
Antisense Oligonucleotide mediated up-regulation of mRNA: Implications for *SLC6A1*

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December 5, 2019



RNA
THERAPEUTICS
INSTITUTE

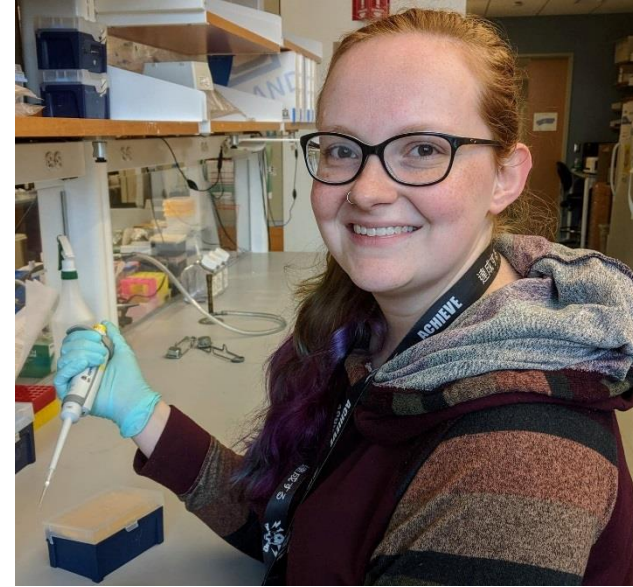


@athmapai



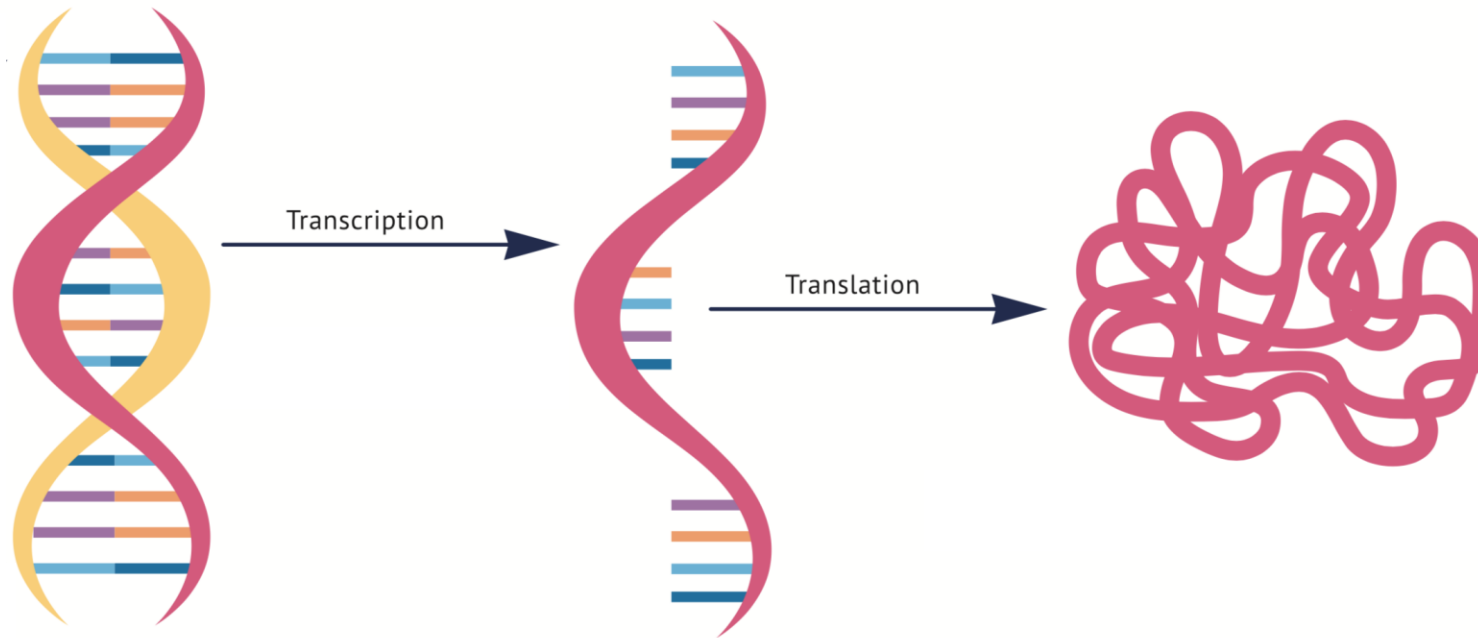


Jonathan K. Watts, PhD
Associate Professor, UMMS RTI

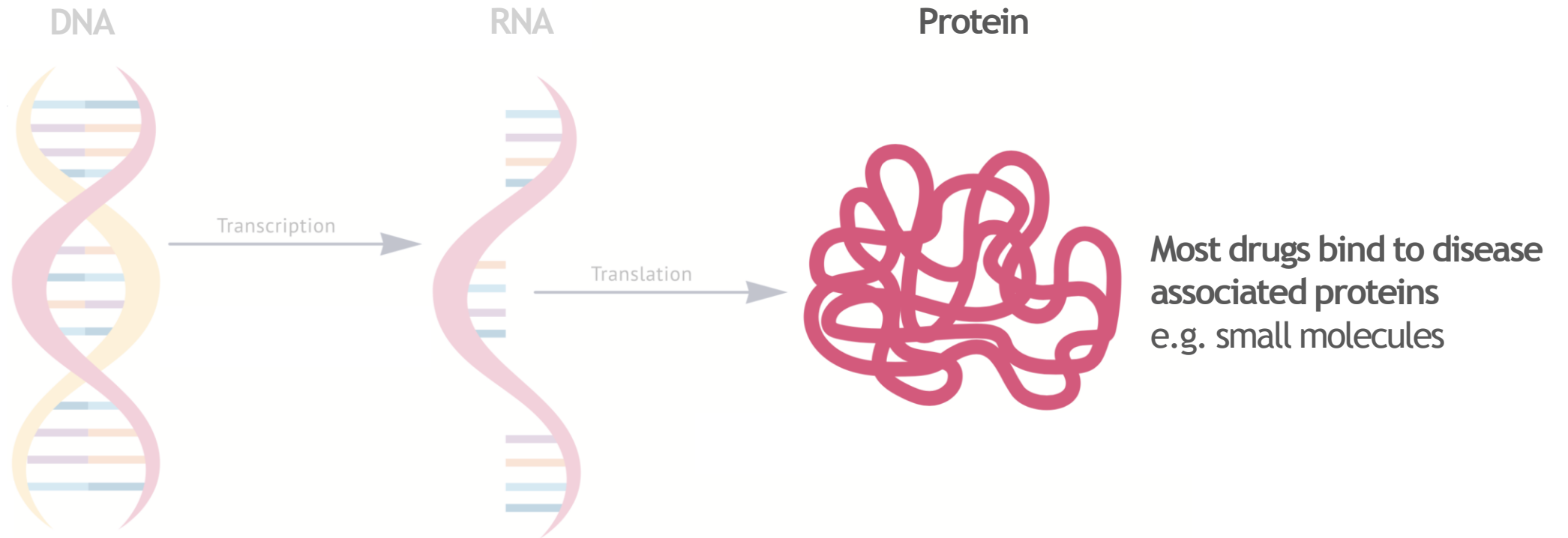


Kaitlyn Valla
Graduate Student, UMMS RTI

The *Central Dogma* of Molecular Biology



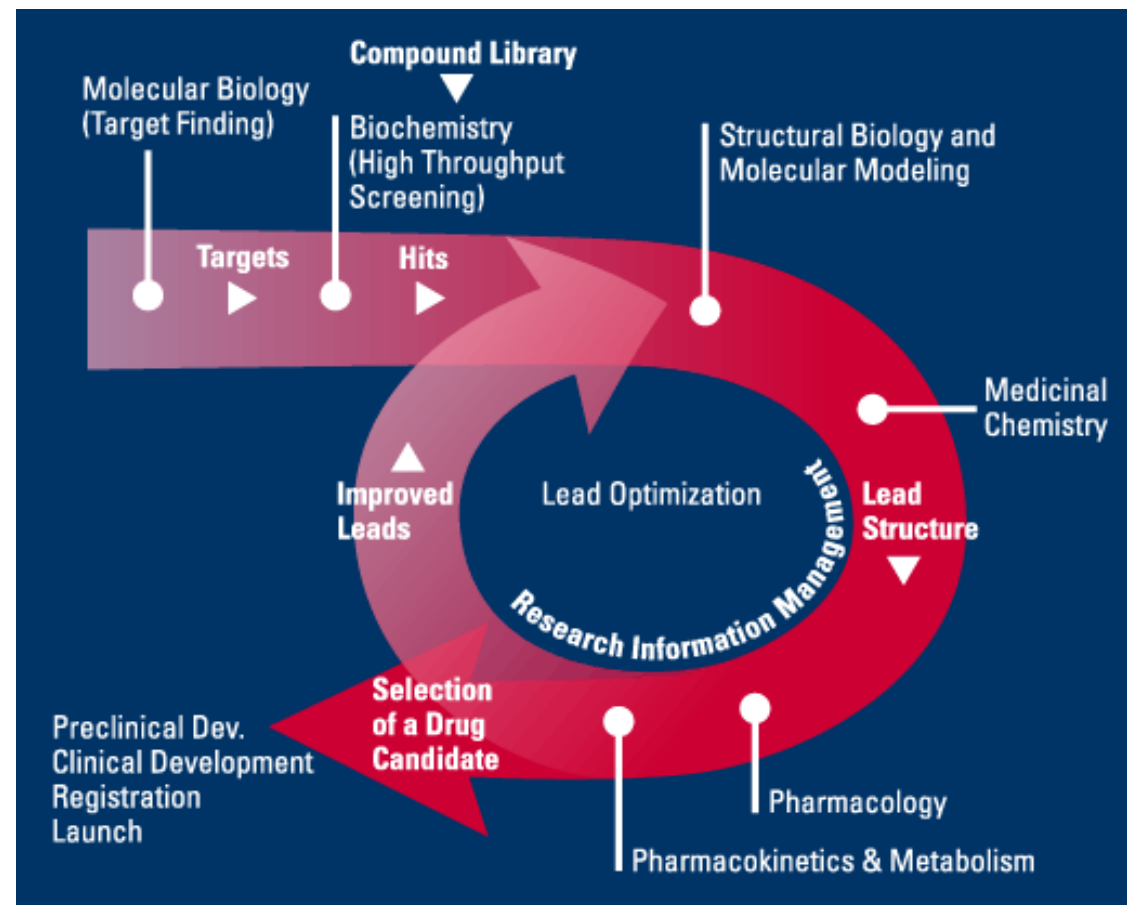
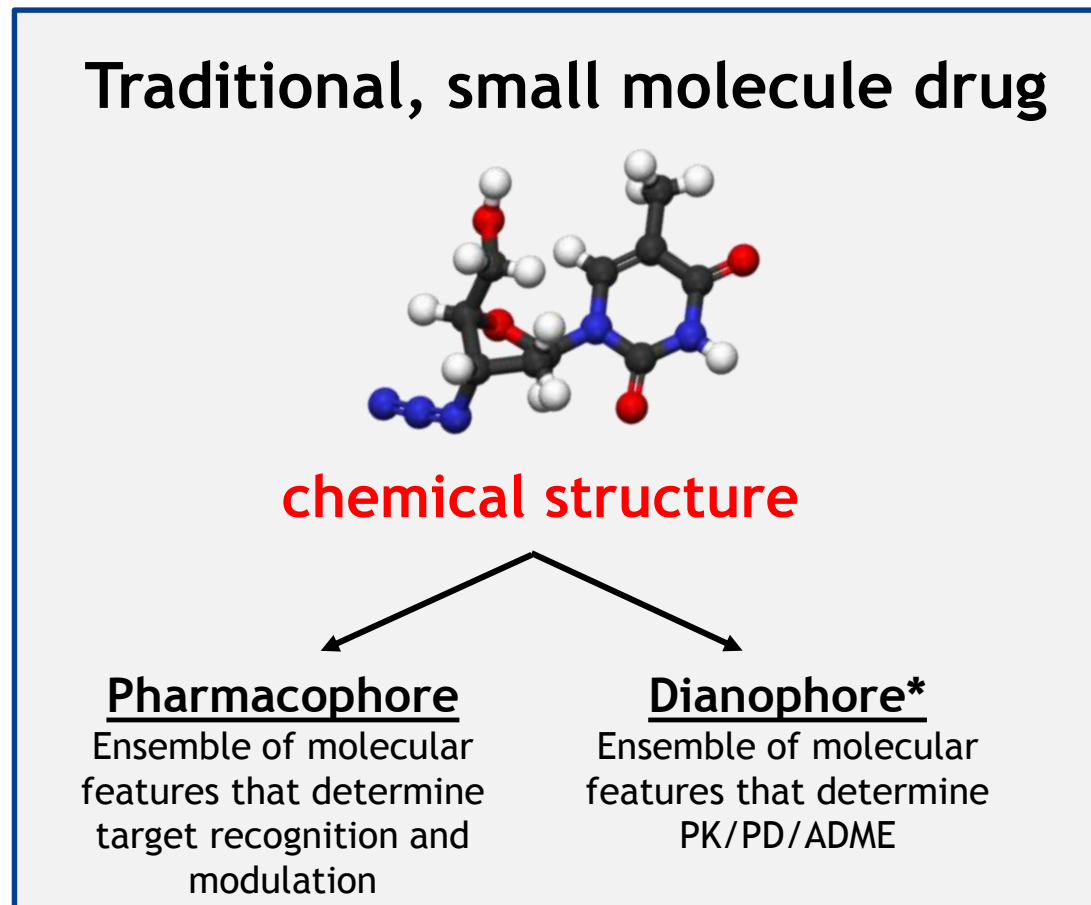
The *Central Dogma* of Molecular Biology



The *Central Dogma* of Molecular Biology

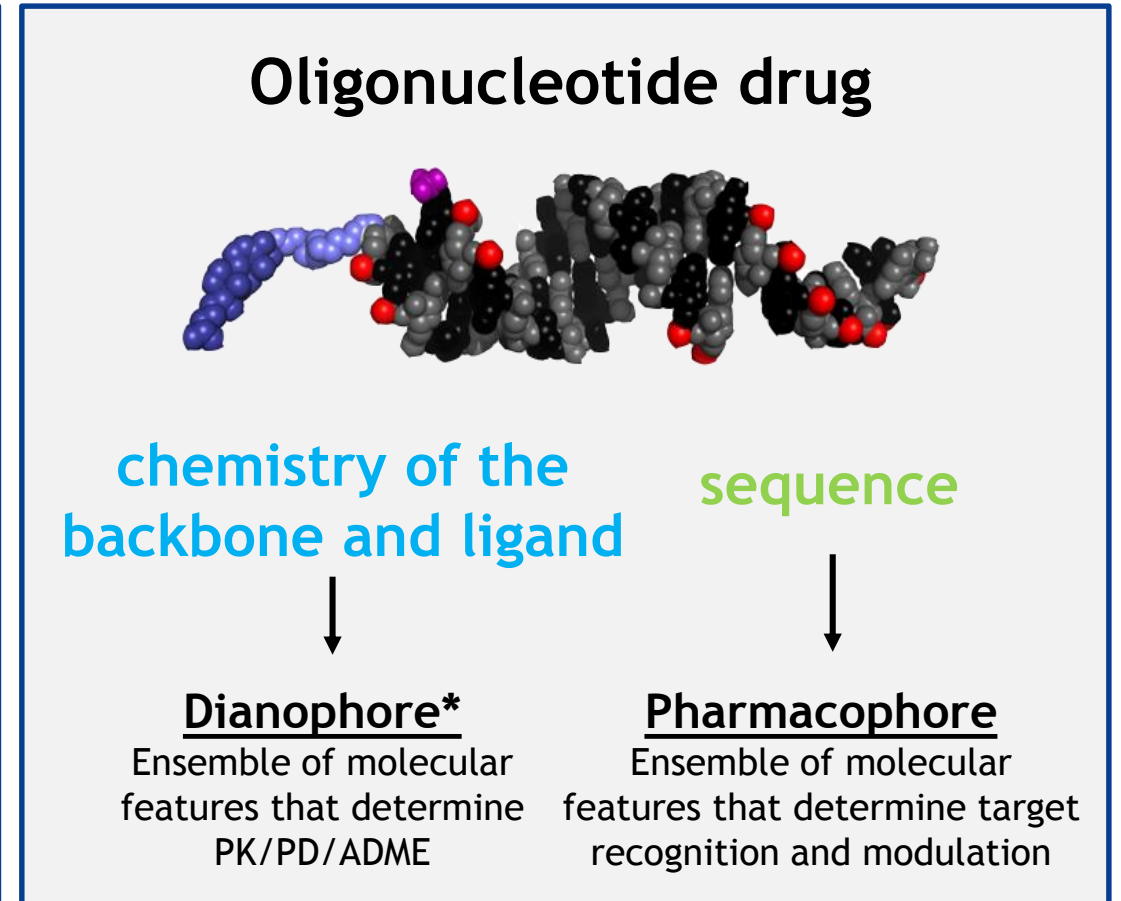
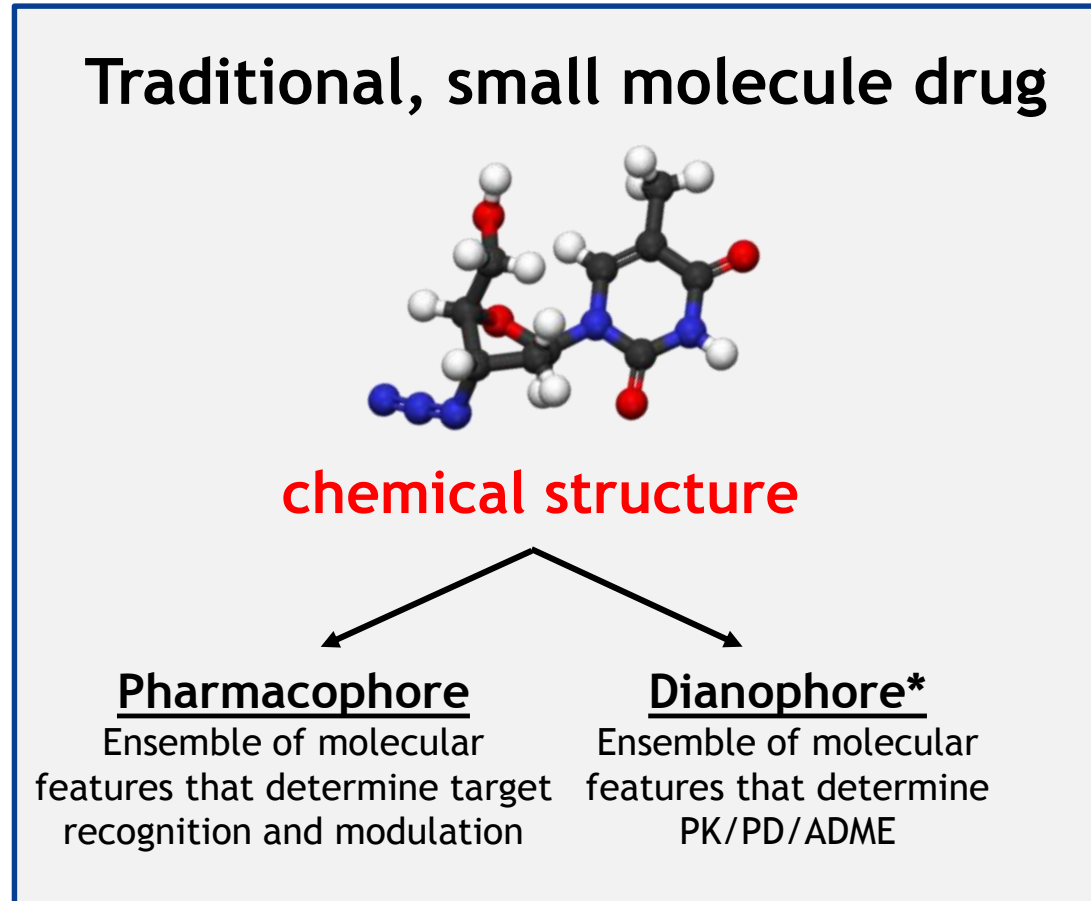


The *dianophore* principle



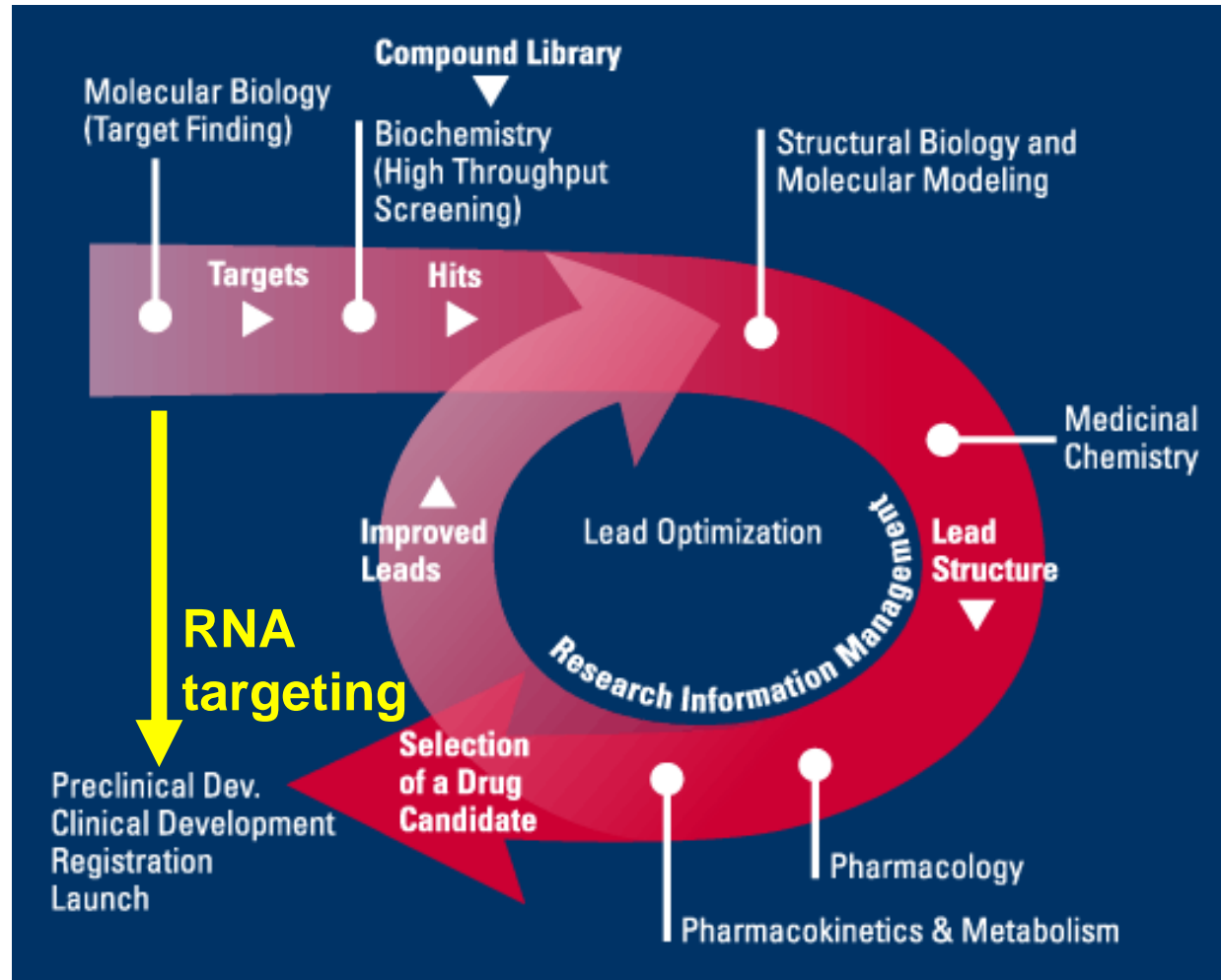
**Dianophore*—from the Greek “διανομή-dianomi” for distribution or delivery

The *dianophore* principle



**Dianophore*—from the Greek “διανομή-dianomi” for distribution or delivery

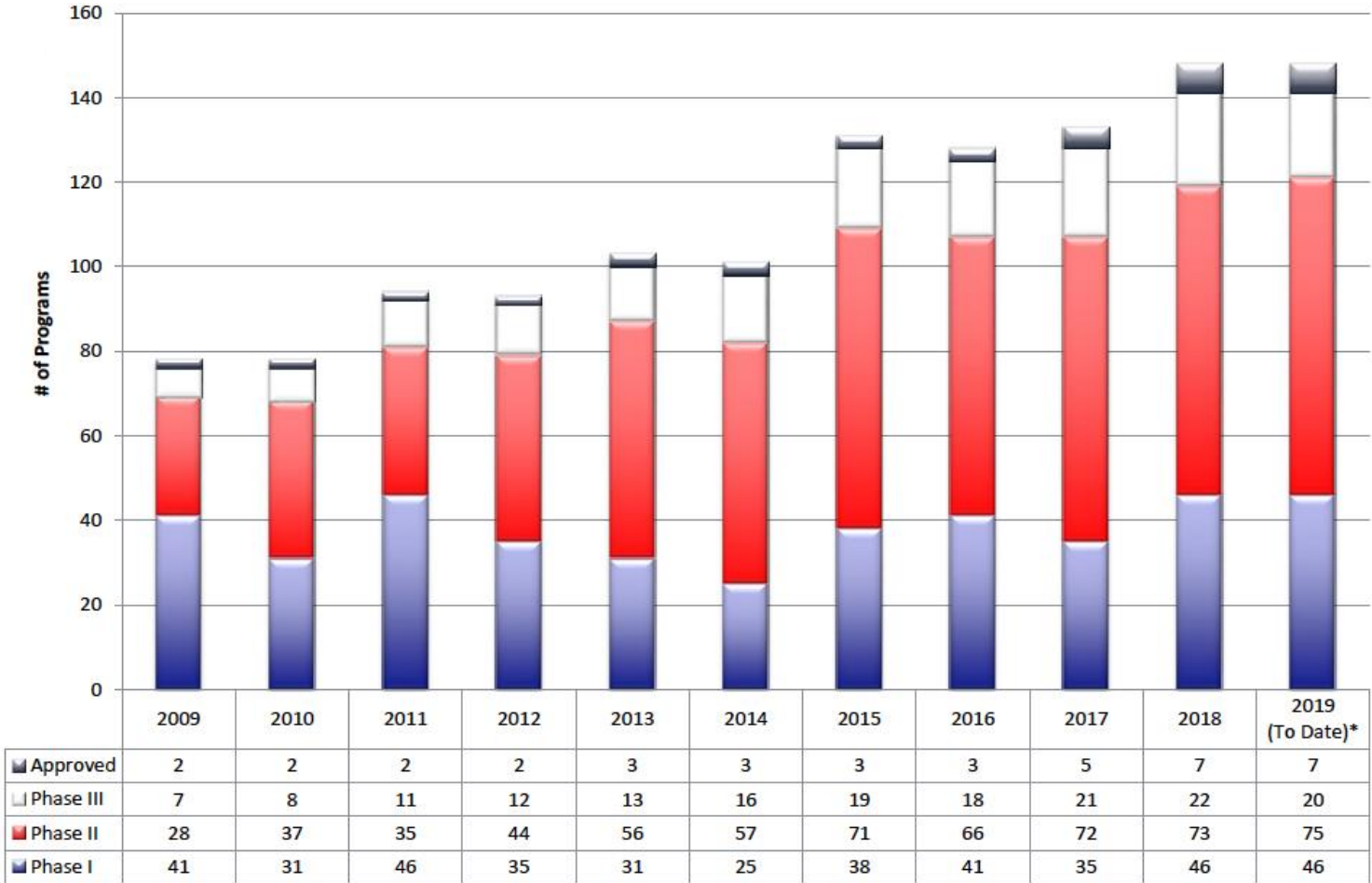
RNA-targeted drug discovery



Oligonucleotide chemistry and ligand drives durable, potent silencing in a given tissue.

Once chemistry is validated, lead compounds for new diseases can be found and validated with a timescale of months rather than years.

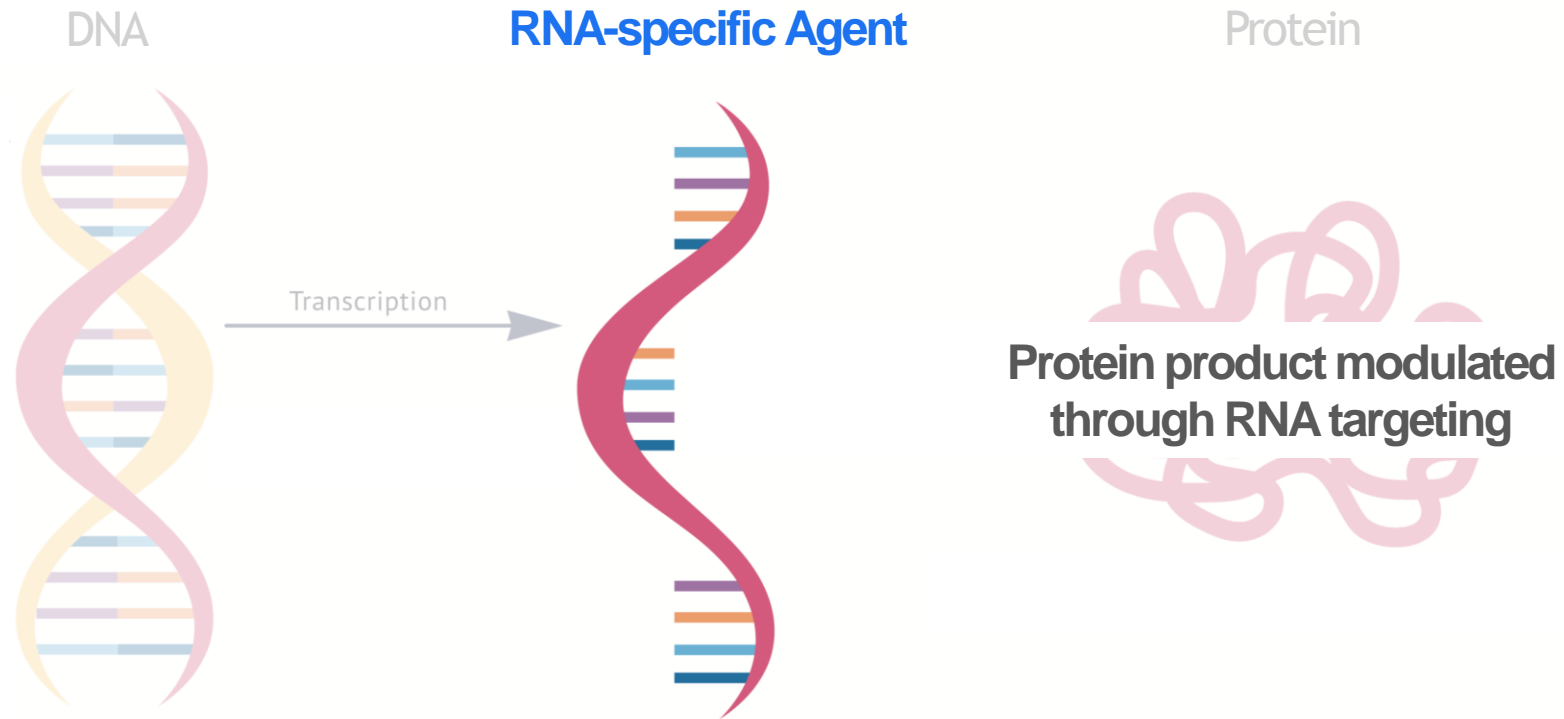
Oligonucleotide Therapies (development & approved)*



Credit: Art Krieg & Jon Watts

* as of March 2019 9

The *Central Dogma* of Molecular Biology

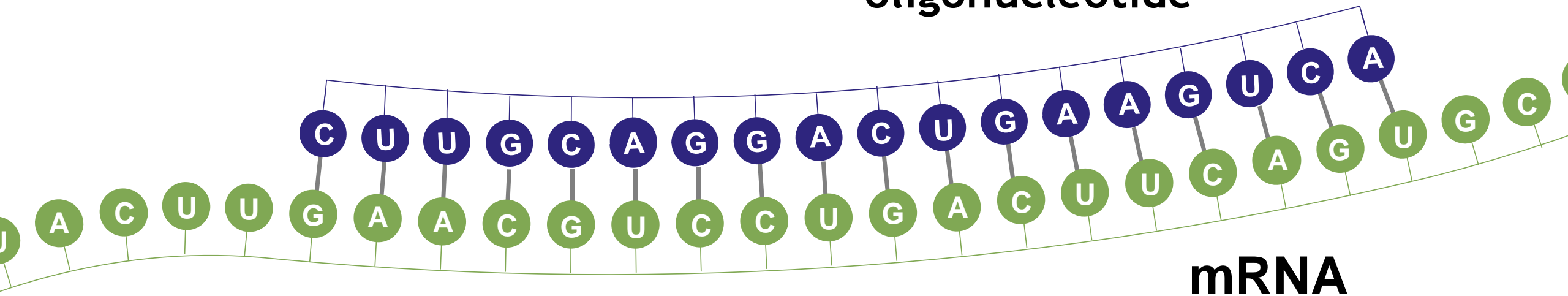


Targeting specific RNA through base pairing

Anti-RNA agent utilizing:

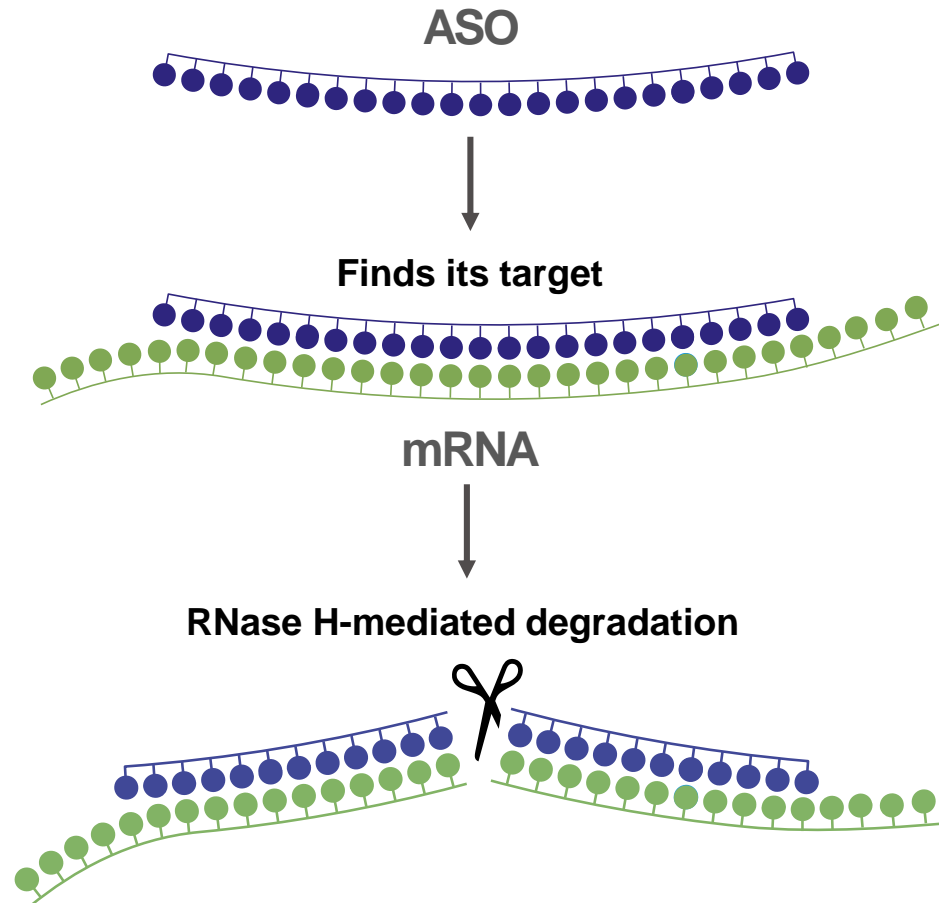
- Watson-Crick Base Pairing
- G-C or A-U or A-T
- Highly conserved
- High specificity

Complementary or “antisense”
oligonucleotide

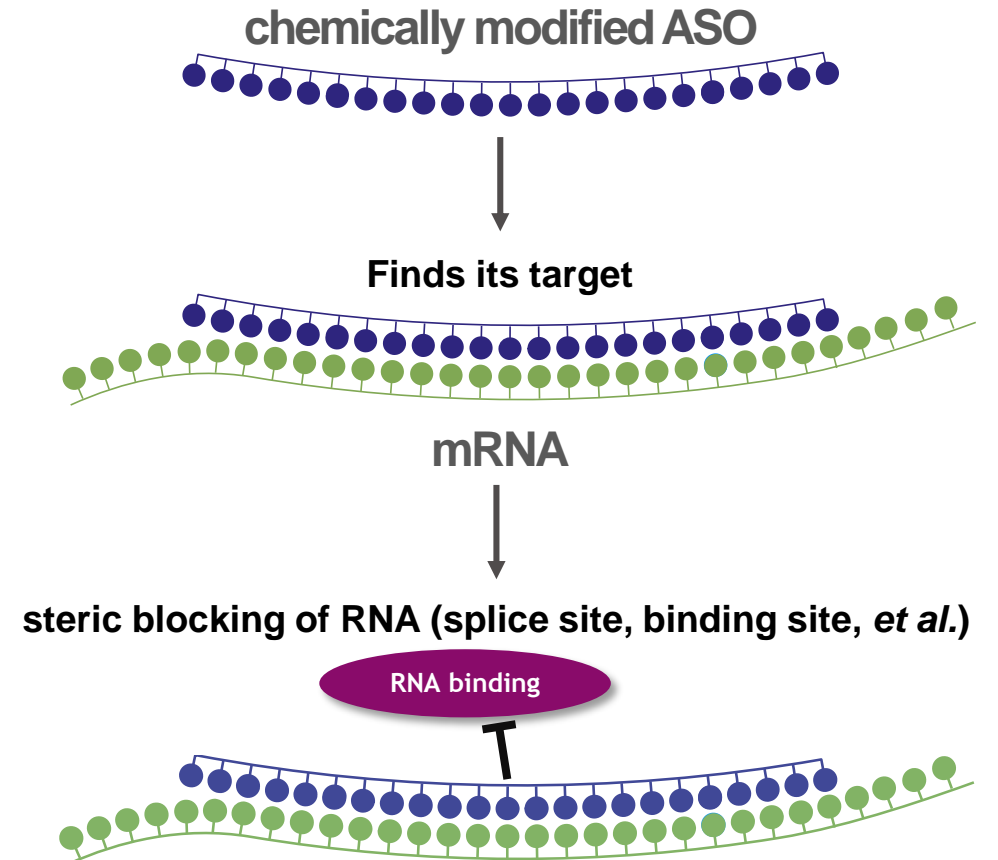


Two mechanisms of action

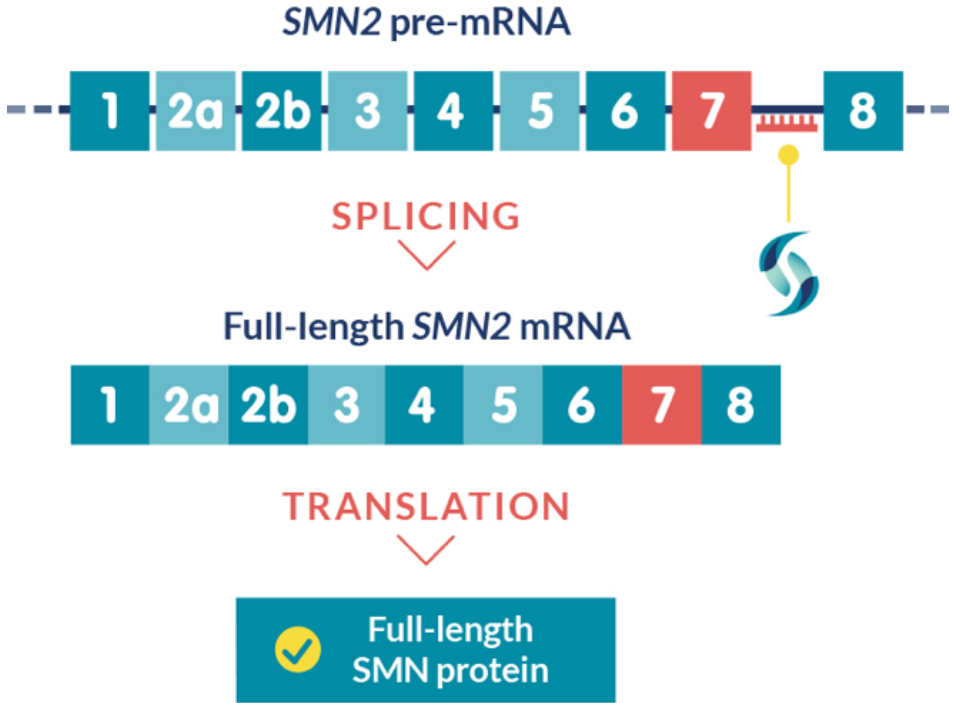
Silencing



Blocking

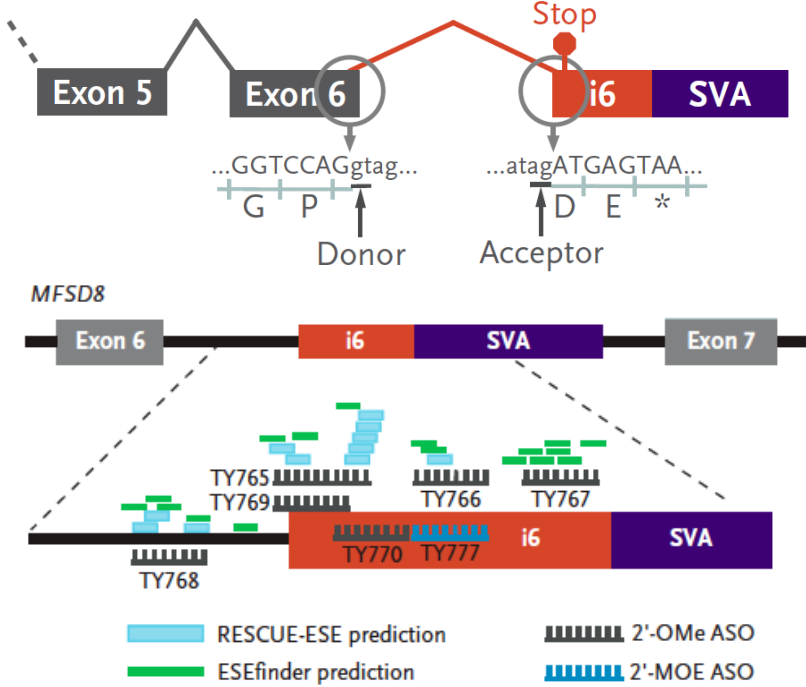


Correcting mis-splicing with ASOs



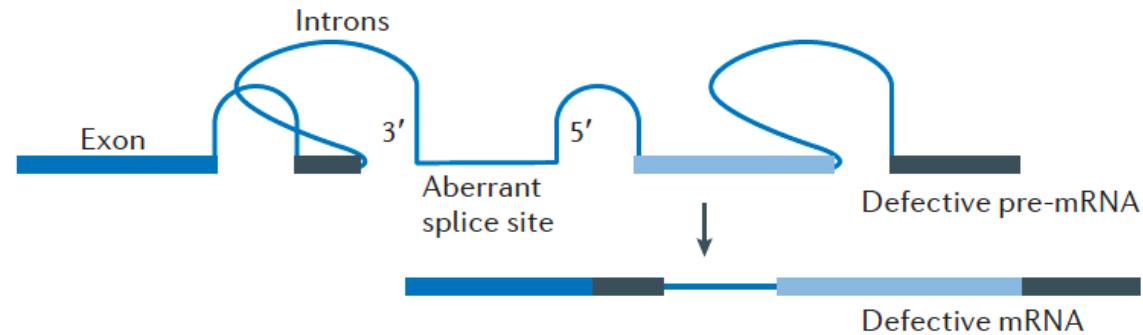
Spinraza/nusinersen

Abnormal MFSD8 Splicing and Translation after SVA Insertion

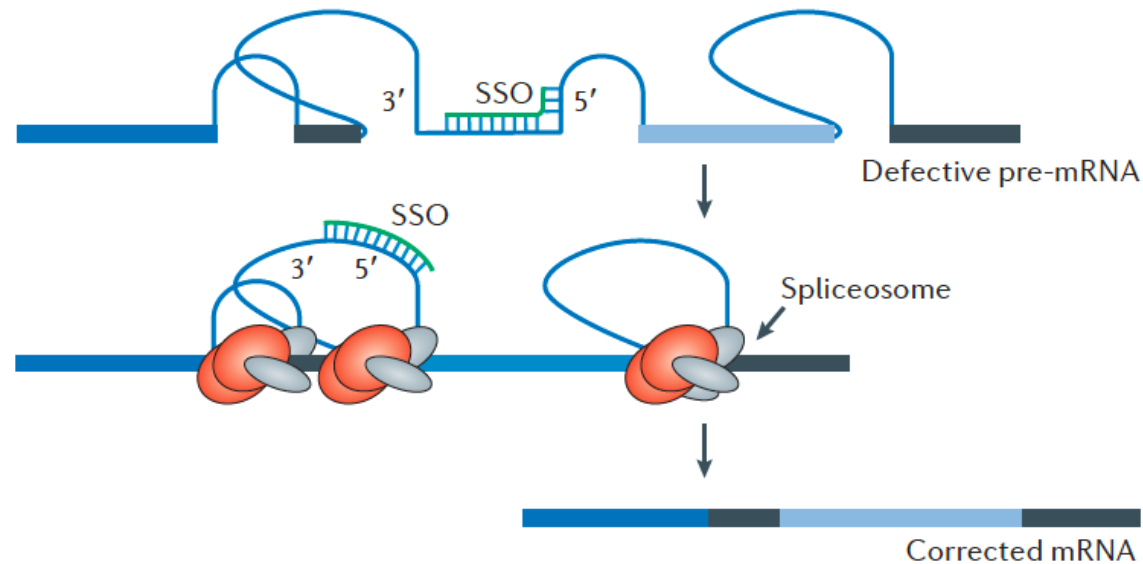


milasen

Upregulation by targeting non-productive splicing



- often degraded (NMD, other mechanisms)
- wasted transcriptional output
- NON-PRODUCTIVE isoform

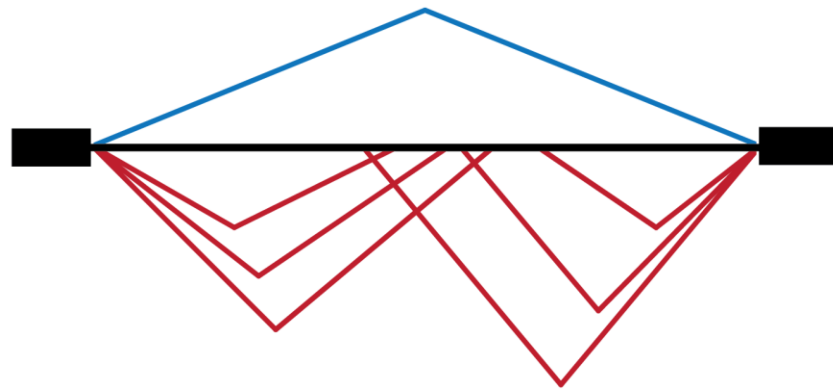


- translated into protein
- PRODUCTIVE isoform

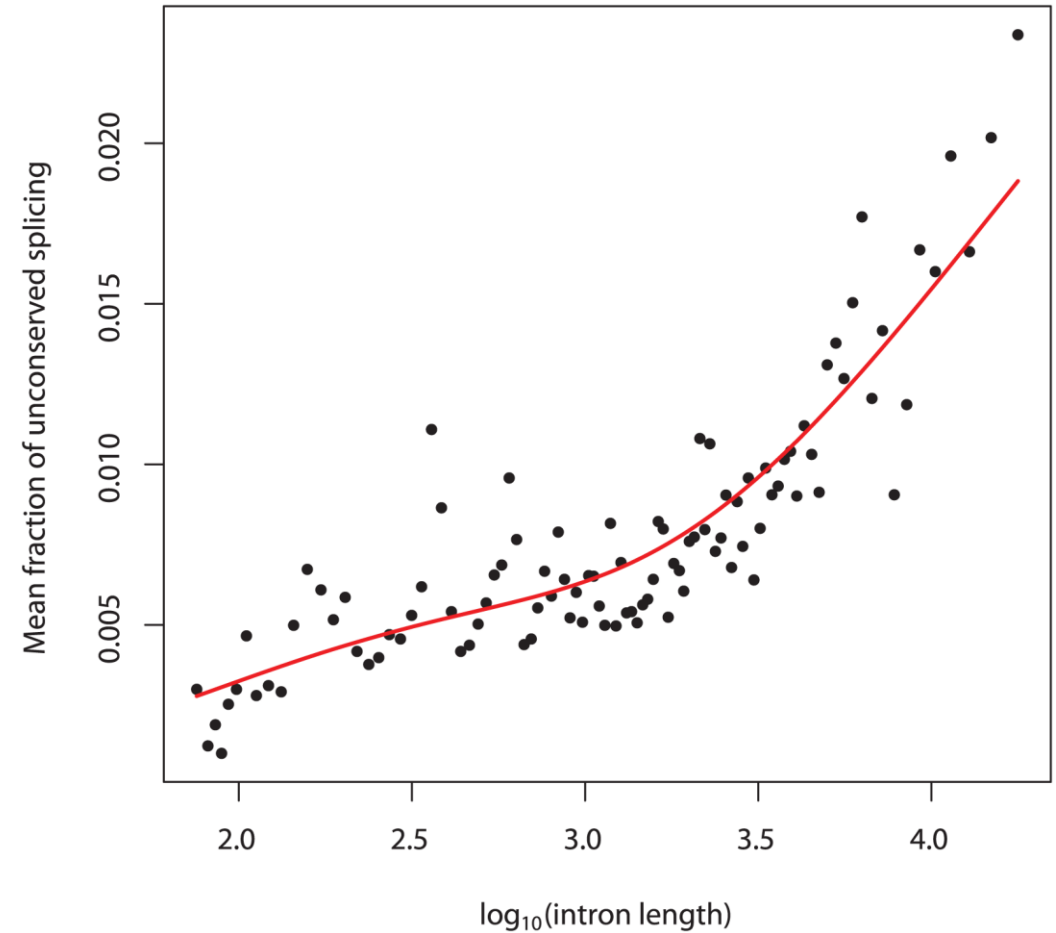
Can we re-direct products of wasted transcription from the healthy allele towards productive mRNA isoforms?

Targeting ASOs to block sites of non-productive splicing

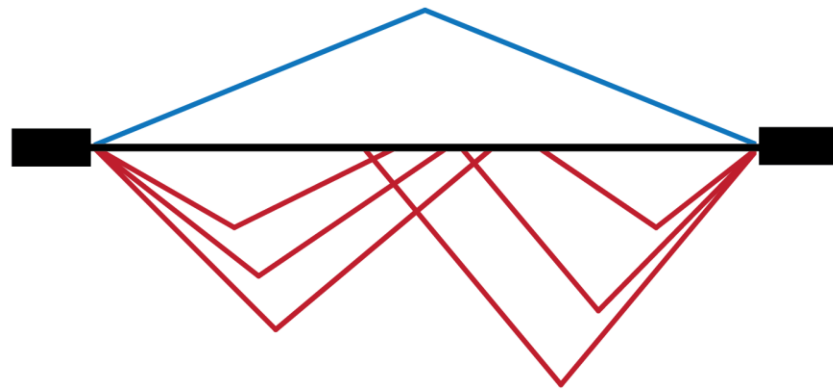
Non-productive splicing is common...



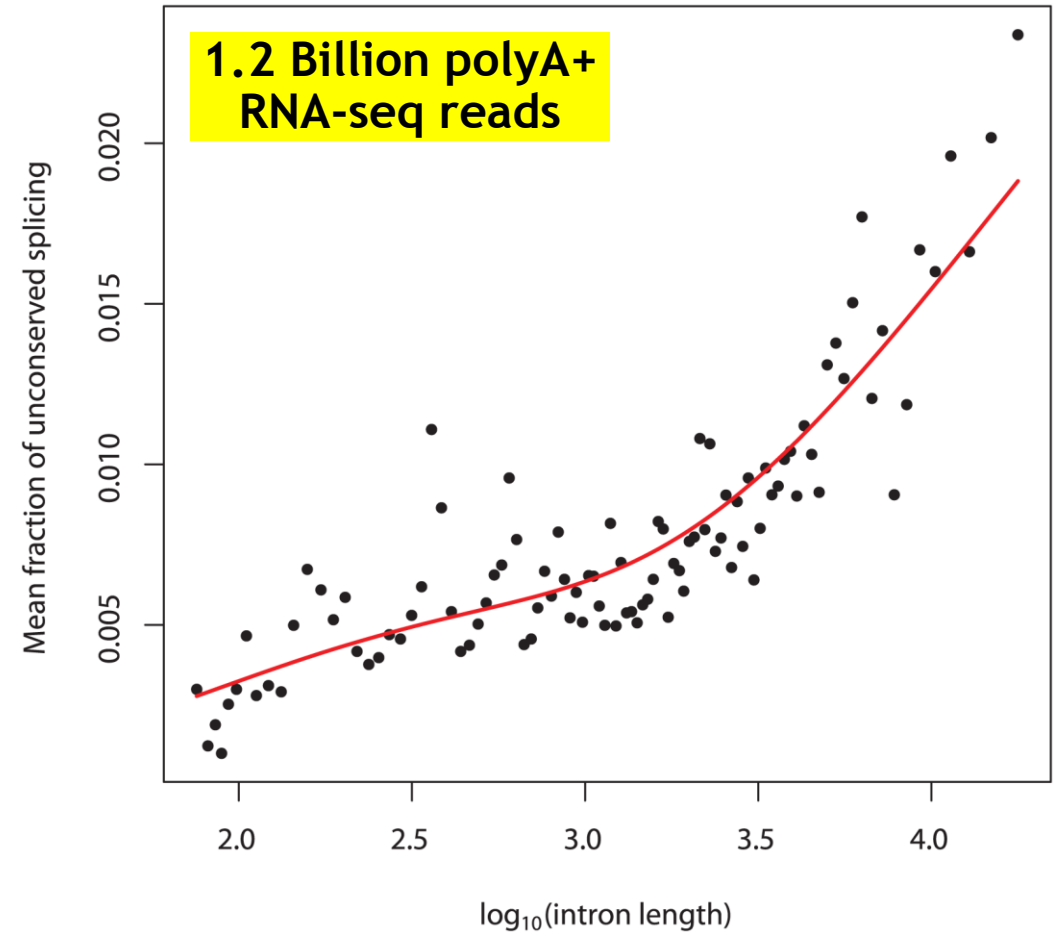
annotated
non-canonical, unannotated



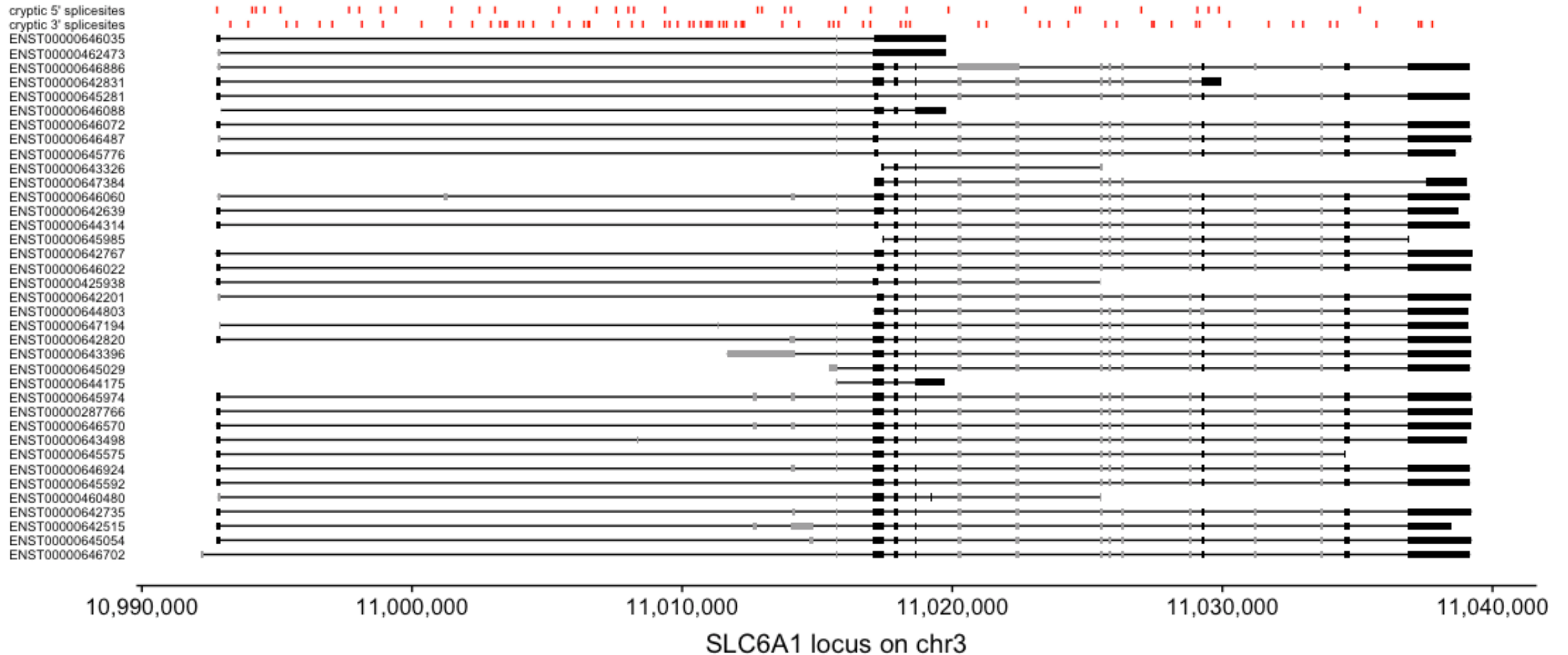
... but hard to find in current RNA profiling datasets



annotated
non-canonical, unannotated

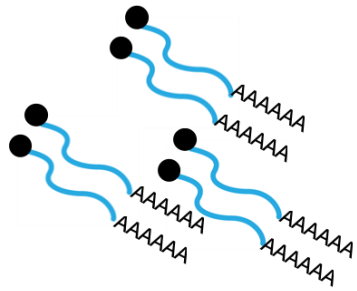


Computational prediction of non-productive splicing

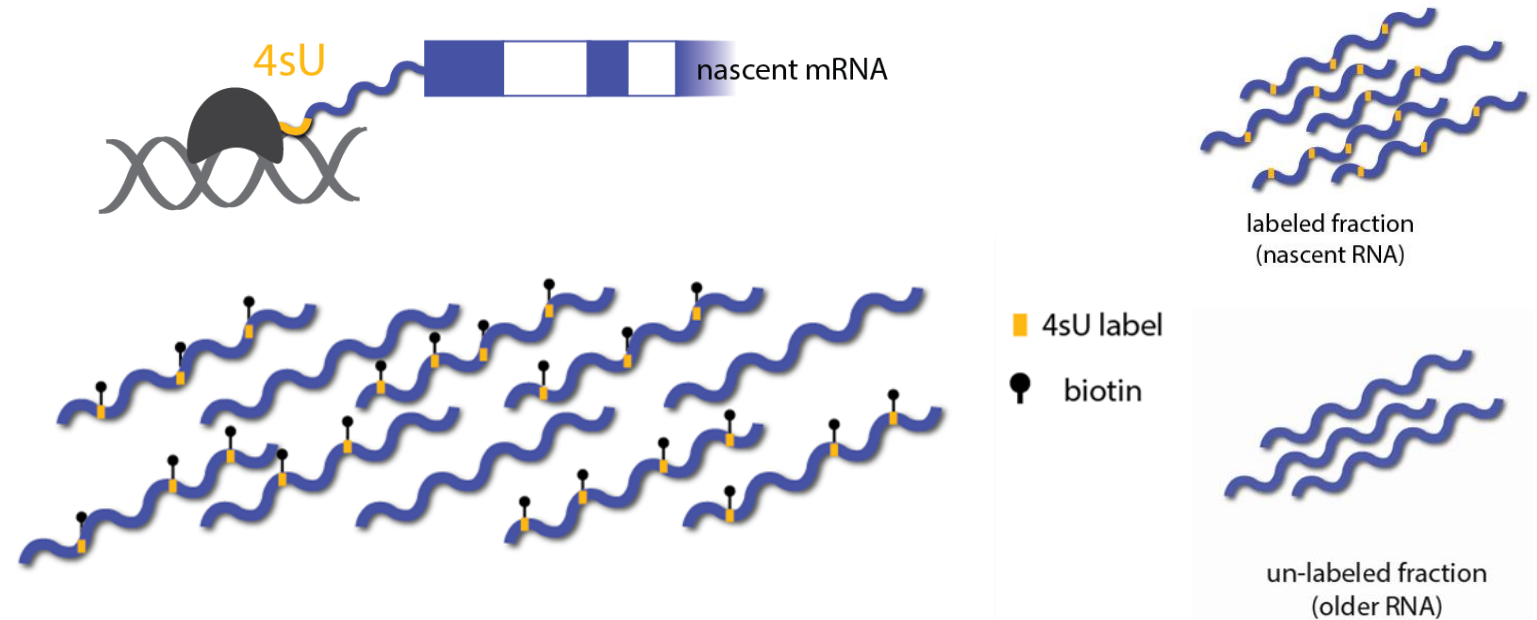
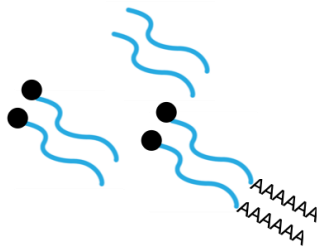


Experimentally identifying sites of non-productive splicing

RNA-sequencing captures mature, stable mRNA



Instead, capture nascent RNA molecules (before maturation & degradation)

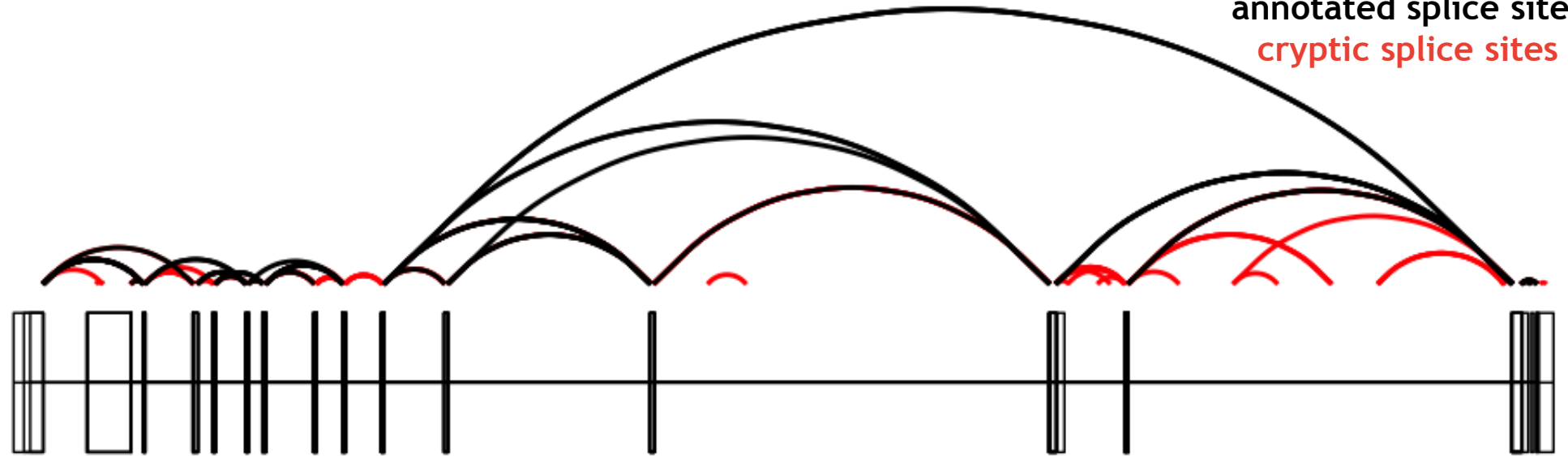


Sites of non-productive splicing in nascent RNA

30m 4sU labeling in SH-SY5Y cells

junction reads from nascent RNA-seq data

annotated splice sites
cryptic splice sites



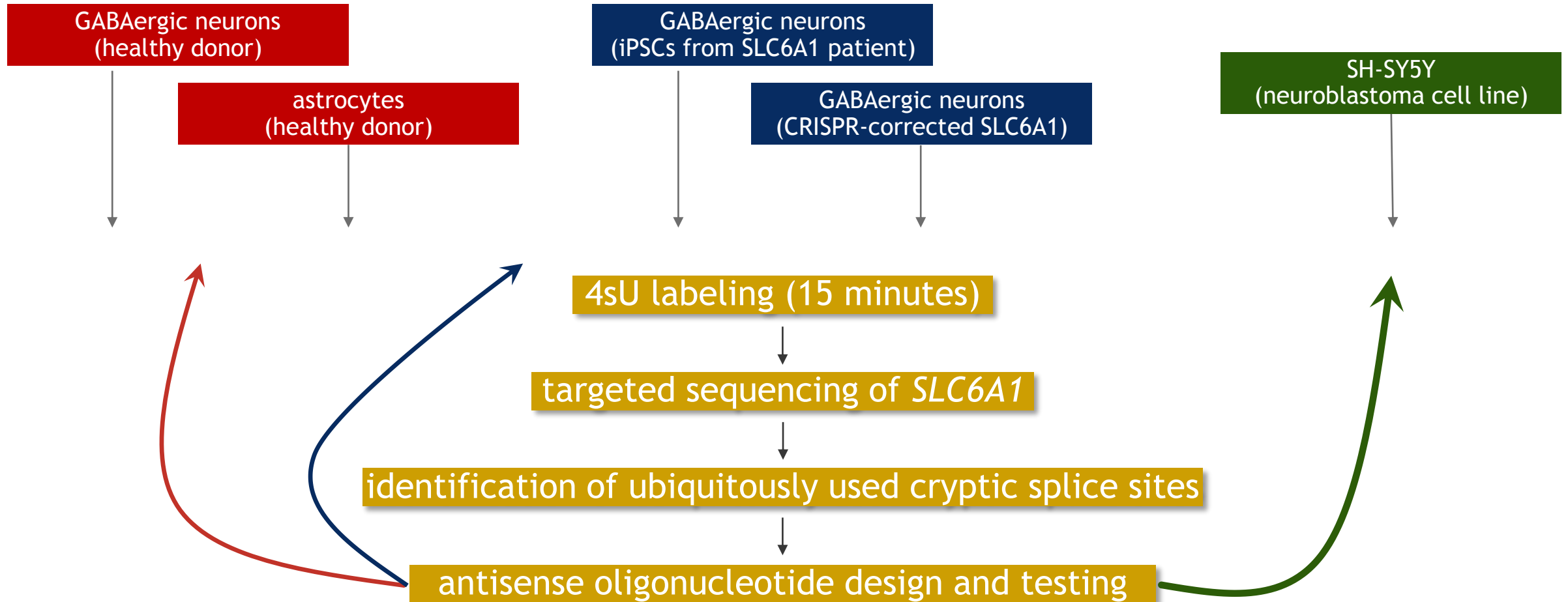
computational predictions (maxEnt)



-55700000 -55690000 -55680000 -55670000 -55660000

isoforms for **SLC6A2** on chromosome 16

Predicting sites of non-productive splicing in *SLC6A1*



Acknowledgements



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Jon Watts, Kaitlyn Valla & lab
Amber & Mark Freed
Myriam Mirza



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