Gene Therapy (III)

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Objectives of this lecture

By the end of this lecture you will be able to:

1. Describe the different strategies for gene therapy
2. Select the suitable strategy based on the clinical case
3. Understand the complexity of clinical application of gene therapy
4. Evaluate proposed strategies according to the therapeutic need

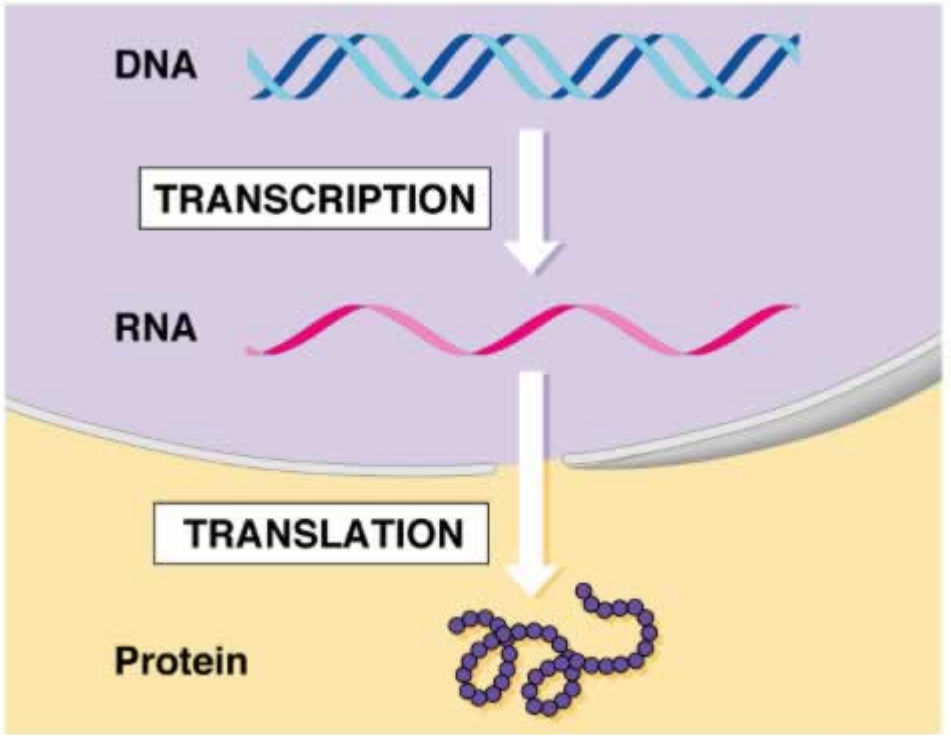
Gene Therapy Strategies

- **Replacement of a missing or defective gene**
- **Introduction of gene(s) to influence cellular process**
- **Interference with gene products**

Interference strategy

- Downregulation of gene expression at the mRNA level

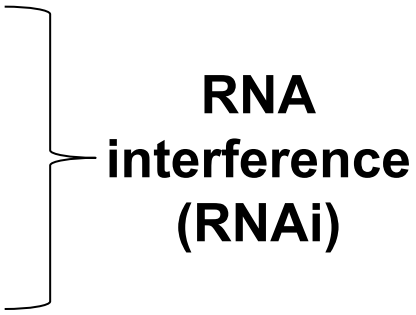
- Inhibition of mRNA translation



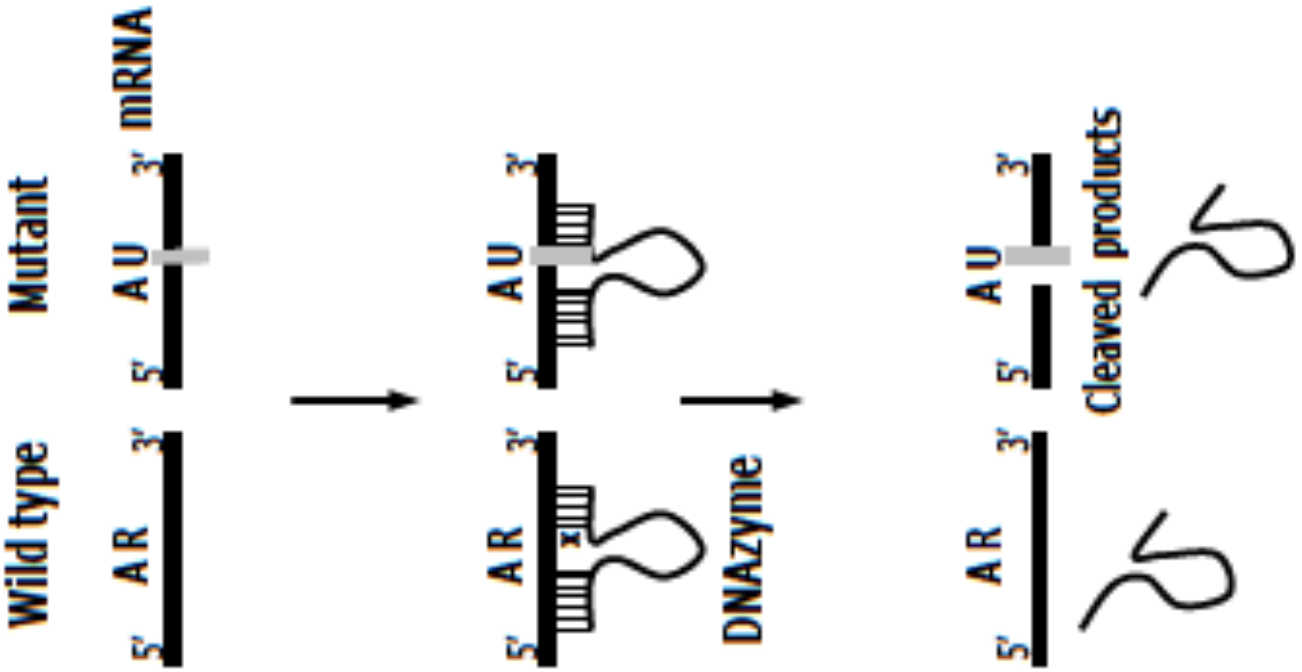
Interference nucleic acids

- DNA
  - Antisense oligodeoxynucleotide (ODN)
  - DNAzyme

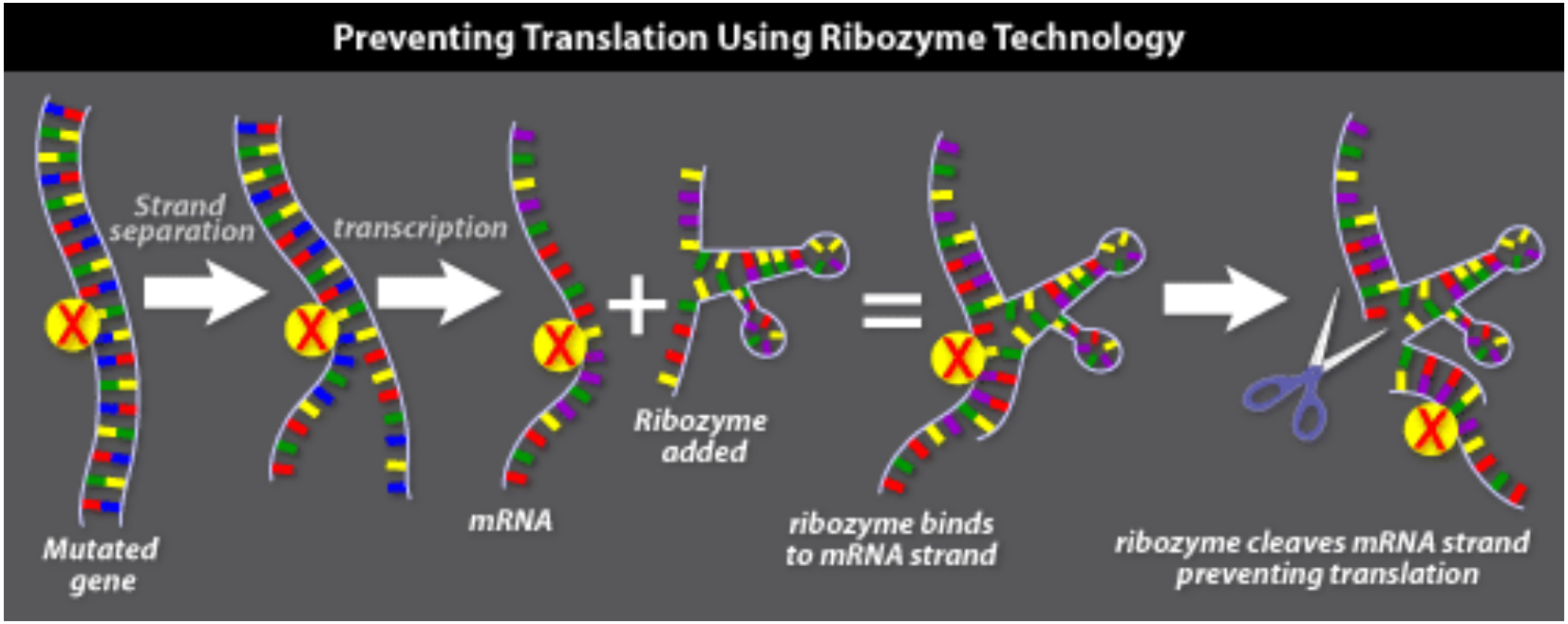
- RNA
  - Antisense RNA
  - Ribozyme
  - Small interfering RNA (siRNA)
  - Short hairpin RNA (shRNA)
  - microRNA (miRNA)



DNAzyme

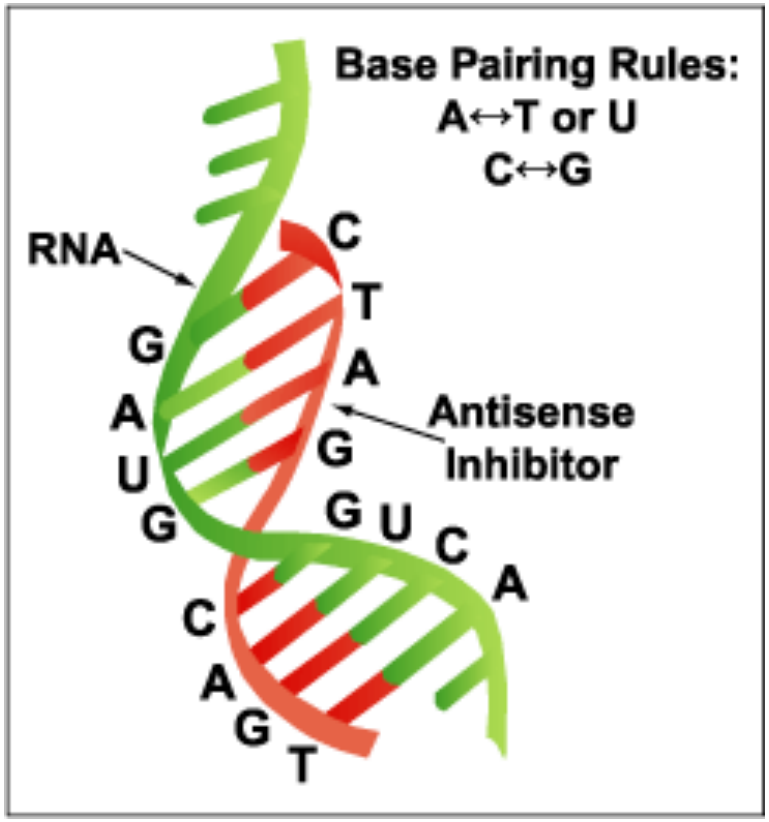


Ribozyme

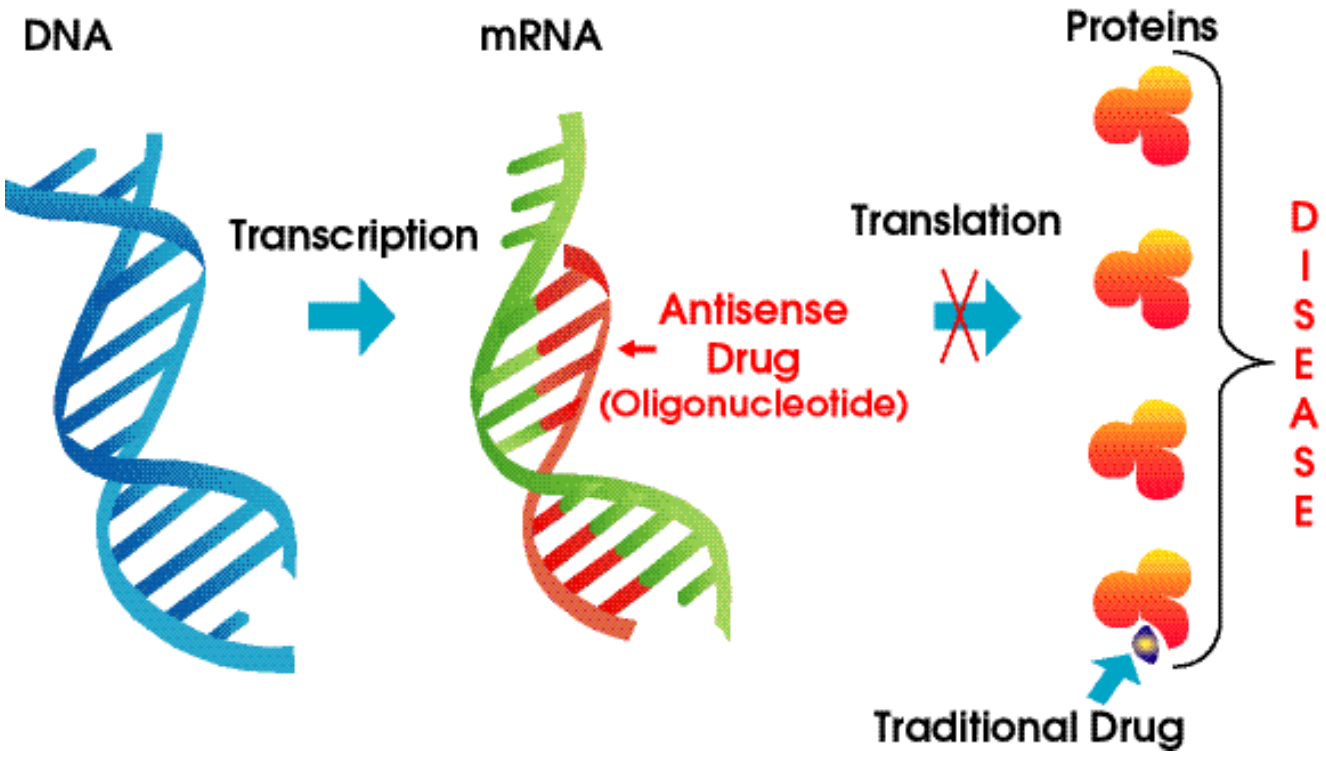

Preventing Translation Using Ribozyme Technology
Strand separation
transcription
Mutated gene
mRNA
Ribozyme added
ribozyme binds to mRNA strand
ribozyme cleaves mRNA strand preventing translation

Antisense ODN

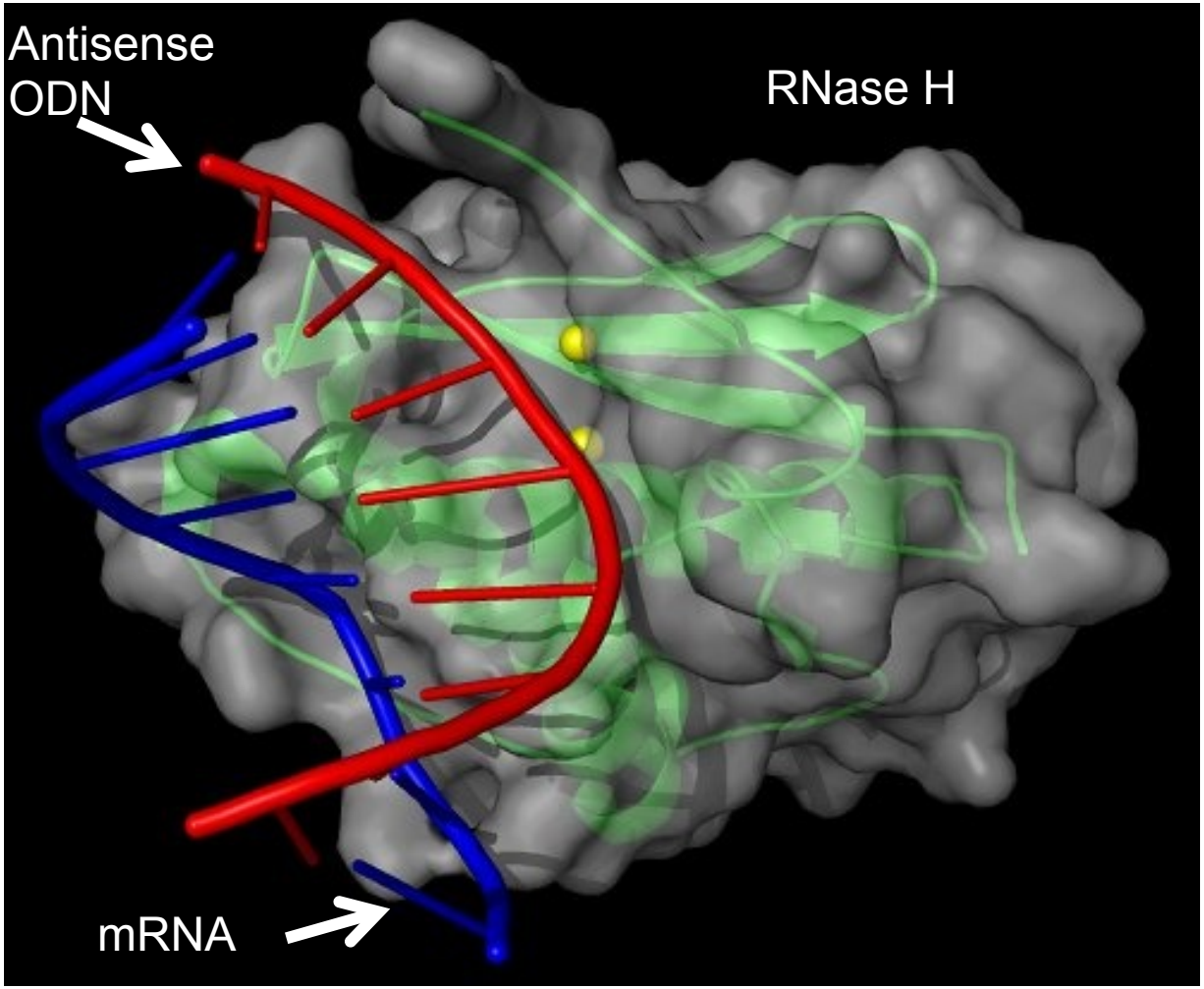
- Sequence-selective oligonucleotide that can bind to a target mRNA to inhibit gene expression i.e. to inhibit translation



Antisense ODN



Antisense ODN



Disadvantages of Conventional Therapy

- Requires screening of thousands of compounds to find an active molecule.

- Lacks specificity of action.

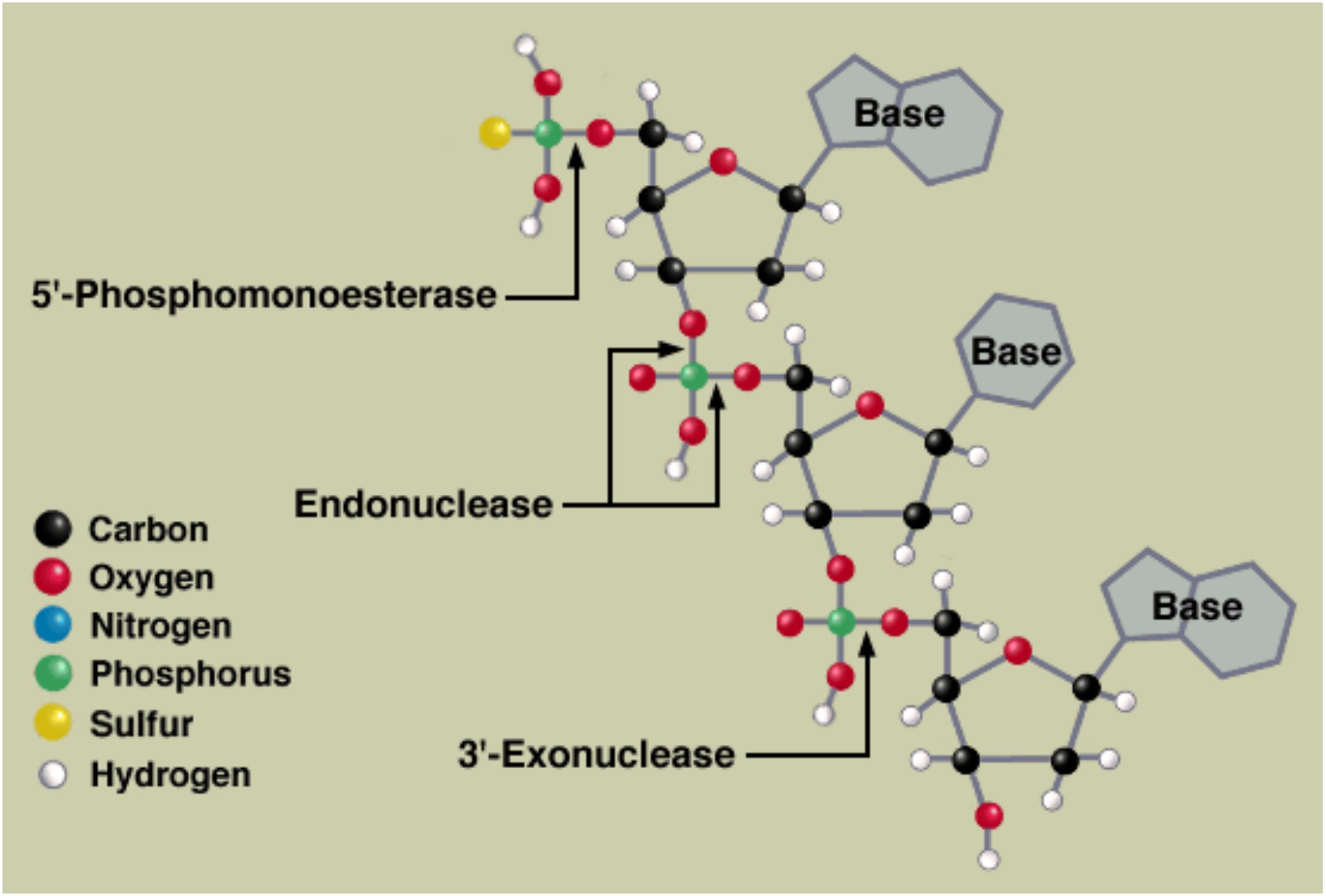
We don't have antisense ODN for every disease

- The main barrier to antisense strategy is ***optimal delivery*** in ***sufficient quantities*** to the ***correct target*** and for the ***desired time*** frame to achieve the desired level of gene inhibition

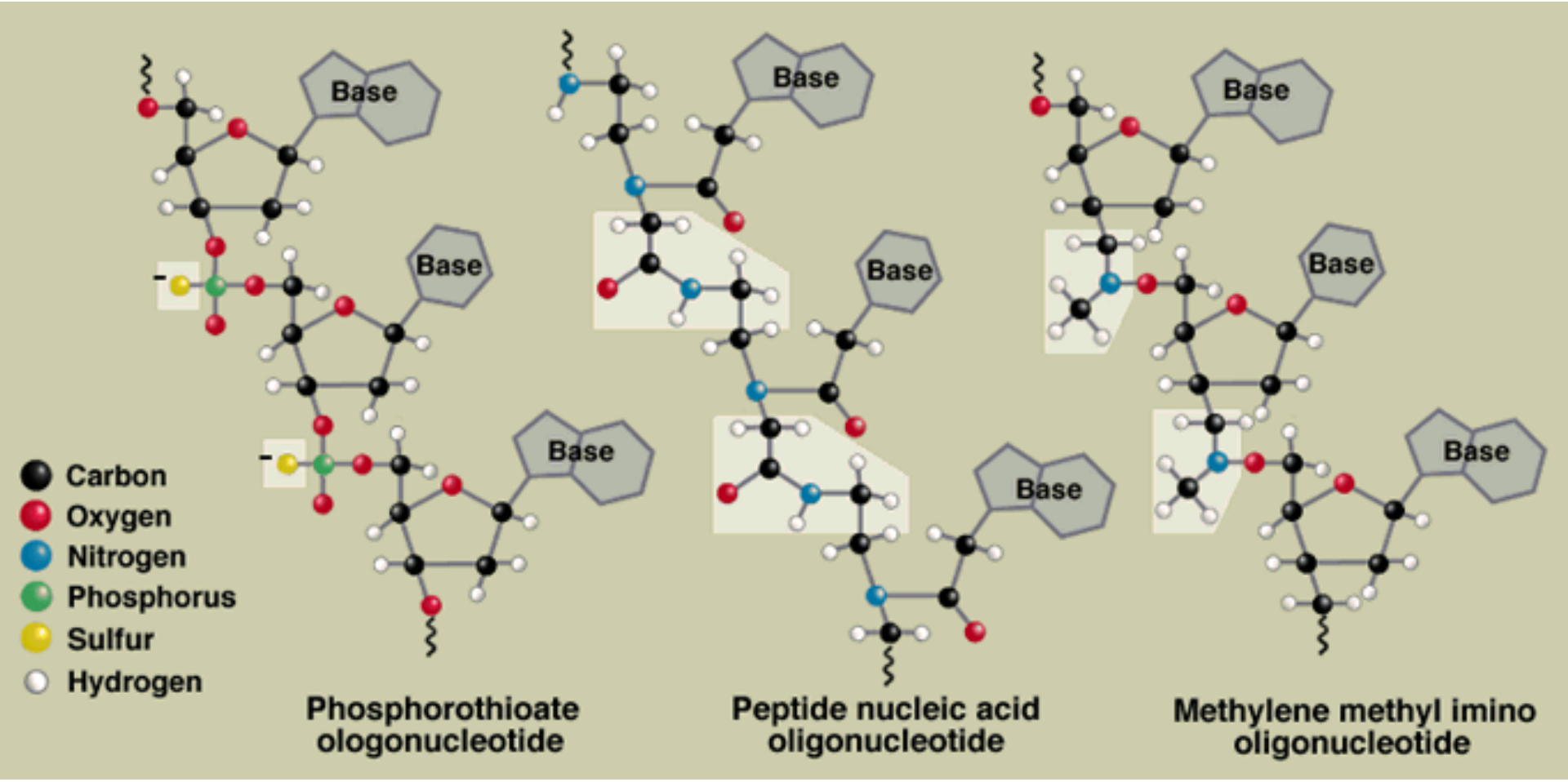
- ODNs are polyanionic macromolecule (large and charge)

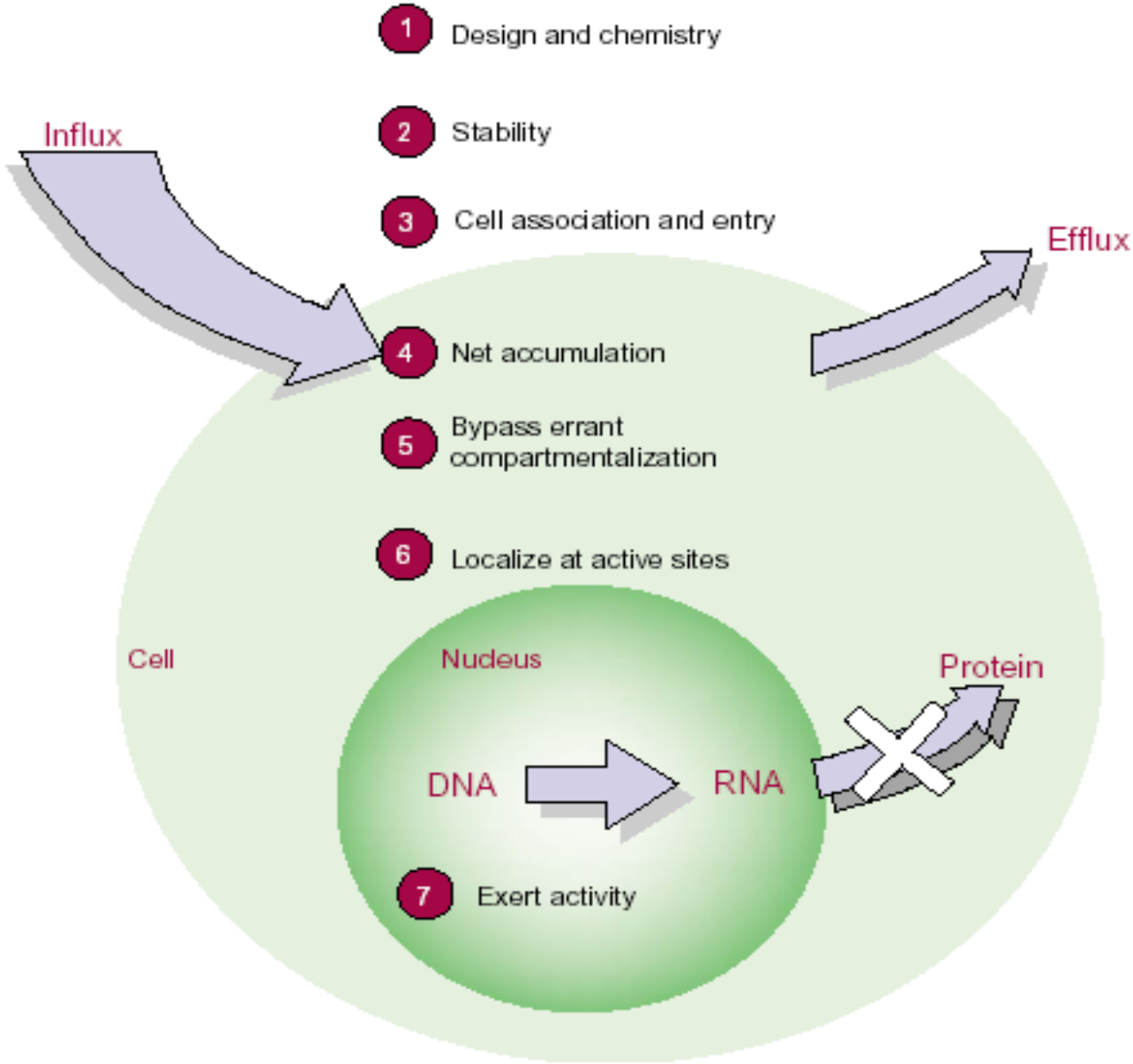
- Stability issues *in vivo*

Designing Biologically Stable ODNs



Designing Biologically Stable ODNs

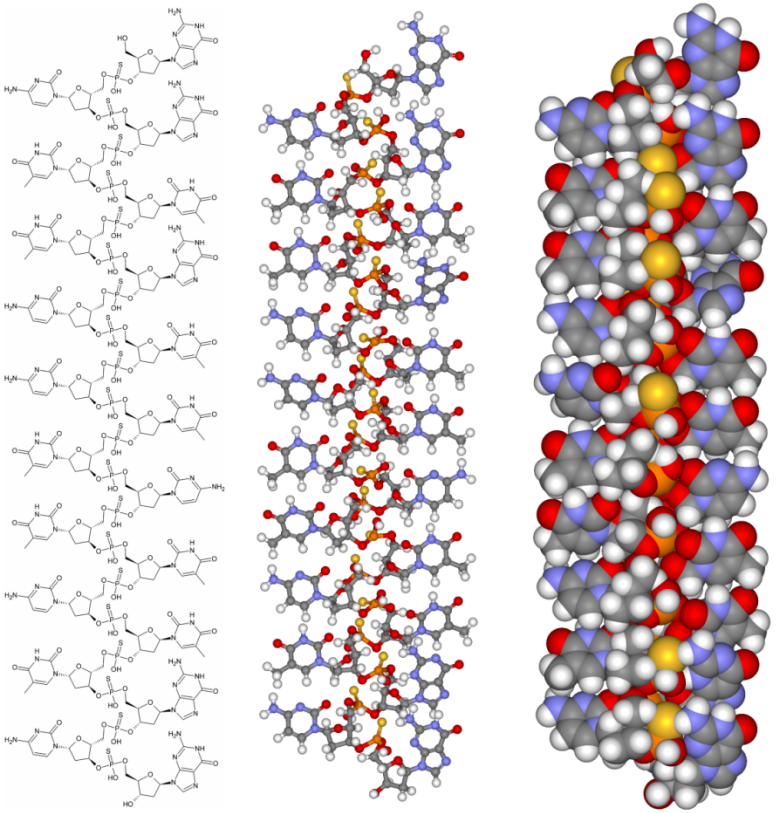



1 Design and chemistry
Influx
2 Stability
3 Cell association and entry
Efflux
4 Net accumulation
5 Bypass errant compartmentalization
6 Localize at active sites
Cell
Nucleus
Protein
DNA
RNA
7 Exert activity

Fomivirsen Sodium (Vitravene)®

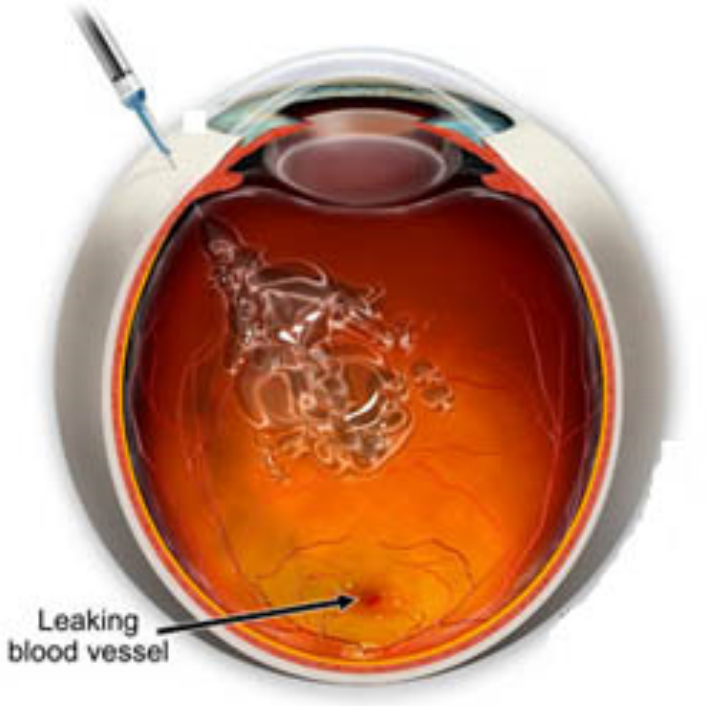
5'-d-[G*C*G*T*T*T*G*C*T*C*T*T*C*T*T*C*T*T*G*C*G]-3'
Sodium salt
*** = racemic phosphorothioate**

- FDA-approved for the local treatment of CMV retinitis in AIDS patients

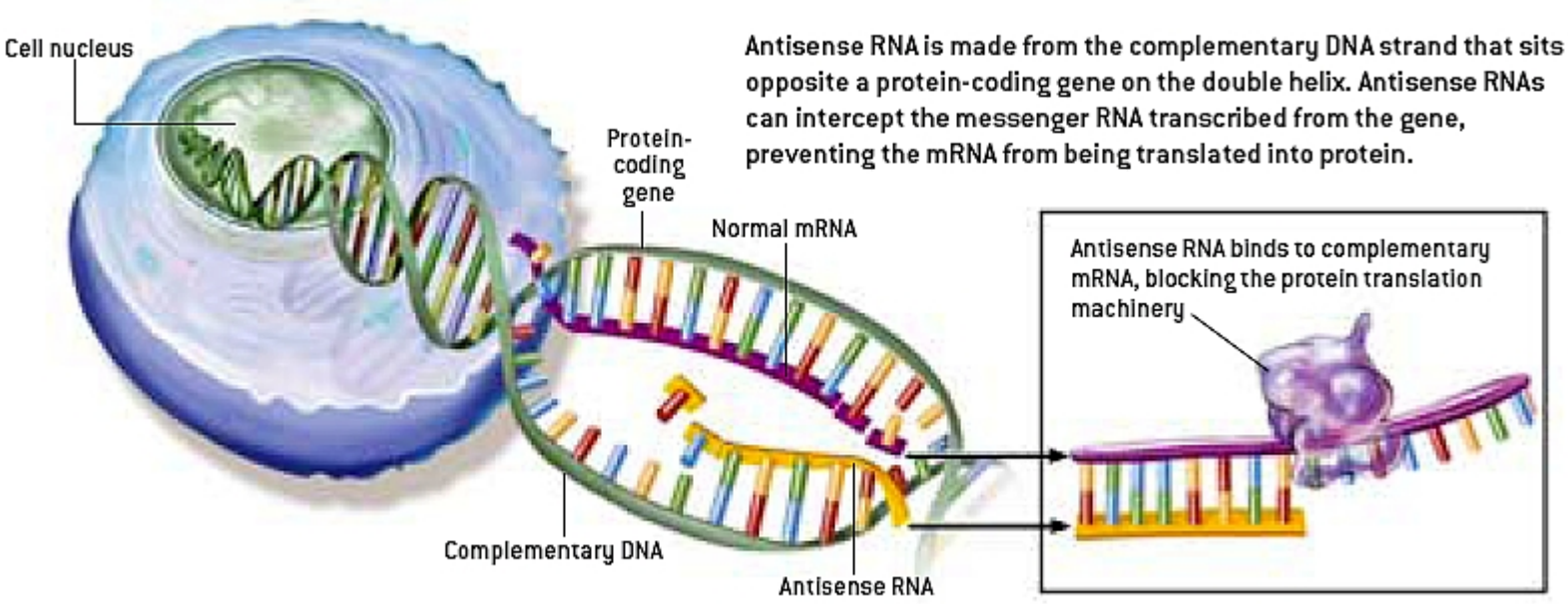


Fomivirsen Sodium (Vitravene)®

- Dose 150-330 µg intravitreal injection
- Every other week for 2 doses
- Cleared locally by exonucleases 1-2 hr after injection



Antisense RNA

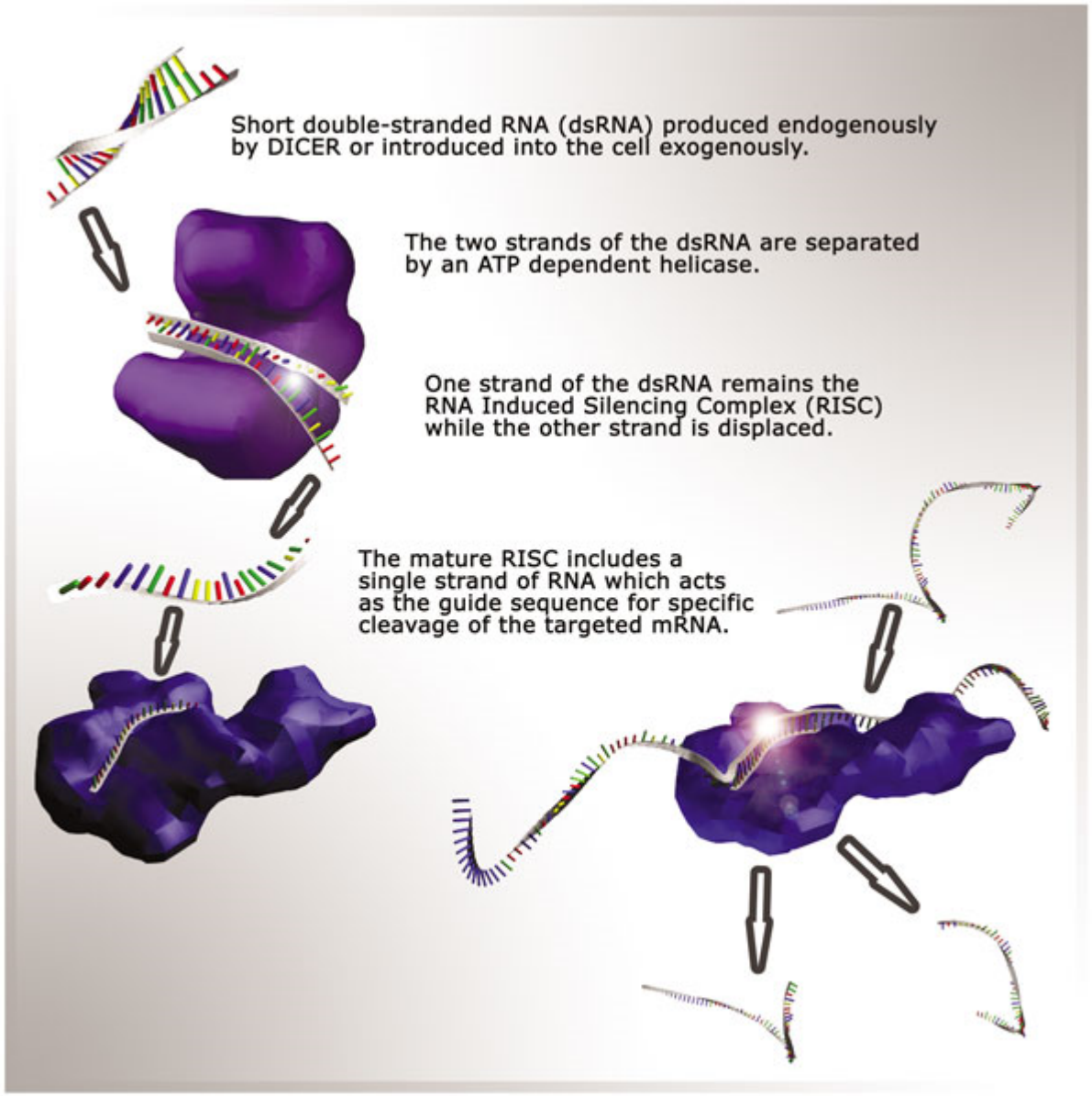


What is RNAi?

- Post-transcriptional phenomenon that was initially discovered in plants
- Mediated by ***double-stranded RNA***



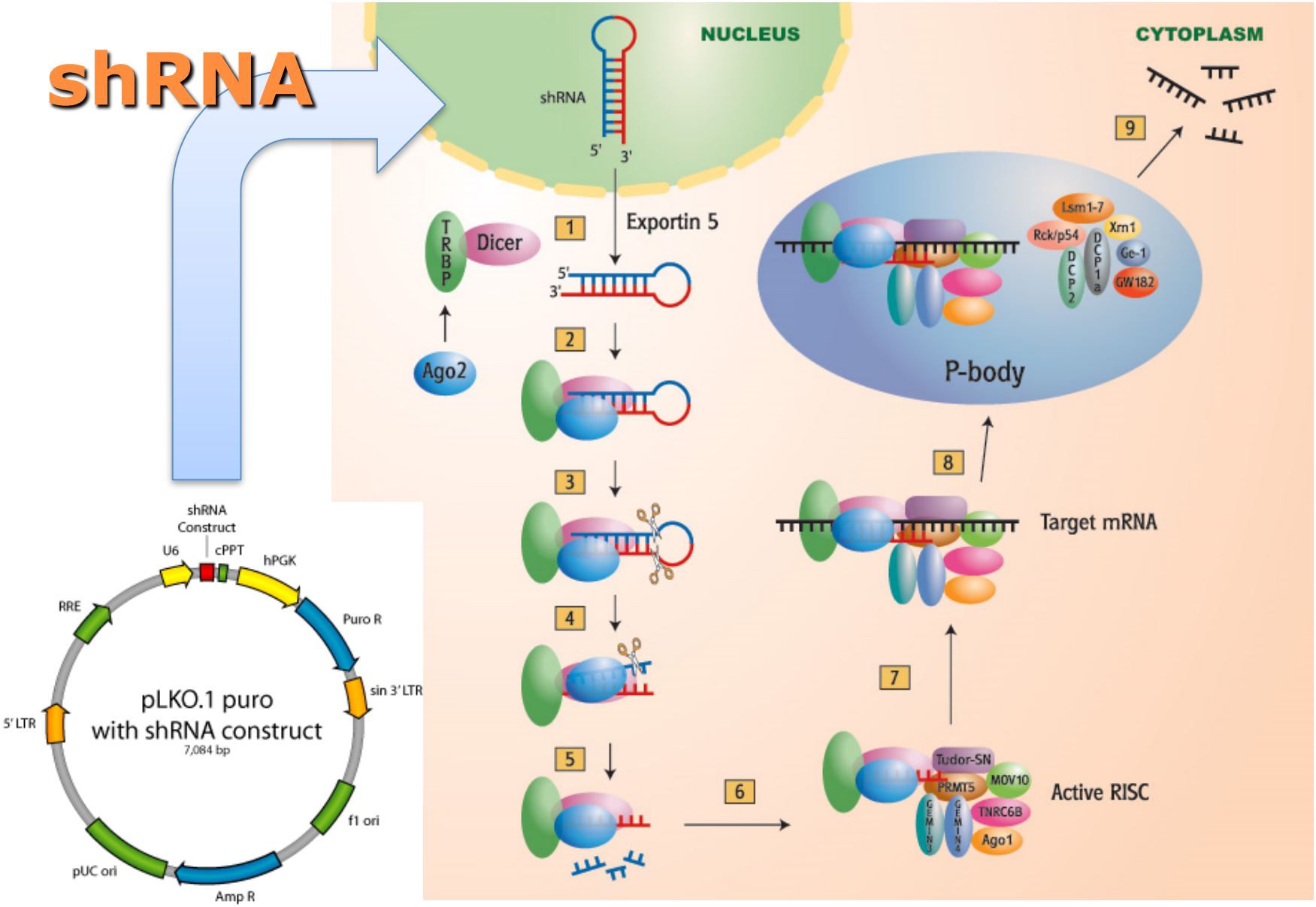
siRNA


Short double-stranded RNA (dsRNA) produced endogenously by DICER or introduced into the cell exogenously.
The two strands of the dsRNA are separated by an ATP dependent helicase.
One strand of the dsRNA remains the RNA Induced Silencing Complex (RISC) while the other strand is displaced.
The mature RISC includes a single strand of RNA which acts as the guide sequence for specific cleavage of the targeted mRNA.

Antisense ODN v.s. siRNA

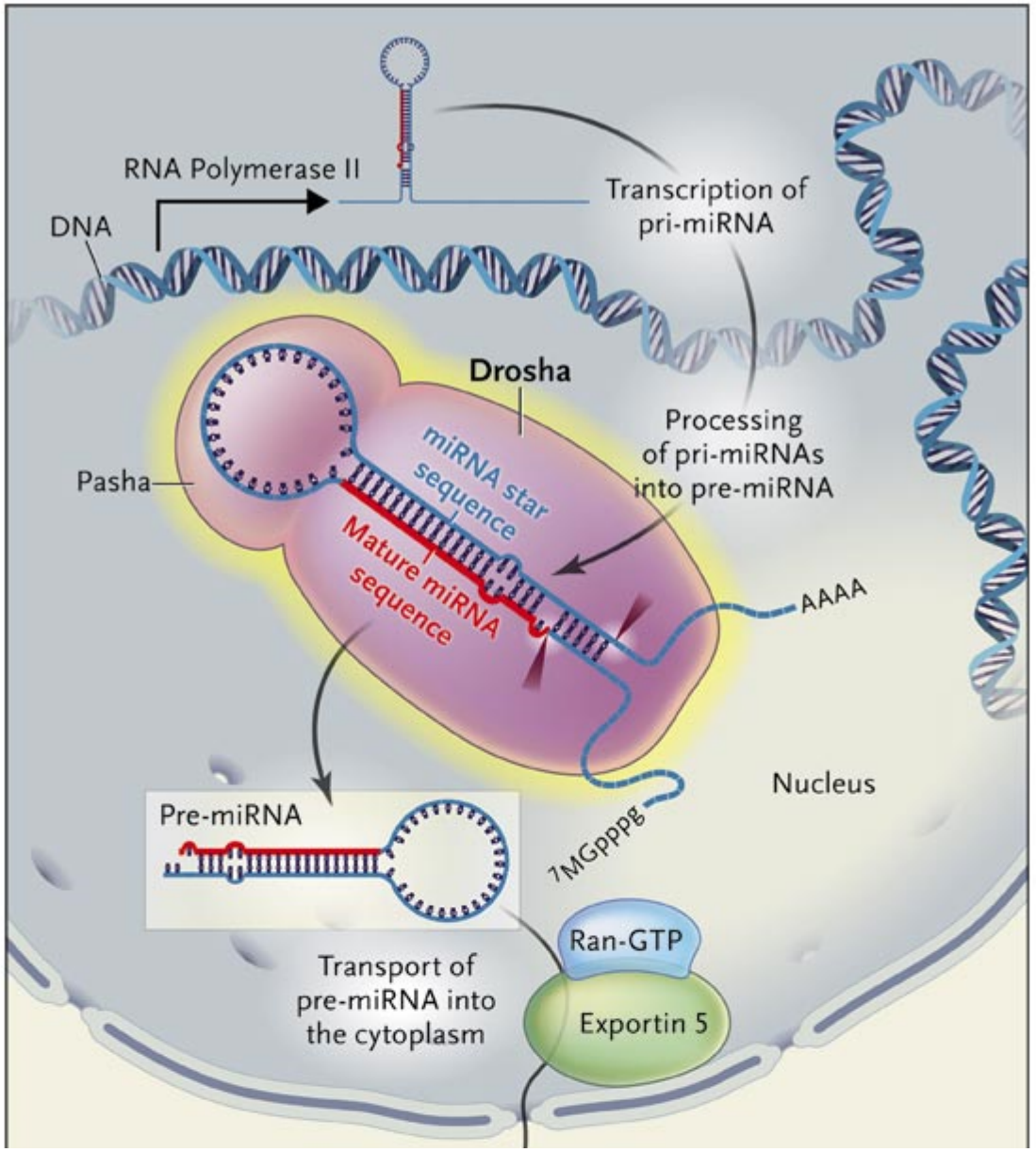
Antisense ODN		siRNA
Nucleotide sugar	Deoxyribose	Ribose
Structure	Single stranded	Double stranded
Length	16-30 bp	19-21 bp
Molecular weight	~ 6-9 kDa	~ 13-14 kDa
Precursor vailability	No	Yes
Site of action	Cytoplasm / Nucleus	Cytoplasm
mRNA cleavage	RNase H	RISC
Degradation upon activity	Yes	No
Effective concentration	50-400 nM	5-100 nM

shRNA

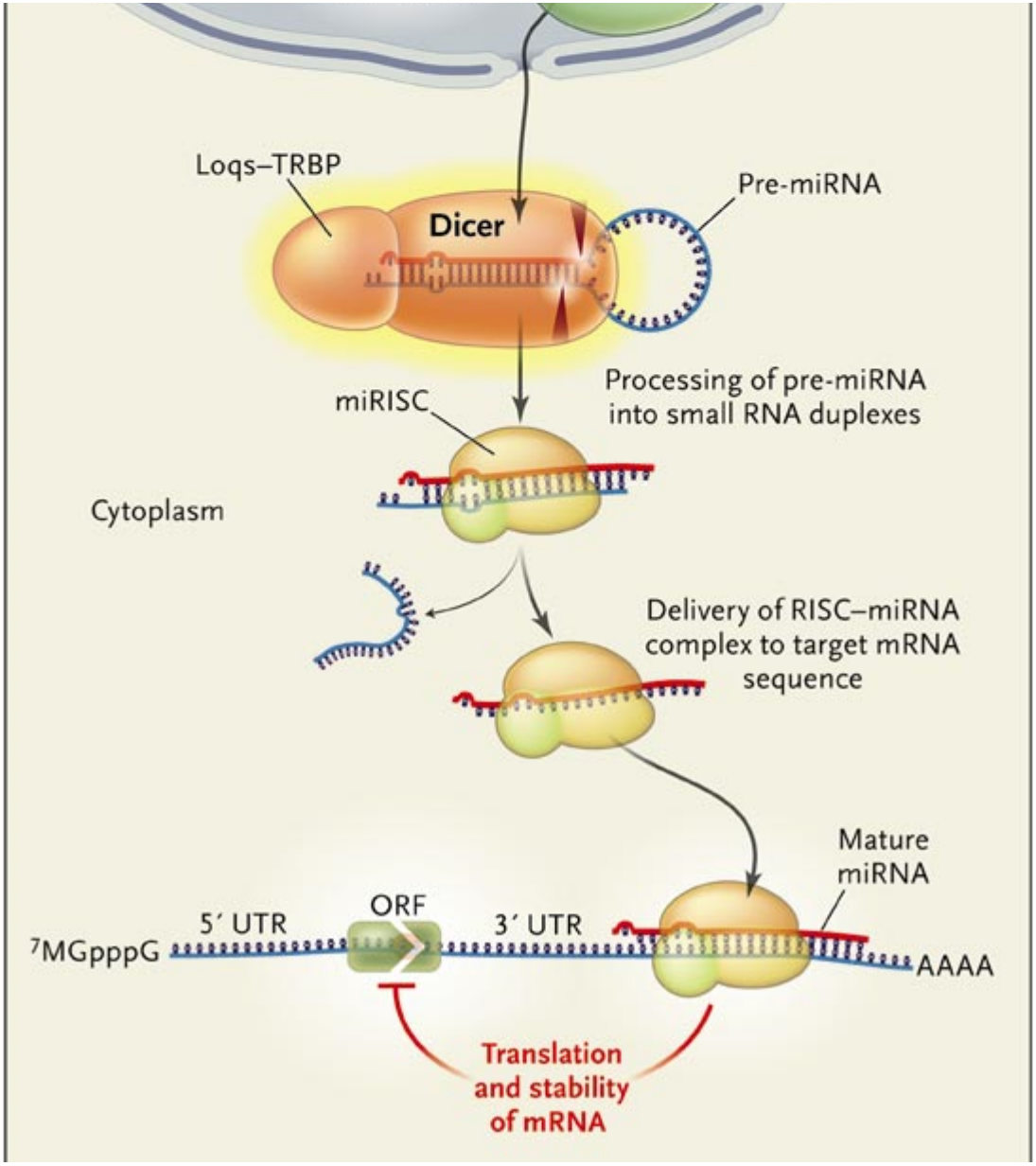


Plasmid DNA

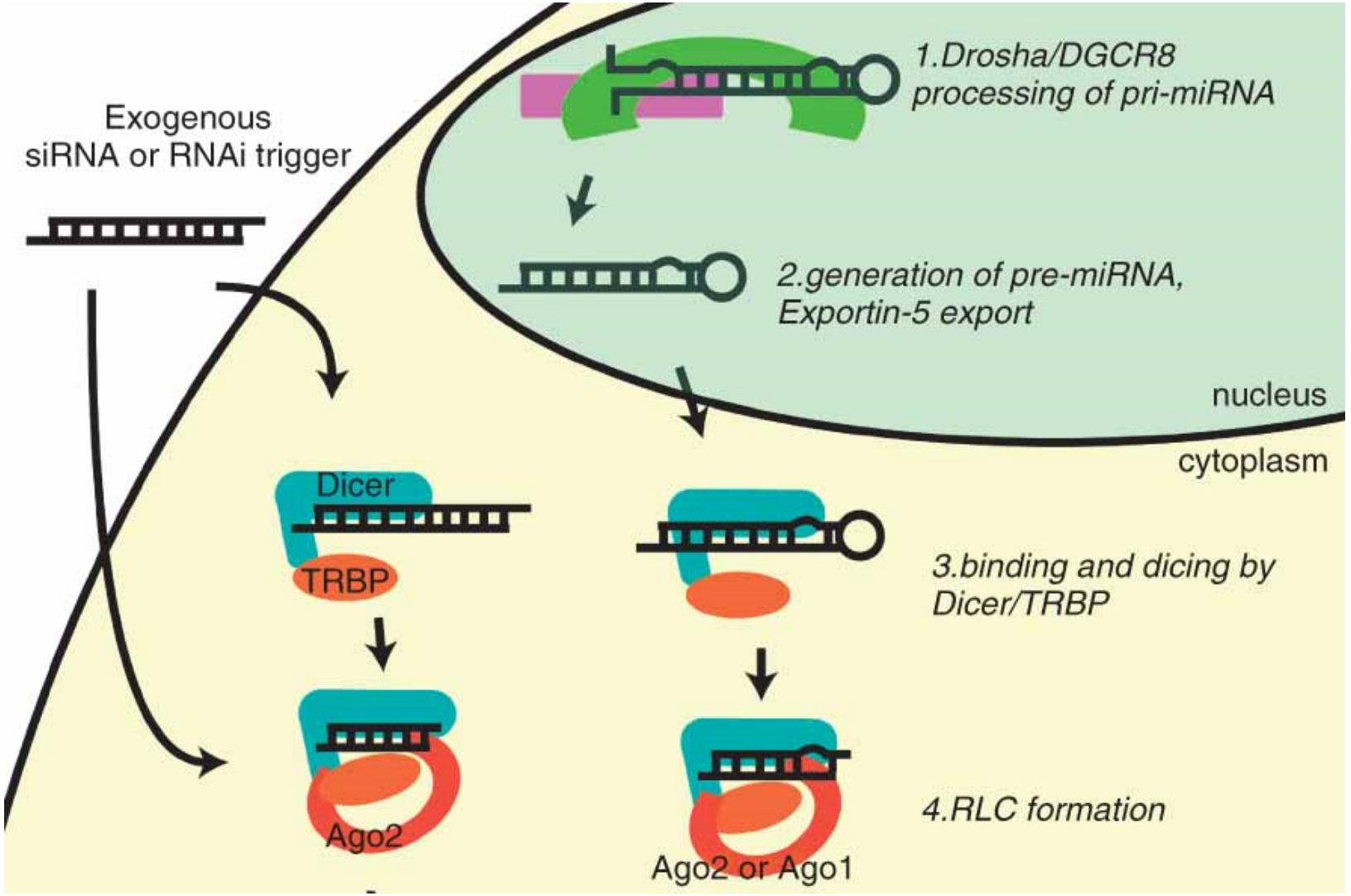
miRNA


RNA Polymerase II
DNA
Transcription of
pri-miRNA
Drosha
Pasha
miRNA star
sequence
Mature miRNA
sequence
Processing
of pri-miRNAs
into pre-miRNA
AAAA
Nucleus
Pre-miRNA
7MGpppg
Ran-GTP
Transport of
pre-miRNA into
the cytoplasm
Exportin 5

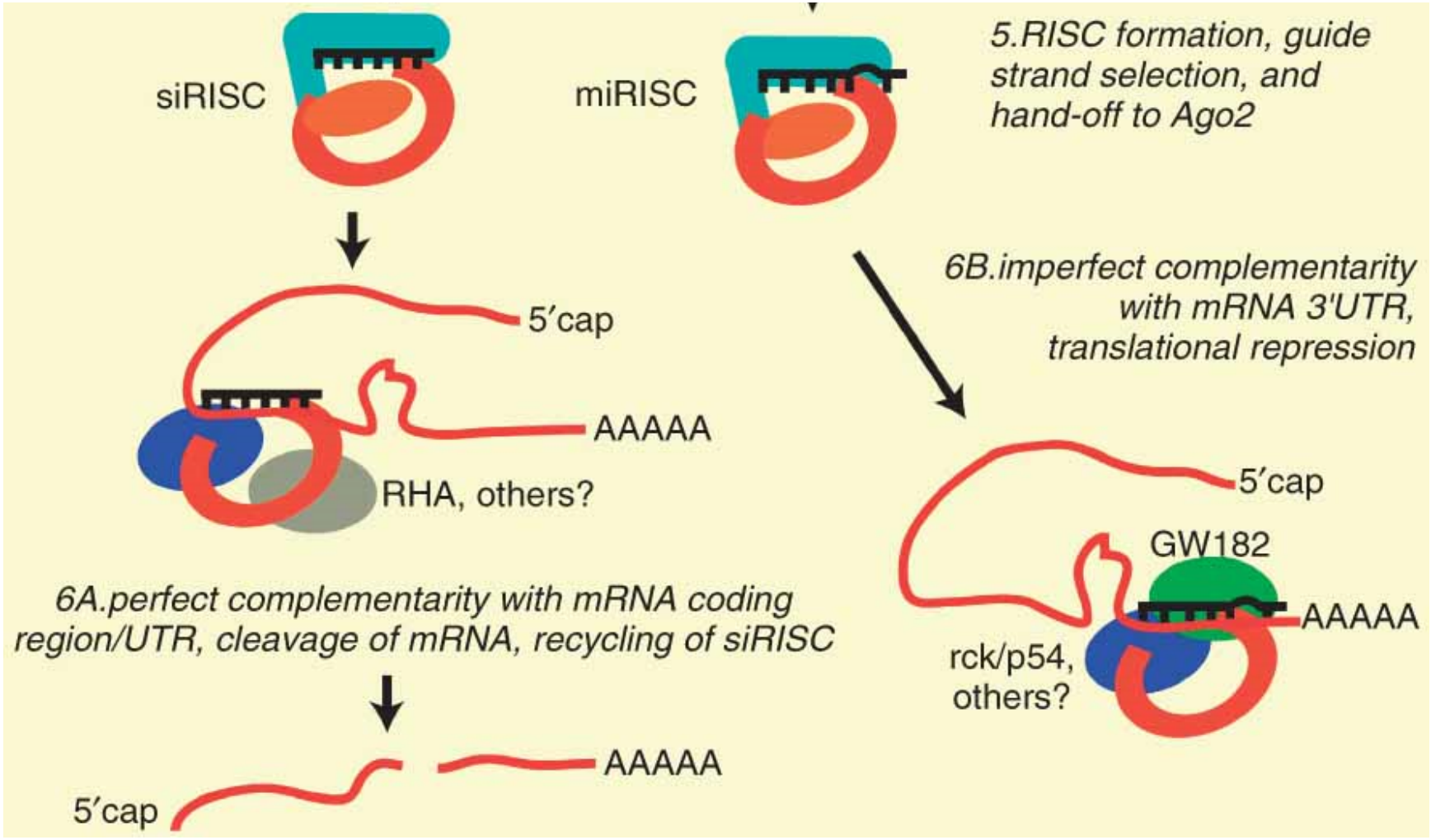
miRNA


Loqs–TRBP
Dicer
Pre-miRNA
miRISC
Processing of pre-miRNA
into small RNA duplexes
Cytoplasm
Delivery of RISC–miRNA
complex to target mRNA
sequence
Mature
miRNA
5′ UTR
ORF
3′ UTR
7MGpppG
AAAA
Translation
and stability
of mRNA

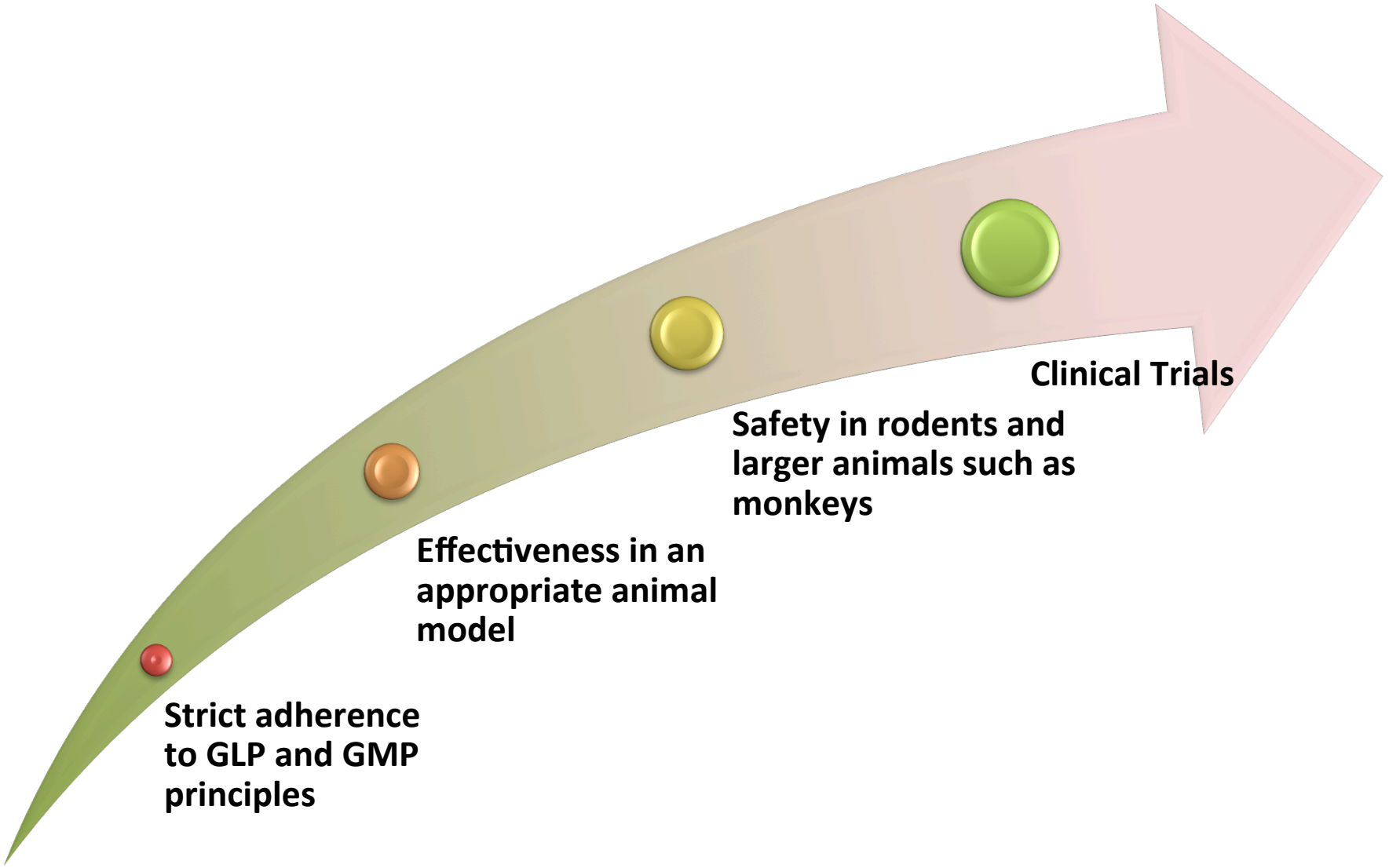
siRNA v.s. miRNA



siRNA v.s. miRNA



Production and Regulation



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