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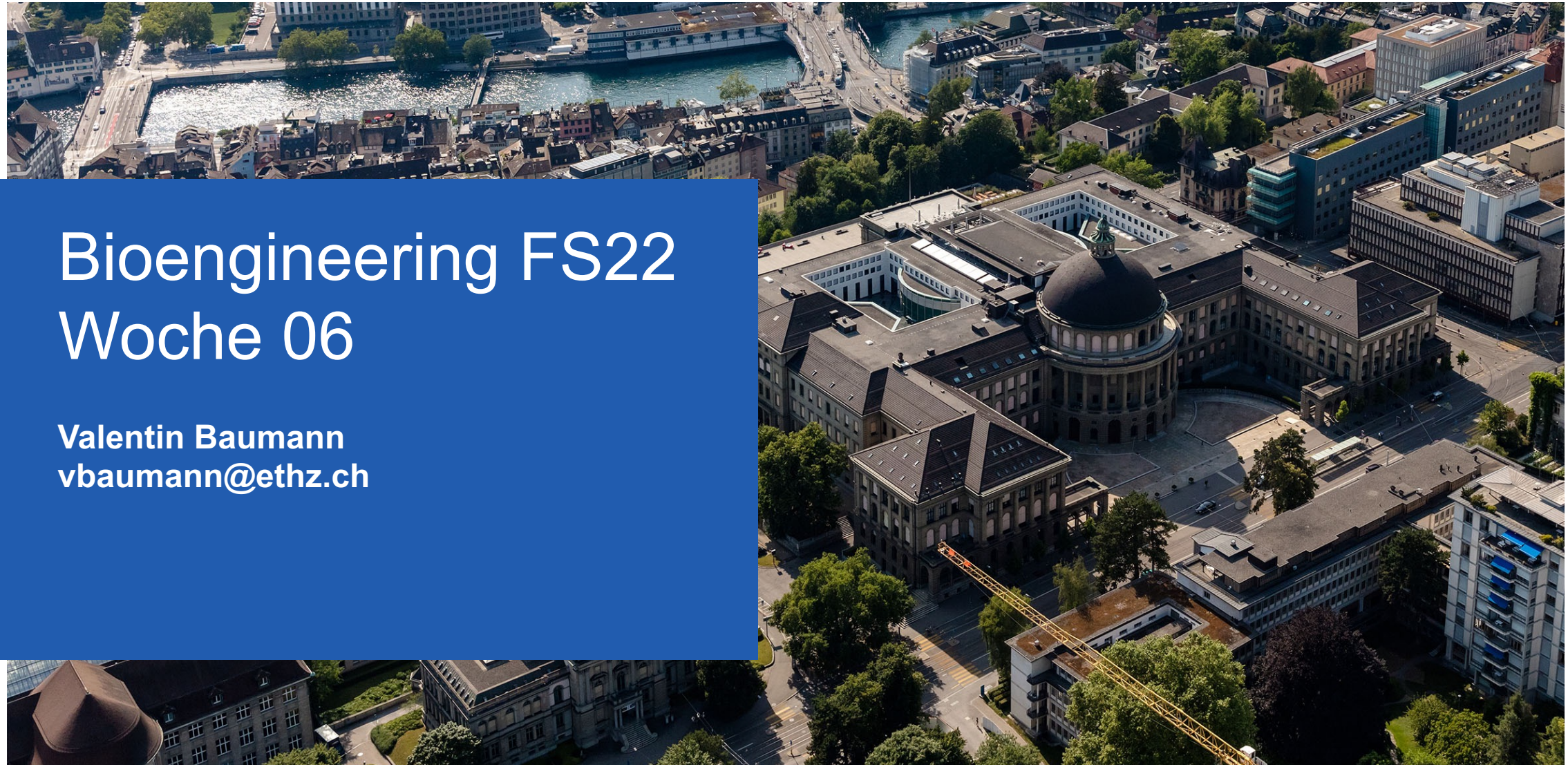

Bioengineering FS22
Woche 06

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Agenda heute

1. Protein-synthesis
2. Regulation of Cell Metabolism

Protein Synthesis Introduction

- Proteins have many functions in the body:
  - Messenger
  - Influence the shape of the cell
  - Driver of cell movement
  - Catalysts
  - Ion pumps
  - Signal transduction
- Proteins are long amino acid chains. The amino-group of the right amino acid connects to the carboxy-group of the amino acid to its left. The arrangement of the amino acids is not random, but determined by genes. The steps from DNA are called the *central dogma* and are the following

1. Transcription from DNA into RNA
2. Translation from RNA into proteins

Protein Synthesis: Transcription – in the nucleus

The process where a genes DNA is copied to make a RNA molecule. The transcription happens due to the transcription enzyme **RNA polymerase**. There are several RNA polymerases, each of them has a different function.

The steps of RNA transcription are:

1. **Initiation**

   RNA polymerase binds to a promoter region on the DNA strand. The promoter is found towards the 5‘ end of the template strand. In eukaryotes, the promoter usually consists of the **TATA box**

2. **Elongation**

   The RNA polymerase starts to move along the template strand (3‘ to 5‘) and along the way synthesises RNA. This is done by adding a nucleotide complementary to the one on the DNA, to the RNA strand.

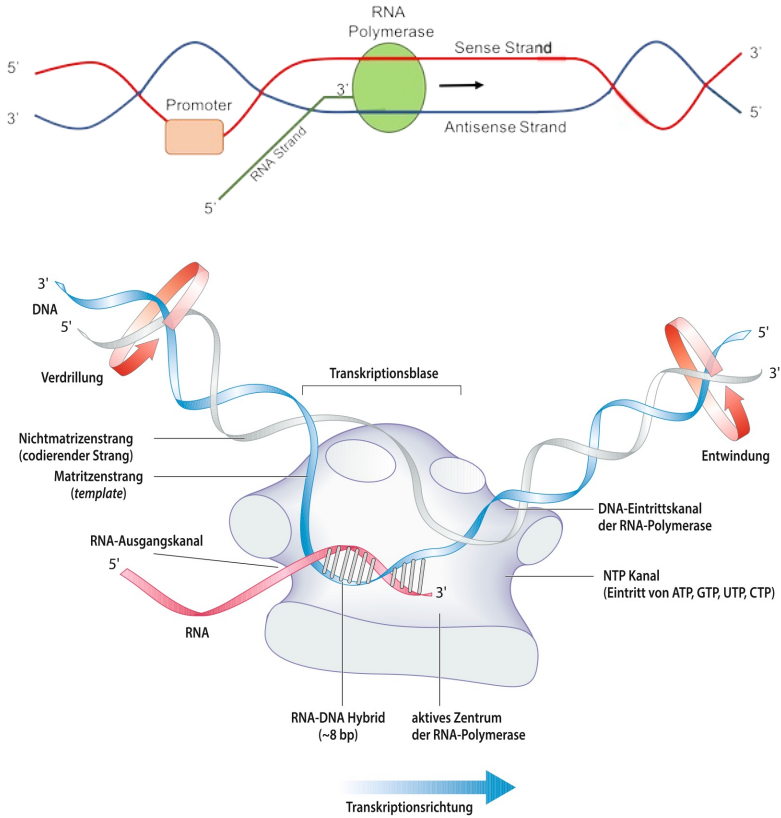
   However, when there is an A-base on the DNA template strand, the RNA polymerase complements it with **Uracil**.

3. **Termination**

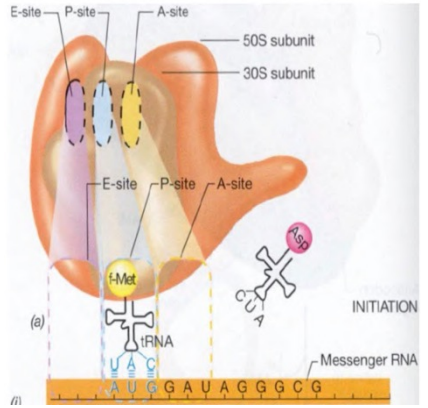
   RNA polymerase will keep transcribing until it gets signals to stop. The process of ending transcription is called **termination**, and it happens once the polymerase transcribes a sequence of DNA known as a **terminator**.

4. Modifications

   The now formed RNA undergoes a couple of post-transcriptional modifications, which usually help to stabilise the RNA strand.



Protein Synthesis: Translation – in the cytosol



The mRNA is exported from the nucleus into the cytosol. There ribosomes attach to the mRNA and start translating the mRNA into a protein. The translation again consists of three parts:

1. Initiation

First, the ribosomal subunits, 50S and 30S, are formed out of rRNA. The ribosome has three parts, the A, P and E site. The tRNA, loaded with an amino acid, enters the ribosomal complex at the A-site, then is pushed into the P-site, where the amino acid is added to the growing protein, and then the empty tRNA leaves the complex and the E-site.

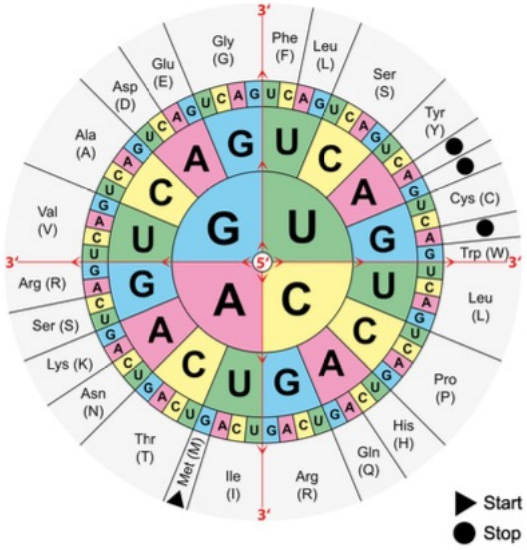
The ribosome binds to the *Shine-Dalgarno* sequence and then the riboosome “scans» the mRNA for the start codon AUG and once found, the translation is initiated. The first amino acid of every protein is Methionine (Met) and the tRNA bound to Met is also the final component of the initiation complex.

2. Elongation

After the first tRNA with the amino acid (Met) has reached the P-site, another tRNA enters the A-site. The only tRNA that enters and binds to the mRNA is the one with complementing RNA sequence. Once the tRNA has been bound in the A-site, the ribosome moves towards the 3’ end of the mRNA. The tRNA previously bound at the P-site is now in the E-site and will detach, whereas the A-site tRNA is now in the P-site. In the P-site, the amino acid from the tRNA will be attached to the amino acid chain by a hydrolysis reaction (slides from the first two weeks)

3. Termination

Once the ribosome has reached one of the stop codons, it detaches and soon after disintegrates into its parts. As soon as it is needed again, it will be reassembled and can be used again.



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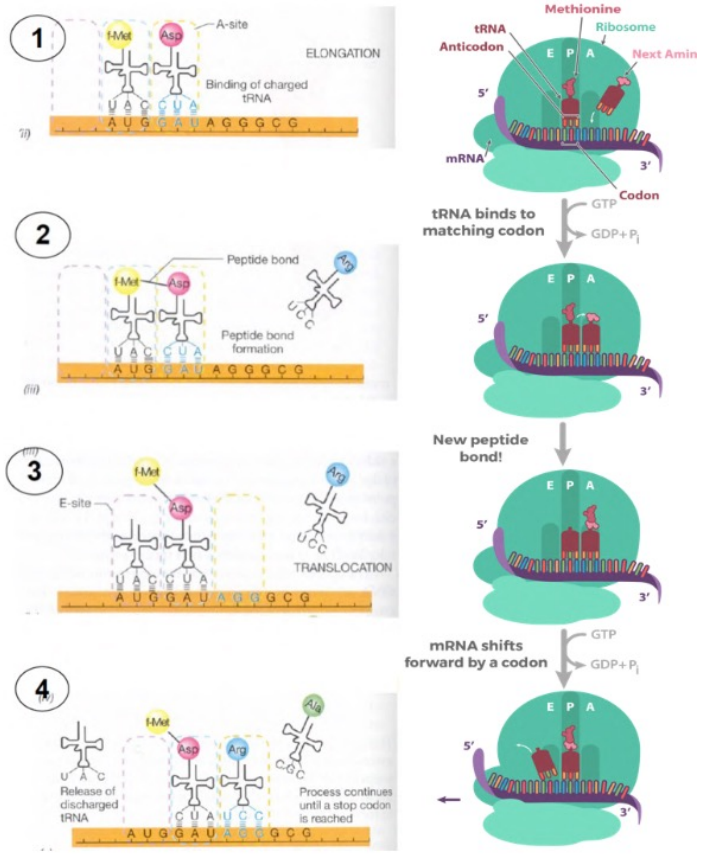
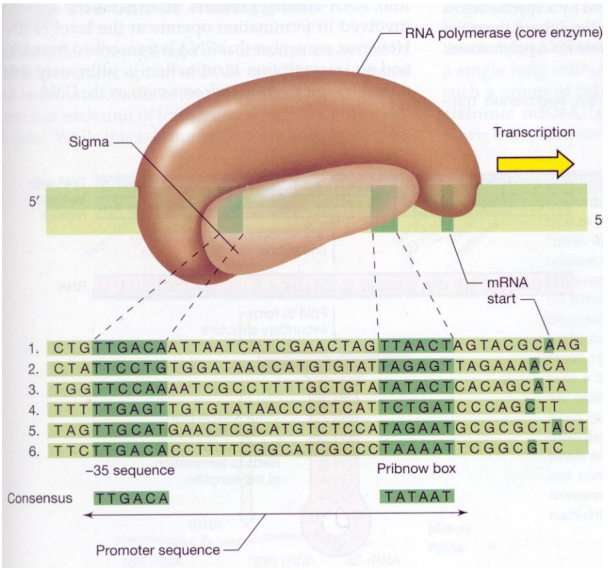
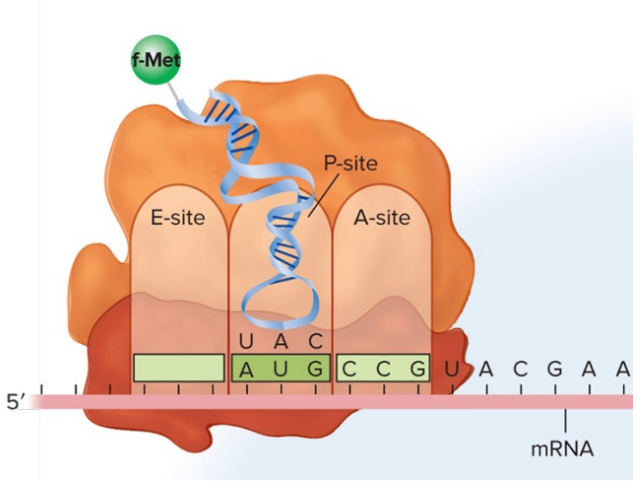


Protein Synthesis: Important players

- **Transcription**
  - *RNA polymerase*
    It consists of two subunits, one containing the core enzyme, which catalyses the synthesis of mRNA and the other being the sigma factor. The sigma factor is responsible for biding to the promoter regions on the DNA and also for detatching from the DNA once the mRNA has been transcribed.
- **Translation**
  - *Ribosome*
    Ribosomes are places of protein synthesis. They also consist of two subunits, 30S and 50S, which are made out of rRNA and proteins. Ribosomes provide the environment for the mRNA to be translated into a protein by tRNA binding.





Protein Synthesis

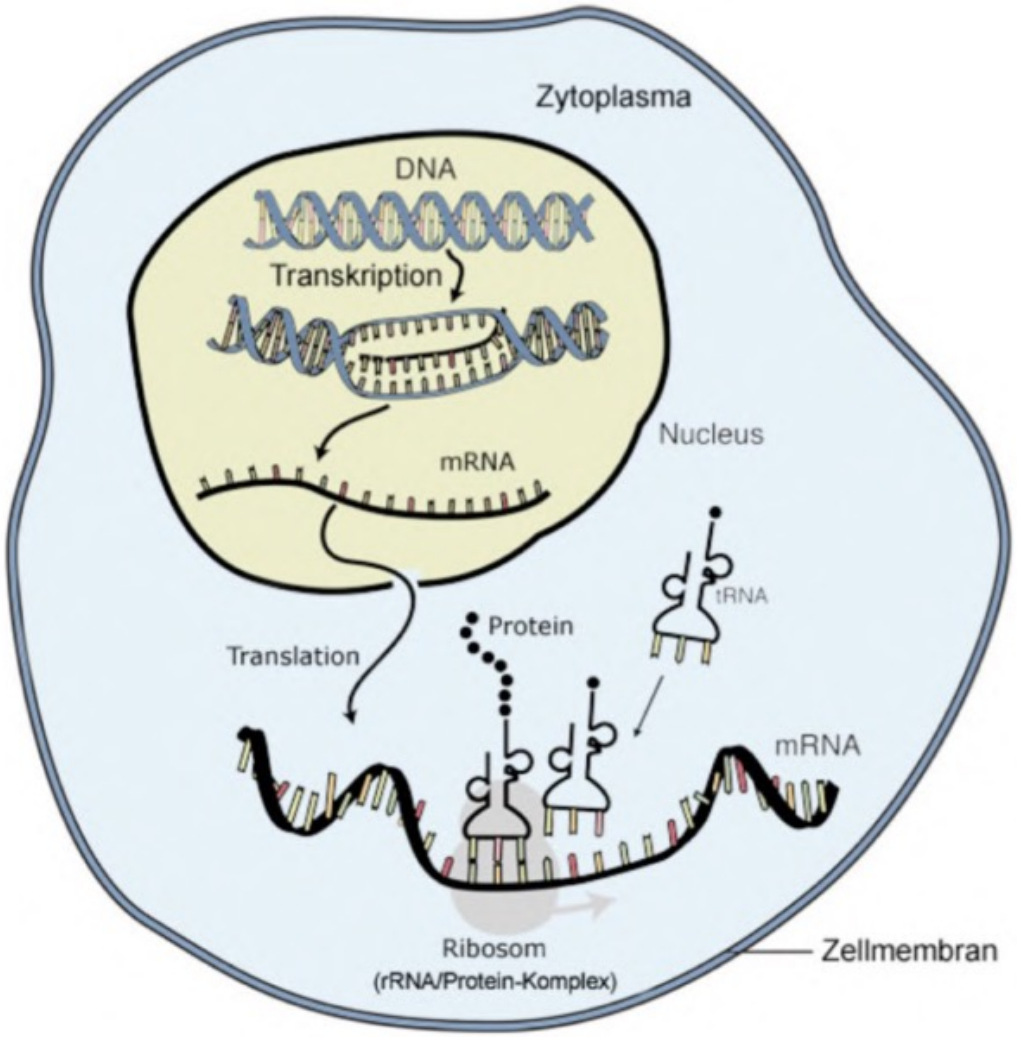


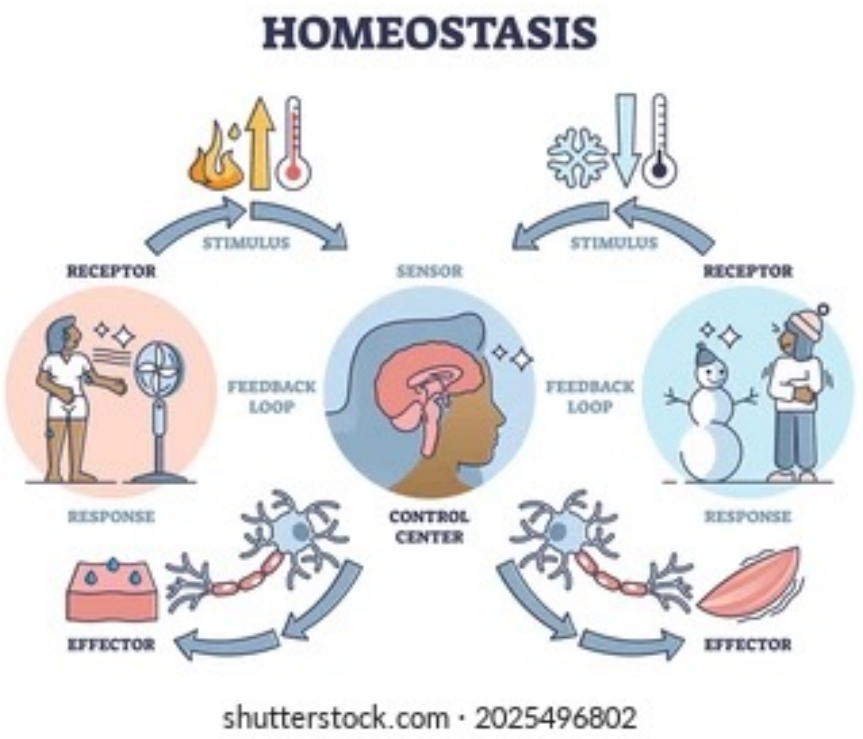
Table 14.2 Types of RNA

Type of RNA	Functions in	Function
Messenger RNA (mRNA) 	Nucleus, migrates to ribosomes in cytoplasm	Carries DNA sequence information to ribosomes
Transfer RNA (tRNA) 	Cytoplasm	Provides linkage between mRNA and amino acids; transfers amino acids to ribosomes
Ribosomal RNA (rRNA) 	Cytoplasm	Structural component of ribosomes

Regulation of Cell Metabolism

- In order to control the chemical reactions in the cell and to maximise the use of the resources to coordinate the processes within the cell, regulation is needed.
- Homeostasis is the self-regulation of a biological system in a dynamic equilibrium to protect against external changes. This is important for the cell survival, because most processes inside the cell happen at their highest velocity of efficiency at cellular conditions.
- The regulation of the cell metabolism happens through regulatory enzymes. These can affect the metabolism on two levels:
  1st metabolic level: regulation of enzymatic activity of the already synthesised enzyme. This serves to fine tune the metabolic processes and acts **fast**

  2nd metbolic level: **slow** regulation of the enzyme concentration by controlling the translation and transcription of the key enzymes.



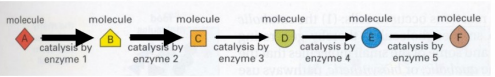
Michaelis-Menten-Kinetik (standard, kompetitiv)

$$r = \frac{r_{\max} \cdot c_S}{K_M + c_S} \qquad r = \frac{r_{max}\, c_S}{K_M\left(1 + \frac{c_I}{K_I}\right) + c_S}$$

$$r_{\max} = k_{+2} \cdot c_{E0}$$

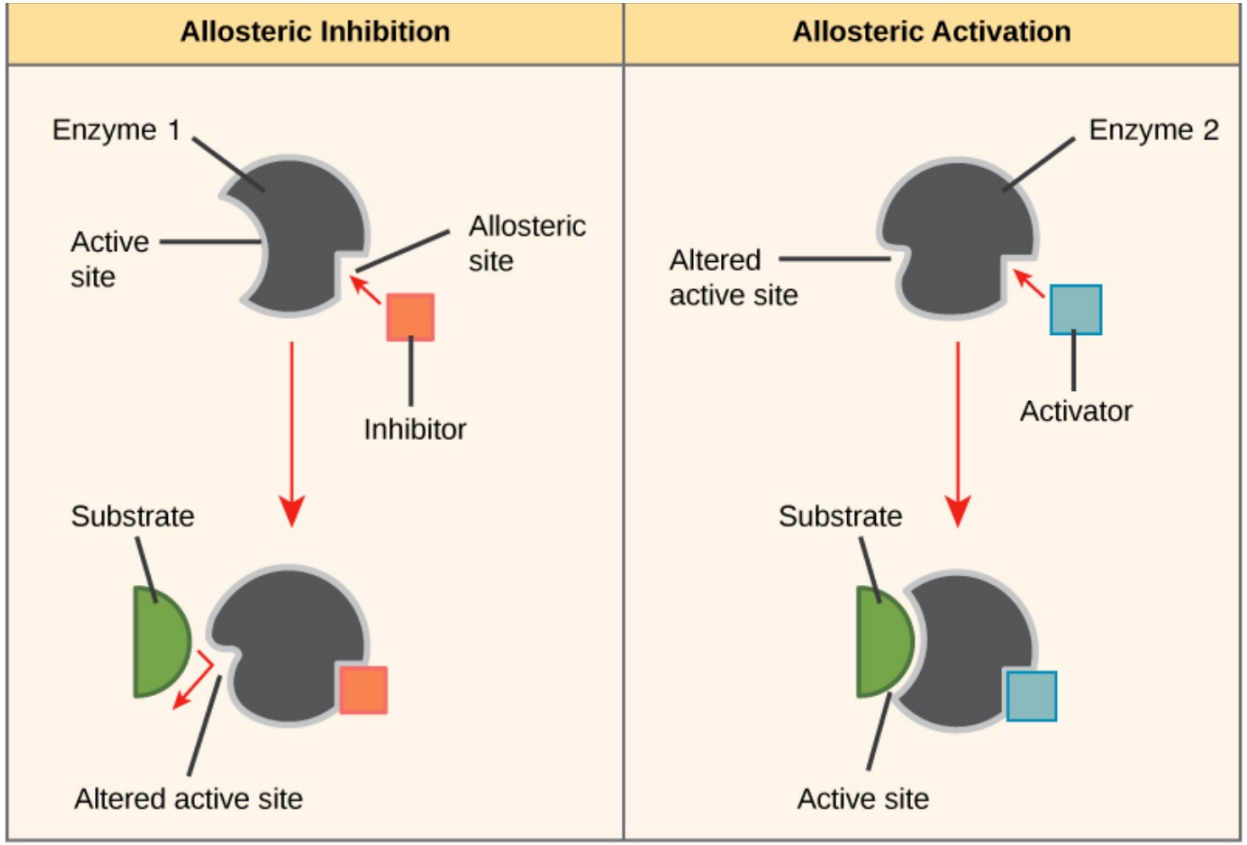
c_I, Inhibitorkonzentration, K_I, Inhibitorkonstante

Reaktionspfad



First Metabolic Level

- Allosteric enzymes are enzymes that are activated or inhibited, upon binding of a regulatory agent at the regulatory site. Once said agent has bound, the enzyme changes its conformation and is now able to fulfill its destined function, or inactive. Once the agent has detatched, the enzyme changes its conformation back into the initial state and is now deactivated.
- The effectors, the regulatory agents that bind to the enzyme, are the regulators of the enzyme activity.
- The curve of allosteric inhibition depends on the number of regulatory substrates that bind to the enzyme.



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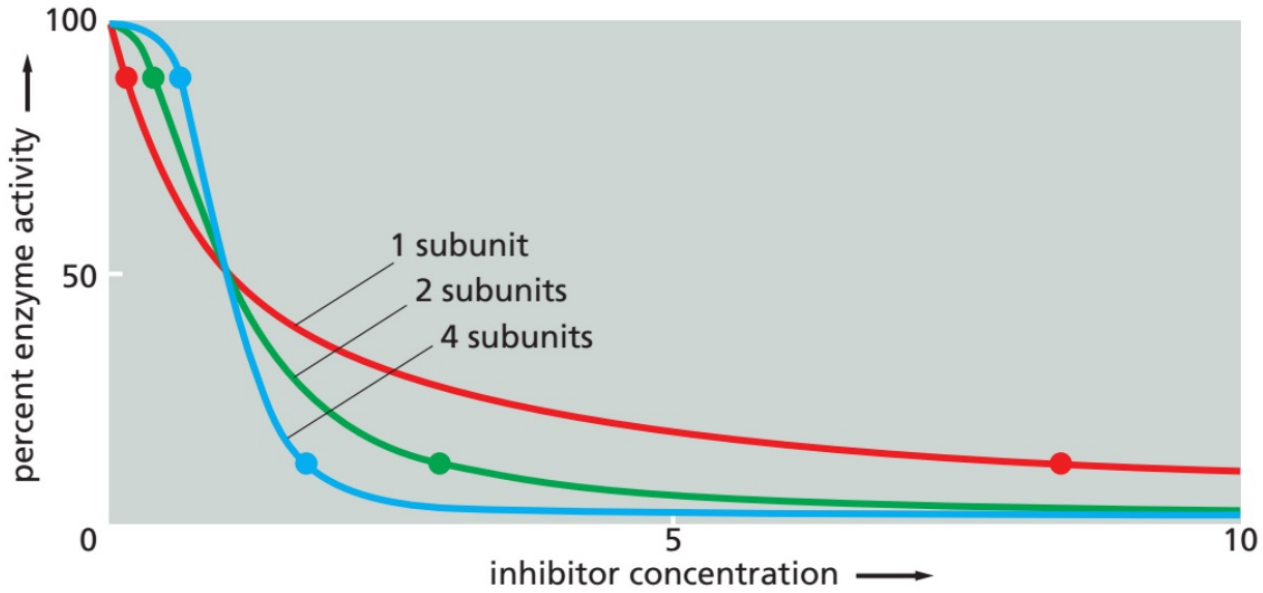
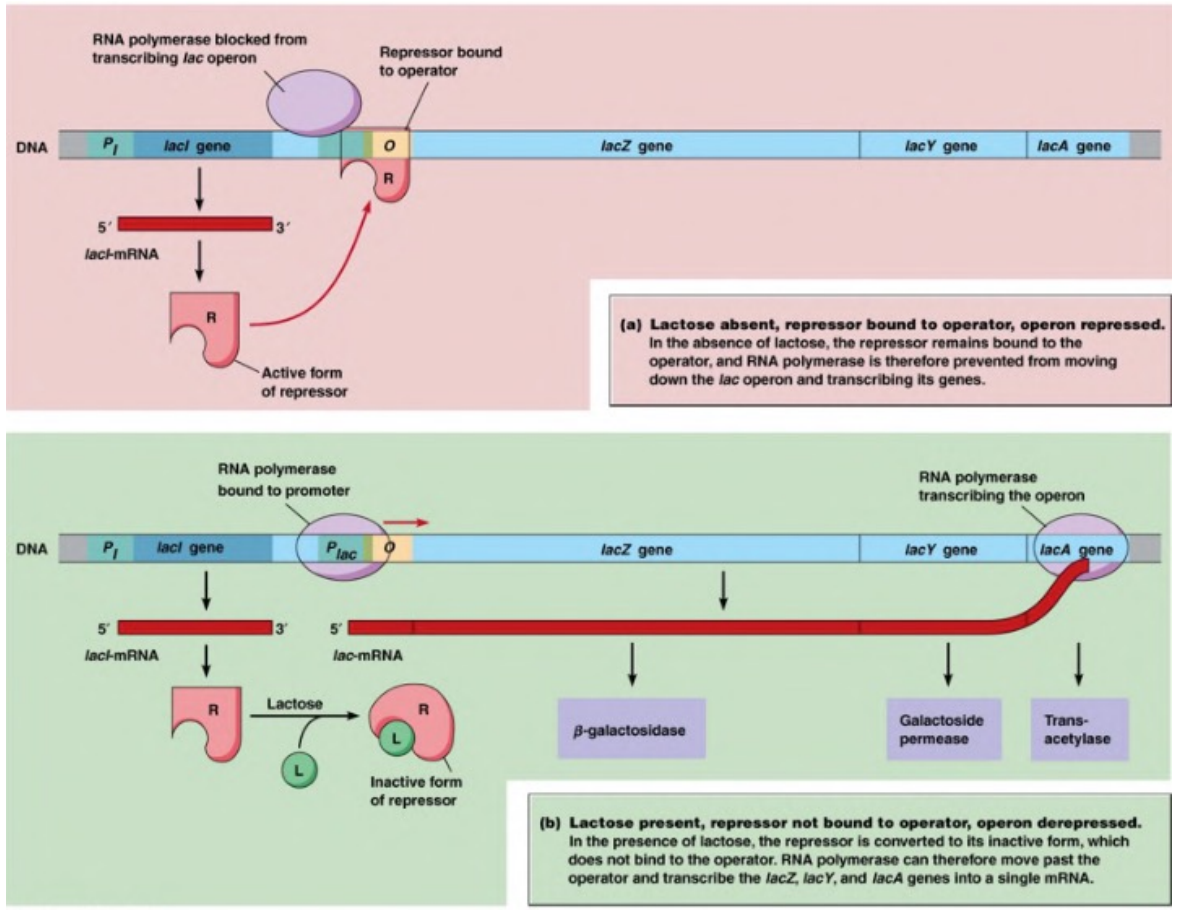


Second Metabolic Level - Operons

- Operons are complete units of gene expression. They contain:
  - **Multiple gene regions**
  - **Operator** – control elements to which regulatory proteins can bind and can control the operon.
  - **Promoter** – part where the RNA polymerase can dock onto and start the synthesis.
- On the DNA in front of the operon, there is also a regulatory gene.
- Operons can have two modes of action
  - Repression
  - Induction

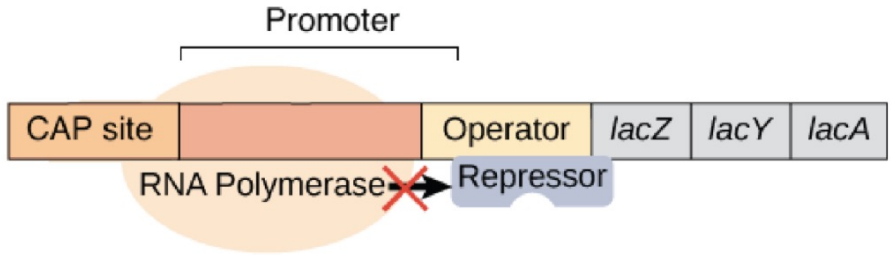


Repression – the transcription is ON by default

- The operon is transcribed if the repressor does not bind to the operator. As soon as a corepressor binds to the repressor, the repressor can bind to the operator and suppress the transcription. The mRNA and the corresponding protein cannot be synthesized.
  - Corepressors are usually end products of a biosynthesis (protein synthesis) or part of the nutrient supply.
- Enzyme repression takes place mainly in metabolic pathways that require many different enzymes (e.g. *biosynthesis of amino acids*). The repression is specific, only this biosynthetic pathway is affected. Enzyme repression is very important because it ensures that no energy is wasted for the synthesis of unused enzymes.
- One important example for repression in operons is the **lac operon**. The *lac* operon of *E. coli* contains genes involved in lactose metabolism. It's expressed only when lactose is present and glucose is absent. Two regulators turn the operon "on" and "off" in response to lactose and glucose levels: the *lac* repressor and catabolite activator protein (CAP). The ***lac* repressor** acts as a lactose sensor. It normally blocks transcription of the operon, but stops acting as a repressor when lactose is present.

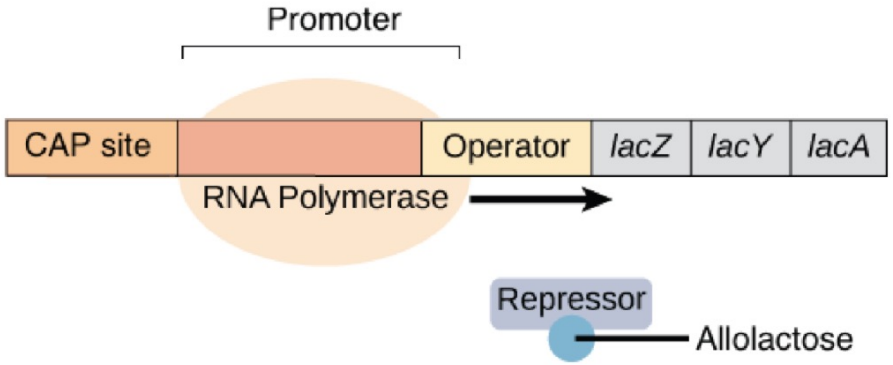
No lactose:

When lactose is absent, the *lac* repressor binds tightly to the operator. It gets in RNA polymerase's way, preventing transcription.



With lactose:

Allolactose (rearranged lactose) binds to the *lac* repressor and makes it let go of the operator. RNA polymerase can now transcribe the operon.



Induction - the transcription is OFF by default

- An inductor binds to activator, which can then dock to the DNA, which in turn enables the RNA polymerase to bind to the promoter, transcription happens.
- Example: regulation of maltose catabolism: Only when maltose is present and binds to activator can the activator bind to DNA and thus activate the synthesis of enzymes for the utilization of maltose
  - Maltose acts as an inductor.
  - Often happens that the molecule, that needs the enzymes produced by the operator in order to be broken down, acts as the inductor (to unblock or activate the enzyme production). So, it can activate only when is needed.