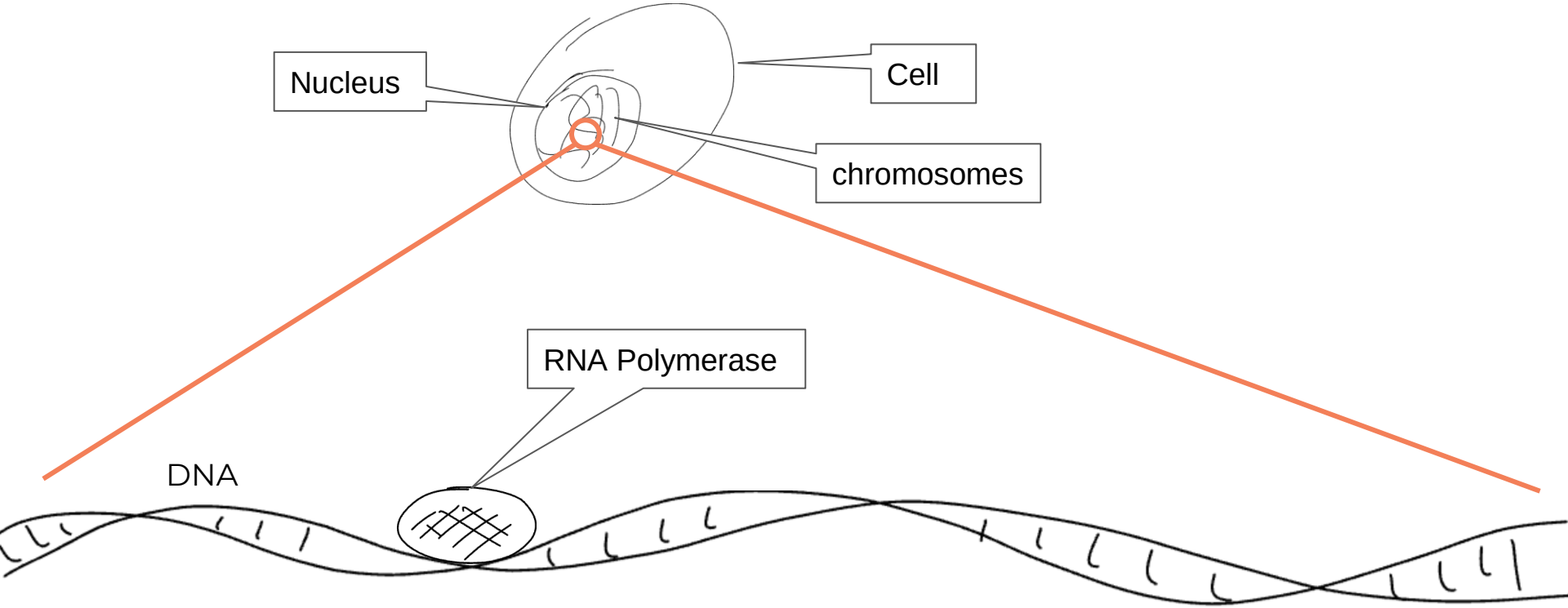
ASOs (and other OBMs) typically target the genetic basis of disease -- typically by modulating RNA



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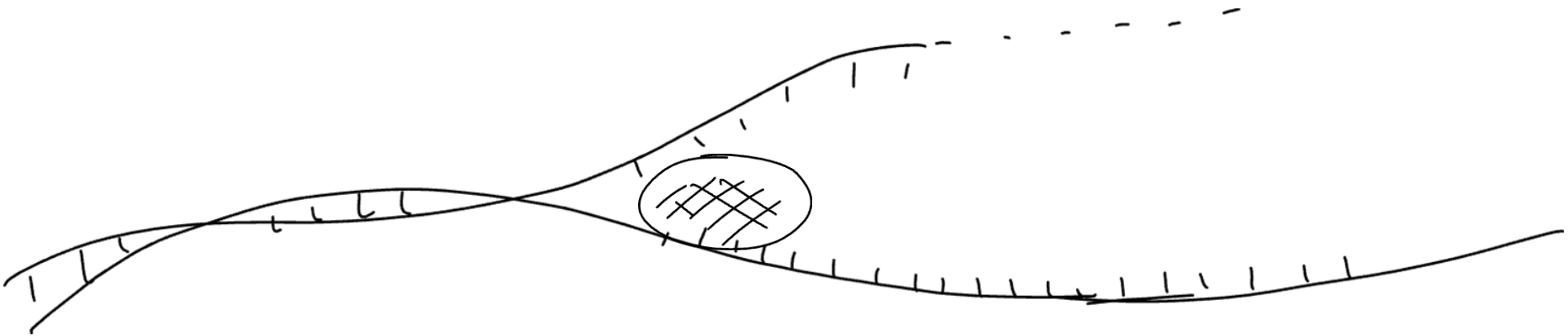

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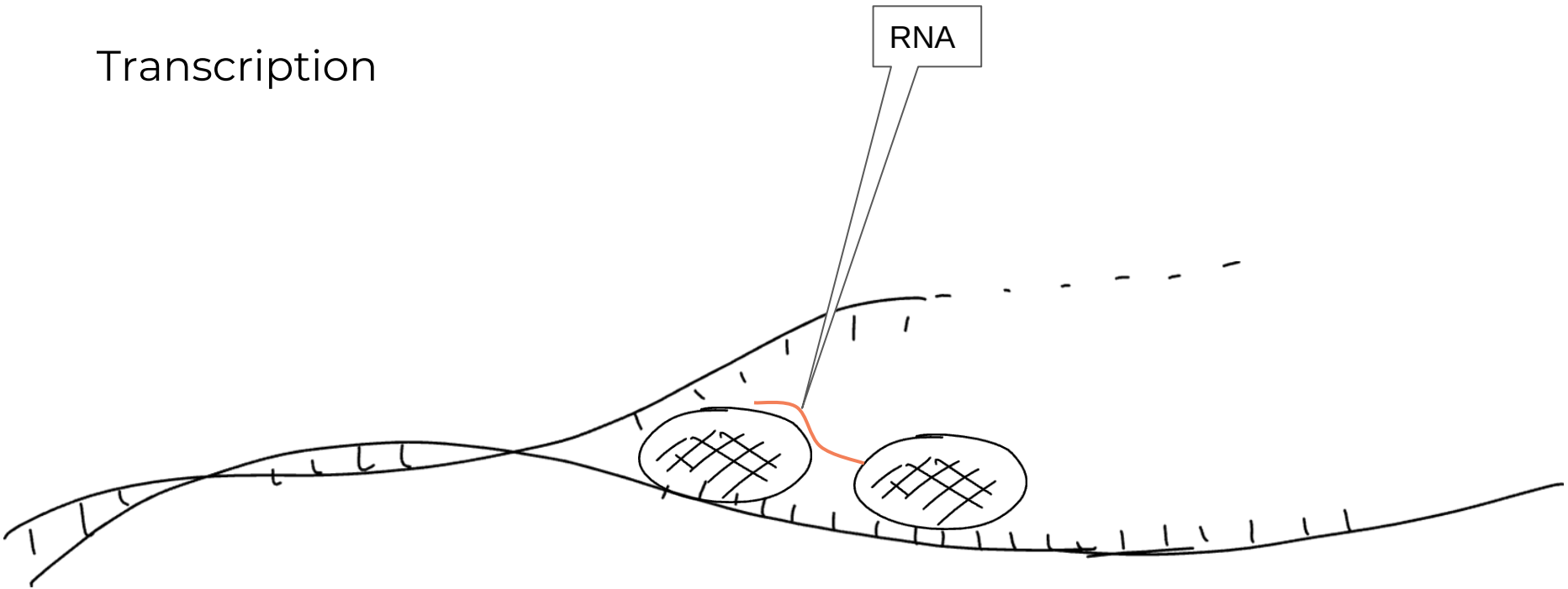

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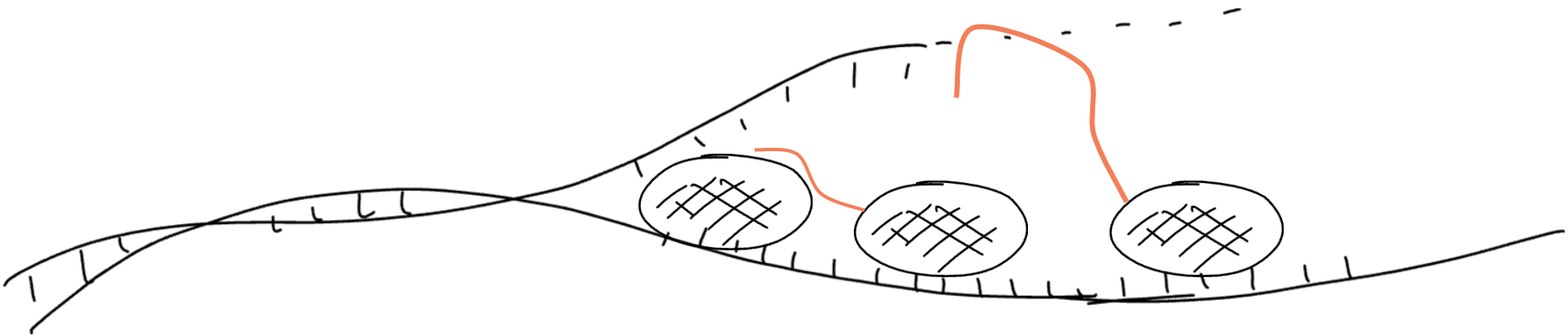

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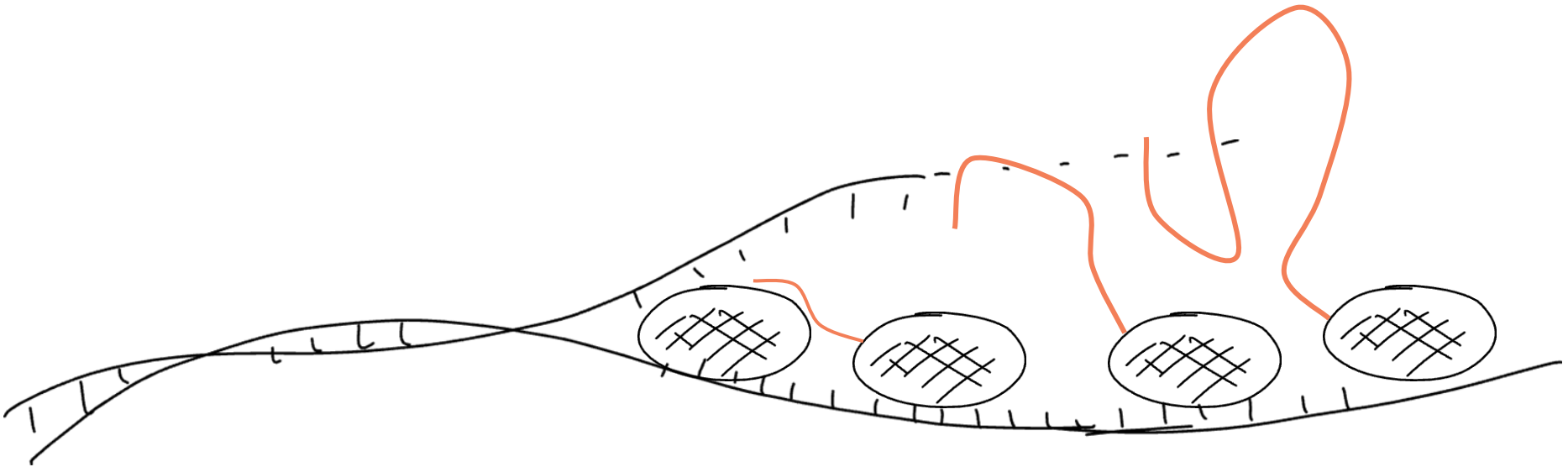

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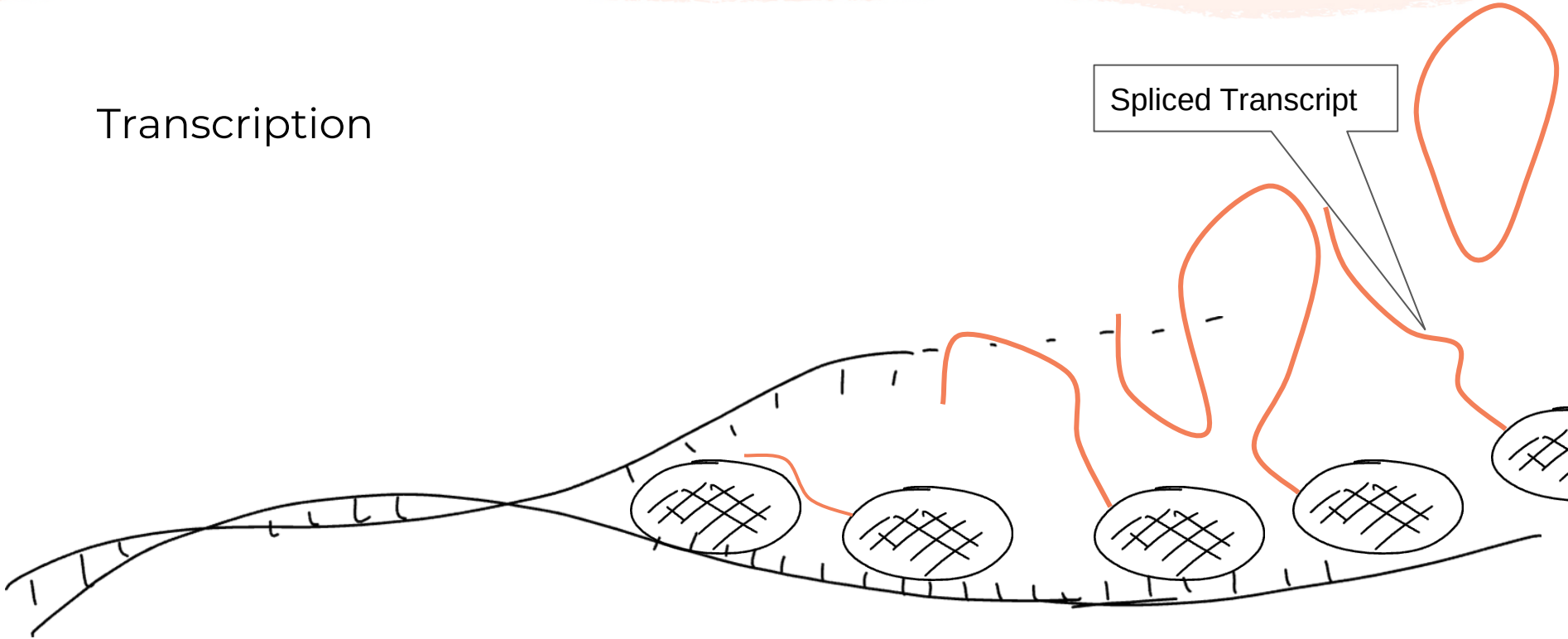

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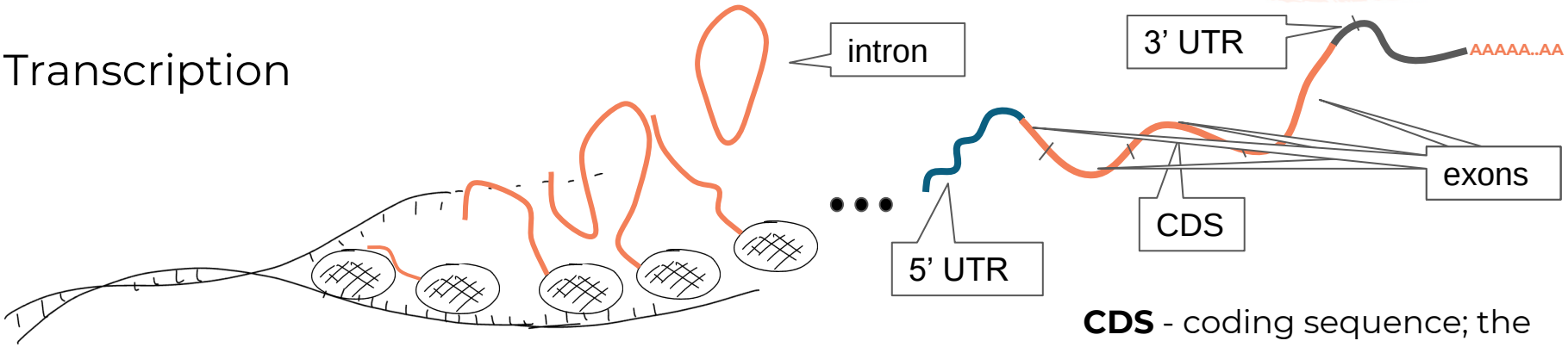
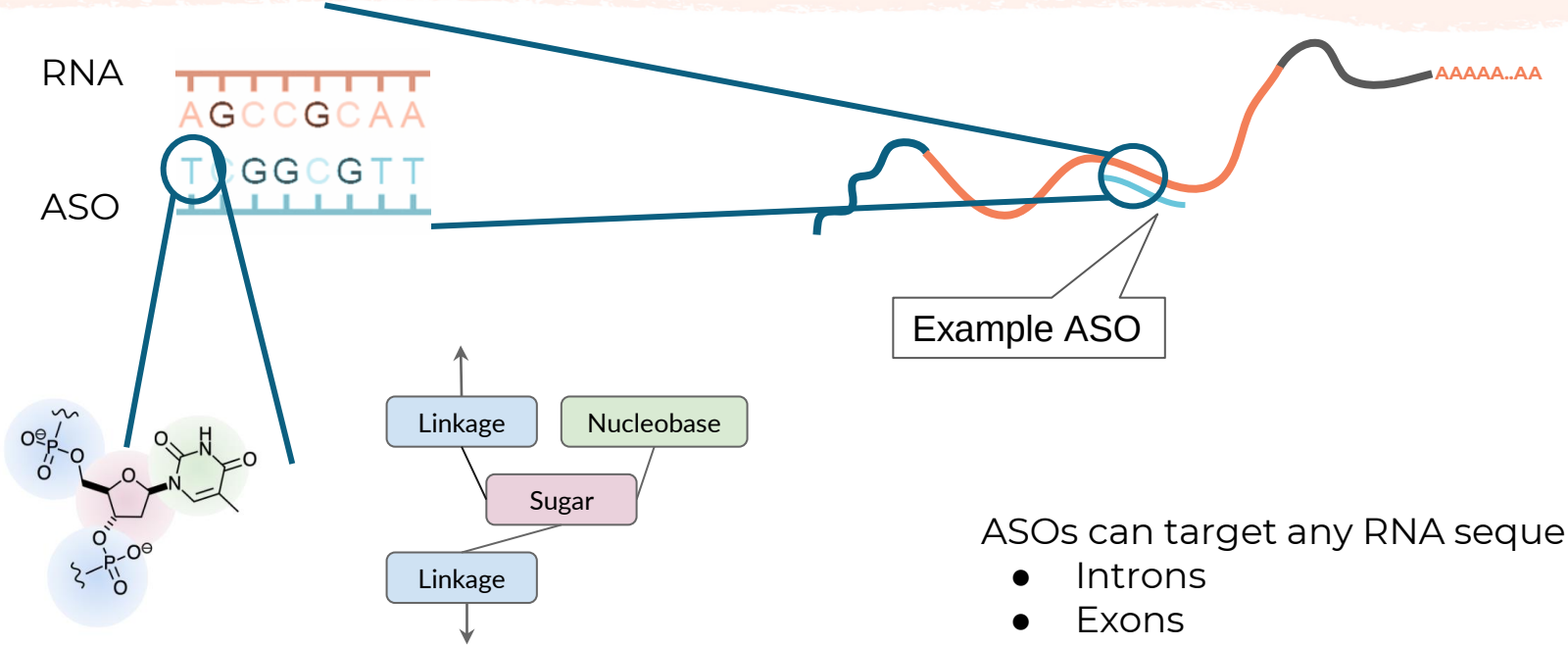


CDS - coding sequence; the region of the RNA that codes for a protein

UTR - untranslated regions; regulatory domains of the RNA

OBMs target RNA through well defined Watson-Crick-Franklin hybridization



ASOs can target any RNA sequence

- Introns
- Exons
- 5’ or 3’ UTRs
- mRNA or pre-mRNA
- Protein coding or non-coding

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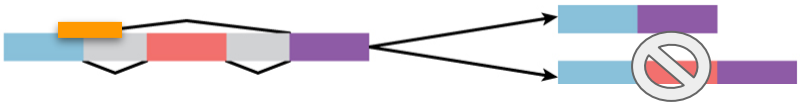
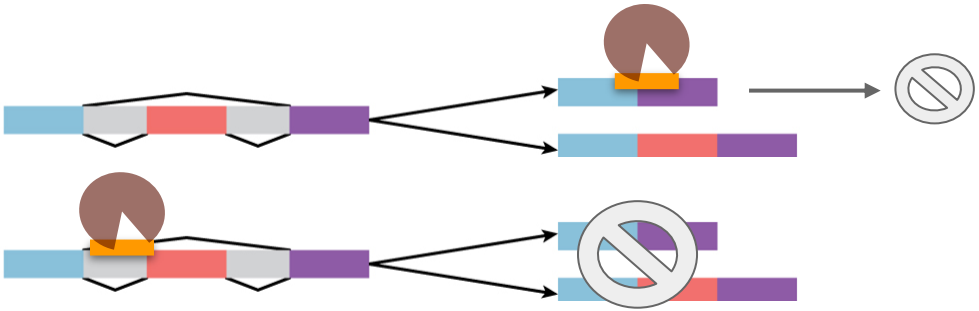
Oligonucleotide based medicines (OBMs) modulate gene expression directly



Occupancy Only
e.g., splice modulating



Enzymatic RNA knockdown
e.g., ASO-directed RNase H1 KD

- Correct aberrant disease causing splicing
- Upregulate gene expression by skipping NMD-inducing transcripts
- Many other occupancy only mechanisms to up and down regulate genes

- Works on mRNA, pre-mRNA, ncRNA (e.g., the druggable and undruggable)
- Allele selectivity achievable allowing for correction of toxic GoF diseases
- Additional enzymatic systems can be engaged (e.g, RISC, ADAR)

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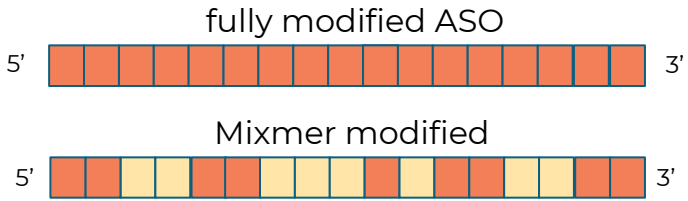
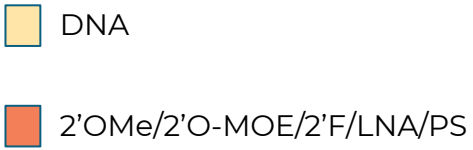
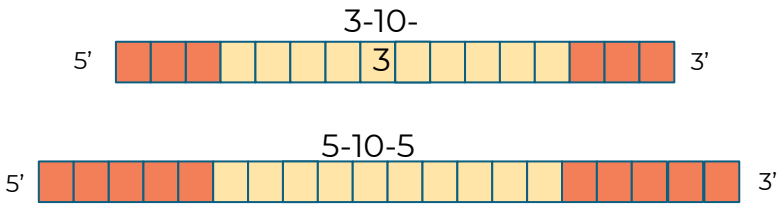
Well established ASO architectures

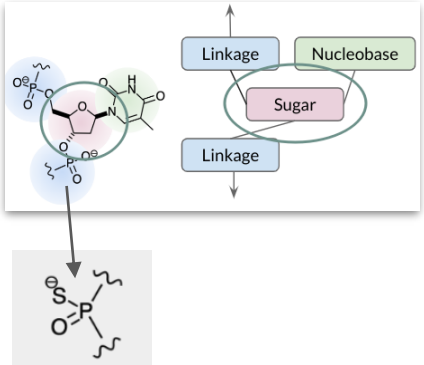
Steric hindrance (splice shifting, …)

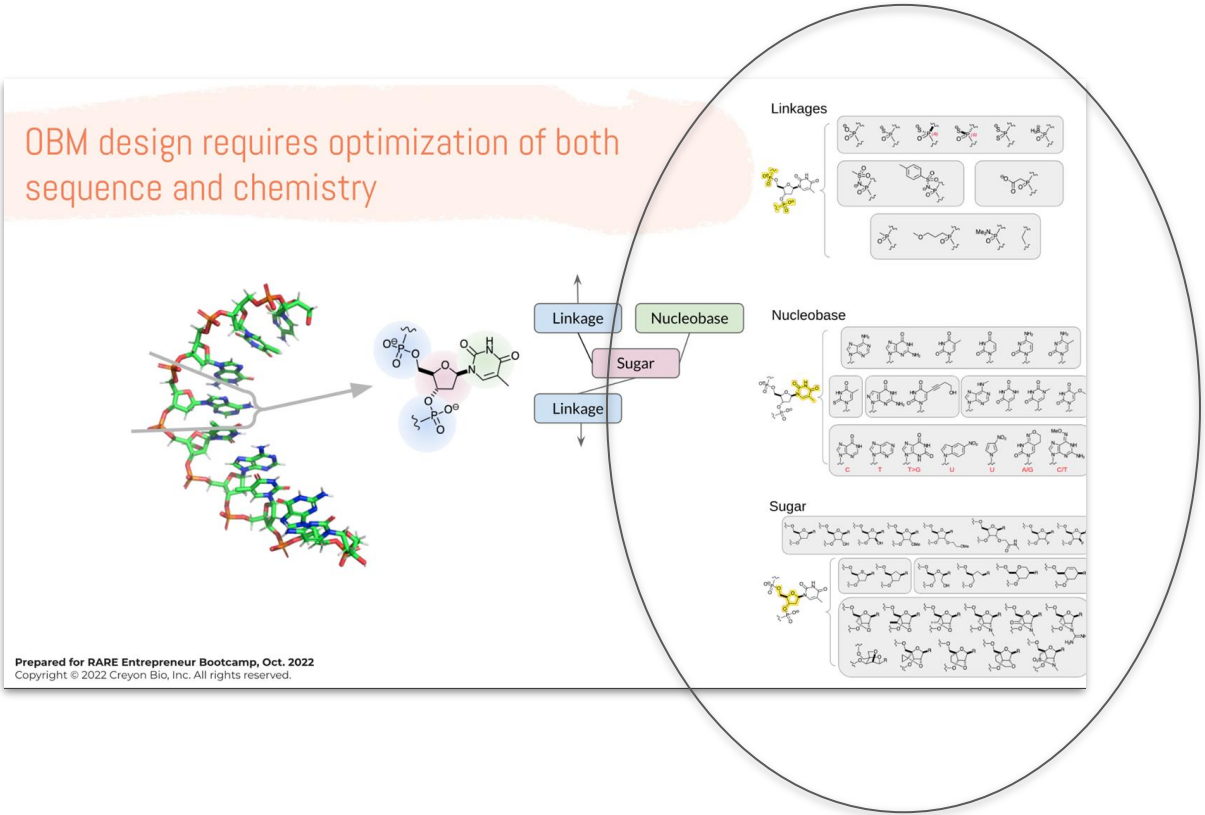




Gapmer modified ASOs (*RNaseH1 engaging*)

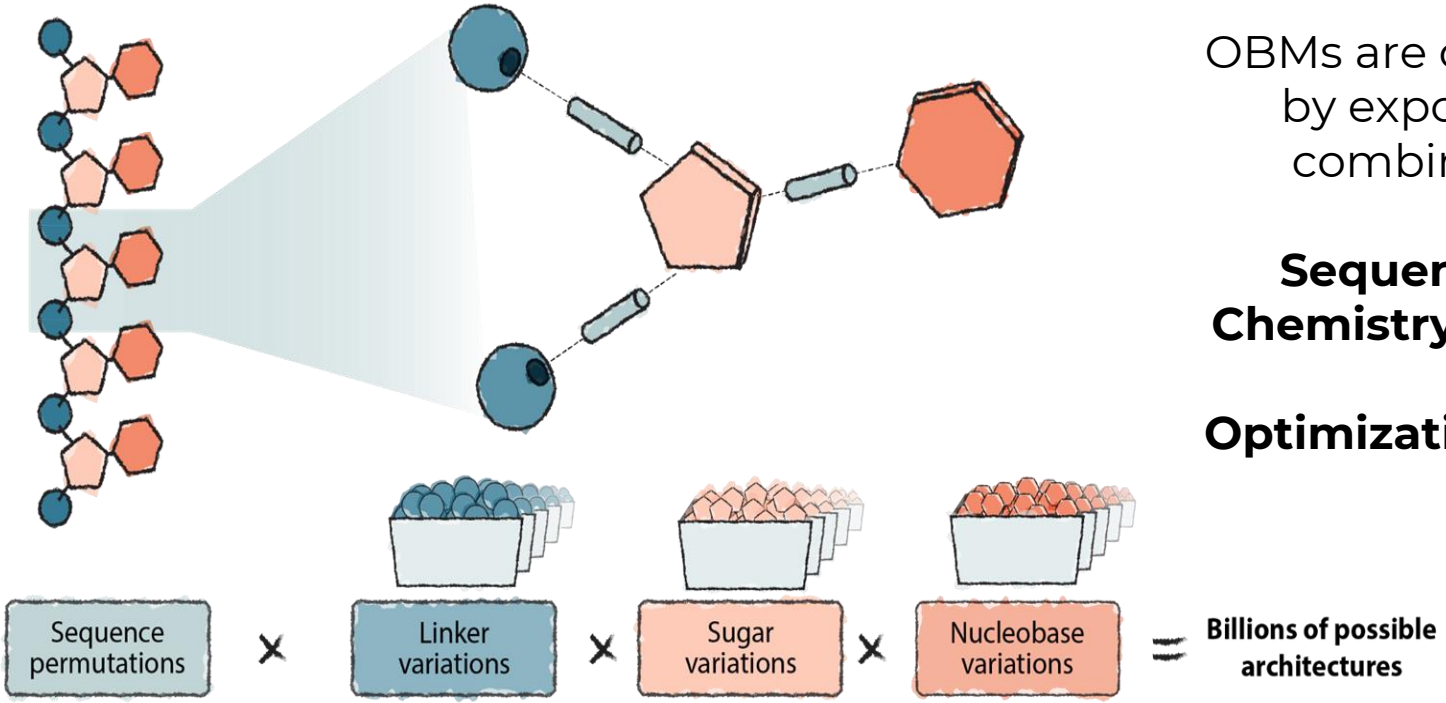






There is a growing number of interesting chemistries that can be used in ASOs and other OBMs.

Design Caveat



OBMs are challenged by exponential combinations

Sequence and Chemistry Collude…

Optimization is Hard

Potential toxicities induced by ASOs

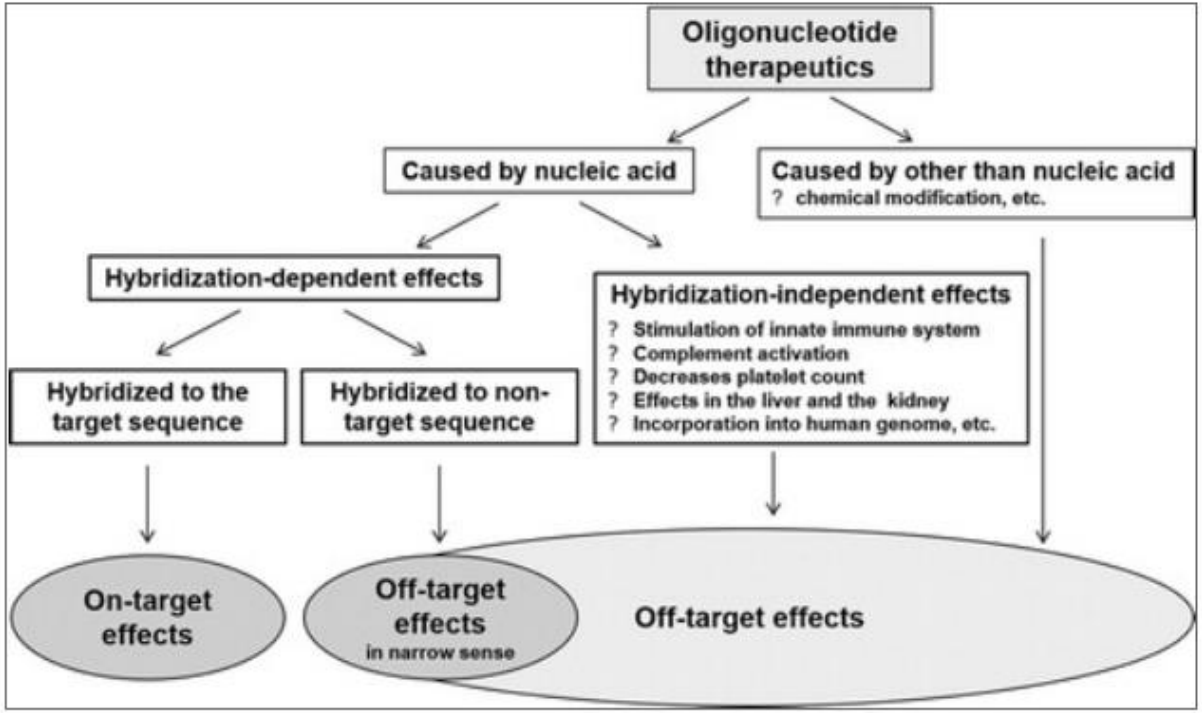


Image from: Hirabayashi et al. Considerations of the Japanese Research Working Group for the ICH S6 & Related Issues Regarding Nonclinical Safety Assessments of Oligonucleotide Therapeutics: Comparison with Those of Biopharmaceuticals.Nucleic Acid Therapeutics. Apr 2021.114-125.http://doi.org/10.1089/nat.2020.0879

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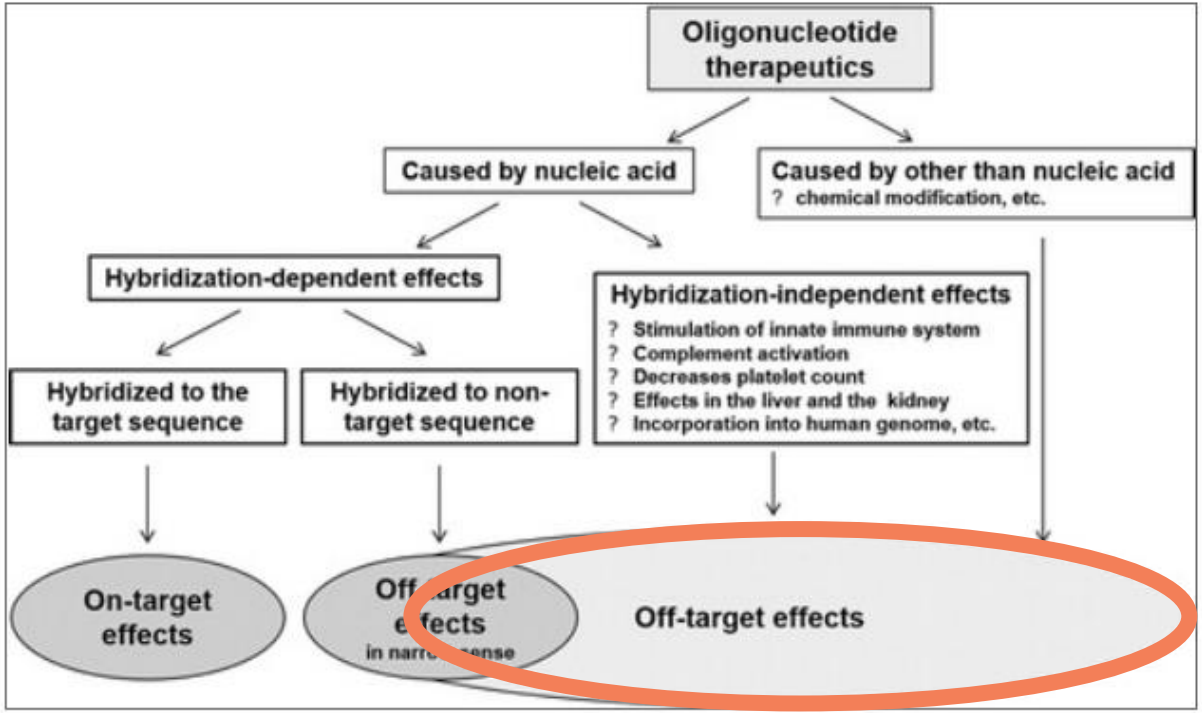

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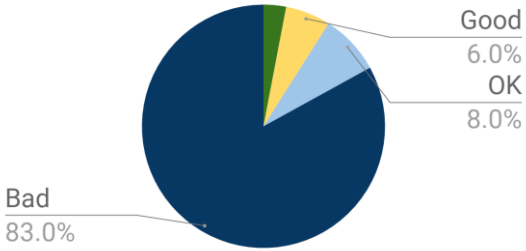
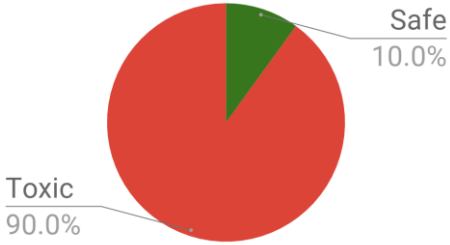
Compound related pharmacology makes developing ASOs (and other OBMs) challenging

Most OBMs are not Active



Most OBMs are Toxic



Shown for 3-10-3 BNA gapmer ASOs
Liver toxicity and *in vitro* activity

Because several safety endpoints to meet…

Cytotoxicity
Liver, kidney, delayed neurotoxicity..

Membrane toxicity
Acute neurotoxicity, depolarization..

Immunogenicity & immunostimulatory
Platelet effects, receptor specific activations…

..and several efficacy contributors…

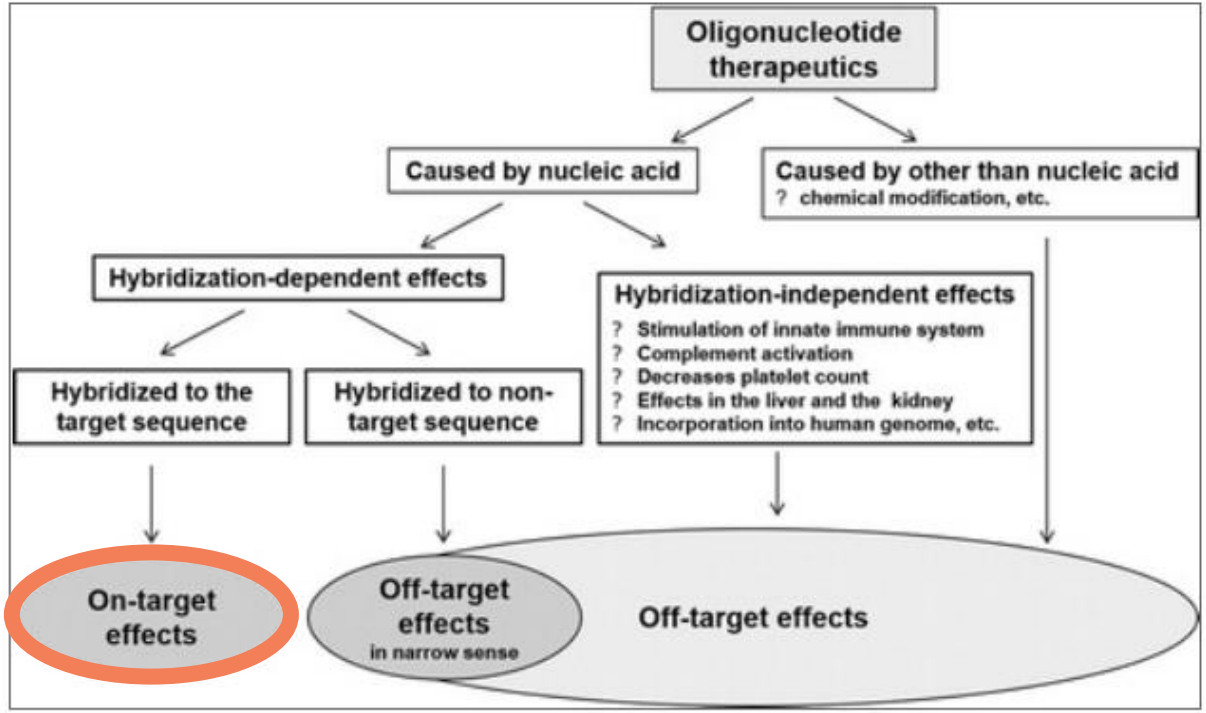
Tissue distribution & productive uptake
Localization in active compartment

Targetability of RNA
Accessibility, (co-)transcriptional dynamics of RNA

Hybridization, Specificity & enzymatic rules
Edit tolerance of RNase H, Ago2… sequence preference

Prepared for RARE Entrepreneur Bootcamp, April 2023

Potential toxicities induced by ASOs



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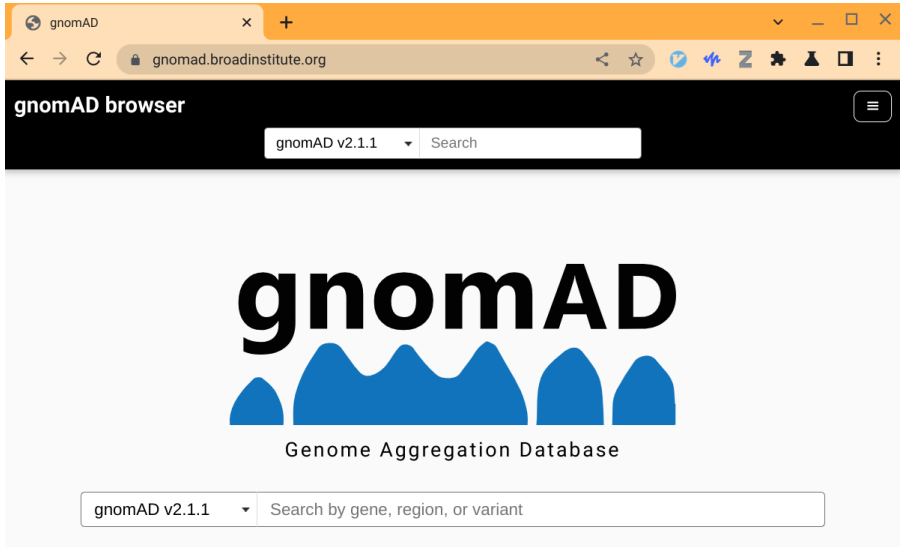
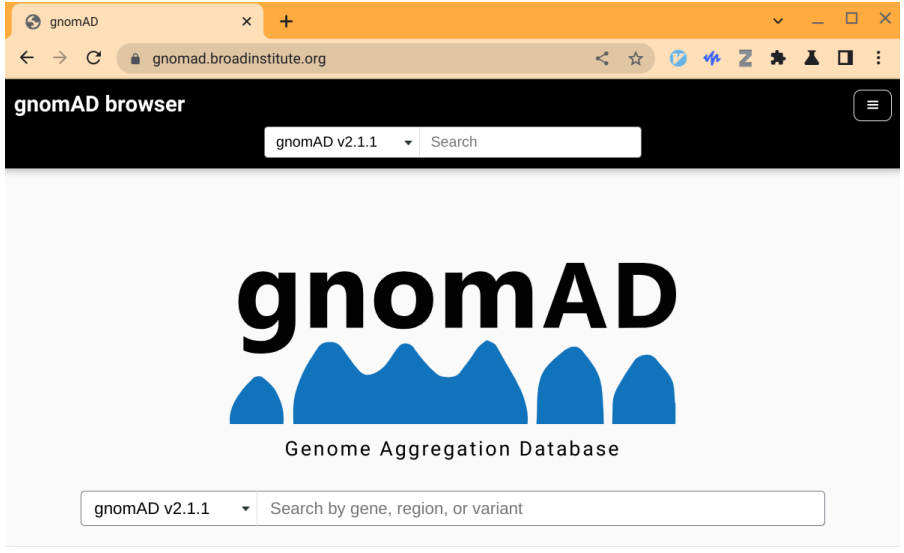
Population genomic databases can derisk on-target risk.. sometimes



Using population genomics will report on how frequently suspected loss-of-function variants are seen. If we see many of them, then the gene is likely loss-of-function tolerant.

Population genomic databases can derisk on-target risk.. sometimes



PCSK9

Constraint

Category	Expected SNVs	Observed SNVs	Constraint metrics
Synonymous	187.5	170	Z = 1.01 o/e = 0.91 (0.8 - 1.03)
Missense	435	419	Z = 0.27 o/e = 0.96 (0.89 - 1.04)
pLoF	26.9	26	pLI = 0 o/e = 0.97 (0.71 - 1.34)

Constraint metrics based on Ensembl canonical transcript (ENST00000302118.5).

HTT

Constraint

Category	Expected SNVs	Observed SNVs	Constraint metrics
Synonymous	709.8	779	Z = -2.04 o/e = 1.1 (1.03 - 1.16)
Missense	1729.3	1404	Z = 2.78 o/e = 0.81 (0.78 - 0.85)
pLoF	158.6	19	pLI = 1 o/e = 0.12 (0.08 - 0.18)

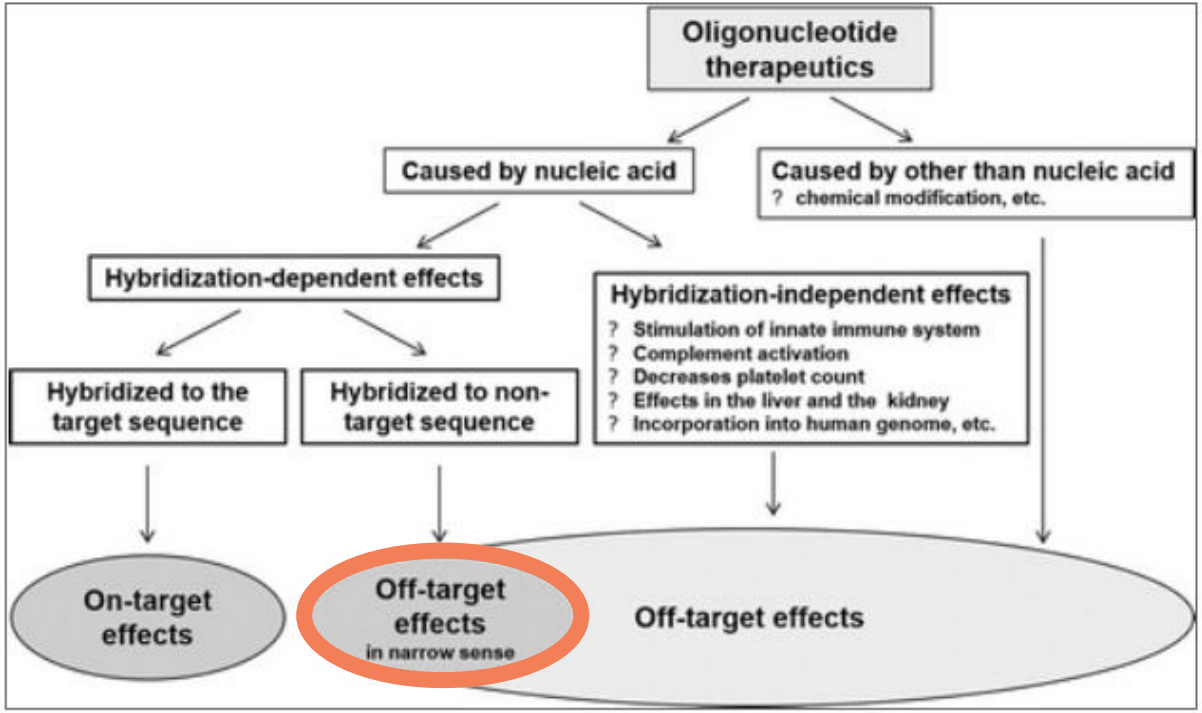
Constraint metrics based on Ensembl canonical transcript (ENST00000355072.5).

Other insights into on-target tolerability concerns

- Mouse or other model organism phenotypes
  - Caveats:
    - Humans ≠ Mouse ≠ fly ≠ worms …

- Modern Biology Tools:
  - High-content imaging
  - Functional genomics
  - iPS derived cellular models (organoids, neurons, …)

Potential toxicities induced by ASOs



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Assessing hybridization-dependent off-target effects

Search for all perfect matched and near-matched sequences in the relevant transcriptome.

- ASOs - active at all expressed RNA (e.g., pre-mRNAs, ncRNAs)

Caveats:

- Sequence analysis tools built for NGS analysis (e.g., bowtie, STAR) or evolutionary searches (BLAST, BLAT) have optimizations that may miss important hits

Perhaps the most profound consequence of the genome revolution in the long run will be the development of targeted therapeutics based on a detailed molecular understanding of pathogenesis.

However, this is also the goal most challenged by long timelines, high failure rates and exorbitant costs.

-Francis Collins, 2010

Collins, F. 2010. “Has the revolution arrived?.” *Nature* 464: 674-675.

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