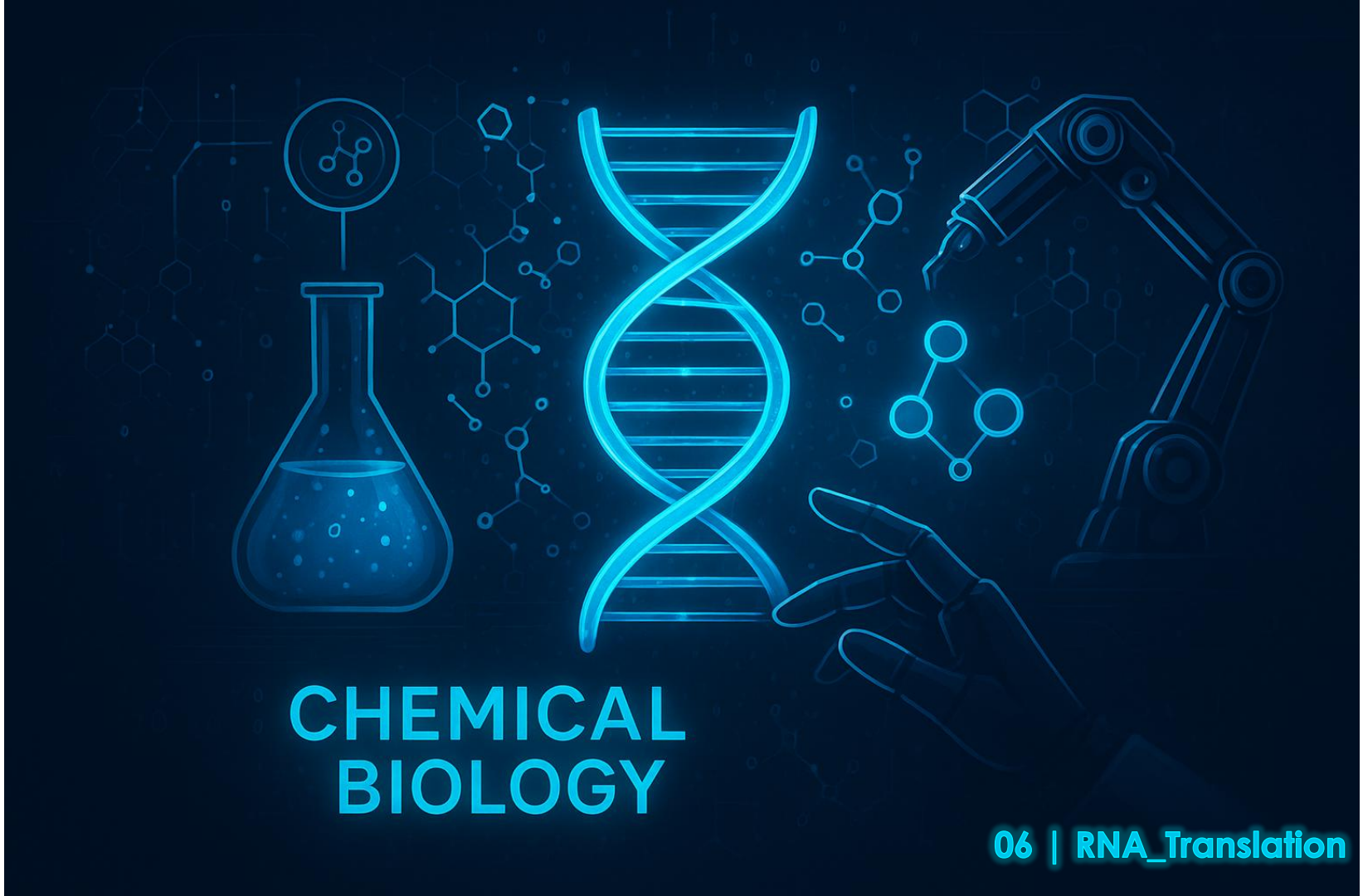

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General Course Information

- Moodle: https://go.epfl.ch/CH-313
  - Lecture slides (evening before the lecture)
  - Distributed presentation topics (assignments)
  - Forum (for questions and announcements)

- Examination (written, graded, detailed information will follow)
- Contact:
  - Moodle forum (for questions)
  - markus.jeschek@epfl.ch

- **“Concepts over details!”**
- **Interact! Ask! Discuss! Anytime!**

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Group Presentations

- Critical discussion of primary literature
- Illustrative examples for topics from the lecture

- Why?
  - Repetition of core concepts, techniques etc.
  - Presentation skills and critical discussion of research
  - Insight into current research topics

- How?
  - Two students per group
  - Assignments distributed one week before delivery of presentation (via Moodle)
  - **Send slides: markus.jeschek@epfl.ch (Mon evening before presentation)**
  - **15 min presentation (both group members should present!)** + Q&A

Tipps for Group Presentations

- Rough structure
  - Short intro on general topic
  - Main presentation according to assignment
  - Brief outlook incl. points of criticism/open questions/personal opinion as kick-starter for the discussion
- Everybody should participate in the discussion, incl. constructive(!) feedback on presentation style
- Questionnaires with different points, feedback by peers
- Typical assignment:
  - You will receive a certain topic including a related publication
  - Introduce the topic using the publication
  - present the motivation behind the research, methodology, key results (not every graph!)
  - Additional questions will be provided hinting towards central points
  - Be encouraged to look/present beyond the questions and the provided paper

Group Presentations – Schedule

#	Name1	Name2	Presentation on…	Assignment on…
1	Wininger Quentin	Jeremy	**Sep 23, 2025**	Sep 16, 2025
2	Ema	Ariane	**Sep 30, 2025**	Sep 23, 2025
3	Benjamin	Matthieu	**Oct 7, 2025**	Sep 30, 2025
4	Ivana	Ipek	**Oct 14, 2025**	Oct 7, 2025
5	Mridhula	Elodie	**Oct 28, 2025**	Oct 21, 2025
6	Abigail	Robin	**Nov 4, 2025**	Oct 28, 2025
7	Eva	Florian	**Nov 11, 2025**	Nov 4, 2025
8	Bastien	**Axel**	**Nov 18, 2025**	Nov 11, 2025
9	Melodie	Siolène	**Nov 25, 2025**	Nov 18, 2025
10	Nicole	Maria	**Dec 2, 2025**	Nov 25, 2025

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Course Topics – Overview

- Week 1 | Introduction + DNA
- Week 2 | DNA
- Week 3 | DNA
- Week 4 | DNA
- Week 5 | DNA/RNA
- **Week 6 | RNA/Translation**
- Week 7 | Enzymes
- Week 8 | Enzymes
- Week 9 | Metabolism
- Week 10 | Metabolism
- Week 11 | Engineering
- Week 12 | Engineering
- Week 13 | Engineering
- Week 14 | LSAM Intro + Exam Preparation

[tentative schedule]

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Questions?

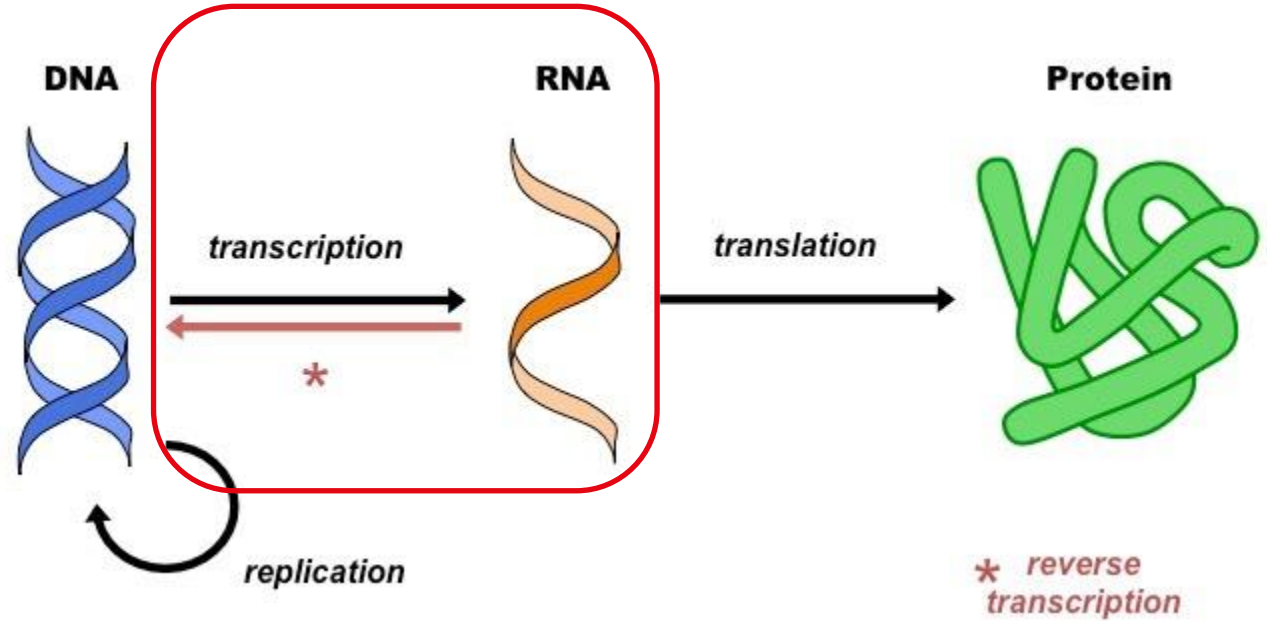
Indicative Feedback Results

technical problems → next time!

RNA

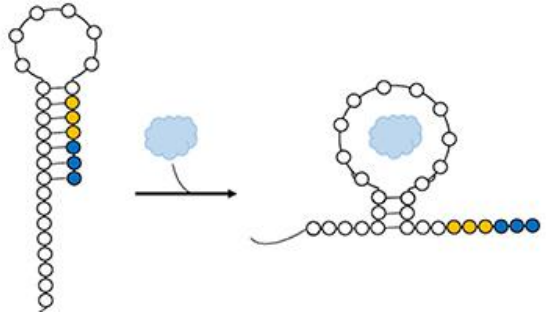
The Central Dogma

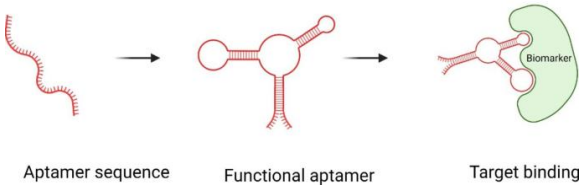
- …of Molecular Biology

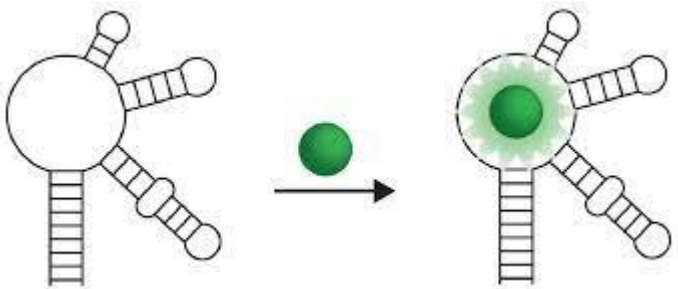


RNA Aptamers

- Short, single stranded RNA molecule
- Defined 3D structure
- Specific binding of molecules with high affinity
- *In vitro* selection for improved binders: SELEX method
- Can be used as versatile sensors/reporters
  - Spinach aptamer
  - catalytic aptamers
  - Quenching-/FRET-based beacons
  - riboswitches







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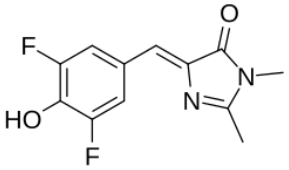
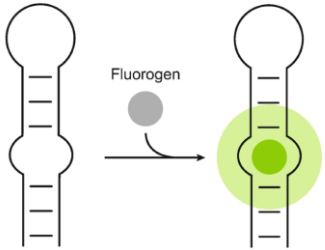
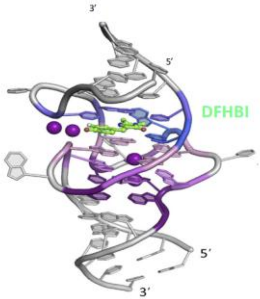
RNA Aptamers (examples)

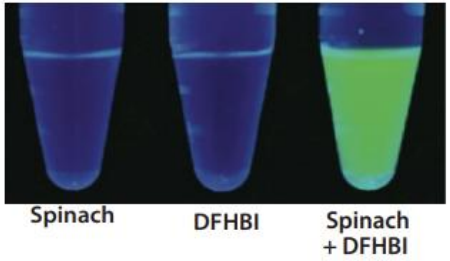


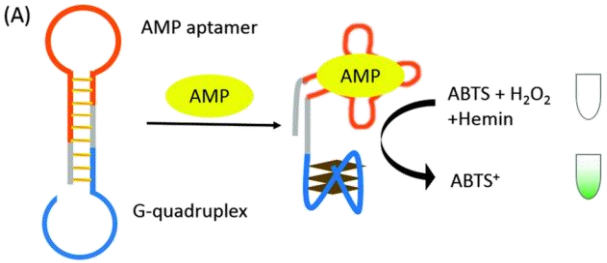
DFHBI
(3,5-Difluoro-4-hydroxy-benzylidene imidazolinone)

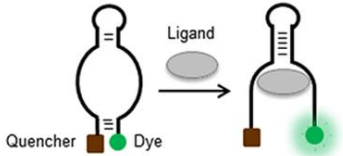


“spinach aptamer”

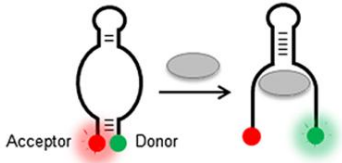








Quenching-based beacon



FRET-based beacon

Q: What could such RNA aptamers be used for?

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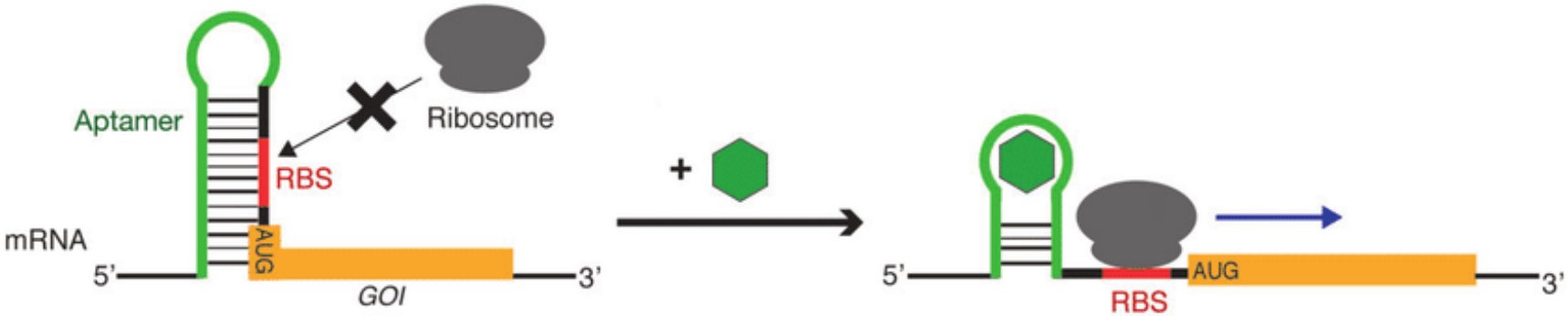
Riboswitches

- part of mRNA
- contain an aptamer
- “expression platform”: interaction with gene expression machinery
- Can act as ON-/OFF-switches via different mechanisms

(e.g. translation, transcription, mRNA degradation etc.)



Q: What could riboswitches be used for in nature and biotechnologically?
What are pros and cons compared to the aptamer examples before (e.g. Spinach)?

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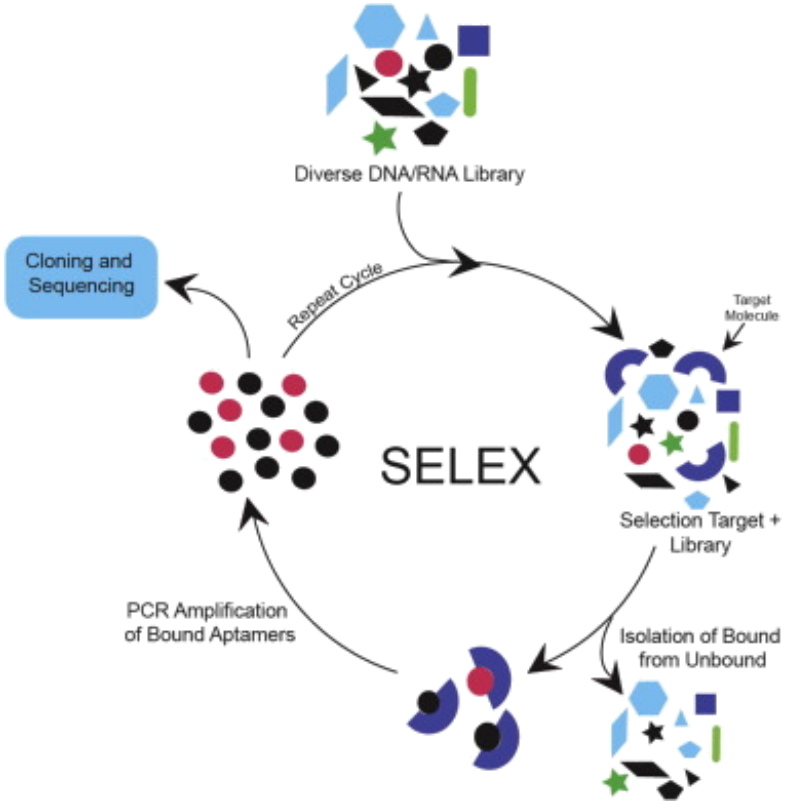
SELEX

- Systematic Evolution of Ligands by EXponential enrichment
- Process of *in vitro* selection for RNA (or DNA aptamers)
- Binders are enriched from large pools of random sequences
- Iterative enrichment, increasing stringency
- Routinely leads to binders with

(sub-)nanomolar affinity



Q: How is binding affinity commonly calculated?

Protein

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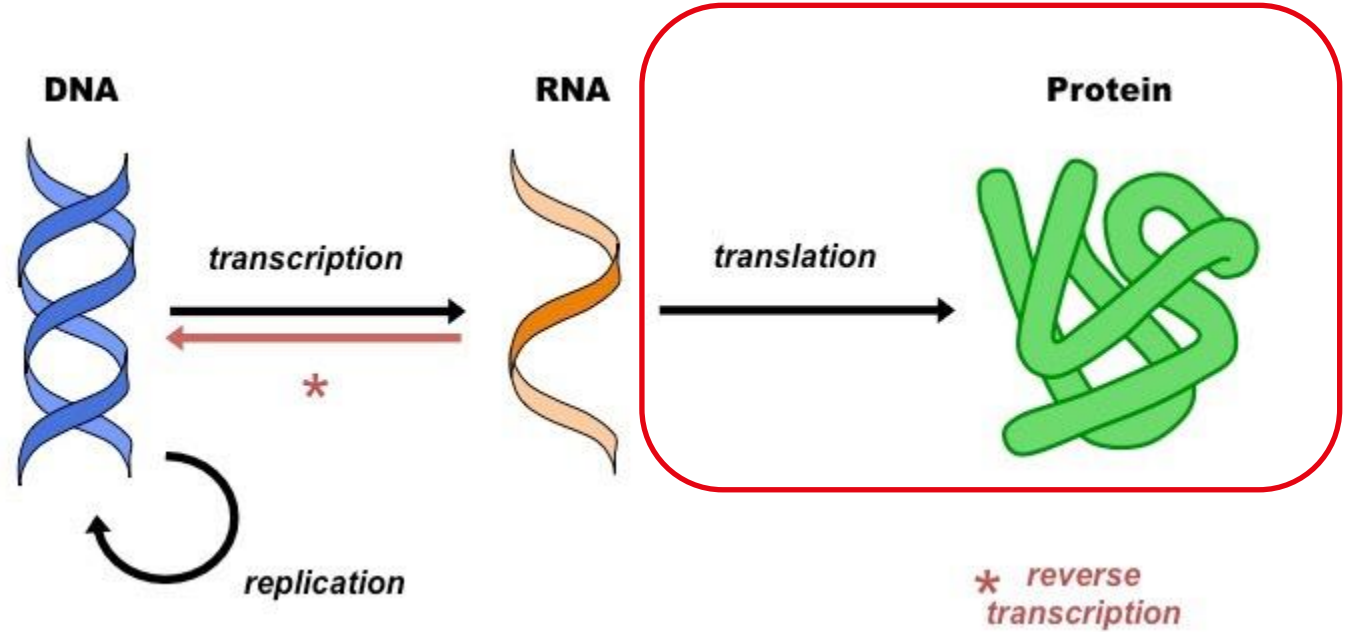
The Central Dogma

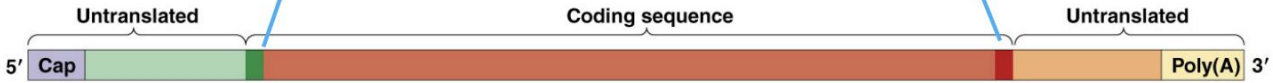
- …of Molecular Biology

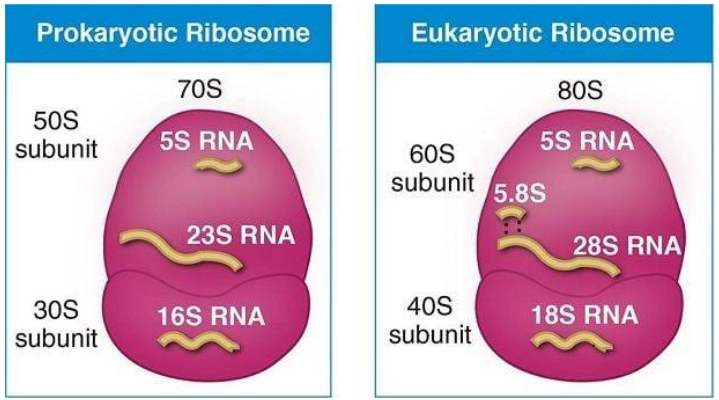


Messenger RNA (mRNA) and Ribosomes

Bacterial mRNA

Eukaryotic mRNA





Q: Name and describe key differences between pro- and eukaryotic mRNAs!

What does the “S” in 70S ribosome stand for?

RECAP

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The Genetic Code and Amino Acids

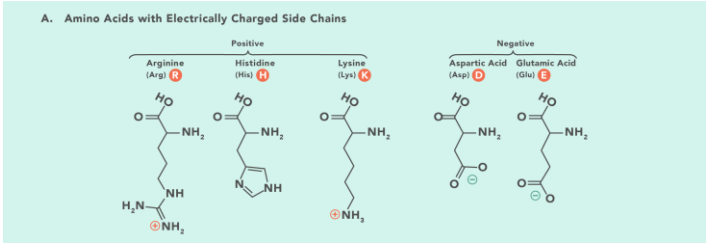
- Universal amongst all forms of life
  - 64 codons
  - 20 amino acids
  - 3x Stop signals
  - Start signals:
    - Bacteria: AUG (also GUG, UUG)

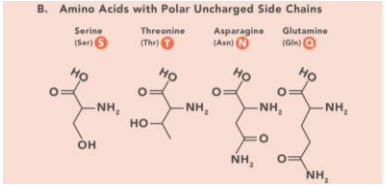
    → N-formylmethionine (fMet)

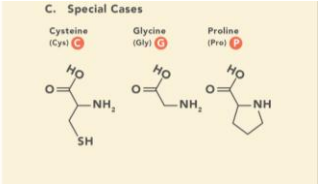
    - Eukaryotes: AUG

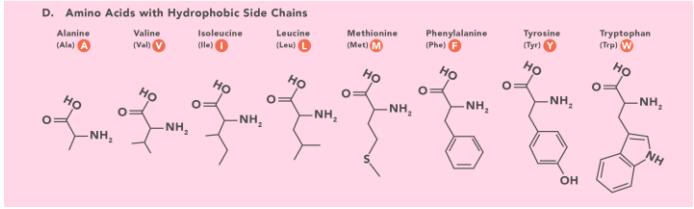
    → Methionine (Met)

First letter	Second letter: U	Second letter: C	Second letter: A	Second letter: G	Third letter
U	UUU Phe UUC Phe UUA Leu UUG Leu	UCU Ser UCC Ser UCA Ser UCG Ser	UAU Tyr UAC Tyr **UAA Stop** **UAG Stop**	UGU Cys UGC Cys **UGA Stop** UGG Trp	U C A G
C	CUU Leu CUC Leu CUA Leu CUG Leu	CCU Pro CCC Pro CCA Pro CCG Pro	CAU His CAC His CAA Gln CAG Gln	CGU Arg CGC Arg CGA Arg CGG Arg	U C A G
A	AUU Ile AUC Ile AUA Ile **AUG Met**	ACU Thr ACC Thr ACA Thr ACG Thr	AAU Asn AAC Asn AAA Lys AAG Lys	AGU Ser AGC Ser AGA Arg AGG Arg	U C A G
G	GUU Val GUC Val GUA Val GUG Val	GCU Ala GCC Ala GCA Ala GCG Ala	GAU Asp GAC Asp GAA Glu GAG Glu	GGU Gly GGC Gly GGA Gly GGG Gly	U C A G









Q: Where is the genetic code encoded?

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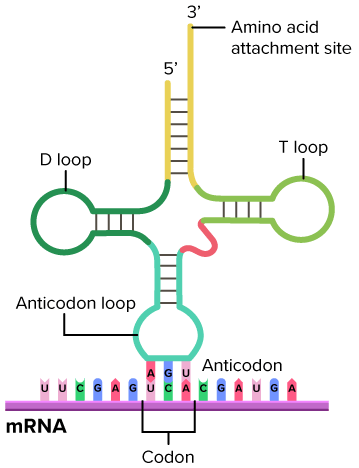
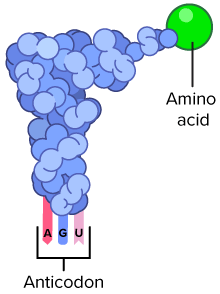
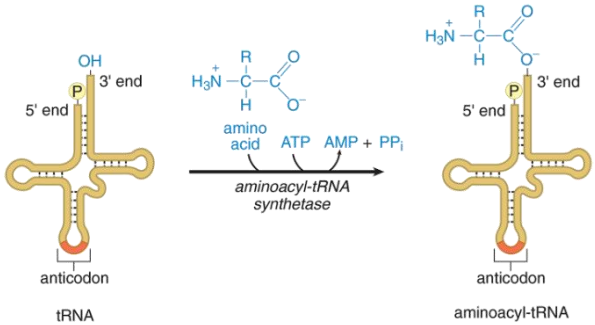
Transfer RNA (tRNA) and tRNA Synthetases

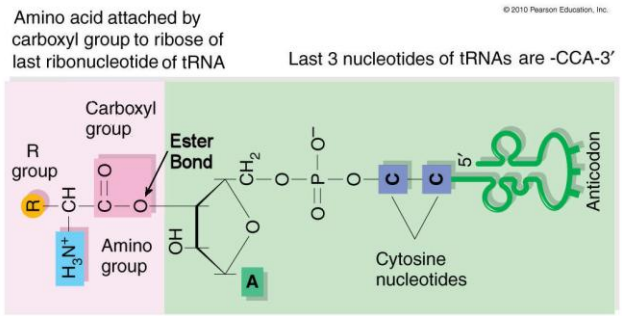
- tRNA
  - approx. 60-100 nt long
  - “adapters” between mRNA and polypeptide



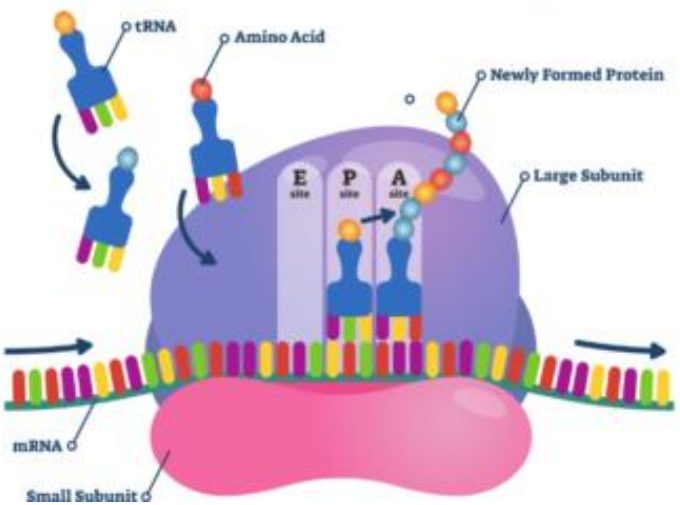


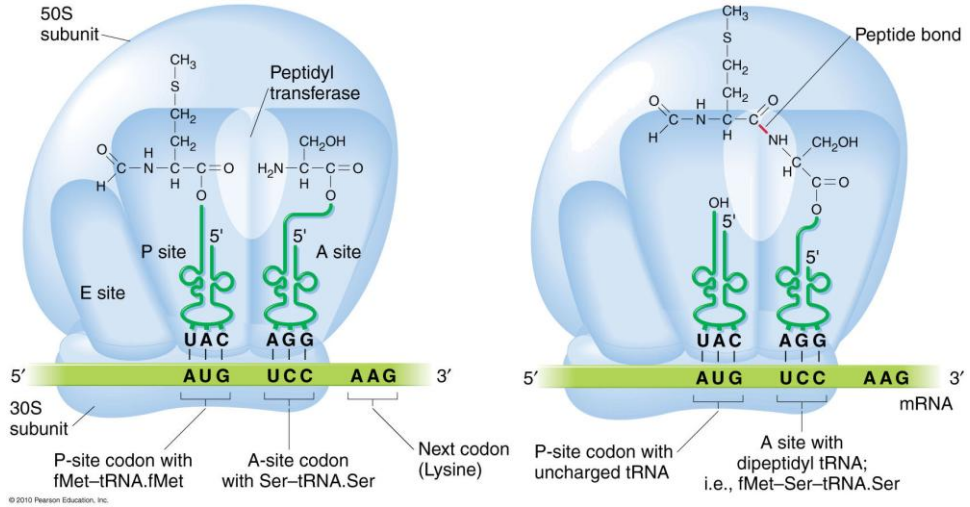
- Amino acyl tRNA synthetases (aaRBS)
  - enzymes “loading” tRNAs with correct amino acids
  - on acceptor arm of tRNA: […]-**CCA**-3’OH





Translation





- Ribosomal translation
  - approx. 10 aa/s
  - N → C direction
  - transamination (ester-amide exchange)
  - error rate approx. 10^{-4} - 10^{-3}
  - prokaryotes: in parallel with transcription
  - eukaryotes: spatial/temporal separation from transcription

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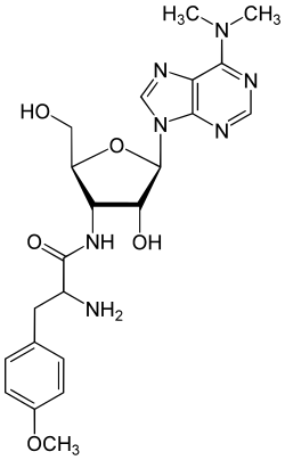
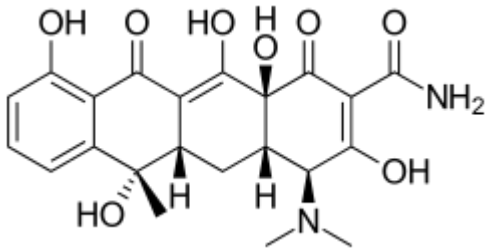
Blocking Translation – Examples

- Puromycin
  - amino nucleoside from bacteria (*Streptomyces*)
  - mimics 3'-end of loaded Tyr-tRNA
  - puromycylated chain → premature termination of translation
  - antibiotic
  - used in mRNA display



- Tetracycline
  - blocks entry of charged tRNAs to A-site
  - several species of *Streptomyces*
  - broad-spectrum antibiotic
  - specific to 30S ribosome



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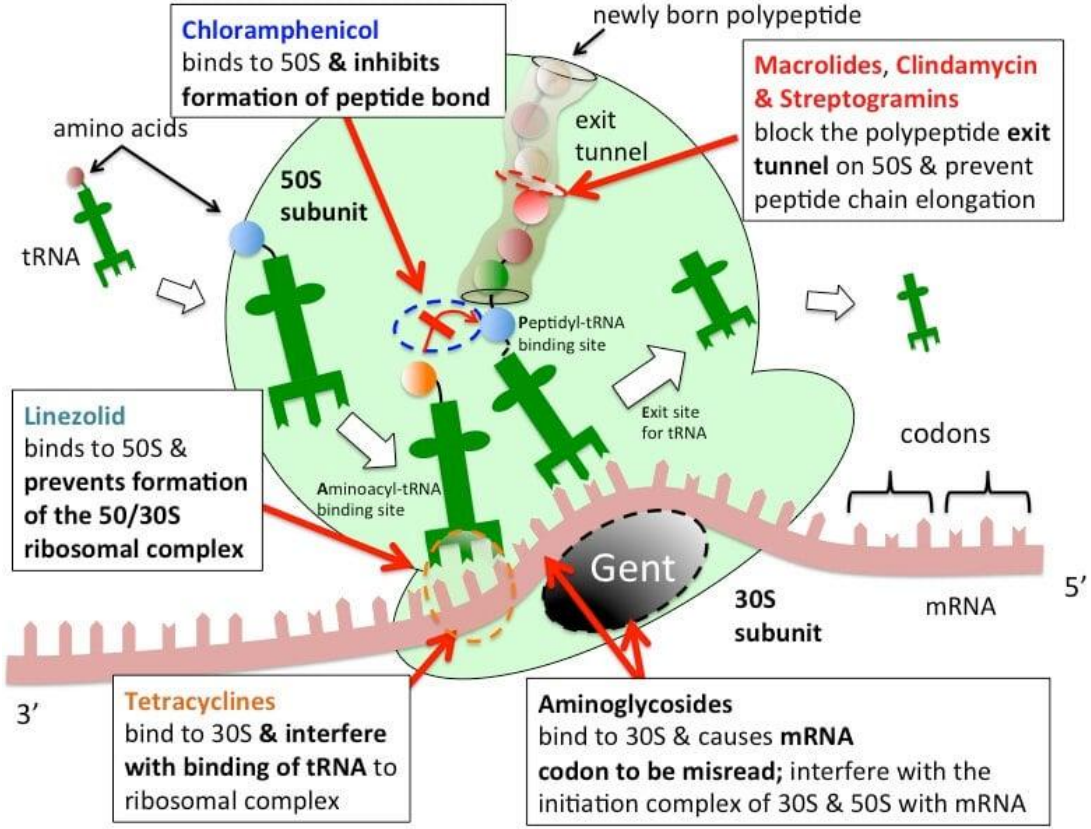
Blocking Translation – Overview



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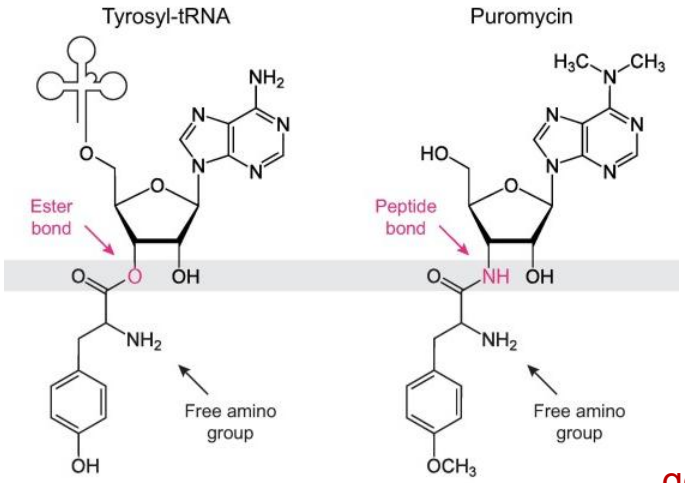
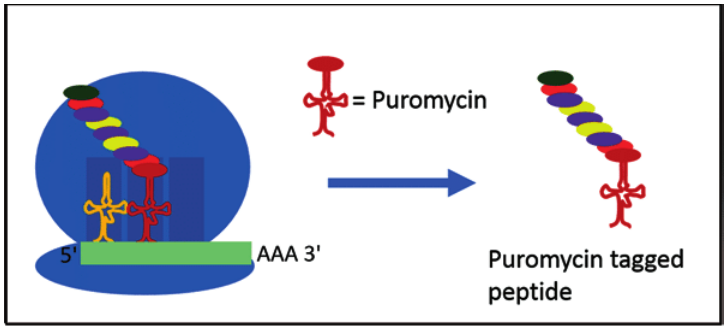
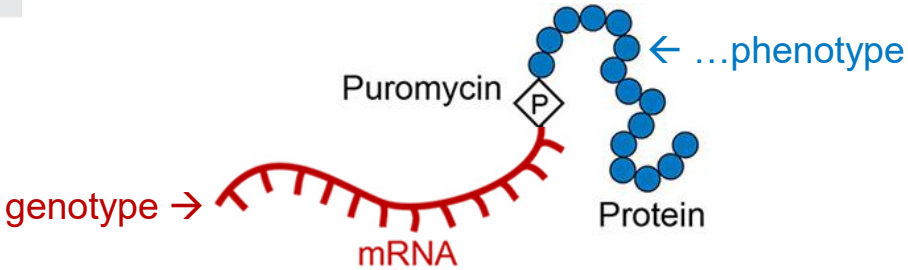
mRNA Display

- Technique for *in vitro* evolution of protein binders (up to 10^{13} variants)
- Important prerequisite: **genotype-phenotype linkage** (see “Engineering” lectures)
- Relies on puromycin







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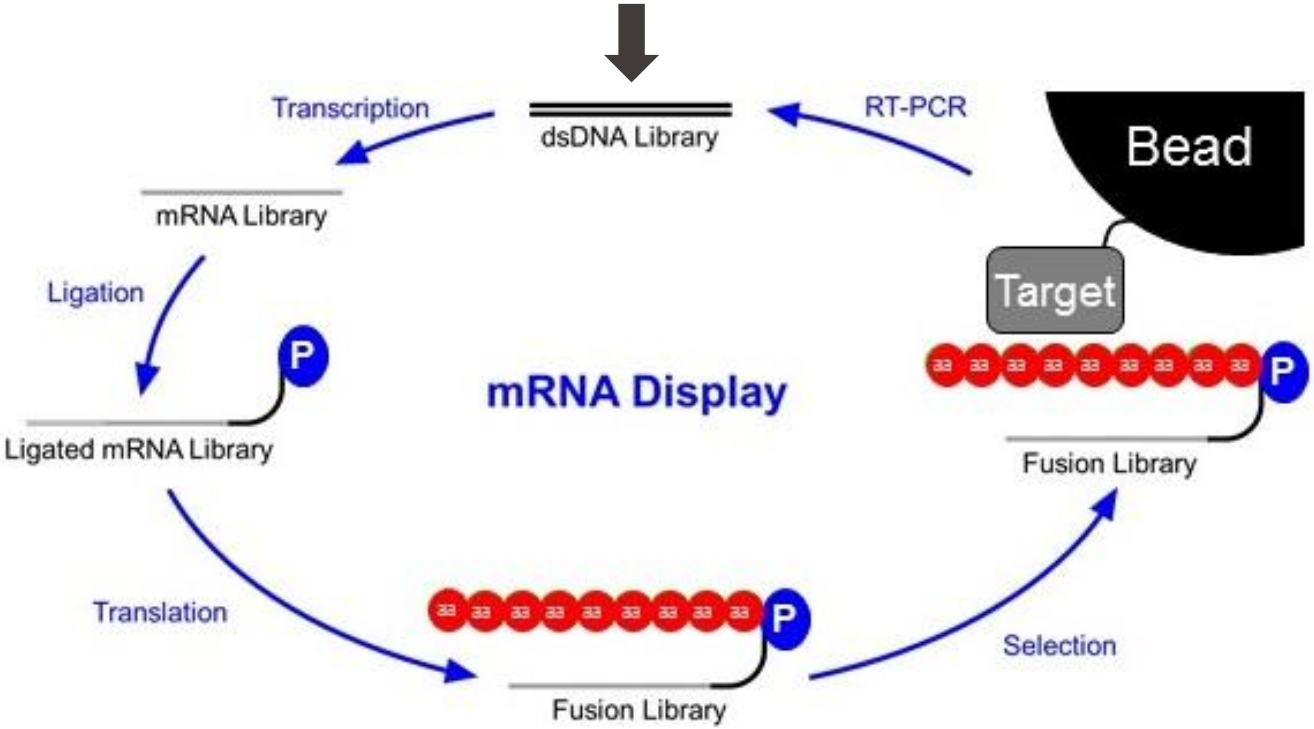
mRNA Display – Procedure

e.g. oligo synthesis,
mutagenic PCR etc.



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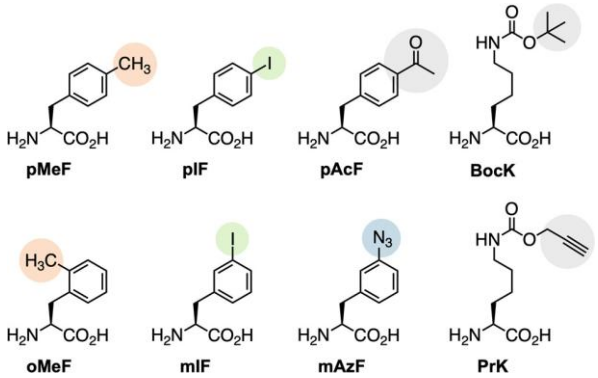
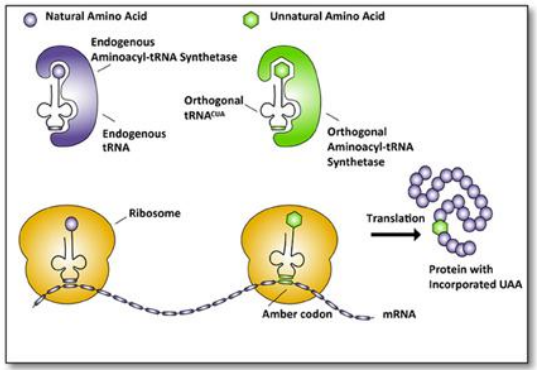
Unnatural Amino Acid Incorporation

- Incorporation of unnatural or “non-canonical” amino acids (ncAAs) into proteins

→ goal: new functionalities

- High-jacking of translation machinery
- So-called **“amber suppression” approach** (exploiting the “amber” stop codon)





In the standard genetic code, there are three different stop codons:

- In RNA:
  - UAG ("amber")
  - UAA ("ochre")
  - UGA ("opal"
- In DNA:
  - TAG ("amber")
  - TAA ("ochre")
  - TGA ("opal" or "umber")

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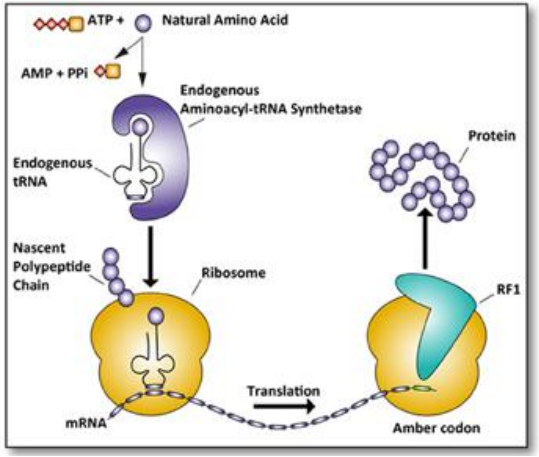
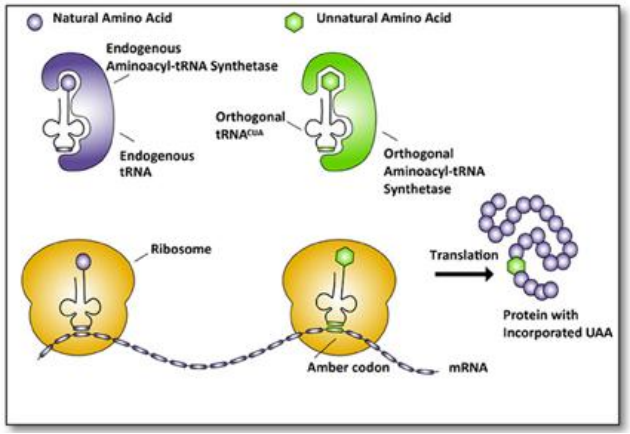
Amber Suppression – Principle

- Natural translation termination: a stop codon is decoded by a release factor (RF) aborting translation
- Amber suppression:
  - (1) a tRNA decoding the amber stop codon (UAG) is loaded with ncAA by engineered tRNA synthetase
  - (2) the ncAA is incorporated upon occurrence of UAG in the mRNA via the ribosome (instead of stopping)
- "orthogonal pair"
  - tRNA + tRNA synthetase
  - Requirements:
    - loading of tRNA with ncAA
    - no loading with other amino acids!





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Engineering Orthogonal Pairs

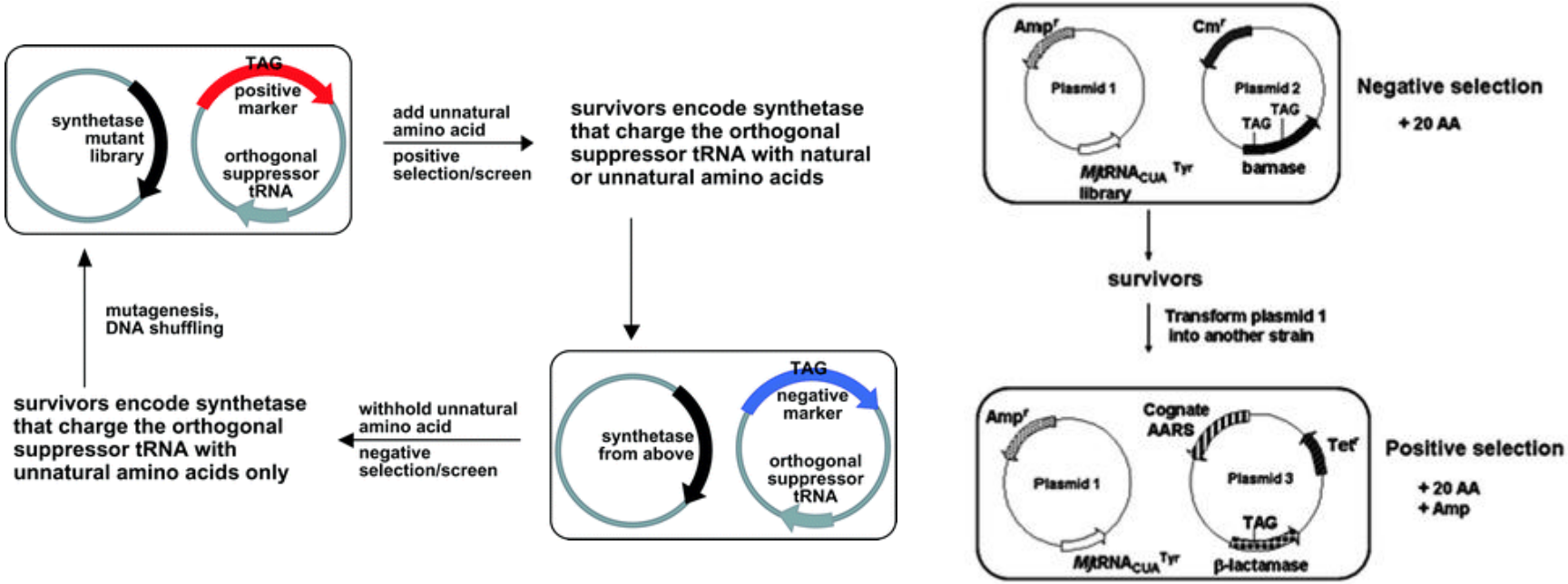
- Through iterative rounds of positive and negative selection
  - Positive selection:

  “A selectable marker gene with a TAG codon is used. Only tRNA synthetase variants that successfully incorporate an amino acid at the stop codon produce the full gene product of the marker (e.g. antibiotic resistance). Upon exposure to the ncAA and the selection pressure (e.g. antibiotic), only successful variants survive.”

  - Negative selection:

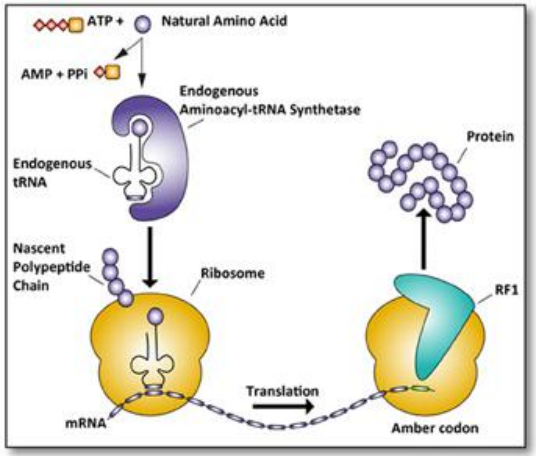
  “Surviving tRNA synthetases from the positive selection are combined with another selectable marker gene with a TAG codon. In this case, the marker (e.g. toxic gene) kills the cell if incorporation at the stop codon occurs. Here the ncAA is not added(!), leading to the depletion of unspecific synthetases”.

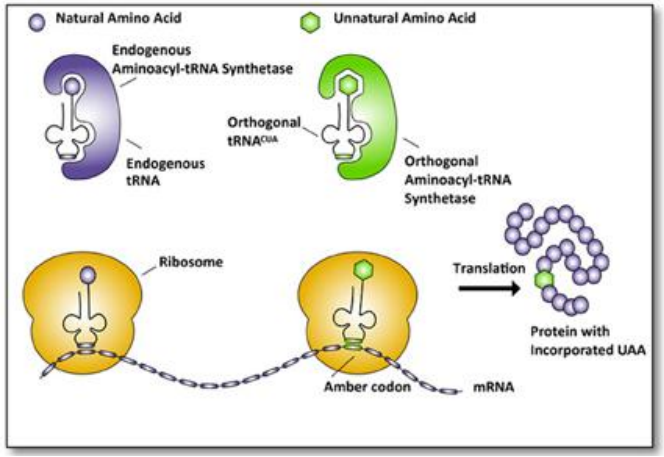
Engineering Orthogonal Pairs

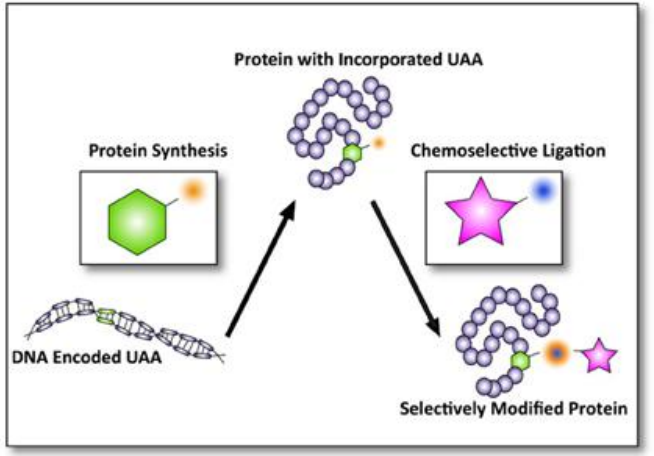


Q: Why is the negative selection step needed?

Problems of ncAA Incorporation







Q: Why could it be difficult to produce ncAA-containing proteins in natural cells (typically *E. coli*)?

Hint 1: Toxicity for cells

Hint 2: Impurity of product

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nature

Total synthesis of *Escherichia coli* with a recoded genome

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