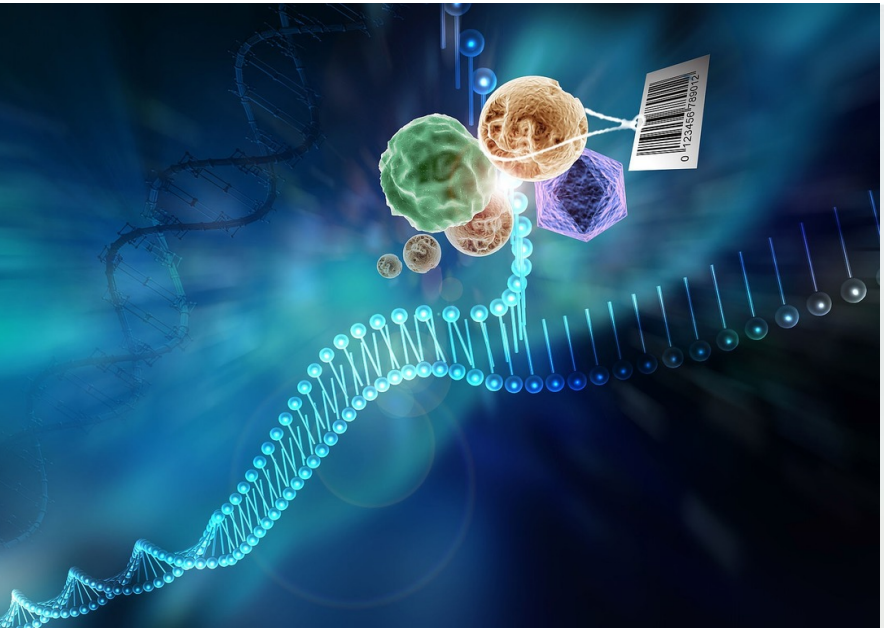
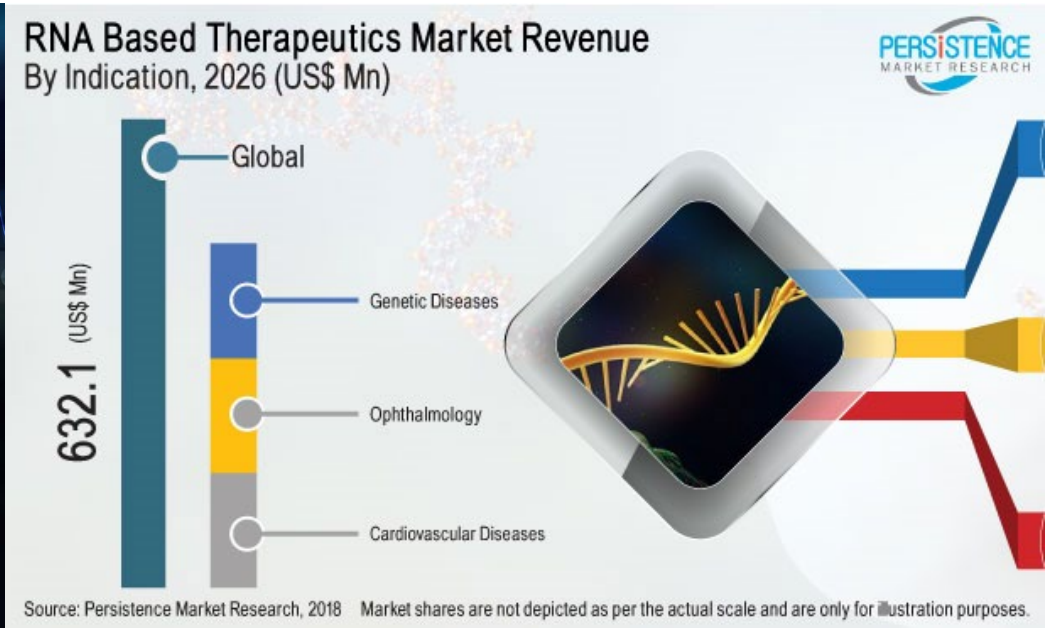
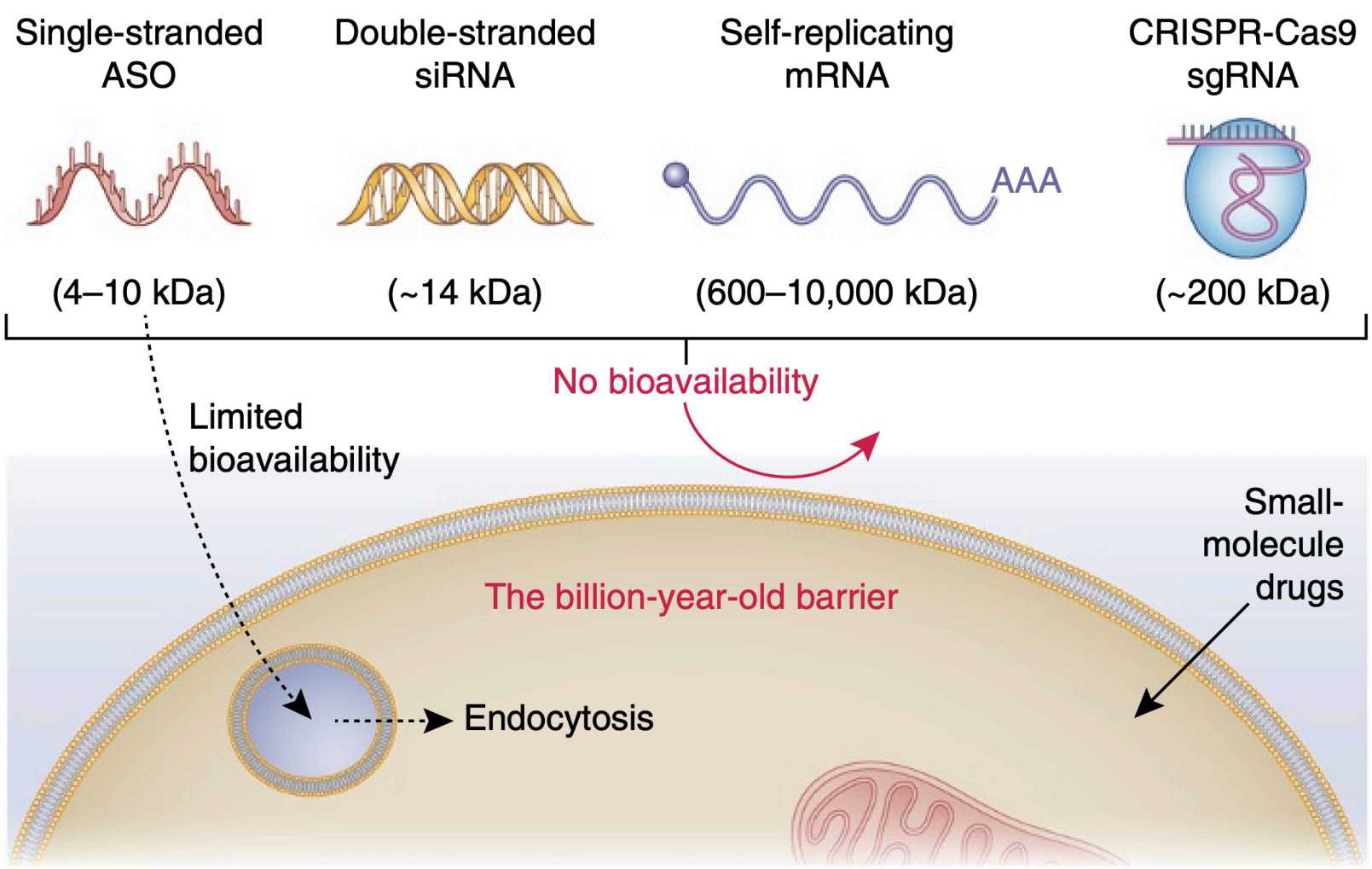
RNA *and* Disease

- known since the late 1800s.
- more than *just* a messenger
- recognized with 33 Nobel Prizes *so far*...





Why was RNA *not* used as a therapeutic before?



Dowdy SF (2017) Nature Biotechnology

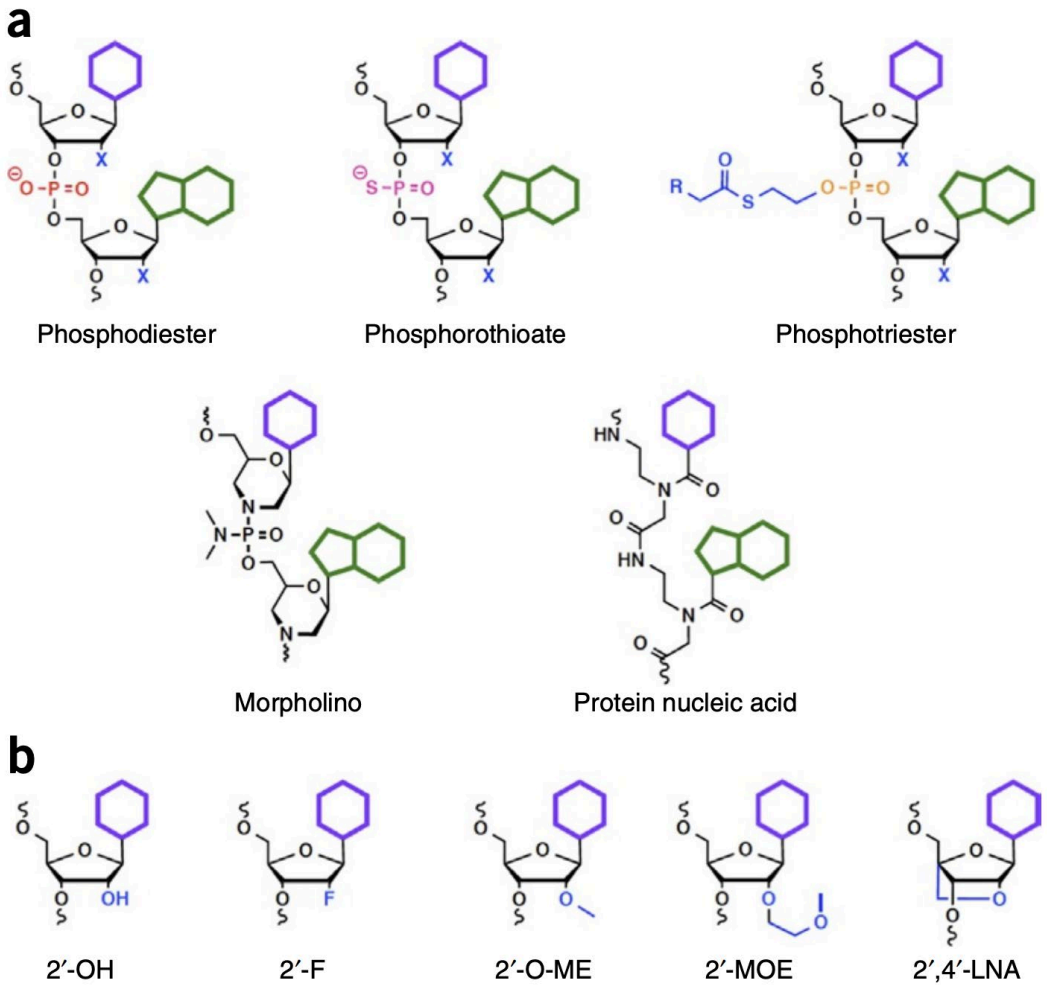
Why was RNA *not* used as a therapeutic before?

Table 1 Challenges erected by evolutionary barriers to RNA therapeutic delivery

Feature	Challenge for delivery
Oligonucleotide size and charge	Too large or too charged to passively diffuse across the lipid bilayer
RNase susceptibility	Rapid degradation by blood and tissue RNases.
Reticuloendothelial system	Rapid clearance from the blood by the kidneys and liver scavenger receptors
Immunogenicity	Oligonucleotides activate extracellular and intracellular innate immune responses
Endocytosis	Oligonucleotides are taken up, but trapped inside endosomes

Dowdy SF (2017) Nature Biotechnology

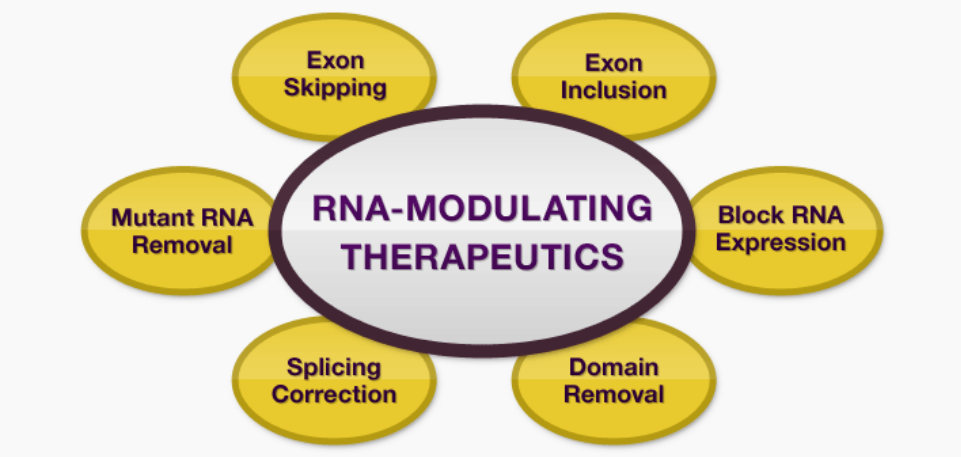
Chemical modifications that have led to RNA *Therapeutics*

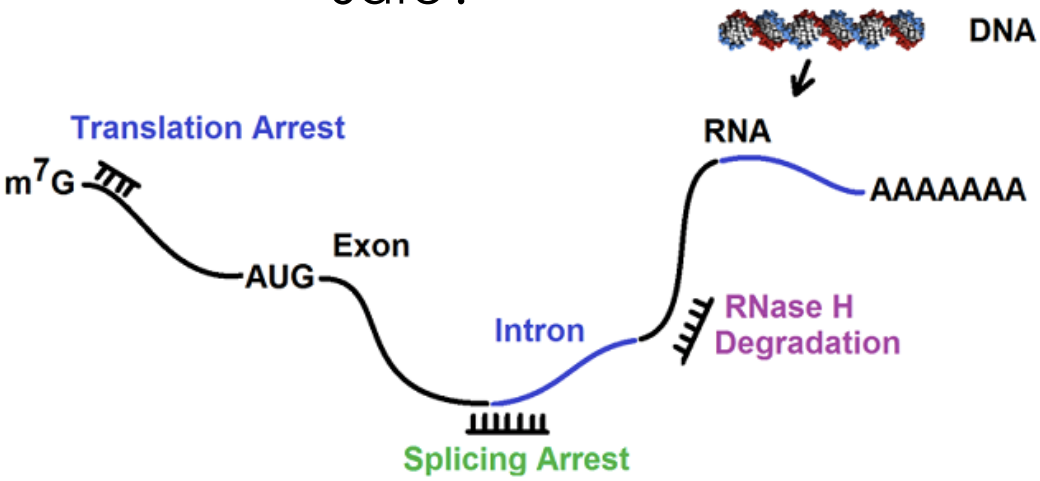


Why is RNA an excellent druggable molecule?

Advantages: Specific
Accessible
Universal

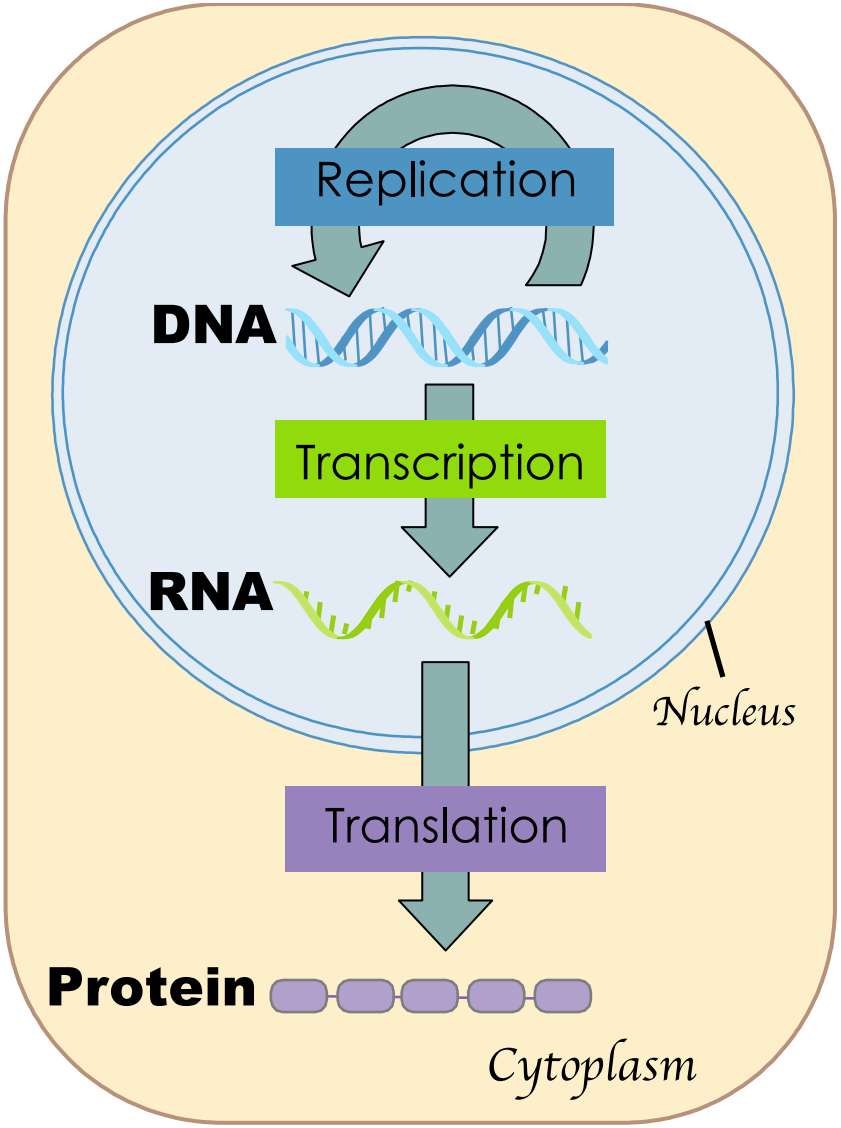
Disadvantages: Delivery
Stability
Safe?



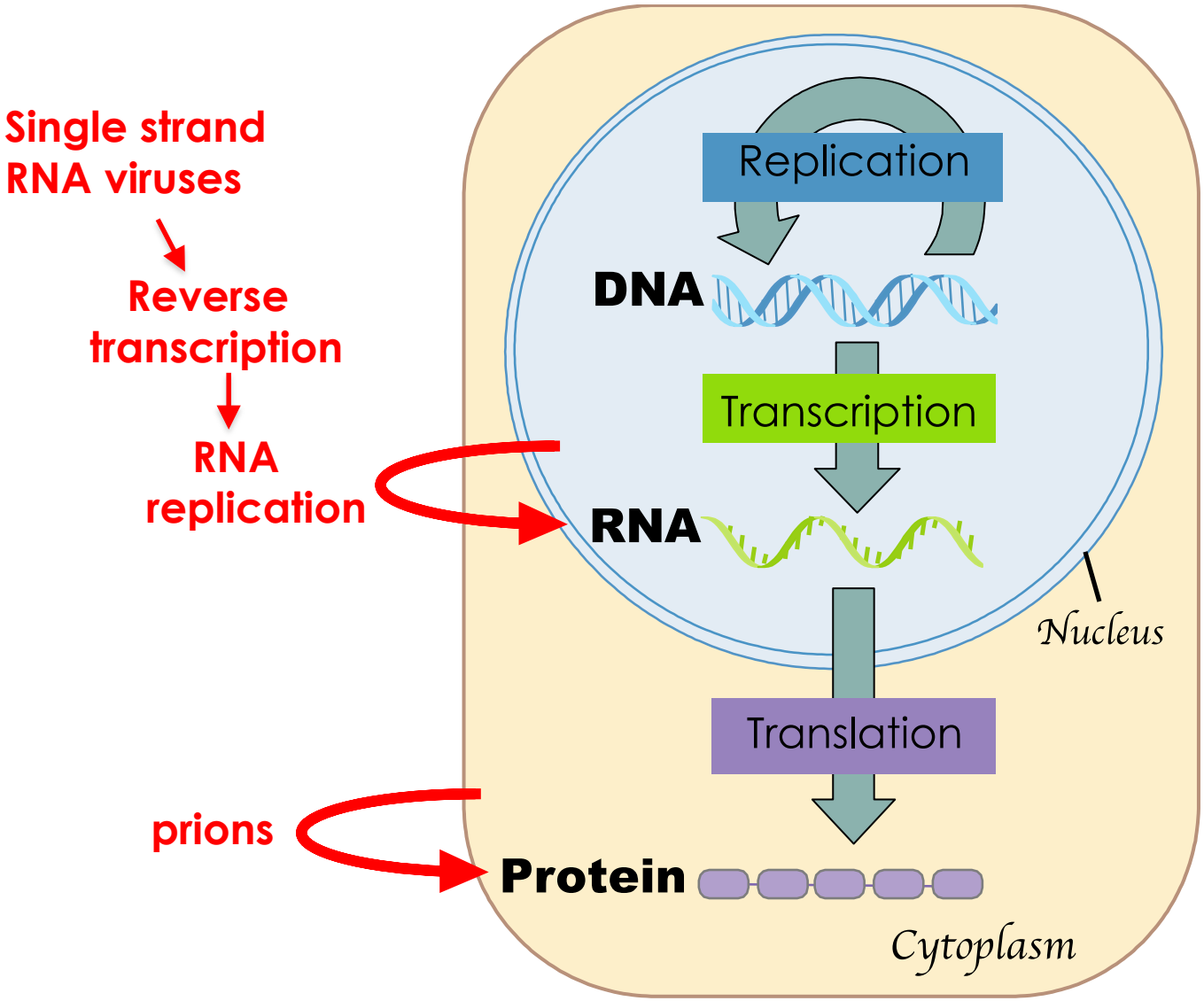


Antisense oligonucleotides

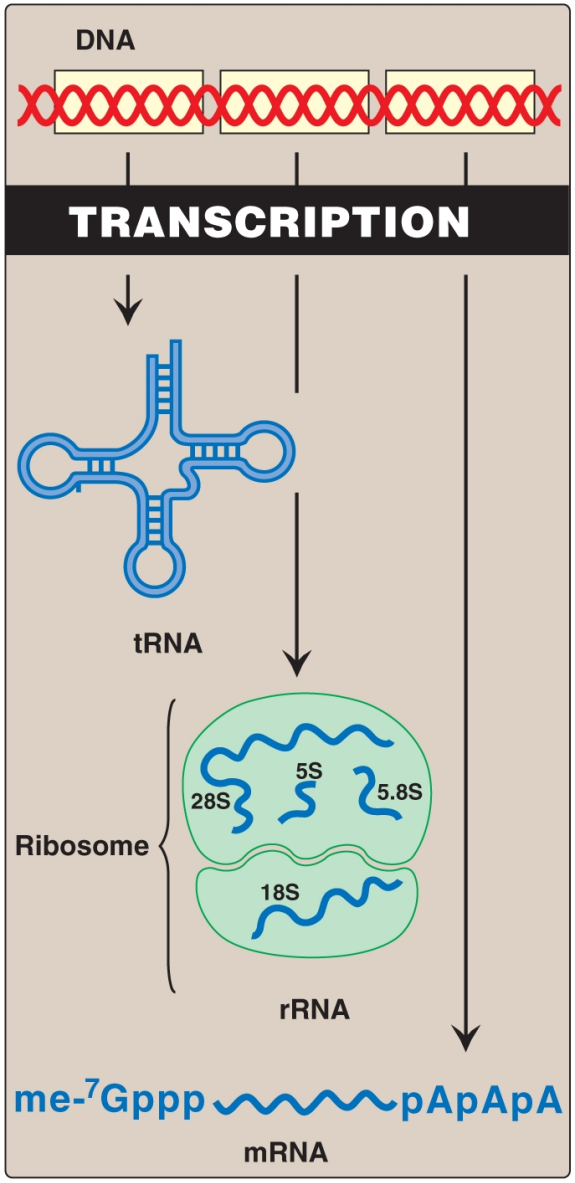
"The Central Dogma"

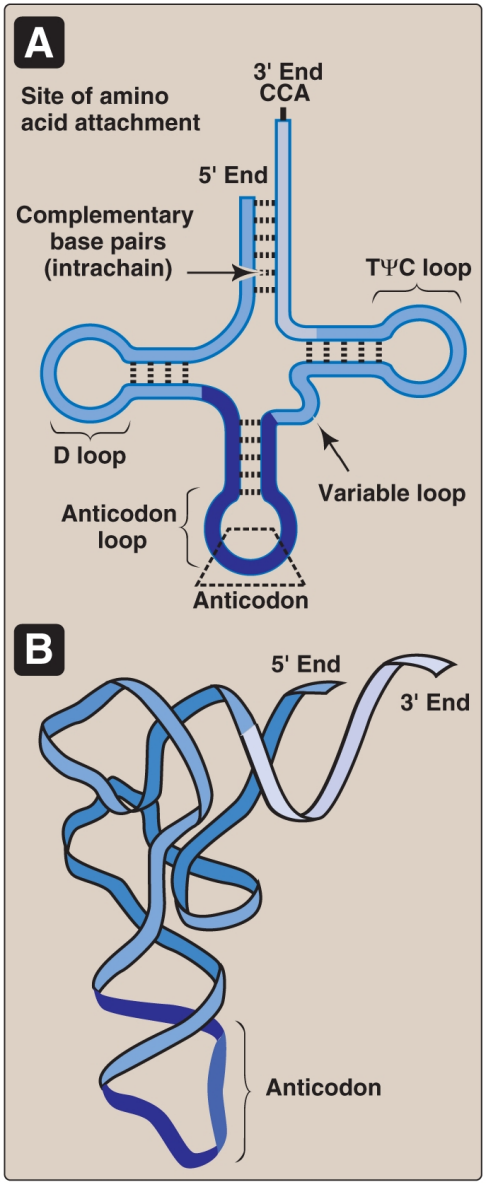


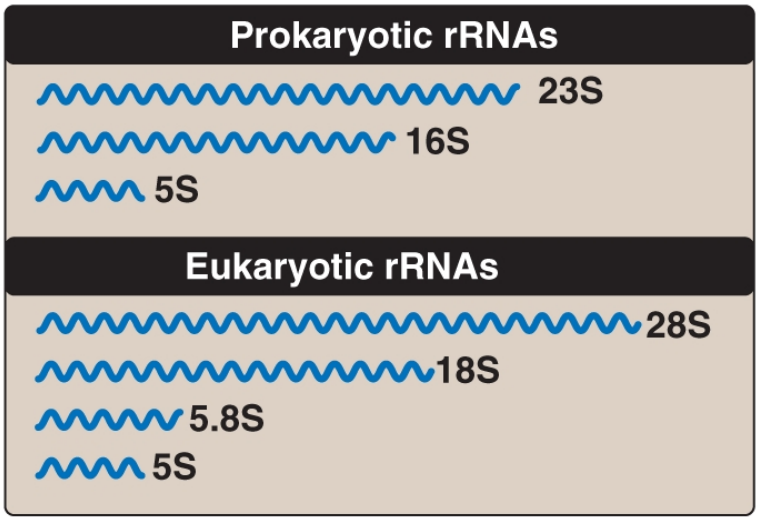
"The Central Dogma *debunked*"

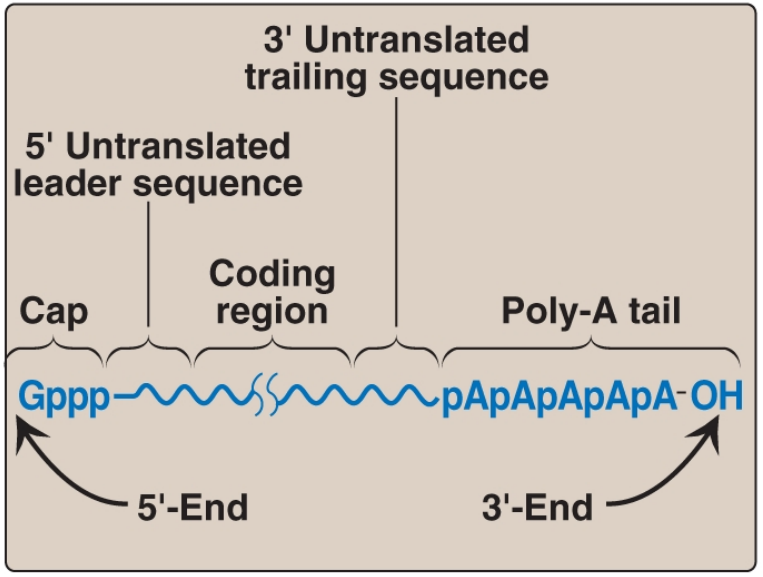


Different classes of RNA

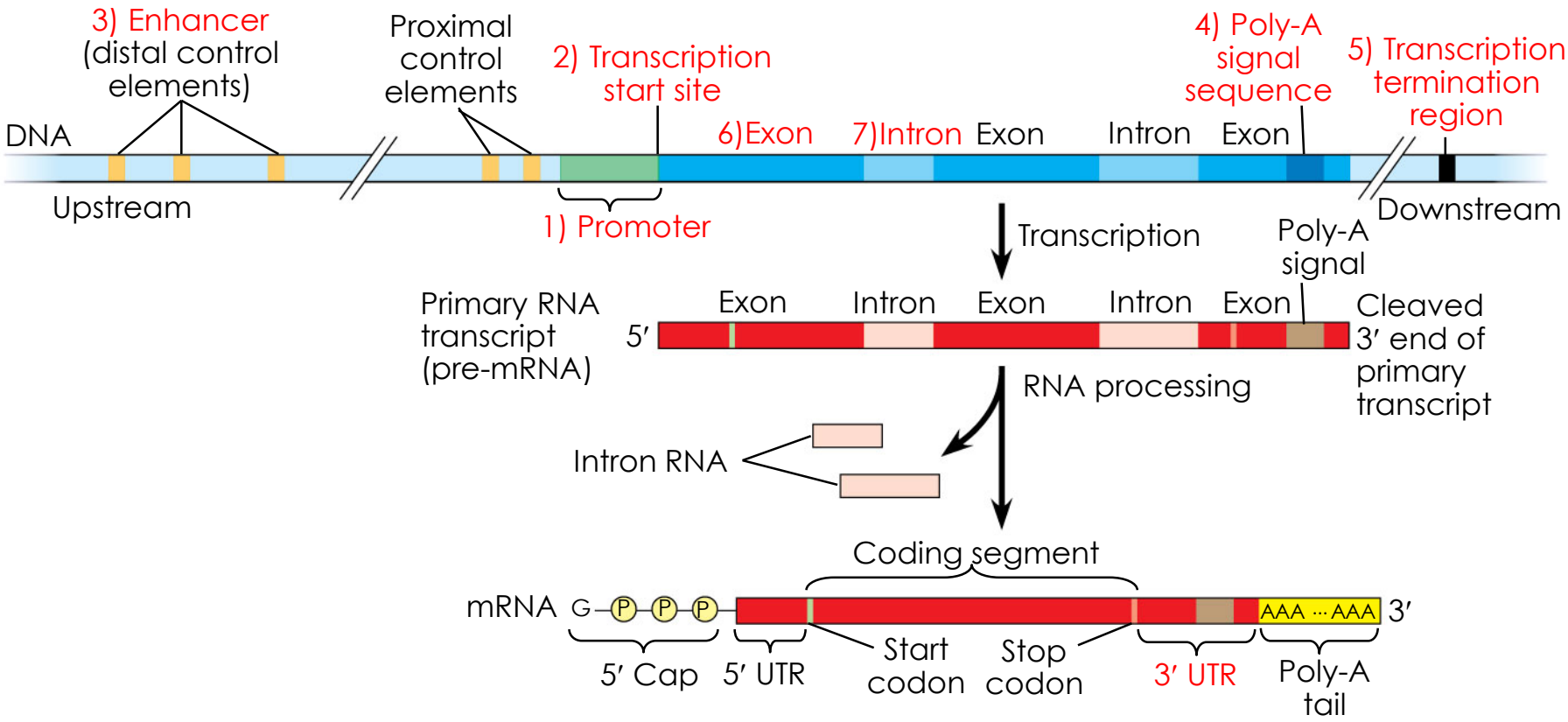








A "typical" human protein-coding gene



Mature mRNAs are selectively exported from the nucleus

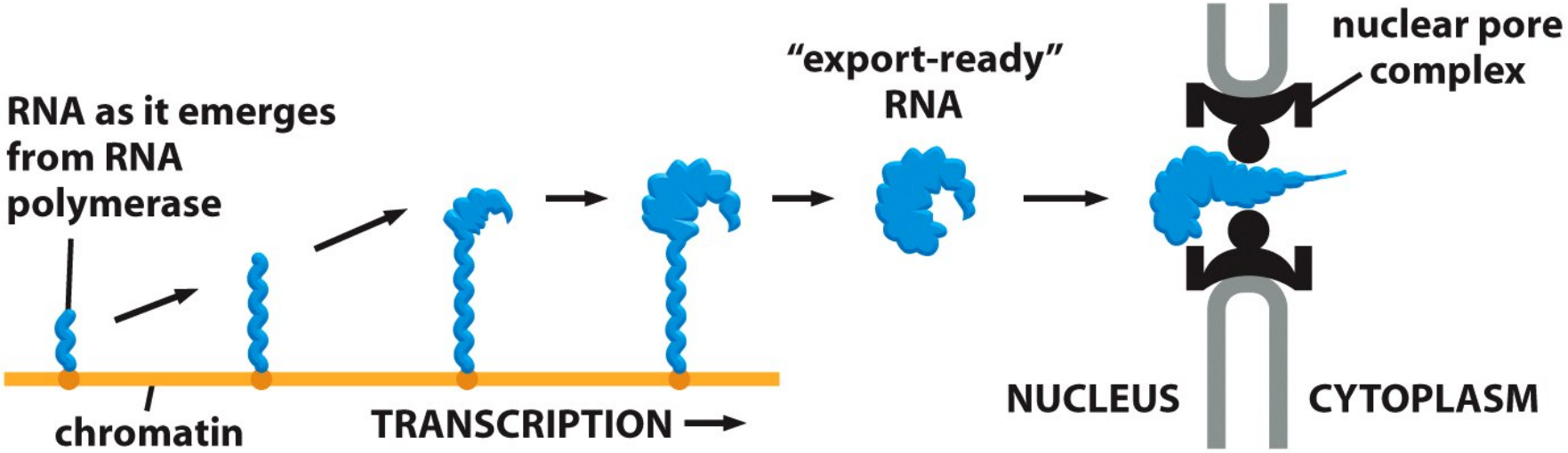


Figure 6-39a Molecular Biology of the Cell 5/e (© Garland Science 2008)

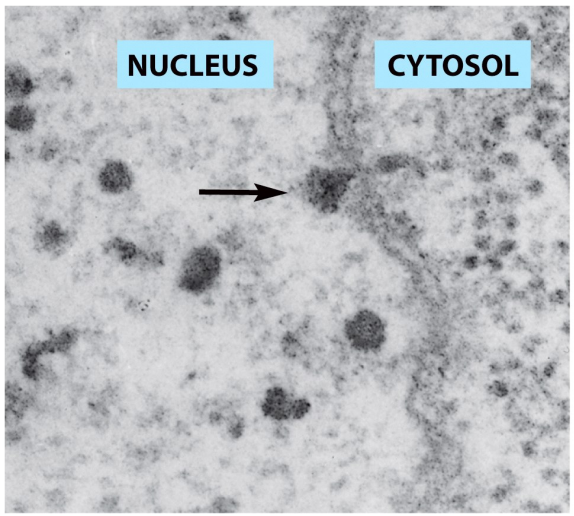


Figure 6-39b Molecular Biology of the Cell 5/e (© Garland Science 2008)

Mature mRNAs are selectively exported from the nucleus

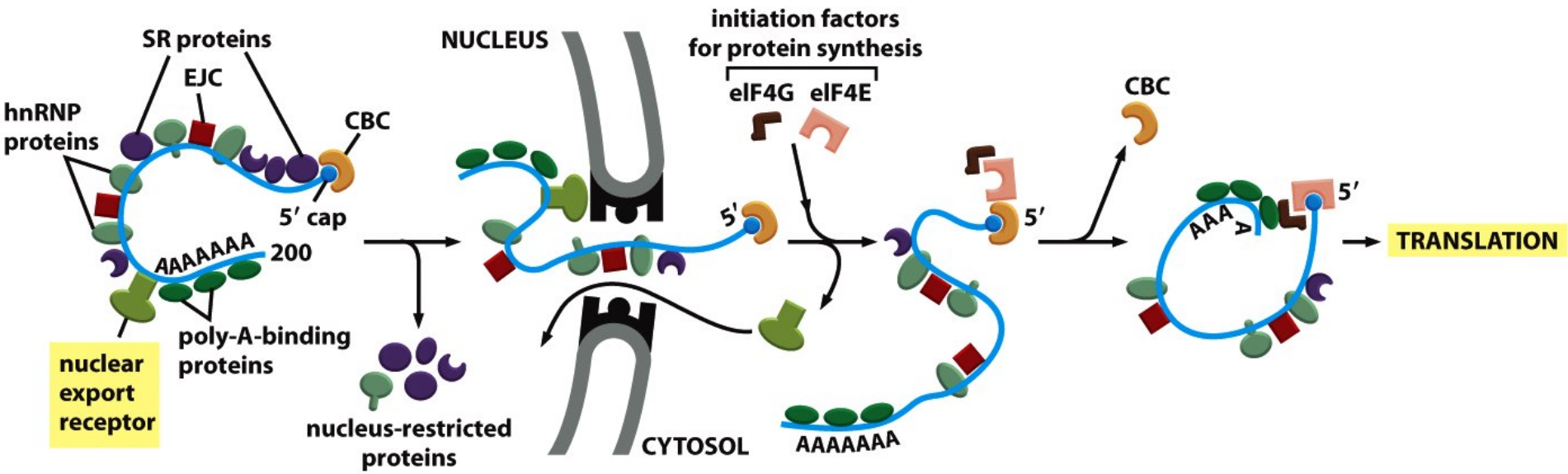
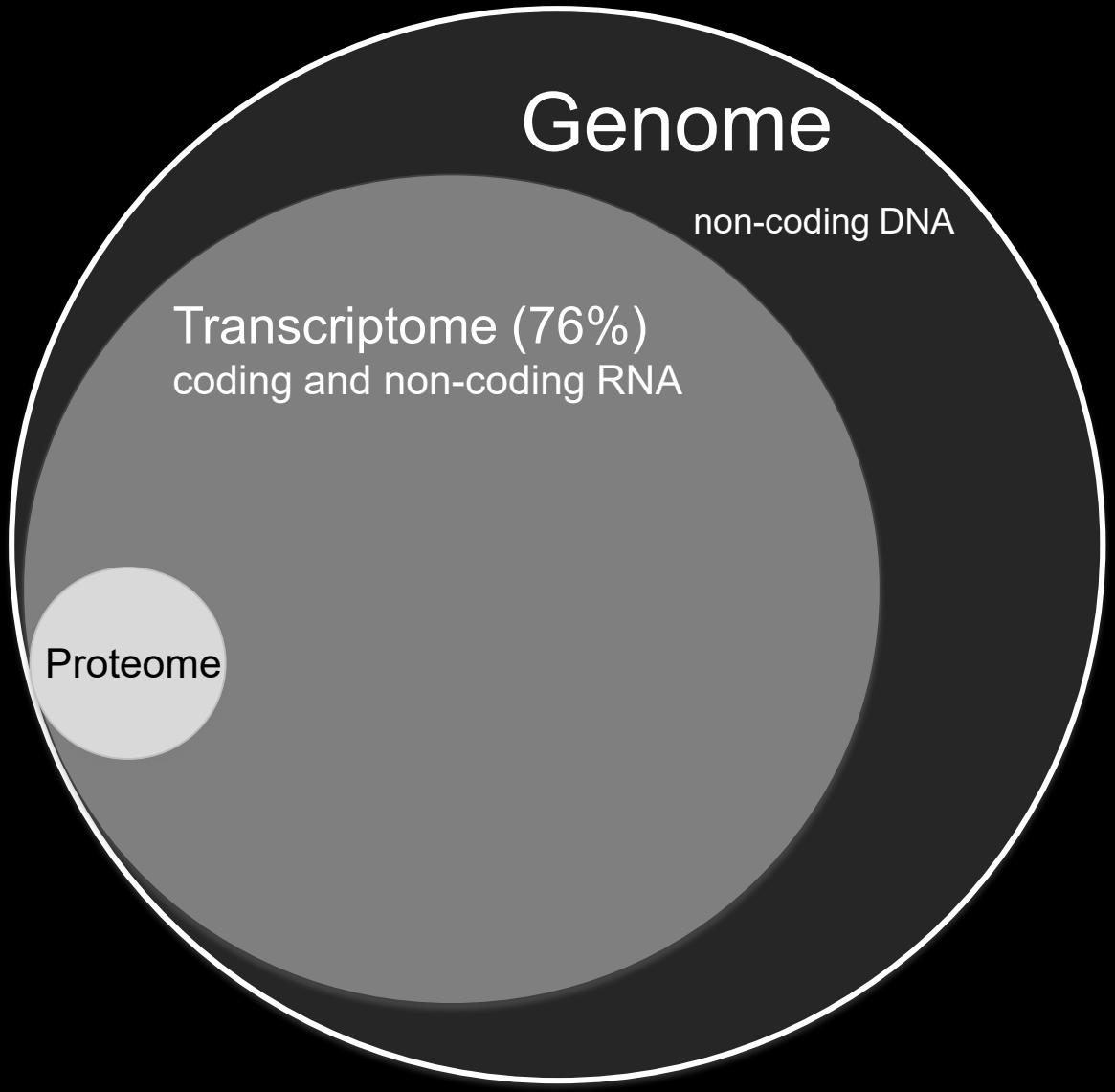
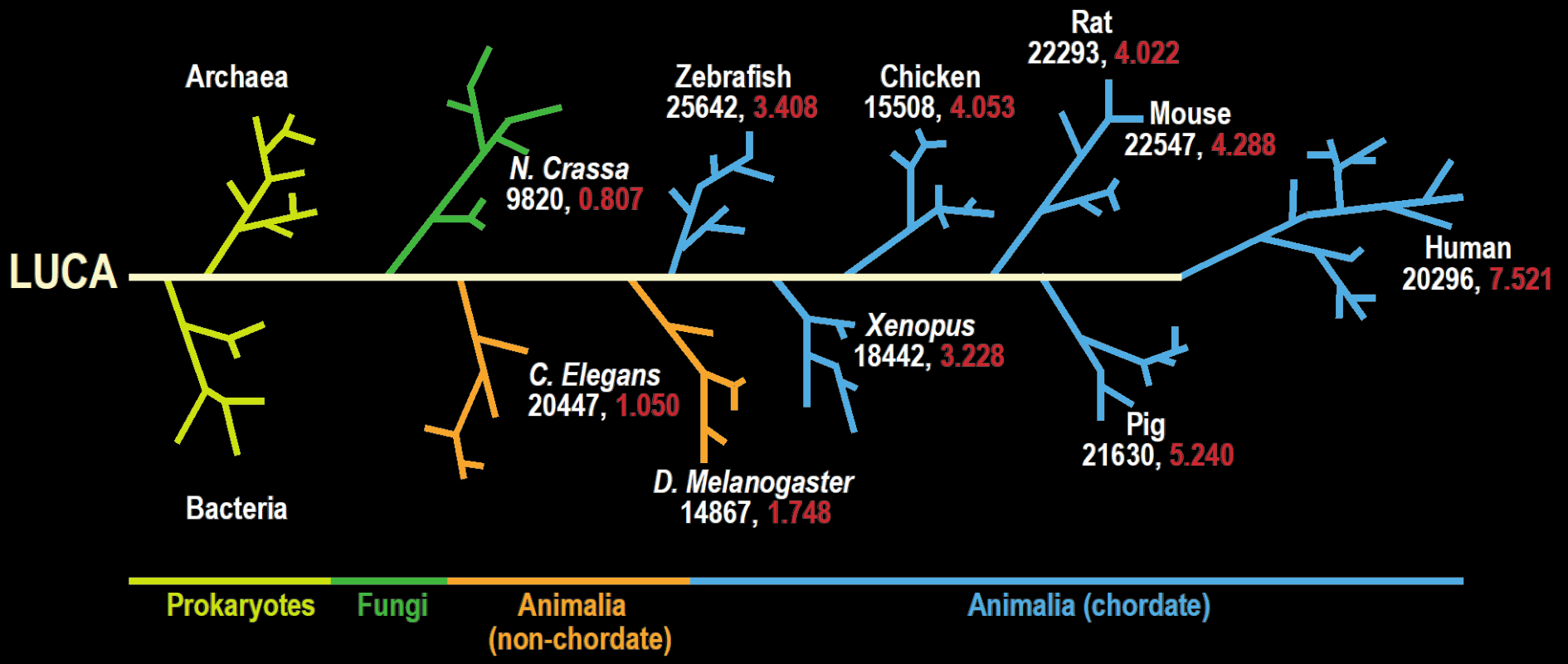


Figure 6-40 Molecular Biology of the Cell 5/e (© Garland Science 2008)

- RNA is never naked.
- Capping, splicing and polyadenylation are export signals.


Genome
non-coding DNA
Transcriptome (76%)
coding and non-coding RNA
Proteome

Transcriptome diversity versus organismal complexity

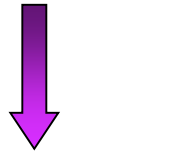


number of genes

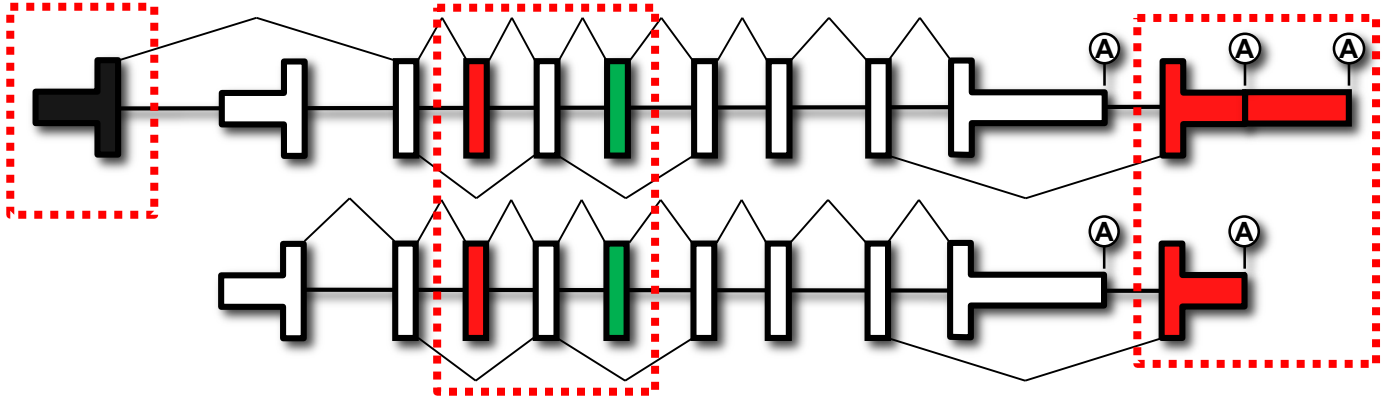
number of transcripts per gene

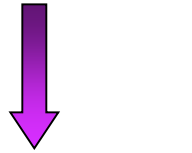
Lewis, Pan and Kalsotra (2017) Nature Reviews Mol Cell Biol

gene

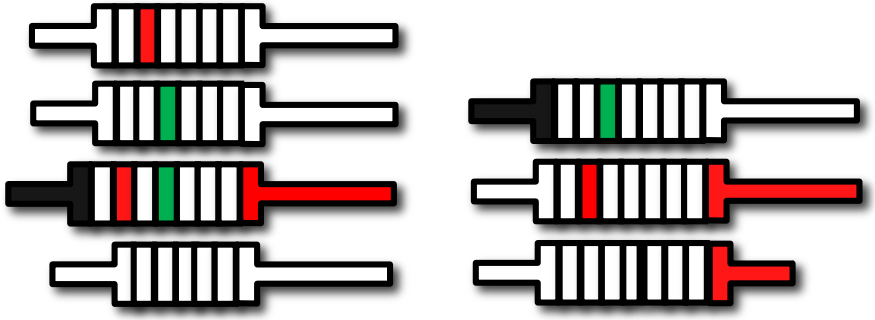


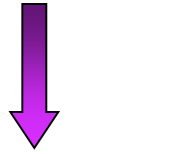
pre-mRNAs



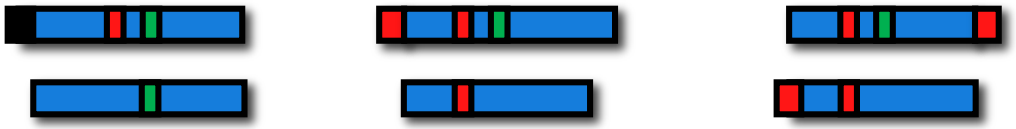


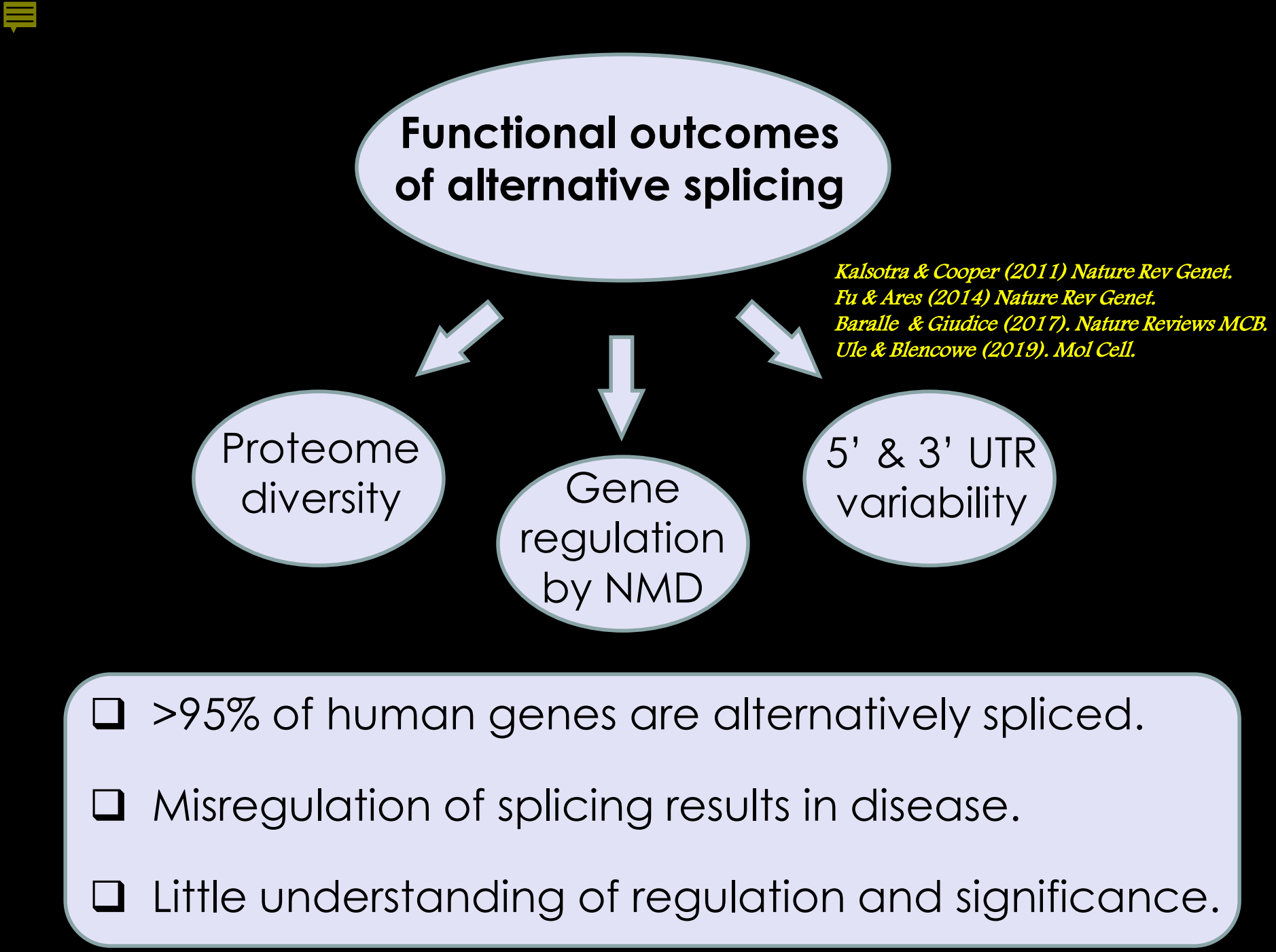
mRNAs
combinatorial
possibilities





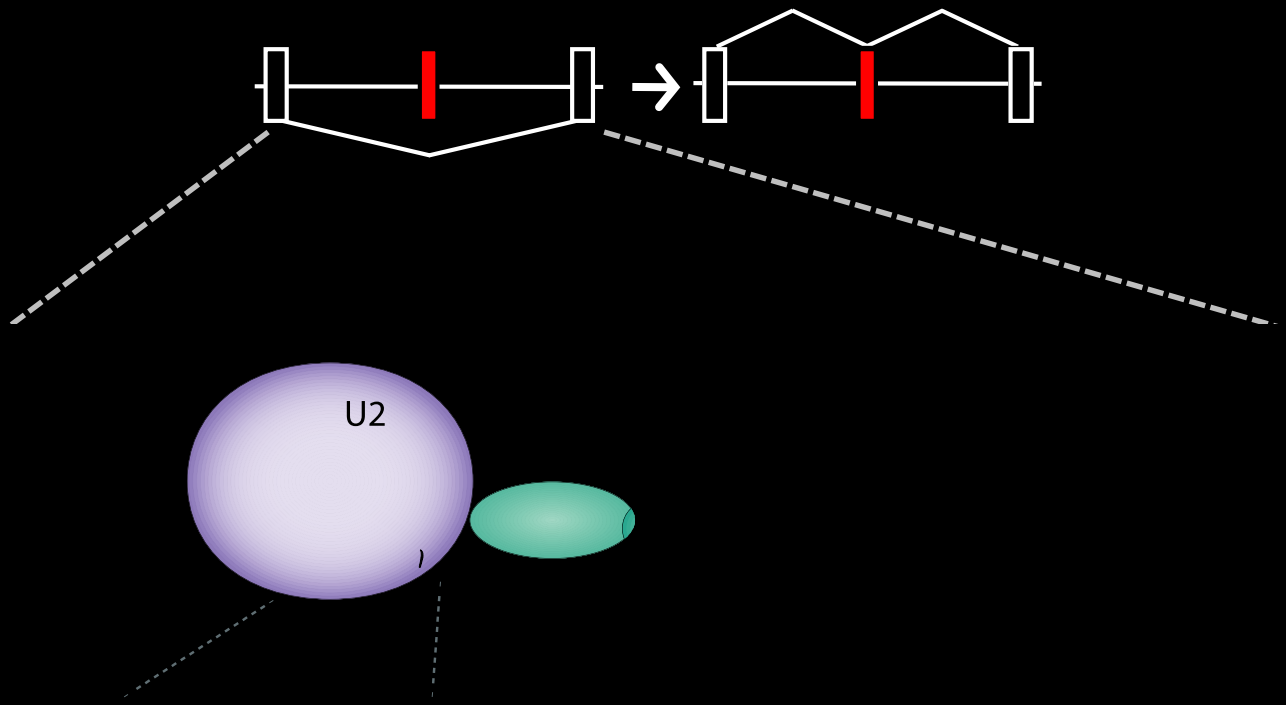
proteins





- >95% of human genes are alternatively spliced.
- Misregulation of splicing results in disease.
- Little understanding of regulation and significance.

How is alternative splicing regulated?

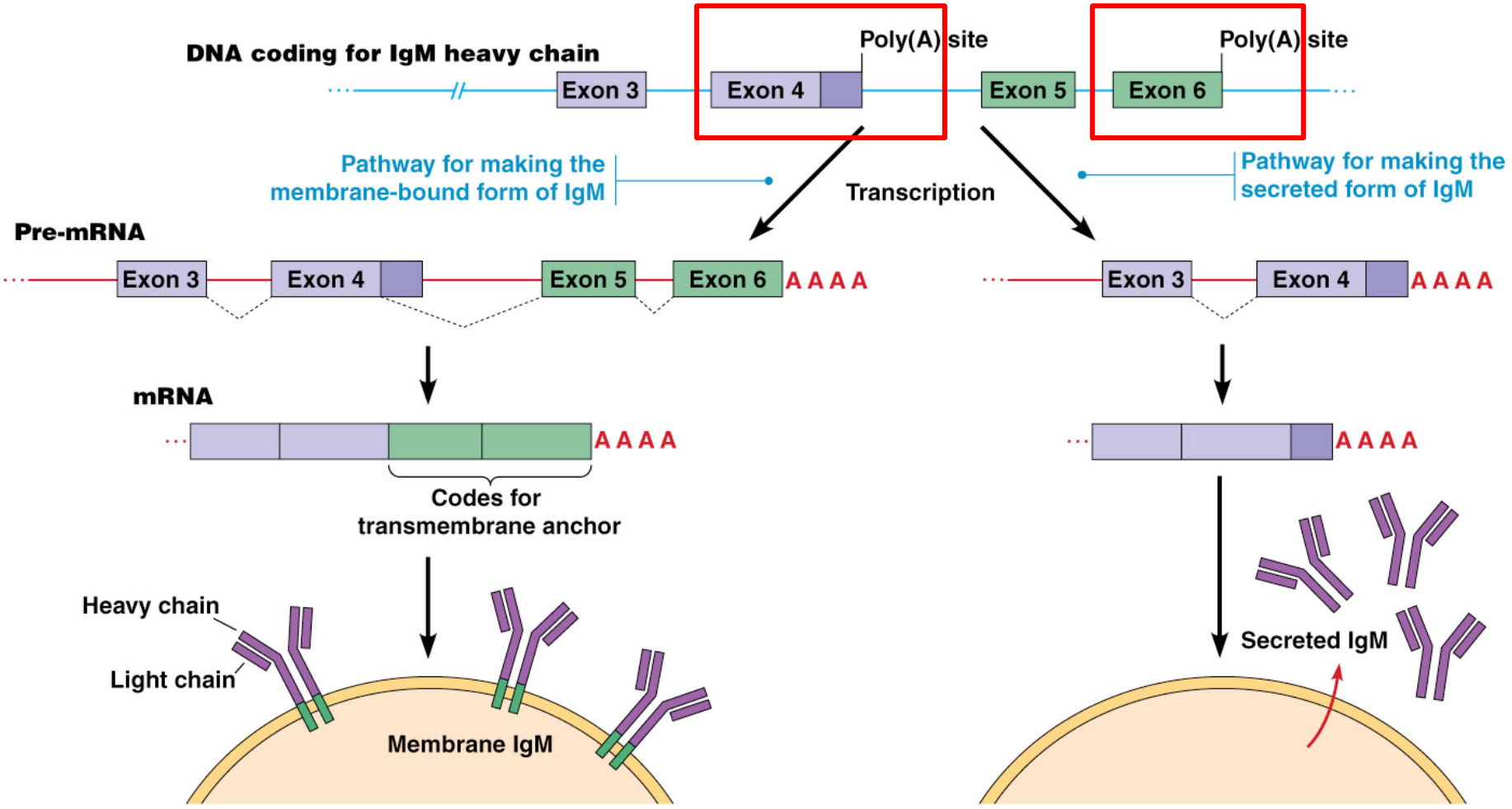


Kalsotra & Cooper (2011) Nat. Rev. Genet.

The most important consequence of organisms having introns is that introns allow for multiple, related protein variants, called *isoforms*, to be produced from one gene through a process termed

"Alternative splicing"

Alternative polyadenylation/splicing produces membrane vs. secreted Abs.

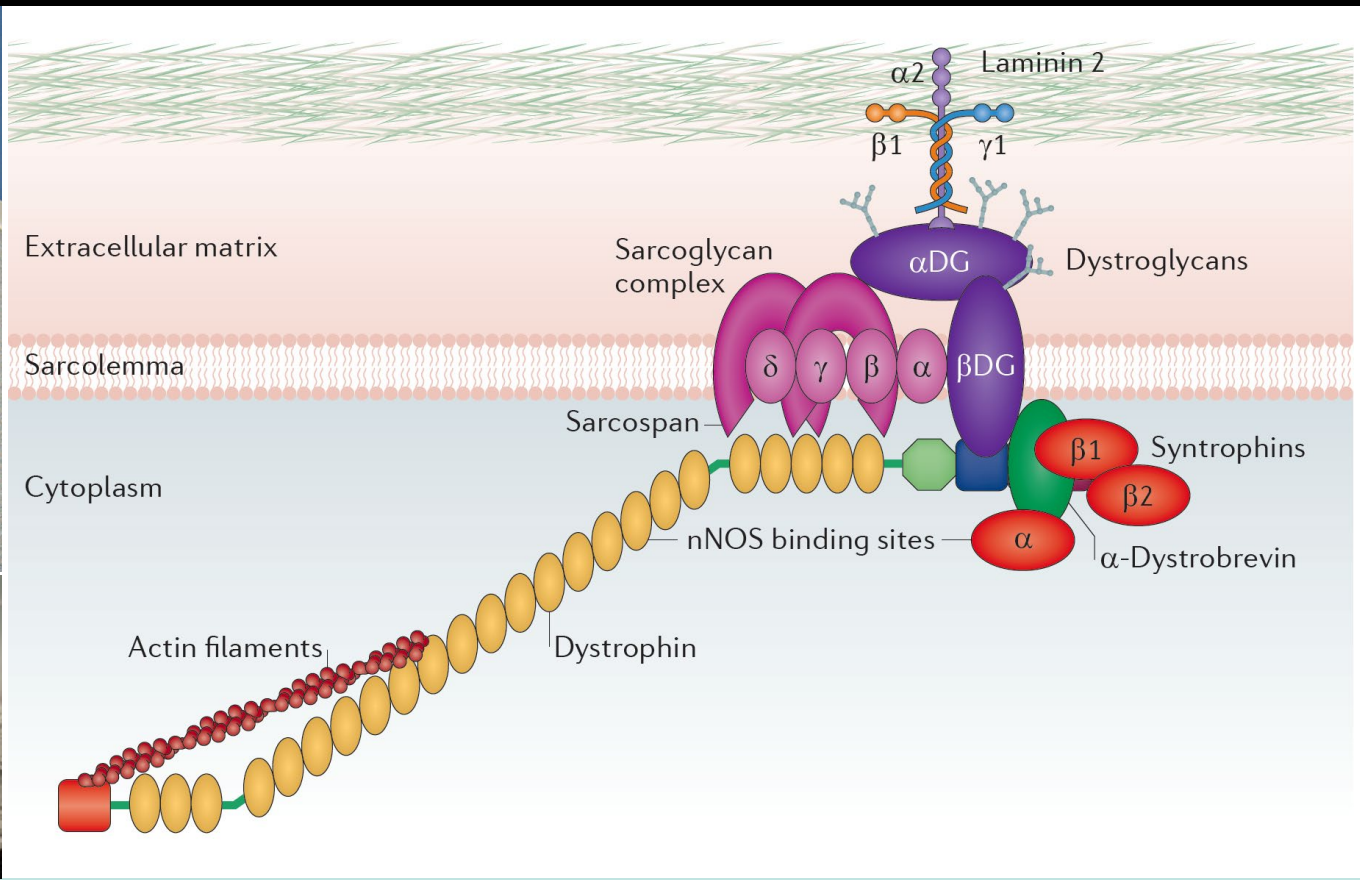


RNA splicing *and* disease

- Up to 50% of mutations that cause disease do so by disrupting splicing.
- Many of these mutations are in consensus splice sites but most are not but rather are in auxiliary elements.
- New realization: large number of exonic mutations that cause disease do so primarily by disrupting splicing and not by disrupting protein function.
- Clue to significance of exonic mutations to splicing was finding of silent mutations that caused disease.

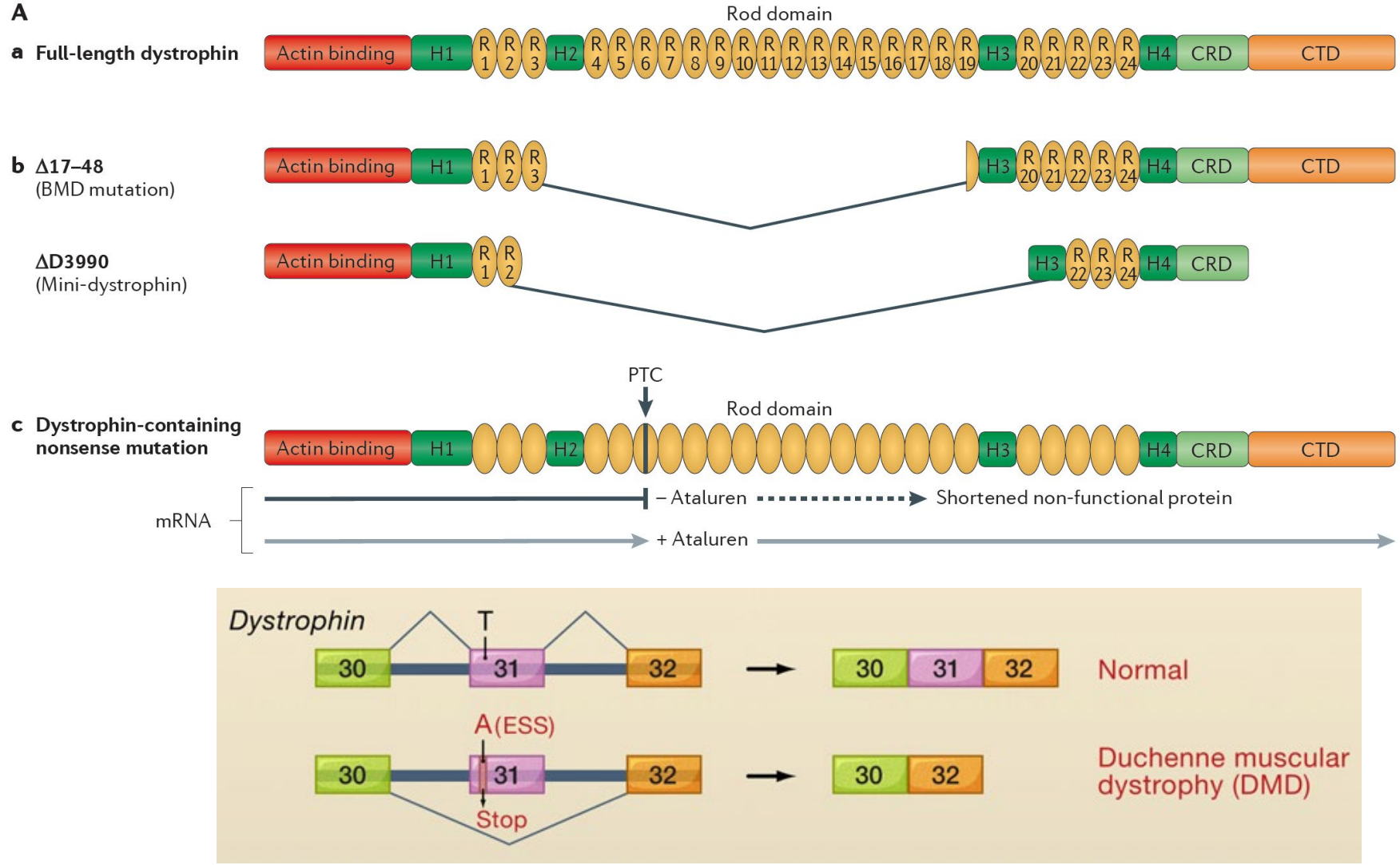
cis vs. *trans* splicing defects

Duchenne Muscular Dystrophy: due to *cis* splicing defects



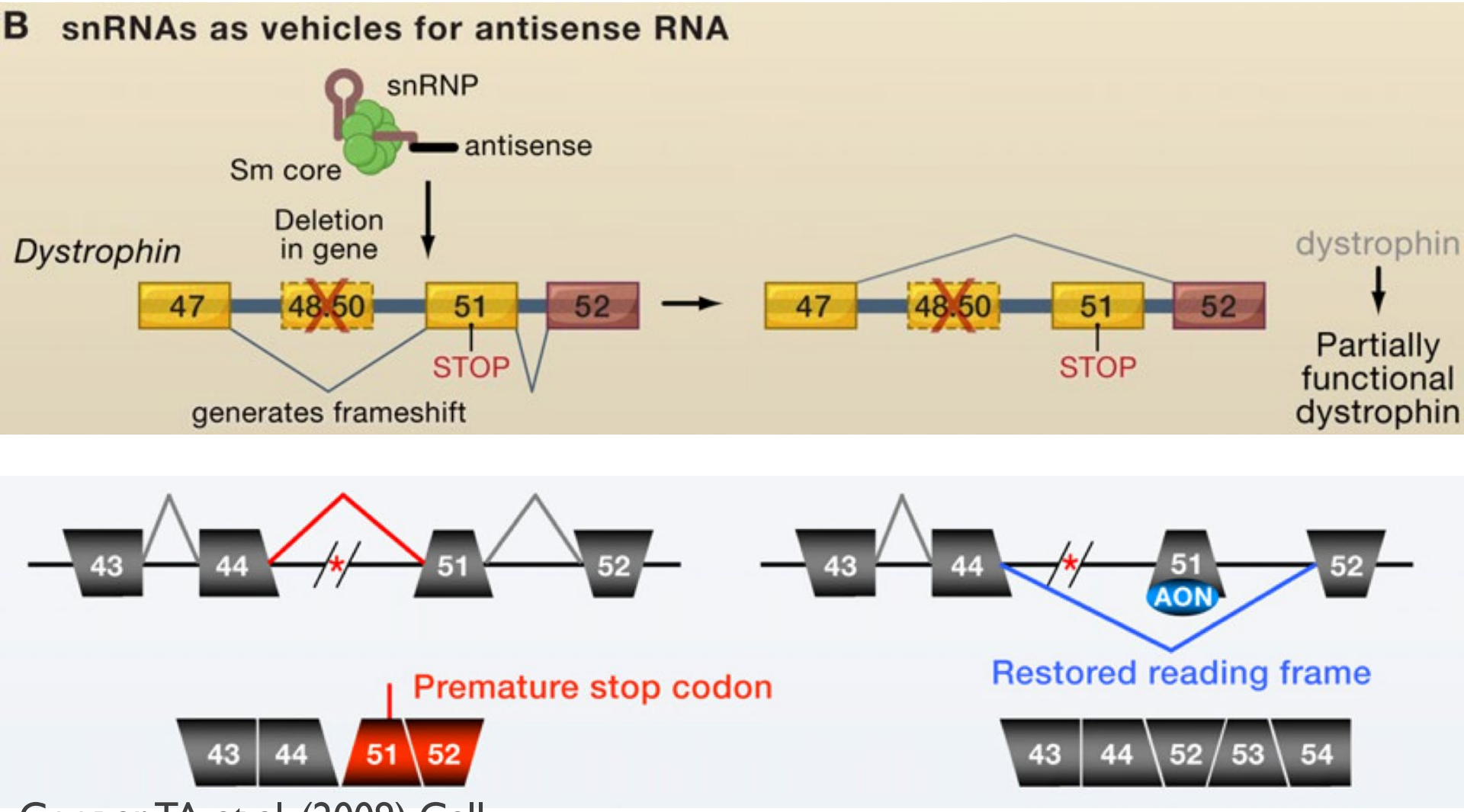
- Dystrophin is a 2.4Mb long X-linked recessive gene and contains 78 introns.
- It is cytoskeletal structural protein which is essential for the muscle membrane stability as it provides an important link between internal cytoskeleton and the extracellular matrix.

Duchenne Muscular Dystrophy: due to *cis* splicing defects



Cooper TA et al. (2009) Cell

Therapeutic approach for Duchenne Muscular Dystrophy



Cooper TA et al. (2009) Cell

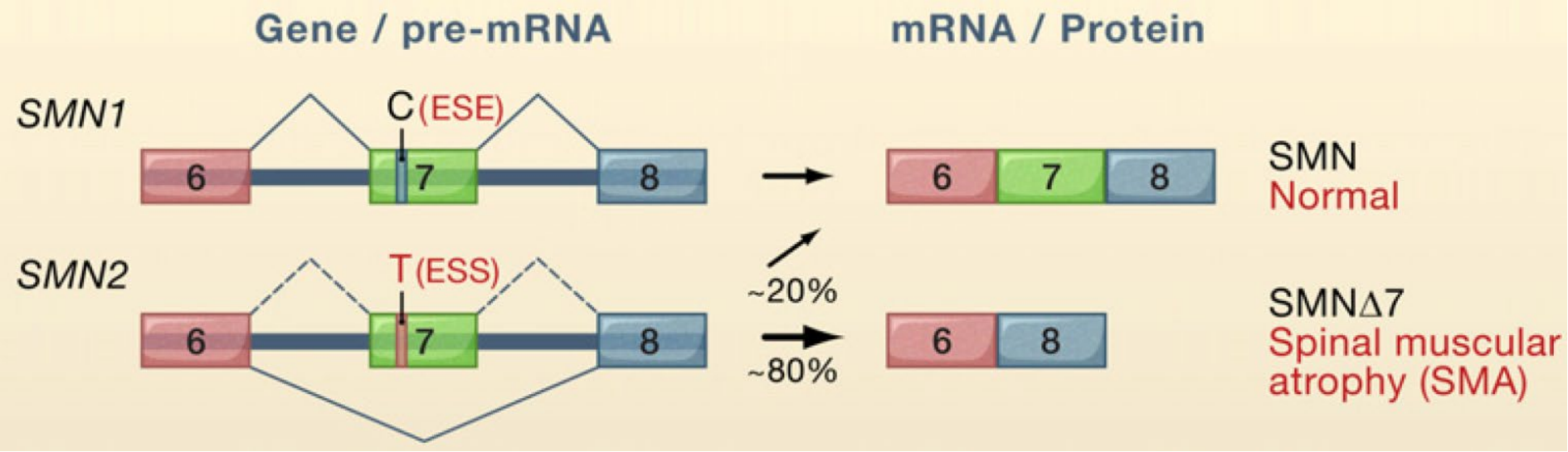
Spinal Muscular Atrophy (SMA) is caused by mutations in the SMN1 gene

A *cis* mutation causing *trans* defects

- SMN proteins are localized in the cytoplasm. Help assemble the spliceosomal U-rich snRNPs). snRNPs are made up of small nuclear RNAs and a group of 7 proteins known as Sm ribonucleoproteins that make up the stable Sm core of the snRNP.

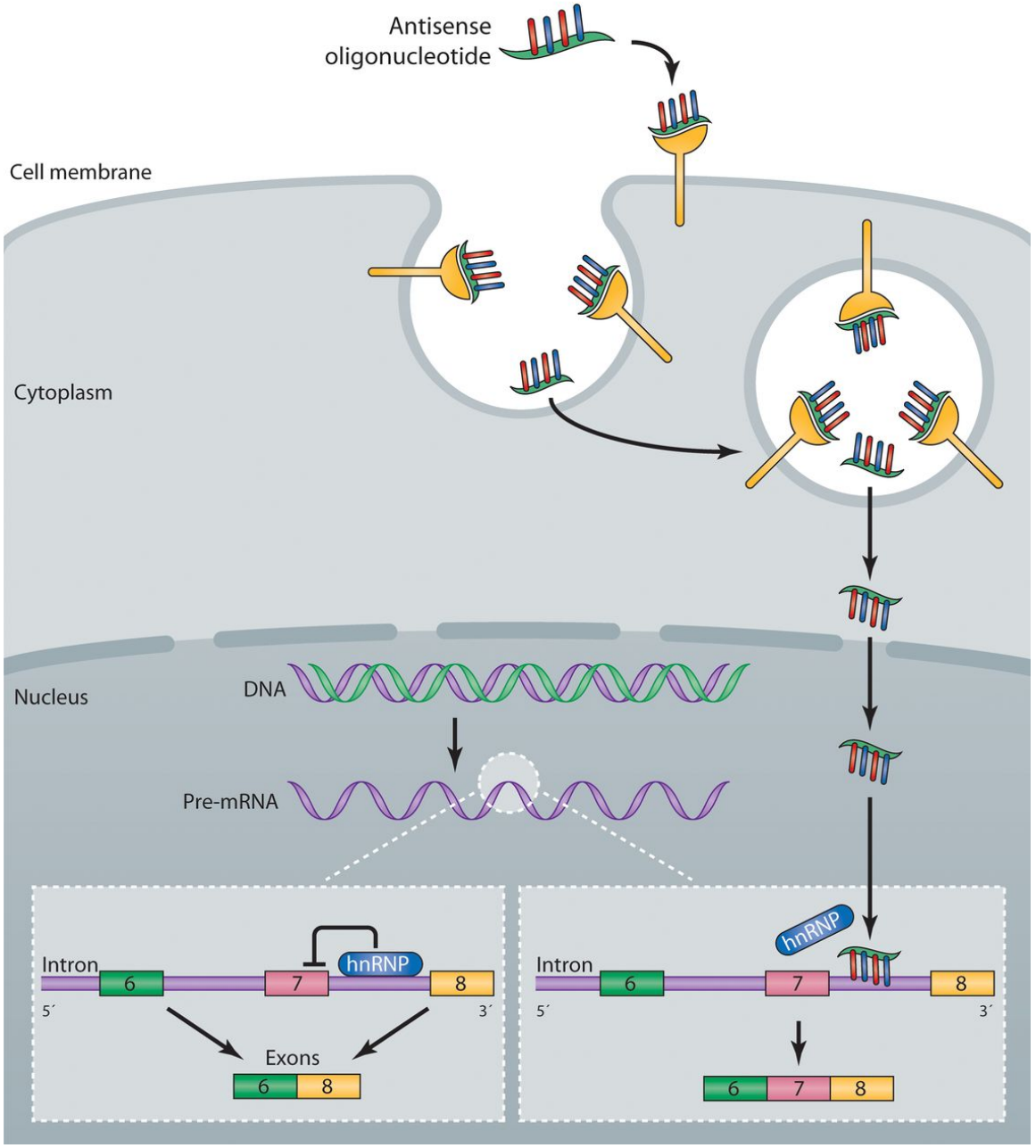
- Two SMN protein paralogues in humans- SMN1 and SMN2. Mutations in SMN1 cause the disease. SMN2 compensates if enough copies are present.

Disease Pathogenesis for SMA

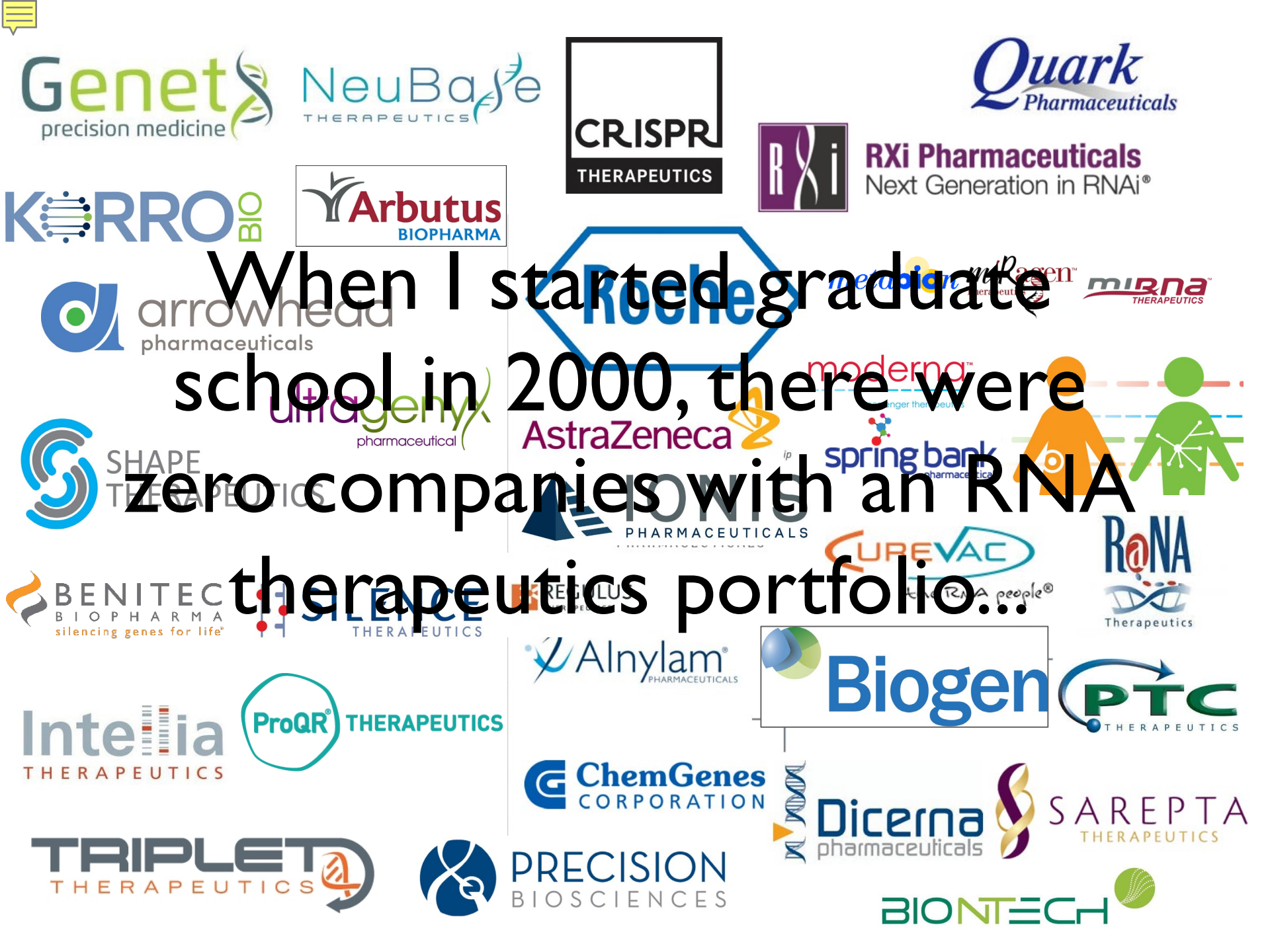


- Homozygous deletions or mutations of the *SMN1* gene result in reduction of full length (fl-SMN). *SMN1* encodes fl-SMN protein, while *SMN2* mostly encodes a protein that is lacking in exon 7 and is highly unstable.
- The severity of the disease can be modified by extra copies of the *SMN2* gene. All patients have reduced levels of fl-SMN protein, but those with the phenotype of SMA type 1 have as little as 9% of the normal amount of fl-SMN, those with SMA type 2 have 14%, and with SMA type 3, about 18%.
- Once fl-protein levels approach 23% of normal levels, motor neuron function appears normal, and carriers usually have 45 to 55% fl-SMN protein.

Therapeutic approach for the treatment of Spinal Muscular Atrophy



Breakthrough Prize awarded to *Krainer* and *Bennett* for SMA research leading to *Spinraza*


When I started graduate school in 2000, there were zero companies with an RNA therapeutics portfolio...
Genetic precision medicine
NeuBase THERAPEUTICS
CRISPR THERAPEUTICS
Quark Pharmaceuticals
RXi Pharmaceuticals Next Generation in RNAi®
KORRO BIO
Arbutus BIOPHARMA
arrowhead pharmaceuticals
Roche
miRNA THERAPEUTICS
moderna
ultragenyx pharmaceutical
AstraZeneca
spring bank pharmaceuticals
SHAPE THERAPEUTICS
IONIS PHARMACEUTICALS
CUREVAC
RaNA Therapeutics
BENITEC BIOPHARMA silencing genes for life
SILENCE THERAPEUTICS
Alnylam PHARMACEUTICALS
Biogen
PTC THERAPEUTICS
Intellia THERAPEUTICS
ProQR THERAPEUTICS
ChemGenes CORPORATION
Dicerna pharmaceuticals
SAREPTA THERAPEUTICS
TRIPLET THERAPEUTICS
PRECISION BIOSCIENCES
BIONTECH