

RNA genes

Functional non-coding RNAs
(ncRNA)

Jan 29th 2020.

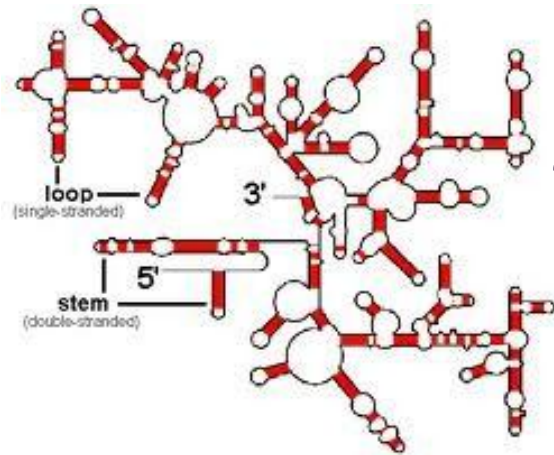
After human genome sequencing it became obvious that human genome consists of many non-protein coding genes, genes that code for different RNAs.

RNA genes

- Unexpectedly large group of non-coding ncRNAs
- At least 85% of human genome is transcribed!
~ 20 000 protein coding genes
over 10 000 RNA coding genes
- Most of the genome is transcribed from both DNA chains – multigenic transcription
- Types of RNA
 - Coding – mRNA - 20%
 - Noncoding – regulatory ncRNAs, rRNA, tRNA - 80%

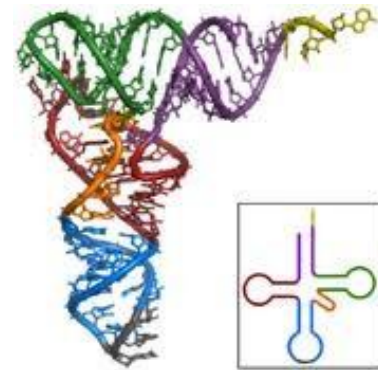
Before HGP known RNA genes

Ribosomal RNA - rRNA



~ 700 genes

Transfer RNA - tRNA



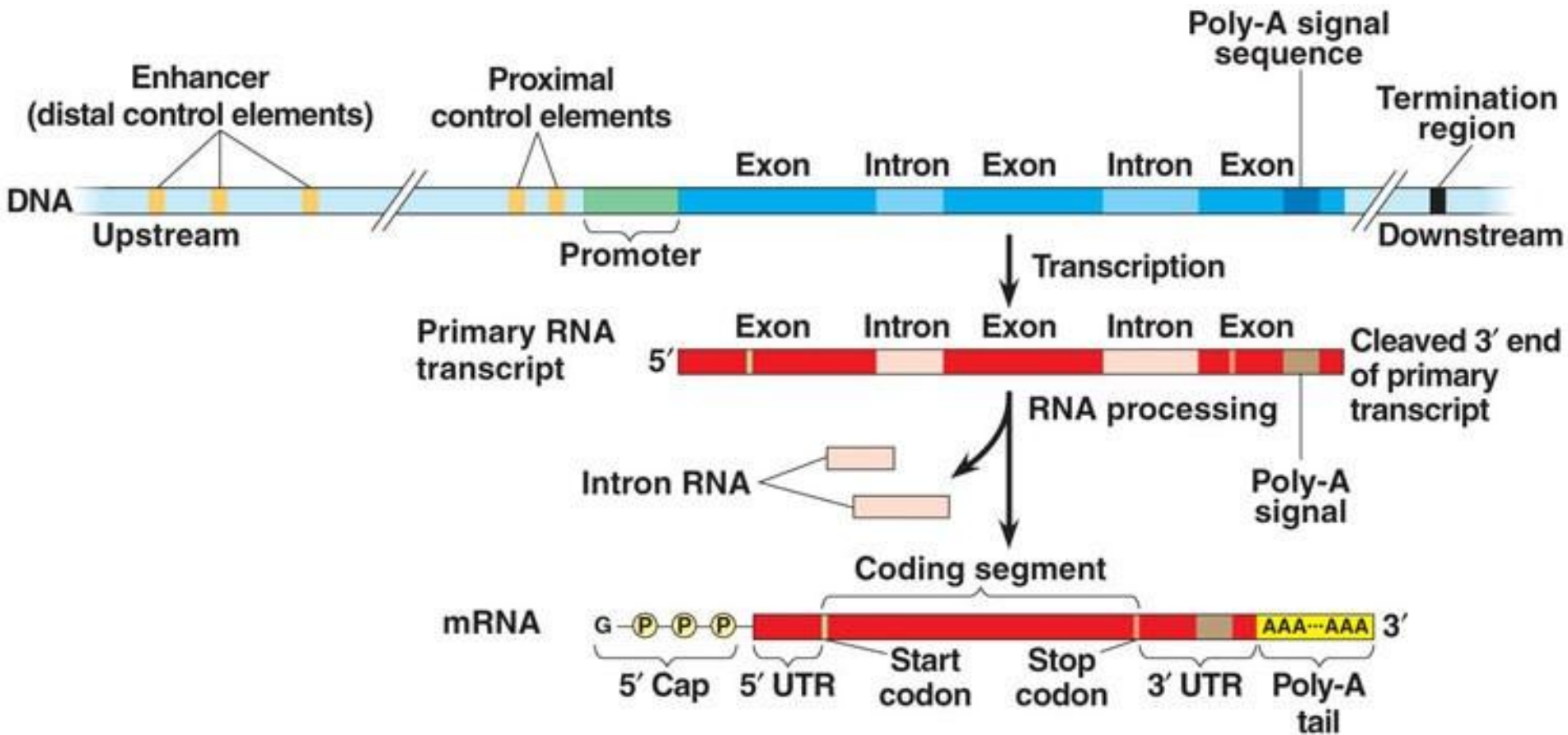
~ 130 genes

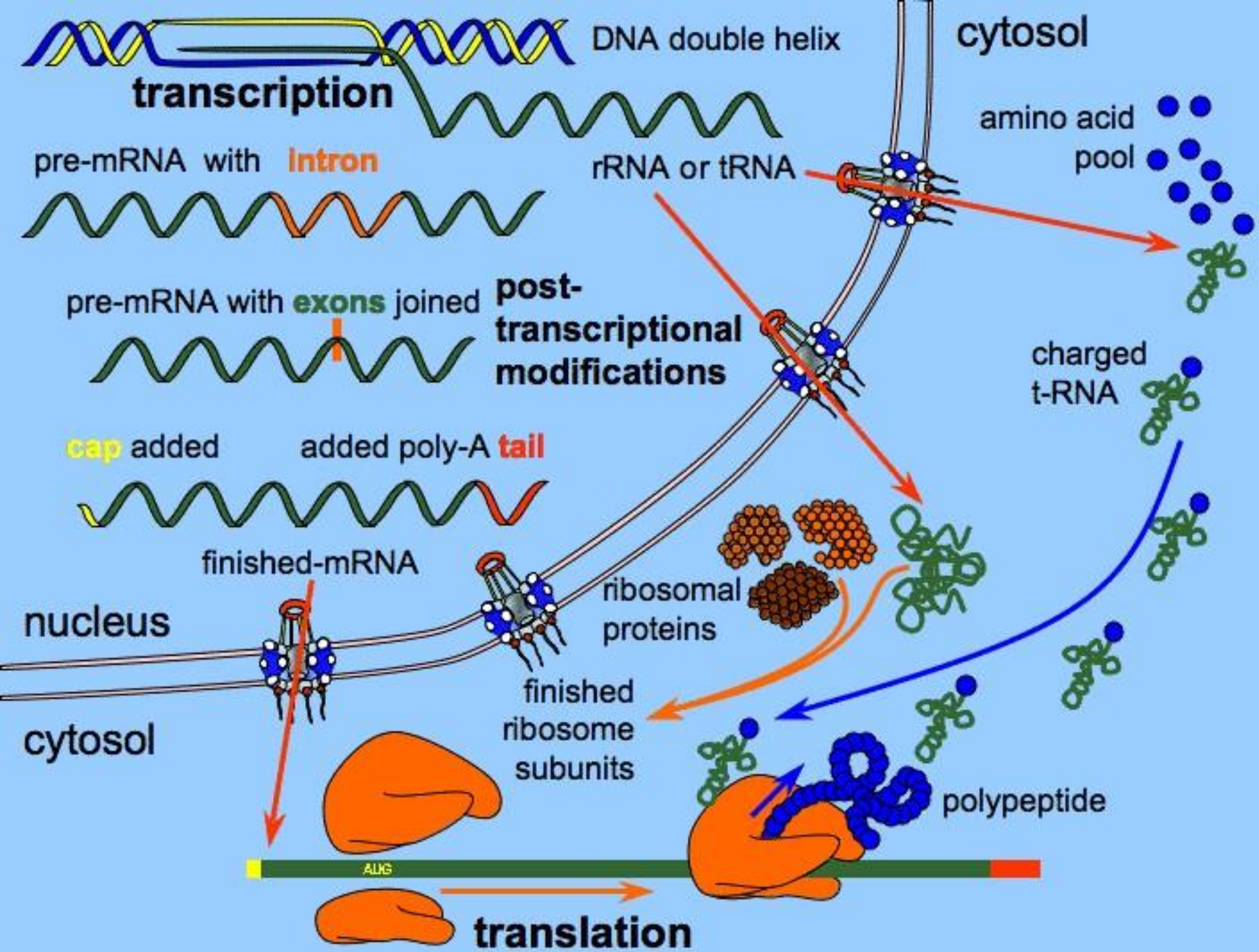
small RNAs –parts of
ribonucleoproteins



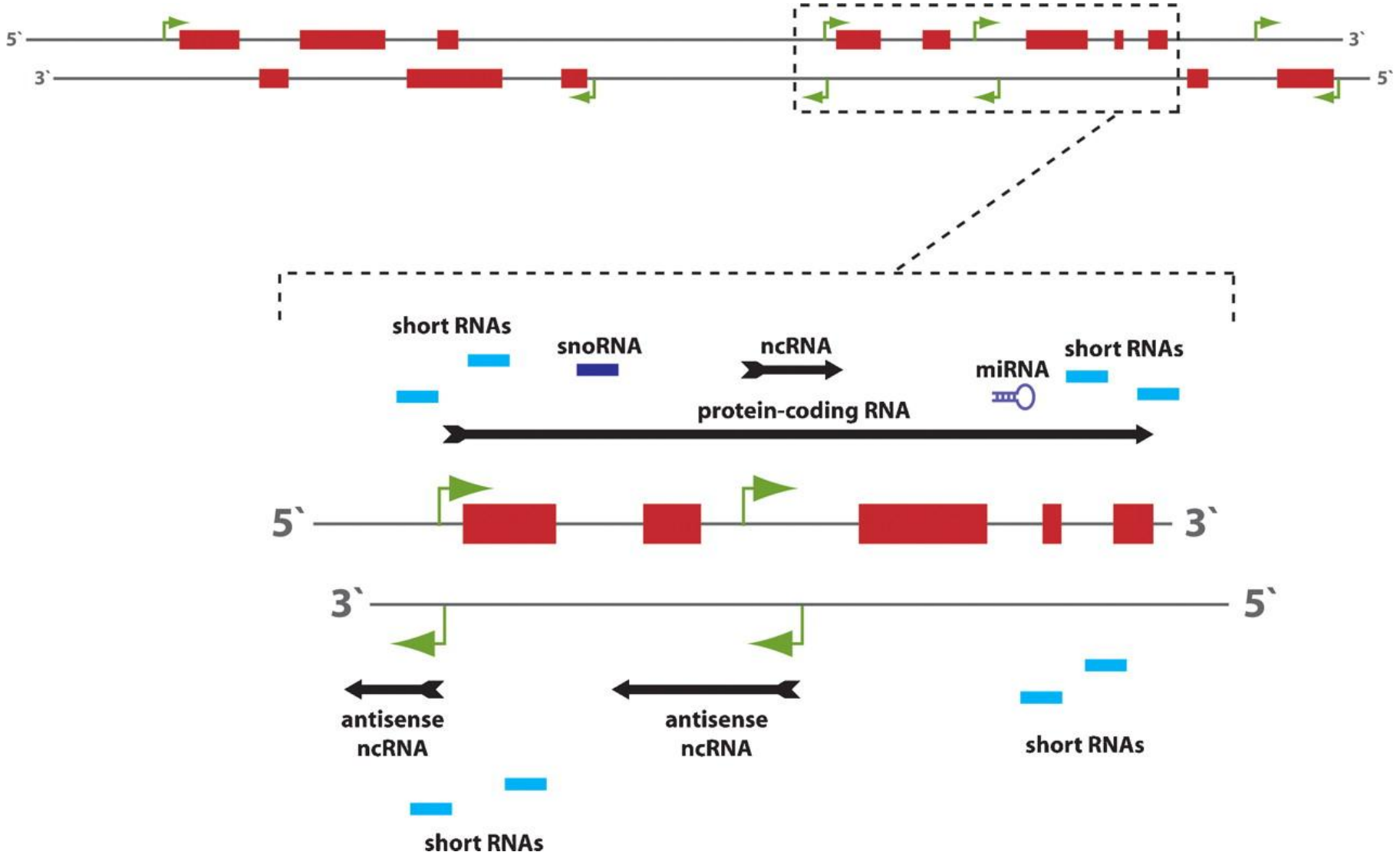
RNA splicing, X-chromosome
inactivation, imprinting,
telomerase

mRNA

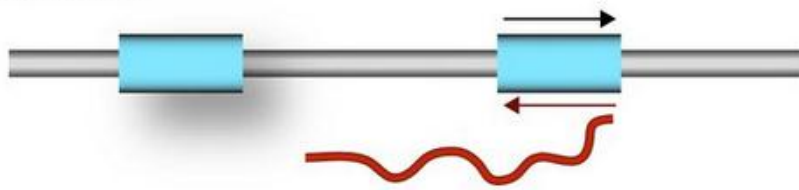




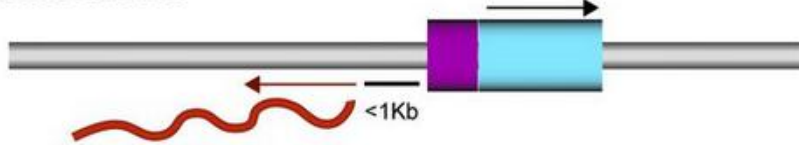
Human genes complexity



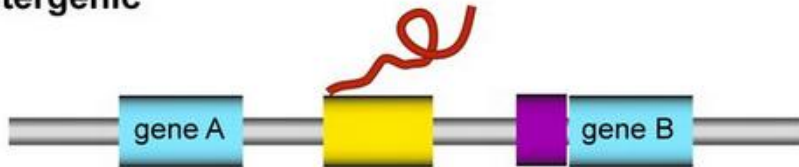
a. Antisense



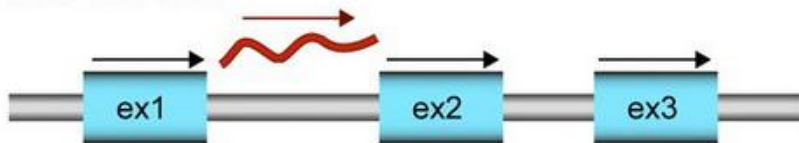
b. Bidirectional



c. Intergenic



d. Sense-intronic



a) Antisense are transcribed from the opposite strand of coding genes

b) Bidirectional are transcribed from the opposite strand, in the opposite direction and within 1 kb of the promoter of coding genes

c) Intergenic are transcribed in the genomic region between two coding genes and usually are located in enhancer regions acting in cis on the promoters of the downstream genes

d) Sense-intronic lncRNAs are transcribed from the sense strand of an intronic region with no overlap of exonic sequence

RNA genes

Ribosomal RNA, rRNA

Transfer RNA, tRNA

Small nuclear RNA, snRNA

Small nucleolar RNA, snoRNA

Small Cajal body RNA, scaRNA

RNA ribonucleases

Small cytoplasmic RNA

Micro RNA, miRNA

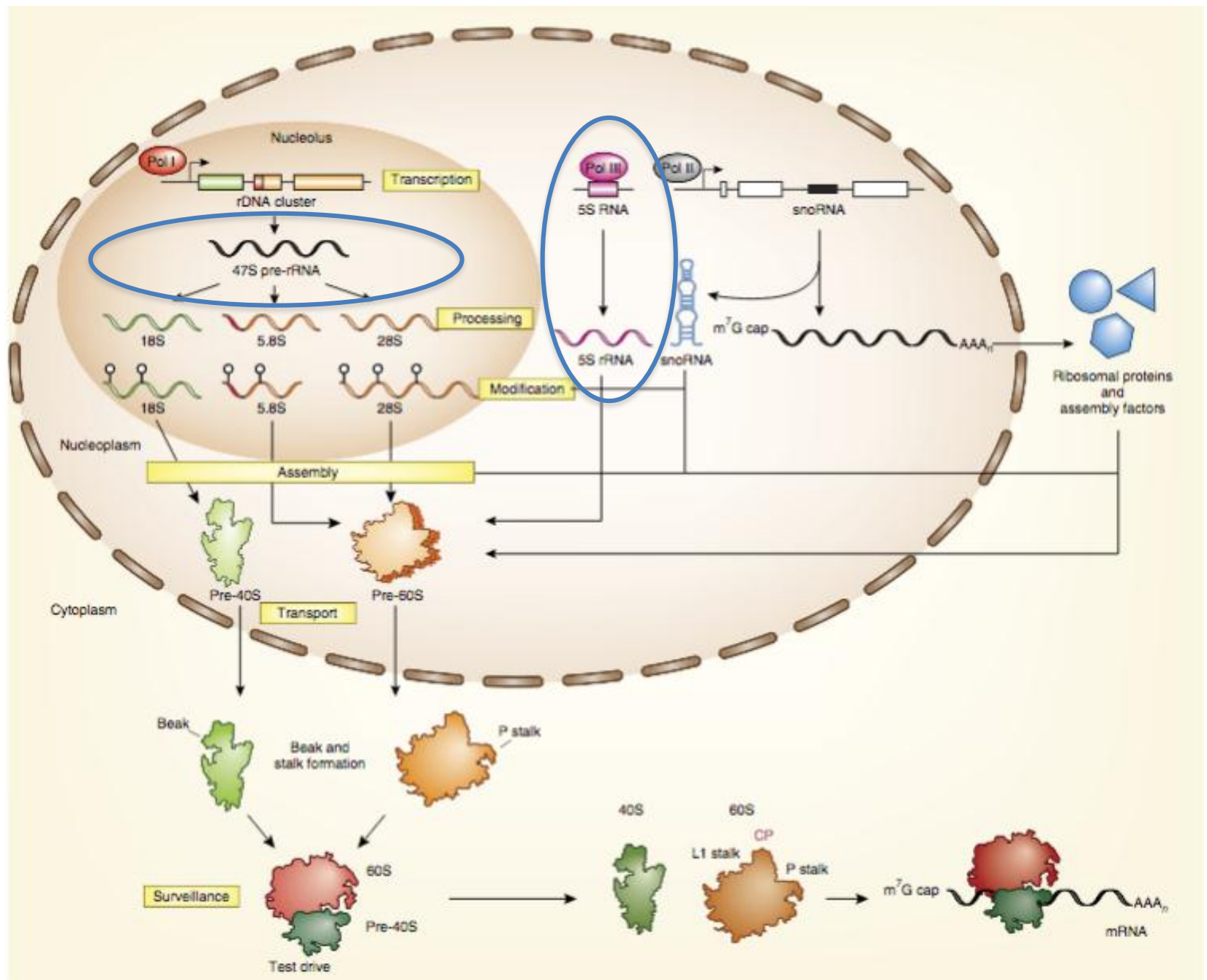
Piwi-binding RNA, piRNA

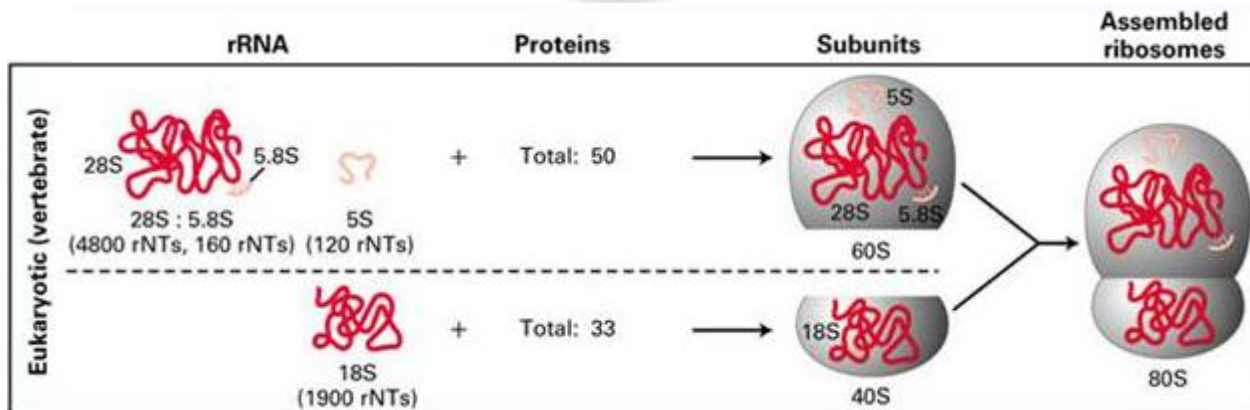
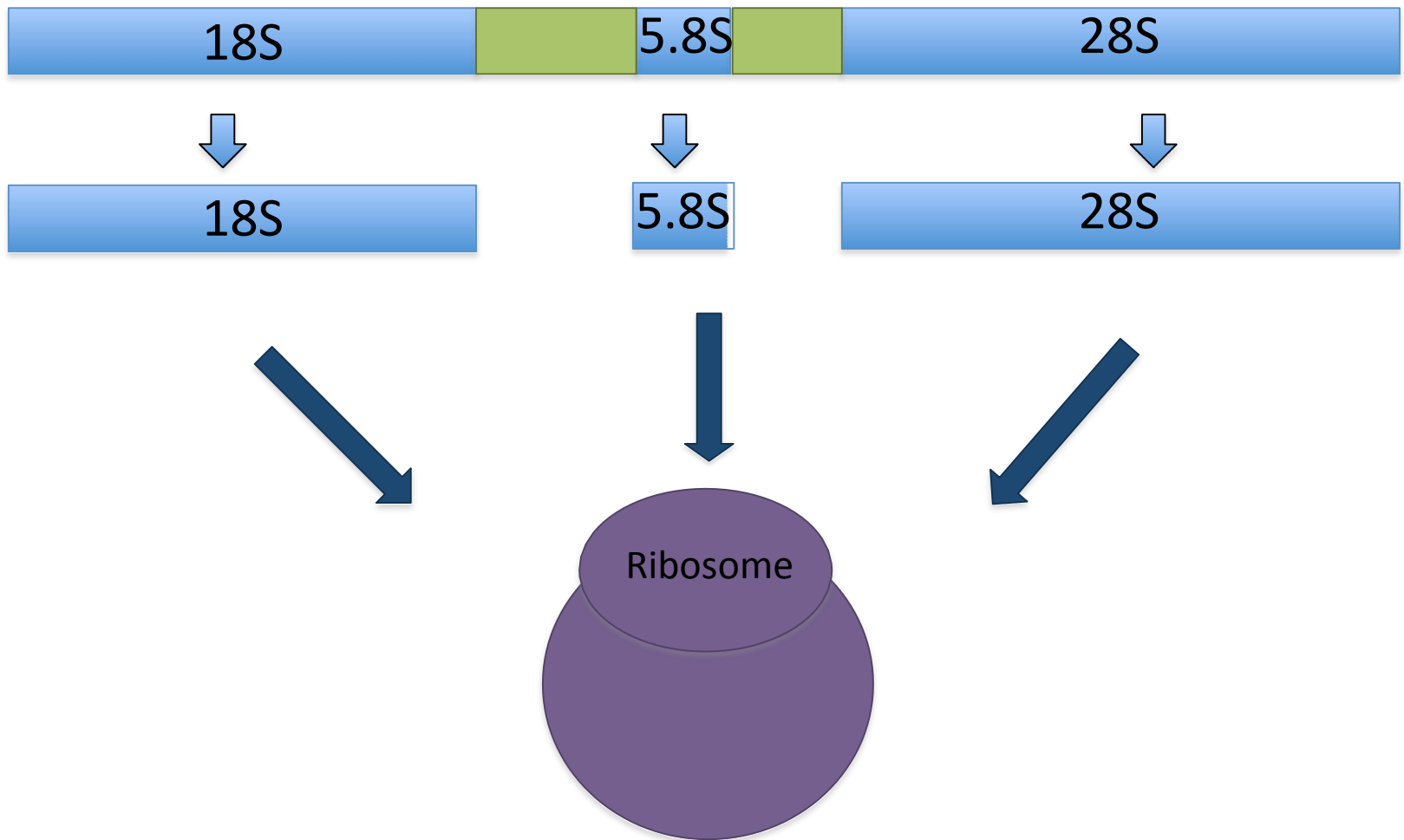
Endogenic small interfering RNA, endo- siRNA

Long non-coding regulatory RNA

Ribosomal RNA genes

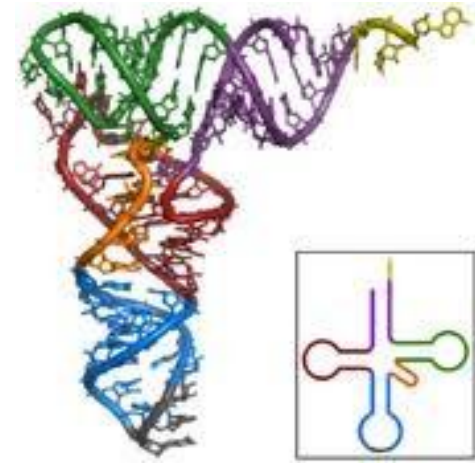
- 2 mitochondrial rRNA (12S and 16S)
- 4 cytoplasmic rRNA (28S, 5.8S, 5S, 18S)
 - 5S - 16 genes on chromosome 1 (a lot of pseudogenes)
 - 28S, 5.8S i 18S – unique multigenic transcription unit (several megabases long)
 - Over 40 repeats of more than 100 rRNA genes on 5 different acrocentric chromosomes





Transfer RNA genes

- 22 mitochondrial tRNAs = 22 mitochondrial genes
- Nuclear tRNA – over 500 genes – mostly on chromosome 1 and 6
 - 49 groups depending on anticodon specificity (61)
 - Weak correlation between number of genes and aa abundance
 - e.g. Cys (2,25%) = 30 genes
 - Pro (6.10%) = 21 genes

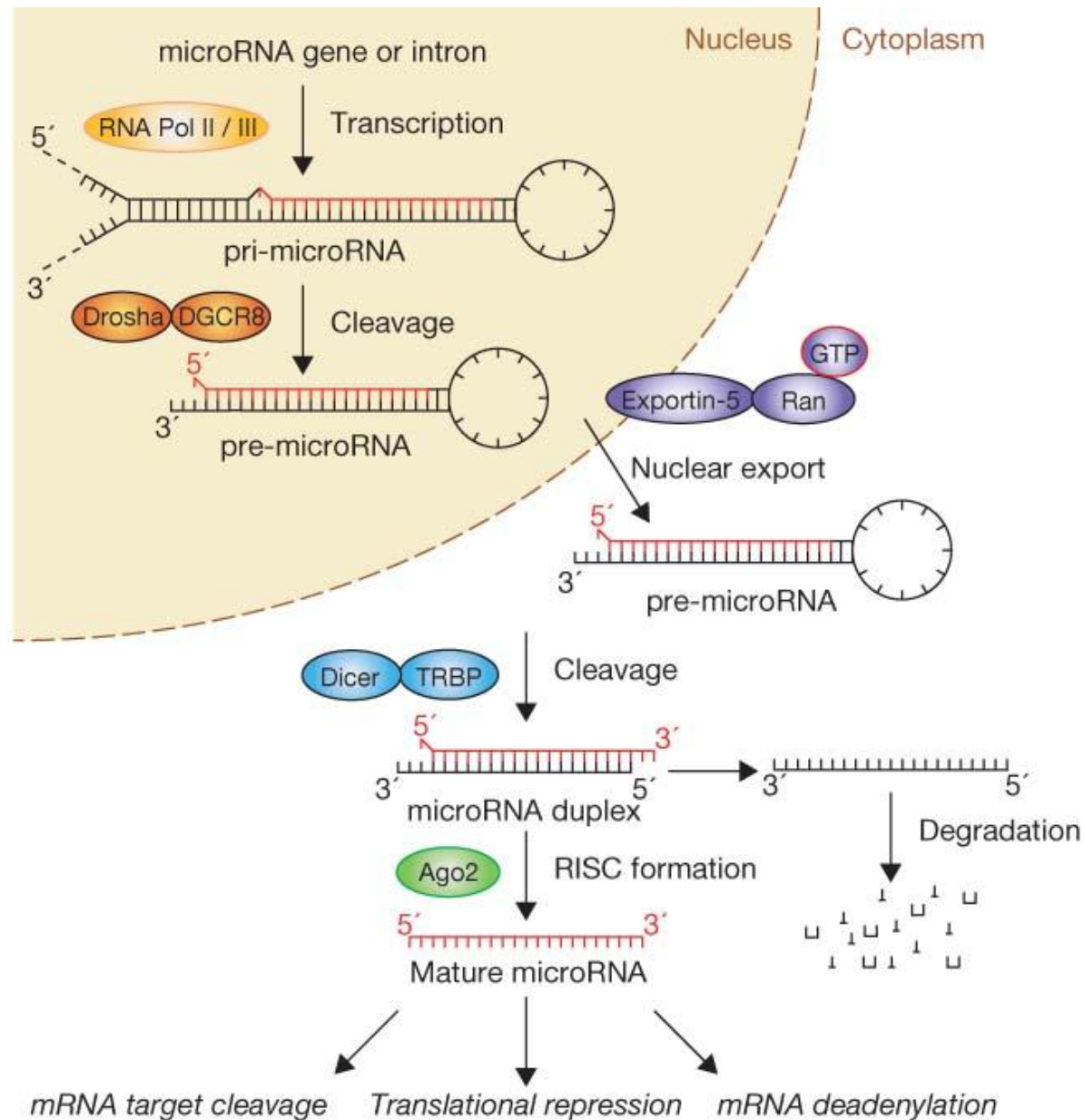


miRNA



- 20-21 nt long
- First described in *C.elegans* & *Drosophila*
- Evolutionary conserved
- Gene expression control and regulation
- Synthesized as longer precursors by RNA polymerase III
- Part of RNA interference process

miRNA processing

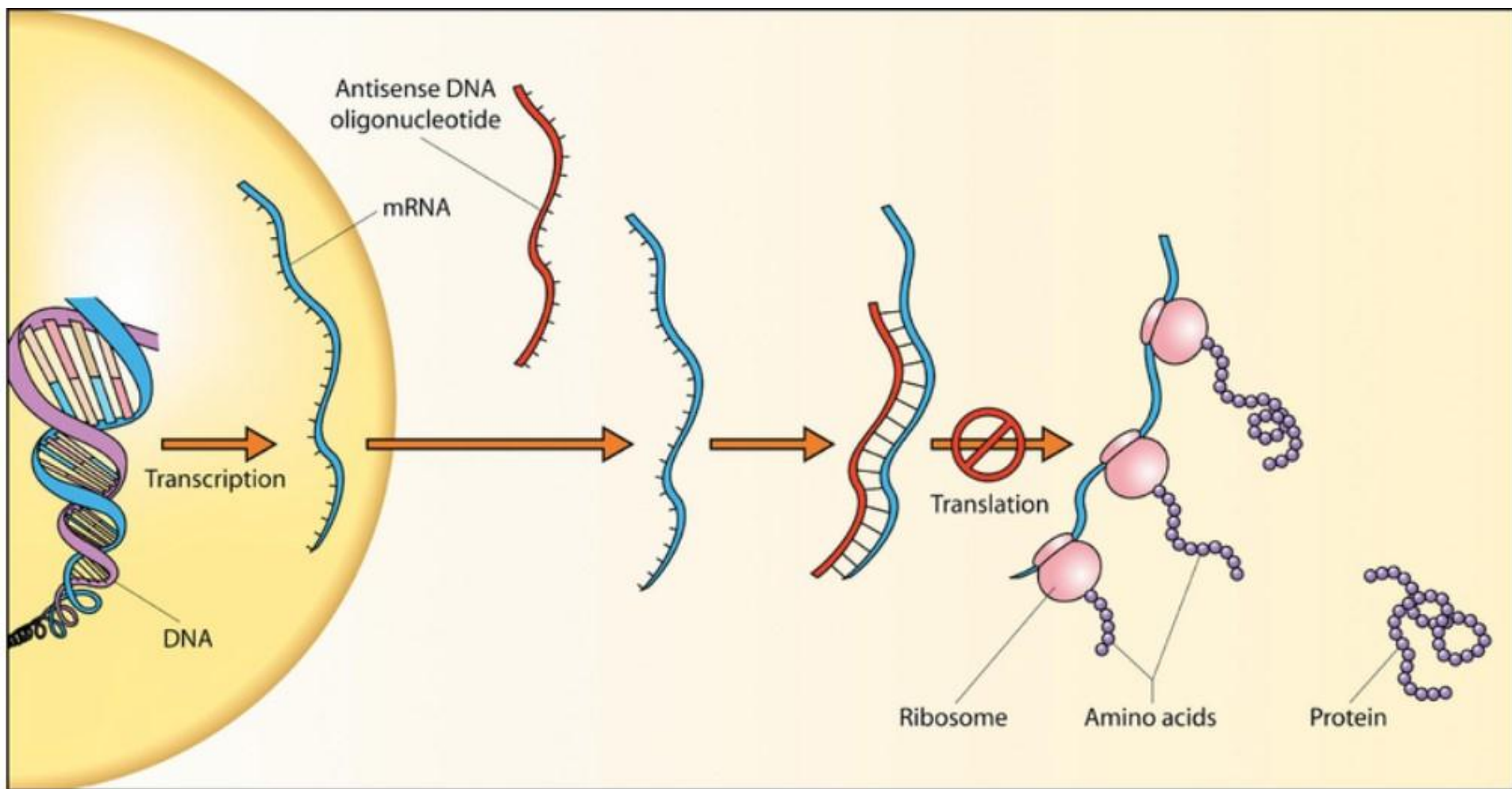


RNA interference

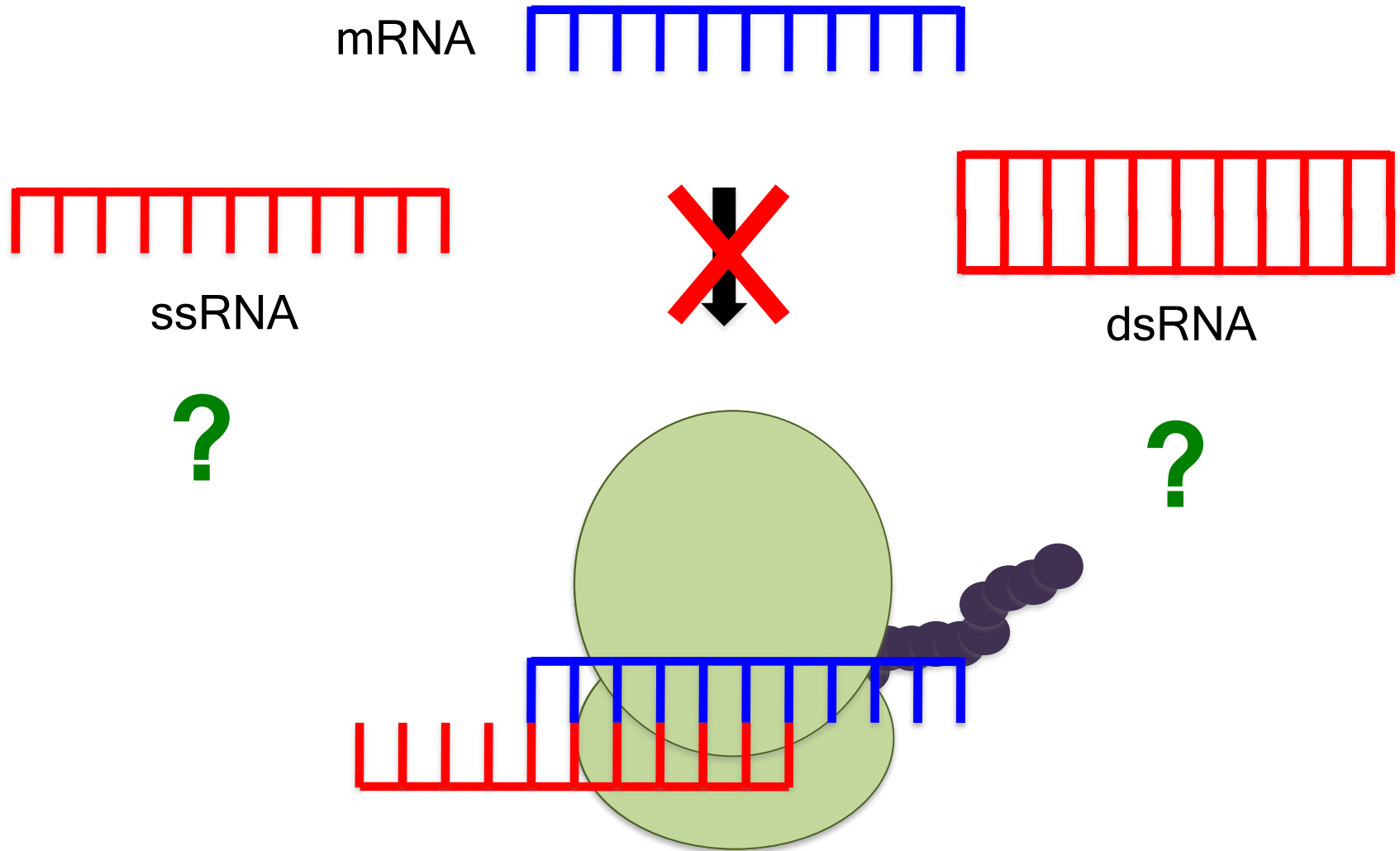


RNA interference

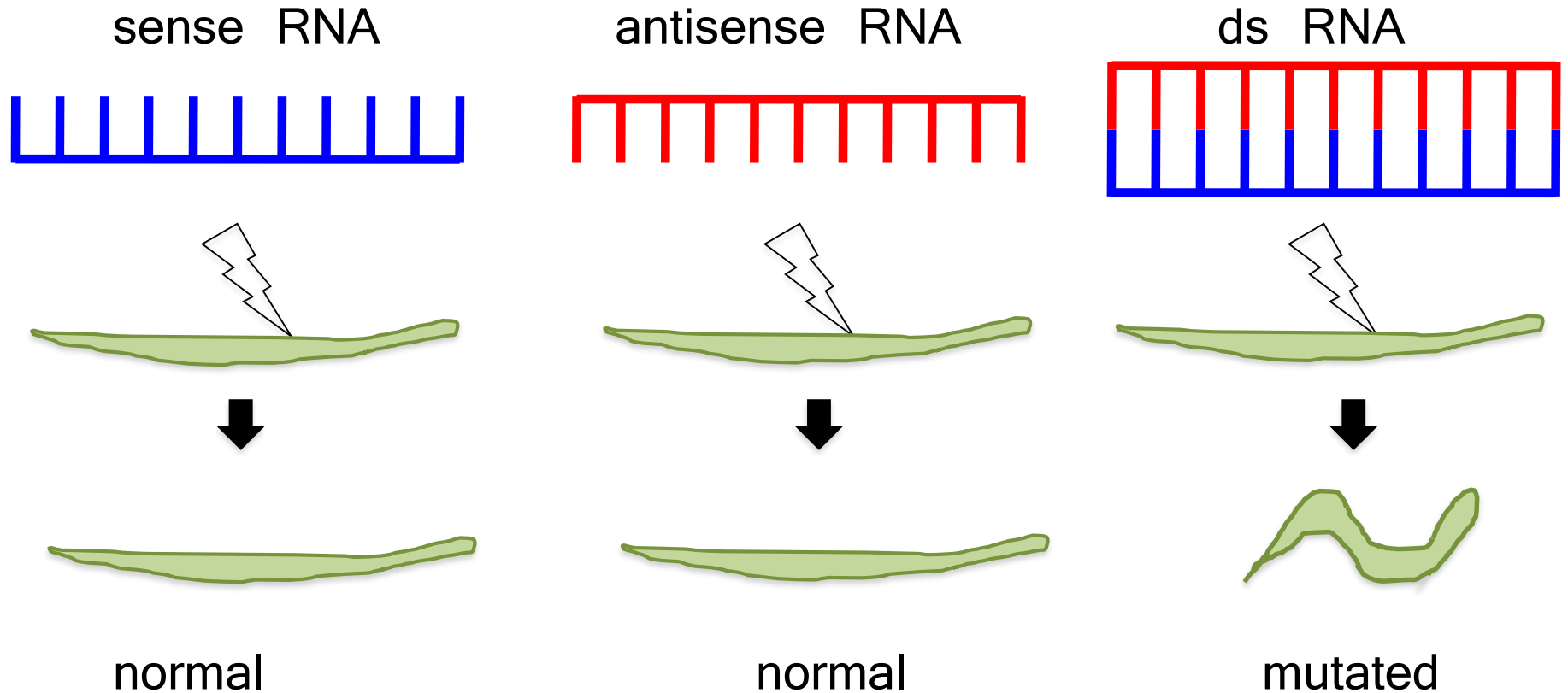
- Natural way of regulation of gene expression
 - 30% of human genome regulated
- Post-transcriptional silencing of protein synthesis
- More than 1000 genes
- Craig Mello & Andrew Fire 1998. (NP 2006)
 - in *C. elegans*
- Important role in defending cells against parasitic genes (viruses and transposons)



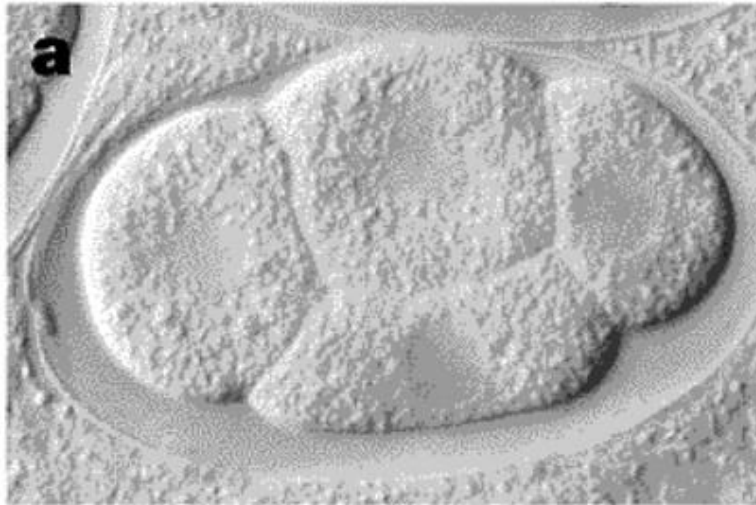
ssRNA ili dsRNA??



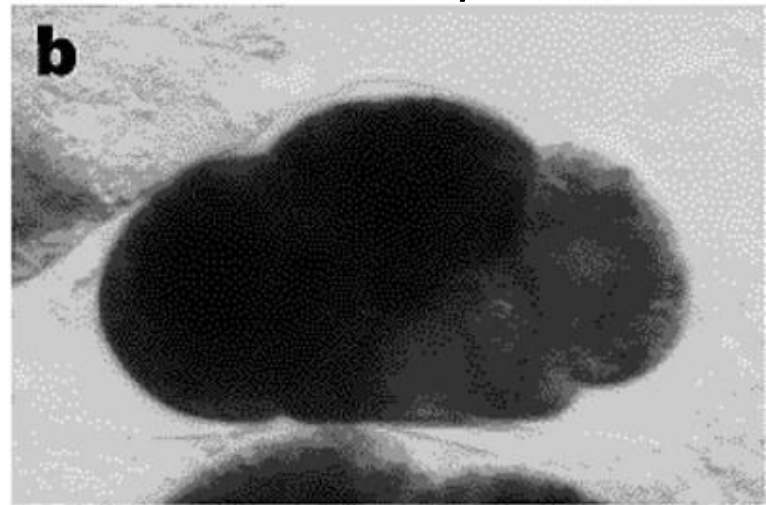
dsRNA??



Negative ctrl.
(no probe)



mex-3 normal
expression



Sense RNA.

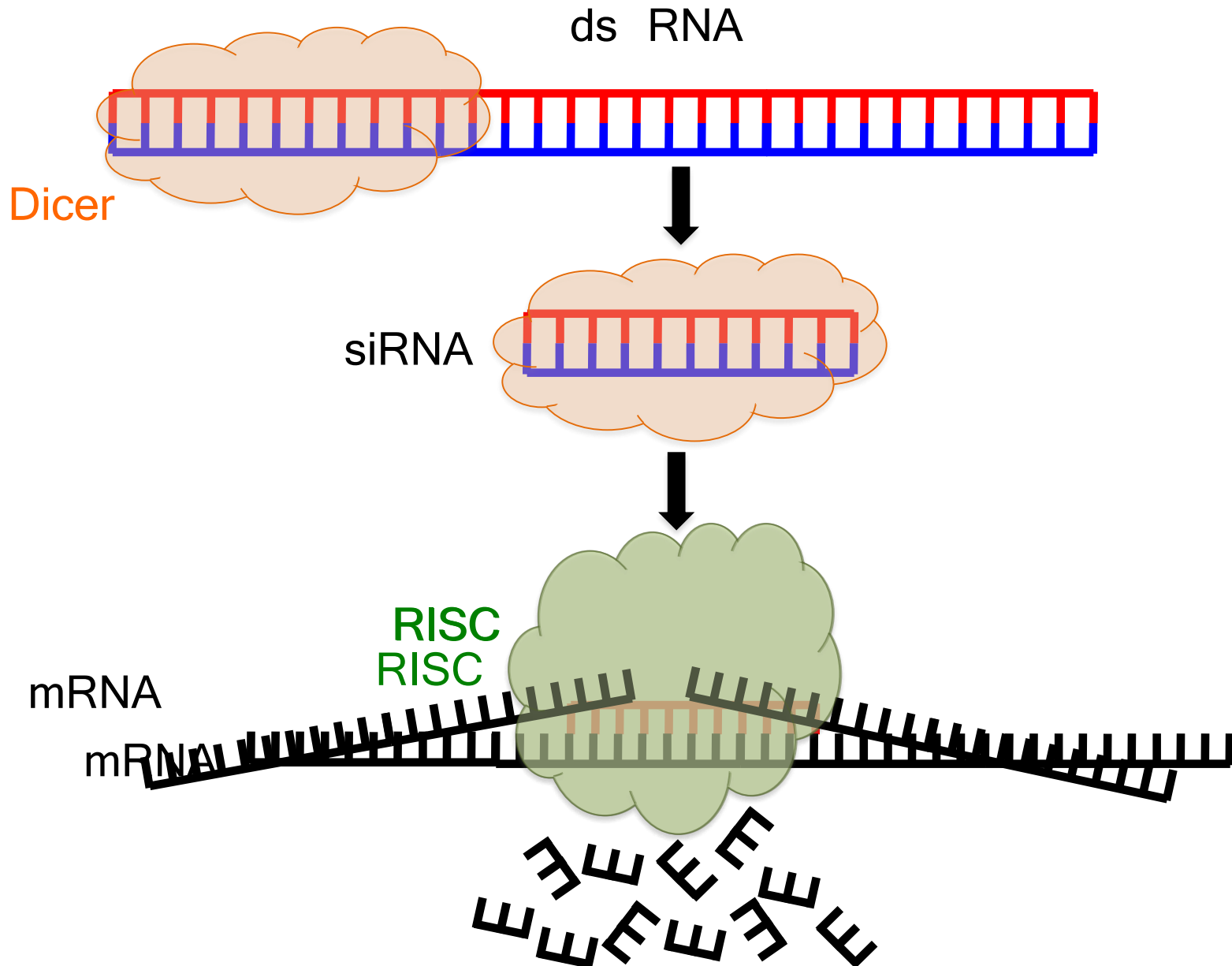


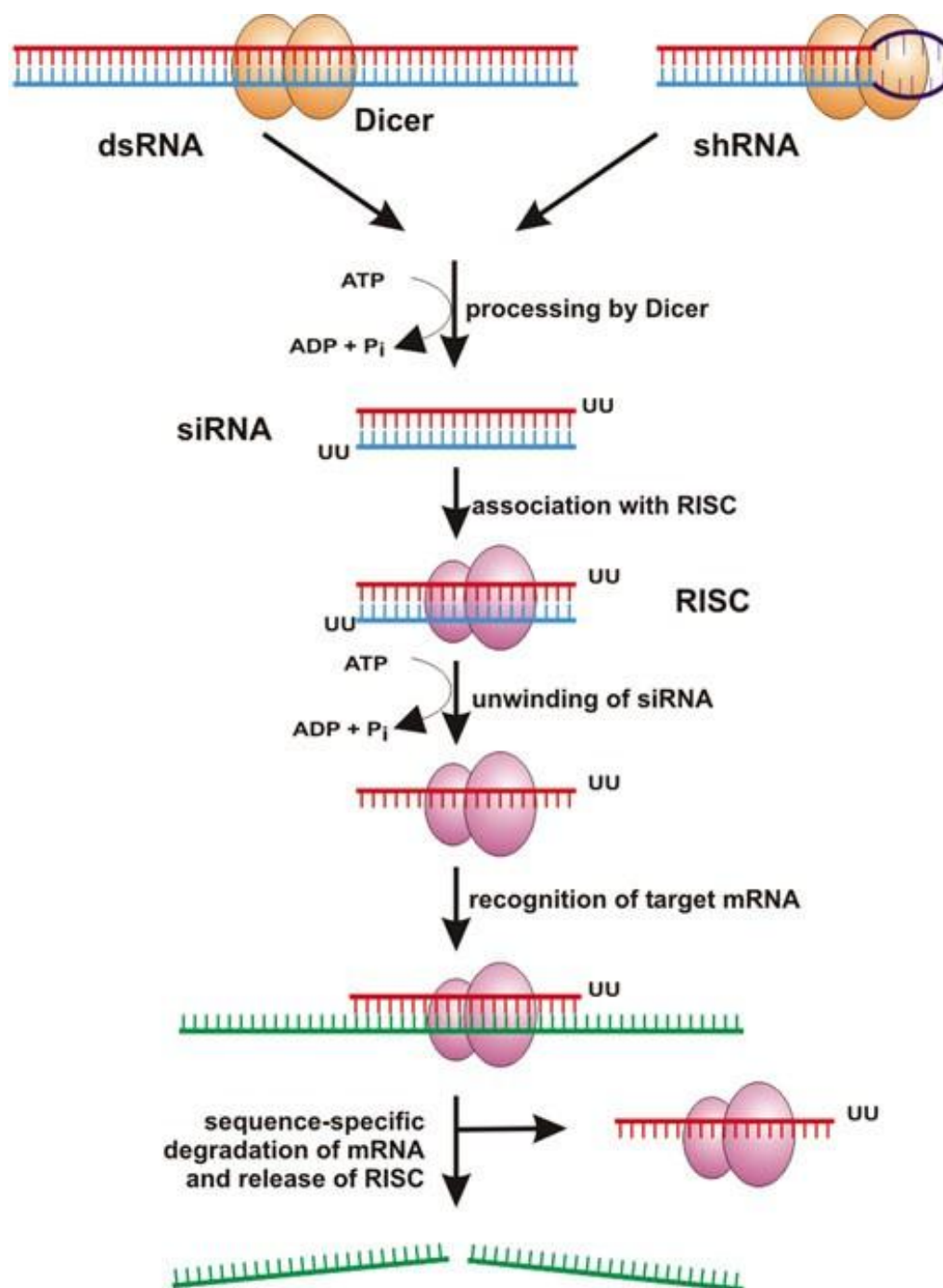
dsRNA

The Players in RNAi

- RNA
 - siRNA: dsRNA 21-22 nt, most commonly a response to foreign RNA (usually viral)
 - miRNA: ssRNA 19-25nt. Encoded by non protein coding genome, regulates post-transcriptional gene expression
- RISC:
 - RNA induced Silencing Complex, that cleaves mRNA
- Dicer (enzyme):
 - produces 20-21 nt cleavages that initiate RNAi

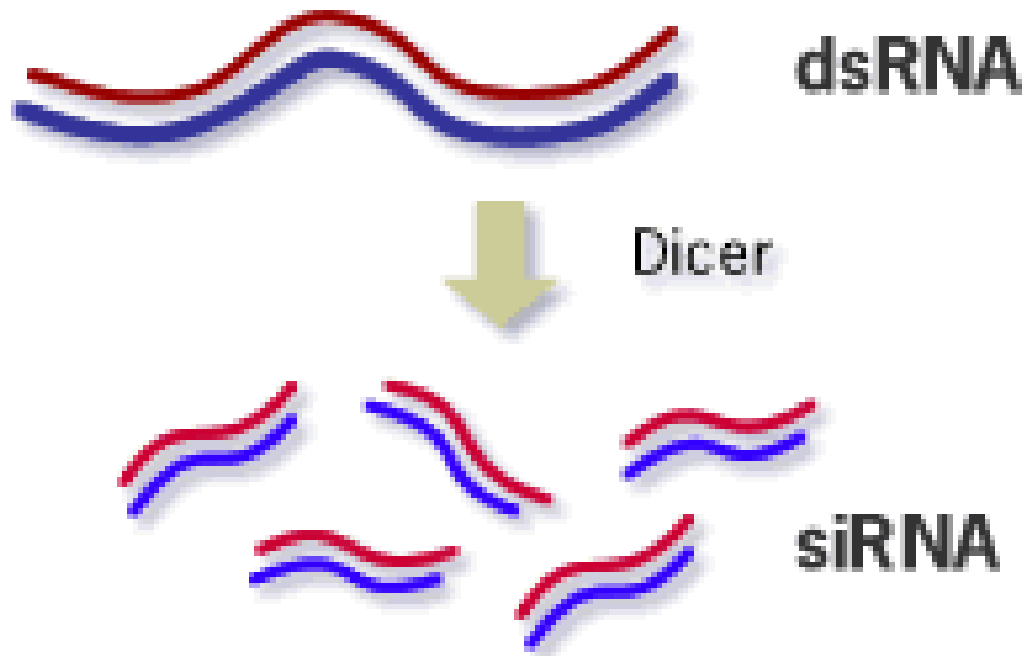
Dicer and RISC - enzymes





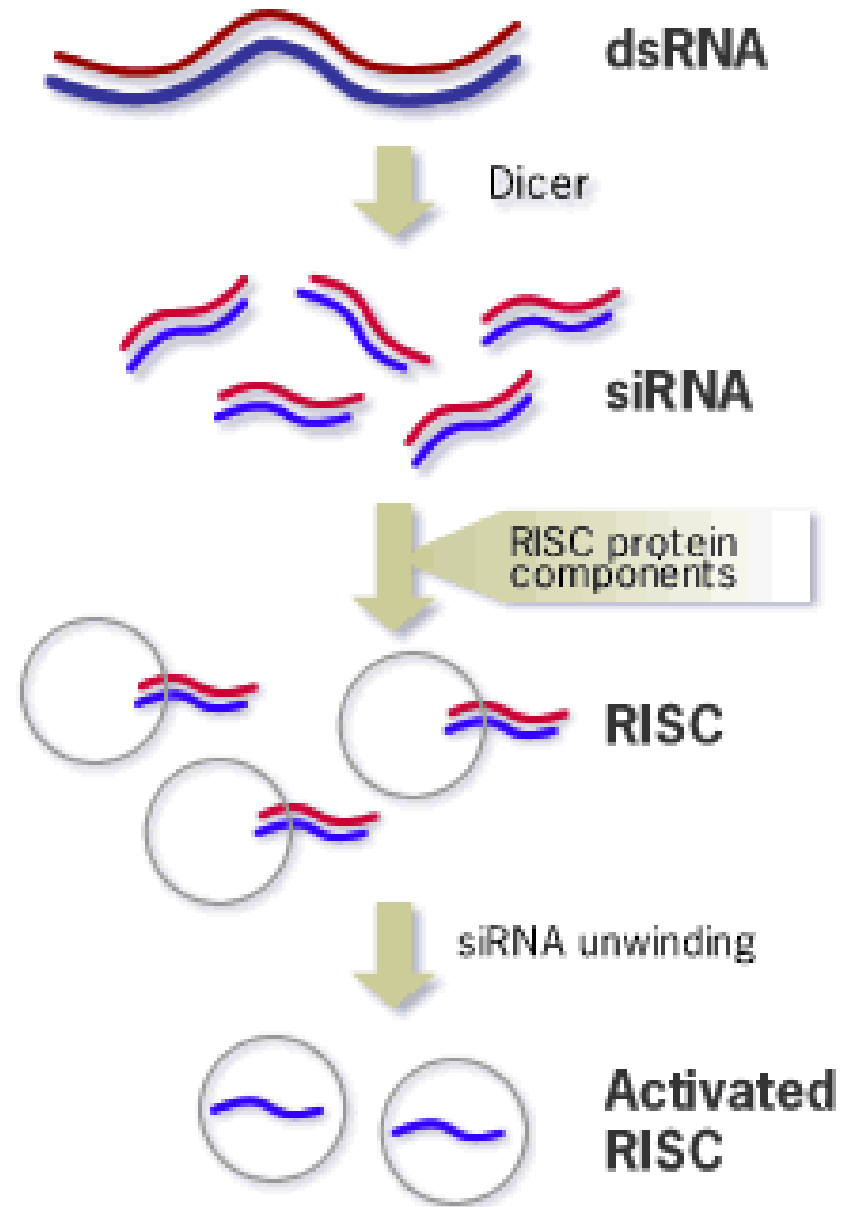
RNAi – 1st step

- dsRNA is cut by Dicer into short siRNA (21-25 bp nucleotides)



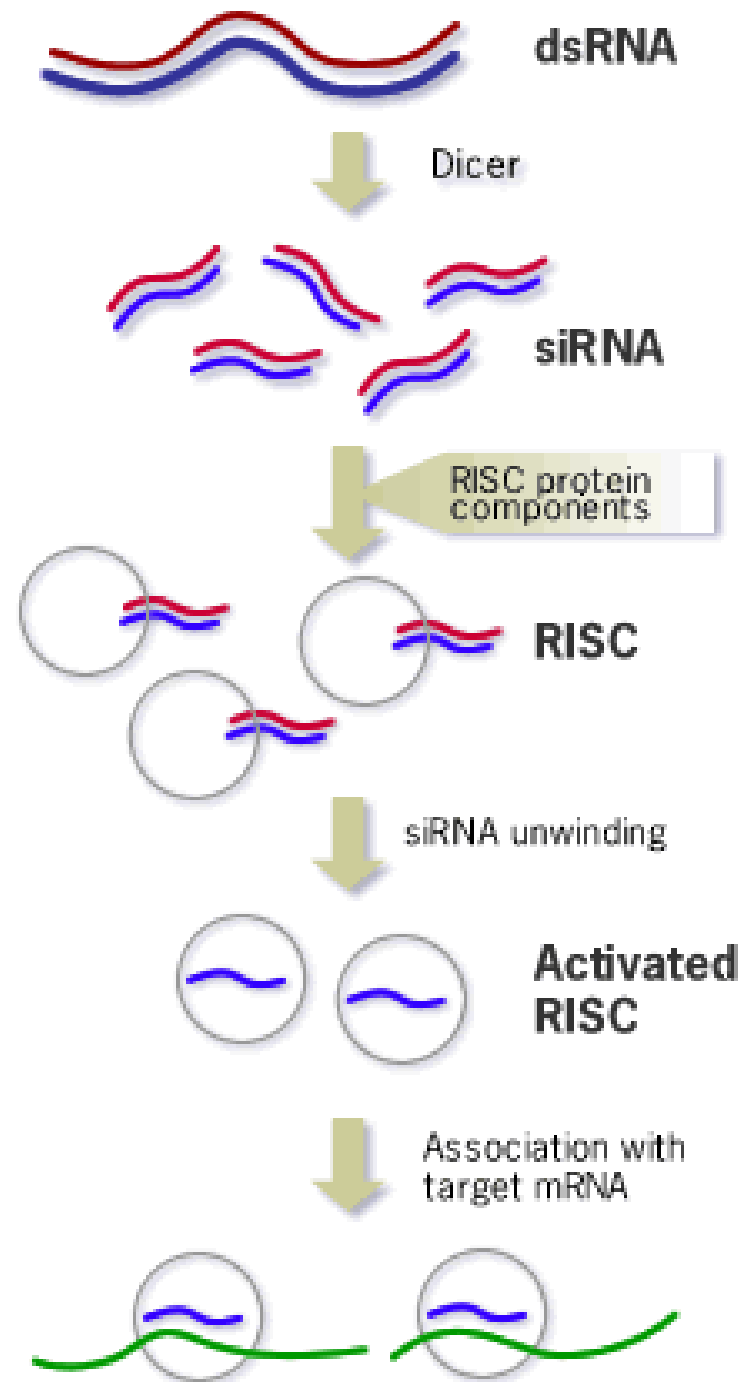
2nd step

- siRNA binds RISC complex (RNA-induced silencing complex)
- Unwinding siRNA



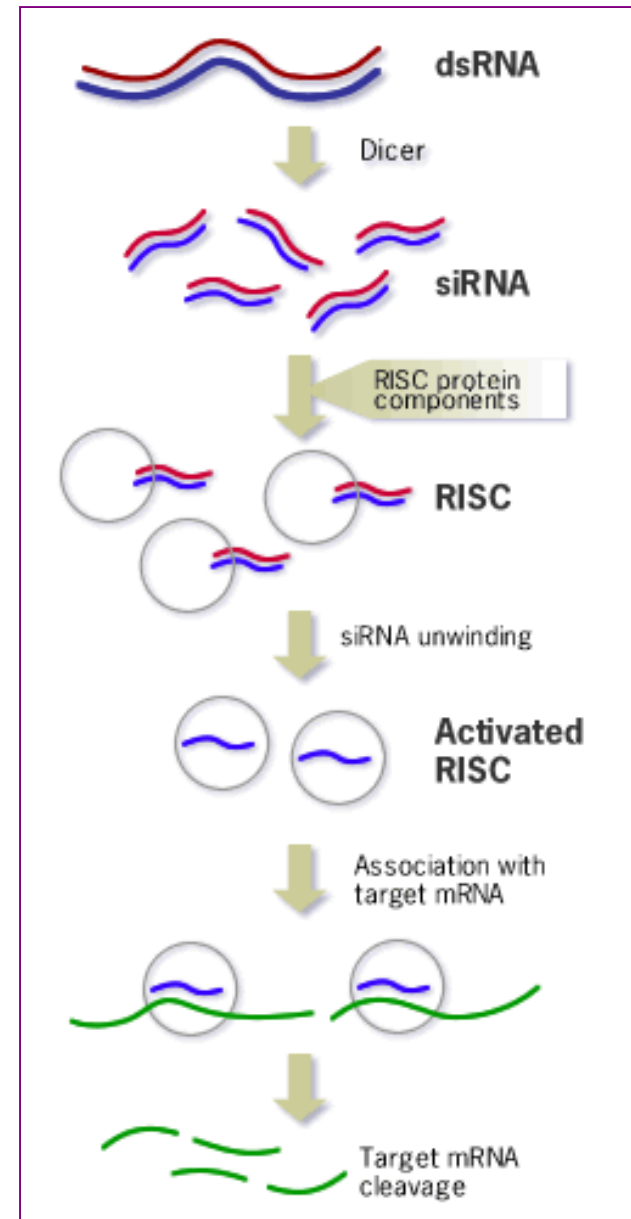
3rd step

- Antisense siRNA helps RISC binding to complementary mRNA

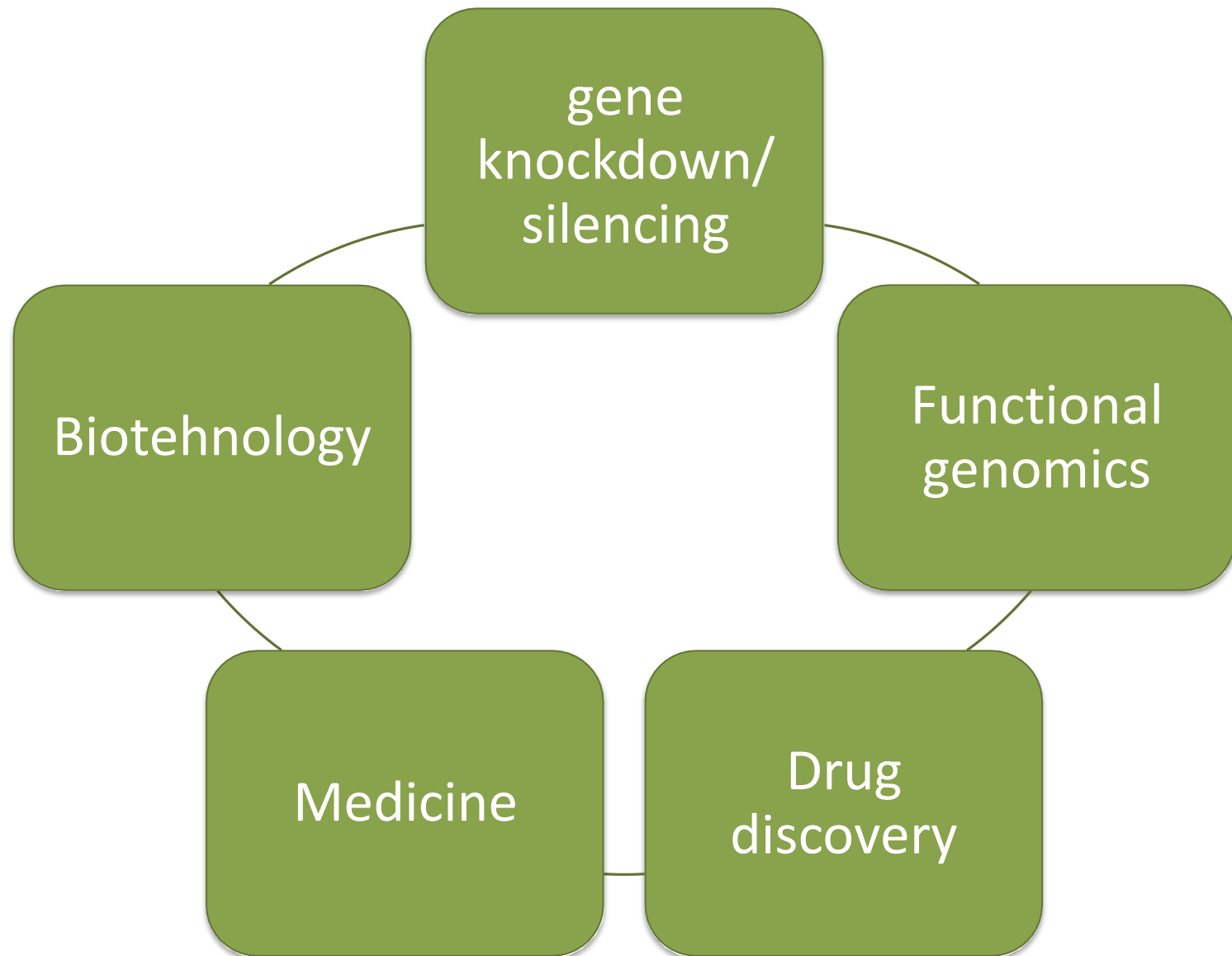


4th step

- RISC cuts mRNA
- mRNA is cleaved by other enzymes, RNases



RNAi applications



RNAi in medicine

- Antiviral therapy
 - Herpes simplex, hepatitis, HIV
- Neurodegenerative diseases
 - Huntington's disease
- Tumors
 - Silencing of genes responsible for cellular proliferation

Problem!

1. “off-target” effects
2. siRNA delivery into cells

First FDA approved RNAi therapy

(in 2018)

- For hereditary amyloidosis (transthyretin-related) polyneuropathy; AD
 - Caused by mutation in transthyretin (carrier protein) that deposits in nervous tissue
 - Affects 50 000 people globally
 - Life expectancy 2,5 - 15 years
- In phase 3 clinical trial: 90% of patients showed a halt in disease progression (while 4% in placebo group)
- Infusion of lipid nanoparticles with siRNA into liver to block translation of abnormal protein
 - Drug (Patisiran) will reach annual sales of 1 billion USD by 2023