Ökophysiologie und Genetik von Mikroorganismen

19. März 2010

http://131.130.57.230/clarotest190/index.php?category=GENECWS09

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Major Modes of Regulation

1. **REGULATION OF ENZYME ACTIVITY**
   a) Noncovalent Enzyme Inhibition
   Feedback Inhibition, Isoenzymes
   b) Covalent Modification of Enzymes
   Adenylylation

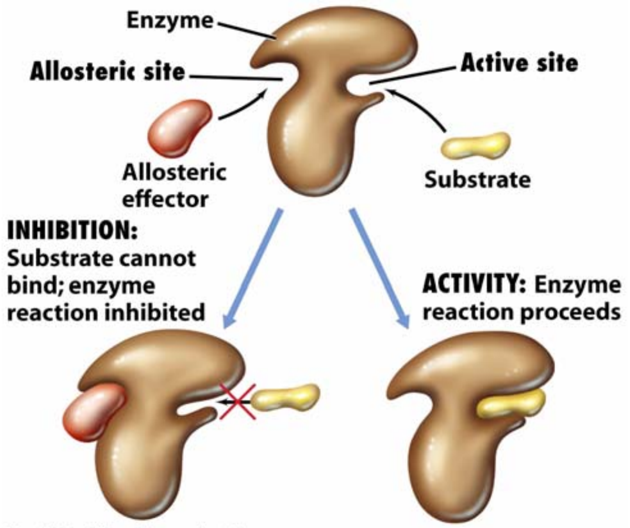
2. **REGULATION OF TRANSCRIPTION BY NEGATIVE AND POSITIVE CONTROL**
   a) Structure of DNA-Binding Proteins
   Helix-turn-helix, Leucin-Zipper, Zn-finger
   b) Negative Control of Transcription: Repression and Induction
   Example: ara and lac operon
   c) Positive Control of Transcription
   Example Maltose Catabolism in *Escherichia coli*

3. **GLOBAL REGULATORY MECHANISMS**
   - **a) Catabolite repression**
   - **b) The Stringent Response**
   - **c) Other Global Control Networks**
   - Alternative Sigma Factors

4. **OTHER MECHANISMS OF REGULATION**
   - **a) Attenuation**
   - **b) Anti-sense RNA**
   - **c) Riboswitches**
   - **d) Quorum sensing**

REGULATION OF ENZYME ACTIVITY

Enzyme inhibition by an allosteric effector



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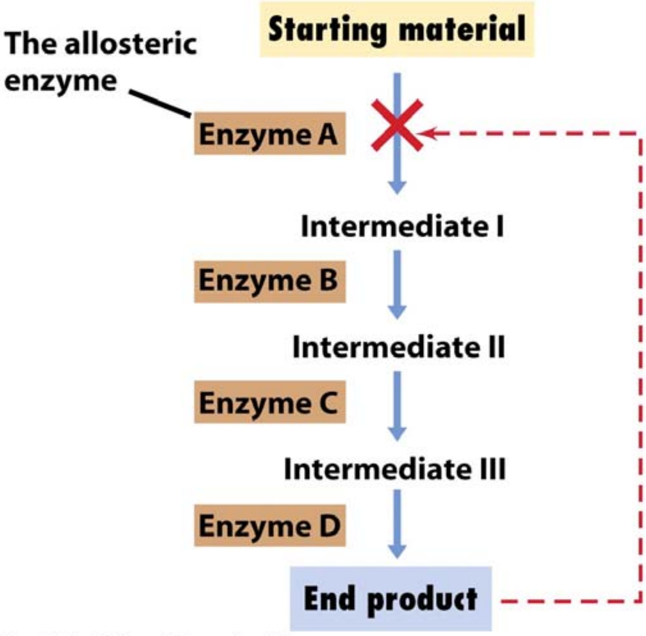

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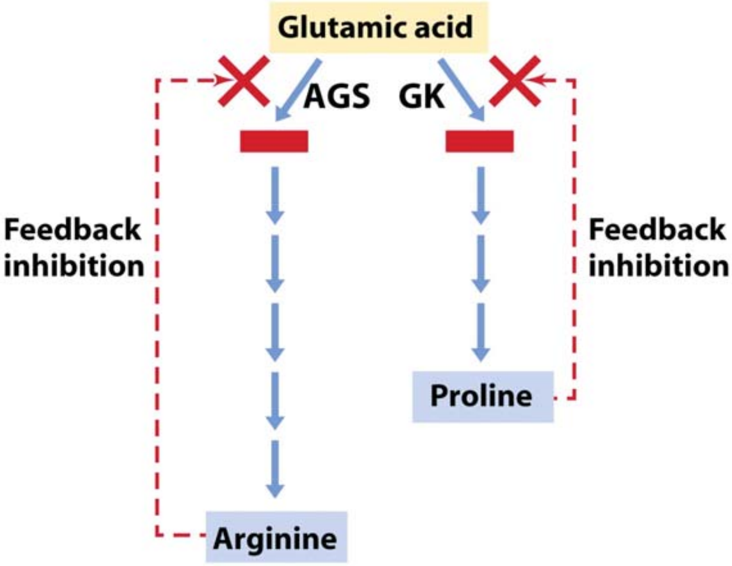
Allosteric Feedback inhibition in a branched biosynthetic pathway of amino acids



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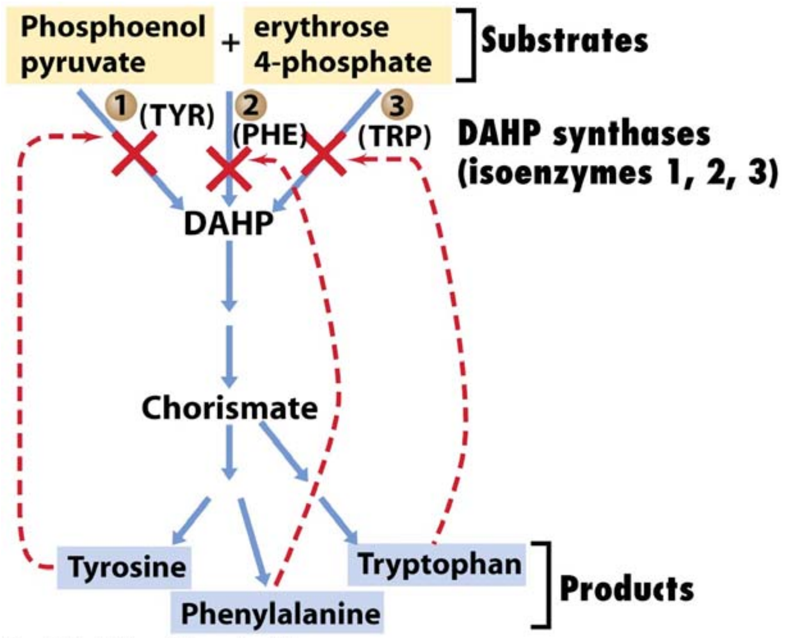
Isoenzymes



Phosphoenol pyruvate + erythrose 4-phosphate

Substrates

① (TYR) ② (PHE) ③ (TRP)

DAHP synthases (isoenzymes 1, 2, 3)

DAHP

Chorismate

Tyrosine

Phenylalanine

Tryptophan

Products

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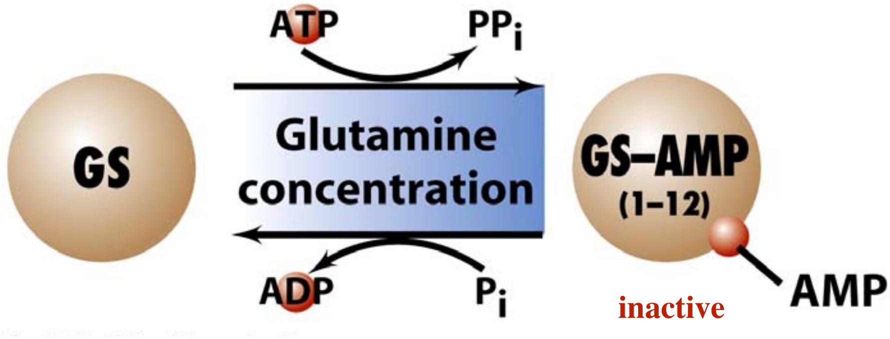
Covalent Modification of Enzymes:

Example Glutamine Synthetase and Adenylylation

Glutamine Synthetase: Rolle in NH_3 Assimilation



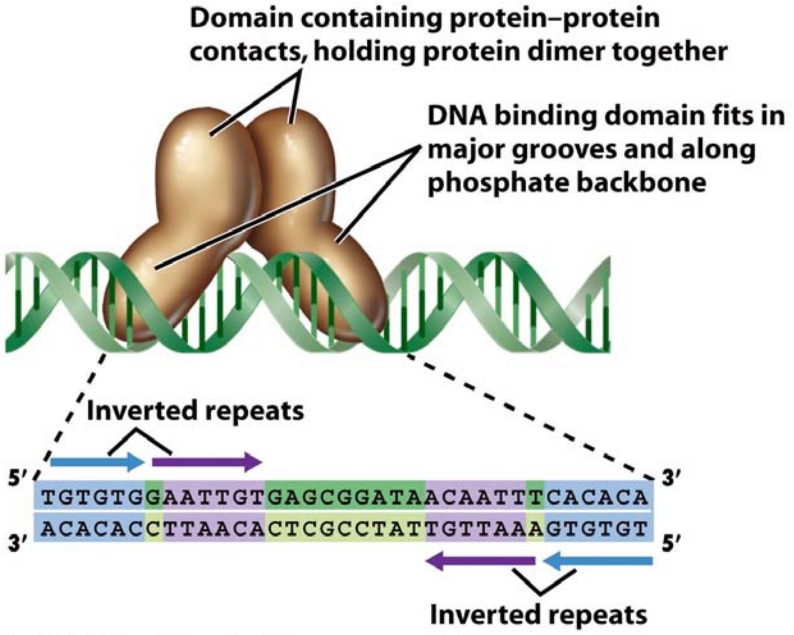
viel NH_3
Glutamin-Synthetase aktiv:

Glutamat + ATP + NH_3 => Glutamin + ADP + Pi

wenig NH_3
Glutamin-Synthetase adenylyliert

Covalent modification is a regulatory mechanism for changing the activity of an enzyme. Enzymes regulated in this way can be reversibly modified. One type of modification is adenylylation (the addition of AMP).

DNA-BINDING PROTEINS AND REGULATION OF TRANSCRIPTION BY NEGATIVE AND POSITIVE CONTROL



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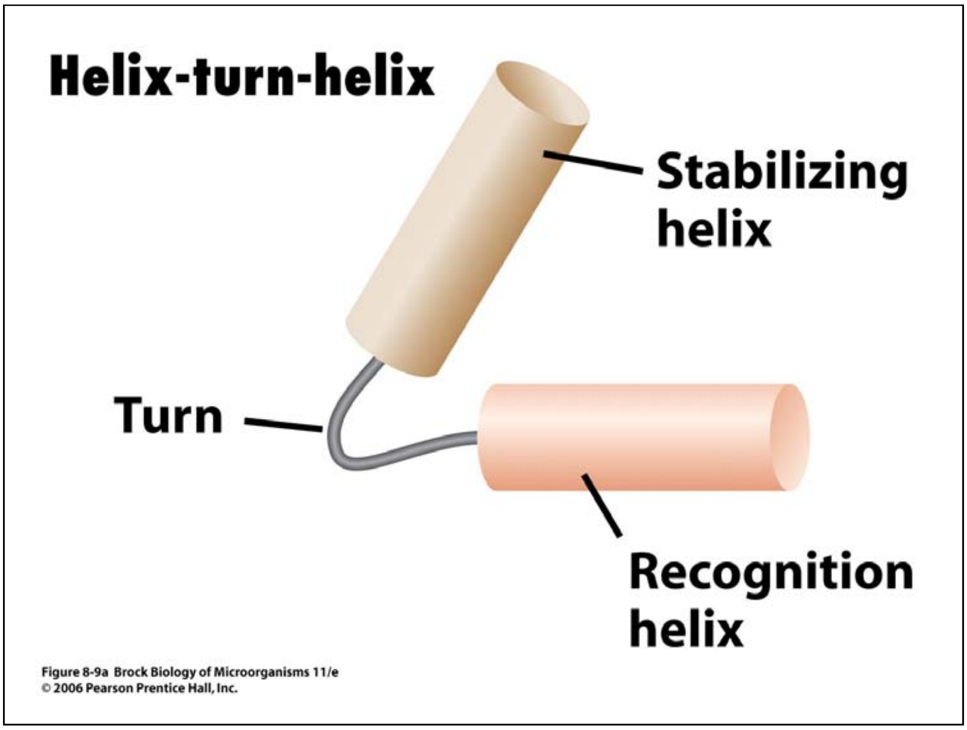

Helix-turn-helix

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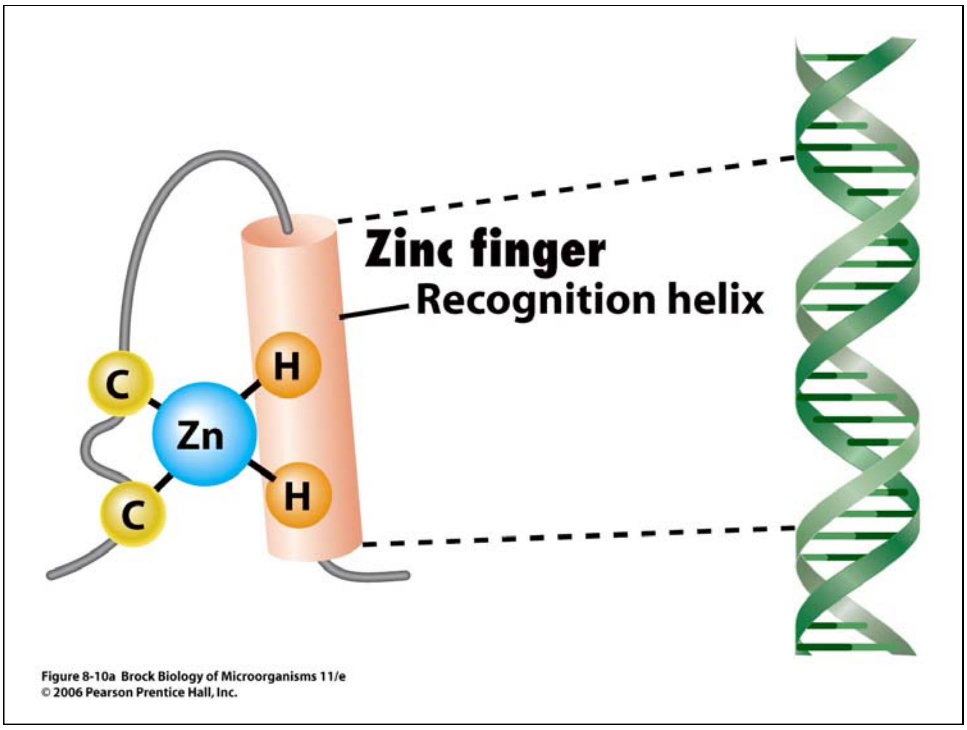

Zinc finger

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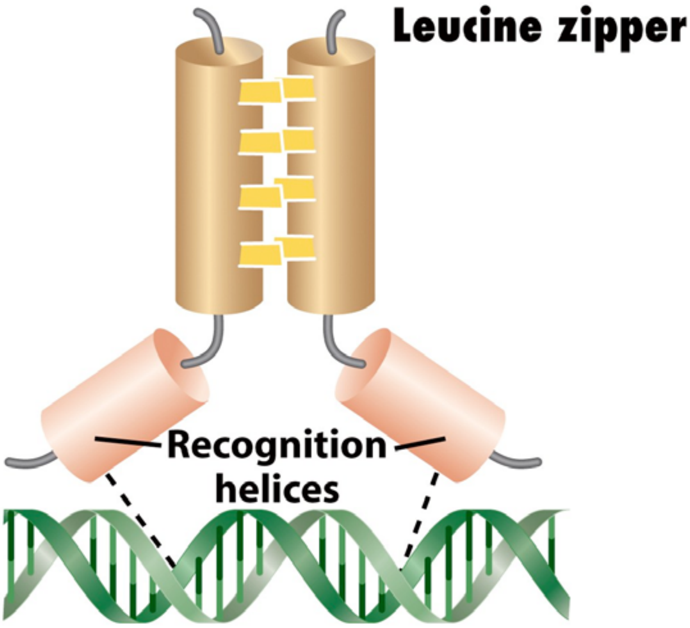

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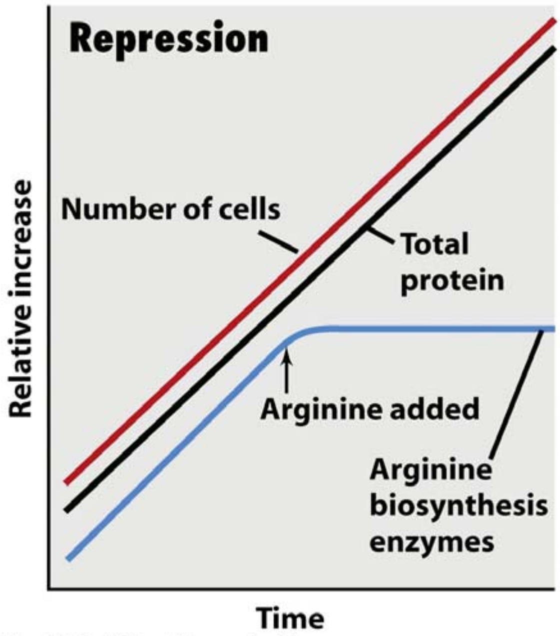
Negative control:

Enzyme Repression and Induction



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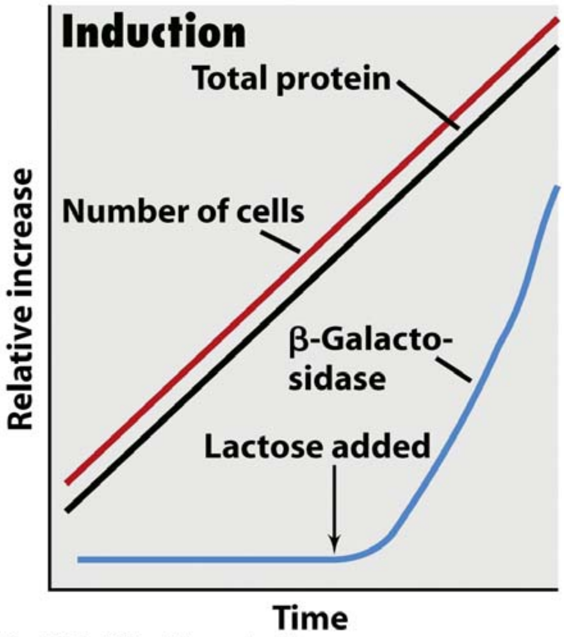

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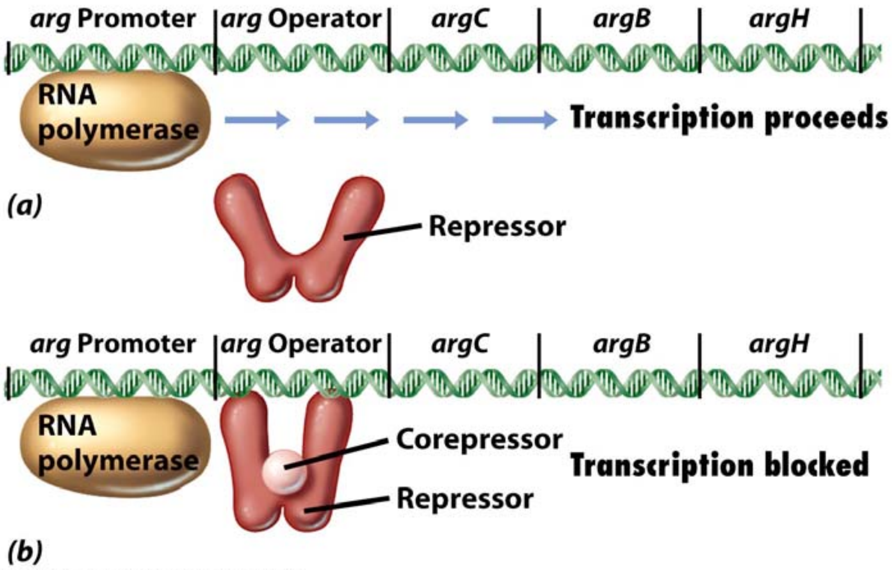
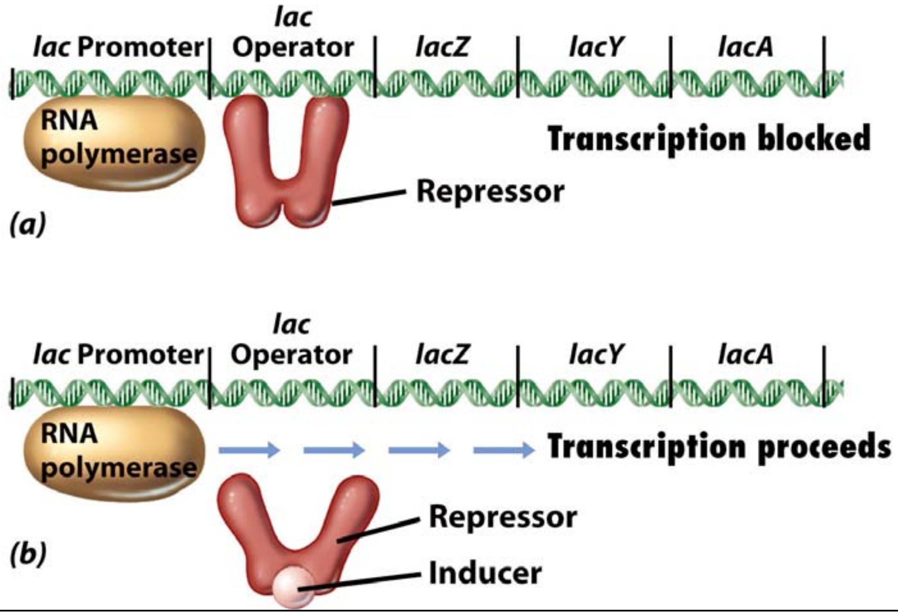
Repression of arginine biosynthesis



Induction

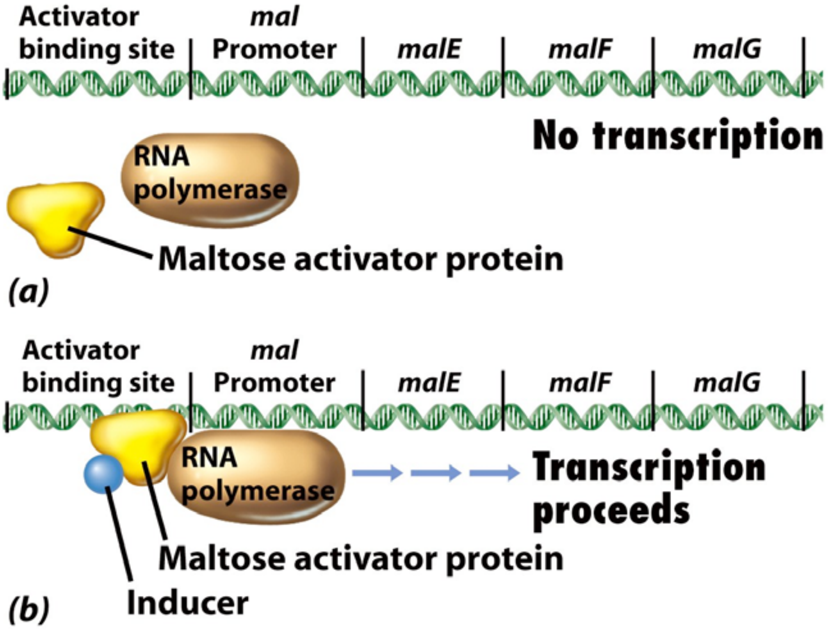


The amount of an enzyme in the cell can be controlled by increasing (induction) or decreasing (repression) the amount of mRNA that encodes the enzyme. This transcriptional regulation involves allosteric regulatory proteins that bind to DNA.

For negative control of transcription, the regulatory protein is called a repressor and it functions by inhibiting mRNA synthesis.

Positive Control of Transcription

Maltose Catabolism in *Escherichia coli*



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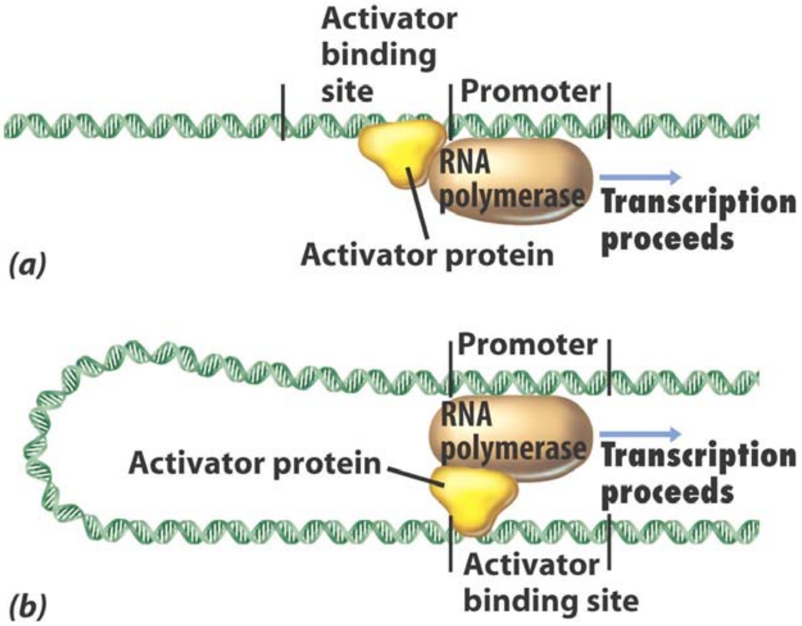

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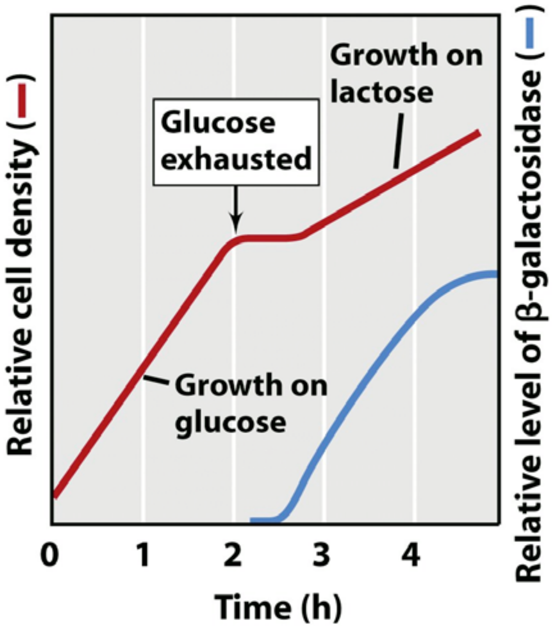
Positive regulators of transcription are called activator proteins. They bind to activator-binding sites on the DNA and stimulate transcription. As in repressors, activator protein activity is modified by effectors. For positive control of enzyme induction, the effector promotes the binding of the activator protein and thus stimulates transcription.

GLOBAL REGULATORY MECHANISMS

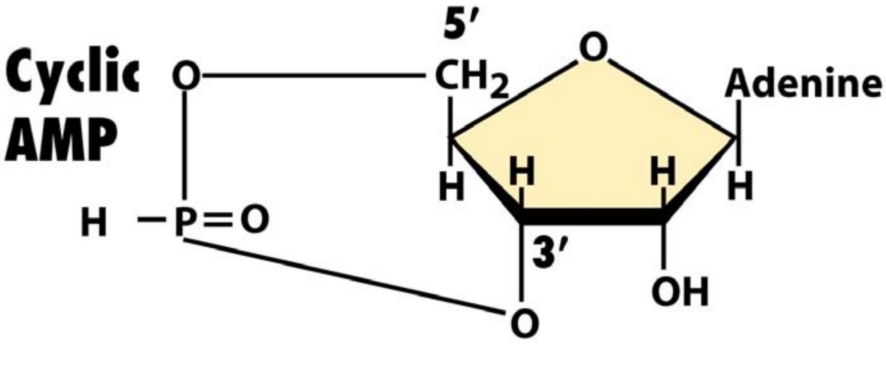
Catabolite Repression

Diauxie: growth on glucose and lactose



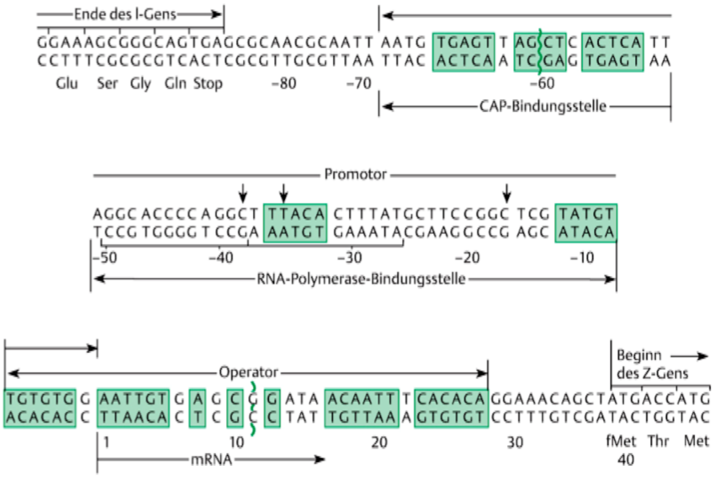
The best carbon source is used first

Cyclic Adenosinmonophosphate - cAMP

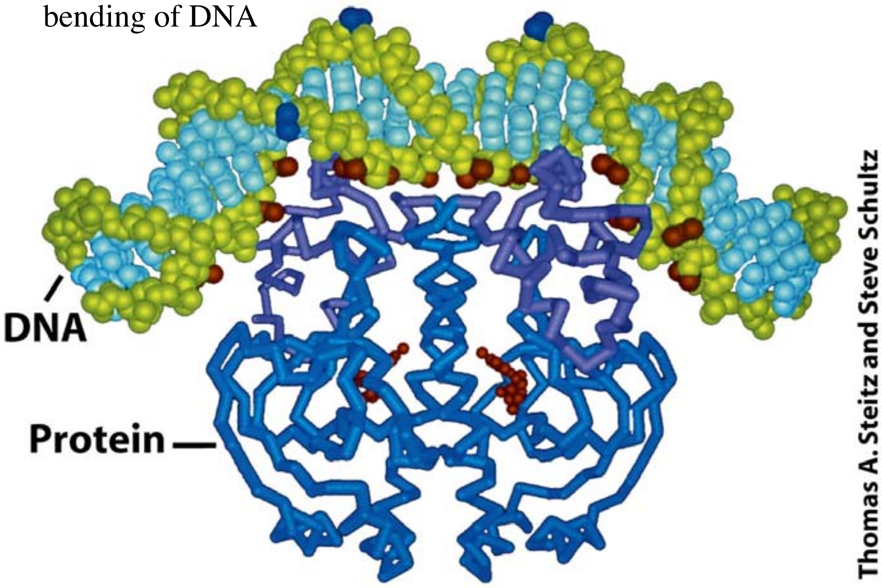


- -Produced by Adenylatcyclase
- -Inhibited by glucose
- -Glucose stimulates also transport of cAMP out of the cell

Kontrollelemente des Lac Operons



cAMP binding protein (catabolite activator protein) CAP; bending of DNA



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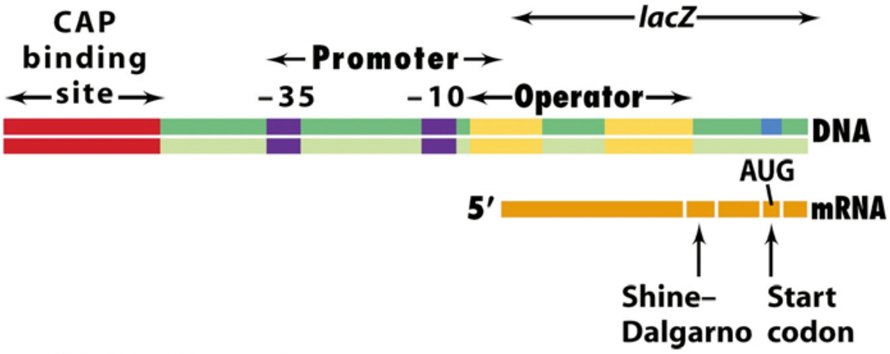
An allosteric protein

Das lacZ operon ist positiv und negativ reguliert!
CAP: positive Kontrolle
lacRepressor: negative Kontrolle



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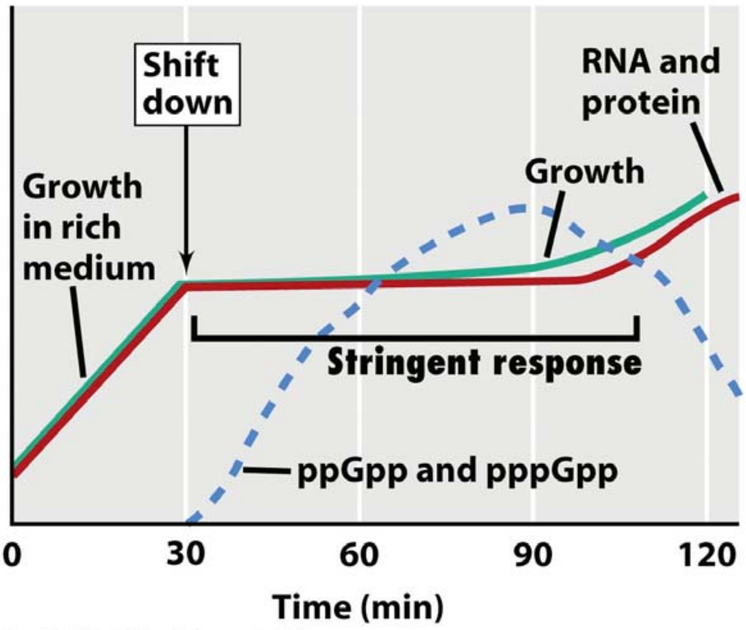
Global control systems regulate the expression of many genes simultaneously. Catabolite repression is a global control system, and it helps cells make the most efficient use of carbon sources. The *lac* operon is under the control of catabolite repression as well as its own specific negative regulatory system.

Another global control:
The Stringent Response



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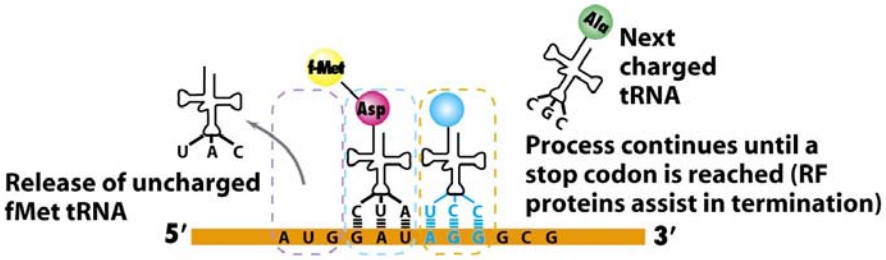
Recognition on the level of translation



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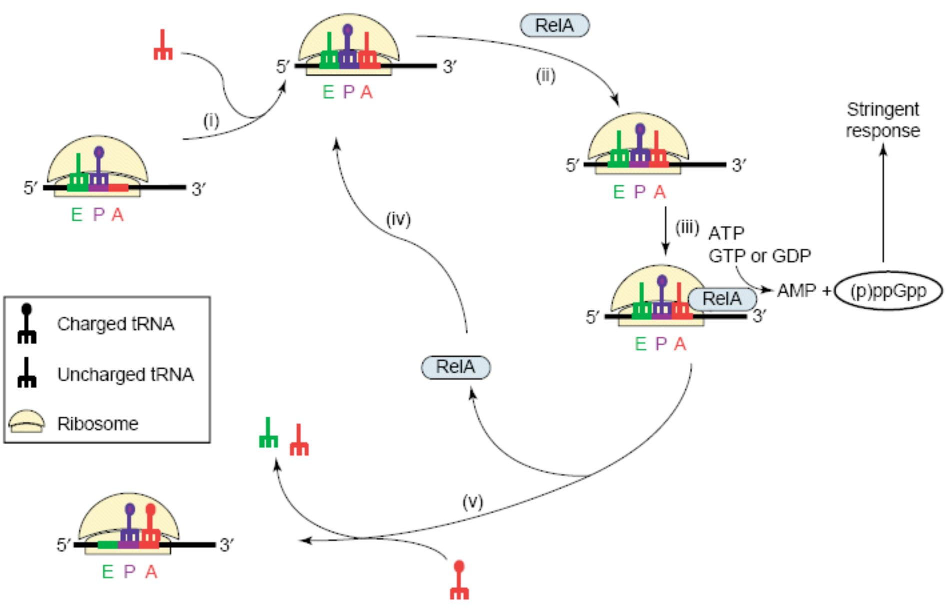
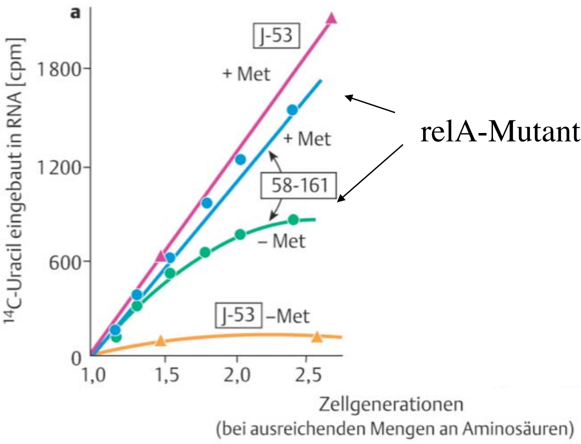


Stringente und relaxierte Kontrolle
Versuch mit einem Methionin-auxotrophen Stamm



relA-Mutante (relaxiert)

The stringent response is a global control mechanism triggered by amino acid starvation. The alarmones ppGpp and pppGpp (hyperphosphorylated nucleotides) are produced by RelA, a protein that monitors ribosome activity. The stringent response achieves balance within the cell between protein production and protein requirements. It is also employed to adjust to stress conditions and establishing symbiosis.

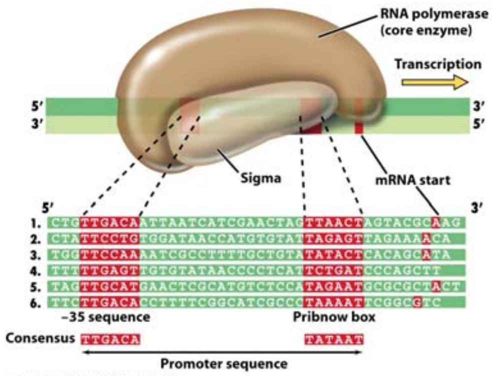
Other Global Control Networks

Regulation durch unterschiedliche Sigma-Faktoren

Konsensus-Sequenzen

	−35-Region		−10-Region
Standard-Promotor:	TCTTGAC	—16–18 bp —	TATAAT
Hitzeschock-Promotor:	CCTTGAA	—13–15 bp —	CCCCAT

Sigma 70
Sigma 32



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Alternative Sigma Factors

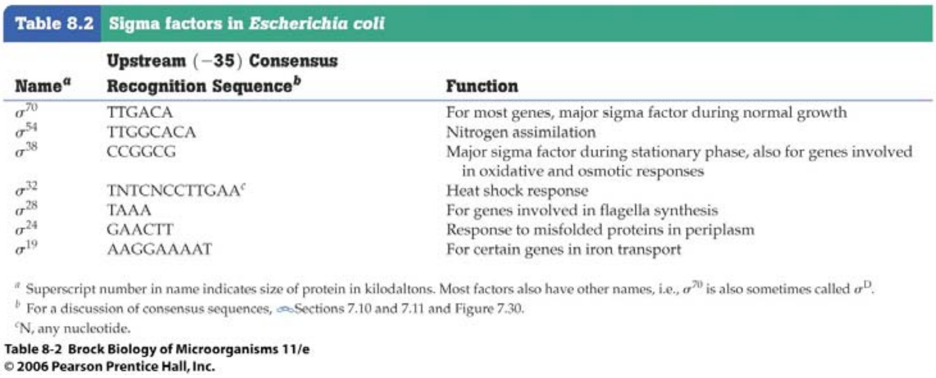

Table 8.2 Sigma factors in *Escherichia coli*

Name[a]	Upstream (−35) Consensus Recognition Sequence[b]	Function
σ^{70}	TTGACA	For most genes, major sigma factor during normal growth
σ^{54}	TTGGCACA	Nitrogen assimilation
σ^{38}	CCGGCG	Major sigma factor during stationary phase, also for genes involved in oxidative and osmotic responses
σ^{32}	TNTCNCCTTGAA[c]	Heat shock response
σ^{28}	TAAA	For genes involved in flagella synthesis
σ^{24}	GAACTT	Response to misfolded proteins in periplasm
σ^{19}	AAGGAAAAT	For certain genes in iron transport

[a] Superscript number in name indicates size of protein in kilodaltons. Most factors also have other names, i.e., σ^{70} is also sometimes called σ^{D}.

[b] For a discussion of consensus sequences, Sections 7.10 and 7.11 and Figure 7.30.

[c] N, any nucleotide.

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Cells can control several regulons by employing alternative sigma factors. These recognize only certain promoters and thus allow transcription of a select category of genes.

Some more global control systems known in *E. coli*

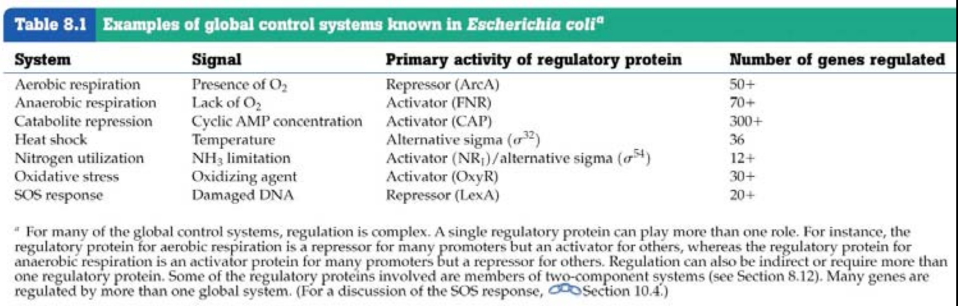

Table 8.1 Examples of global control systems known in *Escherichia coli*[a]

System	Signal	Primary activity of regulatory protein	Number of genes regulated
Aerobic respiration	Presence of O_2	Repressor (ArcA)	50+
Anaerobic respiration	Lack of O_2	Activator (FNR)	70+
Catabolite repression	Cyclic AMP concentration	Activator (CAP)	300+
Heat shock	Temperature	Alternative sigma (σ^{32})	36
Nitrogen utilization	NH_3 limitation	Activator (NR_I)/alternative sigma (σ^{54})	12+
Oxidative stress	Oxidizing agent	Activator (OxyR)	30+
SOS response	Damaged DNA	Repressor (LexA)	20+

[a] For many of the global control systems, regulation is complex. A single regulatory protein can play more than one role. For instance, the regulatory protein for aerobic respiration is a repressor for many promoters but an activator for others, whereas the regulatory protein for anaerobic respiration is an activator protein for many promoters but a repressor for others. Regulation can also be indirect or require more than one regulatory protein. Some of the regulatory proteins involved are members of two-component systems (see Section 8.12). Many genes are regulated by more than one global system. (For a discussion of the SOS response, Section 10.4.)

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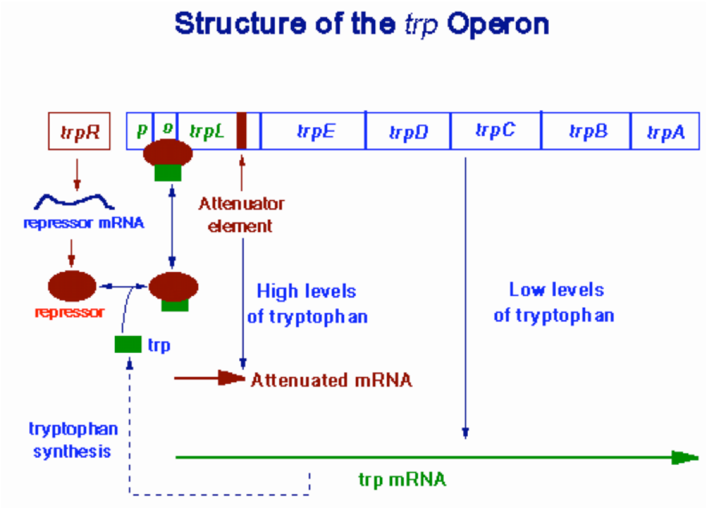
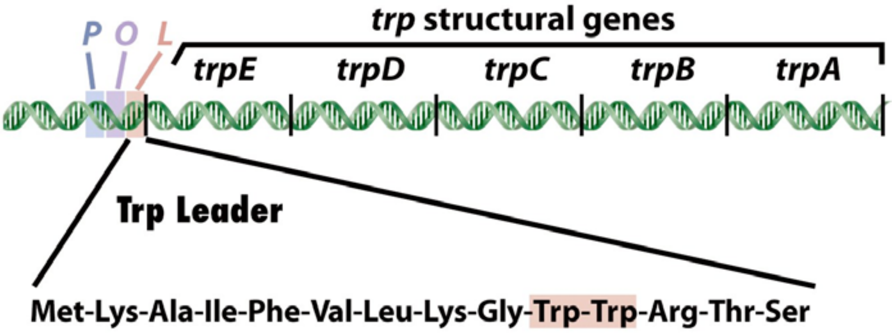
OTHER MECHANISMS OF REGULATION

Attenuation

Amino acid biosynthesis (Tryptophane)



1. Repression der Transkription: Repressor
2. Attenuation!



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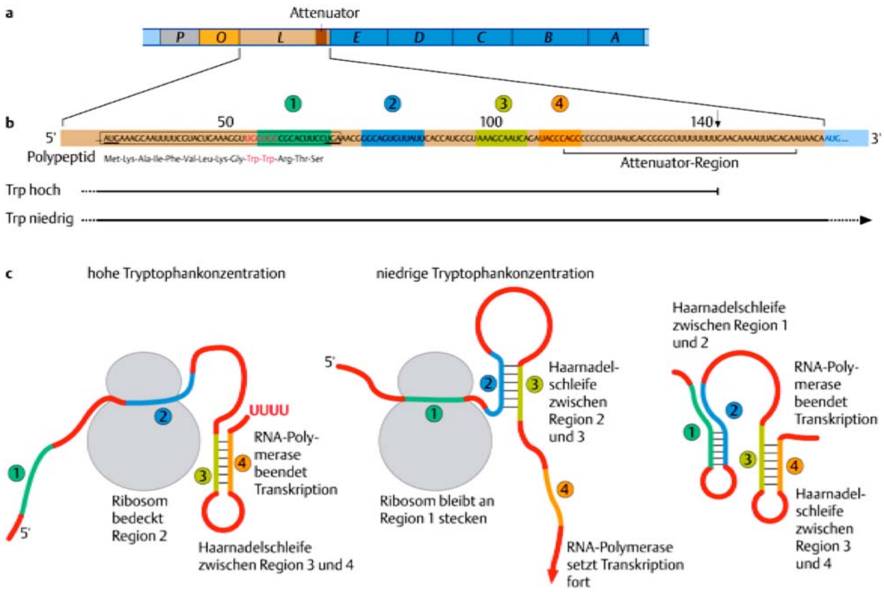
Attenuation der Transkription des trp Operons



Terminator

Antiterminator

Threonine	Met-Lys-Arg-Ile-Ser-Thr-Thr-Ile-Thr-Thr-Thr-Ile-Thr-Ile-Thr-Thr-Gly-Asn-Gly-Ala-Gly
Histidine	Met-Thr-Arg-Val-Gln-Phe-Lys-His-His-His-His-His-His-His-Pro-Asp
Phenylalanine	Met-Lys-His-Ile-Pro-Phe-Phe-Phe-Ala-Phe-Phe-Phe-Thr-Phe-Pro

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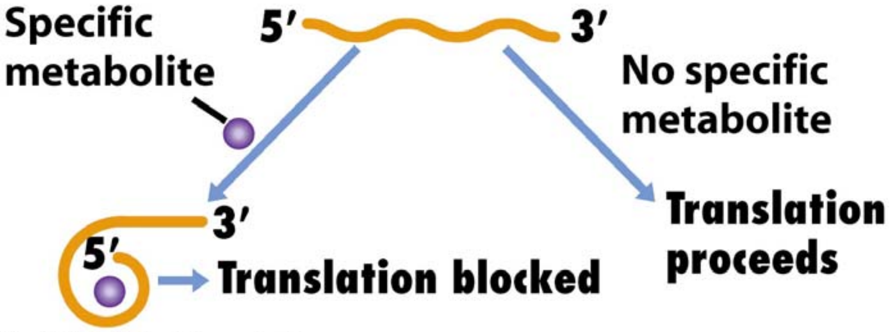
Attenuation is a mechanism whereby gene expression (typically at the level of transcription) is controlled *after* initiation of RNA synthesis. Attenuation mechanisms involve a coupling of transcription and translation and can therefore occur only in prokaryotes.

RNA Regulation and Riboswitches

Regulation durch Riboswitches



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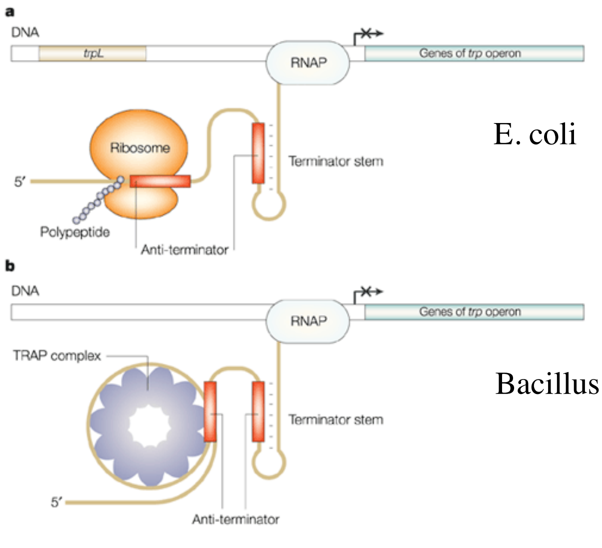
Regulation der Tryptophan-biosynthese in 2 verschiedenen Bakterien



Nature Reviews | Molecular Cell Biology

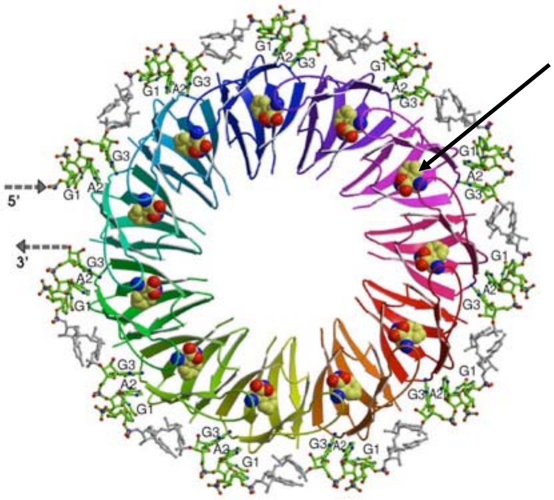
Ribosom als Sensor

E. coli

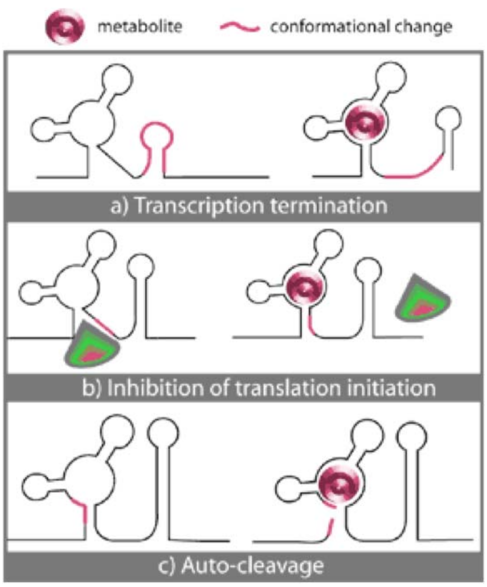
Protein als Sensor

Bacillus subtilis

TRAP protein



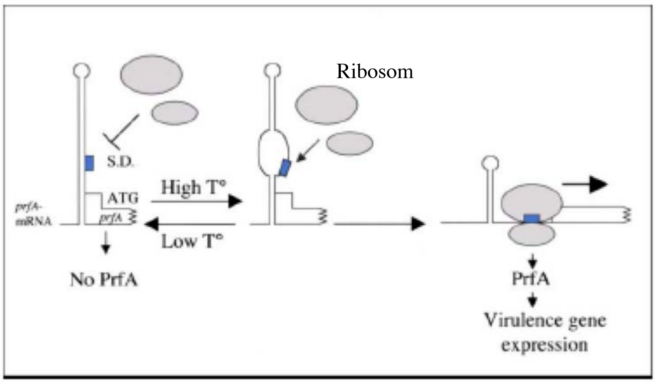
Each subunit binds tryptophane



Cell, Vol. 110, 551–561, September 6, 2002, Copyright ©2002 by Cell Press

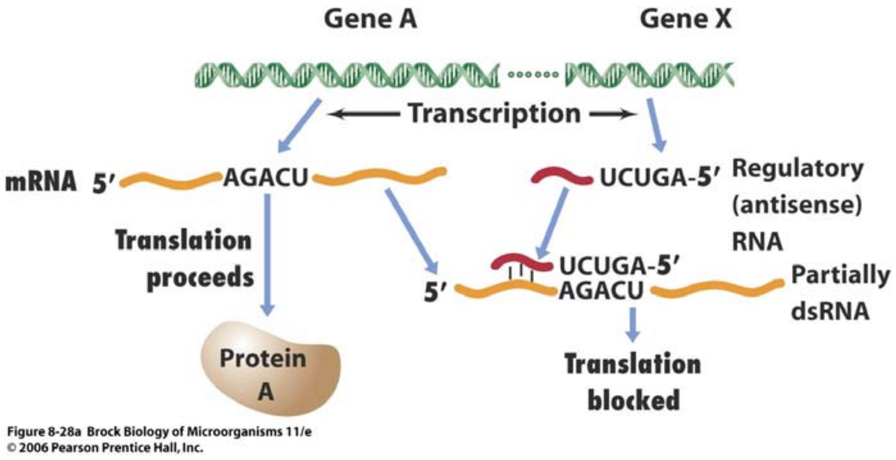
An RNA Thermosensor Controls Expression of Virulence Genes in *Listeria monocytogenes*

„molekulares Thermometer“



30°C 37°C 5-fach erhöhte Transkription

Regulation durch anti-sense RNA Moleküle



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