



EUROPEAN FEDERATION
FOR MEDICINAL CHEMISTRY
AND CHEMICAL BIOLOGY



EFMC
Young Scientists
Network



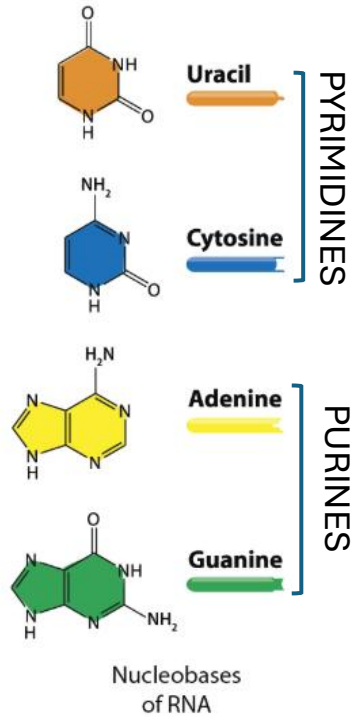
Targeting RNA with Small Molecules

Session Leader: Dr. Federico Riu, PostDoc at Italian Institute of Technology, Italy

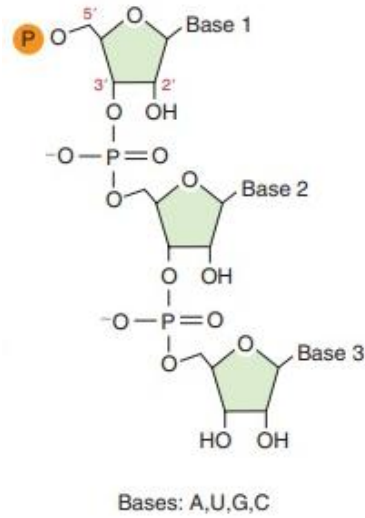
Case Study I: Elena Mencarelli, PhD student in Medicinal Chemistry at Université Côte d'Azur, France

Case Study II: Kateryna Demchenko, PhD student in Medicinal Chemistry at Université Côte d'Azur, France

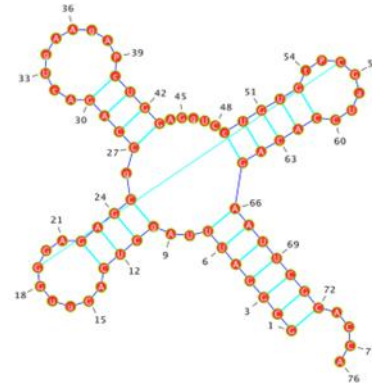
RNA: FROM PRIMARY TO TERTIARY STRUCTURE



PRIMARY



SECONDARY



TERTIARY



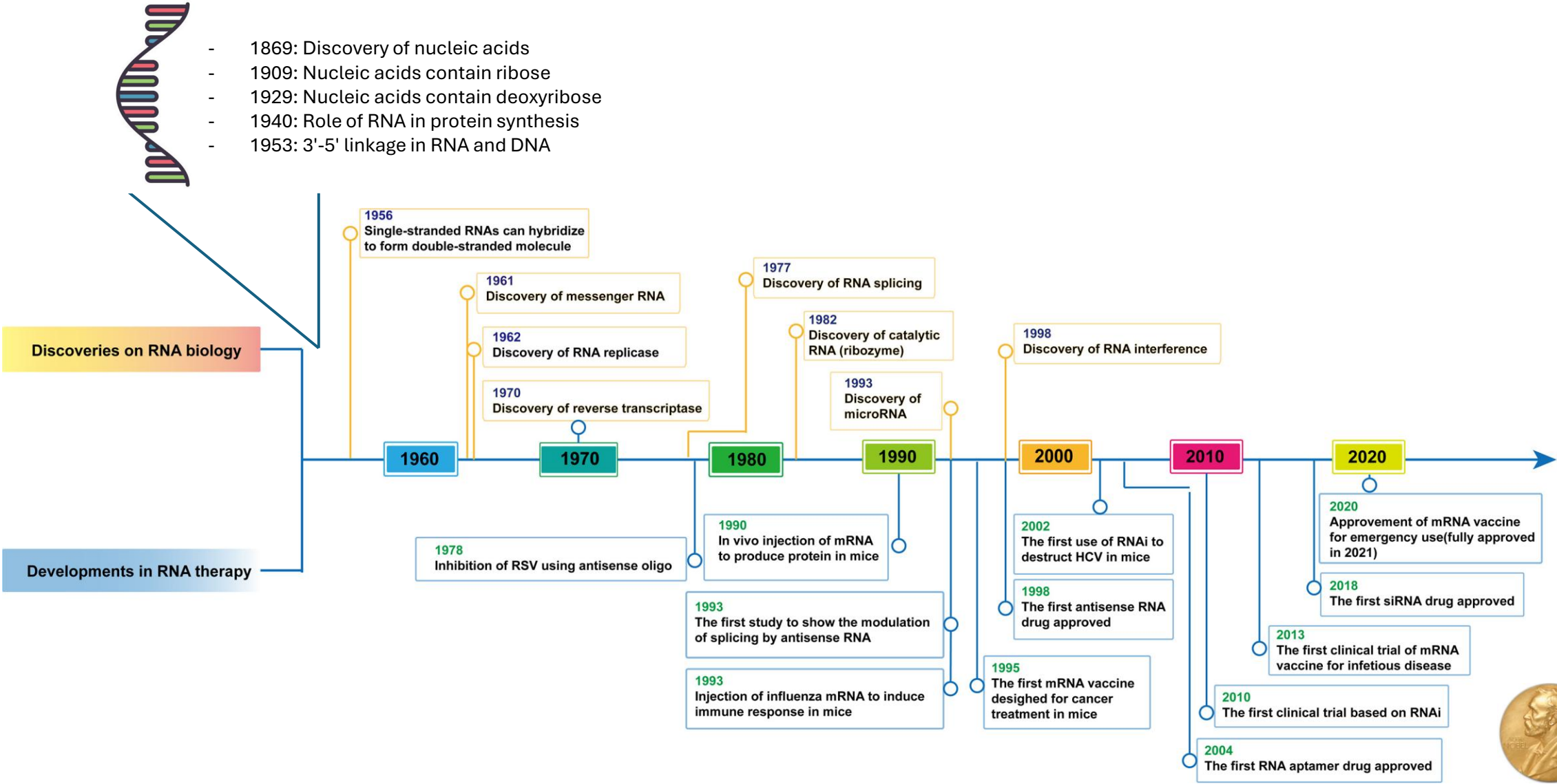
RNA (ribonucleic acid):

- Essential in all living cells
- Role in **protein synthesis** and **gene expression**
- Four bases: **A, G, C** and **U**
- 5-membered ring: **ribose**
- Covalent **phosphodiester** links



- 1869: Discovery of nucleic acids
- 1909: Nucleic acids contain ribose
- 1929: Nucleic acids contain deoxyribose
- 1940: Role of RNA in protein synthesis
- 1953: 3'-5' linkage in RNA and DNA

RNA: DISCOVERIES IN RNA BIOLOGY AND THERAPY DEVELOPMENT



RNA: NOBEL PRIZES RELATED TO RNA RESEARCH



•**1965 Nobel Prize in Physiology or Medicine:** mRNA (F. Jacob, A. Lwoff and J. Monod)



Andrew Z. Fire
Prize share: 1/2



Craig C. Mello
Prize share: 1/2

•**2006 Nobel Prize in Physiology or Medicine:** RNA interference (RNAi) for gene silencing (A. Fire and C. Mello)



•**2020 Nobel Prize in Chemistry:** method for genome editing using the CRISPR/Cas9 system (E. Charpentier and J. Doudna)



•**2024 Nobel Prize in Physiology or Medicine:** microRNA role in gene regulation (V. Ambros and G. Ruvkin)

•**1989 Nobel Prize in Chemistry:** catalytic properties of RNA as ribozymes (S. Altman and T. Cech)



•**2009 Nobel Prize in Chemistry:** ribosome structure and function (V. Ramakrishnan, T. A. Steitz, and A. E. Yonath)

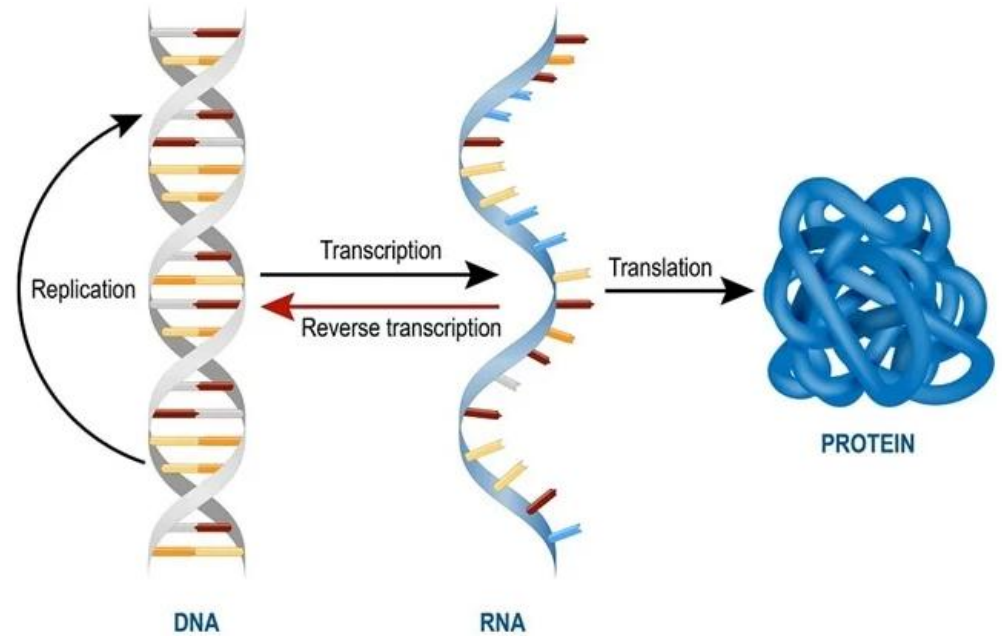


•**2023 Nobel Prize in Physiology or Medicine:** nucleoside base modifications leading to mRNA vaccine against COVID-19 (K. Karikó and D. Weissman)



Coding RNAs:

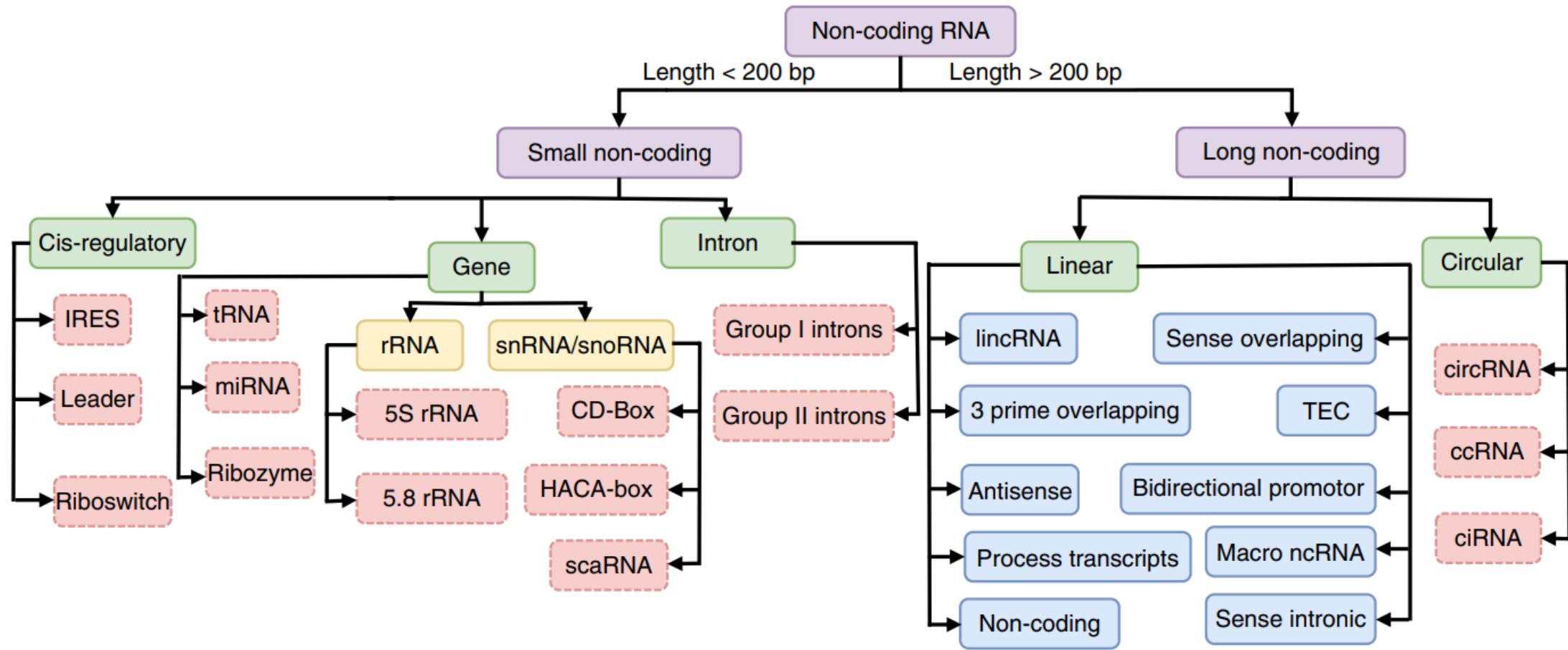
- Blueprint for protein synthesis: **mRNA**
- **Information transfer** from DNA to proteins



RNA: NON-CODING RNAs

Non-coding RNAs:

- Do not encode for proteins but produce **noncoding transcripts**
- Regulate **gene expression** and **protein function**

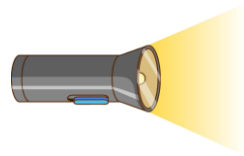


RNA INTO THE SPOTLIGHT: WHY SO IMPORTANT IN DRUG DISCOVERY

20,000
human
proteins

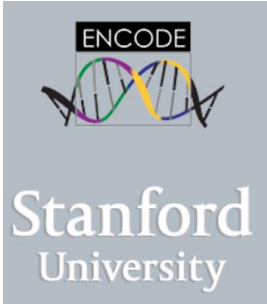
2500 are thought to be disease-related

<30% of these targeted by currently approved drugs



Inspiration to move past a ‘protein-only’ mindset!
RNA: unexpected diverse functions in healthy and diseased cells
e.g., COVID

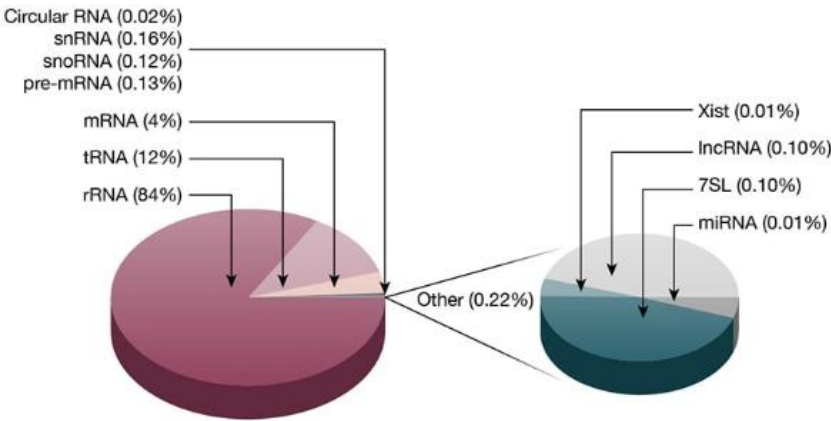
50 years ago, almost no RNA had an established function.....



70–90% of the genome is transcribed to RNA

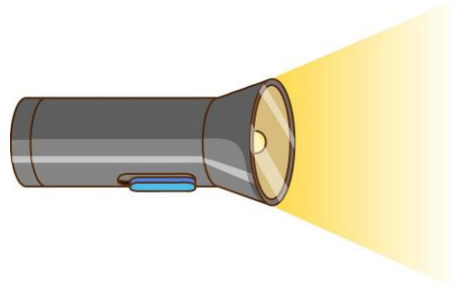
Coding RNAs: **3%** of those transcripts

**Most of the genome yields
ncRNAs, still to be
understood!!**



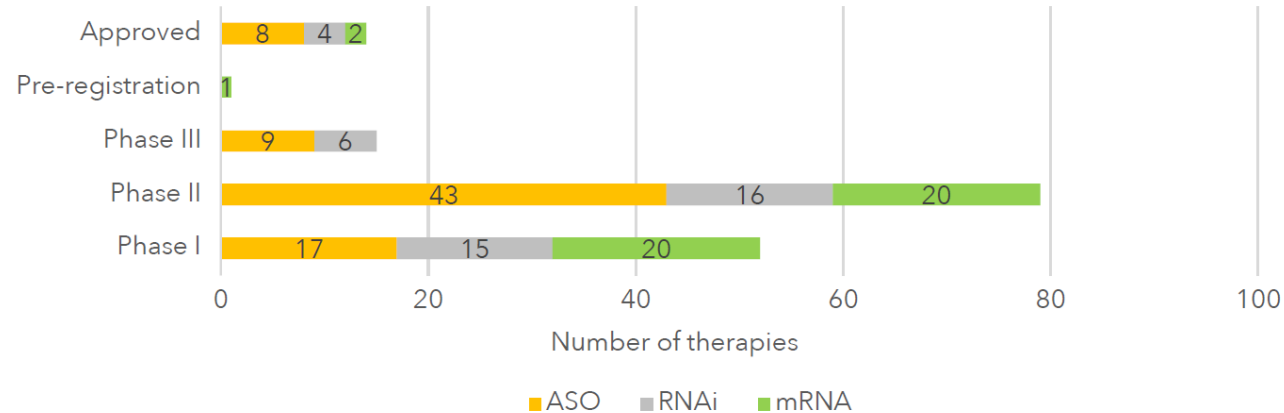
Abundances by mass

RNA-TARGETED THERAPIES



Oligonucleotide-based therapies

RNA-targeting small molecules



- Since 2018, **>30%** of FDA-approved drugs have been biologics
- In **2021**, there were around **700 RNA therapies currently in development** (preclinical to approval)
- **20 in Phase III clinical trials** (50% increase from 2020)

Oligonucleotide-based therapies



Antisense oligonucleotides (ASOs)

RNAi-based therapy

saRNAs promoting RNA activation (RNAa)

miRNA mimics

Aptamers

CRISPR/Cas9-based genome therapy

RNA-based vaccines

PITFALLS OF RNA THERAPEUTICS

- 1) **Susceptible to degradation and systematic clearance**
- 2) **Inability to cross the cell membrane and BBB**
due to their strong negative charge
- 3) **Activation of the innate immune system**



Despite challenges, **15** oligonucleotide-based drugs have been **approved by the FDA in the last two decades** with more being approved each year

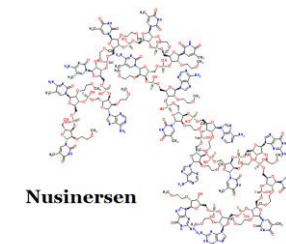
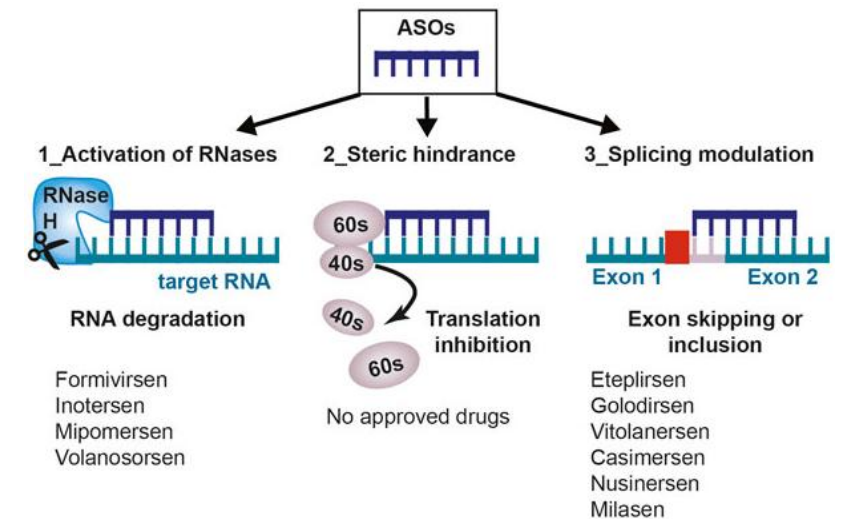
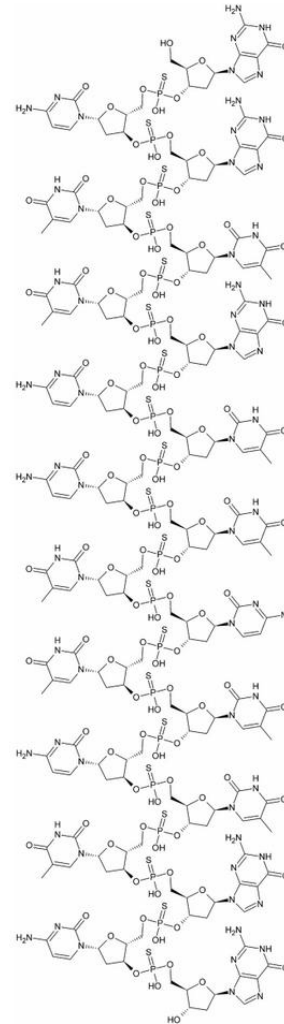
Antisense oligonucleotides (ASOs)

- Among the **first modalities** to be used
- Bind to **complementary sequences** in target RNA
- ASOs **inhibit or alter gene expression**

Fomivirsen

Vitravene, intravitreal injection!

- First FDA-approved ASO (1998), cytomegalovirus retinitis (CMV) in immunocompromised patients (e.g. AIDS)
- **9 ASOs** approved for five separate indications
- Challenges for **delivery, stability** and **hepatotoxicity**



Nusinersen
First FDA-approved Drug for
Spinal Muscular Atrophy, 2016



Eteplirsen for the
treatment of
Duchenne muscular
dystrophy, 2016

RNA-targeting small molecules: an emerging landscape

ADVANTAGES

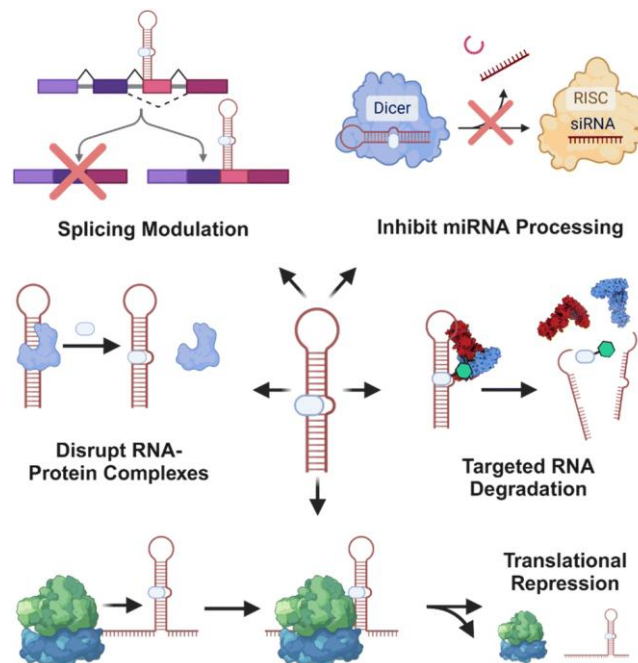
- Druglikeness (better PK features)
- Bind and modulate secondary-tertiary RNAs
- Complementary to ASOs
- Chemical tunability - medchem approach
- More convenient administration
- Lower cost

CHALLENGES

- Potential off-target effects
- Dynamic nature of RNA

 Problematic clinical delivery of oligo-based therapies led to renewed interest in small molecules

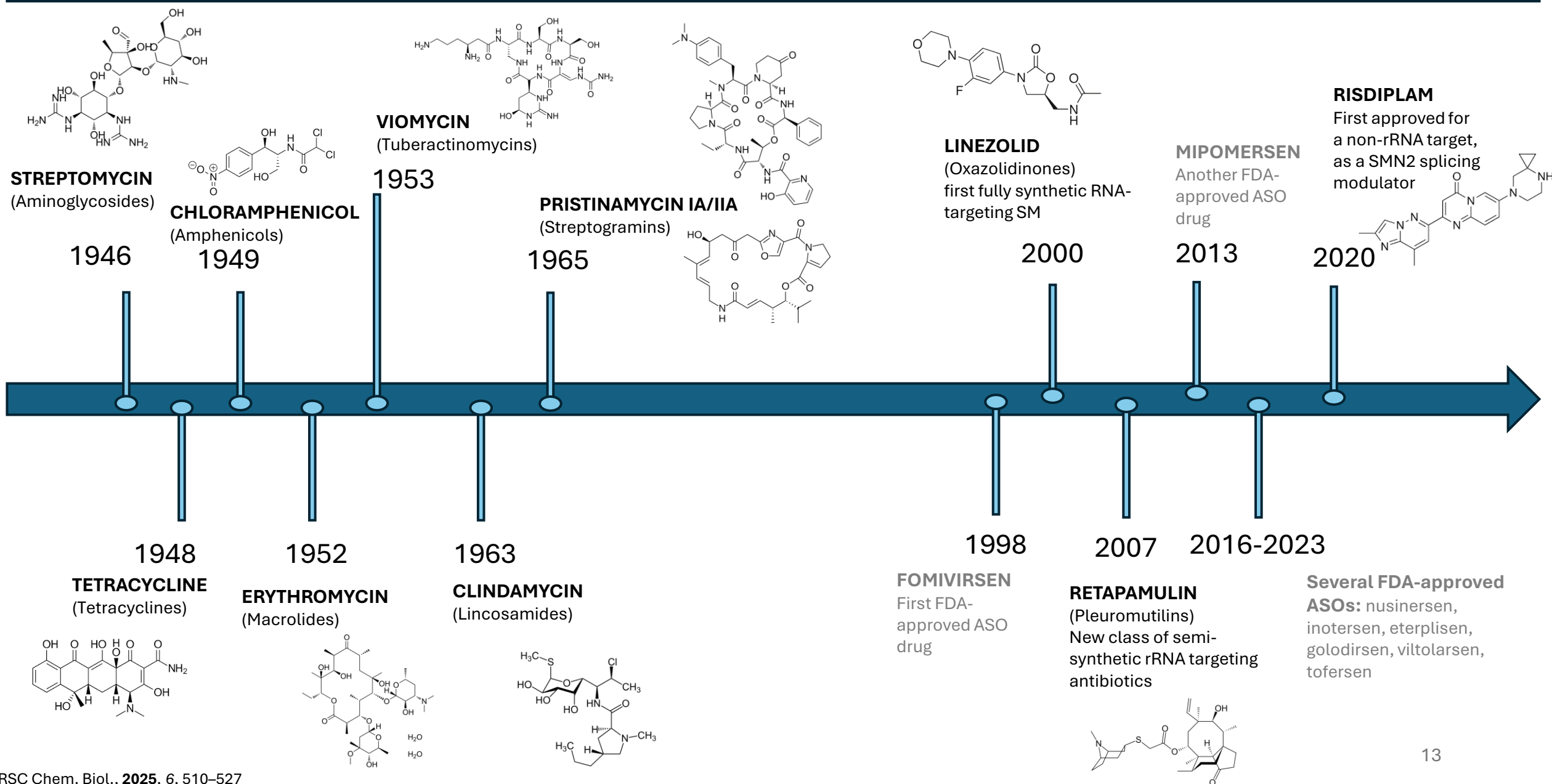
RNA-targeted small molecules remains less advanced than ASOs



RNA Targets for RTSMs:

1. **rRNA** (e.g. 16S rRNA)
2. **viral RNA** (e.g. HCV IRES or HIV TAR-RNA)
3. **mRNA** (e.g. riboswitches)
4. **ncRNA** (e.g. miRNA, lncRNA)
5. **Pre-mRNA** (e.g. introns, spliceosome)

DRUGGING RNA: RNA-TARGETING SMALL MOLECULES (RTSMs)



Small-molecule miRNA inhibitors (SMIRs)

miRNA

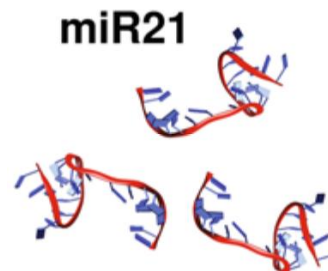
- Small single-stranded, ncRNAs that post-transcriptionally **regulate gene expression**
- Aberrant expression observed in **diseases**: cancer and neurological pathologies
- They were perceived as “**undruggable**”
- **SMIRs** (HTS/structural approach): bind/inhibit miRNAs to prevent interaction with target mRNAs

! Recognition of mRNA through sequence complementarity and recruitment of nucleases to **repress mRNA expression**

Deiters *et al.* (University of Pittsburgh), 2008

- First small molecule miR inhibitor (**miR-21**)

overexpression in **cancers** (*neuroblastoma, colorectal, lung, leukemia and lymphoma...*)



78% decrease in miR-21 levels
87% decrease in pri-miR-21 levels
HeLa cells

- Primary screen of >1000 compounds (10 μ M)
- Hit 1 $\rightarrow$ **optimization** process $\rightarrow$ potent and selective SMIR 2



EC₅₀ = 2 μ M

Long non-coding (lncRNA) targeting SMs

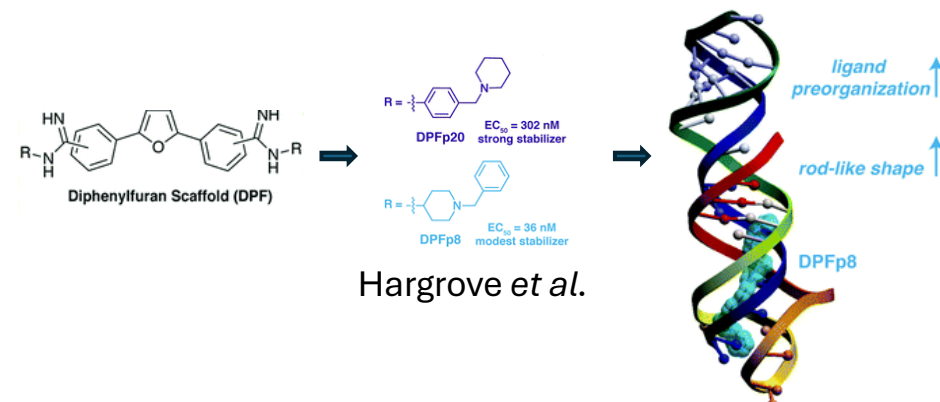
lncRNA

- >200 nucleotides (10000 lncRNAs!)
- **Cellular functions:** regulation of chromatin, transcriptional regulation, ...
- Effort to better understand **binding pockets** within lncRNAs
- Research into SM inhibitors still **under development**

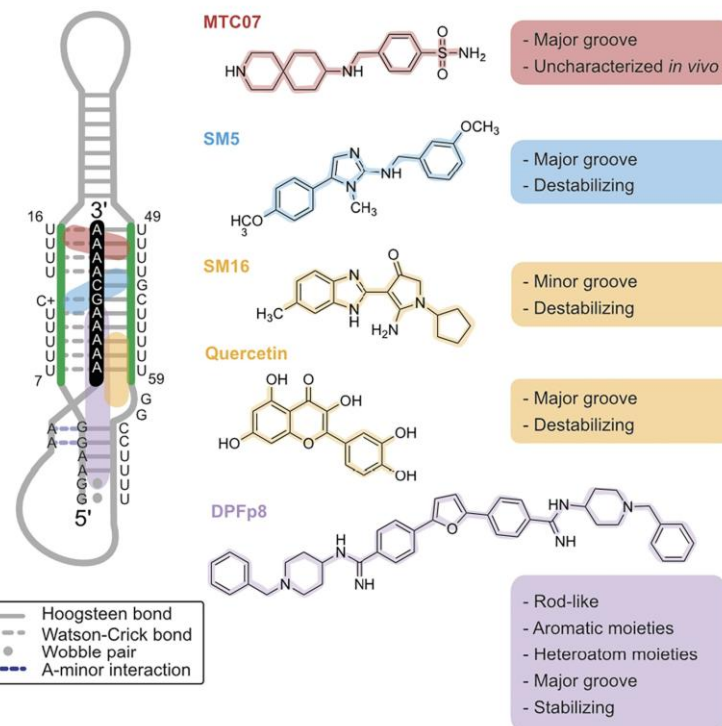
MALAT1 lncRNA

lncRNA metastasis associated lung adenocarcinoma transcript 1 (important in normal pre-mRNA splicing)

- In oncology, MALAT1 lncRNA is **overexpressed** leading to cancer proliferation
- **DPFp8** was the first small molecule targeting the triple helix
- **SM5** and **SM16**: modulate triple helix dynamics
- Reduces MALAT1 transcript abundance in **breast cancer models**



MALAT1 3' triple helix

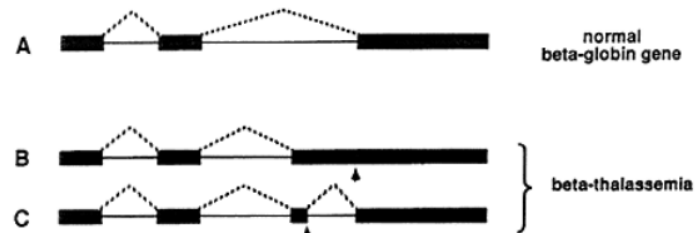


Pre-mRNA targeting SMs - Splice modifiers

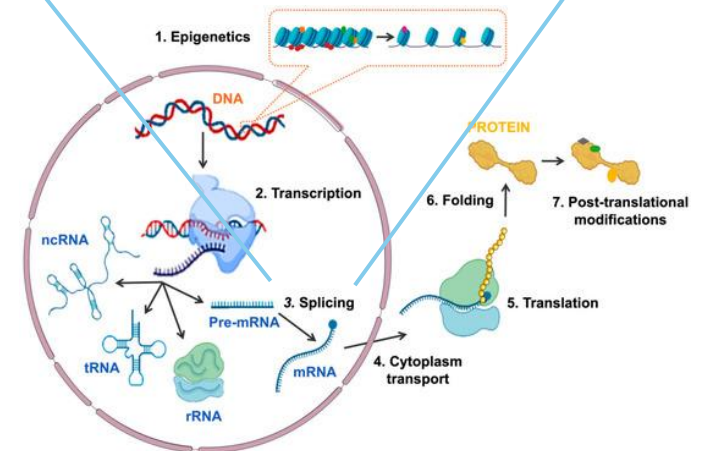
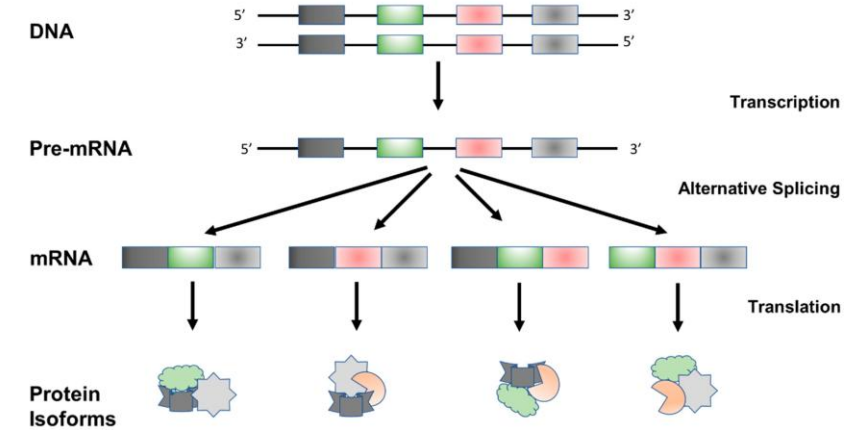
Phillip Sharp and Richard Roberts, 1993
Nobel Prize in Physiology or Medicine -
discovery of "**split genes**"



- Splice means **JOIN**
- **Introns** (*non-coding seq*) and **exons** (*coding seq*)
- In the nucleus, before the mRNA can be exported to the cytoplasm



Defective splicing causing beta-thalassemia
shortage of healthy red blood cells and anemia



DRUGGING RNA: RNA-TARGETING SMALL MOLECULES (RTSMs)

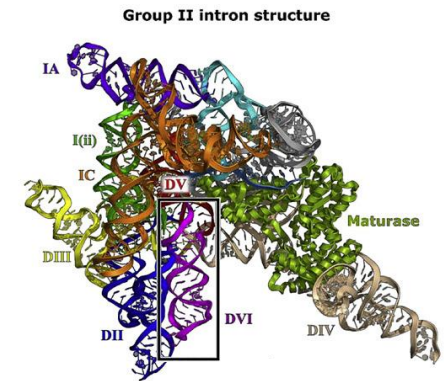
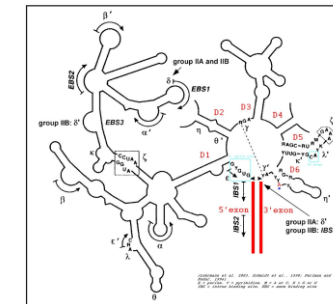
Pre-mRNA targeting SMs - Splice modifiers

Group	Splicing mechanism	Location
Group I	Self-splicing, uses external guanosine cofactor	Bacteria, organelles, bacteriophages, and some eukaryotic nuclei
Group II	Self-splicing, forms a lariat structure during splicing and can involve reverse transcriptase	Chloroplasts, mitochondria, bacteria, and some archaea
Spliceosomal introns	Processed by the spliceosome in the nucleus	Nuclear pre-mRNA in eukaryotes
tRNA (or BHB) introns	Spliced by a specific endonuclease	Nuclear tRNA in eukaryotes and various RNAs in archaea

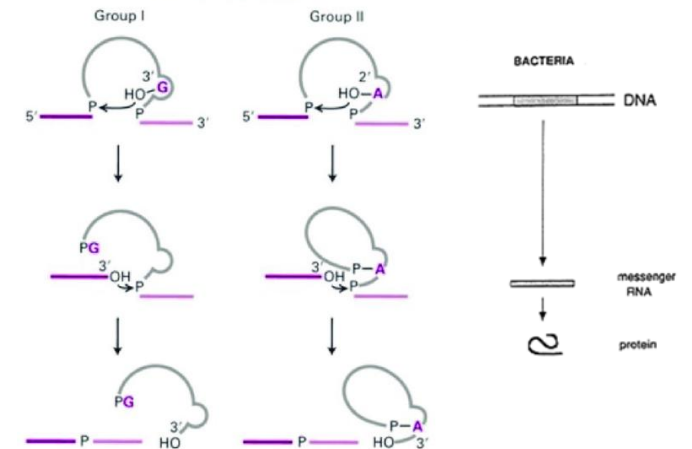
Introns as a Potential Target for Antibacterial or Antifungal Compounds



Intron as autocatalytic self-splicing (2 steps)



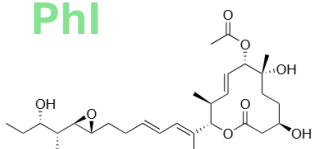
Self-splicing introns



DRUGGING RNA: RNA-TARGETING SMALL MOLECULES (RTSMs)

Pre-mRNA targeting SMs - Splice modifiers

PhI



E7107

(Pladienolide B)

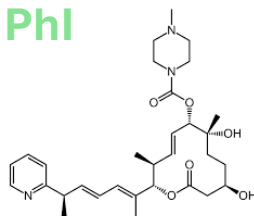
Suspended (Toxicity)

First of a new class of **anticancer** agents targeting the spliceosome

- Interacts with **SF3b**
- **Blocks splicing** of oncogenes



PhI



H3B-8800

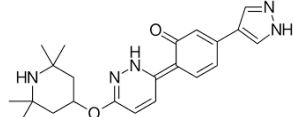
Granted orphan drug status by FDA in 2017 clinical trials for several **types of leukemia**

PhI (2016-2024): **insufficient efficacy** at the tested dose levels

- **Modulator** of a spliceosome complex **SF3b**



PhIIb



Branaplam

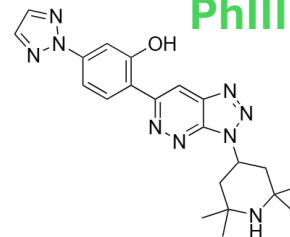
July **2021**: discontinued clinical development for **SMA**

PhIIb trial in **HD**
FDA granted orphan HD
Dosing suspended in **2022**

- **Stabilizes** double-strand structure formed by the SMN2 pre-mRNA and U1 snRNP complex



PhIII



PTC518

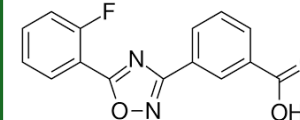
(Votoplam)

PhIII for **Huntington's disease** (HD)
PhII completed positive results in **2024**

- Promoting **inclusion** in mRNA of a 'pseudoexon' containing a **premature stop codon**



Approved
(EMA - Feb 2014)



Translarna

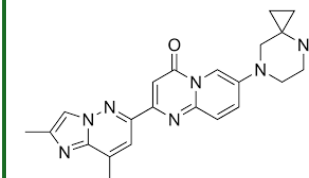
(Ataluren)

In **2025**, EMA discontinued: effectiveness not confirmed. Available in UK.

Reads through a stop codon in **Duchenne's muscular dystrophy (DMD)**



Approved
(FDA - Aug 2020)



Risdiplam

(Evrysdi)

Treatment of **spinal muscular atrophy (SMA)**
Phenotypic screen

Oral medication

- **Stabilizes** an RNA-protein portion between SMN2 pre-mRNA and U1 snRNP, to direct splicing



DRUGGING RNA: GENERAL APPROACHES TO IDENTIFY RNA-BINDING SMALL MOLECULES

HTS of large, diverse libraries against multiple targets

- Substructure-based similarity searching
- Fluorescence-based screening
- Affinity-based screening
- Small molecule microarray
- Fragment-based screening
- DNA-encoded library screening
- Virtual screening (Docking and ML)
- Phenotypic screening

Ligand-based

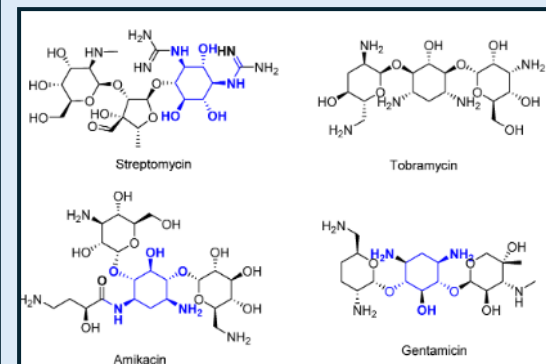
Target-based

"System-based"

2-DOS = 2-deoxystreptamine
DAP = 3,5-diaminopiperidine

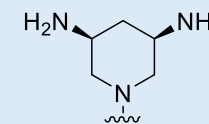
Zhou et al., 2007

Known RNA-targeting SMs



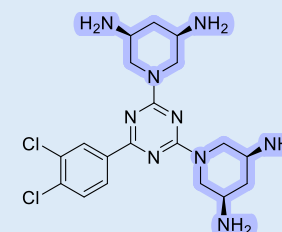
2-DOS as conserved core scaffold between **aminoglycosides** binding 16S rRNA

Identified important substructure



DAP: retained RNA-binding (synthetic mimetic)

Substructure in a new scaffold



Low rRNA affinity increased via attachment to **triazine**

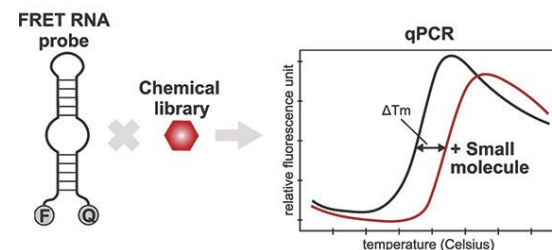
>55 3,5-diamino-piperidine antibacterial **aminoglycoside mimetics**
In vitro transcription-translation assay against *E. coli* (IC₅₀ = 7 μM)

DRUGGING RNA: GENERAL APPROACHES TO IDENTIFY RNA-BINDING SMALL MOLECULES

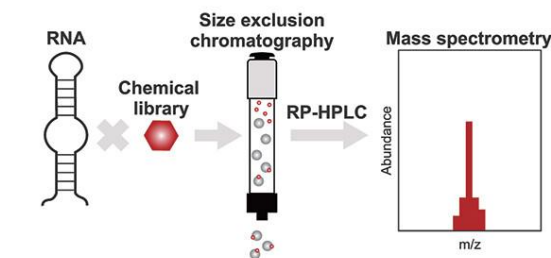
HTS of large, diverse libraries against multiple targets

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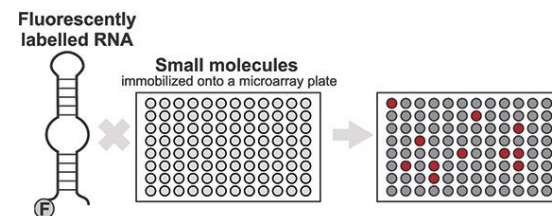
a. Fluorescence-based screening



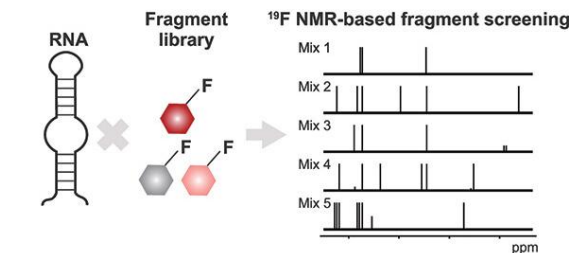
b. Affinity-based screening



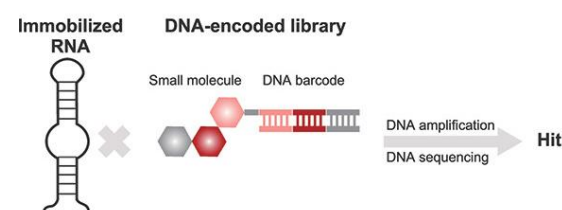
c. Small molecule microarray



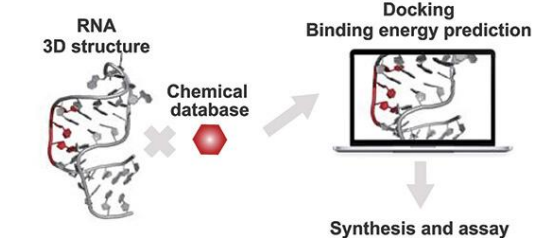
d. Fragment-based screening



e. DNA-encoded library screening



f. Virtual screening



g. Phenotypic screening

