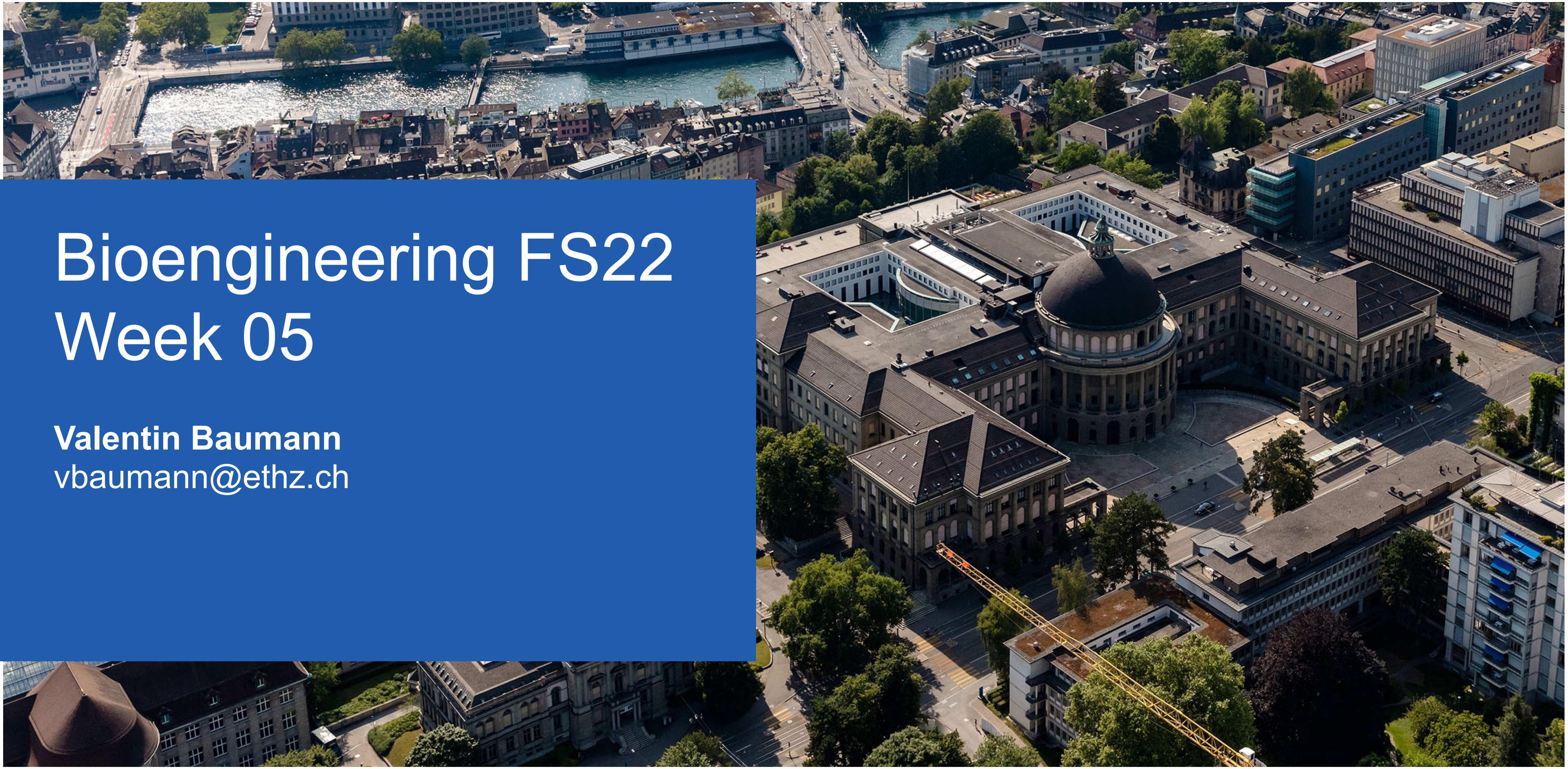


Bioengineering FS22
Week 05

Valentin Baumann
vbaumann@ethz.ch

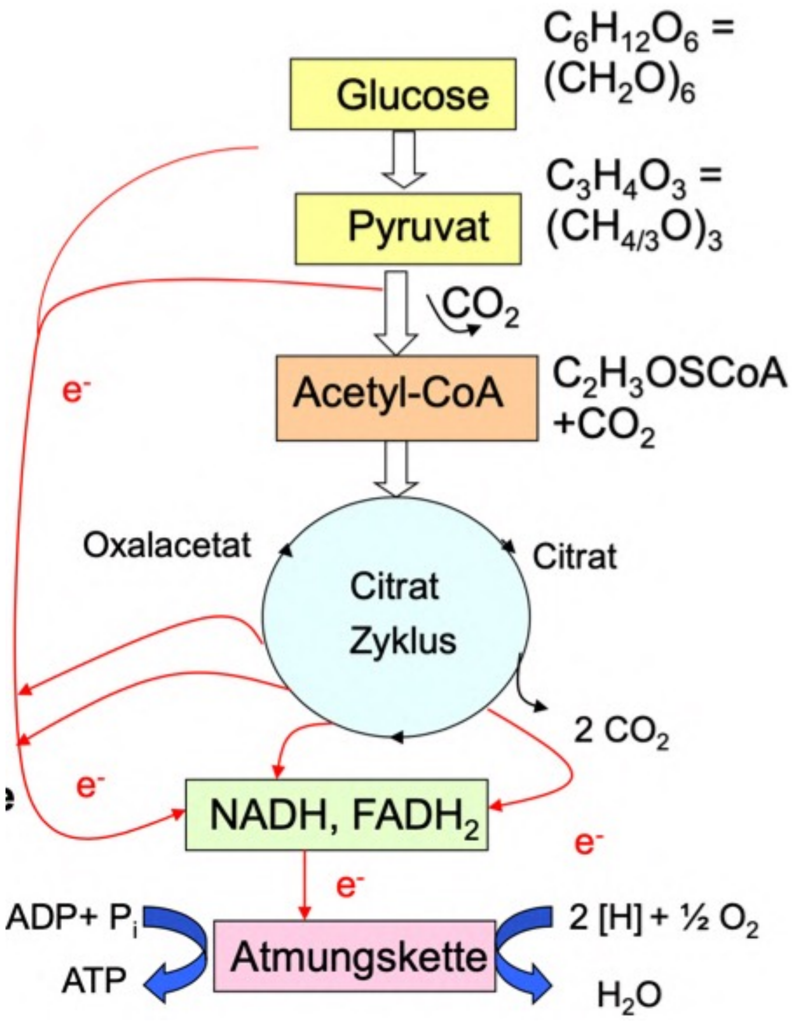
Agenda heute

1. Cellular respiration – Oxidation and reduction-systems (Repetition of lecture)
    1. Glycolysis and gluconeogenesis
    2. Citrate cycle
    3. Respiratory chain/ oxidative phosphorylation
2. Proteinbiosynthesis – Exercise 4
    1. Recap proteins, DNA & RNA
    2. Transcription
    3. Translation

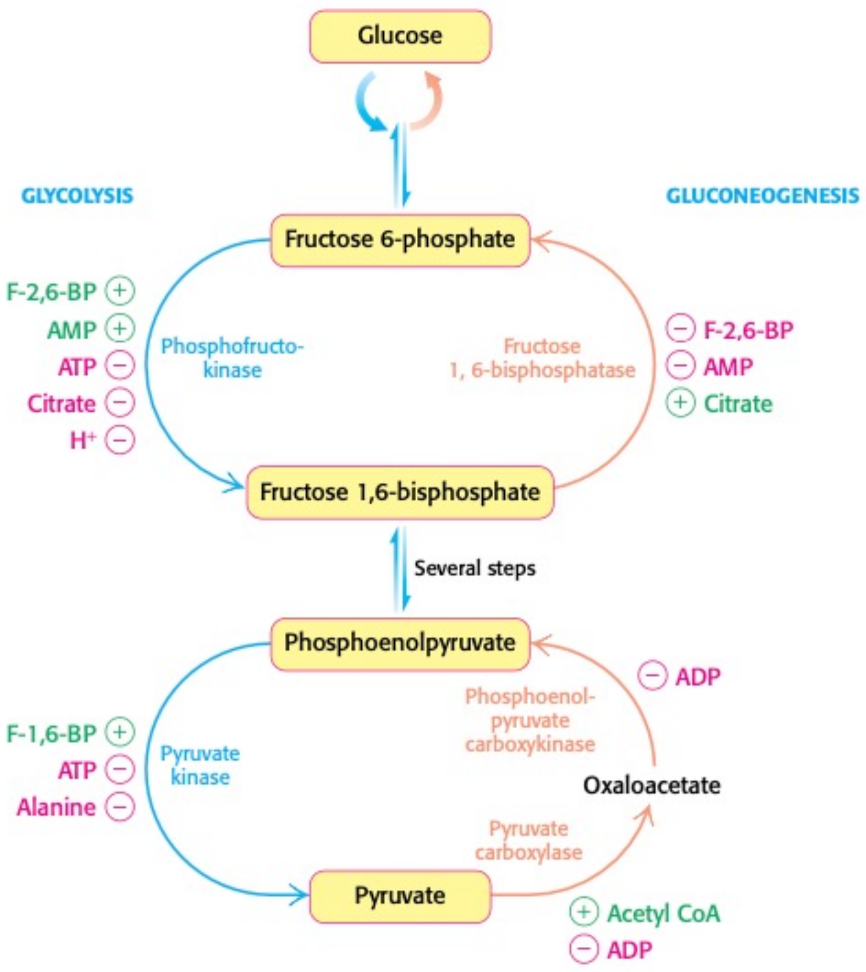
Cell respiration

Cell respiration

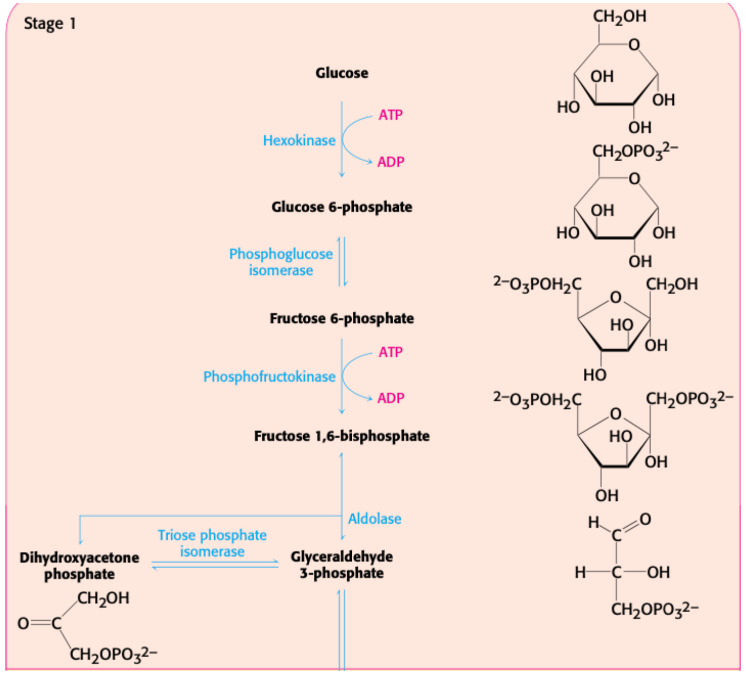
- Uptake of macromolecules that need to be catabolised in order to generate energy and building blocks for new substrates.
- Macromolecules - **carbohydrates, lipids, proteins** – are taken up by eating or drinking them. They are broken down into smaller building blocks in different ways.
- Glycolysis is one of the most important reactions since it generates the reactants for the citrate cycle, which in turn is essential for the respiratory chain. The respiratory chain generates the most energy in the cell and is thus needed for survival.
- When catabolised, macromolecules generate building blocks and energy. Depending on the type of macromolecule that is broken down, the amount of energy differs. The energy is generated during the single steps of metabolism,
- When metabolising a molecule of glucose, the following reactions take place:
  - Glycolysis
  - Citrat cycle
  - Respiratory chain



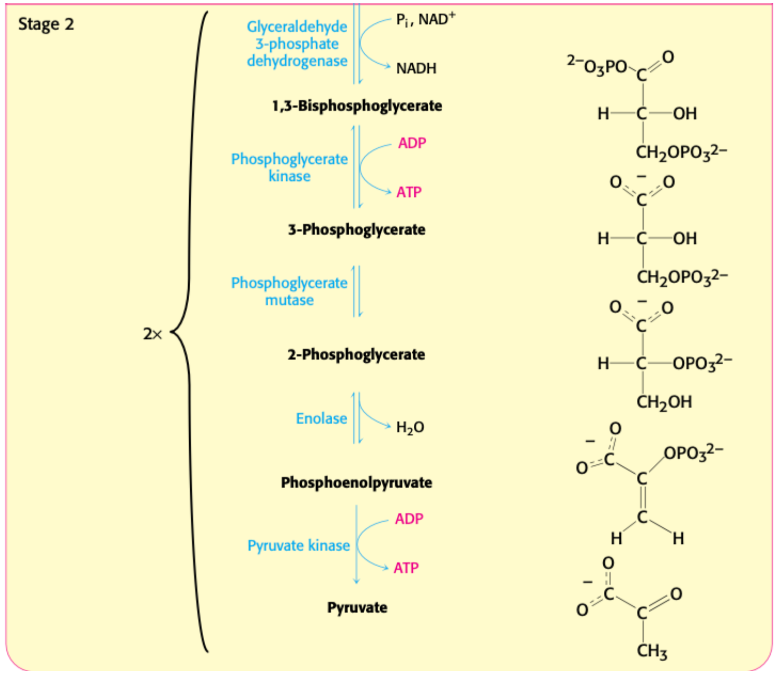
Glycolysis (and gluconeogenesis) simple



Glycolysis (and gluconeogenesis) advanced



In the first stage of glycolysis, 2 ATP molecules are used and Glyeraldehyde-3-phosphate is generated

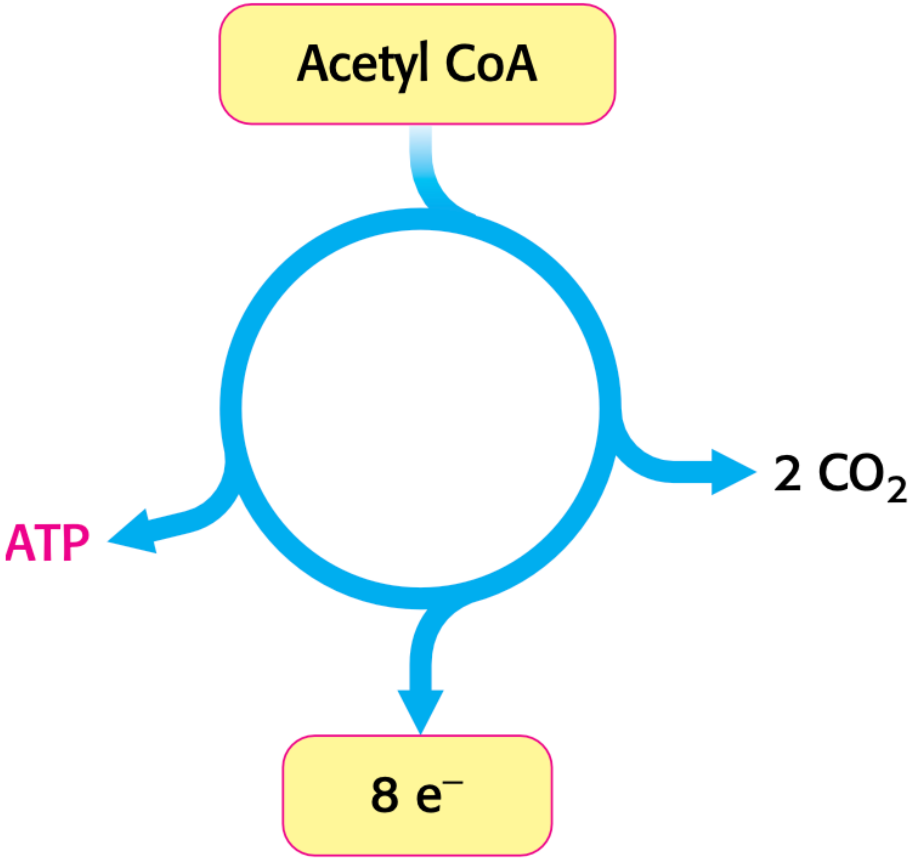


In the second stage of glycolysis, 4 ATP are generated and 2 pyruvate molecules are generated. ATP is generated via **substrate level phosphorylation**. This differs from the ATP generation of the respiratory chain.

Additionally, 2 NADH are generated, these will later be used in the respiratory chain.

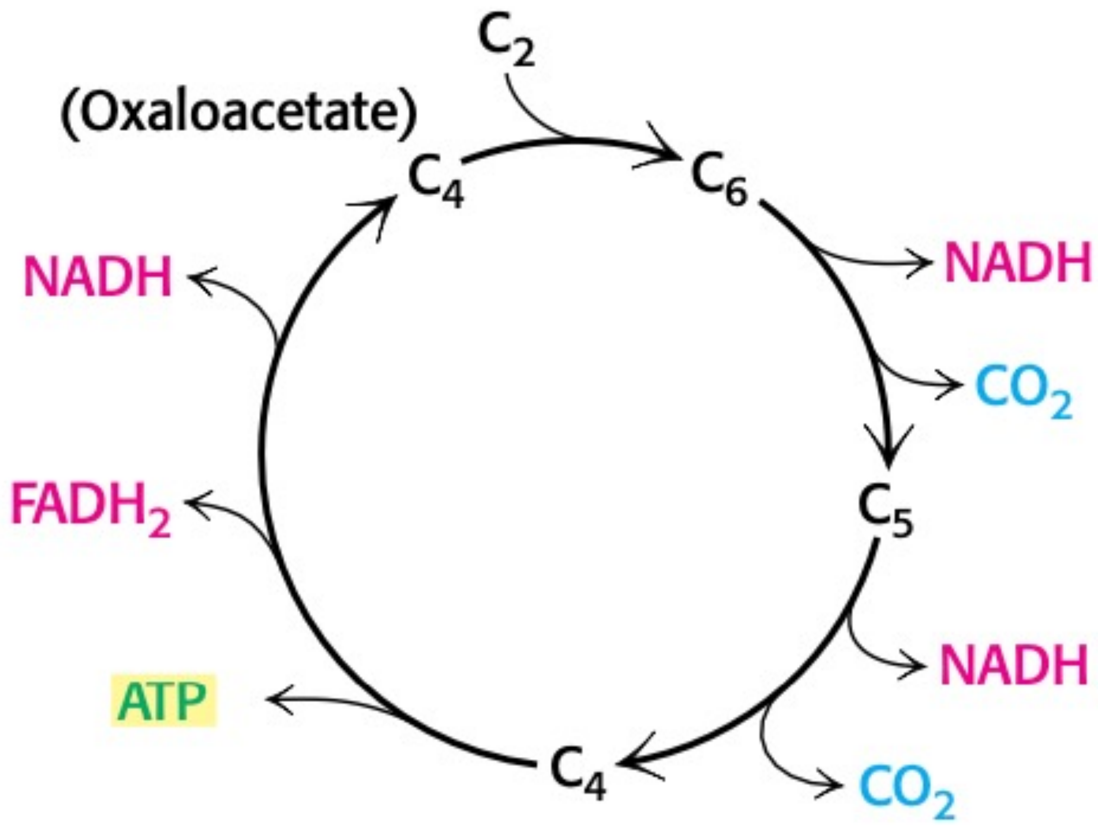
The pyruvate generated will be used in the citrate cycle.

Citrate cycle simple



- Pyruvate that was generated during the glycolysis is converted into Acetyl-CoA.
- Acetyl-CoA then enters the citrate cylcle which generates
  - 2 CO_2
  - 8 e^-
  - ATP

Citrate cycle simple



- The citrate cycle is a sequence of 9 reactions, during which all C atoms of the original glucose molecule are completely oxidised.
- The energy that is generated is stored in the form of NADH and $FADH_2$.

Citrate cycle advanced

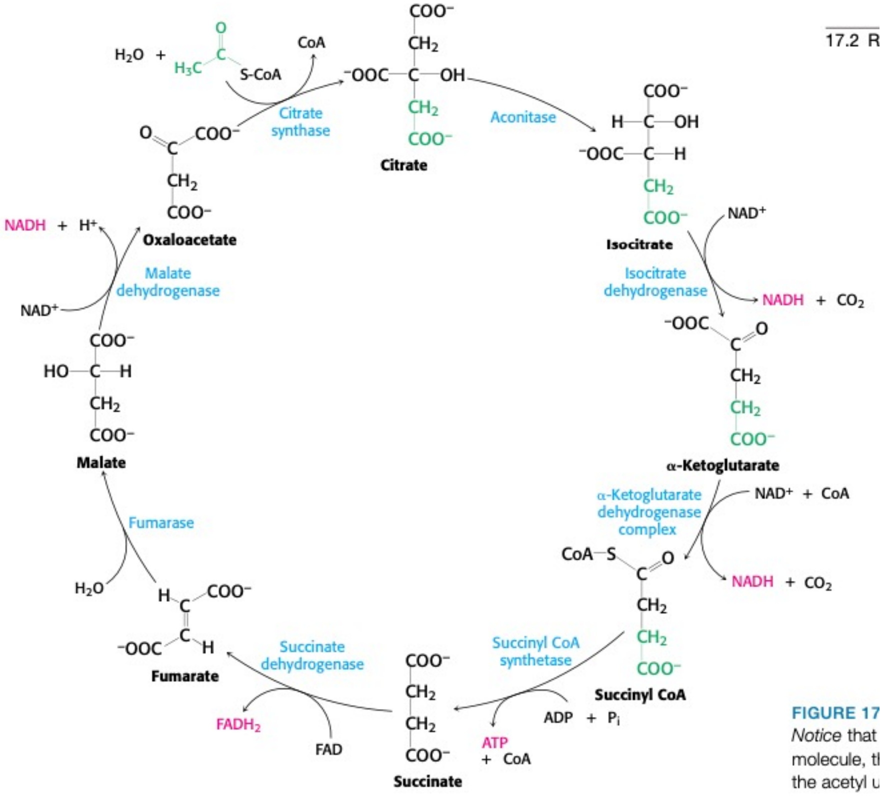
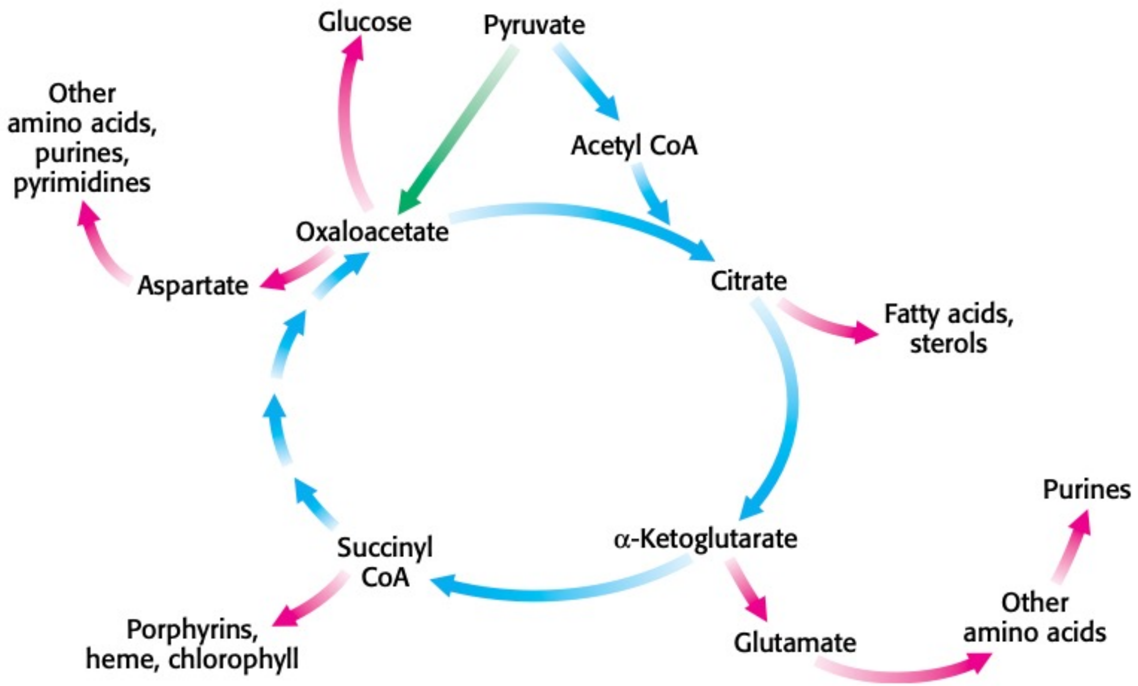


FIGURE 17
Notice that
molecule, tl
the acetyl u

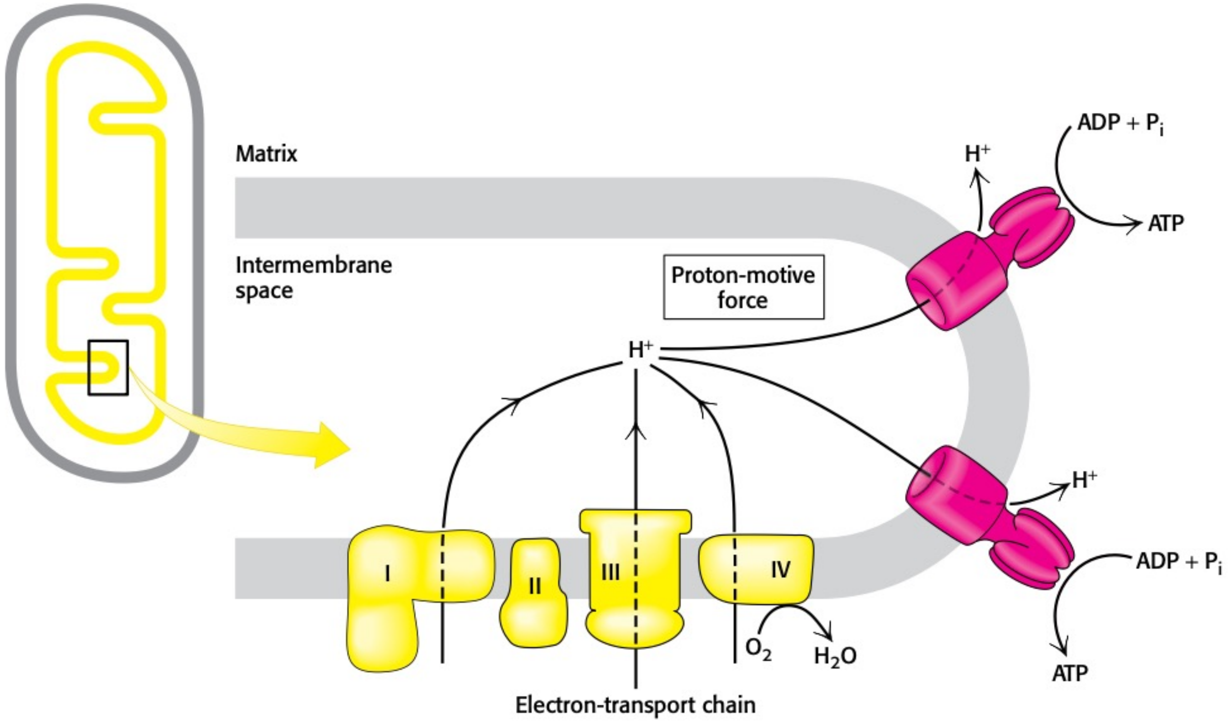
- The name of the **intermediate product**s are black and the enzymes are blue.
- One of the most important things to remember about the citrate cycle is that the first product, **citrate**, is generated from **Acetyl CoA** and **Oxaloacetate**. The latter however is the final product of the citrate cycle, and is therefore always repurposed.
- Per cycle, 3 NADH and 1 $FADH_2$ are generated.
  Since one molecule of glucose generates 2 pyruvate molecules, a total of 6 NADH and 2 $FADH_2$ are generated per glucose molecule.
- The rest of the energy is stored in form of high-enery electrons, which will be needed in the respiratory chain.

Byproducts of the citrate cycle



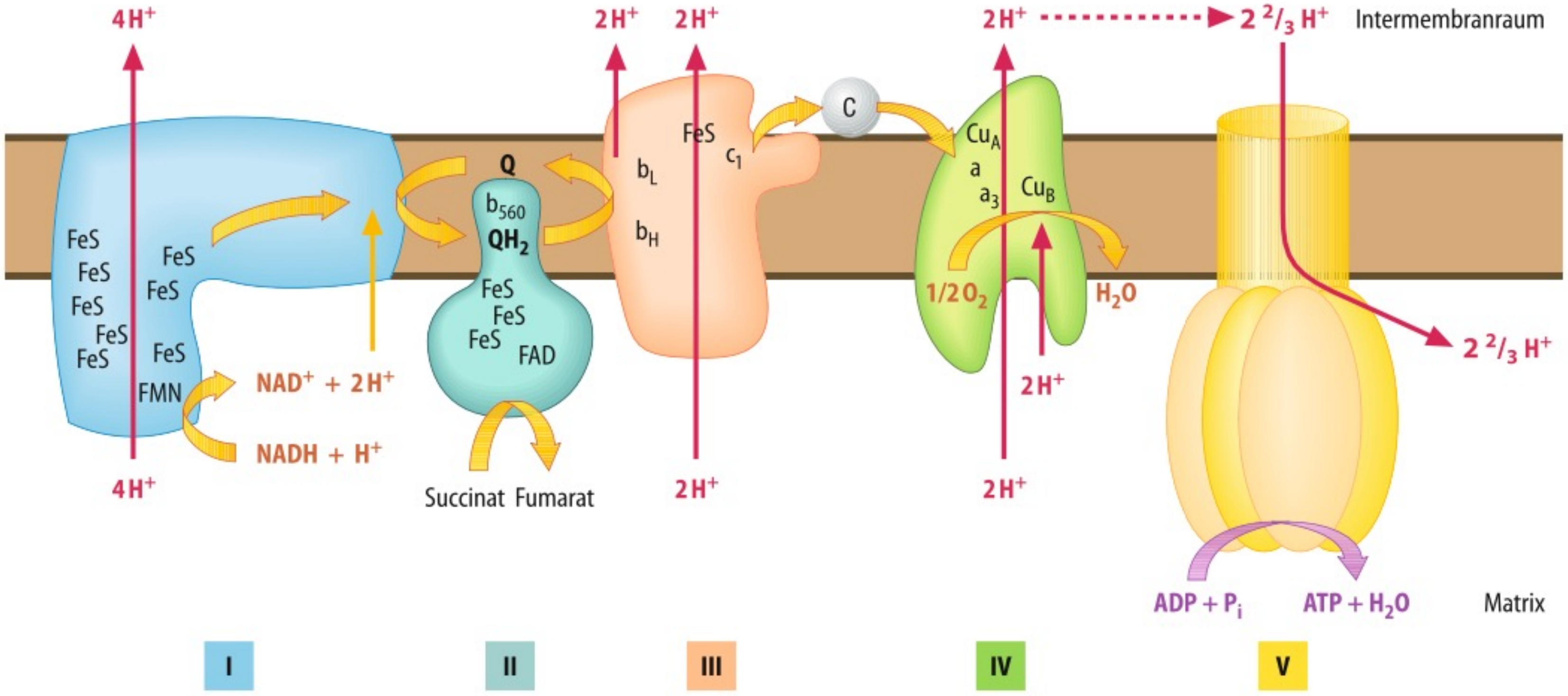
- The citrate cycle not only generates ATP, NADH and $FADH_2$, but also many amino acids and fatty acids as well as the building blocks for DNA.

Respiratory chain simple



- In the respiratory chain, the NAHD, $FADH_2$ are used to power the proton pumps across the membrane.
- The end-goal is to have a structure with a lower energy than NADH, so that H_2O can be produced safely.
- The protons that are pumped across the membrane into the intermembrane space are then used to power the ATPase, where ATP's are generated.

Respiratory chain advanced

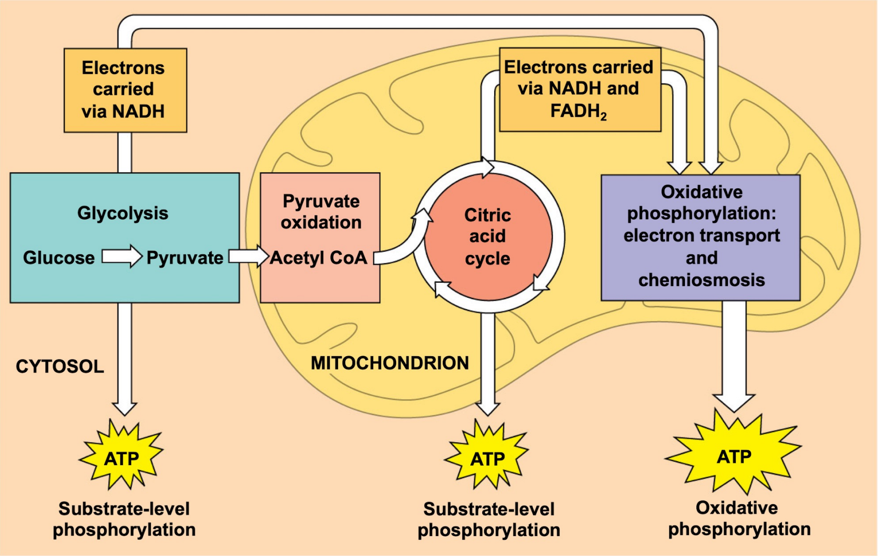


- There are five complexes that are part of the respiratory chain. The first three pump protons into the intermembrane space, the second converts succinate into fumarate. The fourth complex generates H_2O.
- The fifth complex generates ATP by using the electrochemical proton gradient to generate mechanical energy, which in turn generates ATP (chemical energy)

«Everything» put together:

- When catabolised, macromolecules generate building blocks and energy. Depending on the type of macromolecule that is broken down, the amount of energy differs. The energy is generated during the single steps of metabolism,
- When metabolising a molecule of glucose, the following reactions take place:
  - Glycolysis $C_6H_{12}O_6 + 2\,NAD^+ + 2\,ADP + 2\,P_i \rightarrow 2\,C_3H_4O + 2\,NADH_2 + 2\,ATP + 2\,H_2O$
  - Citrat cycle $2\,C_3H_4O + 8\,NAD^+ + 2\,FAD + 2\,ADP + 2\,P_i + 4\,H_2O \rightarrow 6\,CO_2 + 8\,NADH_2 + 2\,FADH_2 + 2\,ATP$
  - Respiratory chain $6\,O_2 + 10\,NADH_2 + 2\,FADH_2 + 34\,ADP + 34\,P_i \rightarrow 46\,H_2O + 10\,NAD^+ + 2\,FAD + 34\,ATP$

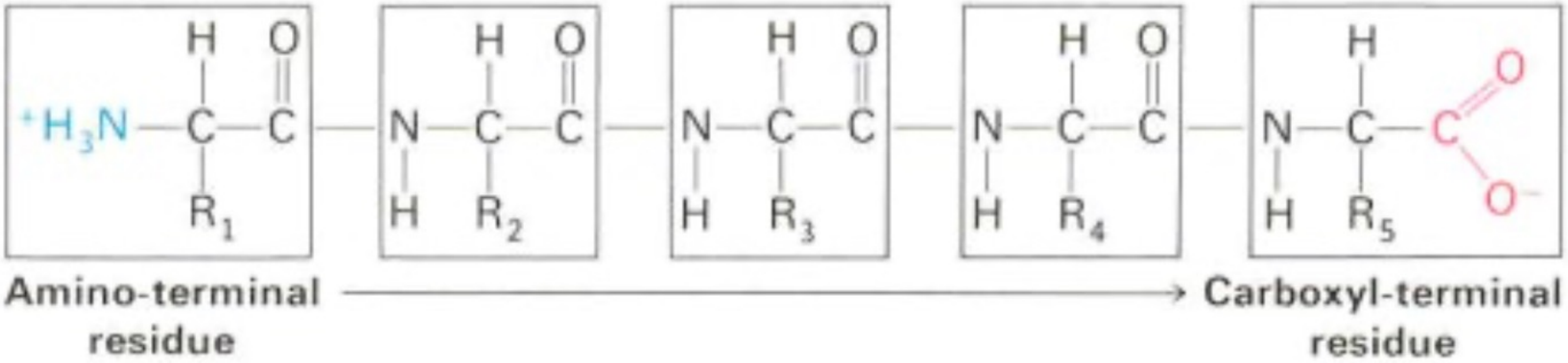
The final fomula is therefore: $C_6H_{12}O_6 + 38\,ADP + 38\,P_i + 6O_2 \rightarrow 6\,CO_2 + 6H_2O + 38\,ATP + 38\,H_2O$



Protein Biosynthesis

Protein Biosynthesis – Protein recap

1. **Primary Structure:** sequence of amino acids



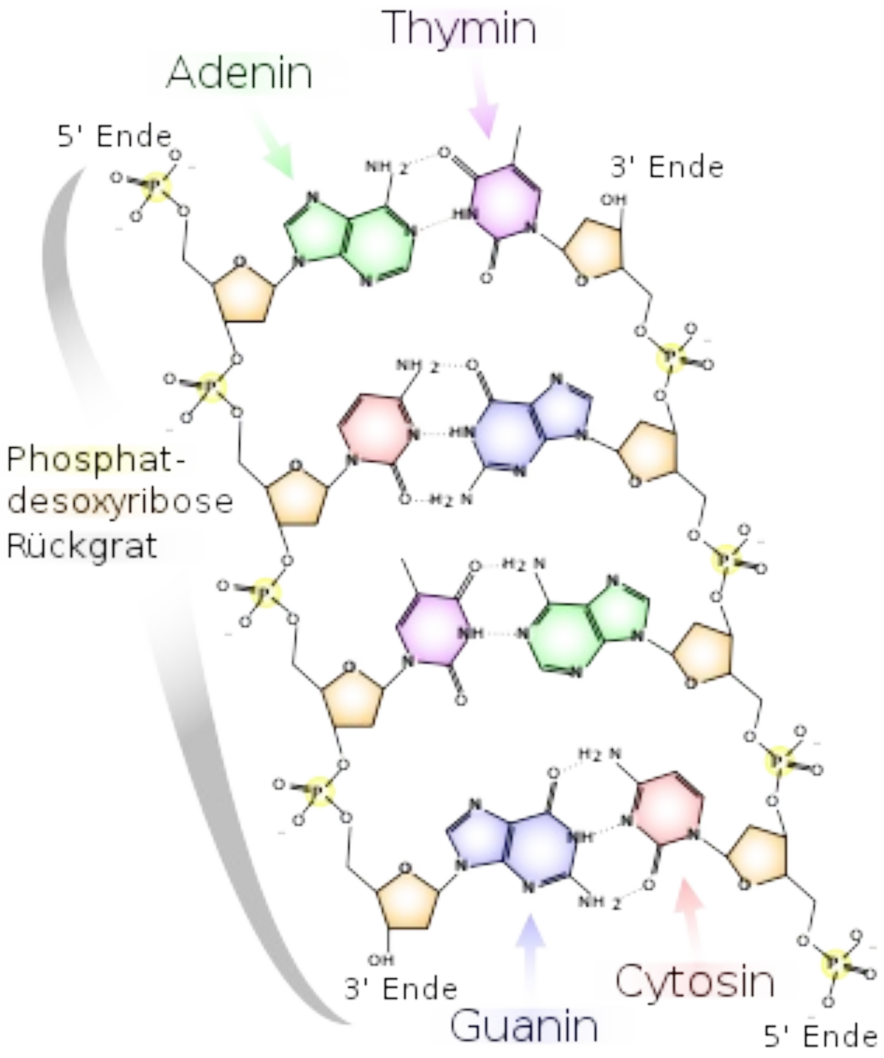
2. **Secondary Structure:** Alpha-helices & Beta-structures
3. **Tertiary Structure:** 3D folding of the amino acid sequence
4. **(Quaternary Structures:** Several protein domains with seperate amino acid chains, interact to make up the whole funcitonal protein)

Protein Biosynthesis – „Instruction manual“ for proteins

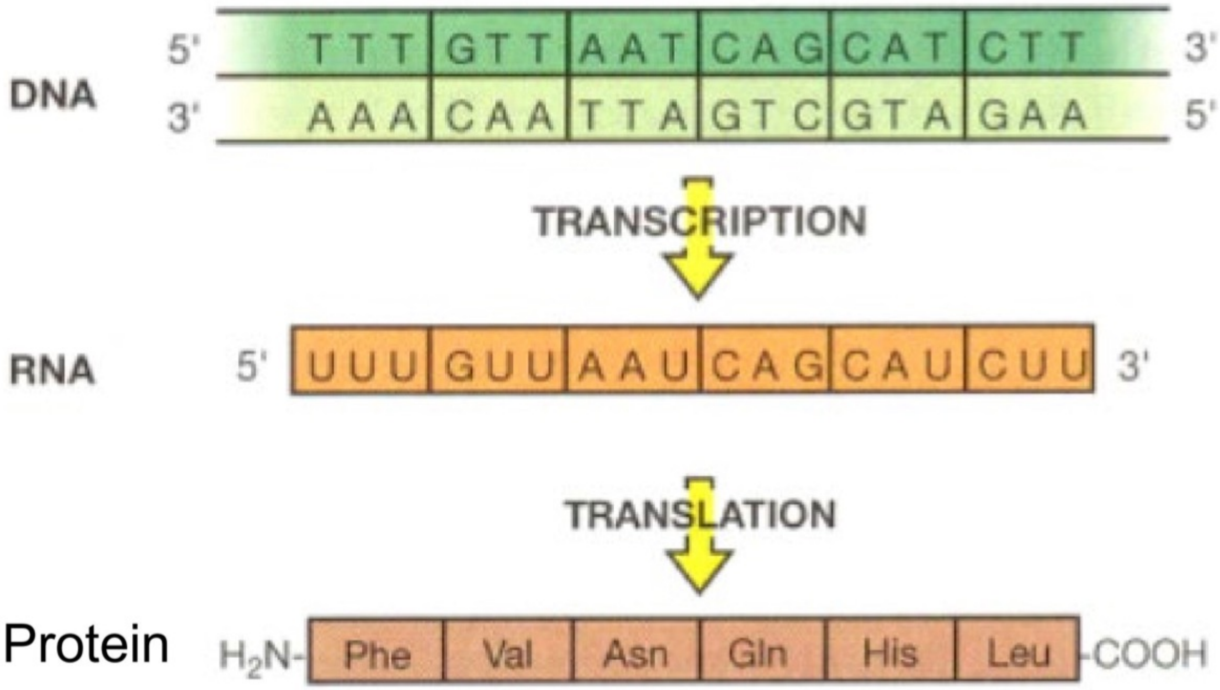
→ Primary Structure encoded in **genome/DNA**

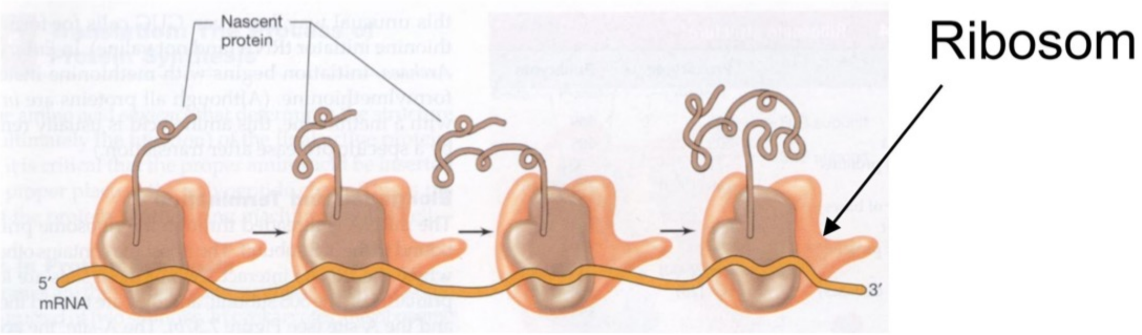
- Bases: A, C, G, T
- DNA sequence, which encodes for a funcitonal protein is a **gene**
- The gene encodes the order of amino acids in the peptide chain of a protein

→ (messenger)RNA used as an intermediary structure during Protein-Biosynthesis (Bases: A, C, G, U; only one strand)



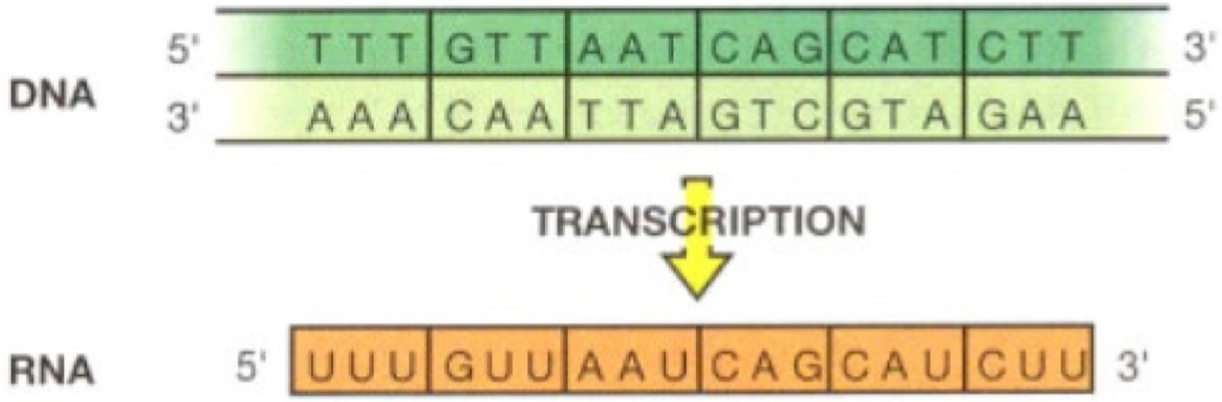
Protein Biosynthesis – Central Dogma

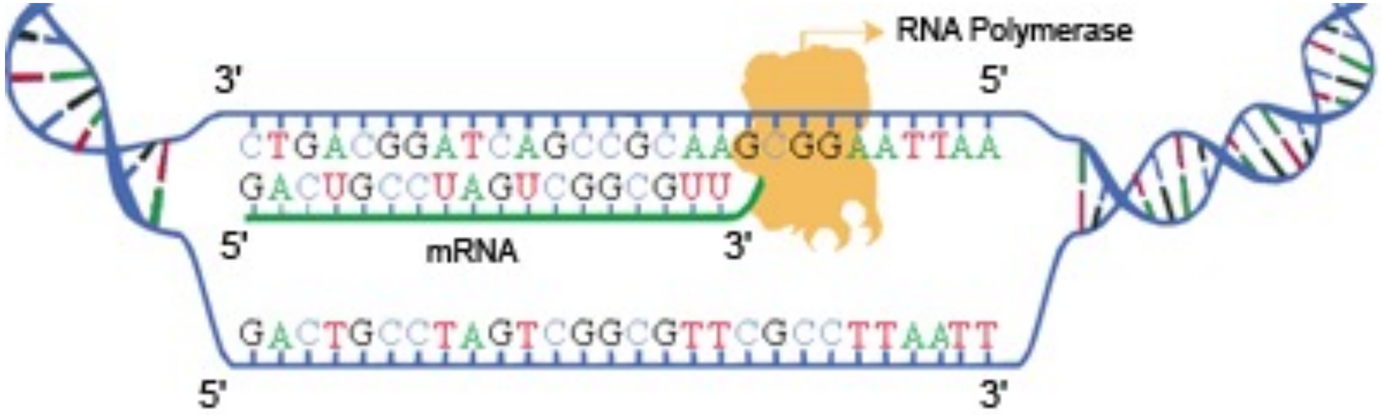




Protein Biosynthesis – Transkription (DNA -> RNA)

- RNA-Polymerase reads DNA (from 3’-5’), producing an mRNA (from 5’-3’)
- DNA strand read by polymerase is called the **template/non-coding strand**
    - mRNA is complementary to it (A&T, C&G)
- Other DNA strand is called the **coding strand**
    - mRNA has same base sequence apart from T being replaced with a U



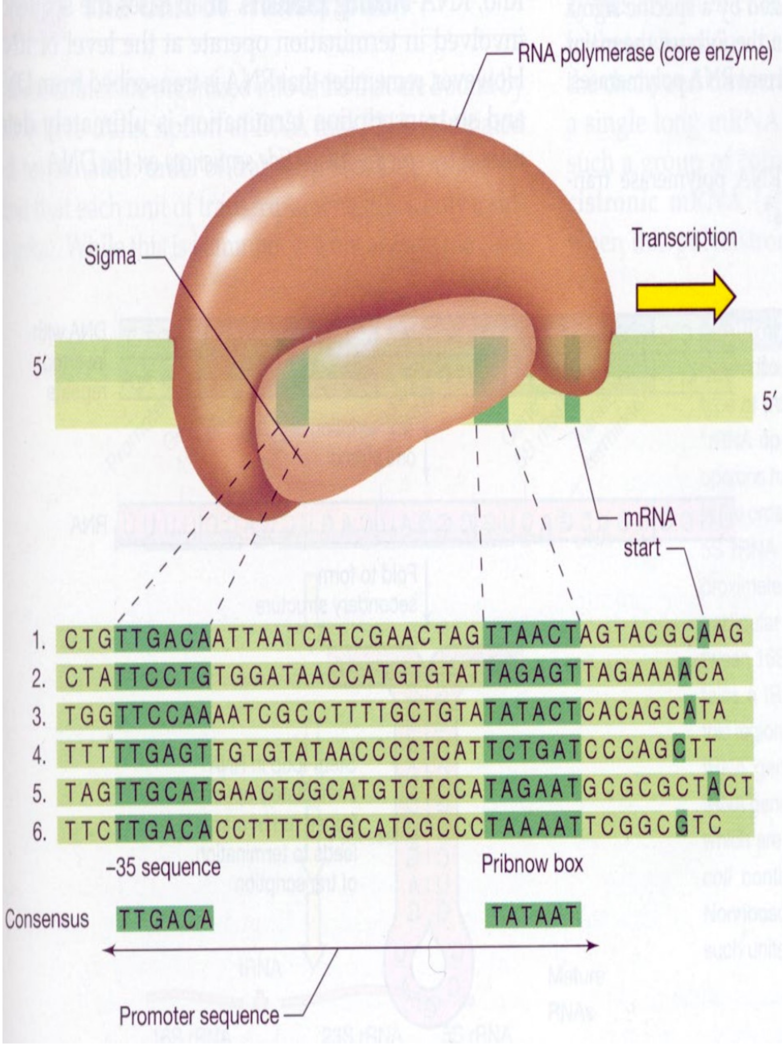


Transcription – Polymerase & Promoters

- RNA Polymerase responsible for reading the template strand 3’-5’ and producing the mRNA strand 5’-3’ according to base pair principles

- Promoter sequences:
  - Upstream of 5’ end of a gene (regarding coding strand)
  - TATA-Box with TATAAT sequence -> recognised by sigma factor

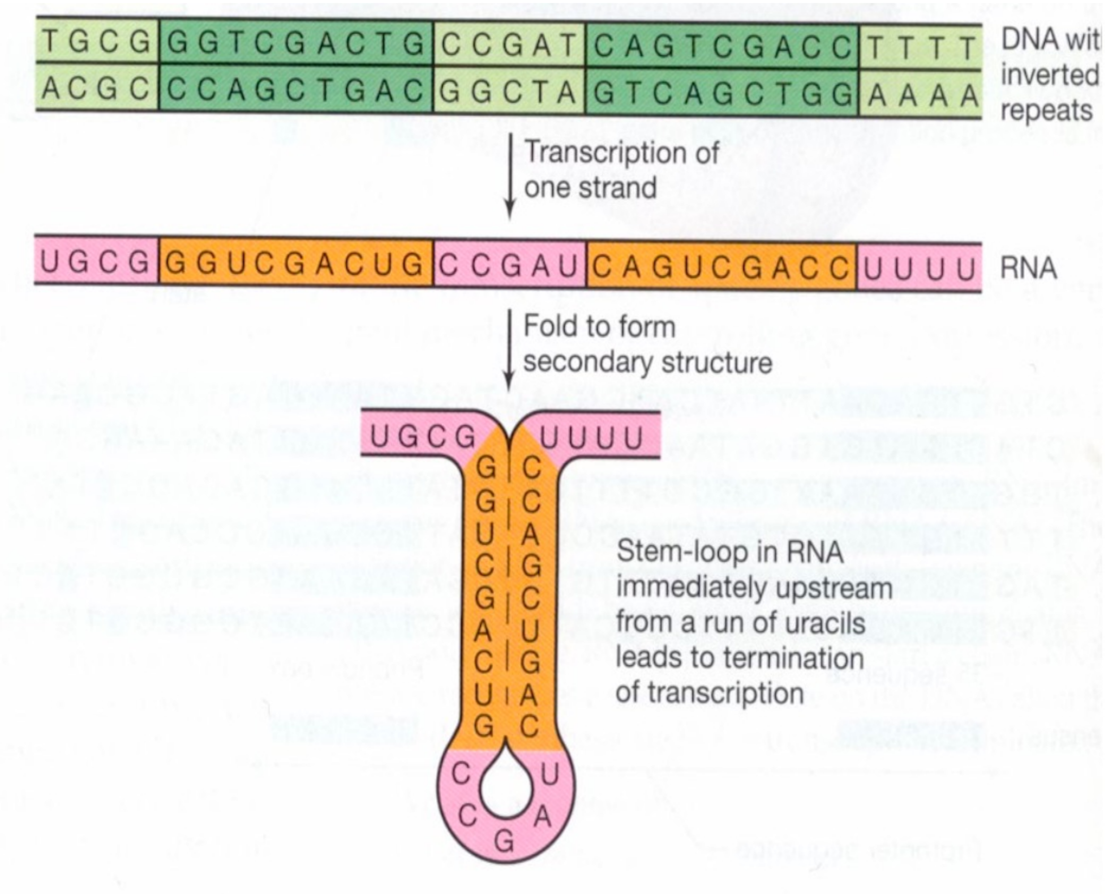
- Initiation process:
  - Sigma factor is only responsible for recognition of the start site for transcription on the DNA **(promoter sequence)**
  - Sigma factor dissociates after a certain transcribed sequence



Transcription – Terminators

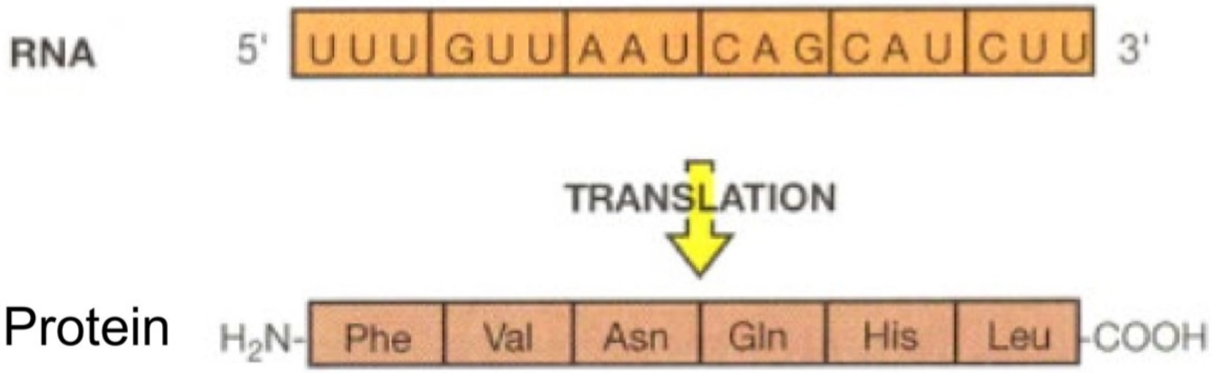
- Terminator sequences:
  - Downstream of the 3' end of a gene (regarding the coding strand)
  - Responsible for the stop of transcription (marks end of pre-mRNA)

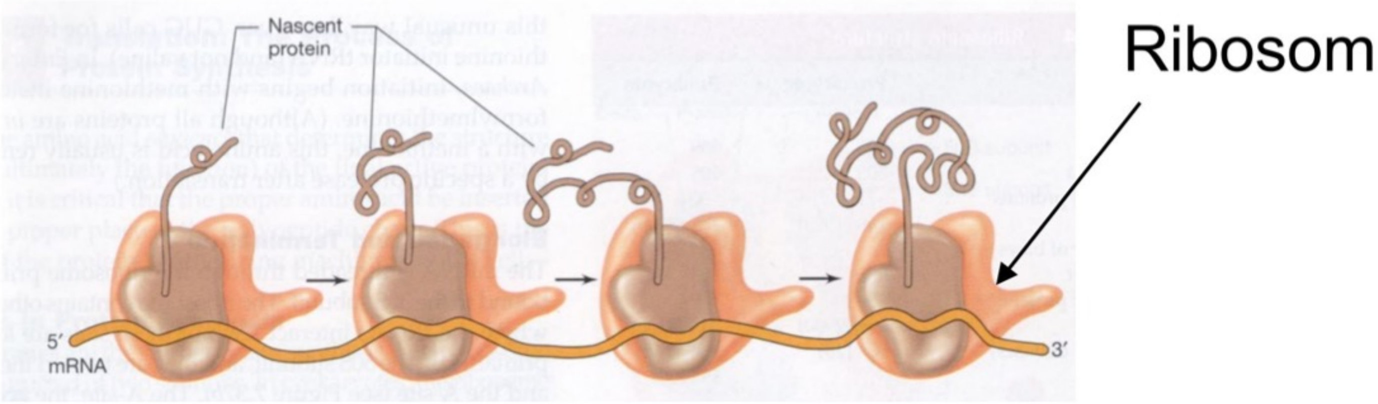
- Termination process:
  - At termination site there is DNA with inverted repeats → synthesised mRNA can form secondary structures called stem-loops
  - Stem-loop structures cause RNA-polymerase to slow down and eventually cease transcription
  - Polymerase dissociates from the DNA



Protein Biosynthesis – Translation (RNA -> Polypeptide chain)

- Ribosome reads mRNA from 5’-3’ (same direction the mRNA is synthesised)
- Polypeptide chain produced from N-terminus to the C-terminus according to genetic code
- Translation can be subdivided into three stages:
  - Initiation
  - Elongation
  - Termination
- Amino acids are ”supplied” to the ribosome by transporter RNAs (tRNAs)





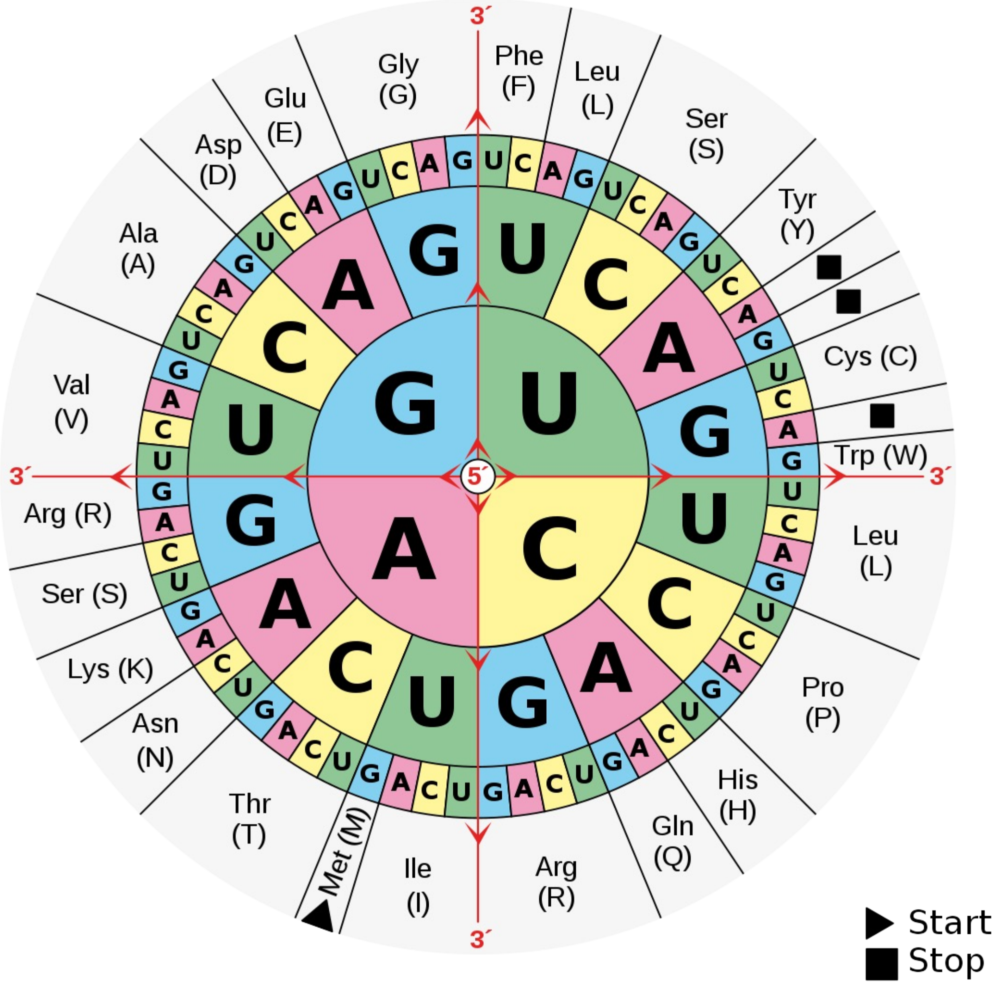
Translation – Genetic Code/ Code Sun

- 3/Triplett of bases of mRNA (= **Codon**) encodes for an amino acid or represents a stop signal

- Genetic code is:
    - Redundant → multiple codons encode the same amino acid (4^3 = 64 codons vs. 20 amino acids)
    - Universal → the genetic code is identical across all species

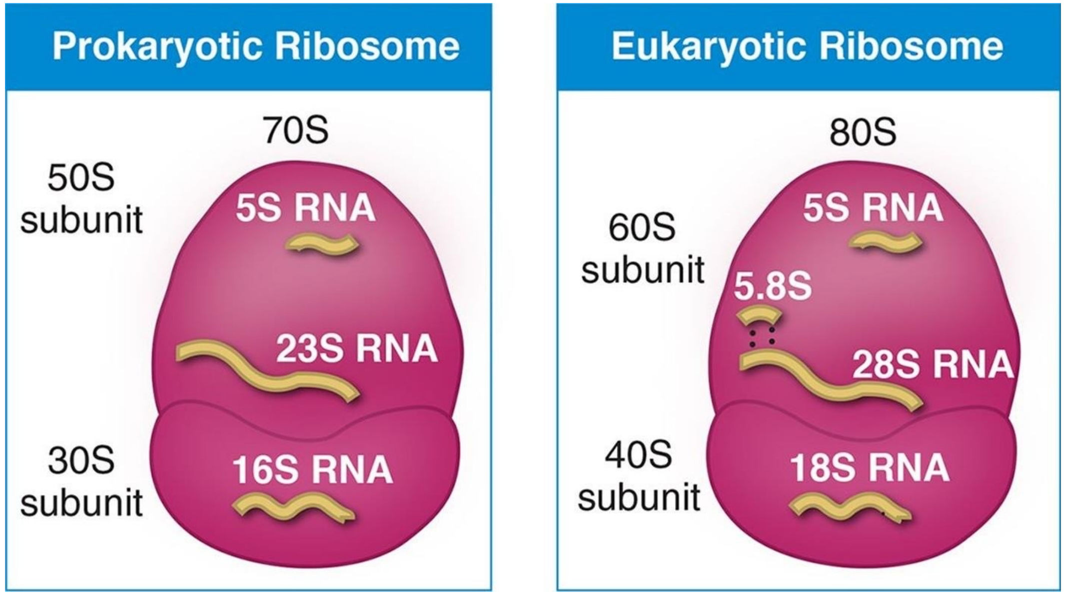
- Start Codon = Methionin (M)

- Every amino acid has a letter assigned for a more convenient way to write amino acid sequences



Translation – Ribosomes & important codons

- Ribosome (prokaryotes) 70S
  - 50S subunit (big subunit)
    - 5S rRNA, 23S rRNA & 34 proteins
  - 30S subunit (small subunit)
    - 16S rRNA & 21 proteins



- 30S subunit of Ribosome recognises and binds so called **Shine-Dalgarno-Sequence (AGGAG)**
- After a few bases the start codon (AUG) follows

→ Initiation of translation

- At the end of translation, ribosome dissociates due to recognition of a **stop codon (UAA, UAG or UGA)**

- Untranslated regions of mRNA at 5’ and 3’ end are of no importance regarding translation

5‘ untranslated leader- AGGAG - (7-9 bp) - AUG - (AS-Codons für das Protein) - UAA / UAG / UGA - 3‘ untranslated leader

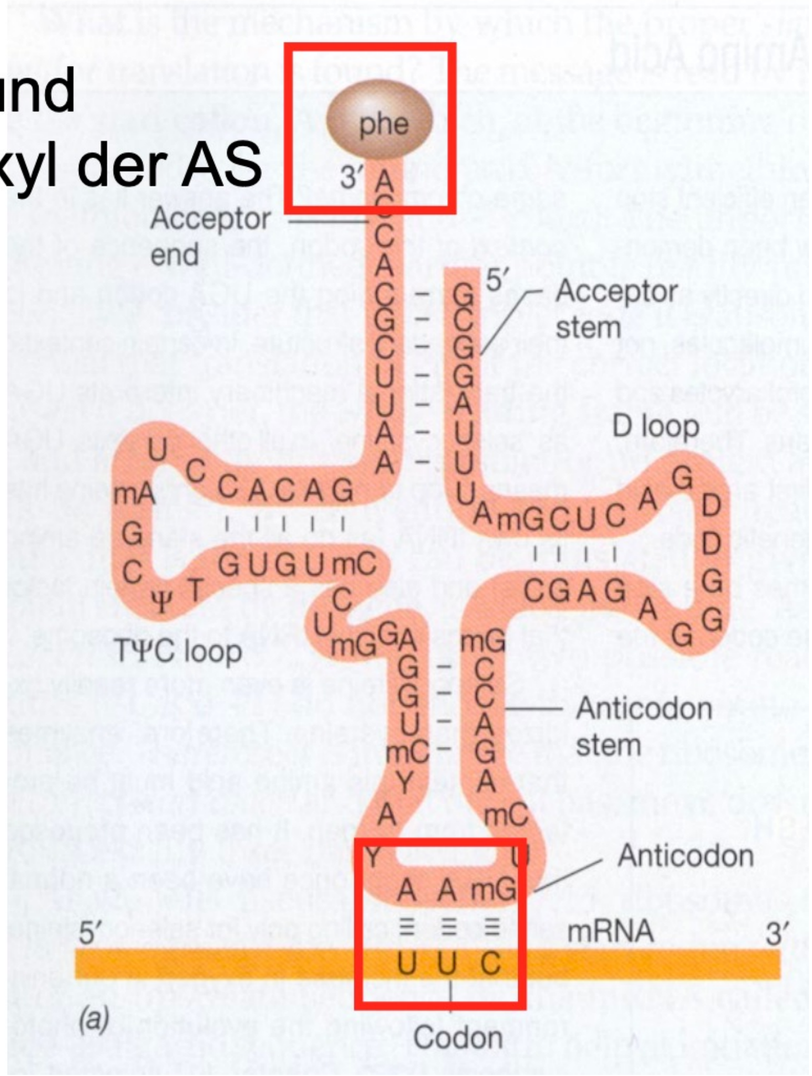
Translation – tRNA

- tRNA - mediator between mRNA and ribosome

- tRNA has cloverleaf form due to secondary structures formed by inverse repeats

- tRNAs has to key aspects:
  - tRNA-Anticodon at the anticodon stem
    - 3 bases complementary to a certain codon on the mRNA → responsible for tRNA binding on the mRNA within the ribosome

  - Bound amino acid at the acceptor end/stem
    - Amino acid corresponds to tRNA anticodon



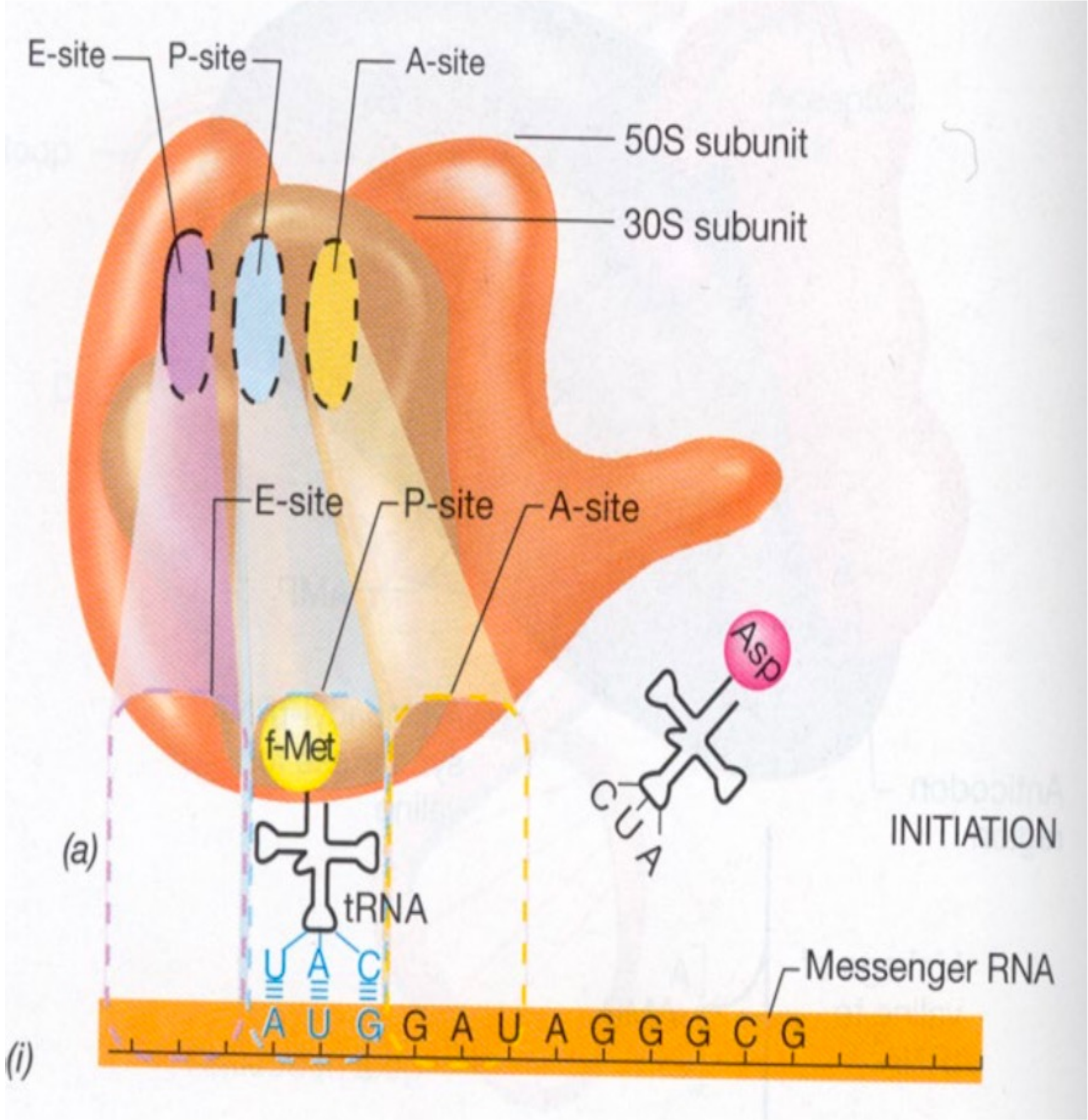
3’OH und Carboxyl der AS

Translation – Initiation

- Initiation complex
  - mRNA
  - 30S subunit of ribosome (bound to Shine-Dalgarno-Sequence of mRNA)
  - Methionin-tRNA (bound to start codon of mRNA)

- 50S subunit binds the initiation complex and initiation is complete

- At the end of initiation, Methionin-tRNA is bound to its codon on the mRNA within the P-site of the ribosome

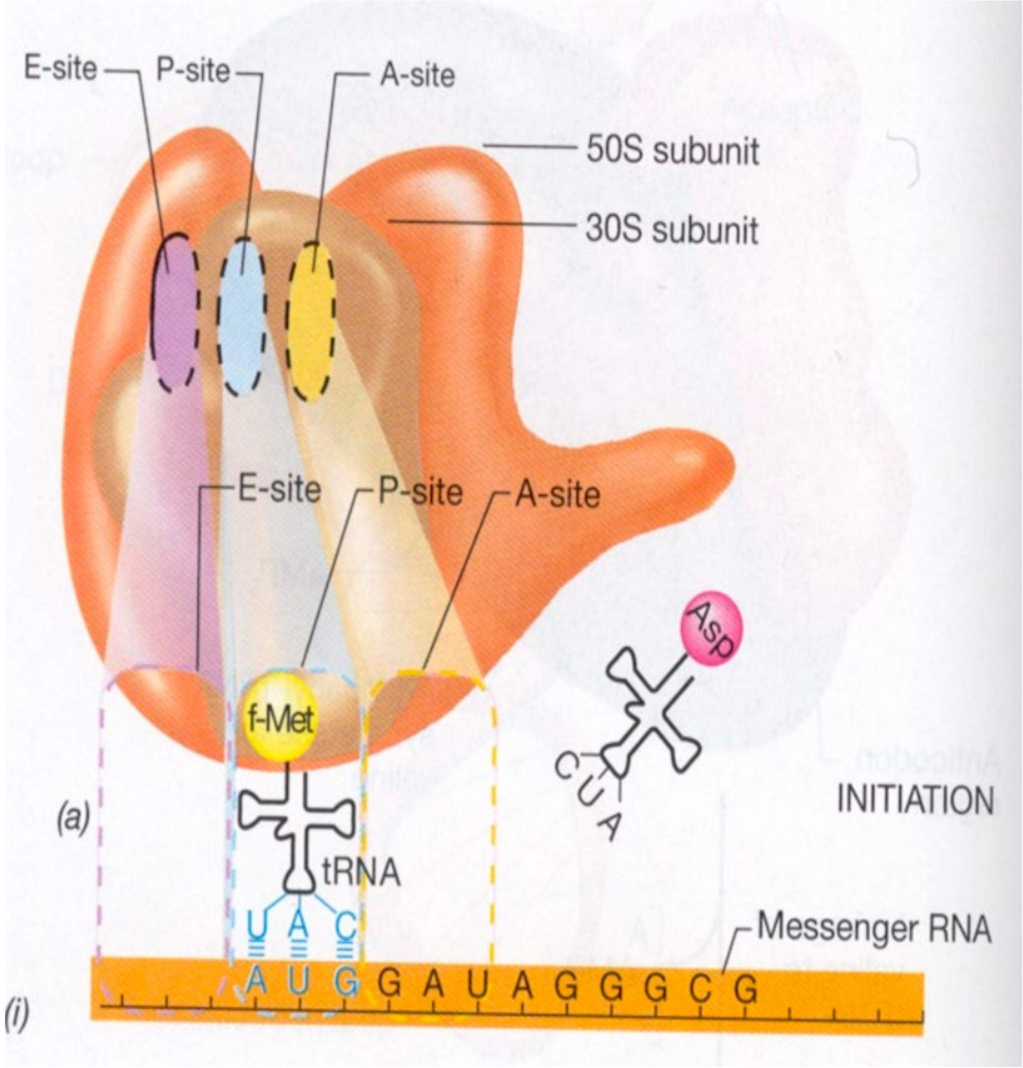


Translation – Elongation (tRNA binding sites)

- At **Acceptor site (A-site)** a new incoming, corresponding tRNA binds the codon presented at the A-site

- At **Peptide site (P-site)** the growing peptide chain is transferred on top of amino acid bound to incoming tRNA in A site

- At **Exit site (E-site)** “empty” tRNAs are dissociated form the ribosome and can be reloaded with the corresponding amino acid to join the loaded tRNA pool within the cytosol



Translation – Elongation

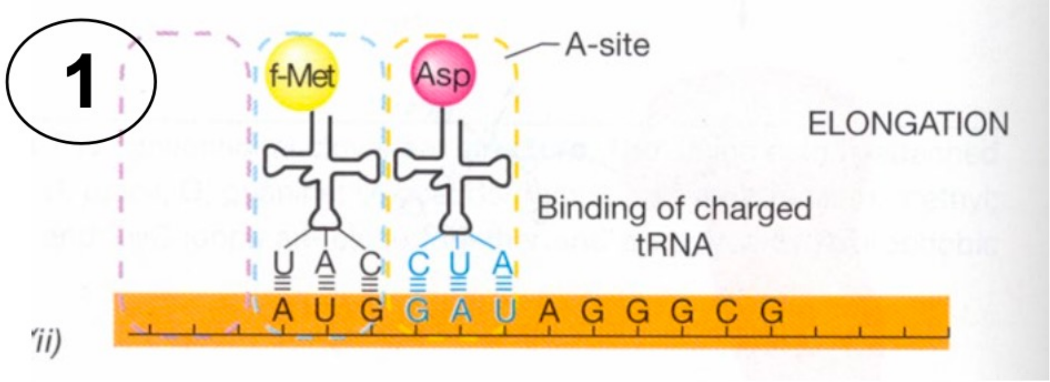
- Elongation consists of 4 sub-processes

1
New tRNA is bound at A-site (here loaded with Asparagin, because of GAU codon)
    - Previous tRNA still loaded with amino acid is boudn in P-site

2
Simultaneously there is:
    - formation of a peptide bond between both amino acids
    - break of the ester bond between the amino acid and tRNA in P-site

→ First amino acid gets transferred onto the amino acid and tRNA in A-site

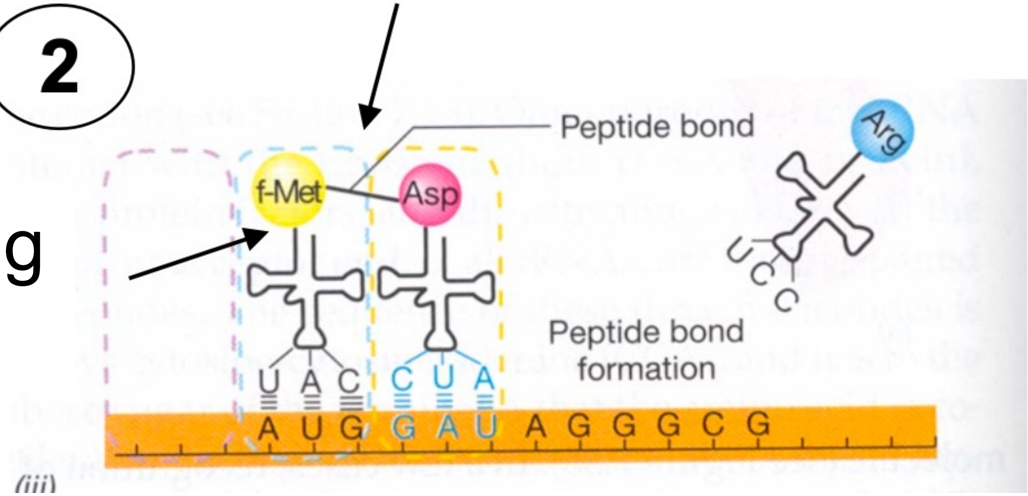
1



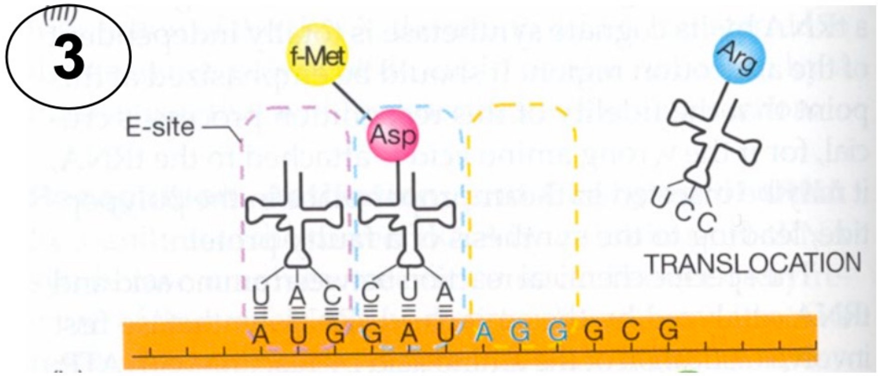
Neue Bindung

2

Bindung gelöst



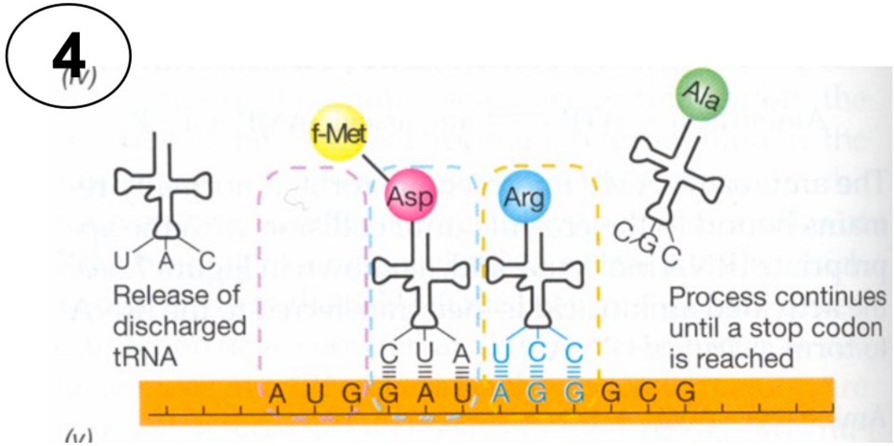
Translation – Elongation



3
Ribosome shifts by one codon towards the 3’ end of the mRNA (translocation)

- Now empty tRNA previously in P-site now in E-site
- tRNA carrying the whole peptide chain previously in A-site now in P-site

The empty tRNA in E-site dissociates from the mRNA and ribosome



4
Acceptor site now free again for the next amino acid loaded tRNA to bind (here Arginin)

→ Steps 1-3 repeated until a stop codon is located in the A-site → triggers termination process

Translation – Termination & Overview

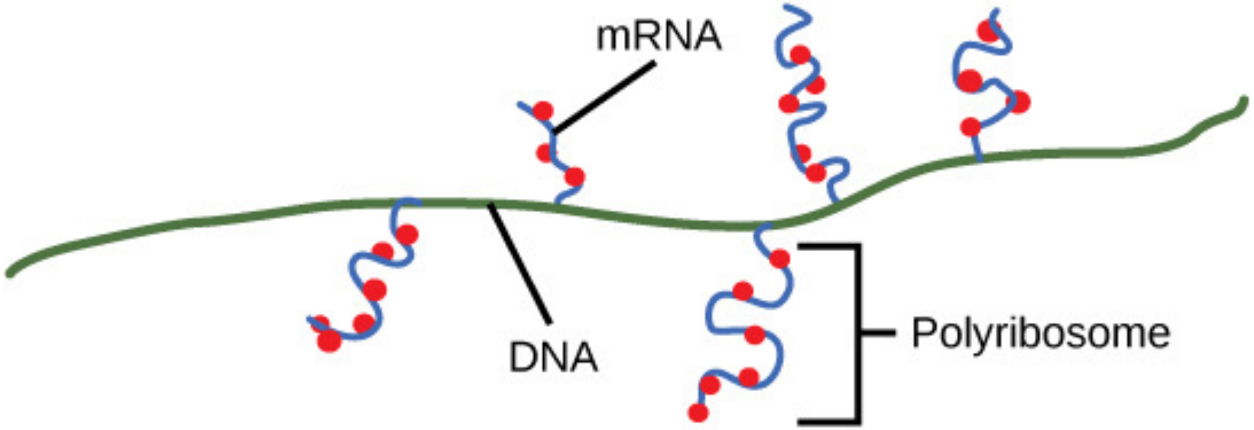
- Translation is terminated
    - As soon as a stop-codon is located in the A-site of the ribosome (UAA, UAG, UGA)
    - Normally no tRNAs for the stop codons → ribosome pauses

- Finished peptide chain is cleaved from the last tRNA located in P-site

- Ribosome dissociates into its subunits freeing up mRNA

- In bacteria Translation starts before transcription is finished

- Multiple ribosomes travel along the still growing mRNA synthesising new polypeptide chains (Polyribosome)



tRNA, rRNA, mRNA

	Grösse	**Funktion**
tRNA	80-95 Nukleotide	Übertragung von Aminosäuren zum Proteinsyntheseapparat
rRNA	drei Arten aus etwa 120, 1540 bzw. 2900 Nukleotiden	Struktur- und Funktions-Elemente von Ribosomen
mRNA	sehr verschieden (von einigen hundert bis zu mehreren tausend Nukleotiden)	die Boten-(messenger)-RNAs enthalten Abschriften der Gene und programmieren den Proteinsynthese-Apparat