PROPEL 101: Investigating Molecular Mechanisms

Adrianna Dabrowska, PhD

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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?

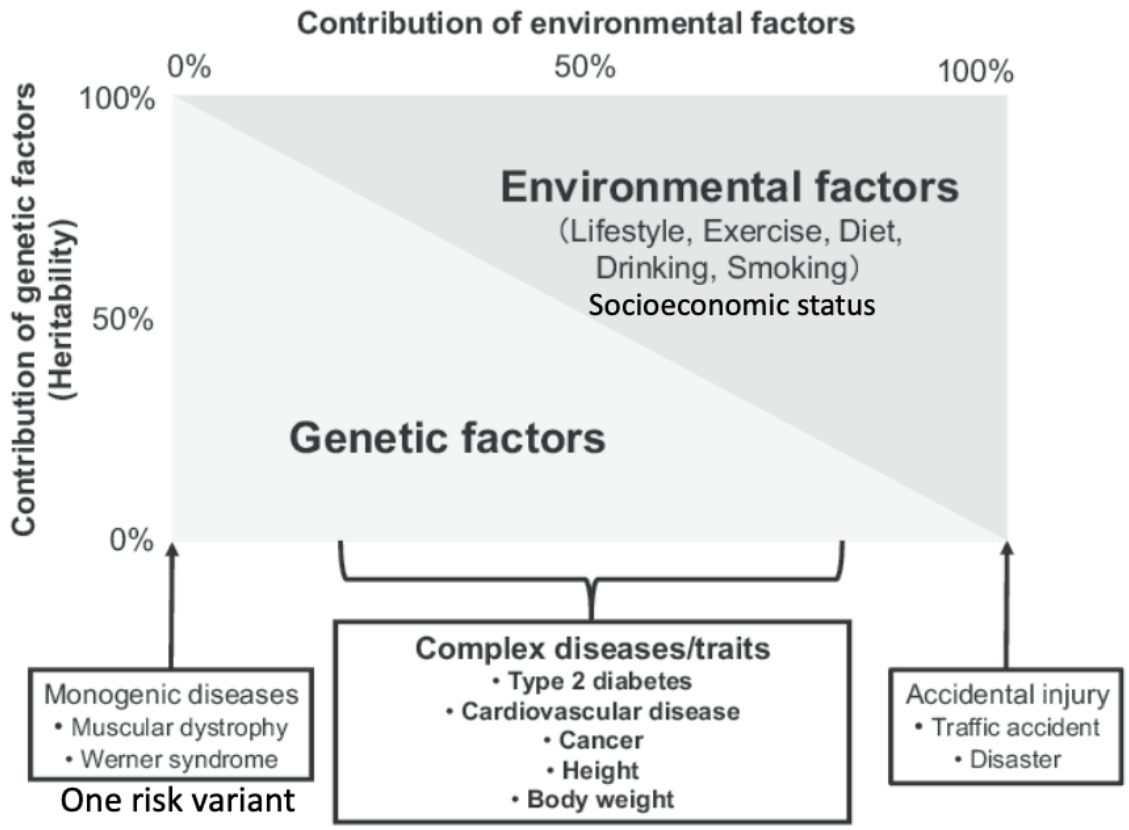
From the perspective of a journal club:

- 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?



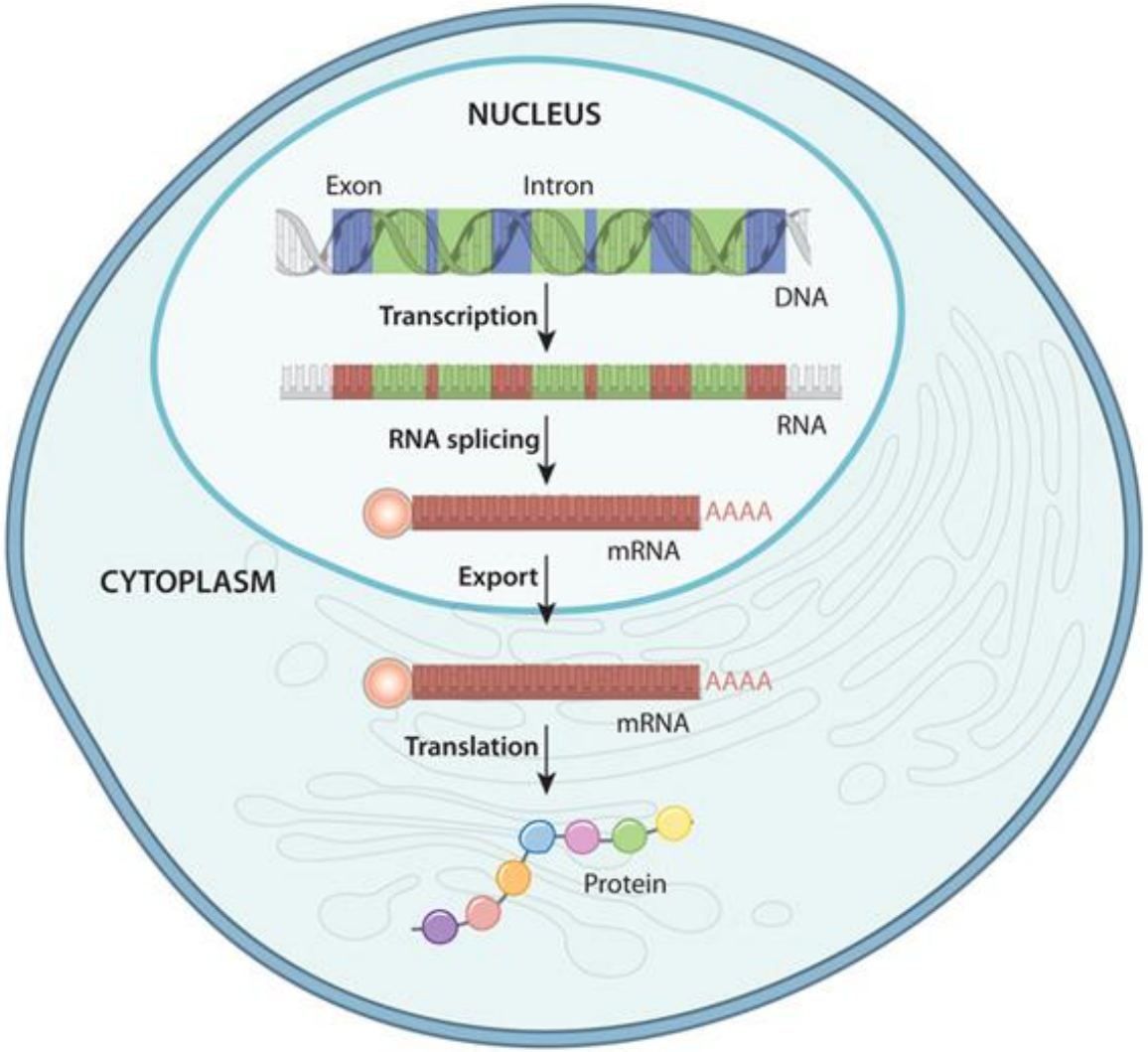
Tanisawa et al., 2016

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.

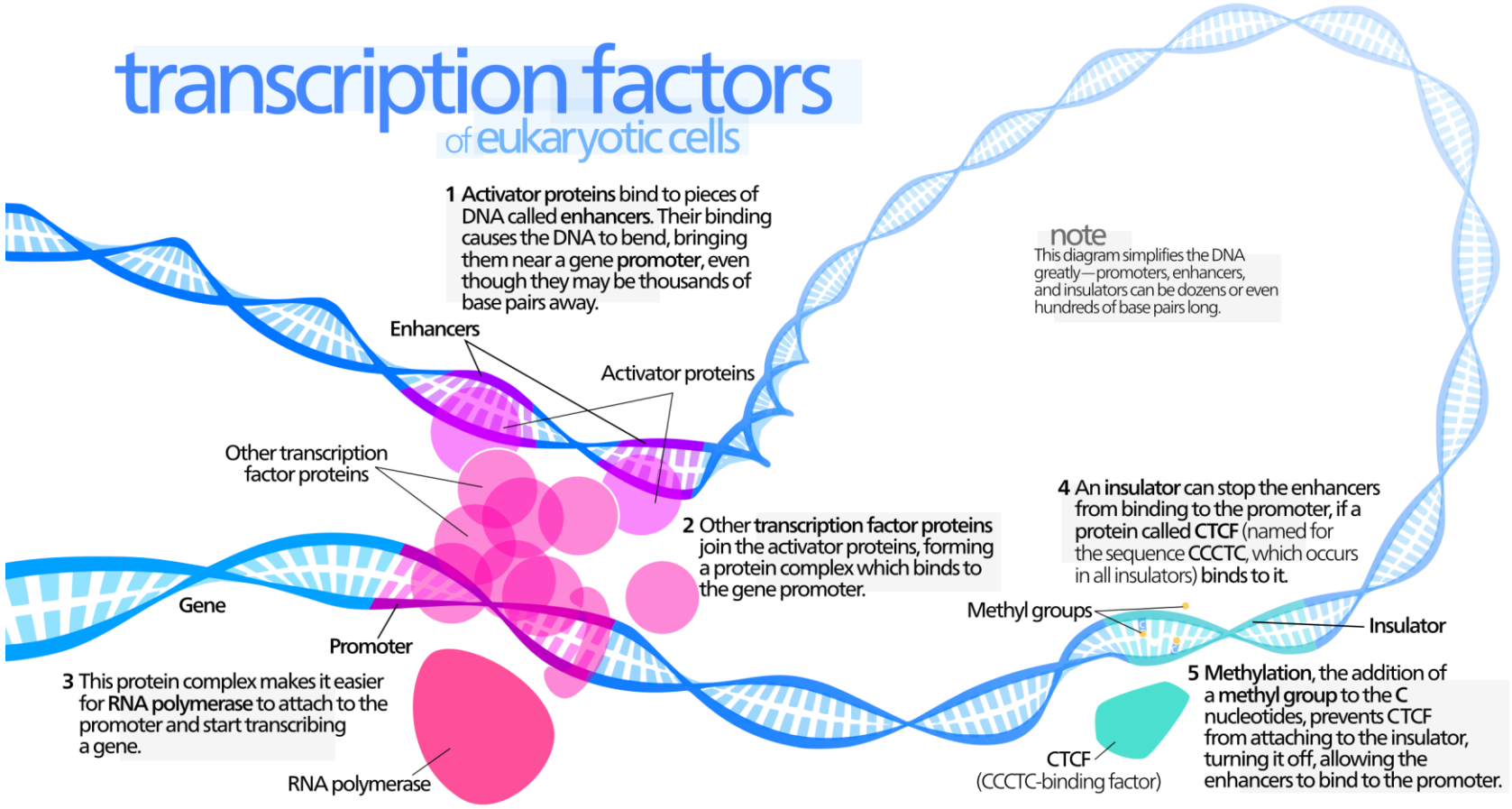
- ✓ Molecular mechanisms are the processes that explain how **genetic variations lead to observable disease phenotypes**. They also refer to the underlying processes that contribute to cell and organismal physiology
- ✓ The **molecular processes that underlie the pathogenesis of diseases**
- ✓ **Alterations in mRNA translation or protein stability that affect a phenotype**
- **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
- **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 - Central Dogma



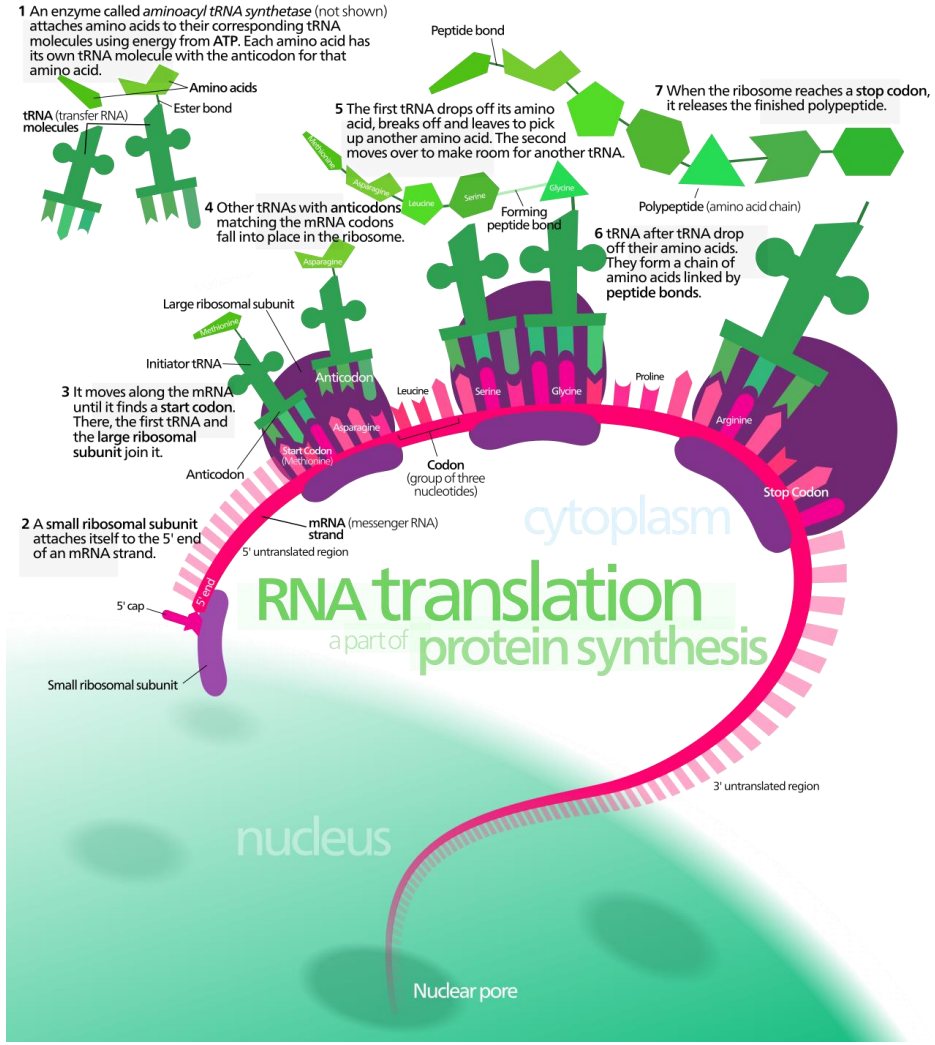
Nature Education, 2010

Transcription -> DNA into RNA



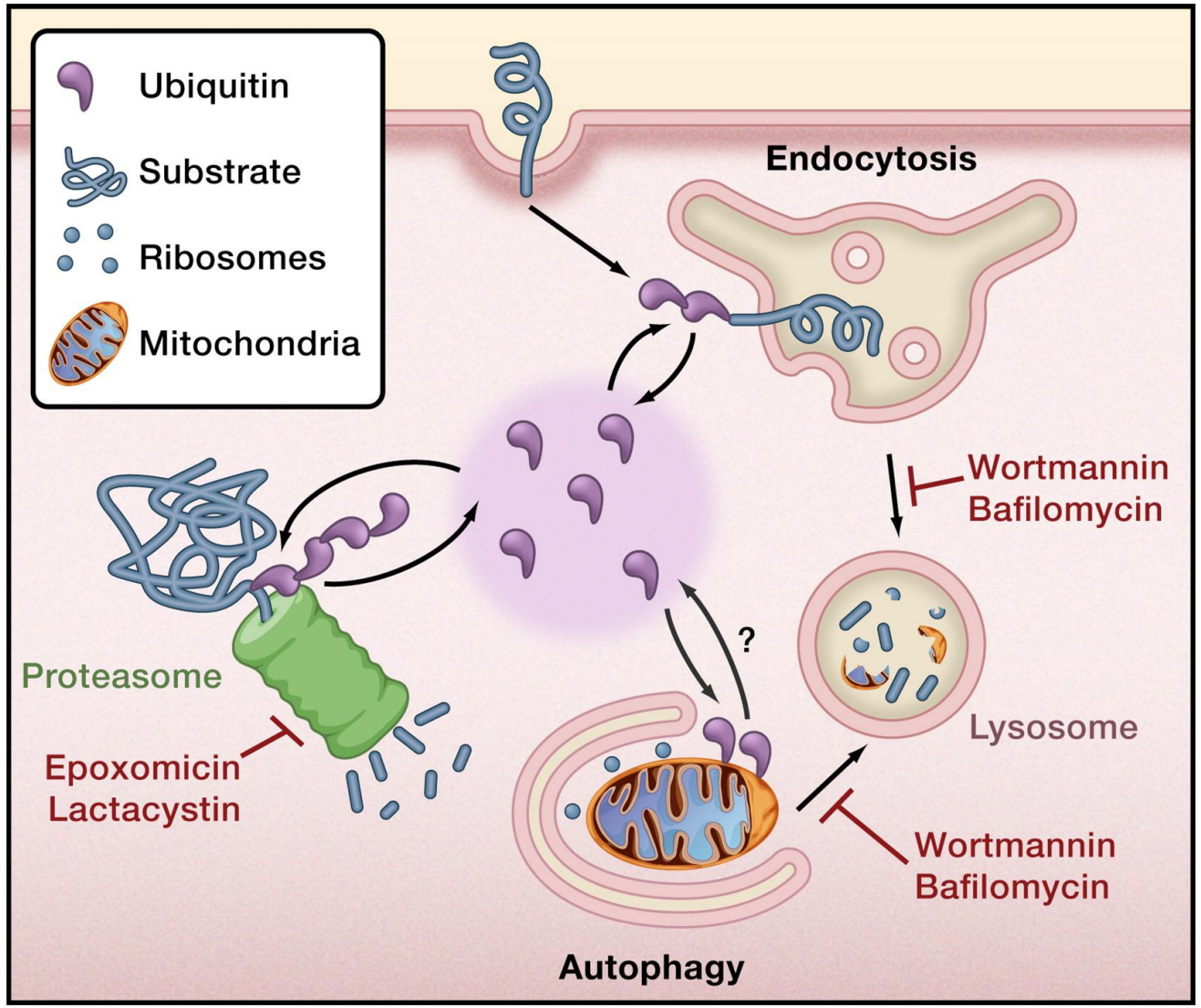
Wikimedia Commons

Translation –> mRNA into protein



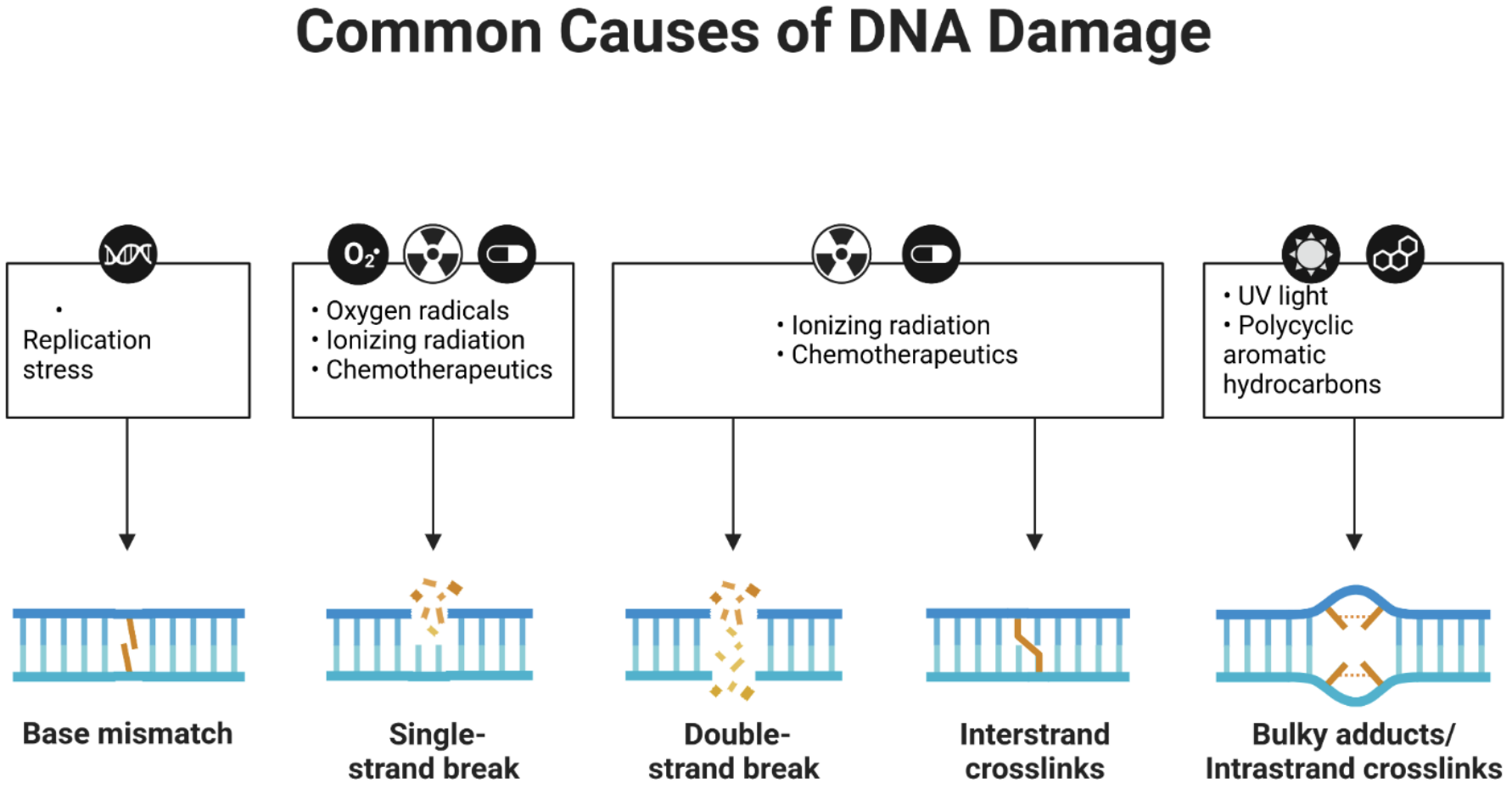
Wikimedia Commons

Protein turnover



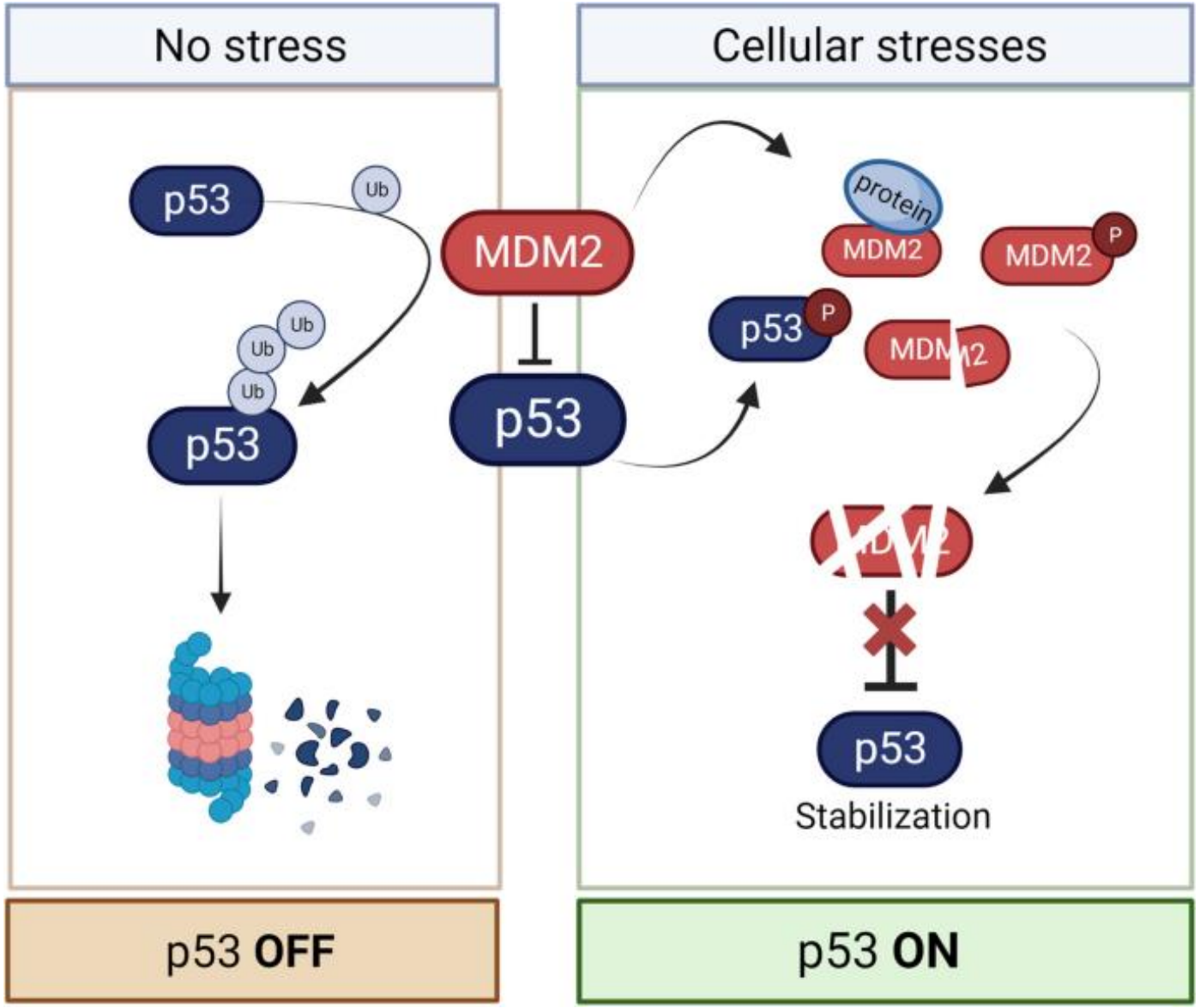
Clague MJ, Urbe S, Cell Minireview, 2010

DNA damage


Common Causes of DNA Damage
•
Replication stress
• Oxygen radicals
• Ionizing radiation
• Chemotherapeutics
• Ionizing radiation
• Chemotherapeutics
• UV light
• Polycyclic aromatic hydrocarbons
Base mismatch
Single-strand break
Double-strand break
Interstrand crosslinks
Bulky adducts/ Intrastrand crosslinks

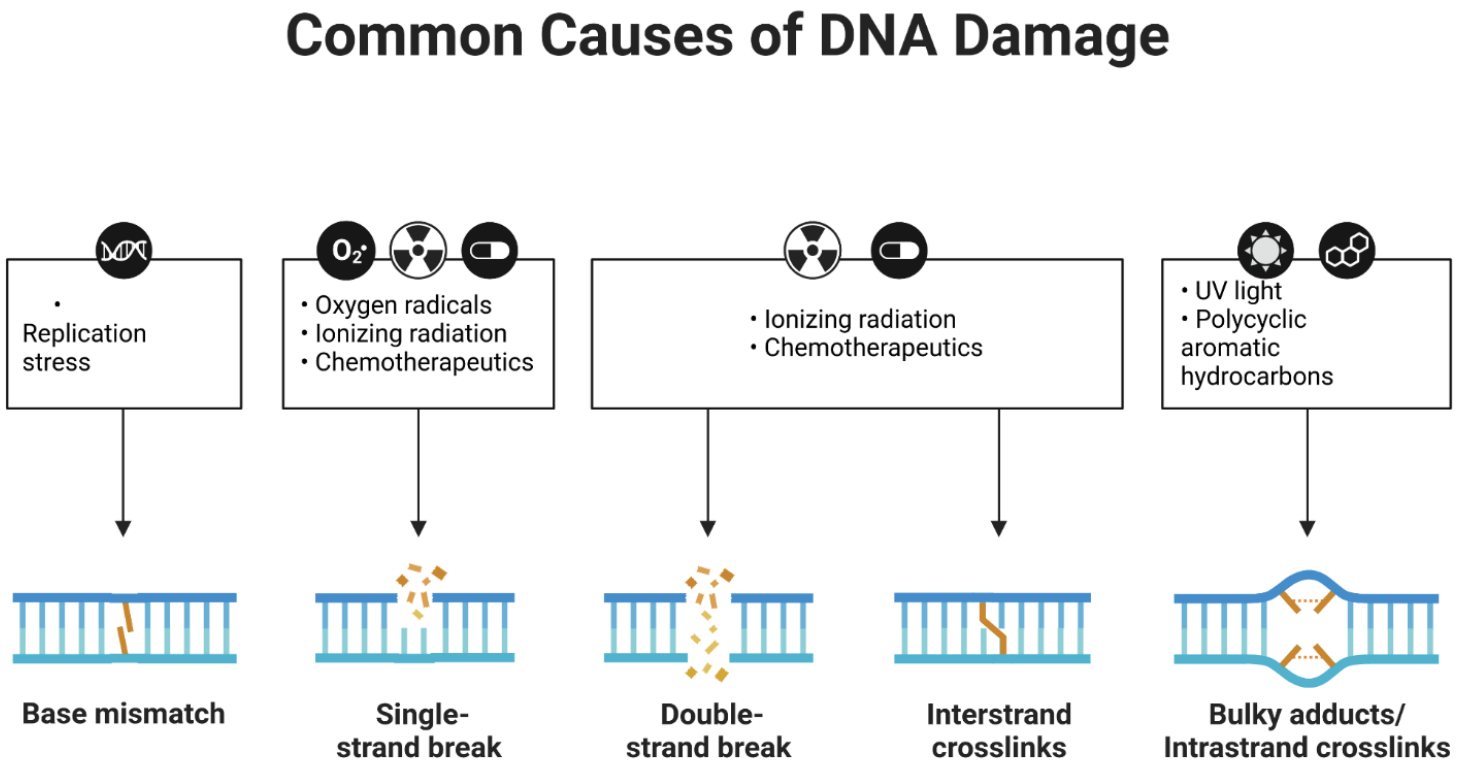
p53 (gene TP53)

- ”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

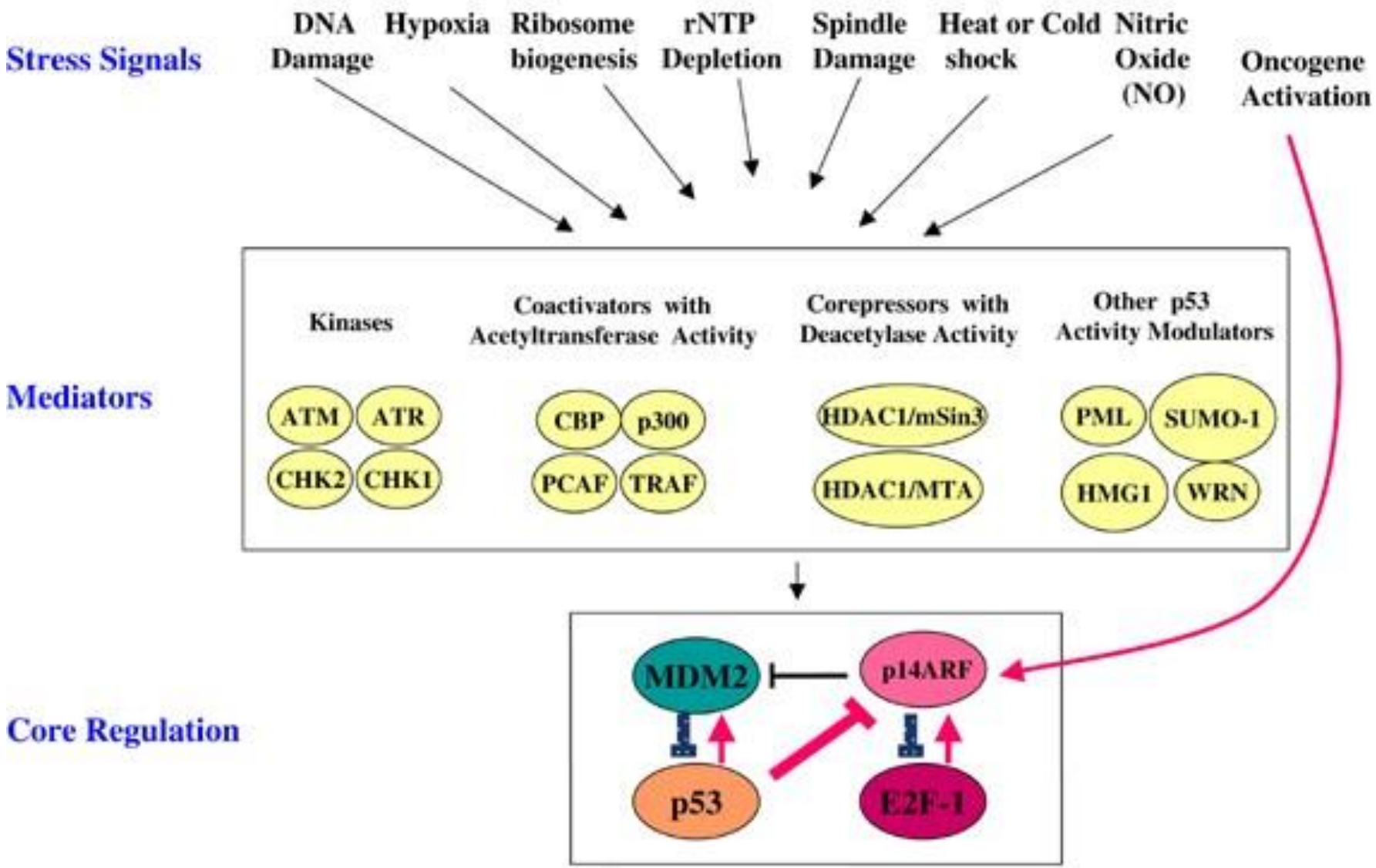
DNA damage – cancer treatments



- Many cancers = TP53 mutated
- Yet DNA-damaging chemotherapies still induce apoptosis

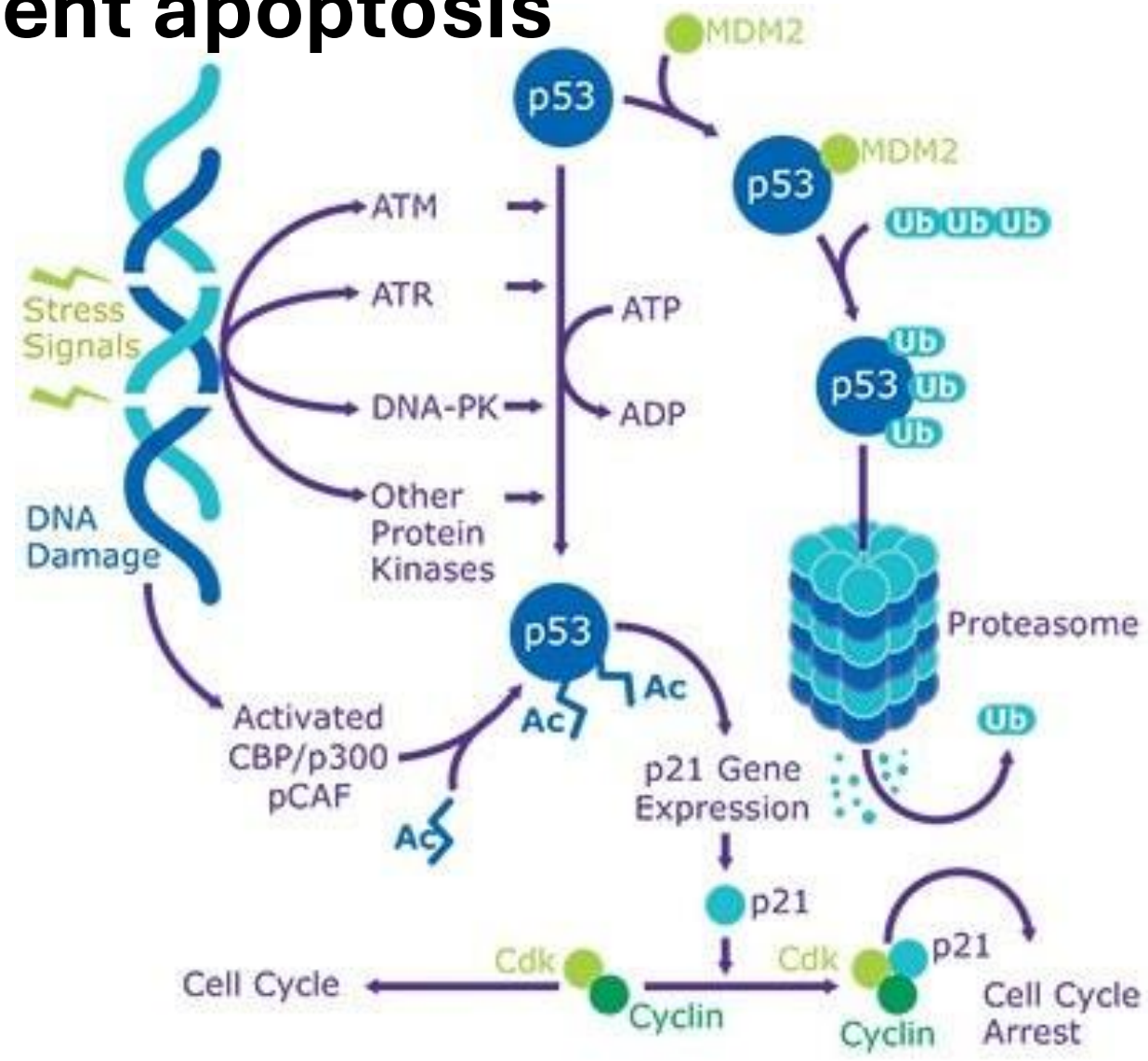
→ There must be alternative pathways

Activation of p53



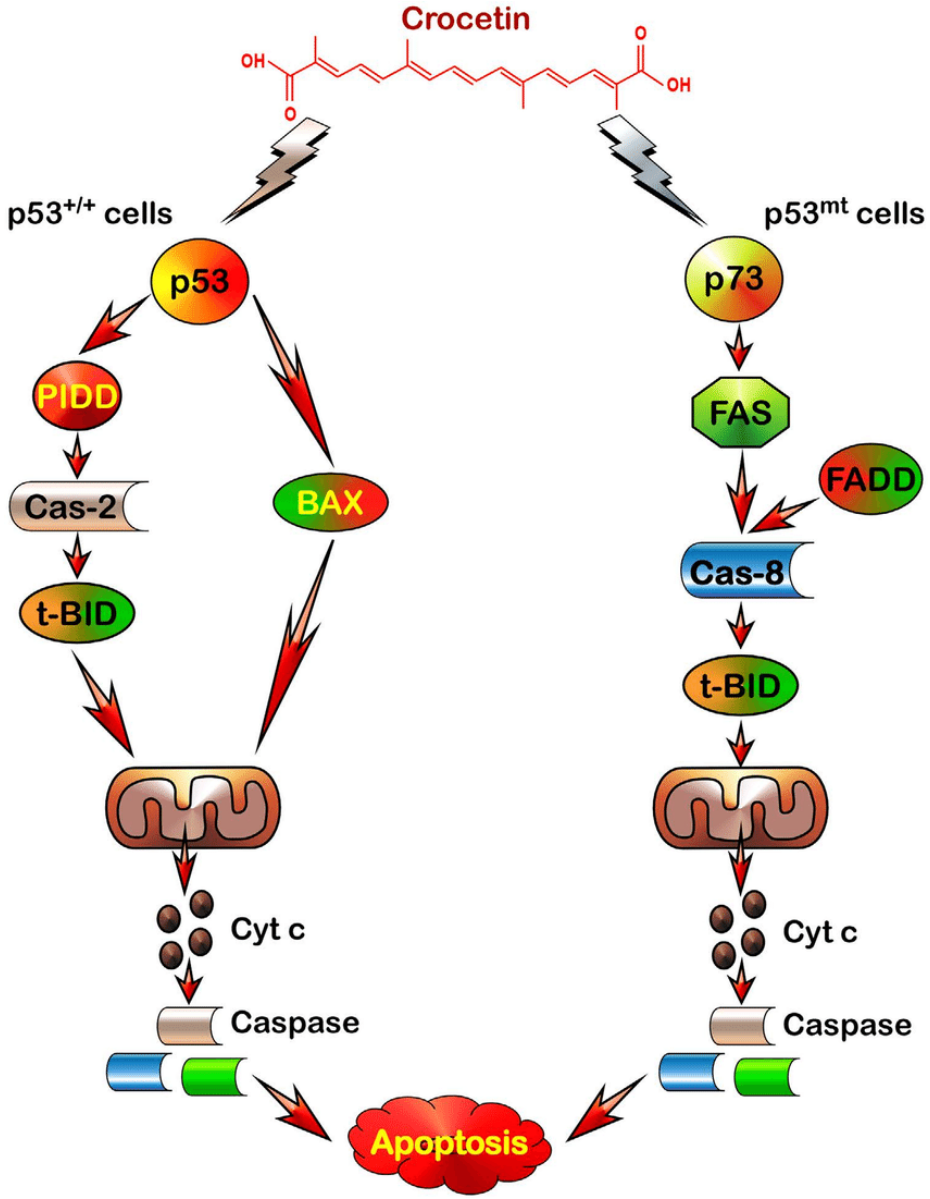
Harris SL, Levine AJ, Oncogene, 2005

p53 – dependent apoptosis



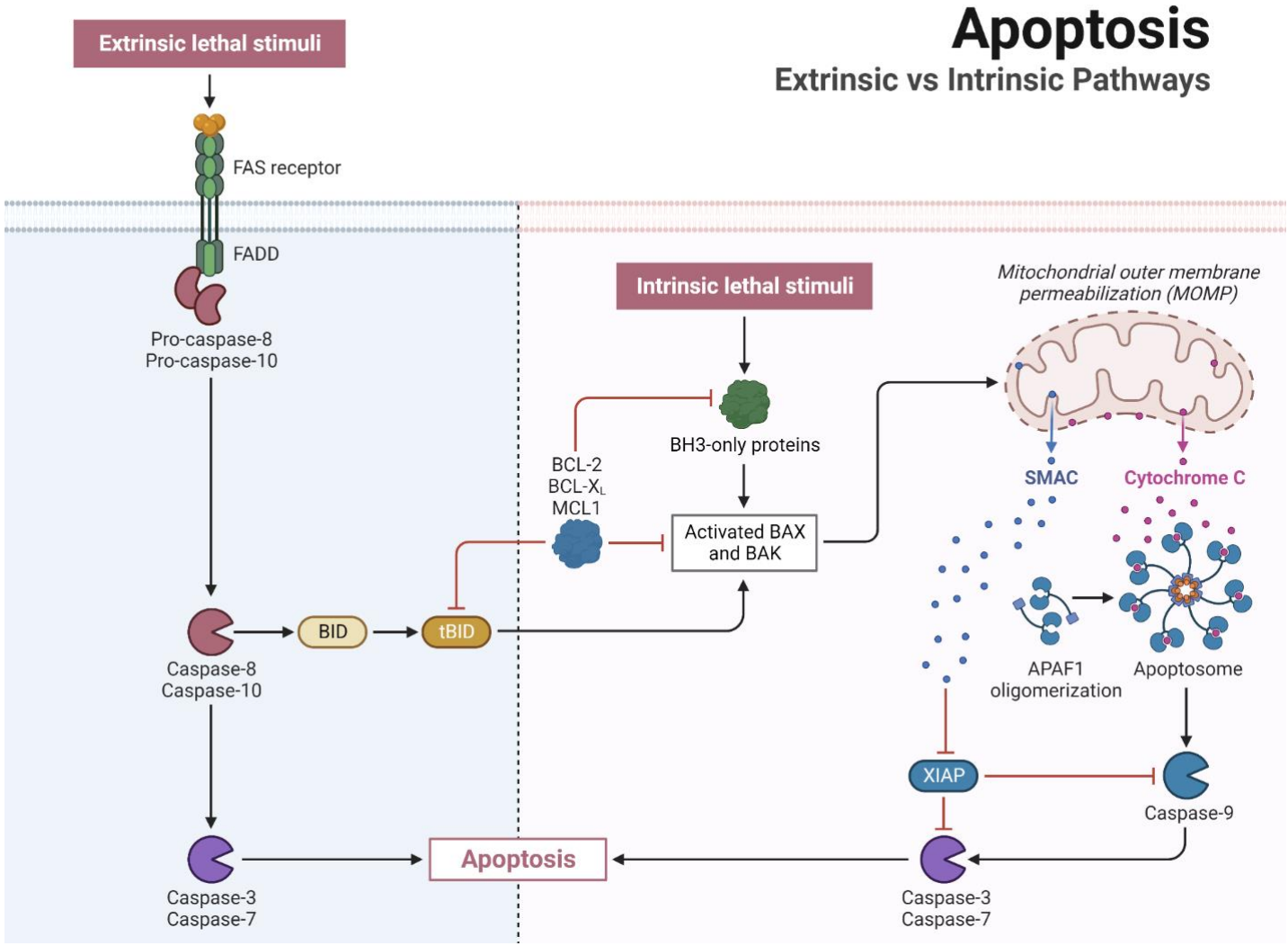
Sigma Millipore

p53 – independent apoptosis

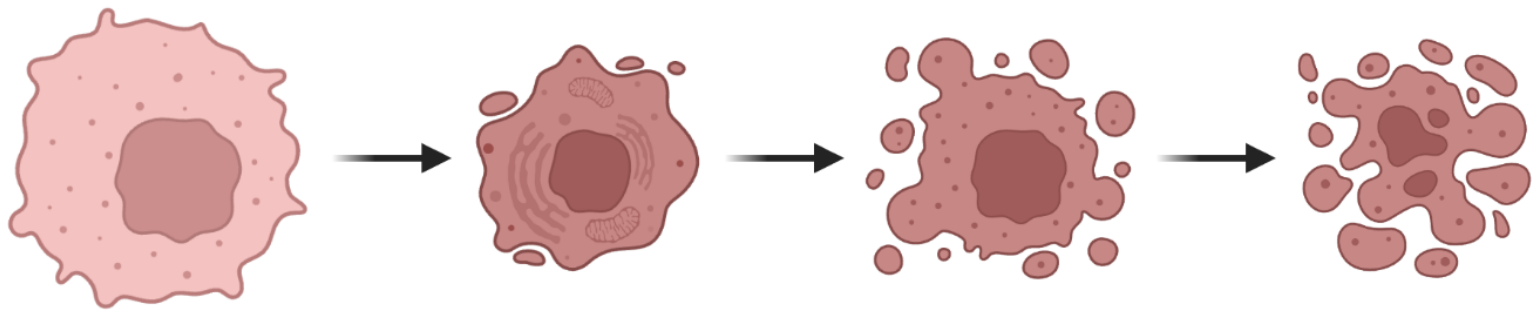


Ray P, Scientific Reports, 2016

Apoptosis


Apoptosis
Extrinsic vs Intrinsic Pathways
Extrinsic lethal stimuli
FAS receptor
FADD
Pro-caspase-8
Pro-caspase-10
Caspase-8
Caspase-10
BID
tBID
Caspase-3
Caspase-7
Apoptosis
Intrinsic lethal stimuli
BH3-only proteins
BCL-2
BCL-XL
MCL1
Activated BAX
and BAK
Mitochondrial outer membrane
permeabilization (MOMP)
SMAC
Cytochrome C
APAF1
oligomerization
Apoptosome
XIAP
Caspase-9
Caspase-3
Caspase-7

Stages of Apoptosis in Cancer Cells



Stage 1

Non-apoptotic cancer cell

Stage 2

Cell shrinkage and chromatin condensation

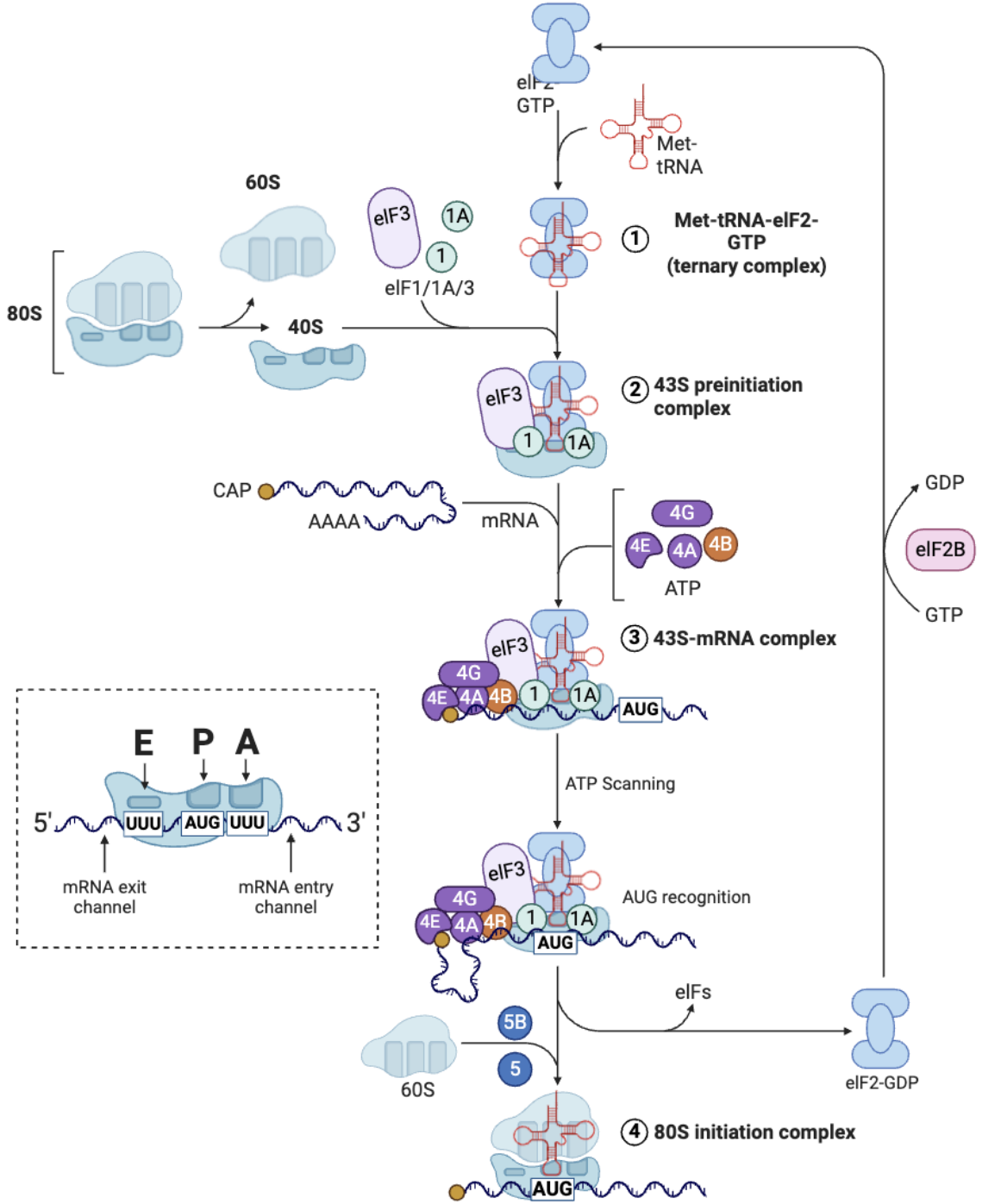
Stage 3

Nuclear fragmentation and membrane blebbing

Stage 4

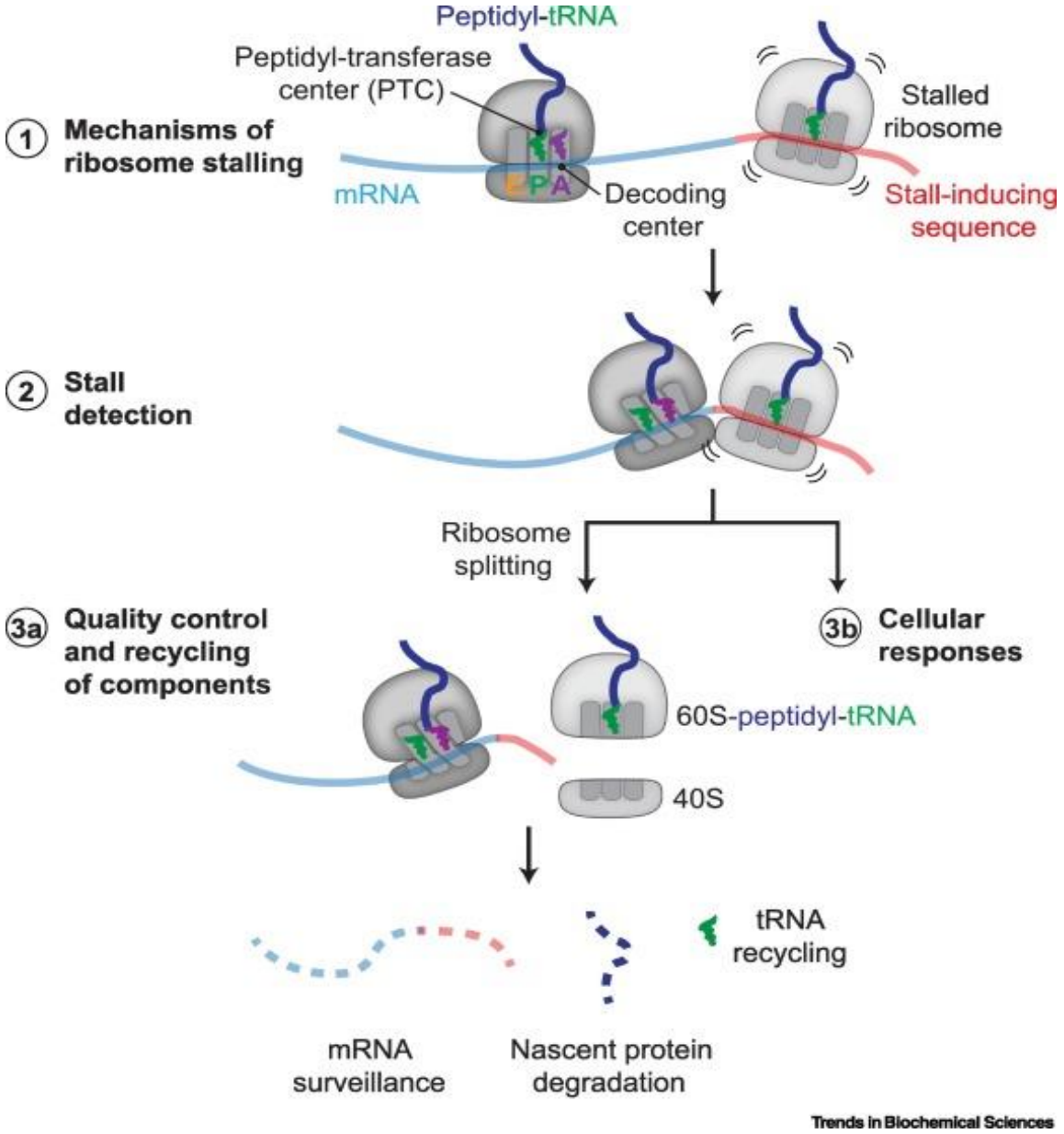
Apoptotic body formation

mRNA translation



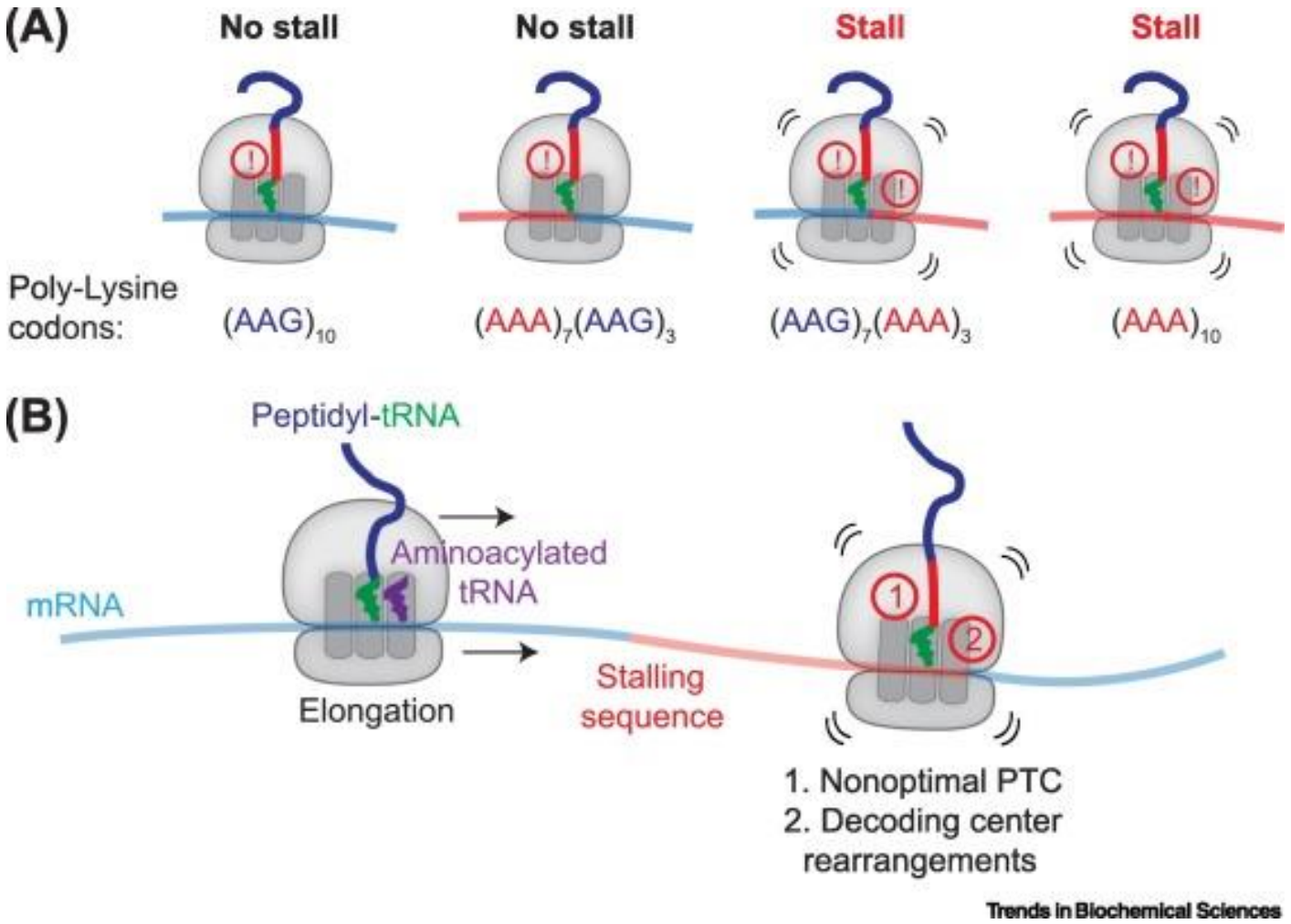
Ribosome stalling

- Ribosome pauses at a codon it cannot decode
- Causes ribotoxic stress
- Can activate downstream signaling pathways



Yip MCJ, Shao S, Trends in Biochemical Sciences, 2021

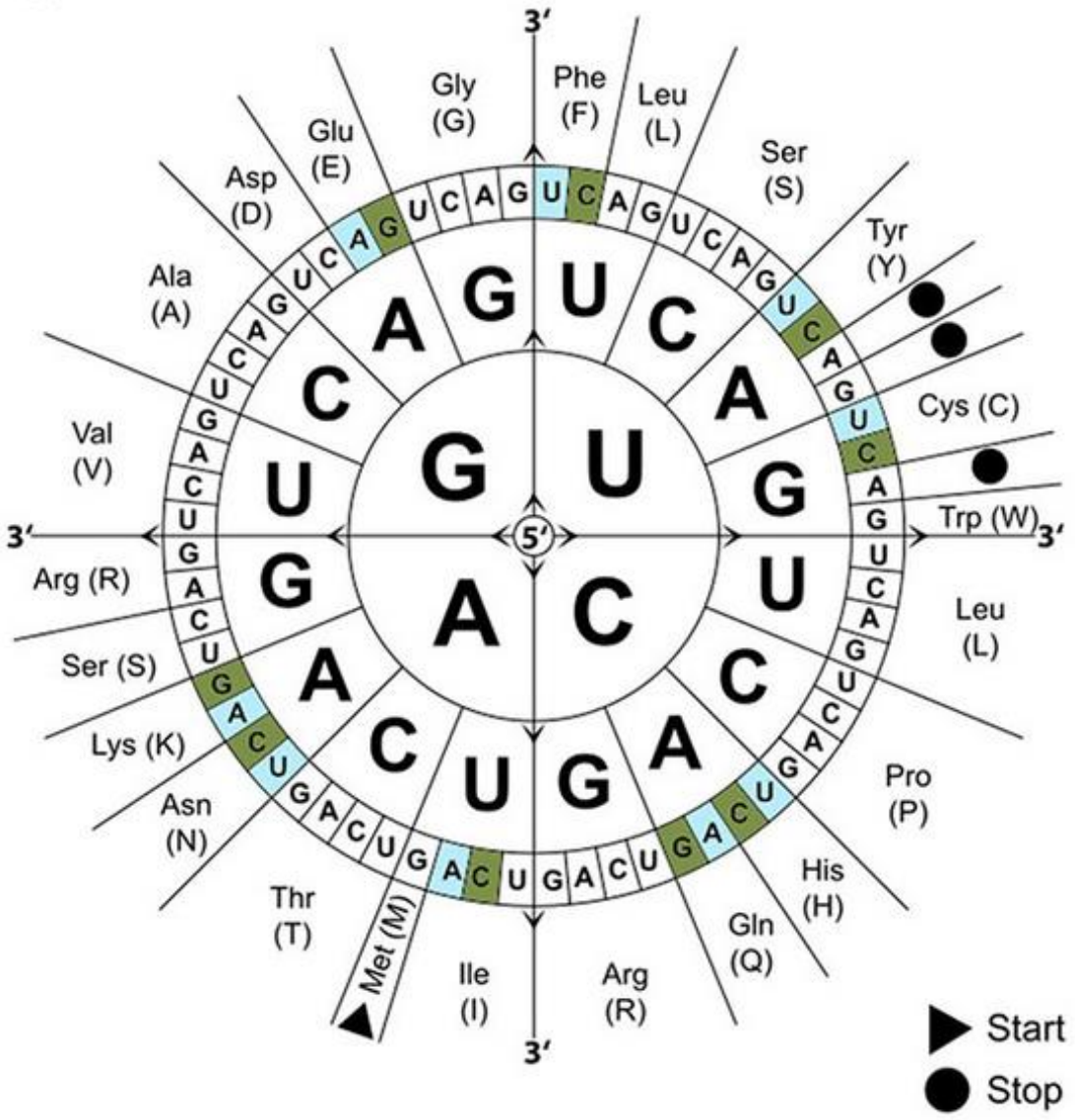
Ribosome stalling



Yip MCJ, Shao S, Trends in Biochemical Sciences, 2021

Codon usage

- Common codons = abundant tRNA
- Rare codons (e.g., UUA) = low tRNA
- Missing tRNA → ribosome stalls

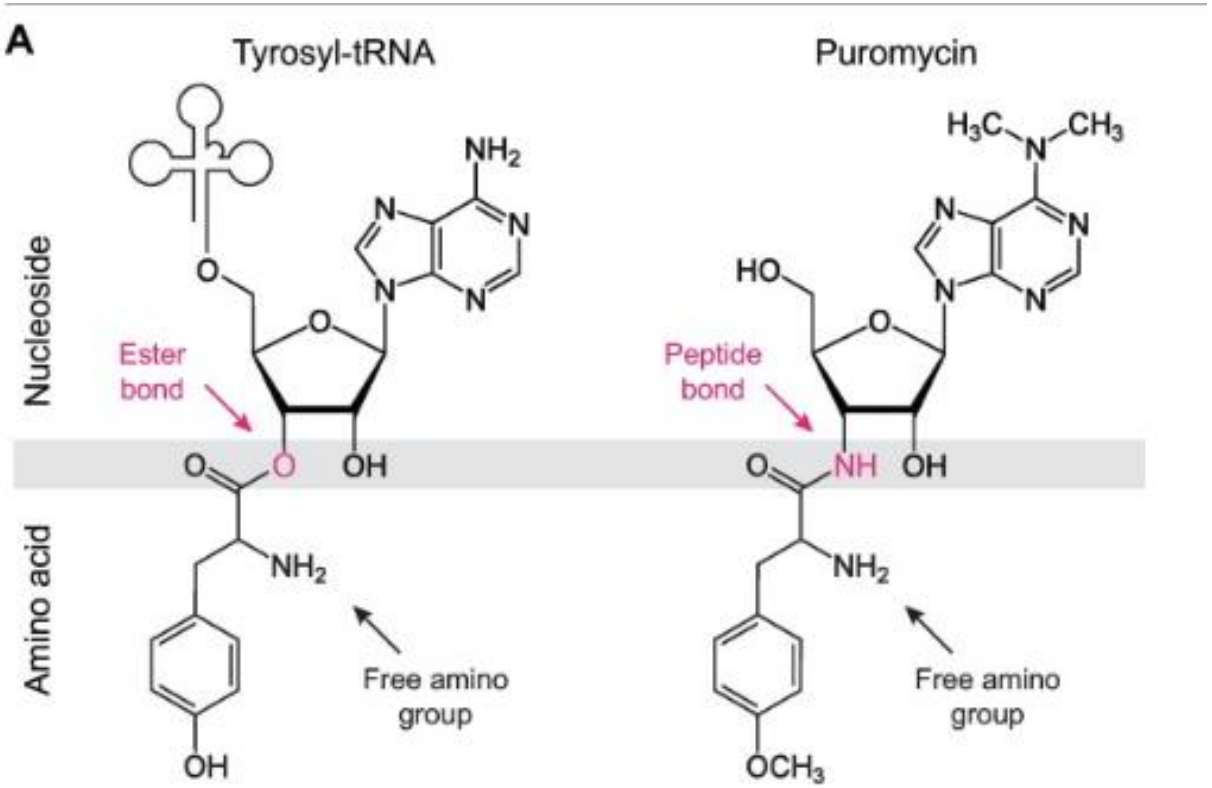


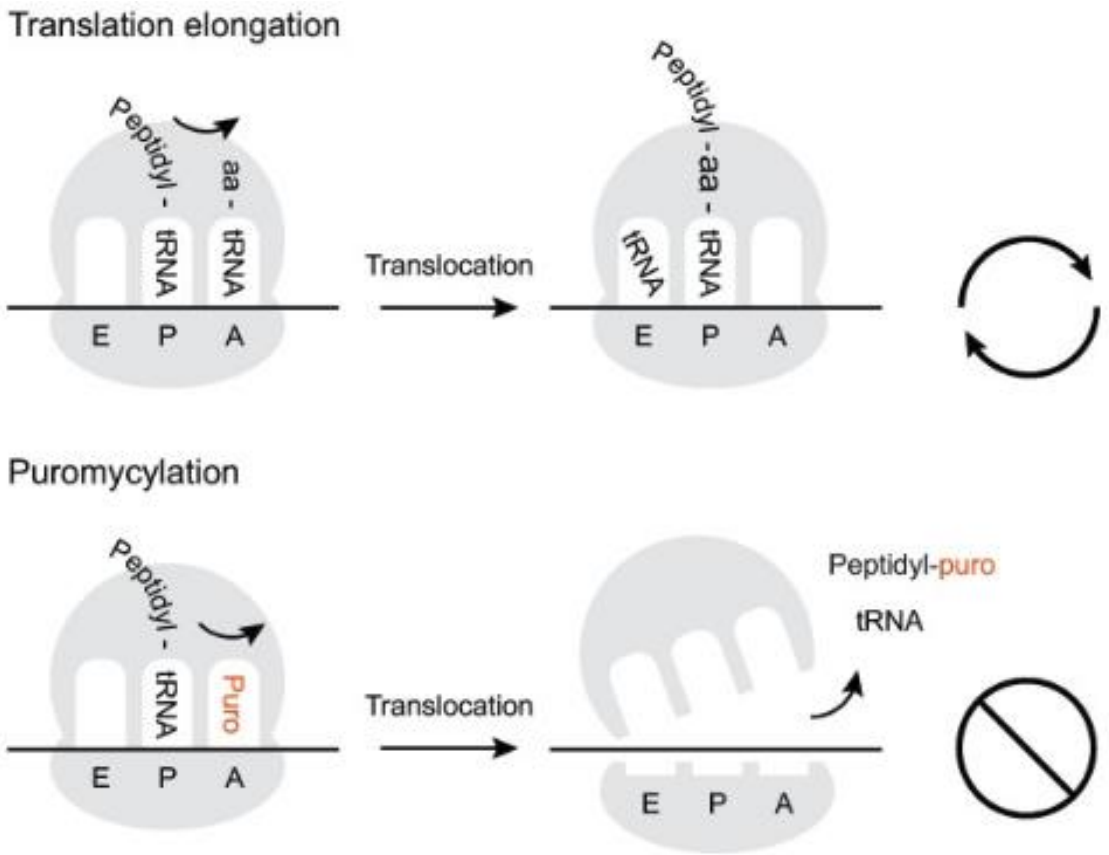
Methods

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



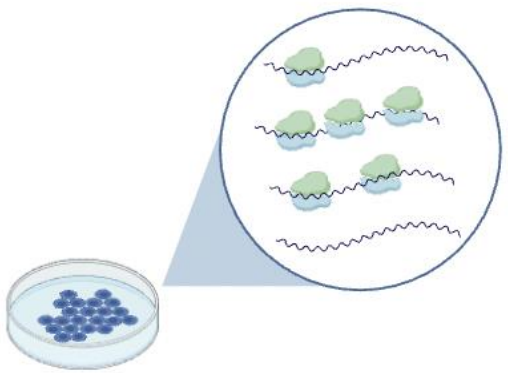


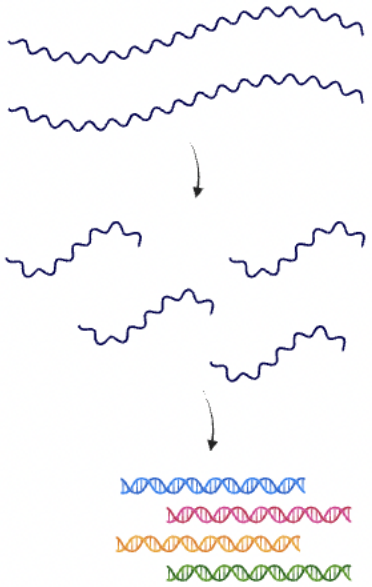
- Low puromycin = low translation

Aviner R, Computational and Structural Biotechnology Journal, 2020

Methods to measure translation efficiency

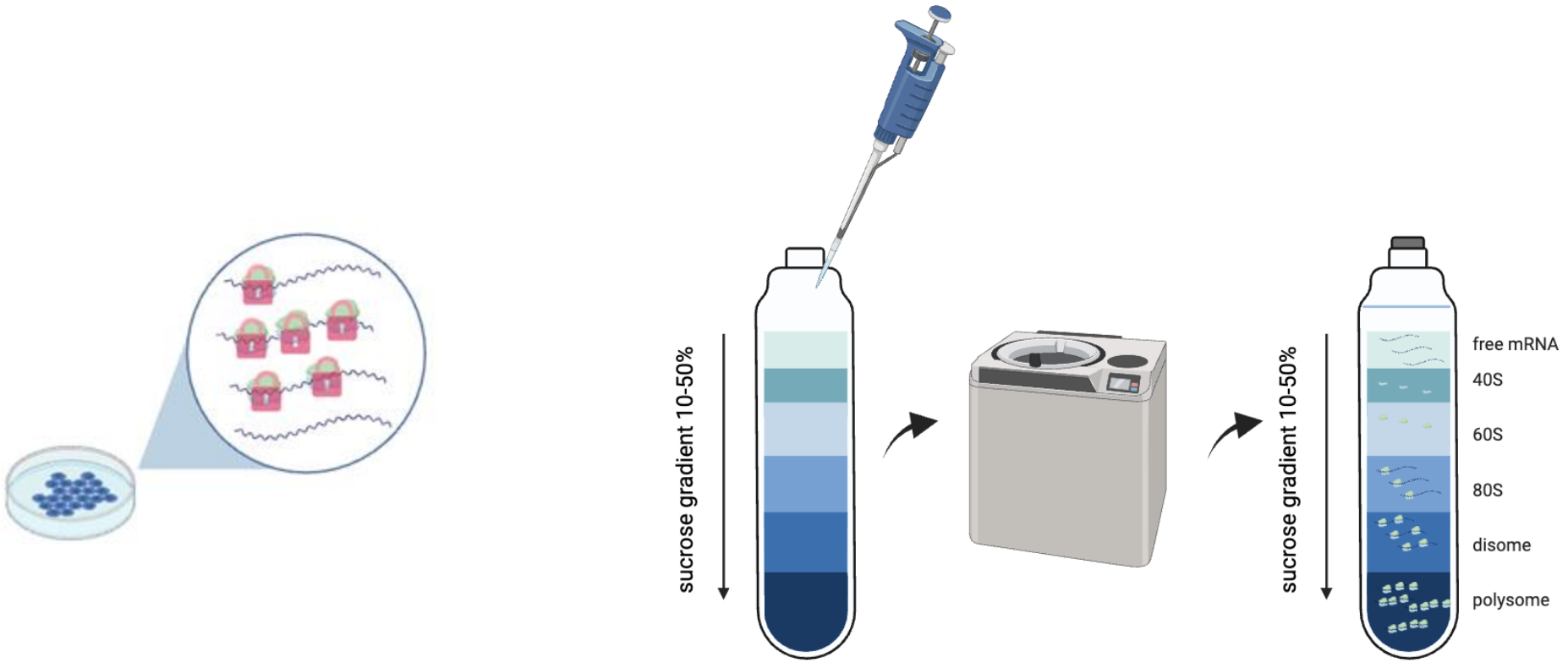
RNA-seq -> total RNA from the cells -> transcriptional changes





Methods to measure translation efficiency

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



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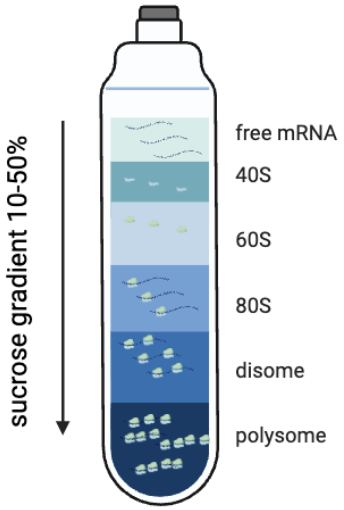
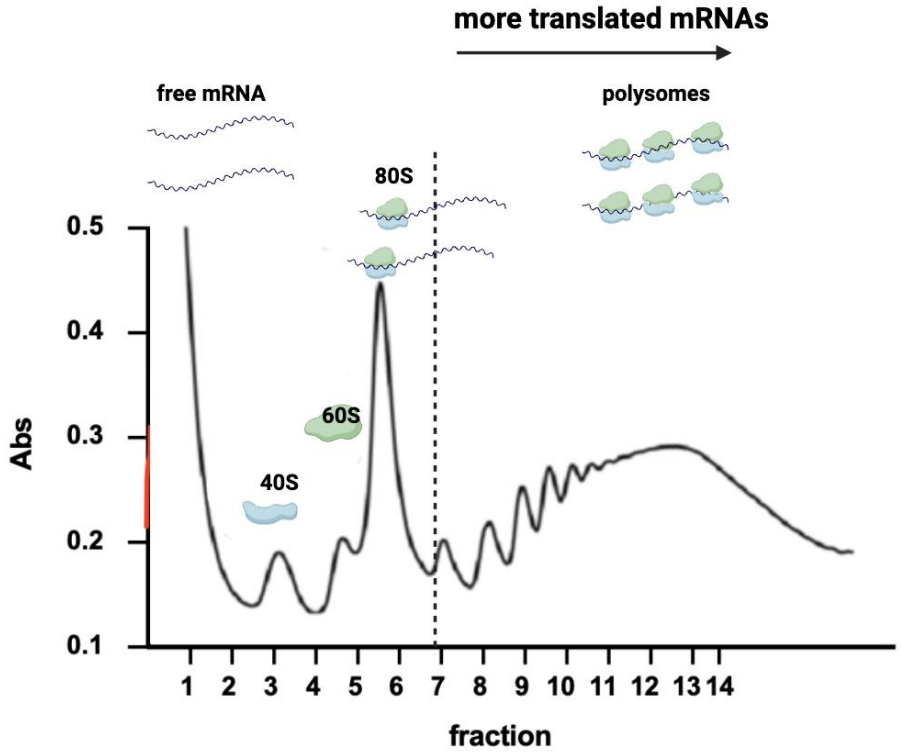
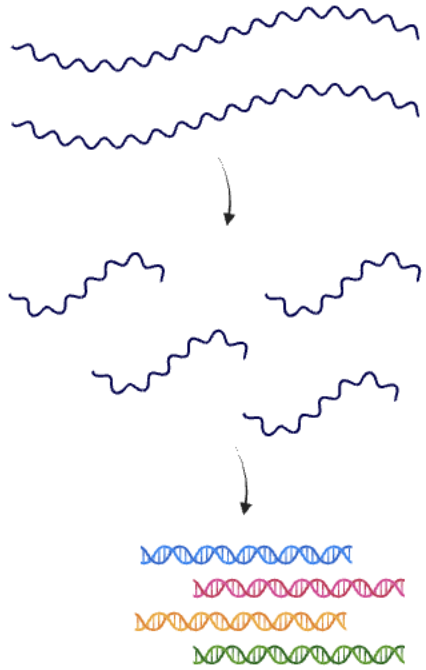
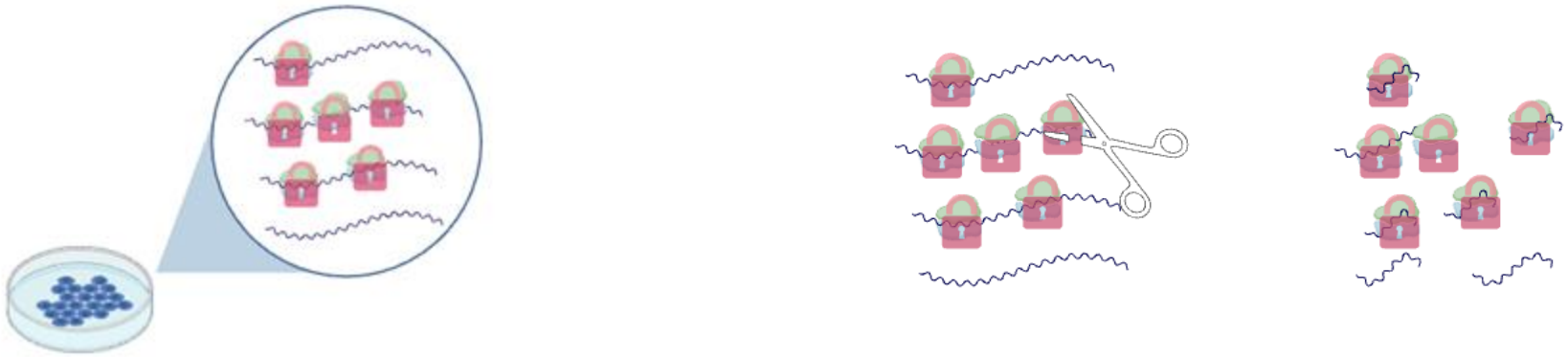






Methods to measure translation efficiency

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



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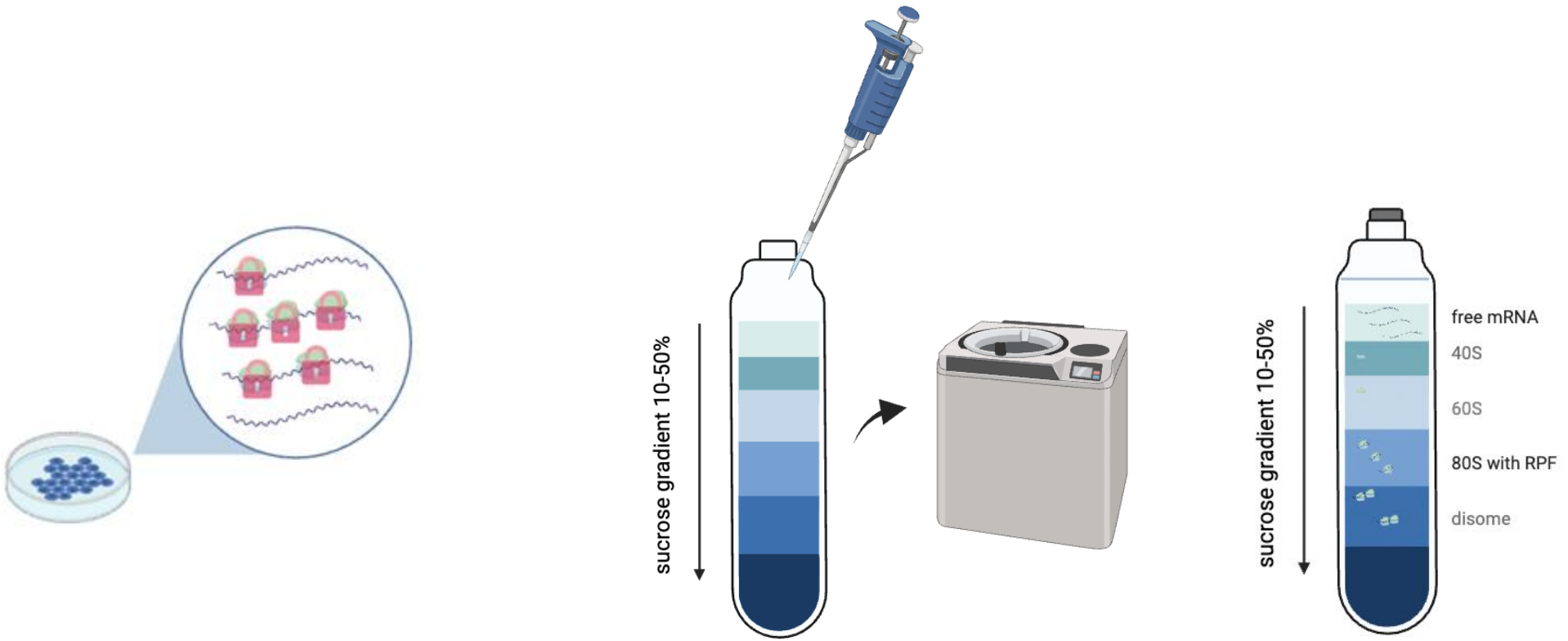

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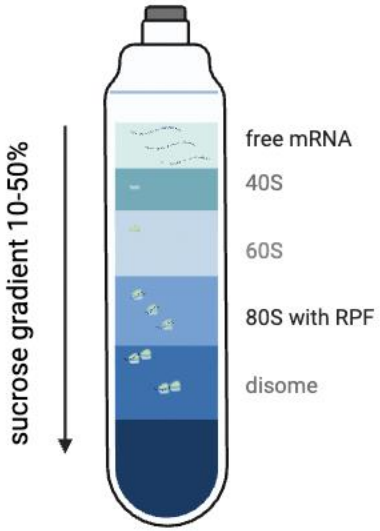
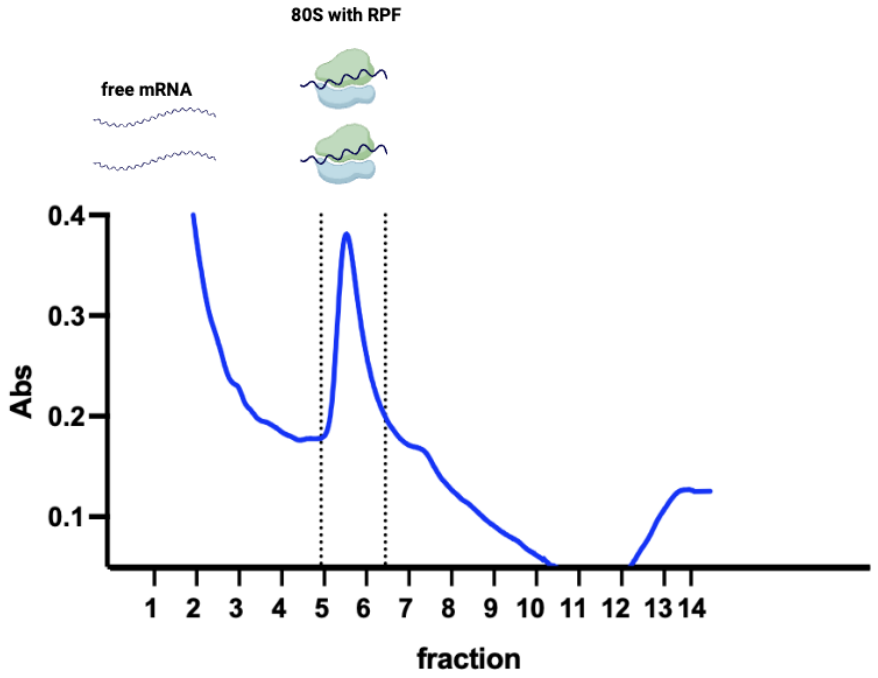
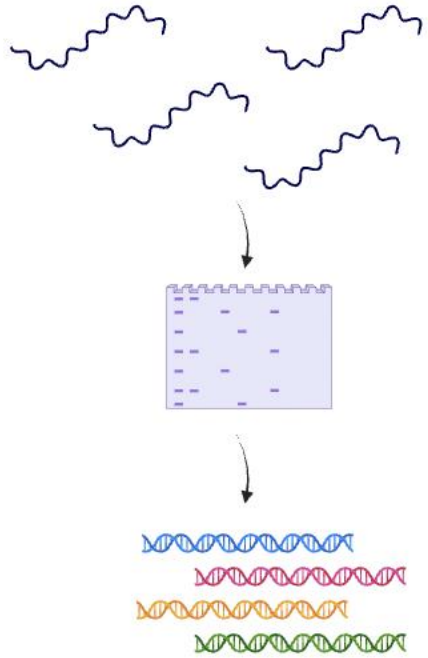






Combined with RNA-seq to adjust translational changes for transcriptional changes

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)

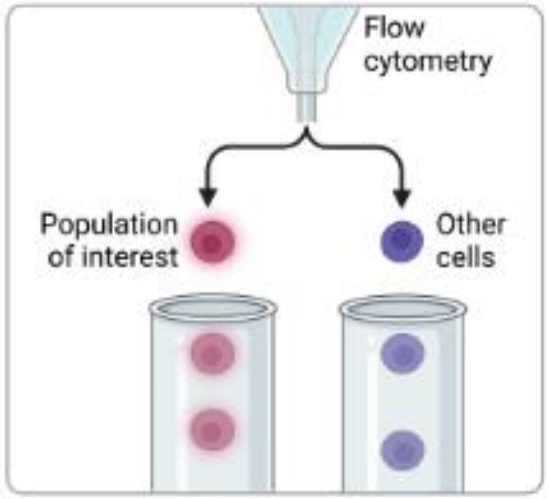
Cell sorter (BD FACSAria II)

Cell analyzer (BD FACSymphony)

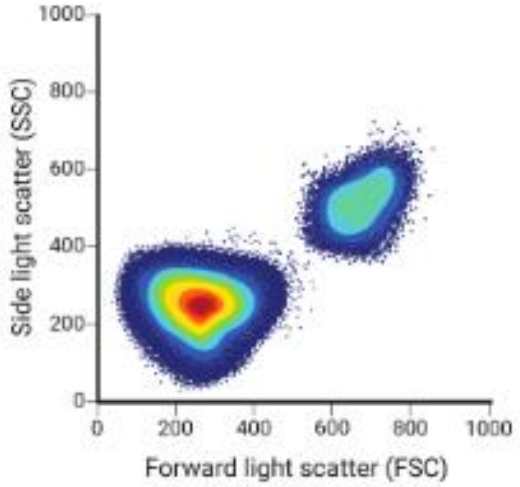
Flow cytometry (Intellicyt iQue Screener)

Test tube

Flow cytometry

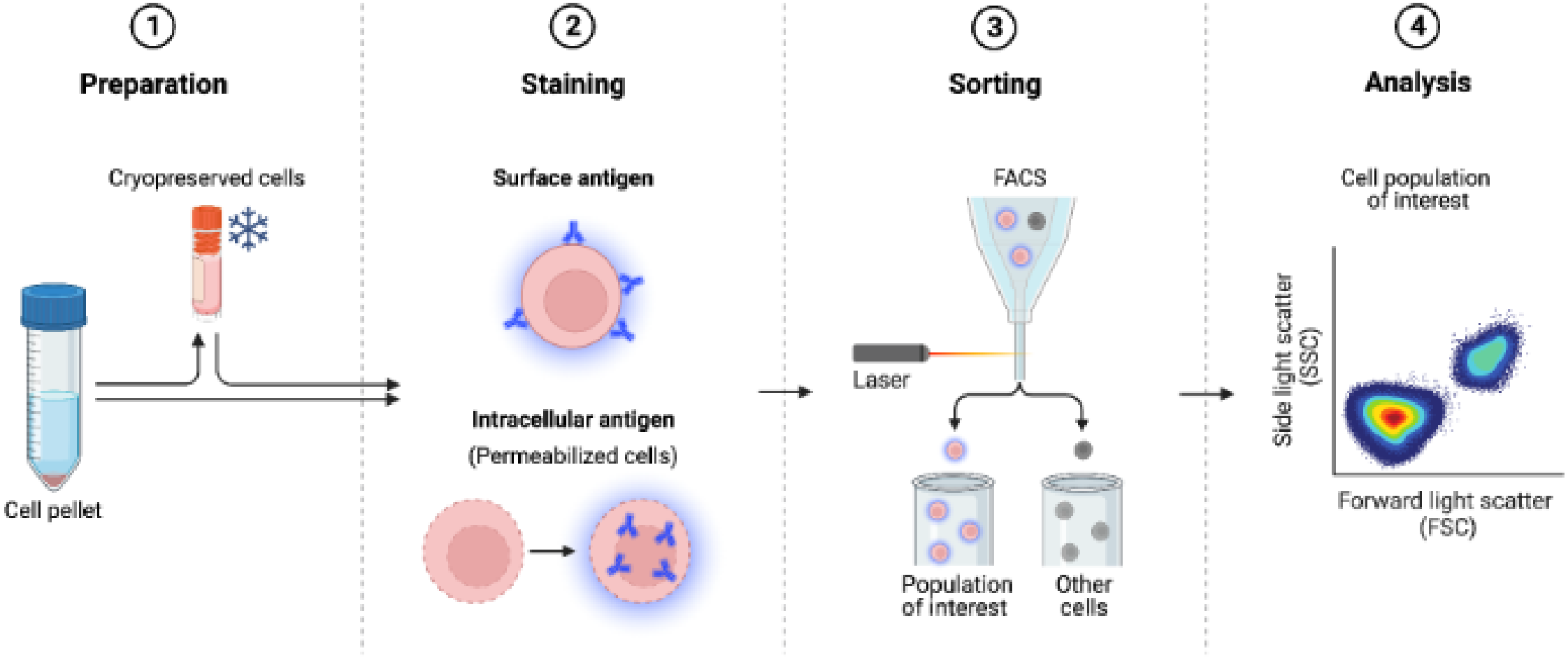


Cell sorting

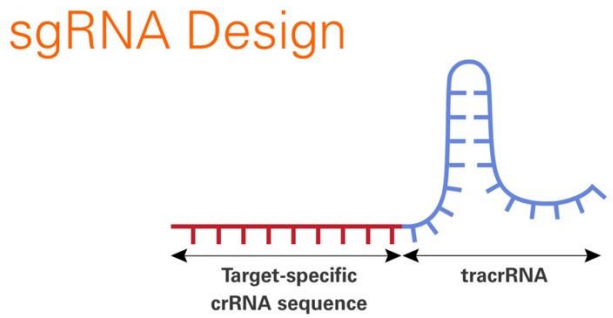


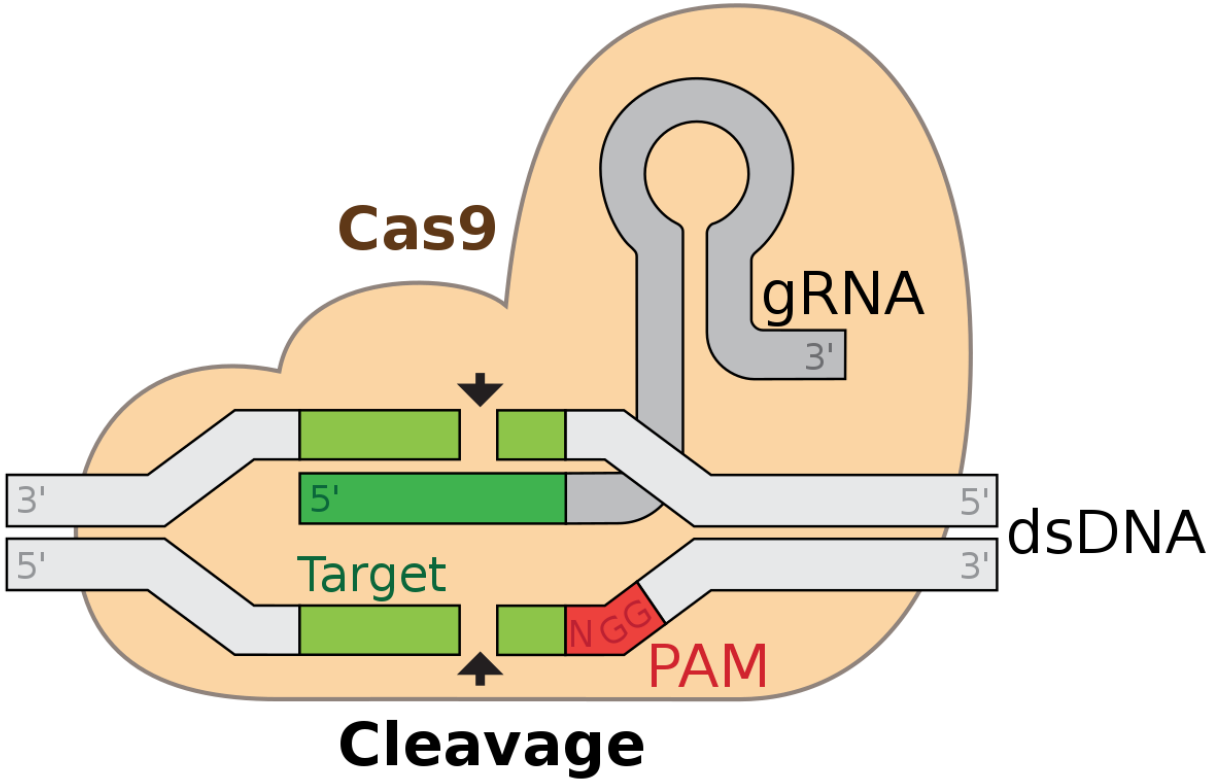
Flow cytometry graph

Flow cytometry



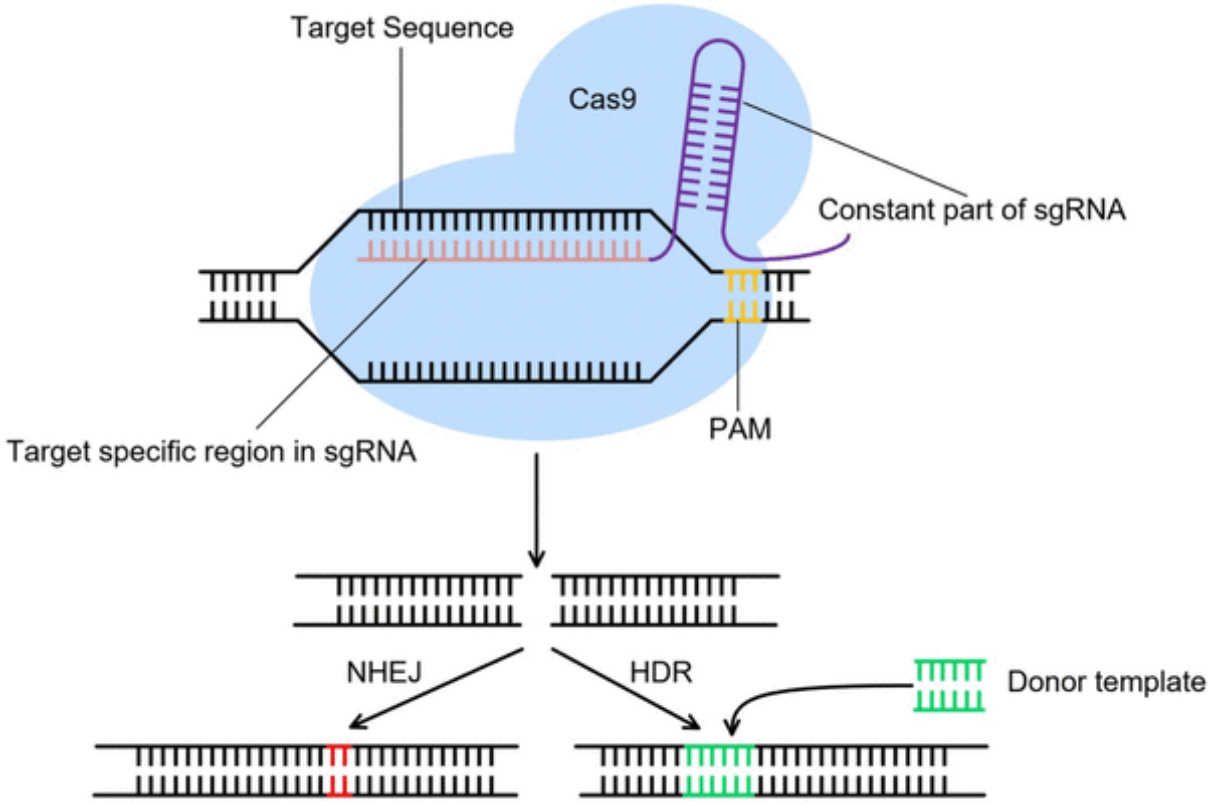
CRISPR





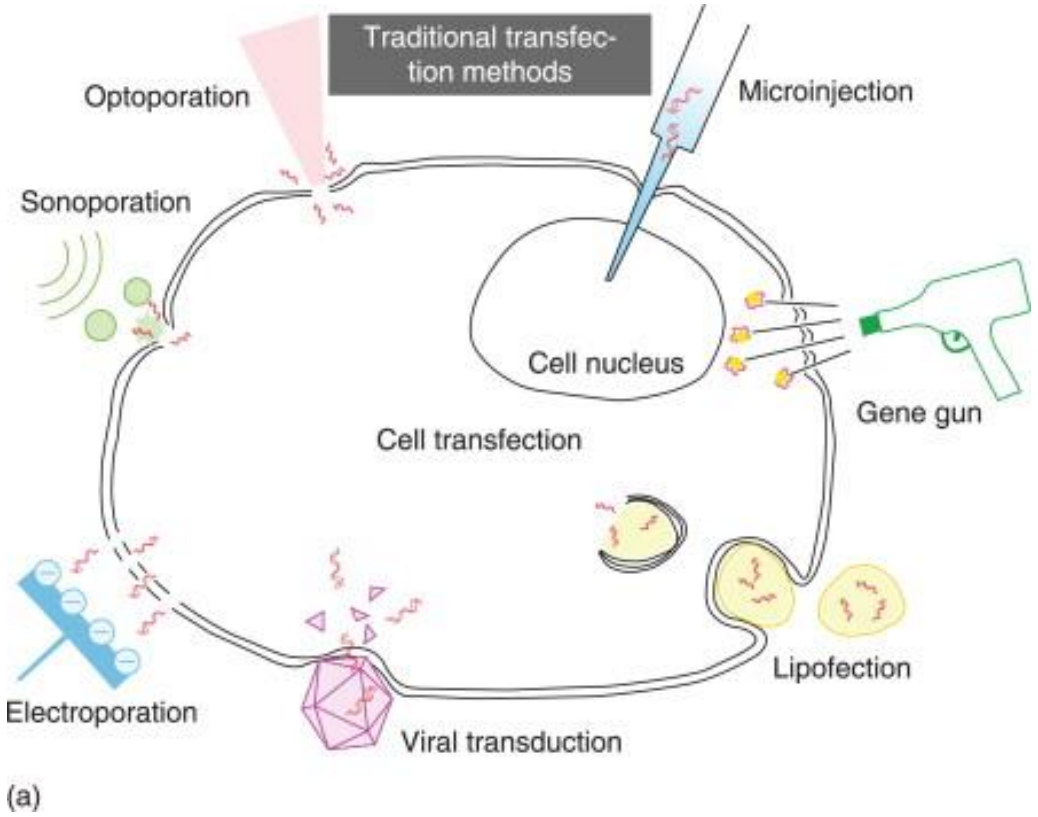
The protospacer adjacent motif (or PAM for short) is a short DNA sequence (usually 2-6 base pairs in length) that follows the DNA region targeted for cleavage by the CRISPR system, such as CRISPR-Cas9. The PAM is required for a Cas nuclease to cut and is generally found 3-4 nucleotides downstream from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

CRISPR



Cui Y, et al., Interdisciplinary Sciences: Computational Life Sciences, 2018

CRISPR



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