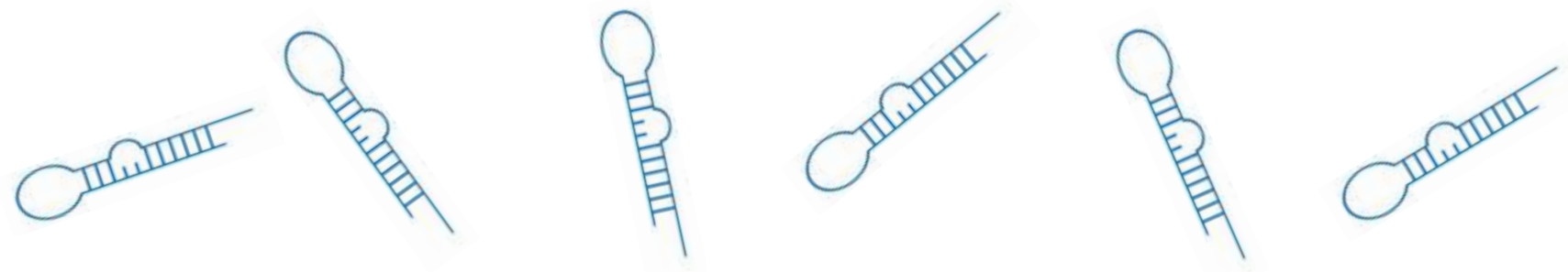




Introduction to RNA Interference (RNAi) Technology

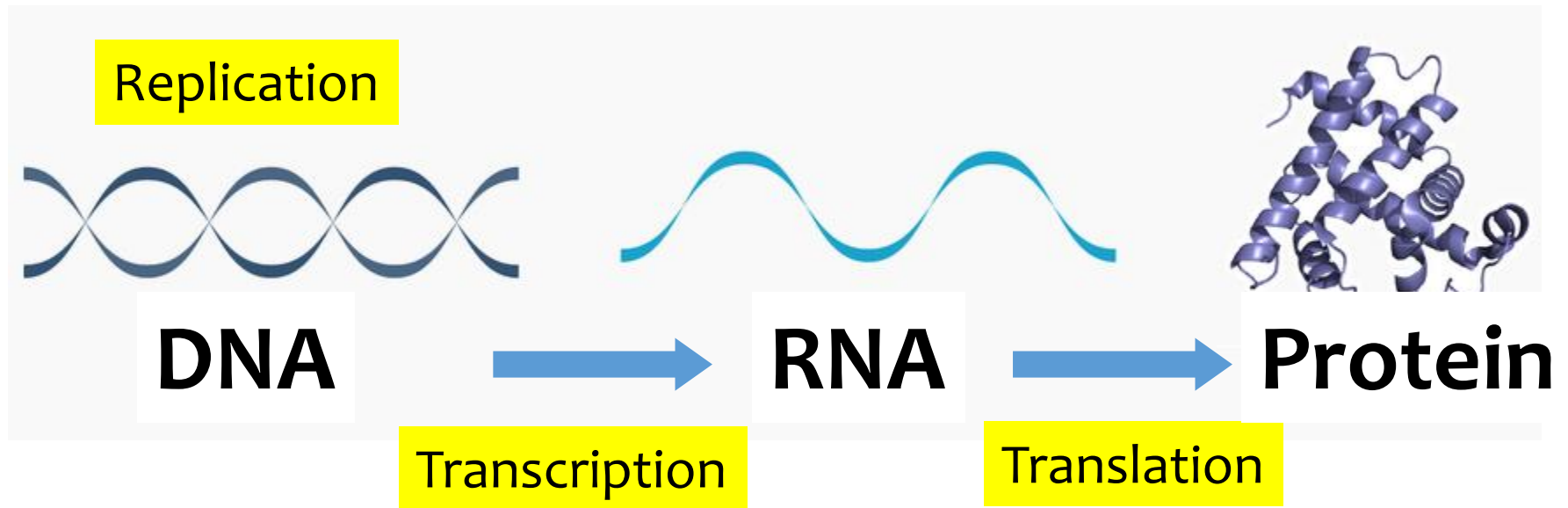
Patipan Jaipeng (DVM)



Introduction to RNA Interference (RNAi) Technology

The Central dogma of molecular genetic

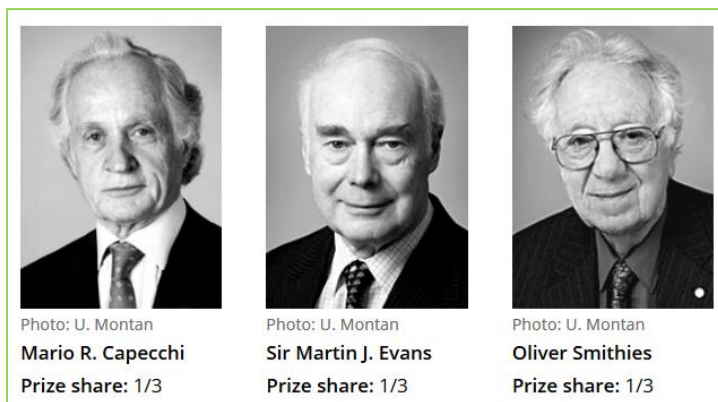
- Replication
- Transcription
- Translation



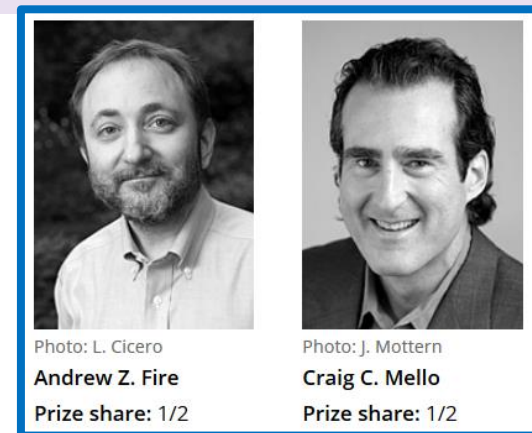
Introduction to RNA Interference (RNAi) Technology

Study of gene function

- Forward genetic (Mutation phenotype → Mutant allele → Gene or Protein sequence)
- Reverse genetic (Gene or Protein sequence → Mutant allele → Mutant phenotype)
 - **Gene replacement or gene knockout** [1st recorded in 1989]
 - **Gene silencing or RNA interference** [1st recorded in 1998]
 - **CRISPR/Cas9 and Targeted Genome Editing** [Function confirmed in 2007]



They were awarded the 2007 Nobel Prize
in Physiology or Medicine

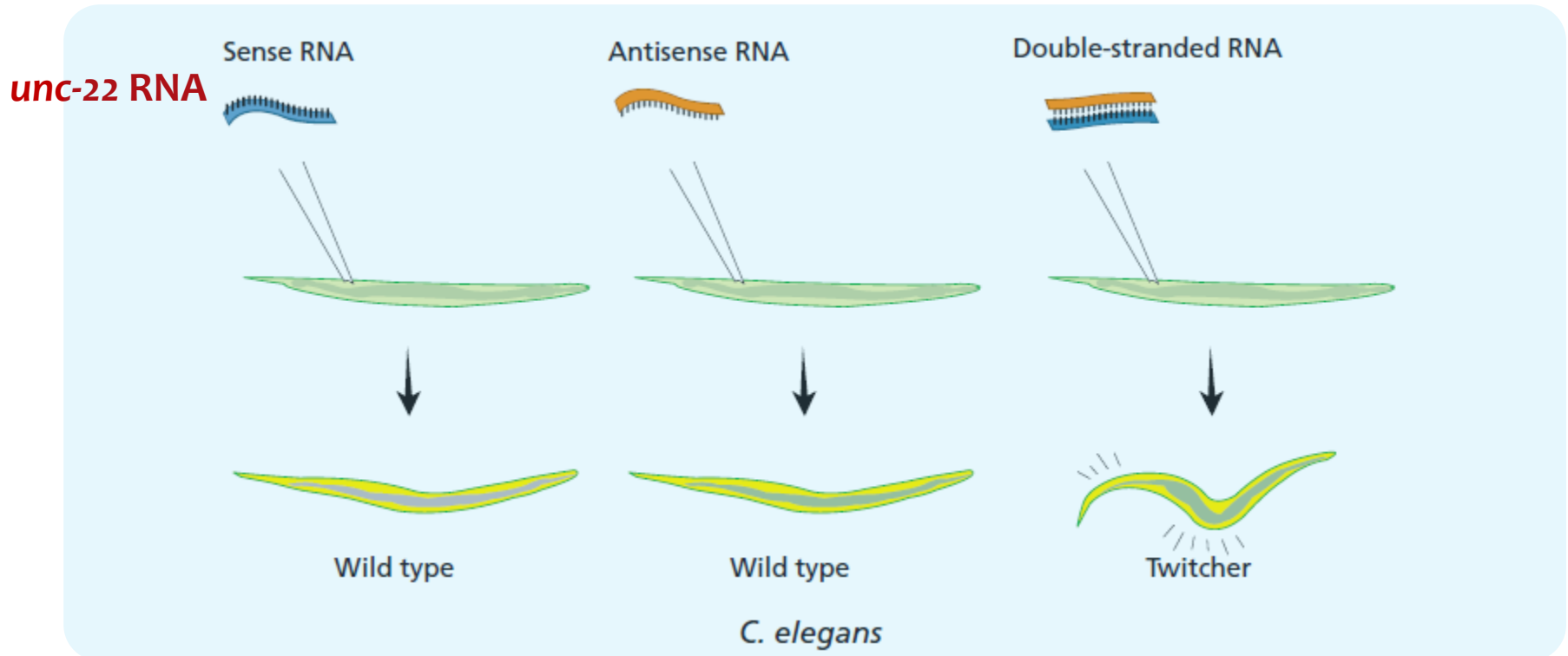


They were awarded the 2006 Nobel Prize
in Physiology or Medicine

Introduction to RNA Interference (RNAi) Technology

RNA interference (RNAi)

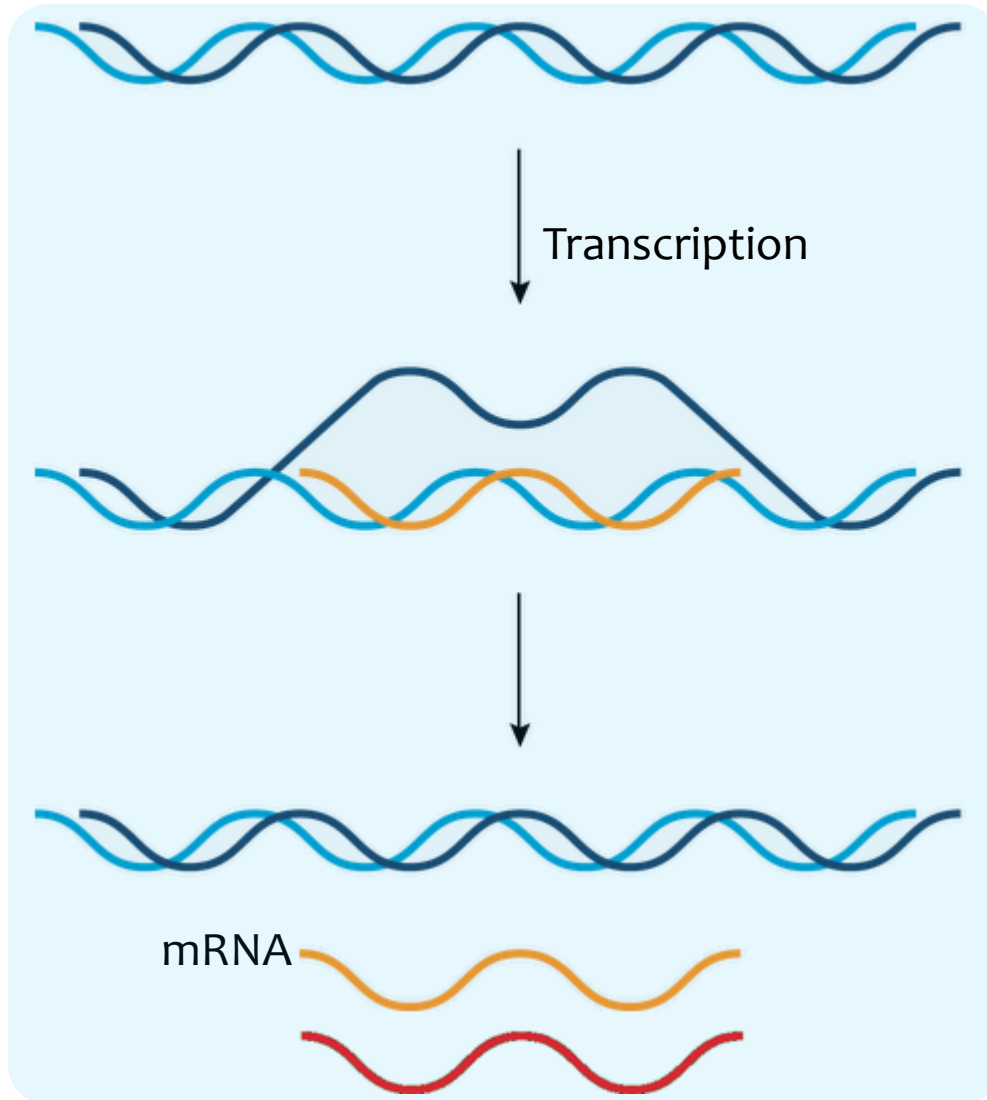
- Phenotypic effect after injection of single-stranded or double-stranded **unc-22 RNA** into the gonad of *C. elegans*.
- The **unc-22 gene** encodes a myofilament protein.
- Decrease in **unc-22** activity is known to produce severe twitching movements.



- Injected double-stranded RNA, but not single-stranded RNA, induced the twitching phenotype in the progeny.

Introduction to RNA Interference (RNAi) Technology

What are sense strand and antisense strand?



Sense DNA strand

Anti-sense DNA strand (Template)

Sense DNA strand

Anti-sense DNA strand

Sense RNA strand

Sense DNA strand

Anti-sense DNA strand

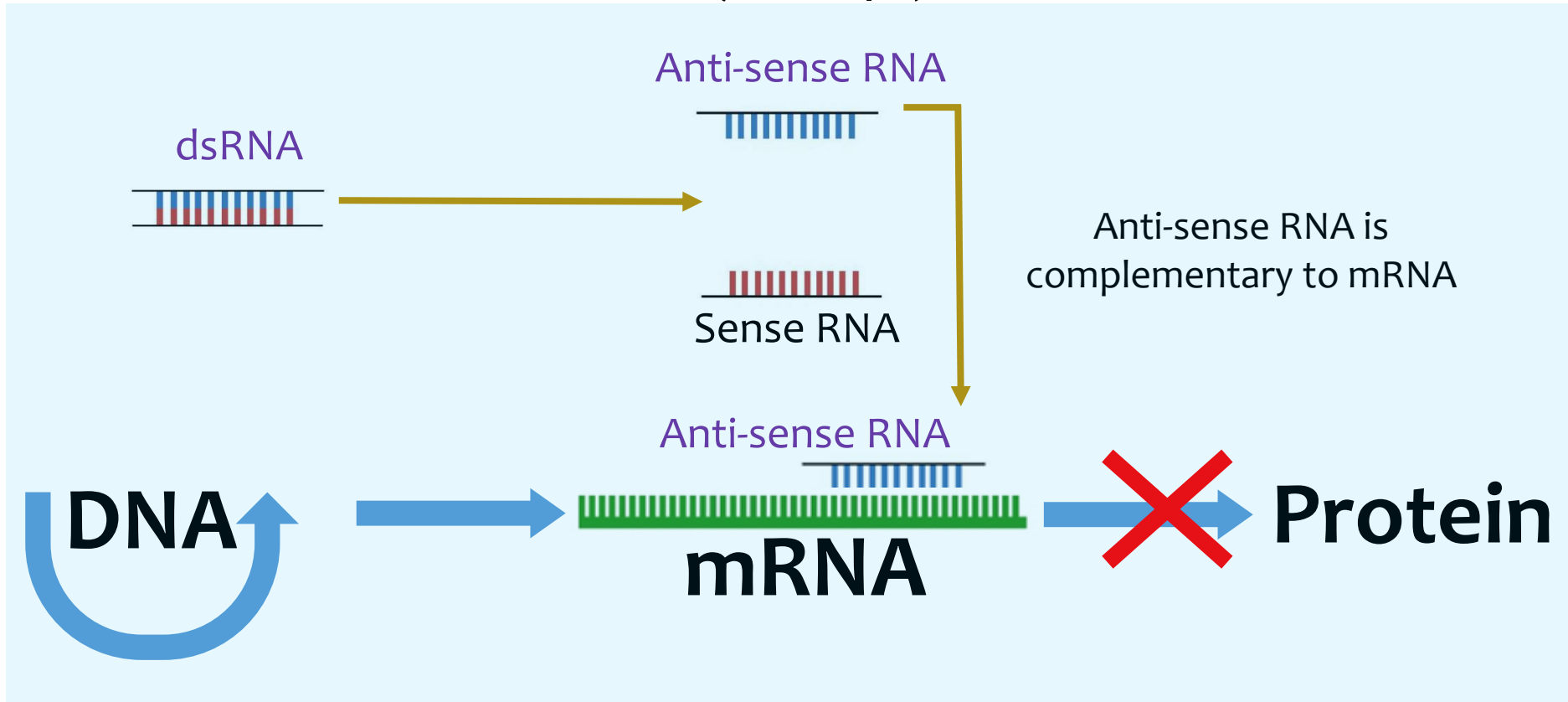
Sense RNA strand

Anti-sense RNA strand

Introduction to RNA Interference (RNAi) Technology

RNA interference (RNAi)

- Anti-sense RNA mechanism (concept)



The anti-sense RNA hybridizes to the mRNA → mRNA degradation or blocking translation

Introduction to RNA Interference (RNAi) Technology

RNA interference (RNAi)

- RNA interference is a posttranscriptional specific gene silencing (PTGS) pathway or specific gene knockdown mechanism in eukaryotes.
- Double strand RNA (dsRNA)
- Degradation or translation inhibition of the mRNA target

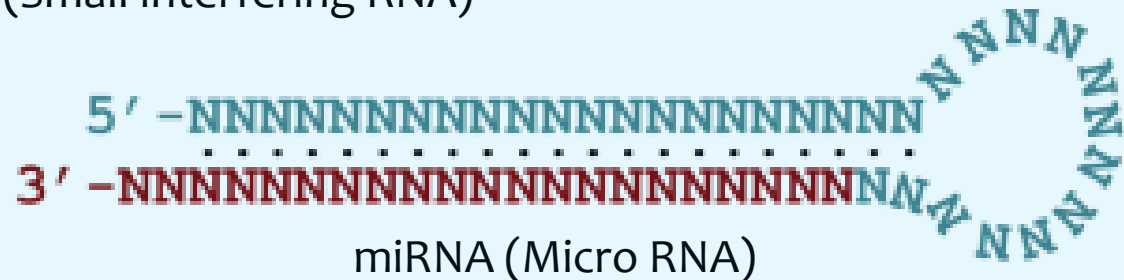
RNA interference (RNAi) application

- Basic research (Gene function)
- Medicine (Tumor suppression, Antiviral replication)
- Agriculture technology

Introduction to RNA Interference (RNAi) Technology

Main Type of RNA interference (origin, structure)

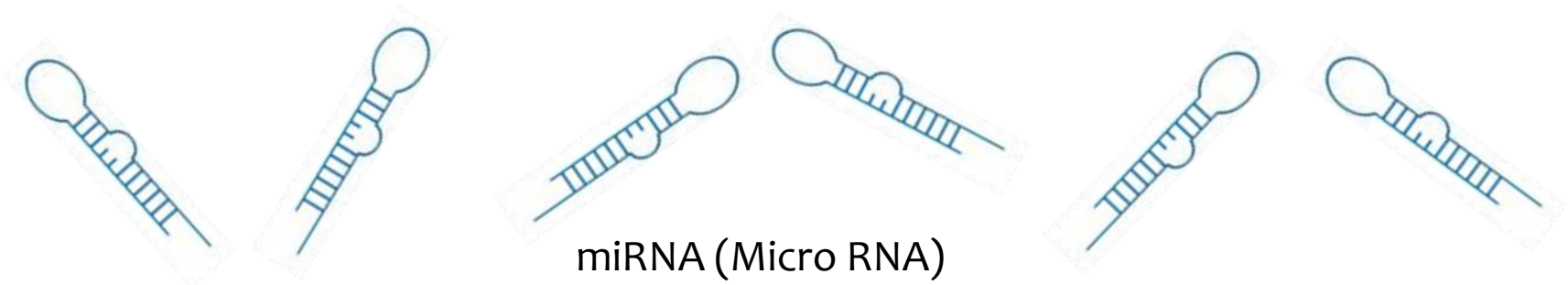
- **MicroRNA (miRNA)**
- **Small interfering RNA (siRNA)**
- Piwi-interacting RNA (piRNA); (functional most clearly in germline)



Introduction to RNA Interference (RNAi) Technology

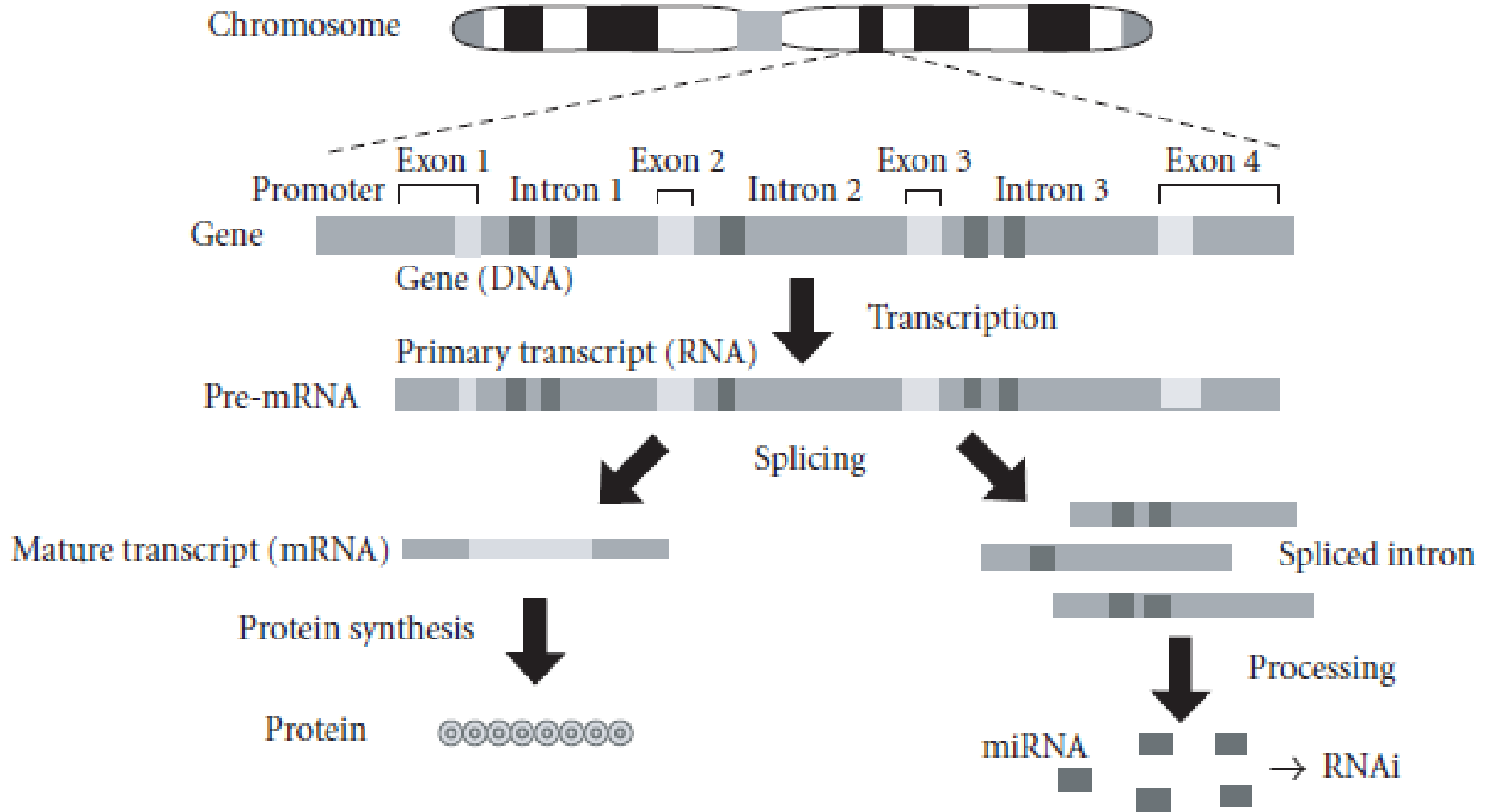
MicroRNA biogenesis

- Nearly 97% of the human genome is composed of non-coding DNA.
- Numerous non-coding DNA have been recently found to encode **miRNAs** (Lin et al., 2006)



Introduction to RNA Interference (RNAi) Technology

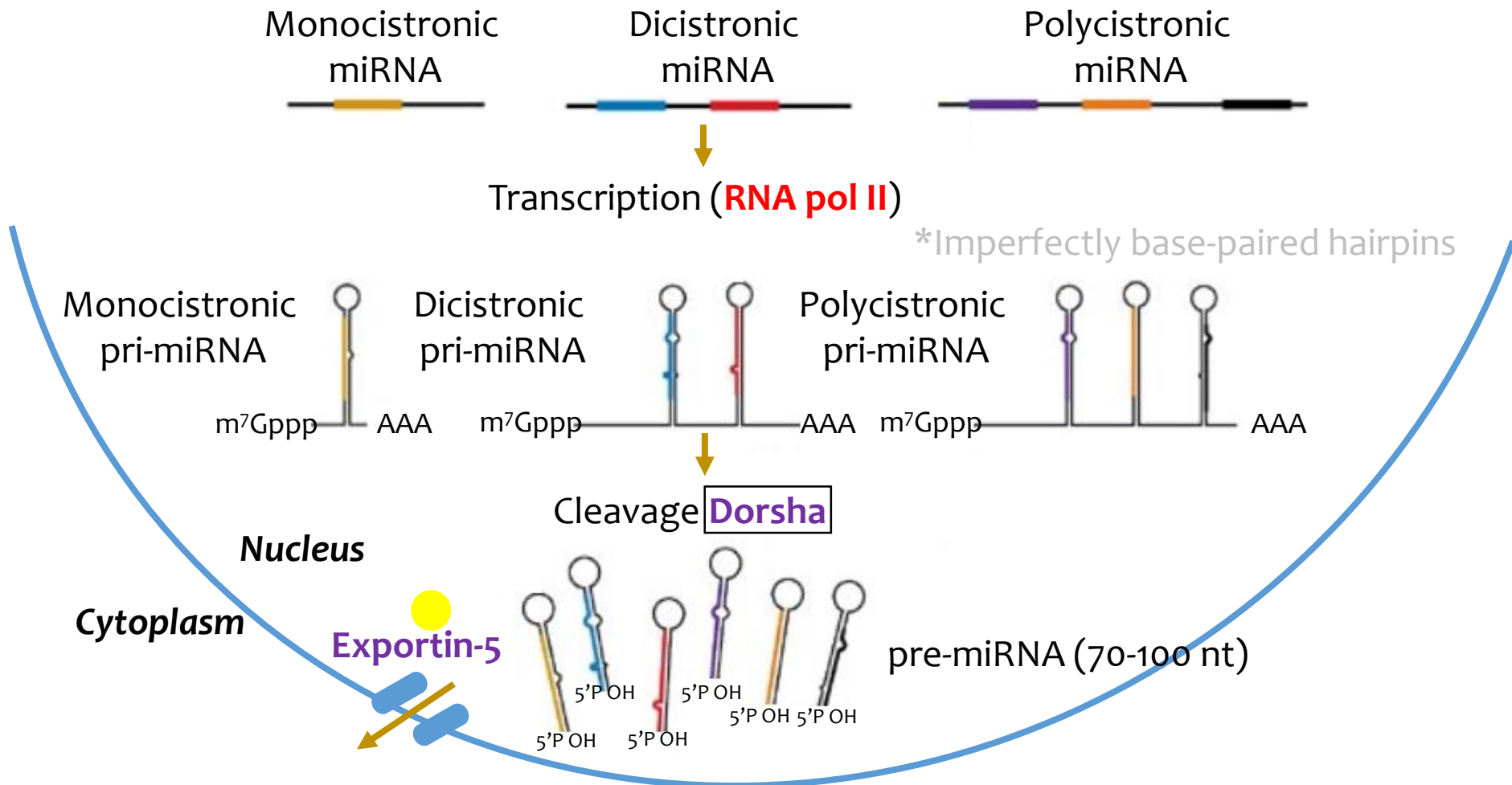
MicroRNA biogenesis in mammalian cells



(Lin et al., 2006)

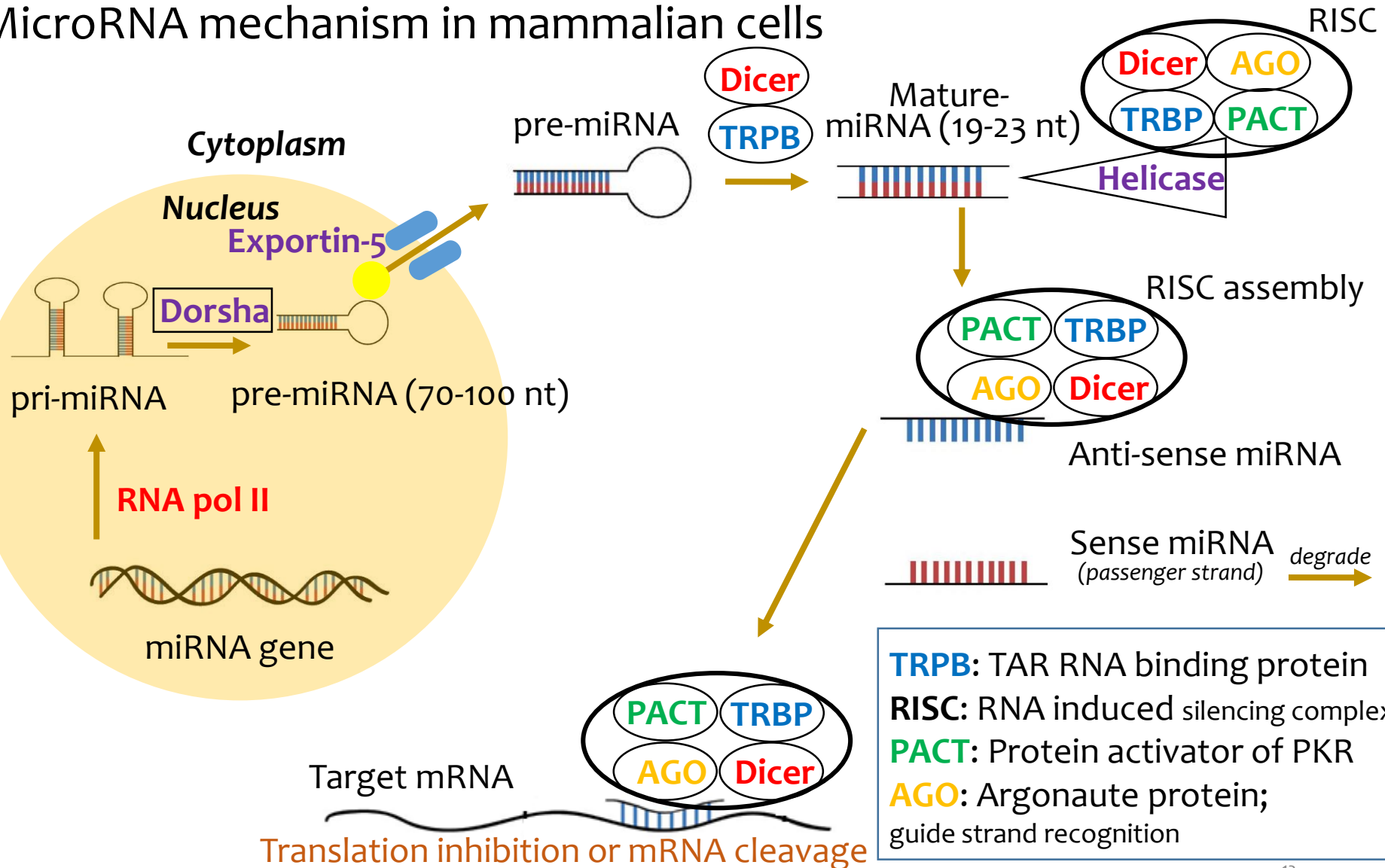
Introduction to RNA Interference (RNAi) Technology

MicroRNA biogenesis in mammalian cells



Introduction to RNA Interference (RNAi) Technology

MicroRNA mechanism in mammalian cells



Introduction to RNA Interference (RNAi) Technology

Step of an RNAi experiment

i. RNAi design

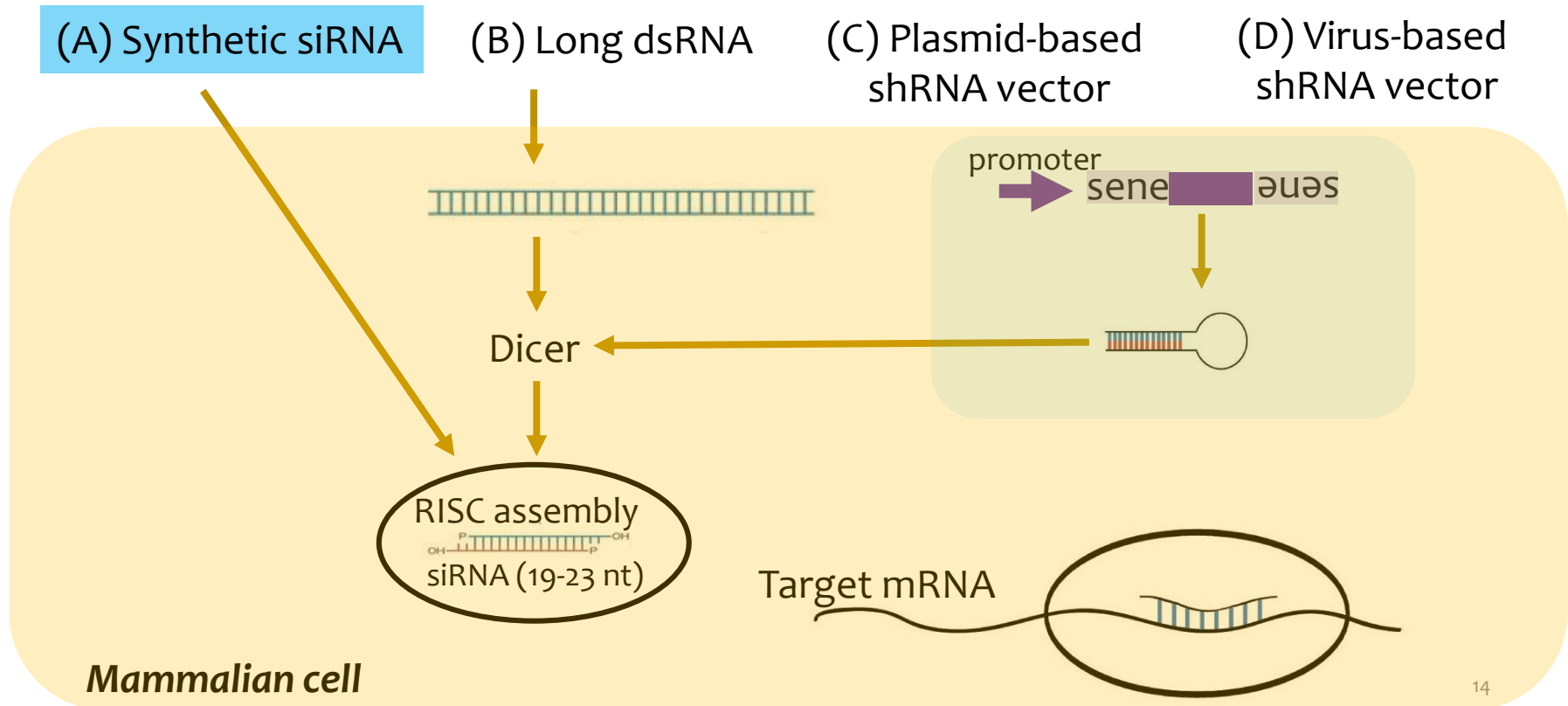
- GenBank[®]; nucleotide sequence database
- DNA Sequencing

Introduction to RNA Interference (RNAi) Technology

Step of an RNAi experiment

i. RNAi design

- Non-vector base RNAi (synthetic siRNA/Long dsRNA); A, B
- Vector base RNAi (synthetic shRNA; short-hairpin RNA) C, D



Introduction to RNA Interference (RNAi) Technology

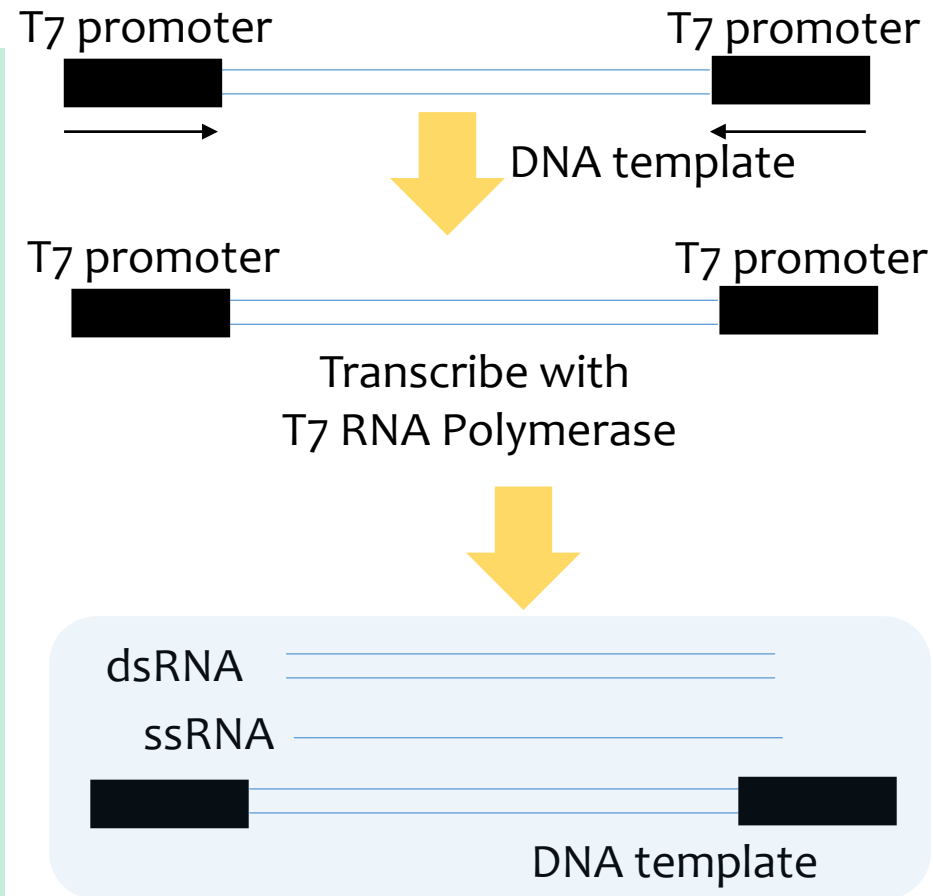
Step of an RNAi experiment

i. RNAi design

- Long double-stranded RNA

In-vitro transcription double-stranded RNA

1. Target gene
2. Primer design; contained T7 promoter (GAATTAATACGACTCACTATAGGGAGA)
3. PCR amplification for making DNA template
4. Synthetic dsRNA in a tube at 37°C for overnight
5. Long-dsRNA purification



Introduction to RNA Interference (RNAi) Technology

Step of an RNAi experiment

- i. RNAi design
 - RNAi design; online tool

Company or Lab	URL
Ambion	www.ambion.com/techlib/misc/siRNA_finder.html
Dharmacon	design.dharmacon.com/rnadesign/default.aspx
Hannon Lab	katahdin.cshl.org:9331/portal/scripts/main2.pl
Integrated DNA Technologies	scitools.idtdna.com/RNAi
Invitrogen	rnaidesigner.invitrogen.com/sirna
McManus Lab	web.mit.edu/mmcm Manus/www/home1.2files/siRNAs.htm
Qiagen	www1.qiagen.com/Products/GeneSilencing/CustomSiRna/SiRnaDesigner.aspx
Sfold Algorithm	sfold.wadsworth.org/index.pl
Tuschl Lab	www.rockefeller.edu/labheads/tuschl/sirna.html
Whitehead Institute	jura.wi.mit.edu/siRNAext

siRNA, small interfering RNA; shRNA, short hairpin RNA.

Introduction to RNA Interference (RNAi) Technology

Step of an RNAi experiment

ii. Delivery system

- Micro-injection; in worm and animal model
- Feeding of artificial diet; in worm model
- Soaking; in worm model
- **Transfection; in cell culture model**
- Viral transduction; in cell culture and animal model
- Electroporation; in cell culture model
- Inhalation; in animal model

Introduction to RNA Interference (RNAi) Technology

Step of an RNAi experiment

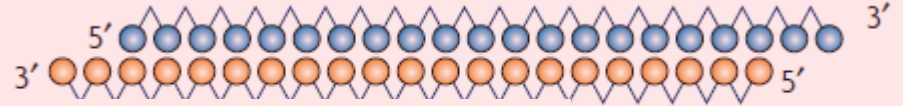
ii. Delivery system

- **Transfection; in cell culture and animal model**
 - Chemically modified structure of nucleic acid
 - Enhanced stability (resistance to nuclease activity)
 - Higher target specificity
 - Ligand-based targeting molecule
 - High affinity for cell specific targeting
 - Lipid-based delivery
 - Protection for nuclease degradation

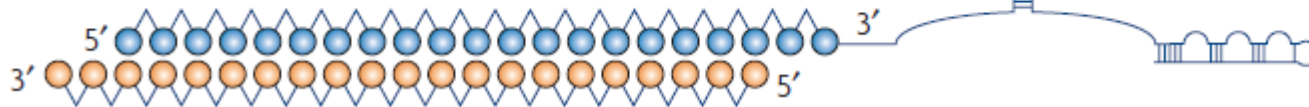
Introduction to RNA Interference (RNAi) Technology

Step of an RNAi experiment ii. Delivery system

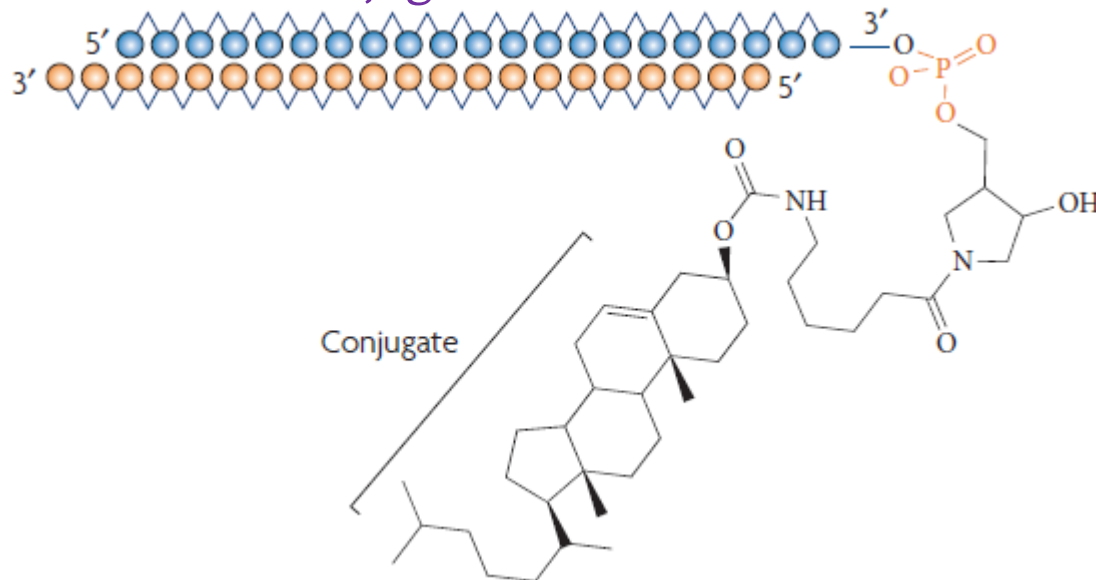
Direct injection (Ex. Ocular, CNS system)
Necked siRNA



Direct injection (Ex. Intra-tumor)
Aptamer-conjugated siRNA



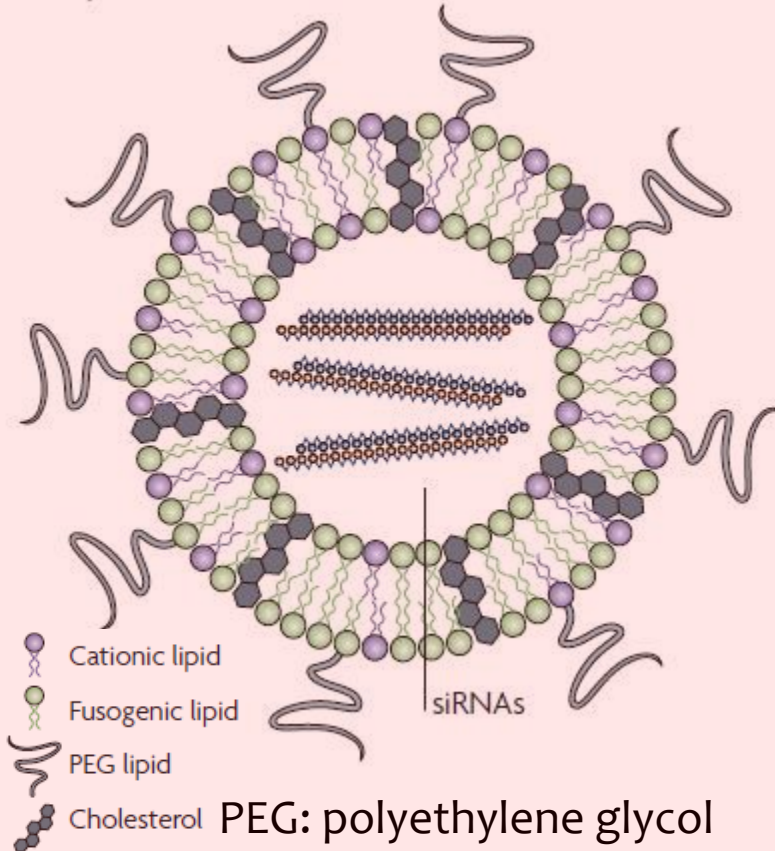
Systemic administration (Ex. Target: hepatocyte)
Cholesterol-conjugated siRNA



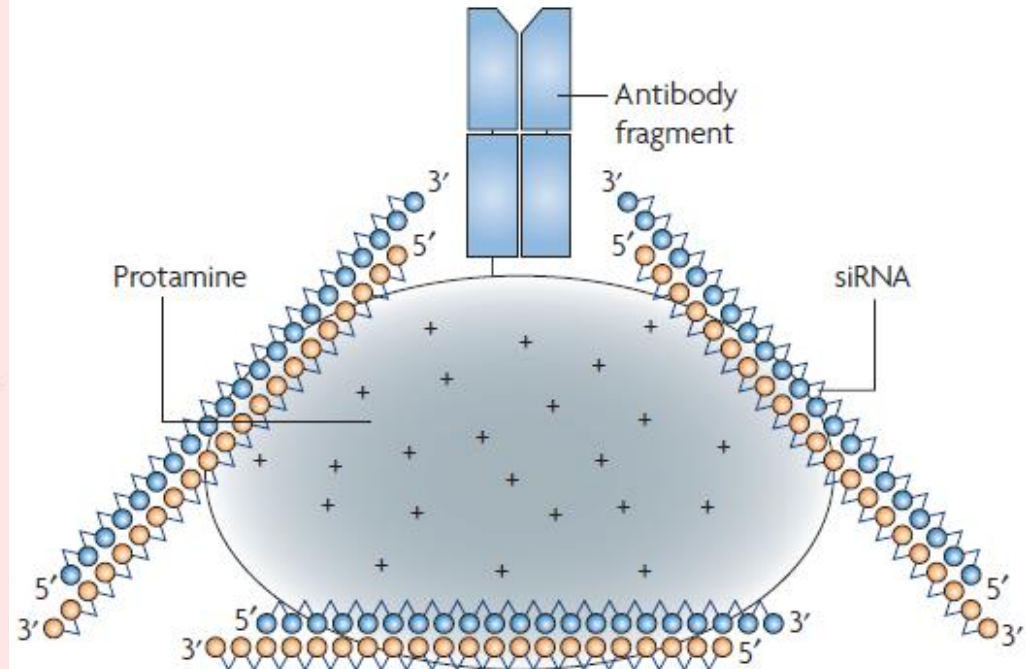
Introduction to RNA Interference (RNAi) Technology

Step of an RNAi experiment ii. Delivery system

Systemic administration
Liposome formulated siRNA



Systemic administration
(Specific cell expressed surface protein)
Antibody-protamine complex siRNA



Introduction to RNA Interference (RNAi) Technology

Step of an RNAi experiment

iii. Validation and measurement

Method	Detection Level	Advantage	Disadvantage	Throughput
Northern blot	Endogenous mRNA	Easy	RNA isolation	Low
qRT-PCR (TaqMan® or SYBR® Green)	Endogenous mRNA	Sensitive, quantitative	RNA isolation, primer design	High
QuantiGene® www.genospectra.com	Endogenous mRNA	Sensitive, quantitative, works on crude lysate	Cost	High
Western blot, IF, ELISA, FACS, etc.	Endogenous protein	Easy	Antibody availability	Low
Western blot, IF, etc., on epitope tag	Exogenous fusion protein	Same antibody for detection	In-frame cloning, restricted target region	High
Fluorescent/enzymatic reporter assay	Exogenous protein (translated from chimeric mRNA)	Entire cDNA can be targeted, only reporter is translated		High

mRNA, messenger RNA; qRT-PCR, quantitative reverse transcription PCR; IF, immunofluorescence; ELISA, enzyme-linked immunosorbent assay; FACS, fluorescence-activated cell sorting.

#Phenotypic analysis

Thank you for your attention