PROPEL 101: Investigating Molecular Mechanisms

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How to read a paper?

- Not about memorizing/learning ALL the different mutations, etc.
- Read the abstract, read the figures -> what is the paper trying to present
- Then do a deep dive -> The introduction should explain the previous work and why this is important for the field – if it is very novel you can check additional short reviews
- Pick a couple of sections that sound most interesting and do a deep dive into those.
- Think about why they performed these experiments, have they explained everything?
- What is the key message

How to understand and convey information?

From the perspective of a journal club:

- What is the key message?

- What are the main findings? (you don't have to show all the figures and all the supplementary figures)

- What is innovative about it?

- Is there anything that remains unanswered?

- What is the impact of this publication on a larger scale (let's say cancer field).

Why do we perform research?

- To understand ->
- basic biology (how things work),
- to discover underlying mechanisms,
- to be able to advance science,
- cure diseases

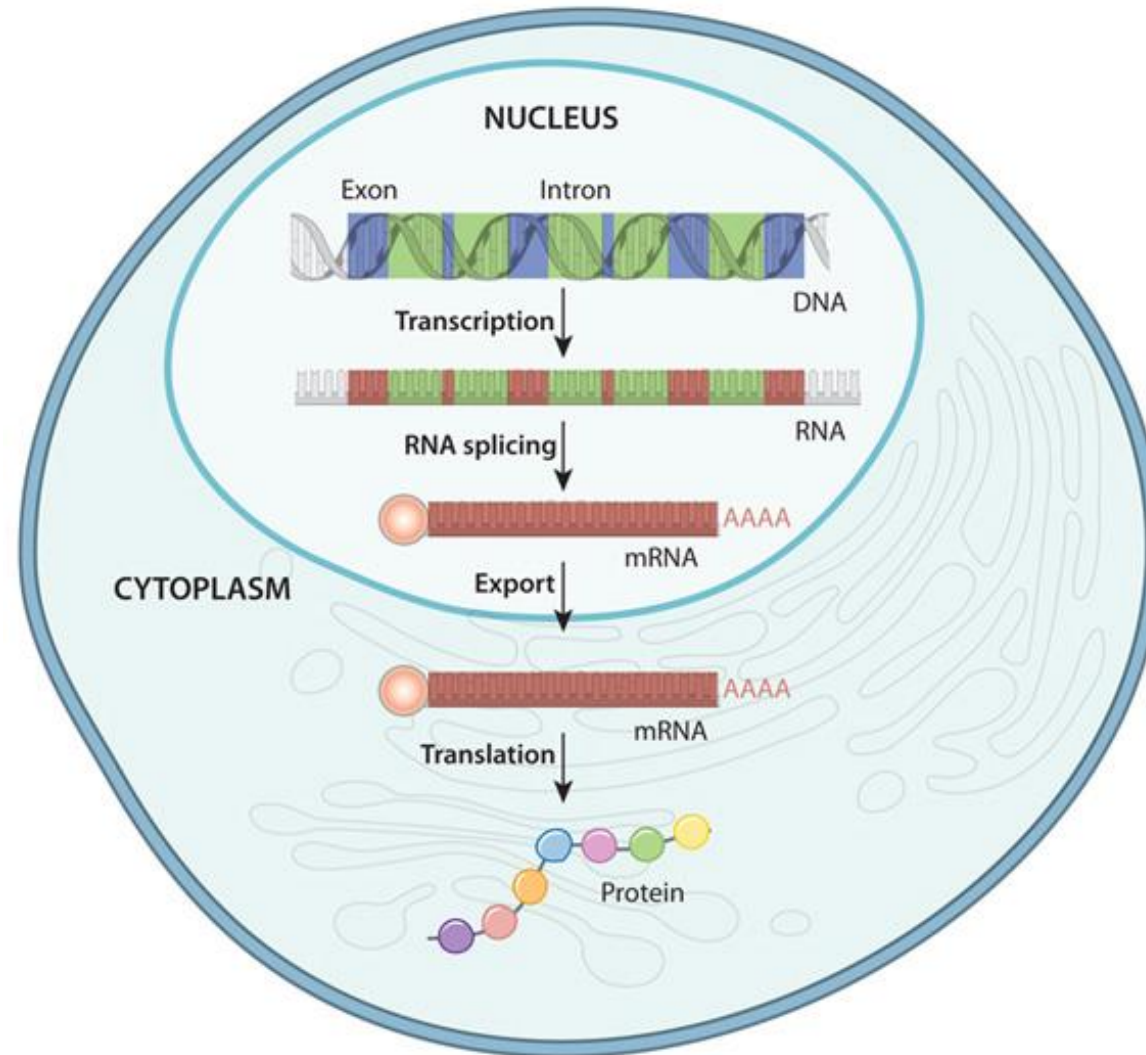# What is a disease?

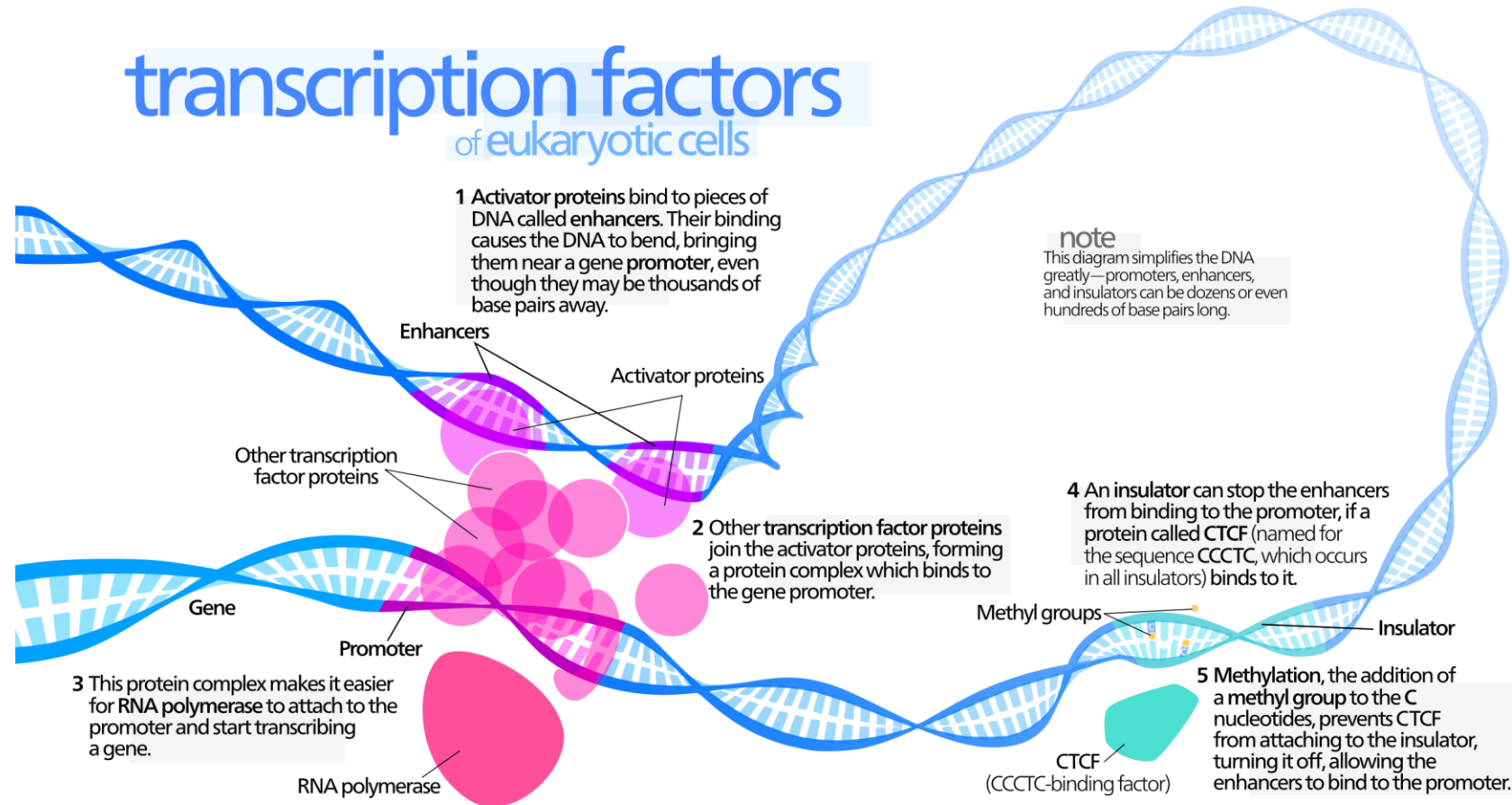Tanisawa et al., 2016
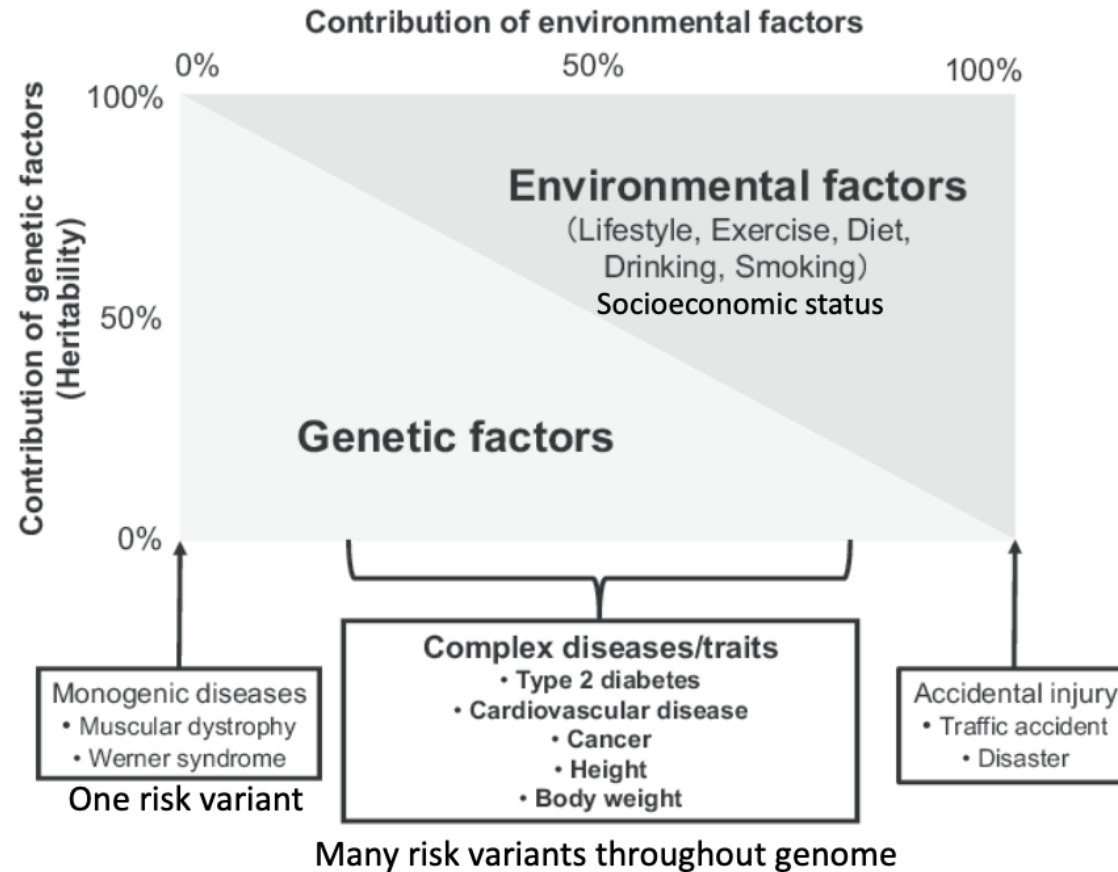
What is a molecular mechanism?

A molecular mechanism describes the detailed, step-by-step sequence of events that occurs at the molecular level to carry out a biological process.

- ✓ Molecular mechanisms are the processes that explain how **genetic variations lead to observable disease phenotypes**. They also refer to the underlying processes that contribute to cell and organismal physiology
- ✓ The **molecular processes that underlie the pathogenesis of diseases**
- ✓ **Alterations in mRNA translation or protein stability that affect a phenotype**
- o **Sickle Cell Anemia**: Normal: Gene makes correct hemoglobin → round red blood cells → good oxygen delivery Mechanism disrupted: One letter change in gene → abnormal hemoglobin → sickle-shaped cells → poor oxygen delivery
- o **Insulin and Diabetes**: Healthy mechanism: Insulin gene → proper insulin → glucose enters cells Type 1 Diabetes mechanism: Immune system attacks insulin-producing cells → no insulin → high blood sugar
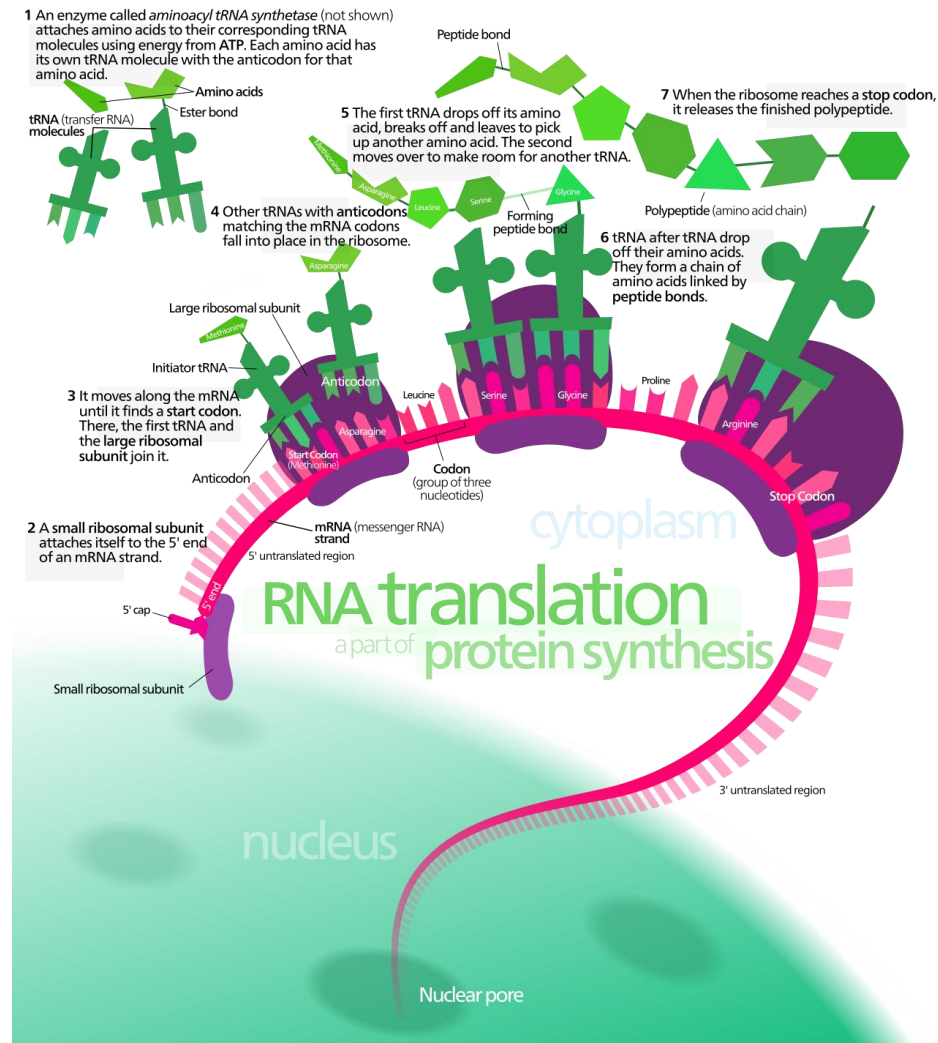
Gene expression pathway
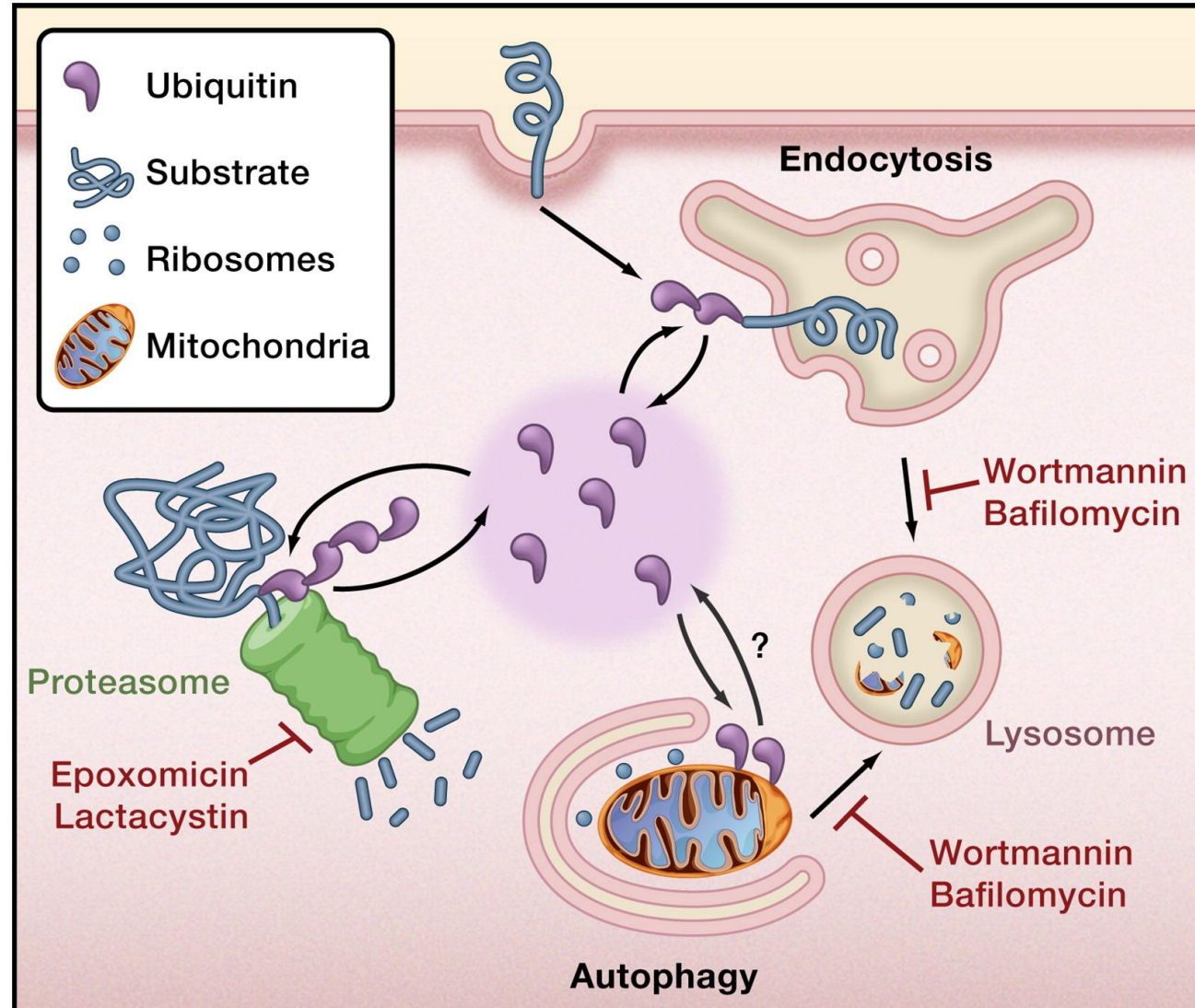
Transcription -> DNA into RNA
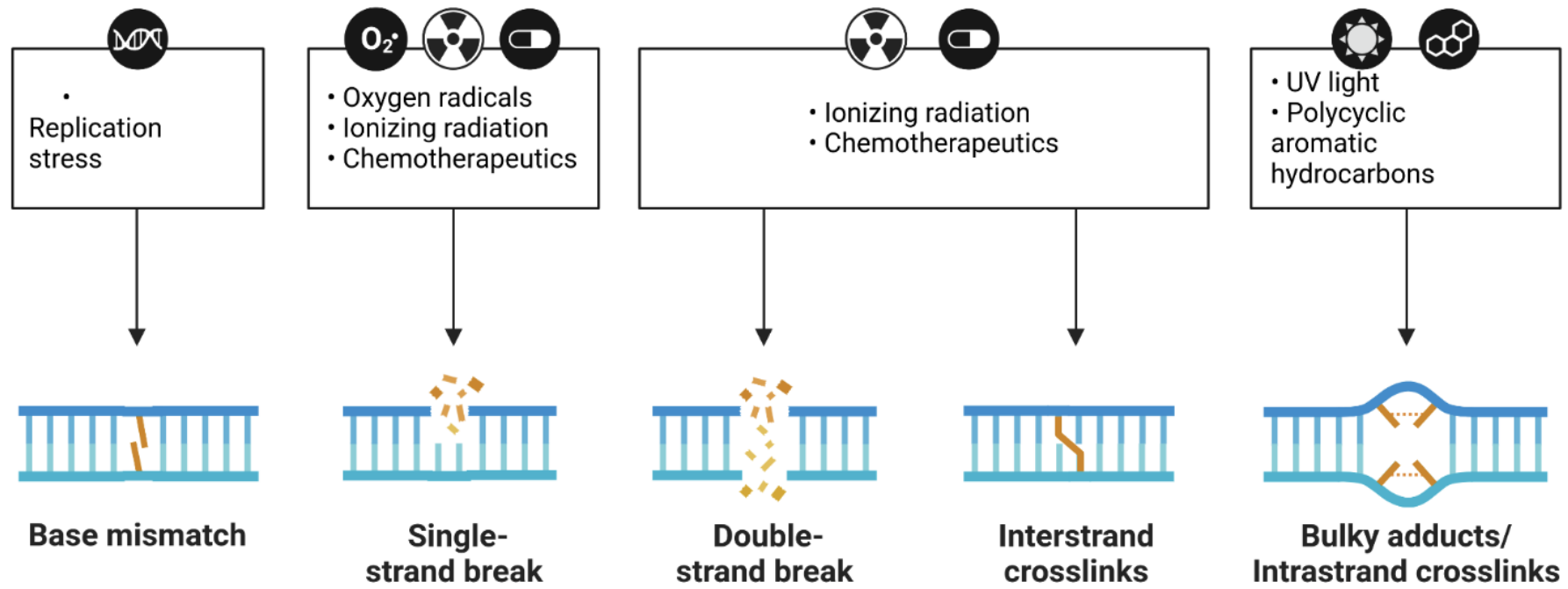
Translation –> mRNA into protein

1 An enzyme called *aminoacyl tRNA synthetase* (not shown) attaches amino acids to their corresponding tRNA molecules using energy from **ATP**. Each amino acid has its own tRNA molecule with the anticodon for that amino acid.

Amino acids

tRNA (transfer RNA) molecules

Ester bond

Peptide bond

5 The first tRNA drops off its amino acid, breaks off and leaves to pick up another amino acid. The second moves over to make room for another tRNA.

7 When the ribosome reaches a **stop codon**, it releases the finished polypeptide.

4 Other tRNAs with **anticodons** matching the mRNA codons fall into place in the ribosome.

Asparagine

Leucine

Serine

Glycine

Forming peptide bond

Polypeptide (amino acid chain)

6 tRNA after tRNA drop off their amino acids. They form a chain of amino acids linked by **peptide bonds**.

Large ribosomal subunit

Methionine

Initiator tRNA

Anticodon

3 It moves along the mRNA until it finds a **start codon**. There, the first tRNA and the **large ribosomal subunit** join it.

Asparagine

Start Codon (Methionine)

Anticodon

Leucine

Codon (group of three nucleotides)

Serine

Glycine

Proline

Arginine

Stop Codon

cytoplasm

2 A small ribosomal subunit attaches itself to the 5' end of an mRNA strand.

mRNA (messenger RNA) strand

5' untranslated region

5' cap

5' end

Small ribosomal subunit

RNA translation
a part of **protein synthesis**

3' untranslated region

nucleus
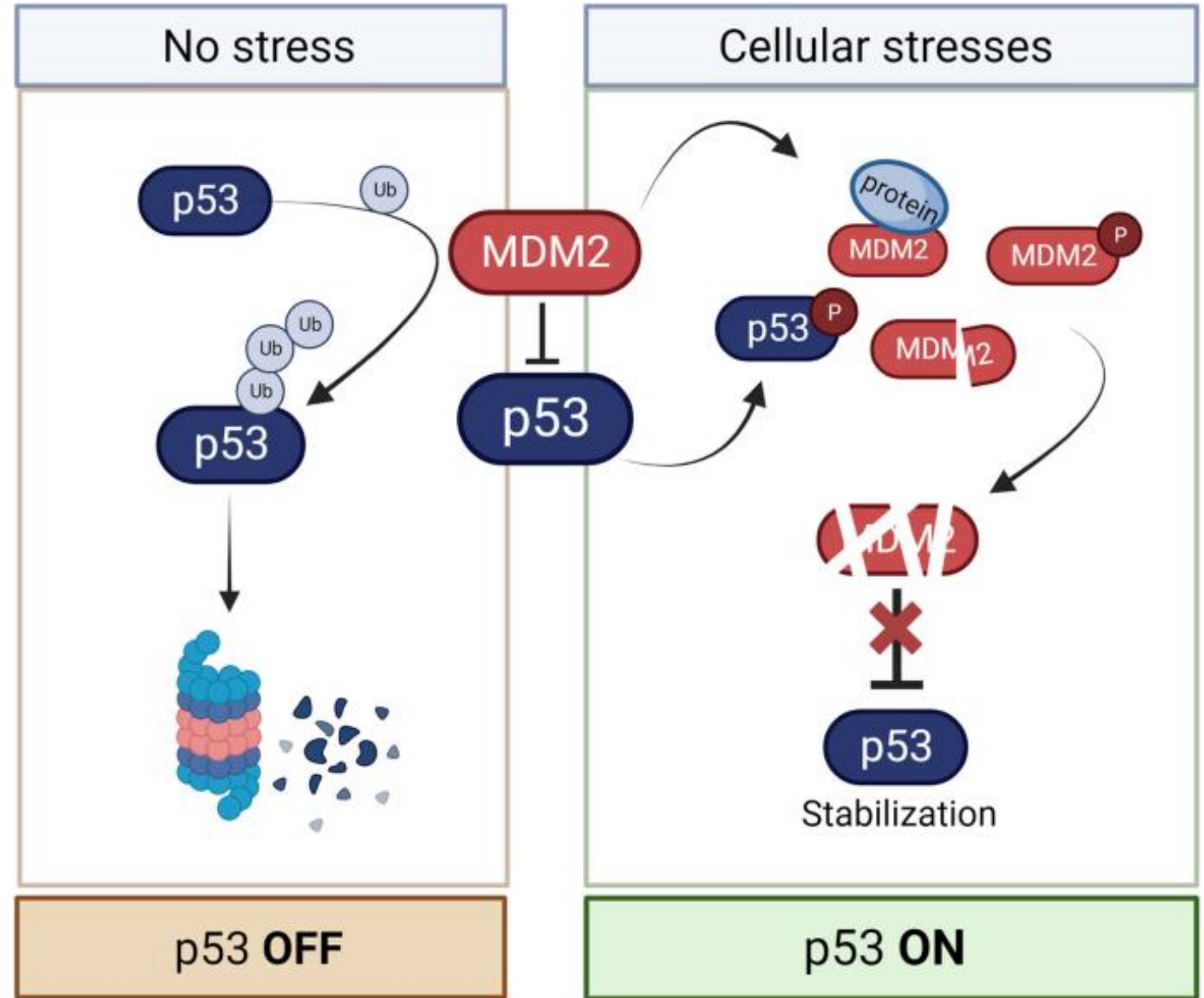
Nuclear pore

Protein turnover
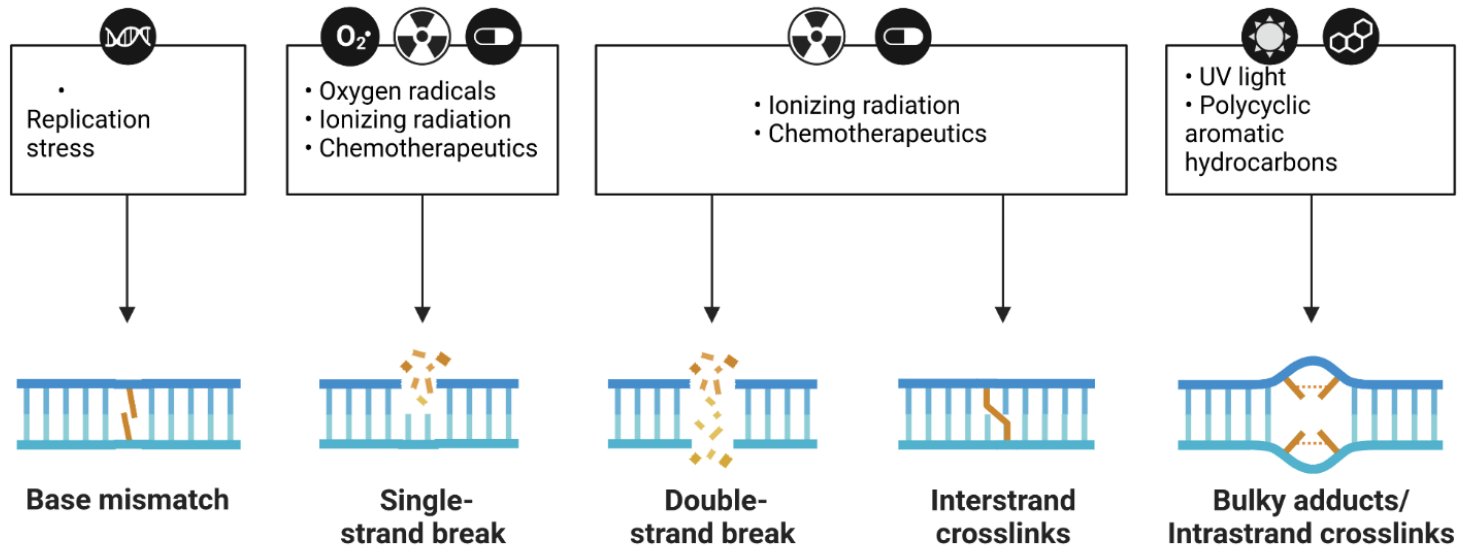
DNA damage

Common Causes of DNA Damage

p53 (gene TP53)

- "guardian of the genome"
- Activated by DNA damage
- Transcription factor
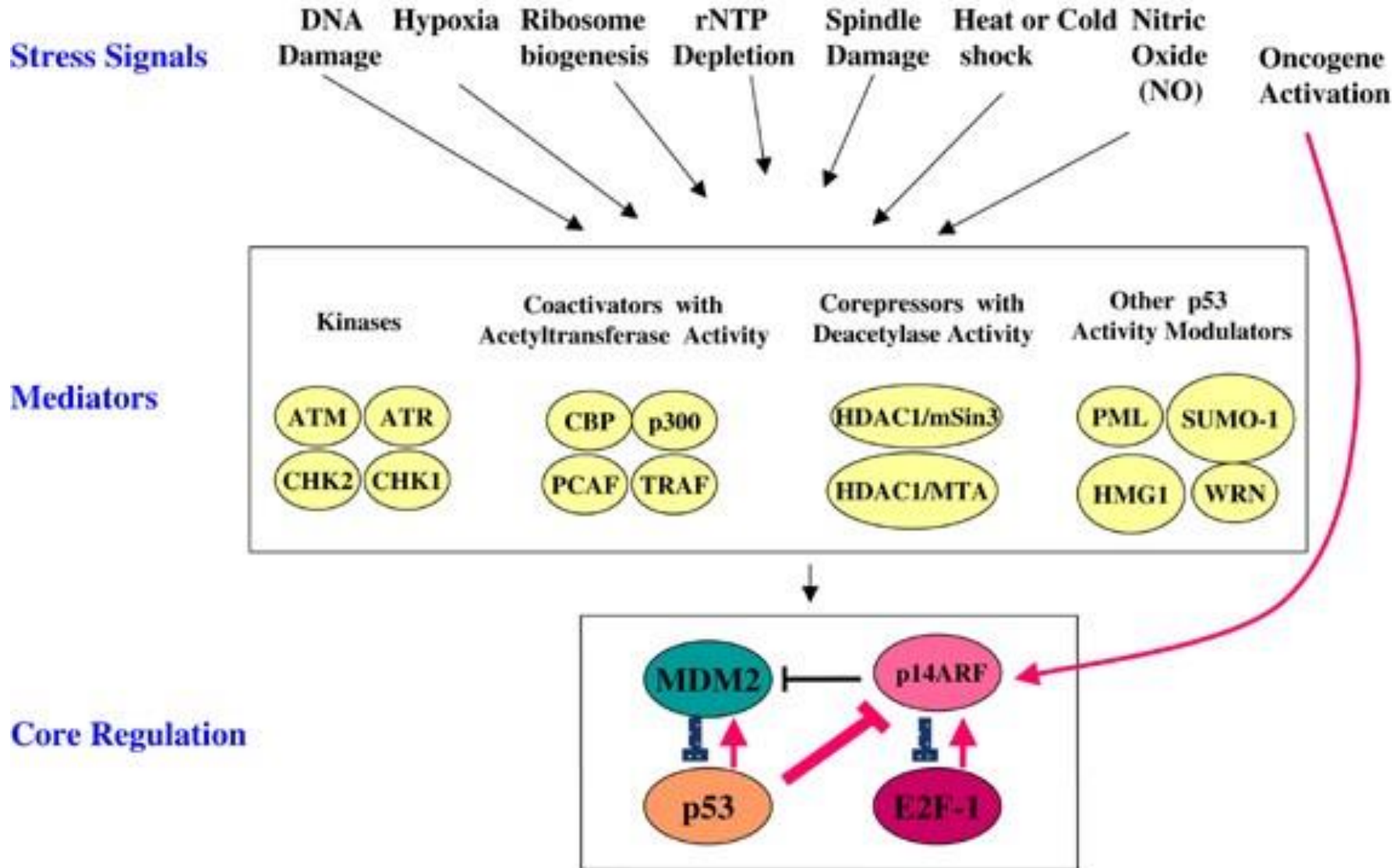- Induces cell cycle arrest, allowing damage repair or inducing apoptosis

Marques JF, Kops GJPL, Chromosome Res, 2023

DNA damage – cancer treatments

Common Causes of DNA Damage

Replication stress → **Base mismatch**

• Oxygen radicals
• Ionizing radiation
• Chemotherapeutics → **Single-strand break**

• Ionizing radiation
• Chemotherapeutics → **Double-strand break** / **Interstrand crosslinks**

• UV light
• Polycyclic aromatic hydrocarbons → **Bulky adducts/ Intrastrand crosslinks**

cells with a defective p53 pathway can undergo apoptosis in response to DNA damage

Harris SL, Levine AJ, Oncogene, 2005

p53 – dependent apoptosis
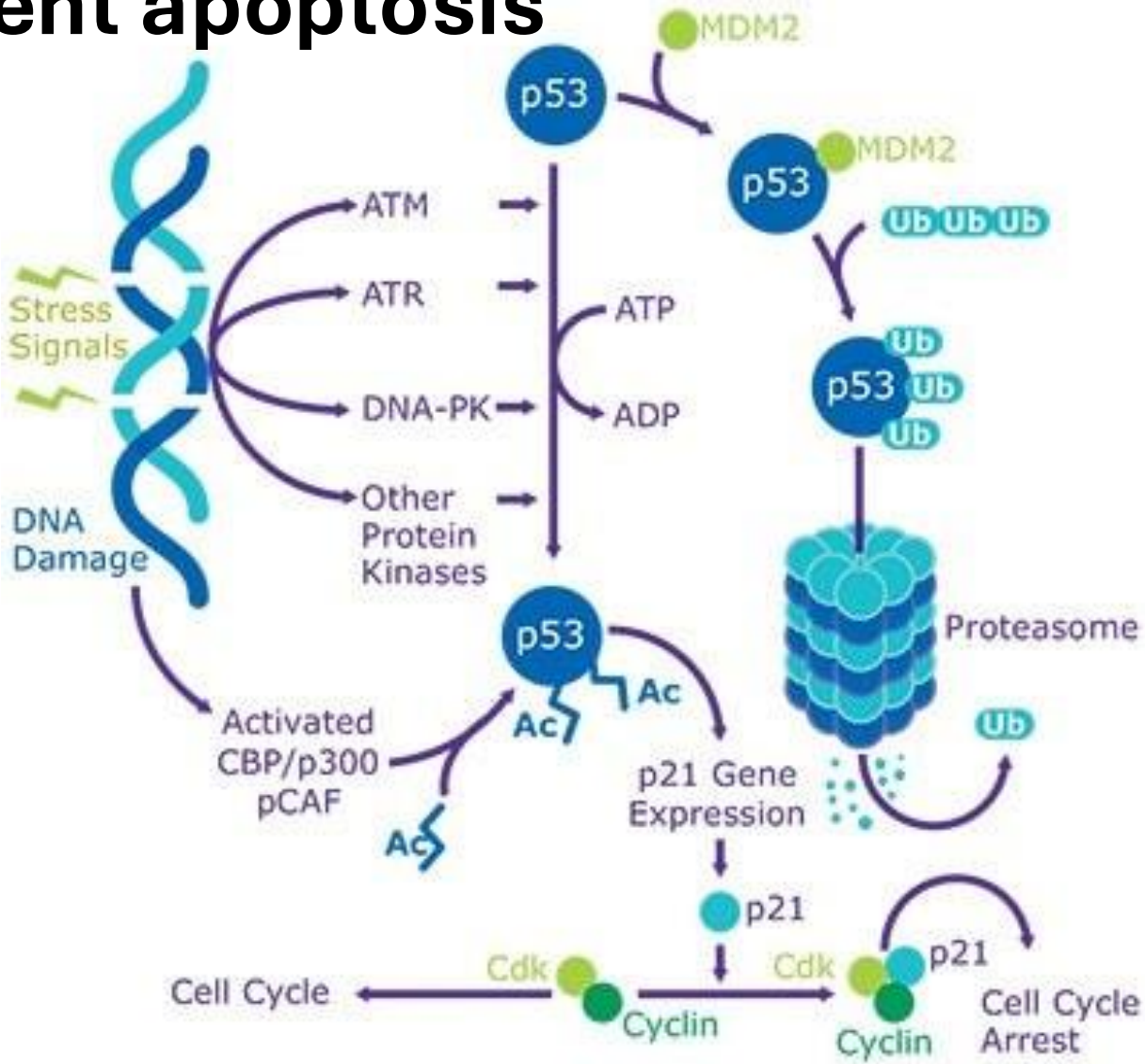
p53 – independent apoptosis
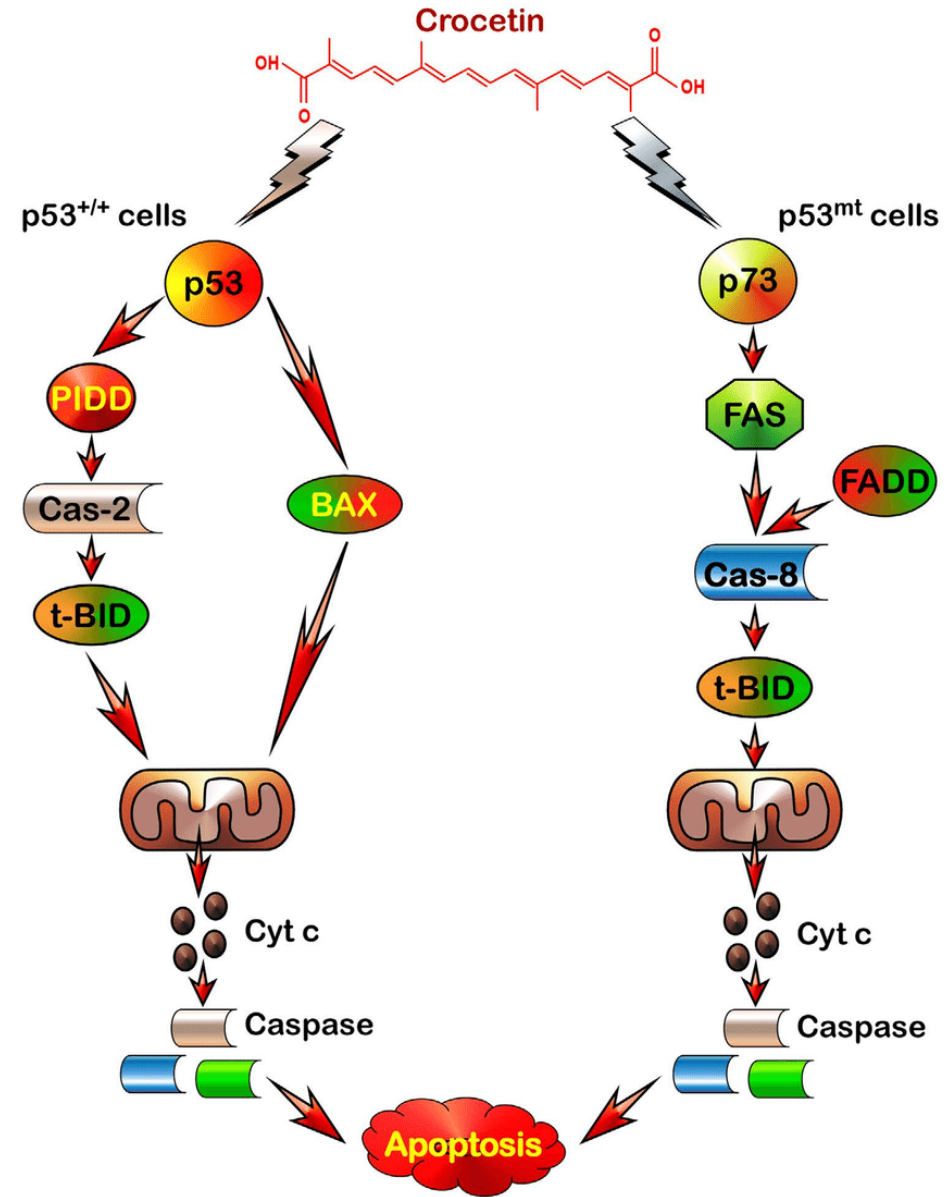
Apoptosis
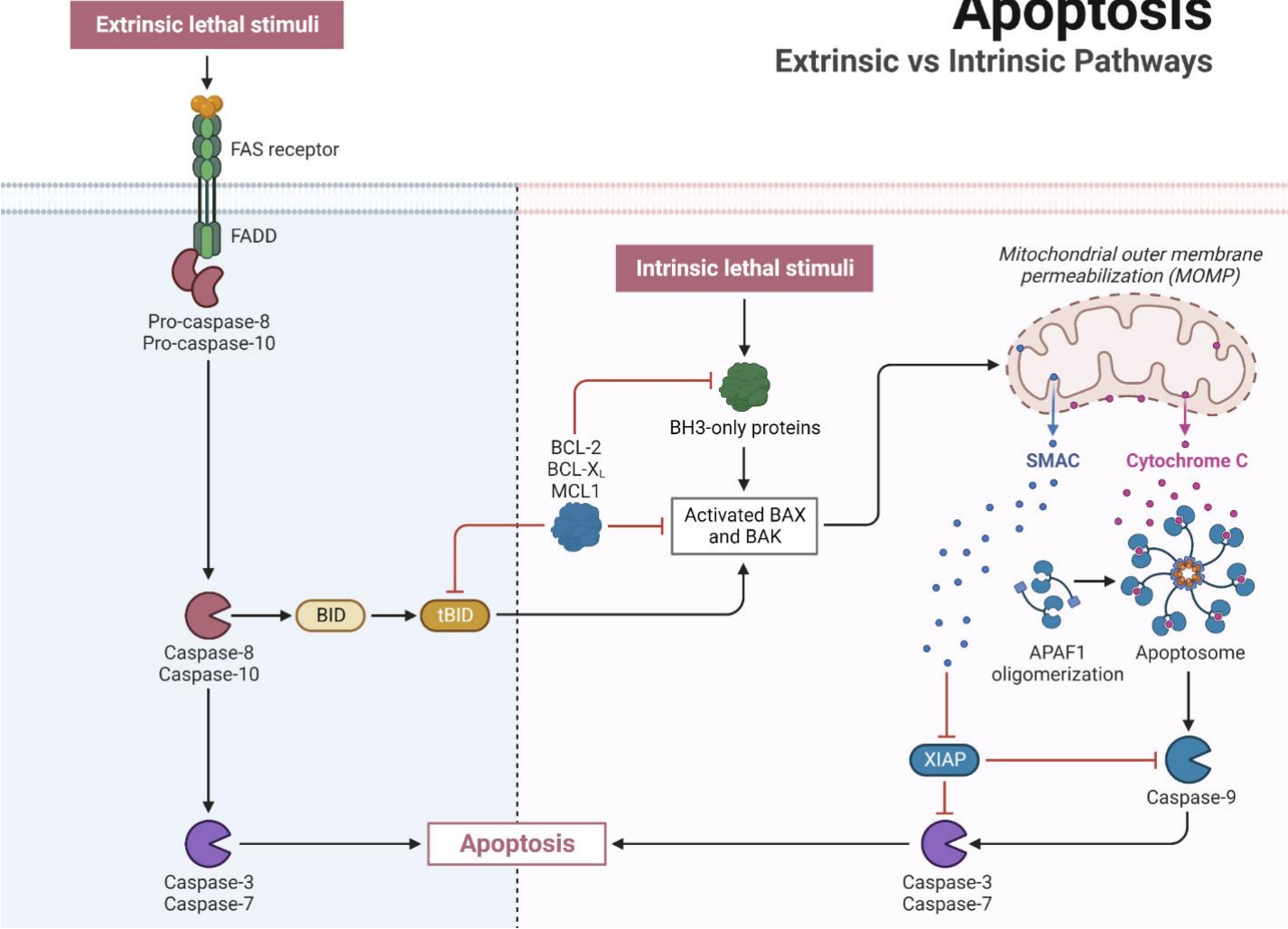
Stages of Apoptosis in Cancer Cells

Stage 1

Non-apoptotic
cancer cell

Stage 2

Cell shrinkage
and chromatin
condensation

Stage 3

Nuclear
fragmentation
and membrane
blebbing

Stage 4

Apoptotic body
formation
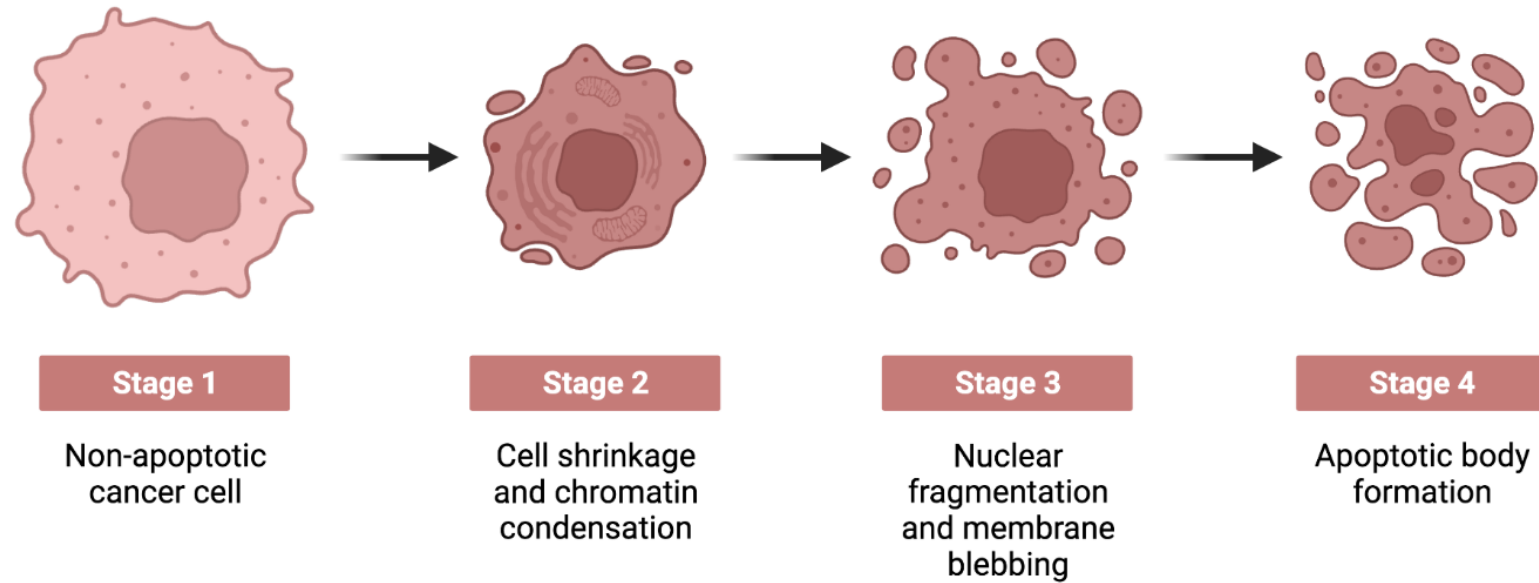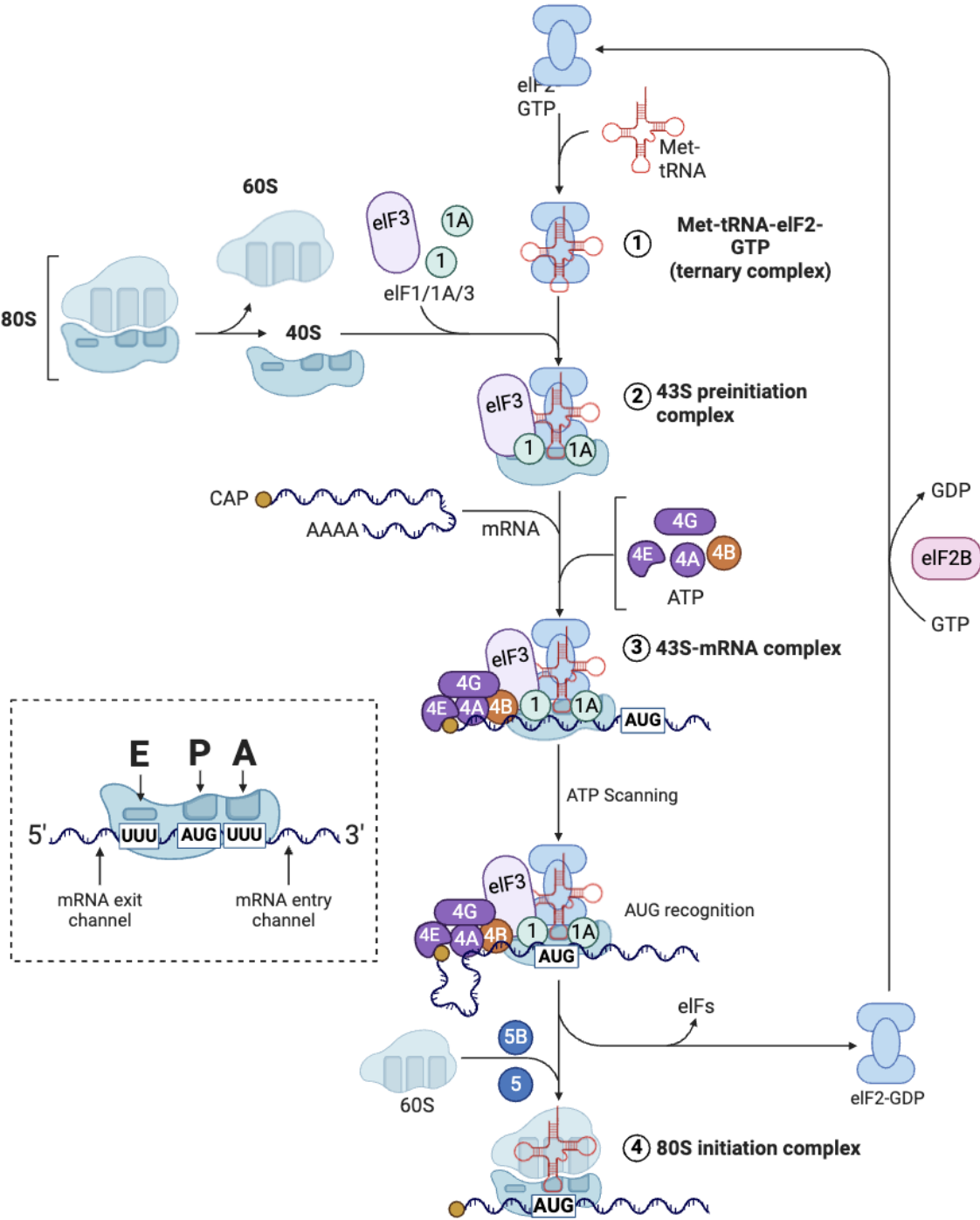
mRNA translation

Ribosome stalling

Ribosome stalling

Codon usage
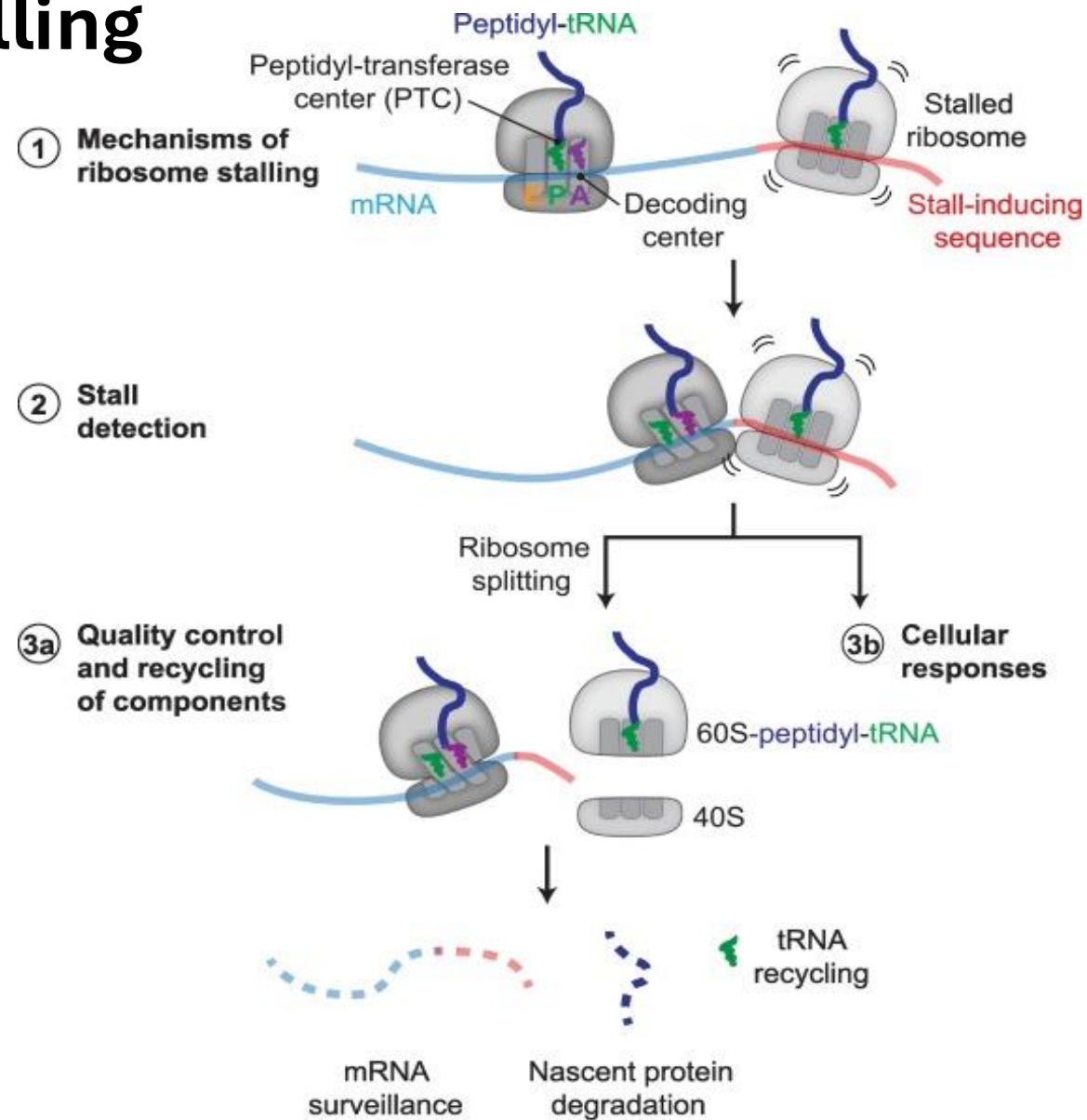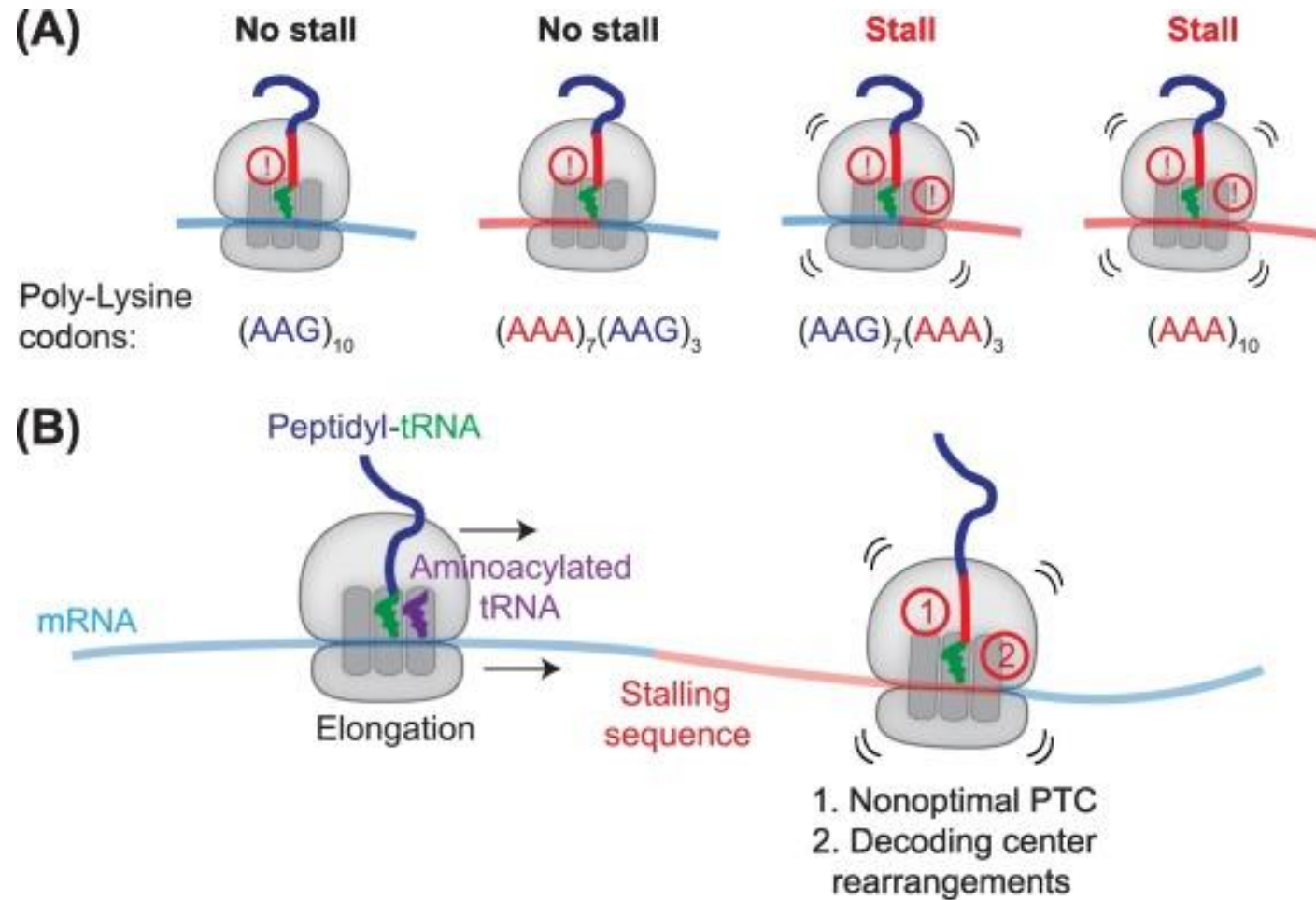
Methods

Methods to measure translation

- ## Puromycin incorporation:
  Puromycin is an antibiotic that prevents protein synthesis by binding to the C-terminus of nascent peptide chains. This causes premature chain termination, releasing the polypeptide chain
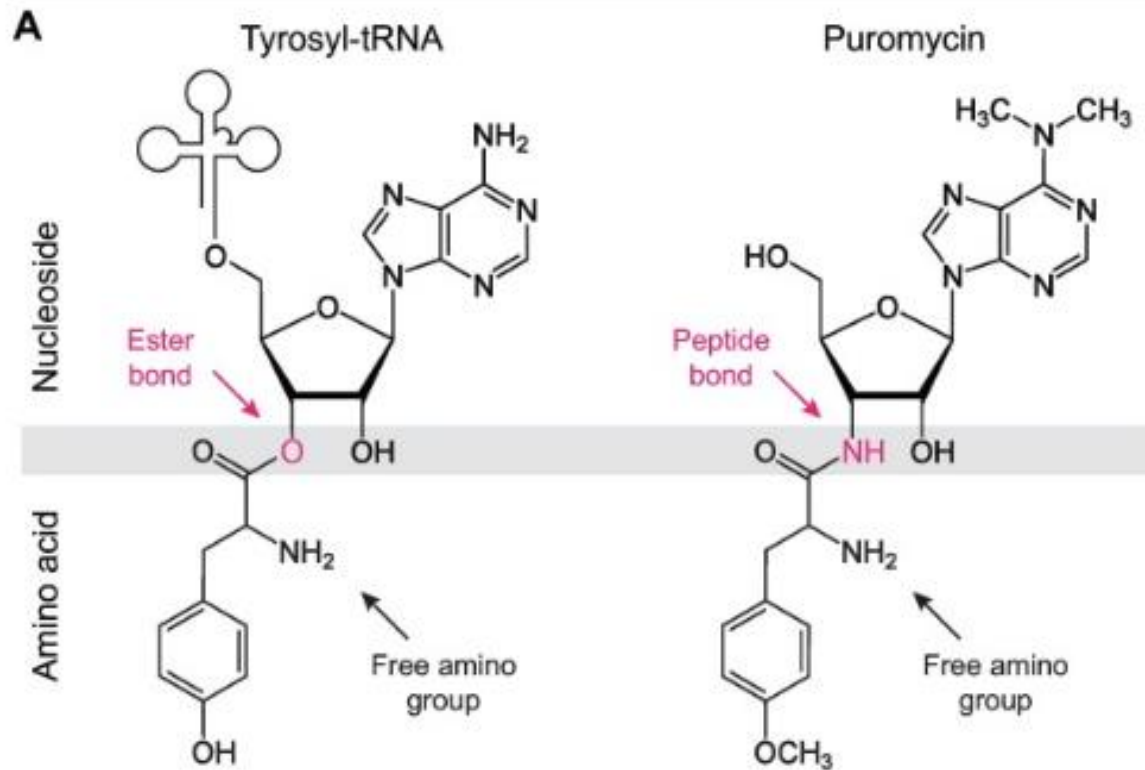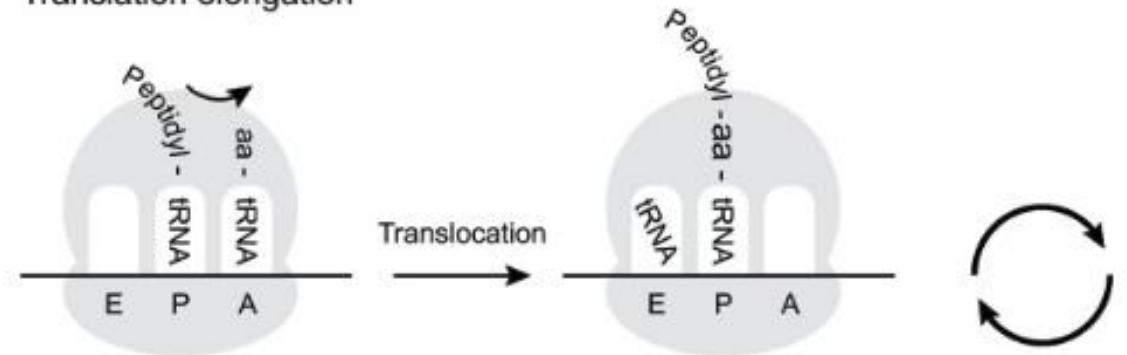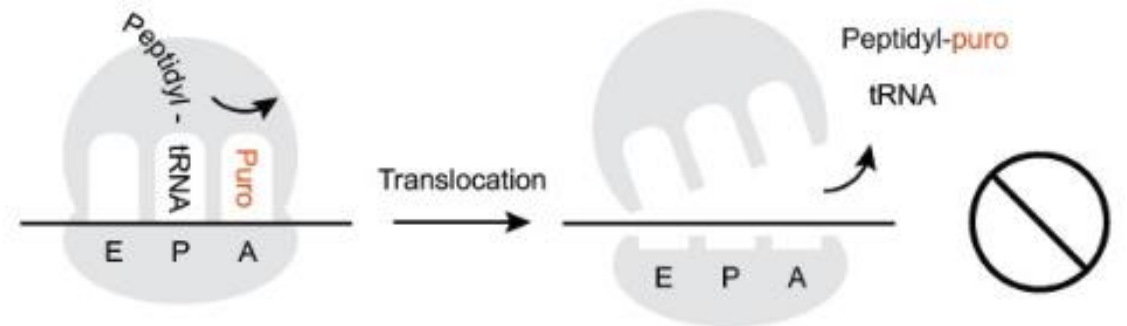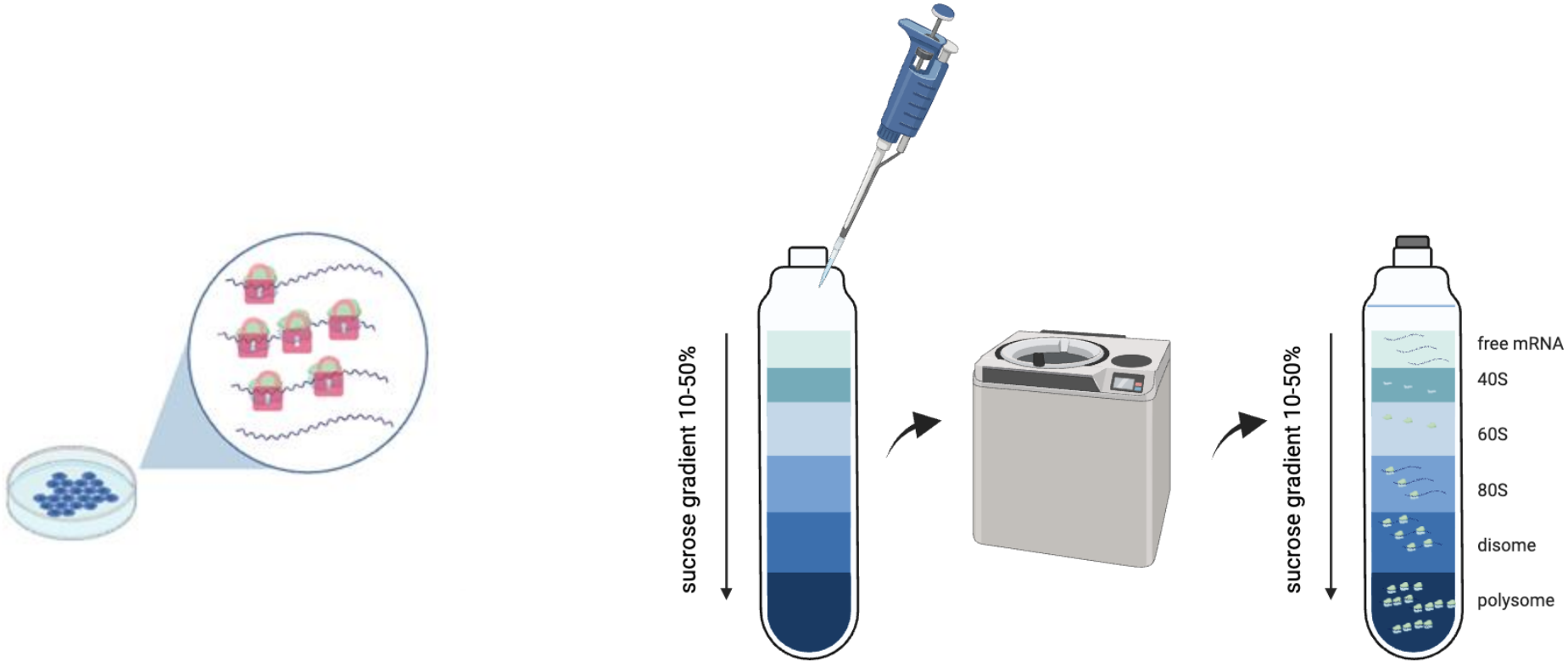
Methods to measure translation efficiency

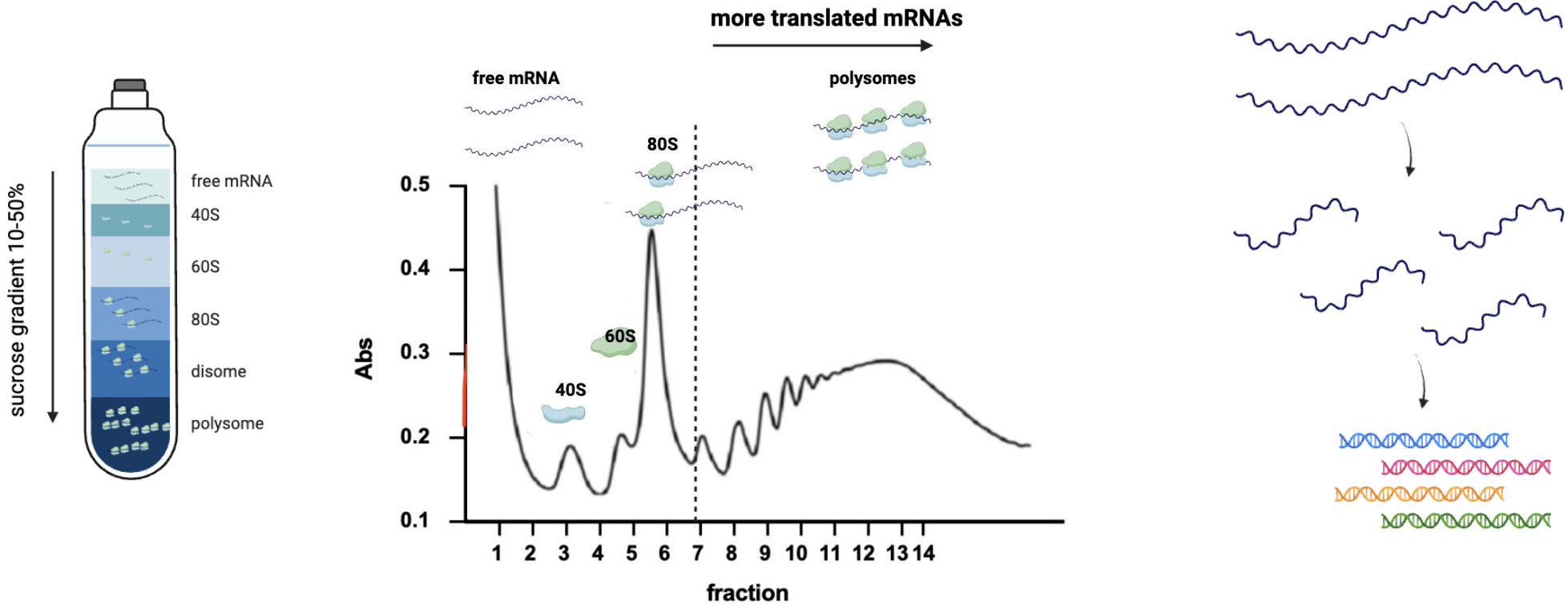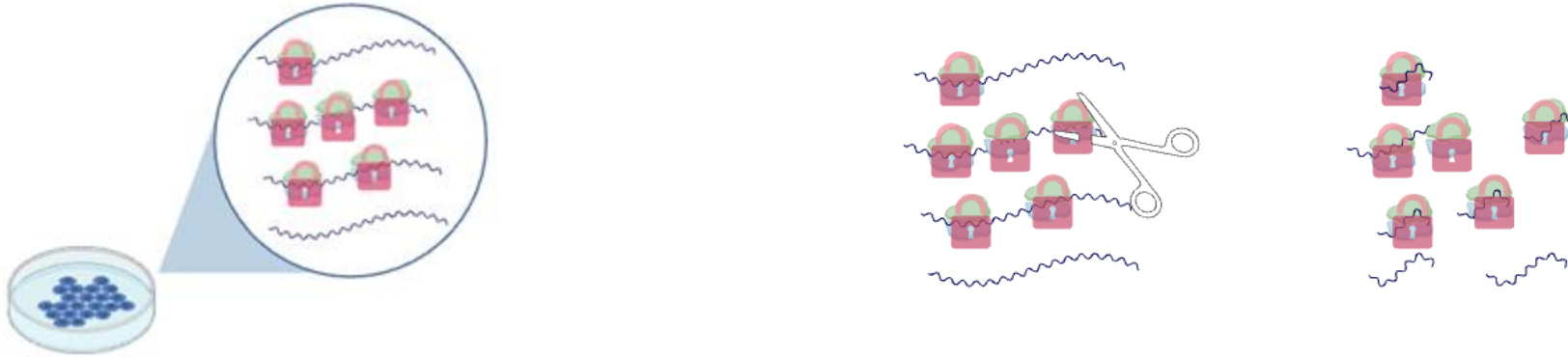**RNA-seq -> total RNA from the cells -> transcriptional changes**

Methods to measure translation efficiency

Polysome profiling -> translational efficiency changes -> high polysomal fractions over free mRNA / low translated

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Polysome profiling -> translational efficiency changes -> high polysomal fractions over free mRNA / low translated
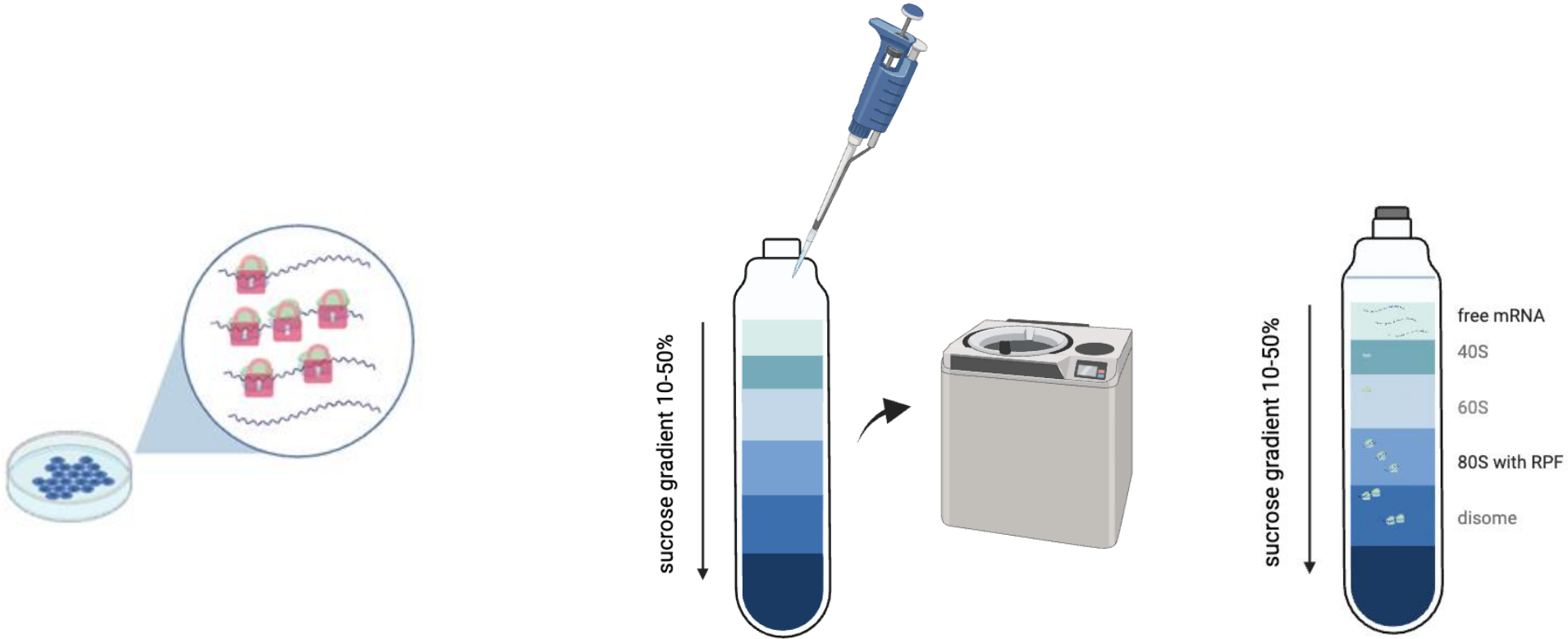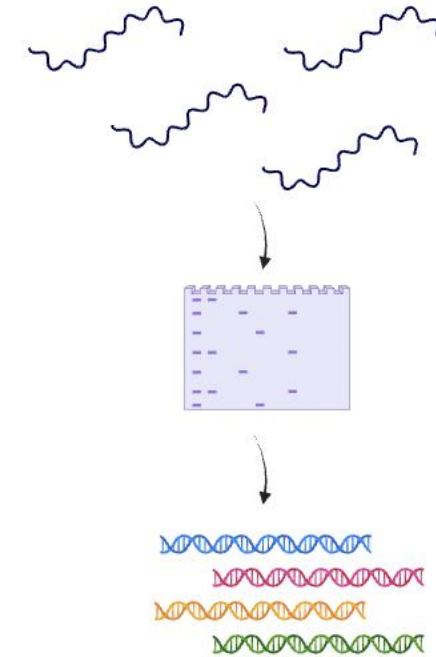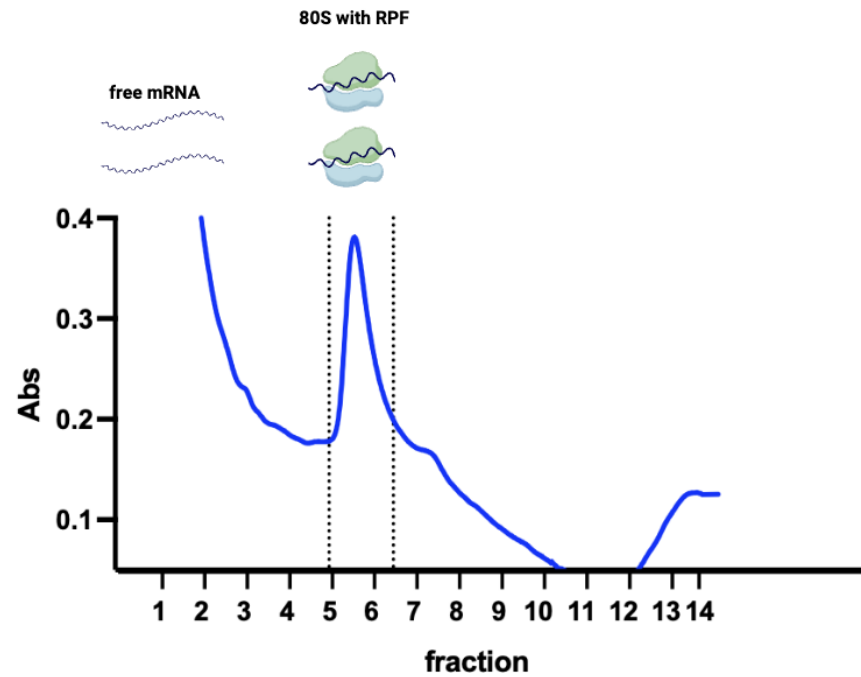
Methods to measure translation efficiency

Ribosomal profiling (ribo-seq) -> translational efficiency changes and positional information about the ribosome

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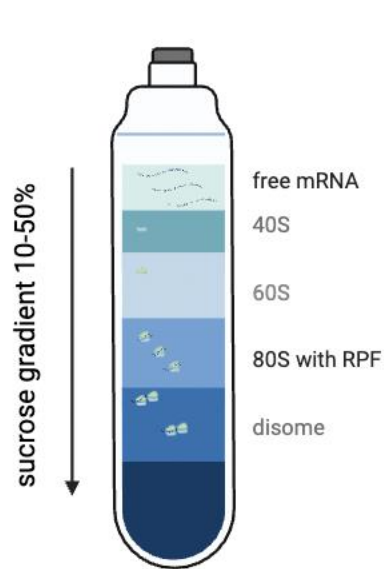
Methods to measure translation efficiency

Ribosomal profiling (ribo-seq) -> translational efficiency changes and positional information about the ribosome

Combined with RNA-seq to adjust translational changes for transcriptional changes

Flow cytometry

Flow cytometer (BD LSR, with pxONE)

Flow cytometer (BD LSR II)

Flow cytometer (BD Accuri C6)

Flow cytometer (BD FACSLyric)

Automatic micro-sampling system (Cytek Aurora)

Flow cytometry (MACSQuant analyzer)

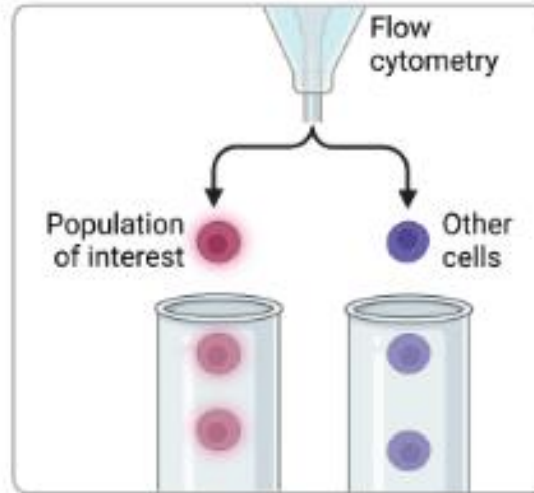Cell sorter (BD FACSAria II)

Cell analyzer (BD FACSymphony)
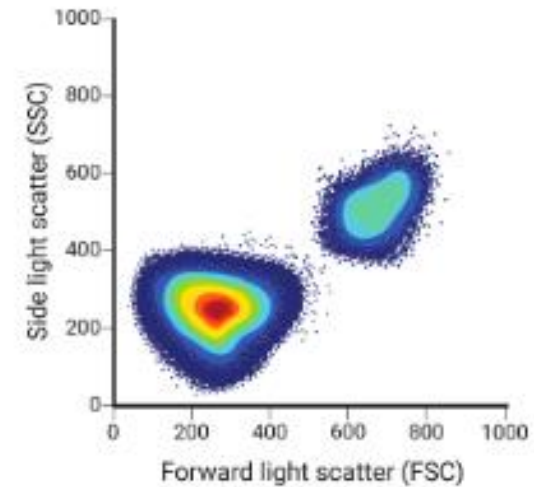
Flow cytometry (Intellicyt iQue Screener)
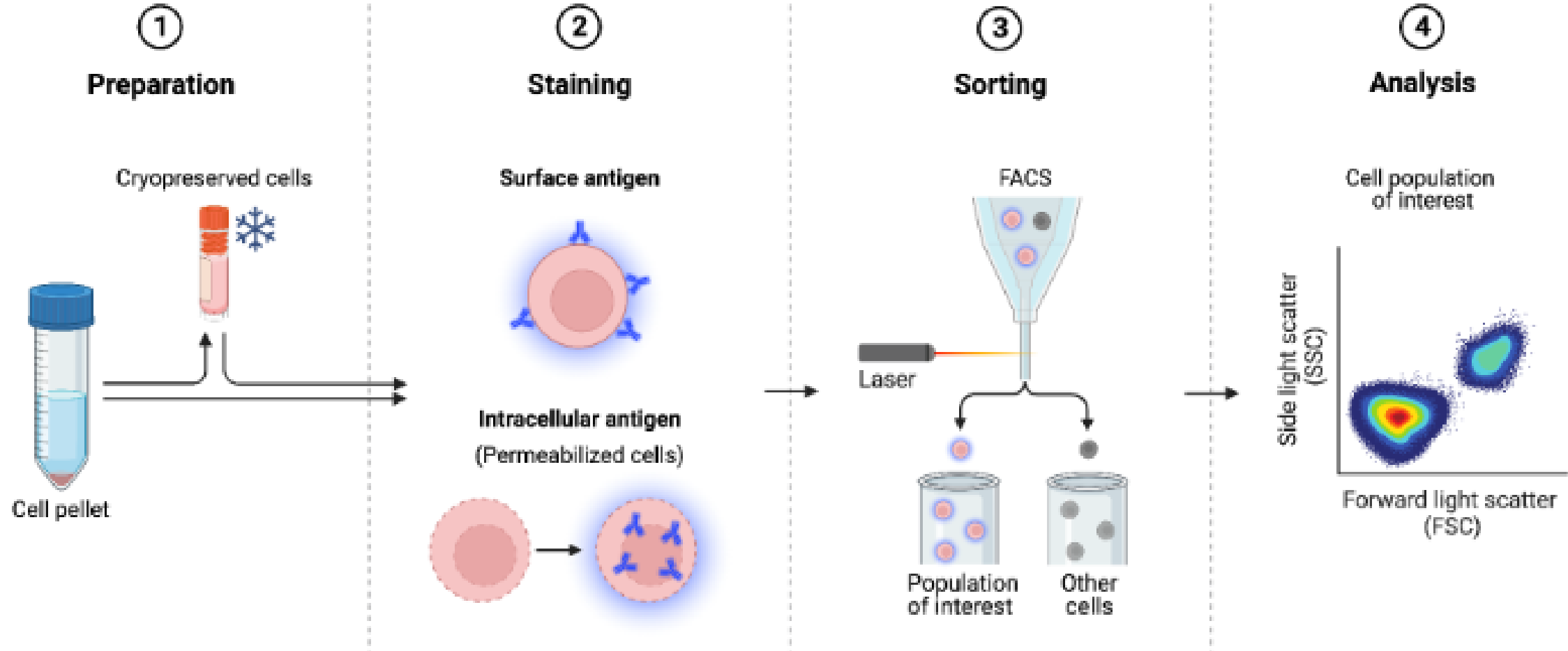
Test tube

Flow cytometry

Flow cytometry

Population of interest

Other cells

Cell sorting

Side light scatter (SSC)

Forward light scatter (FSC)

Flow cytometry graph

Flow cytometry

① **Preparation**

② **Staining**

③ **Sorting**

④ **Analysis**

Cryopreserved cells

Cell pellet

Surface antigen

Intracellular antigen
(Permeabilized cells)

FACS

Laser

Population
of interest

Other
cells

Cell population
of interest

Side light scatter
(SSC)

Forward light scatter
(FSC)

CRISPR

sgRNA Design

Target-specific crRNA sequence — tracrRNA
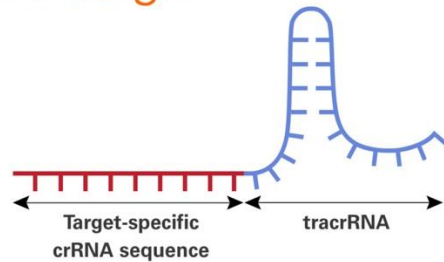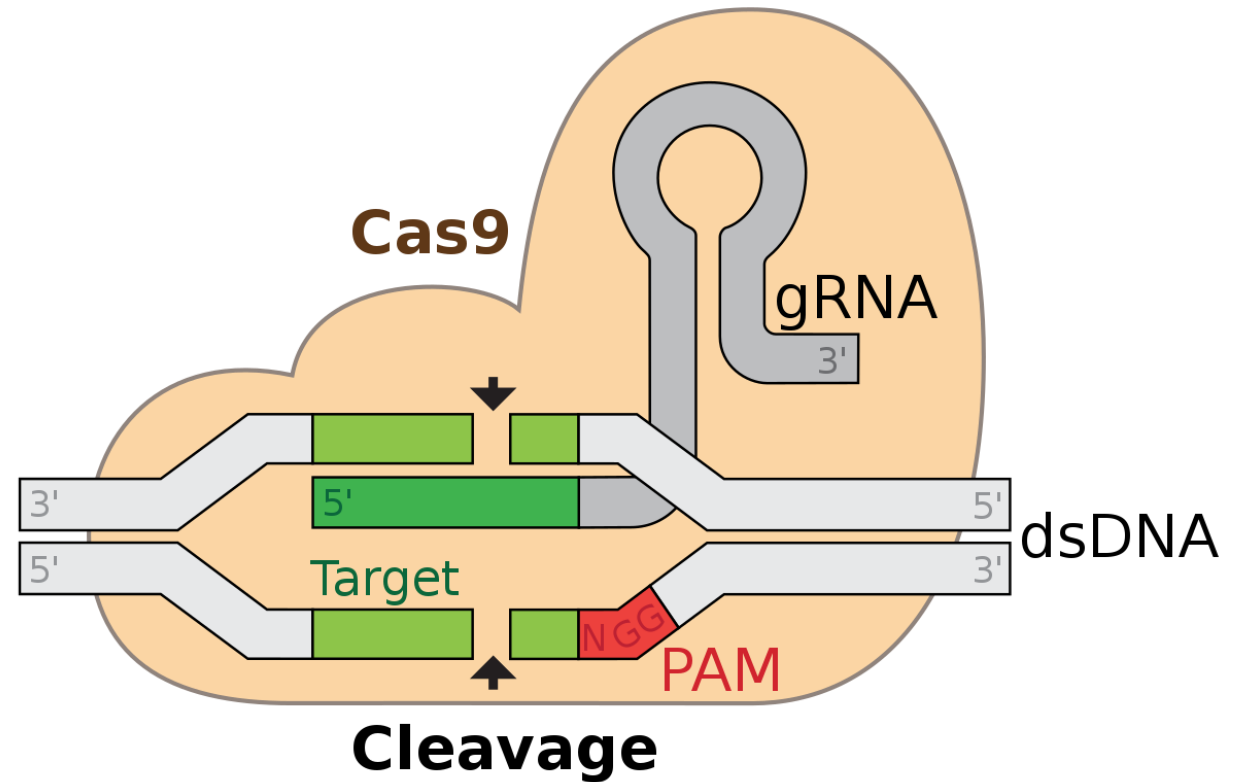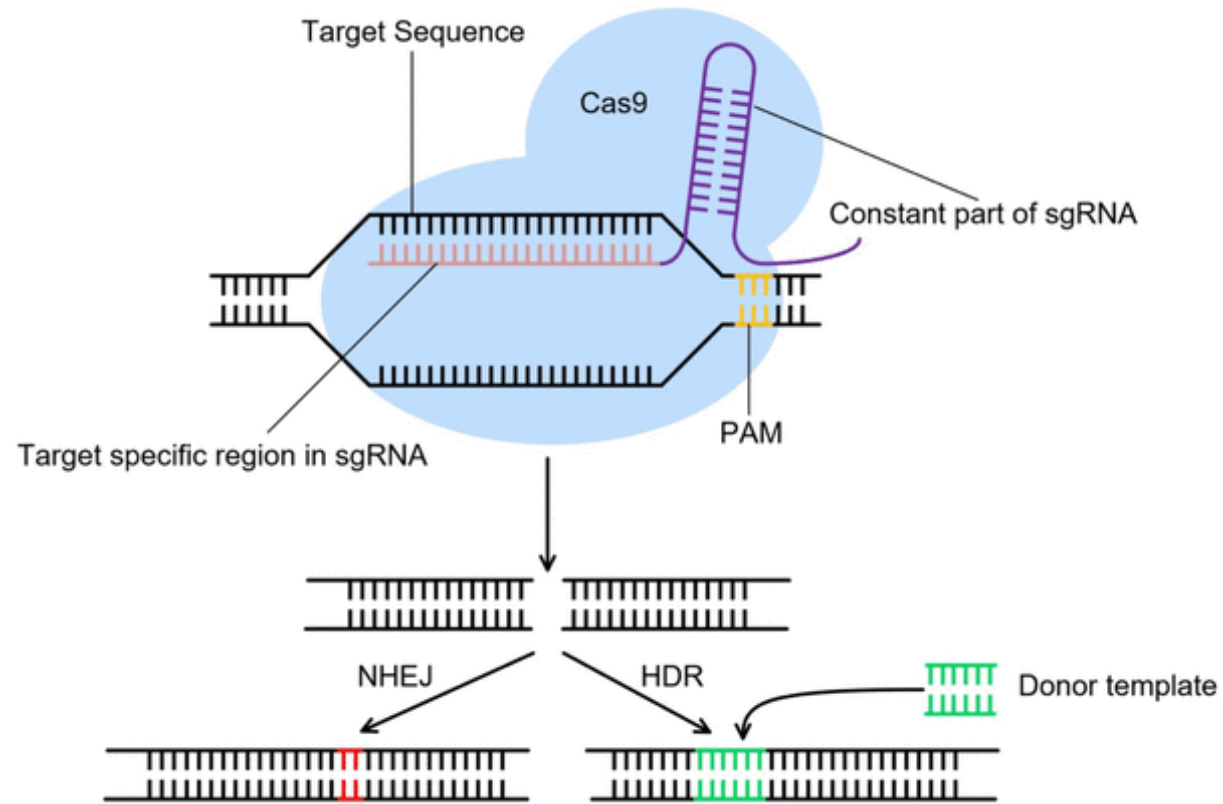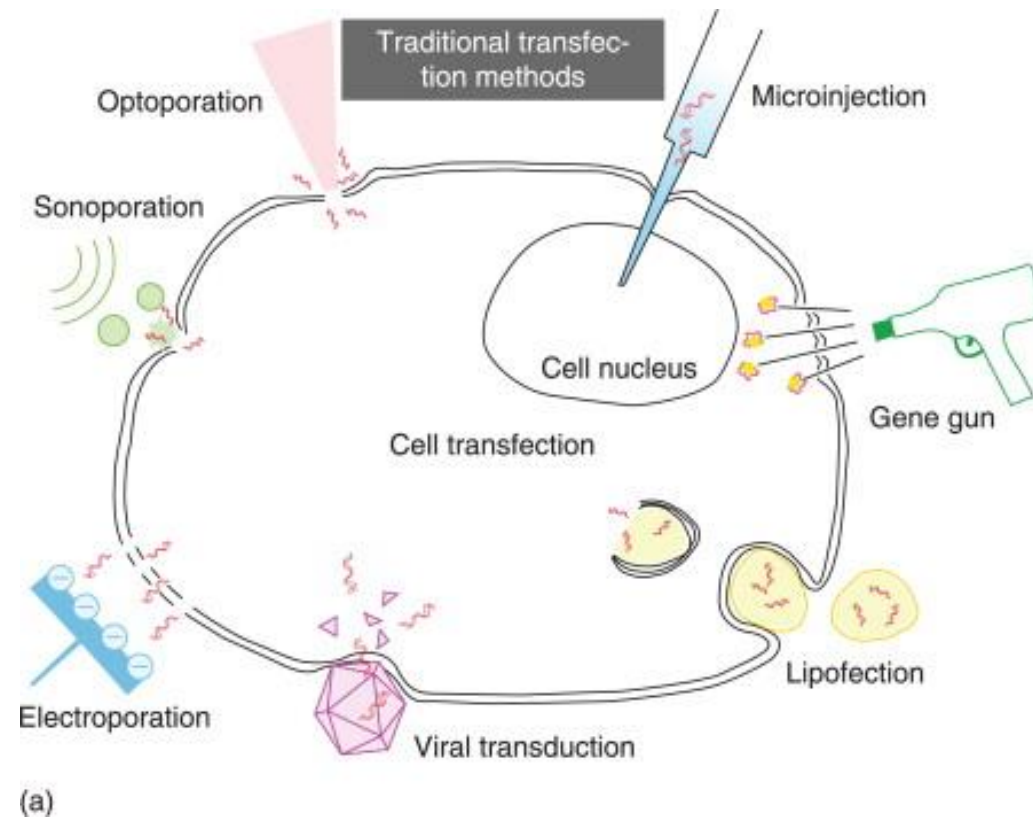
The protospacer adjacent motif (or PAM for short) is a short DNA sequence (usually 2-6 base pairs in length) that follows the DNA region targeted for cleavage by the CRISPR system, such as CRISPR-Cas9. The PAM is required for a Cas nuclease to cut and is generally found 3-4 nucleotides downstream from the cut site.

Cas9

gRNA

3'

dsDNA

3'   5'

5'   3'

Target

5'

N GG

PAM

Cleavage

CRISPR

CRISPR

(a)

Ding, W, et al., Applications of Nanoscience in Photomedicine, 2015

Thank you for your attention.
Any questions?

Please feel free to contact me @
adrianna.dabrowska@ucsf.edu