L06a

Recap

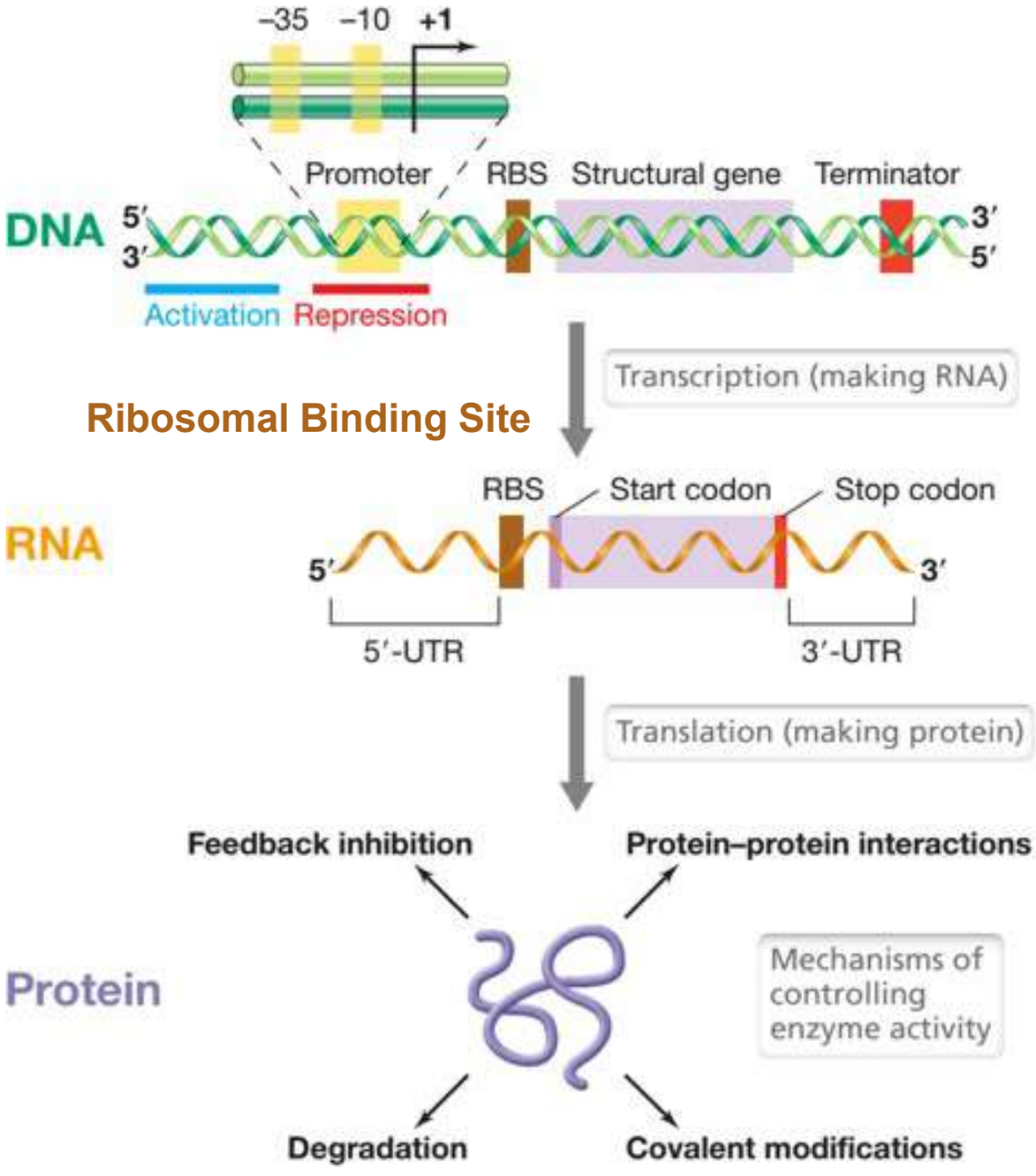
Microbial Regulatory Systems

- Regulatory system **couples growth with available resources**
- Some proteins and RNAs are needed in the cell at about the same level under all growth conditions: **constitutive expression**
- 2 major approaches to regulate protein function:

  A. Control protein **amount**

  B. Control protein **activity**

- *Amount of protein synthesized can be **regulated** at either the level of transcription, by varying the amount of mRNA made, at the level of translation, by translating or not translating the mRNA—> **gene expression***
- After the protein has been synthesized, **post-translational regulatory** processes



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Gene regulation within environmental context

- Microbes need to **adapt** to **changes** in environmental conditions in order to **survive**

- **Adaptation** requires to quickly express the **genes** necessary to **cope** with specific **environmental stimuli** and **maximize energy saving** in any conditions

- rRNAs, tRNAs, ribosomal proteins, RNA polymerases genes are essential —> always expressed —> **constitutive** expression

- Other genes whose activity is **regulated** (i.e. **activation**, **repression**) according to the need of the microbe in a **coordinate** fashion —> **OPERON** indicates a **cluster of genes** with **related functions** and regulated in a **coordinated** manner

RNA synthesis: Transcription_recap

- RNA polymerase (multicomplex enzyme)
- **σ recognizes the appropriate site on DNA** for transcription to begin (σ dissociates from holoenzyme once a short sequence of RNA has been formed)
- **Several σ**, most used σ^{70}
- **Several promoters** w. 2 highly conserved regions
- Upstream the transcription start site:

A. 10 bases upstream, the -10 region, or Pribnow box; consensus sequence of TATAAT

B. 35 bases upstream consensus sequence is TTGACA, -35 region

TABLE 4.3 Sigma factors in *Escherichia coli*

Name[a]	*Upstream recognition sequence*[b]	*Function*
σ^{70} RpoD	TTGACA	For most genes, major sigma factor for normal growth
σ^{54} RpoN	TTGGCACA	Nitrogen assimilation
σ^{38} RpoS	CCGGCG	Stationary phase, plus oxidative and osmotic stress
σ^{32} RpoH	TNTCNCCTTGAA	Heat shock response
σ^{28} FliA	TAAA	For genes involved in flagella synthesis
σ^{24} RpoE	GAACTT	Response to misfolded proteins in periplasm
σ^{19} FecI	AAGGAAAAT	For certain genes in iron transport

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Jacob F, Perrin D, Sanchez C, Monod J (1960) L'operon: Groupe de genes a l'expression coordonne par un operateur. C R Acad Sci 245: 1727–729

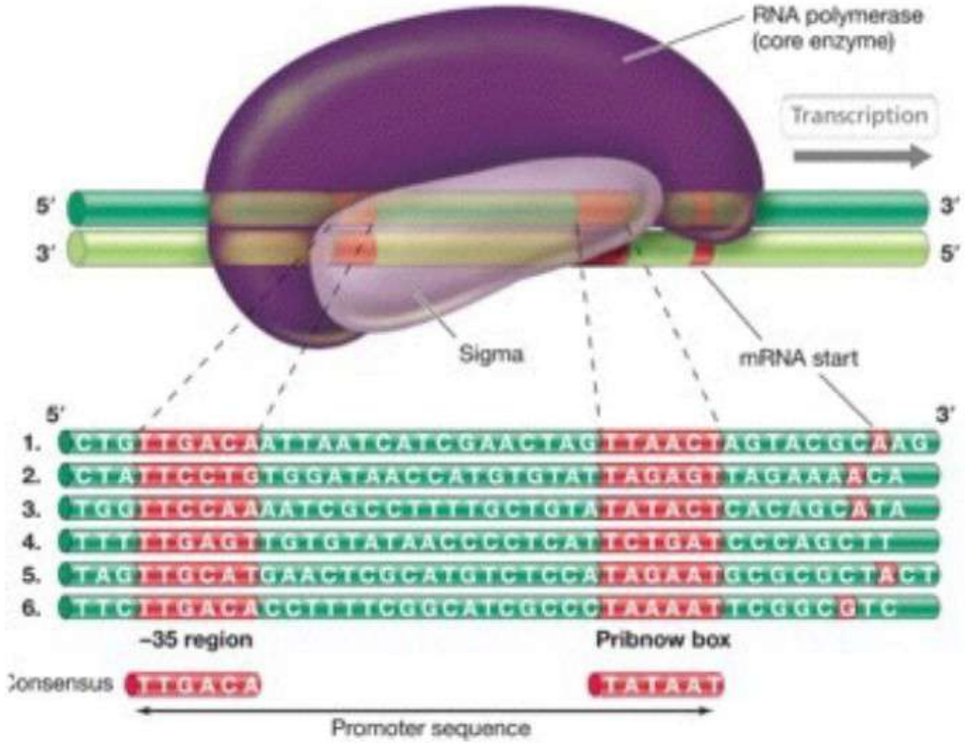
Jacob F, Monod J (1961) On the regulation of gene activity. In: Cold Spring Harbor Symposium Quantitative Biology 26, pp 193–211

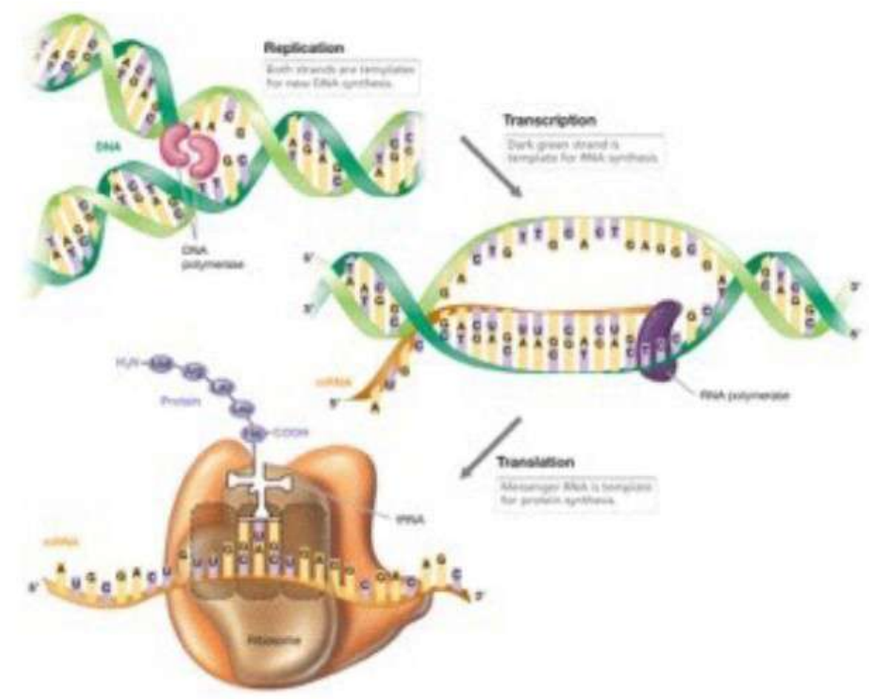
OPERON STRUCTURE: PROG: promoter, repressor, operator and genes

https://youtu.be/10YWgqmAEsQ

RNA synthesis: Transcription_recap II

- Transcription begins at a unique base just downstream from -35 and the Pribnow box
- **Sigma recognizes the promoter sequences on the 5′—>3′ (dark green) strand of DNA**
- **RNA polymerase** core enzyme will actually transcribe the light green strand (that runs 3′—>5′) b/c **core enzyme synthesizes 5′—>3′ direction**





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The Nobel Prize in Physiology or Medicine 1965

Photo from the Nobel Foundation archive.

François Jacob

Prize share: 1/3

Photo from the Nobel Foundation archive.

André Lwoff

Prize share: 1/3

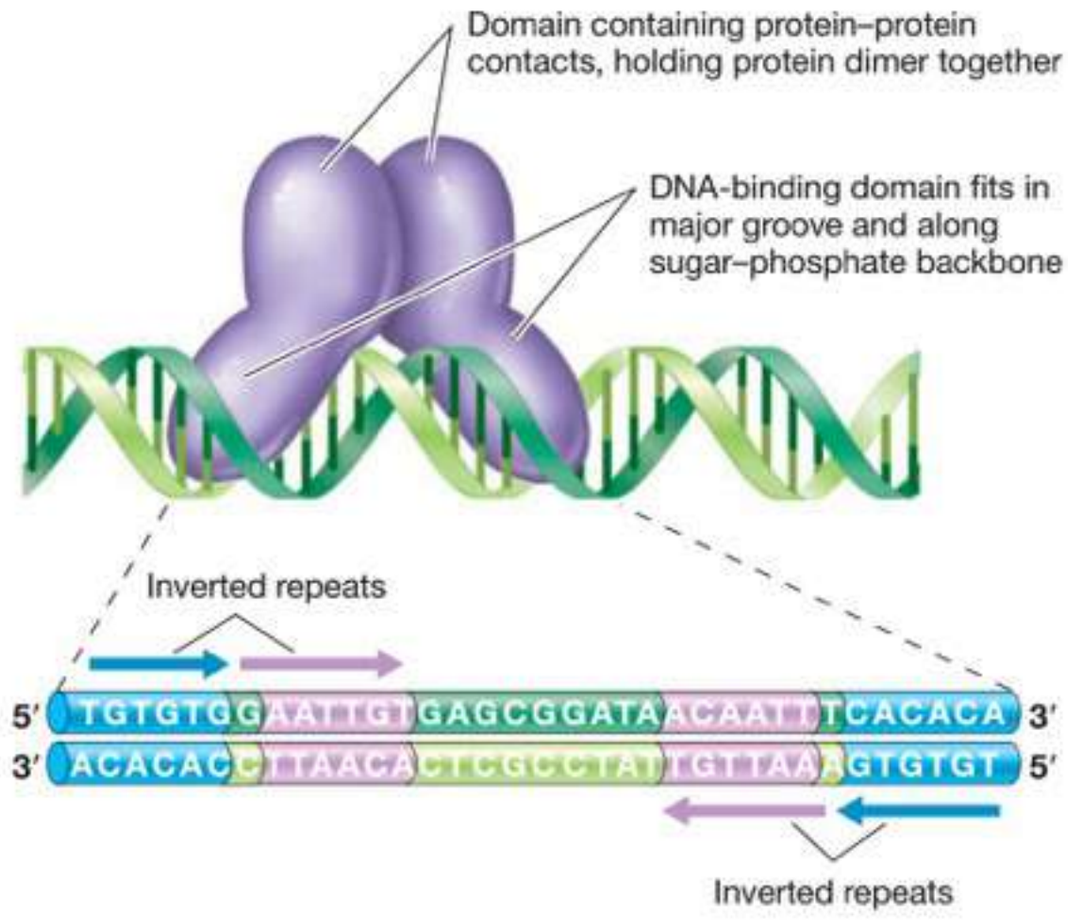
Photo from the Nobel Foundation archive.

Jacques Monod

Prize share: 1/3

DNA-Protein Interaction: Regulation

- For a gene to be transcribed: **RNA pol & σ must recognize a specific promoter site on the DNA**
- **Regulatory proteins influence protein binding** to specific DNA sites —> gene expression by turning transcription on or off
- **Protein-nucleic acid interactions are central to replication, transcription, translation, their regulation**
- **DNA-binding proteins are often homodimeric, 2 identical polypeptide subunits w. domains** (= regions of the protein with a specific structure and function)



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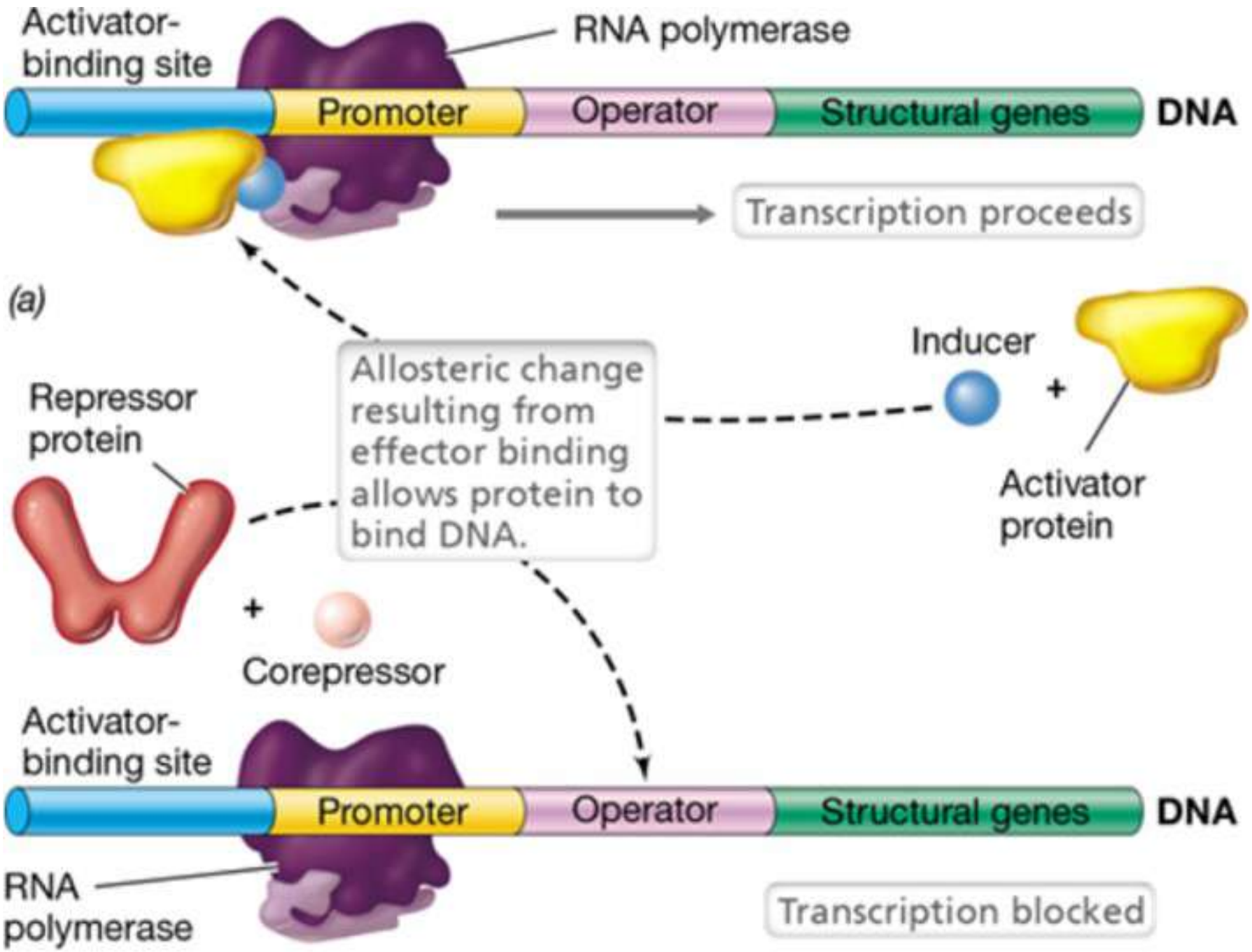
Transcription factors: DNA-protein interactions



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Binding of effector molecules to activator and repressor proteins results in an allosteric change that affects the DNA-binding ability of the transcription factors. *(a)* Binding of an activator to the DNA results in recruiting RNA polymerase and turning transcription on. *(b)* Binding of a repressor protein to the operator region of the DNA results in blocking RNA polymerase and turning transcription off.

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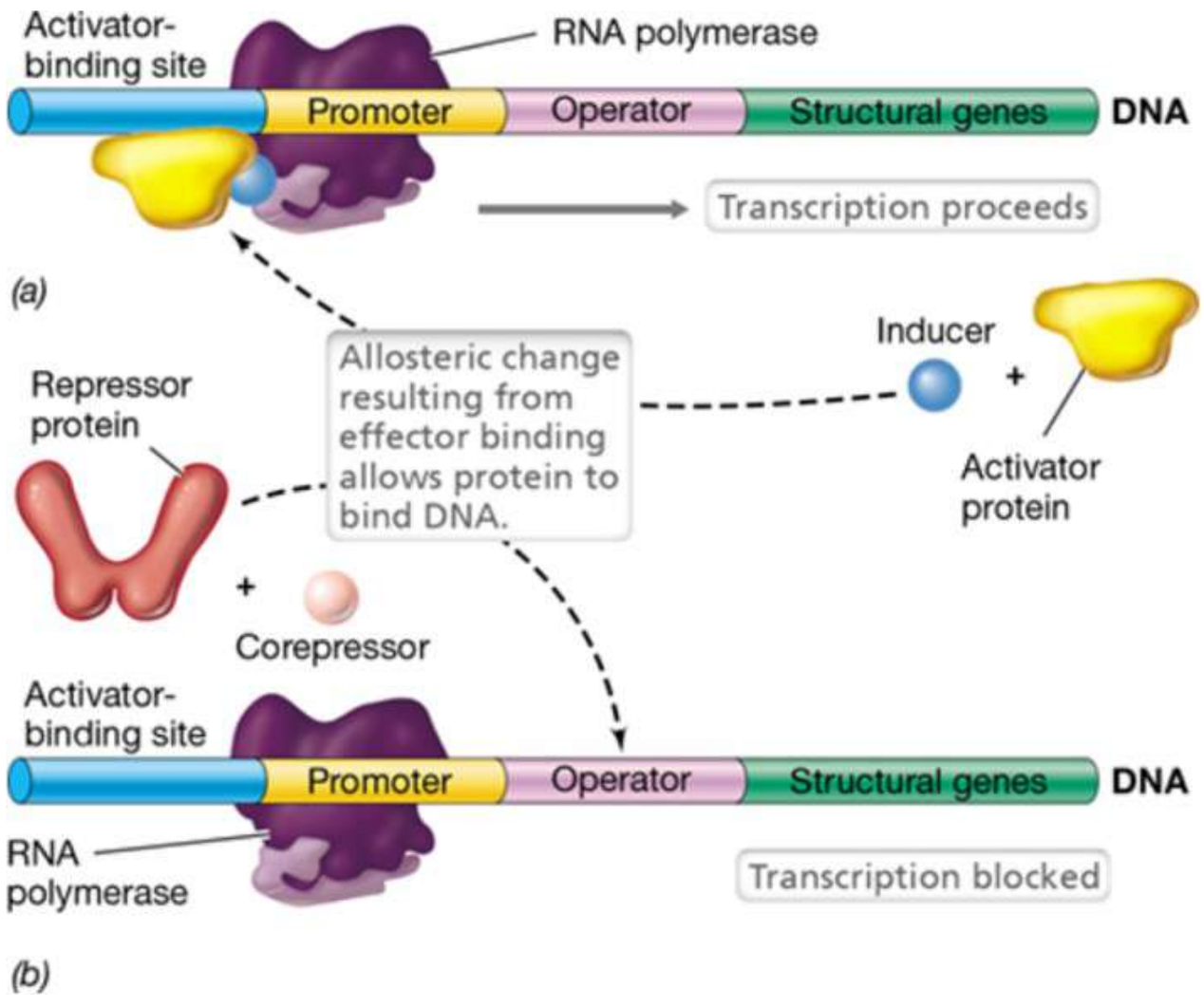
OPERON STRUCTURE

Promoter: RNA-σ binding site (Promoter sequences are DNA sequences that define where transcription of a gene by RNA polymerase begins)

Repressor

Operator: Repressor binding site

Genes



Positive control

Activator proteins help RNA polymerase to recognize promoter site

Negative control

Change in 3D structure of repressor favor (REPRESSION) or prevent (INDUCTION) binding to operator

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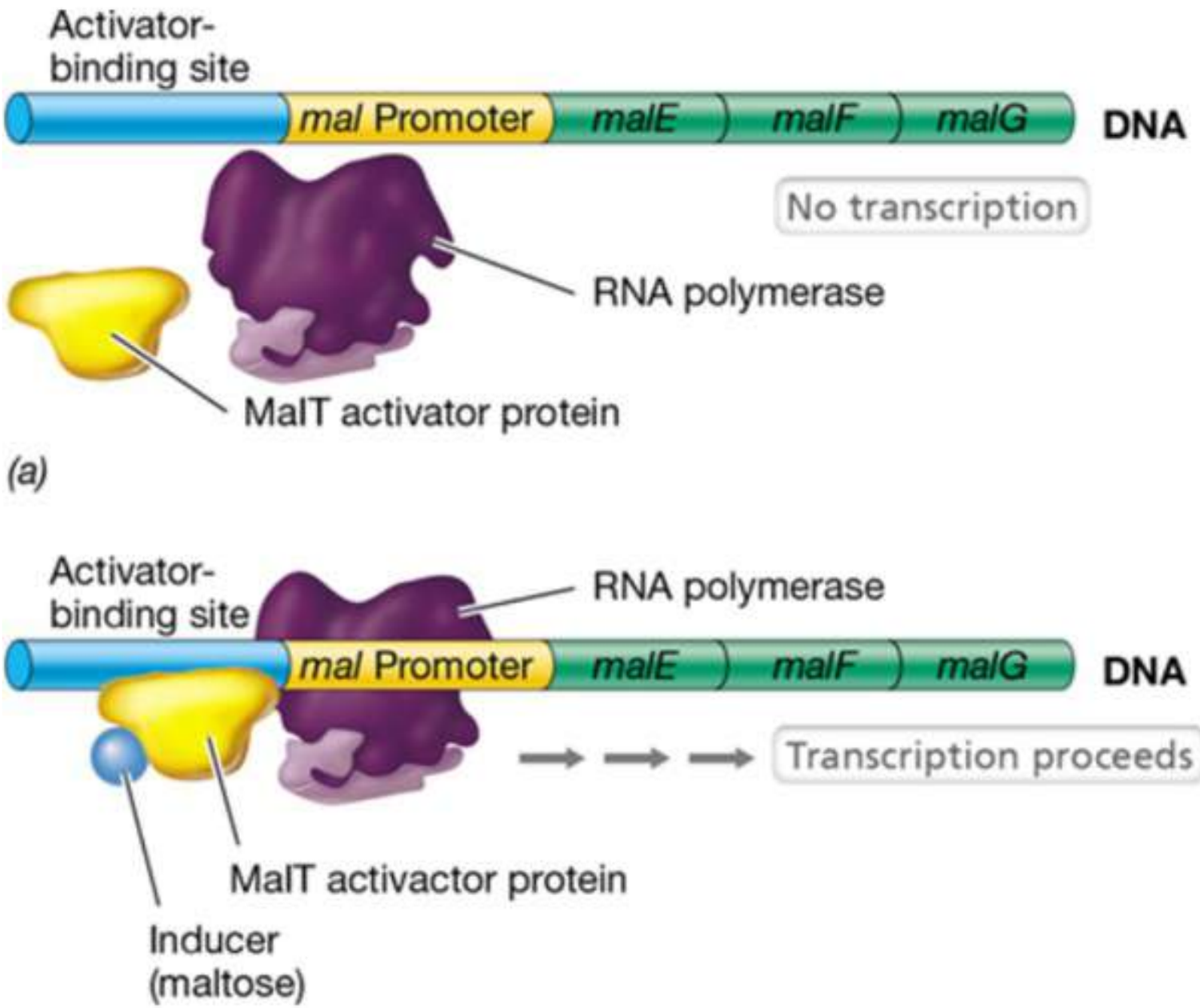
POSITIVE CONTROL

Figure 7.9 Positive control of enzyme induction in the maltose operon.



- Enzymes for maltose (disaccaride) catabolism in *E. coli* are synthesized only after maltose addition to medium
- Maltose activator protein cannot bind to DNA unless it first binds maltose (inducer)
- When maltose activator protein binds to DNA, it allows RNA polymerase to begin transcription

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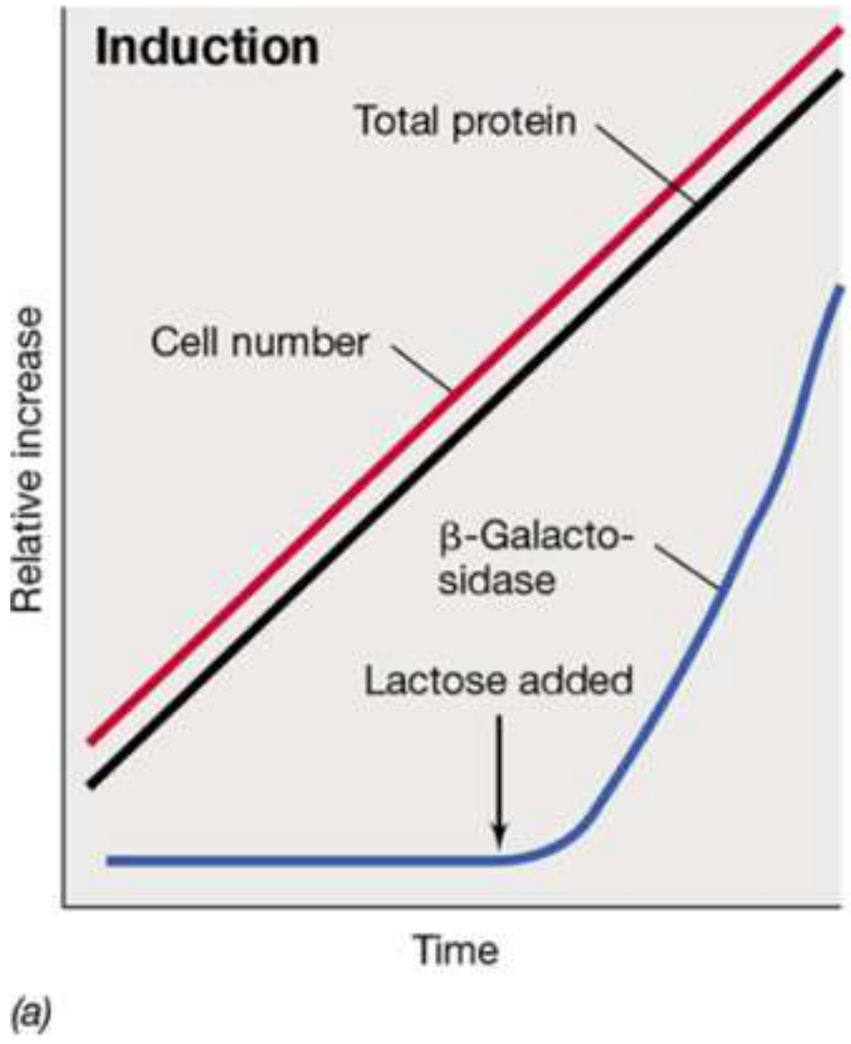
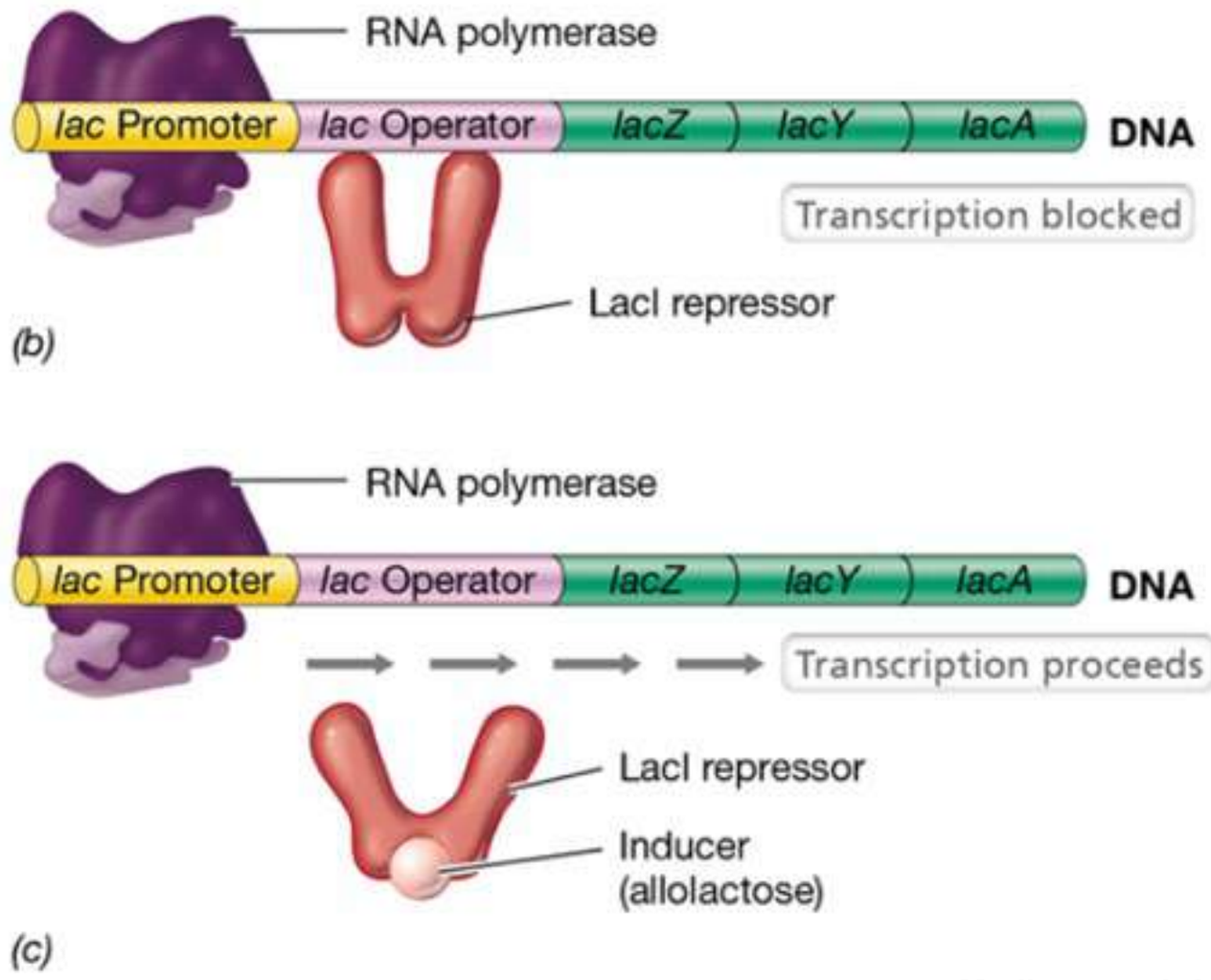
NEGATIVE CONTROL

Figure 7.6 Enzyme induction and expression of the lactose operon.





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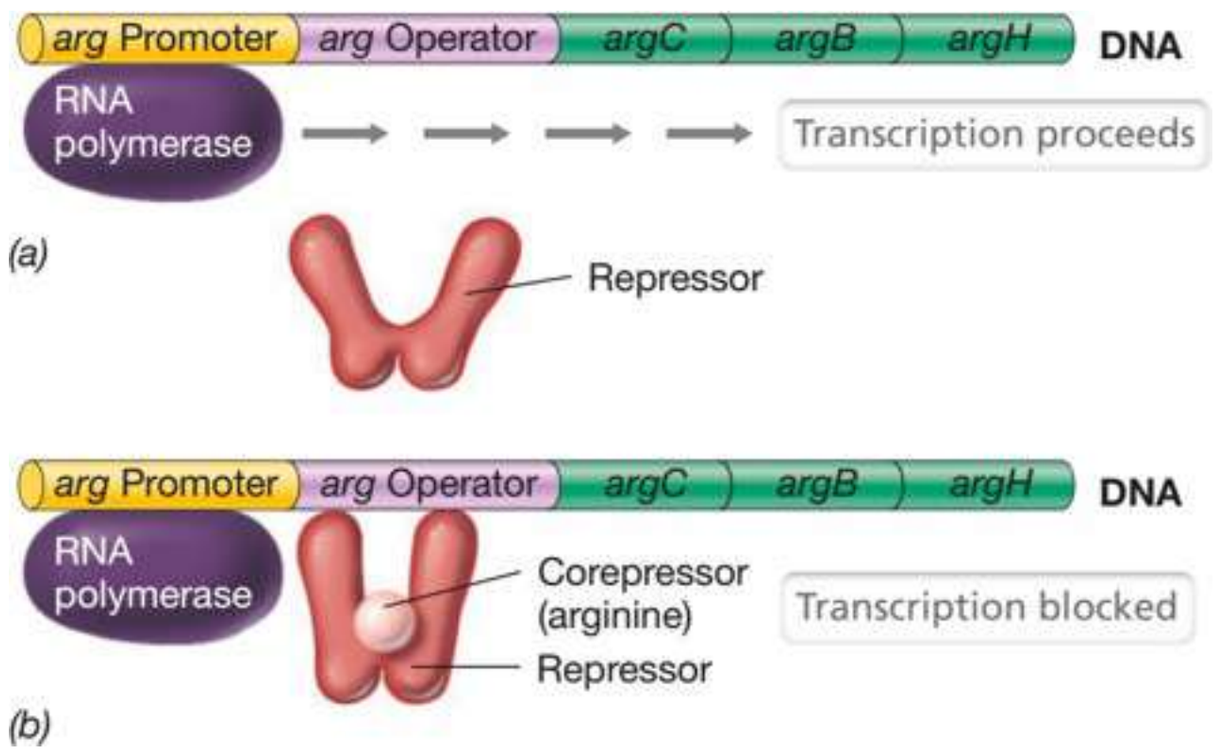
Enzyme repression of arginine biosynthetic pathway

- In *E. coli,* enzymes for **Arg** synthesis are made **only when Arg is absent**—> an excess of Arg decreases synthesis of these enzymes: **enzyme repression**
- *Final product of a particular biosynthetic pathway represses the enzymes of the pathway —> organism does not waste energy and nutrients synthesizing unneeded enzymes*
- A substance that represses enzyme synthesis is called a **corepressor, Arg (effector)**
- **Repressor protein is allosteric** —> its **conformation is altered when effector binds to it**



- By binding its effector, **repressor** protein is activated —> bind to a specific region **Operator (near the promoter of the gene)**

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Global Networks

- When **more than one operon** is **under the control of a single regulatory protein**, these operons are collectively called a **regulon**
- **Global control systems regulate many genes** comprising **more** than one **regulon**
- Global control networks may include activators, repressors, signal molecules, two-component regulatory systems, regulatory RNA & alternative sigma factors

TABLE 6.2 **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 level	Activator (CRP)	> 300
Heat shock	Temperature	Alternative sigma factors (RpoH and RpoE)	36
Nitrogen utilization	NH_3 limitation	Activator (NRI)/alternative sigma factor (RpoN)	> 12
Oxidative stress	Oxidizing agents	Activator (OxyR)	> 30
SOS response	Damaged DNA	Repressor (LexA)	> 20
General stress response	Stress conditions	Alternative sigma factor (RpoS)	> 400

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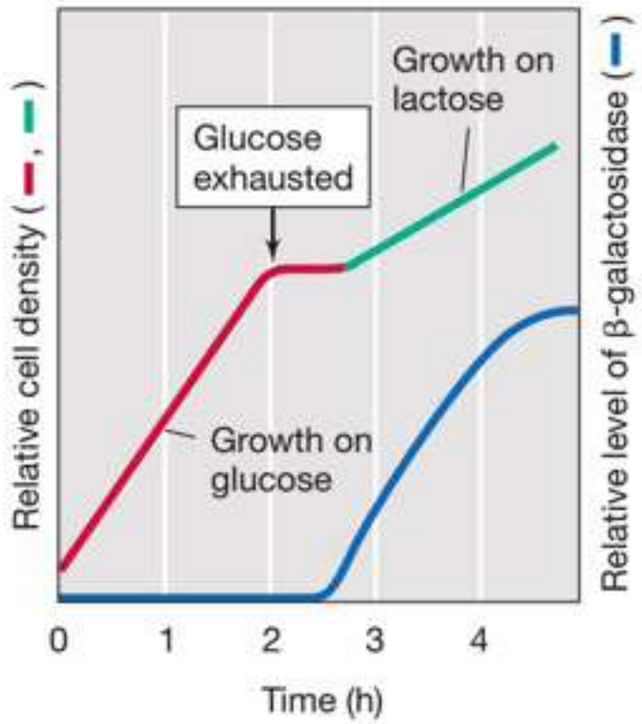
Global Control System

- An organism needs to **regulate many unrelated genes simultaneously** in response to a change in its environment
- Global control systems: **regulatory mechanisms responding to environmental signals by regulating the transcription of many different genes**
- In a complex environment, the **presence of a favored carbon** source represses the induction of pathways that catabolize other carbon sources
- **Catabolite repression** ensures that the organism uses the **best carbon and energy source first** (e.g. glucose)
- Catabolic operons: **lac, malt, genes for the synthesis of flagella (bc if bacteria have a good carbon source available, no need to swim around)**
- One consequence of catabolite repression —> 2 exponential growth phases: **diauxic growth**
- If two usable energy sources are available, the cells first consume the better energy source



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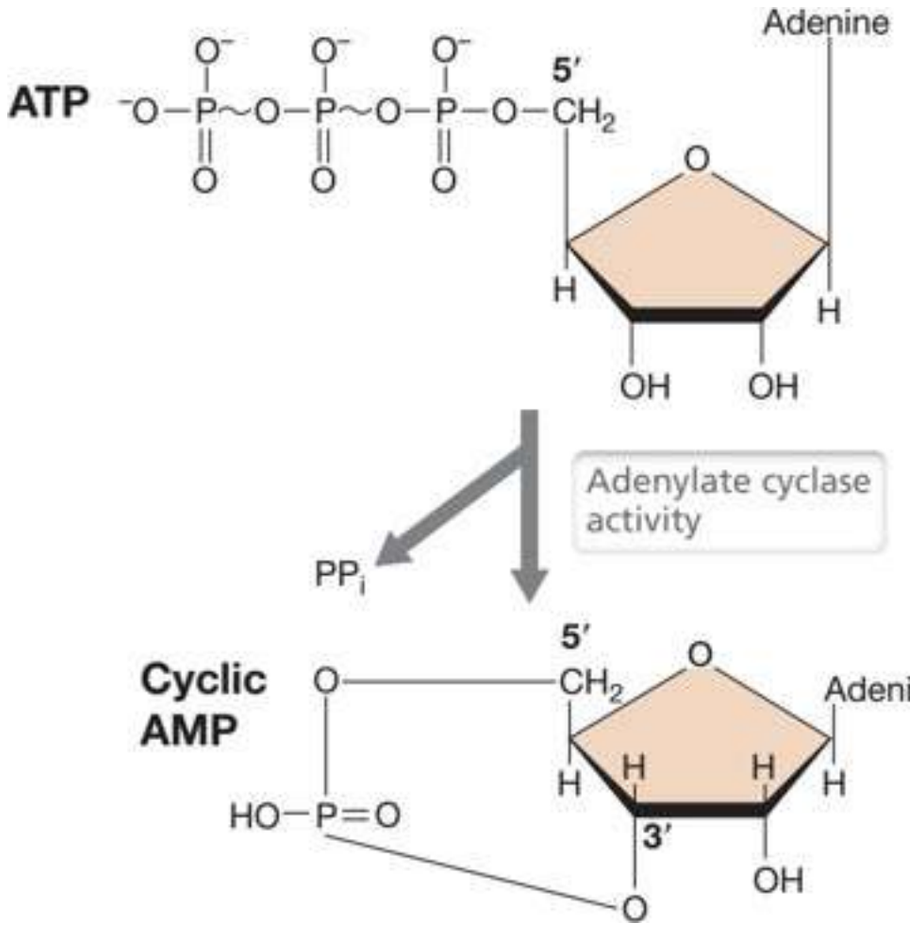
Catabolite repression, I

- **Catabolite repression** relies on an **activator protein** (**positive control**): cyclic AMP receptor protein (CRP) a dimer
- A gene that encodes a **catabolite-repressible enzyme is expressed only if CRP binds to DNA promoter region —> allowing RNA polymerase binding to promoter**
- **Effector** is **cAMP** derived from a nucleic acid precursor, it is a **regulatory nucleotide**
- **Cyclic di-GMP** (biofilm formation)
- **Guanosine tetraphosphate (ppGpp**, stringent response)
- Cyclic AMP is synthesized from ATP by an enzyme called adenylate cyclase
- **Glucose inhibits cyclic AMP synthesis and stimulates cyclic AMP transport out of the cell**
- Direct cause of catabolite repression is low level of cyclic AMP



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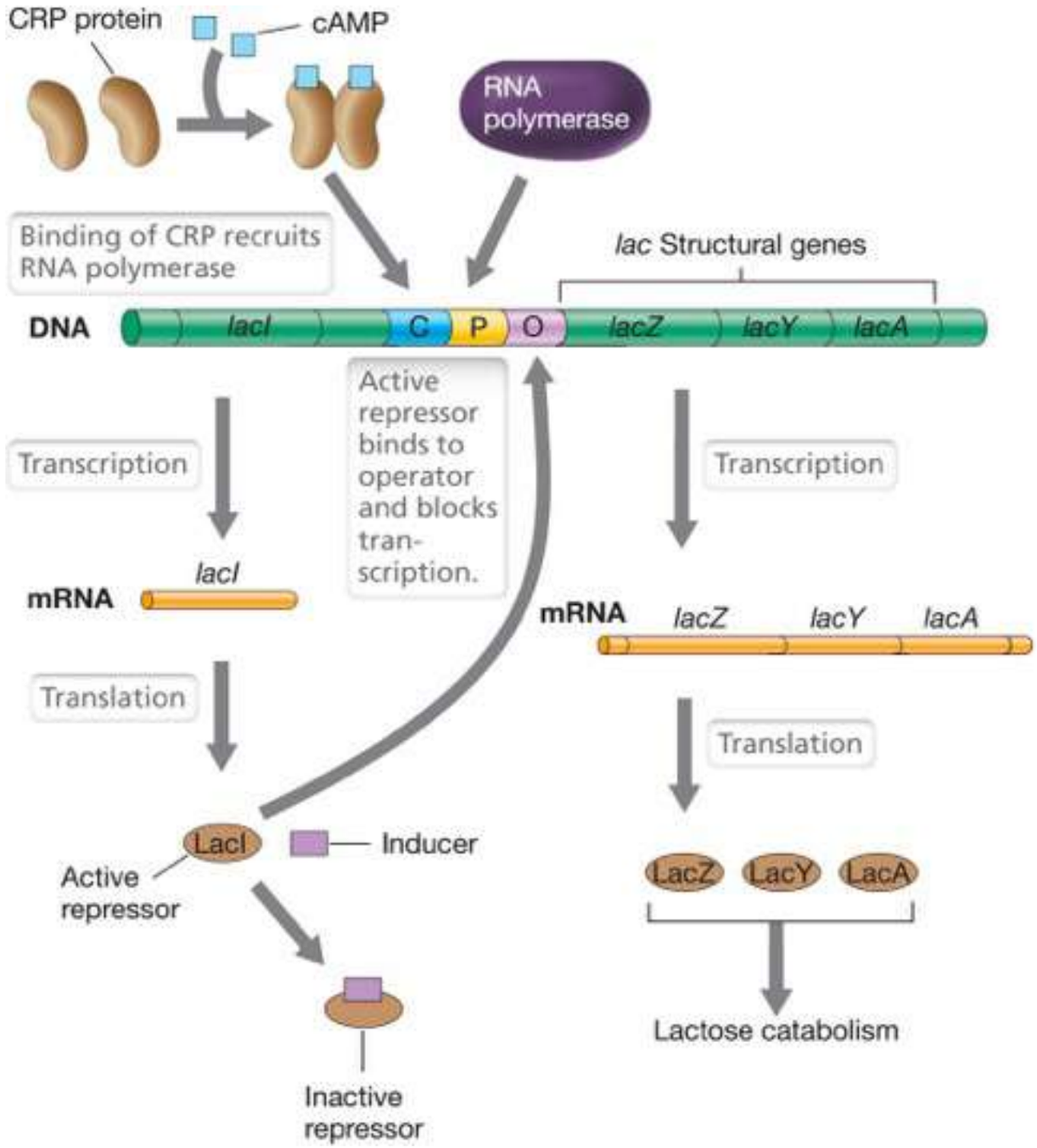
Catabolite repression, II

For **lac** genes to be **transcribed**:

(1)Level of cyclic AMP must be high enough for the CRP protein to bind to the CRP-binding site (positive control)

(2)Lactose or another suitable inducer must be present so that the lactose repressor (LacI protein) does not block transcription by binding to the operator (negative control)



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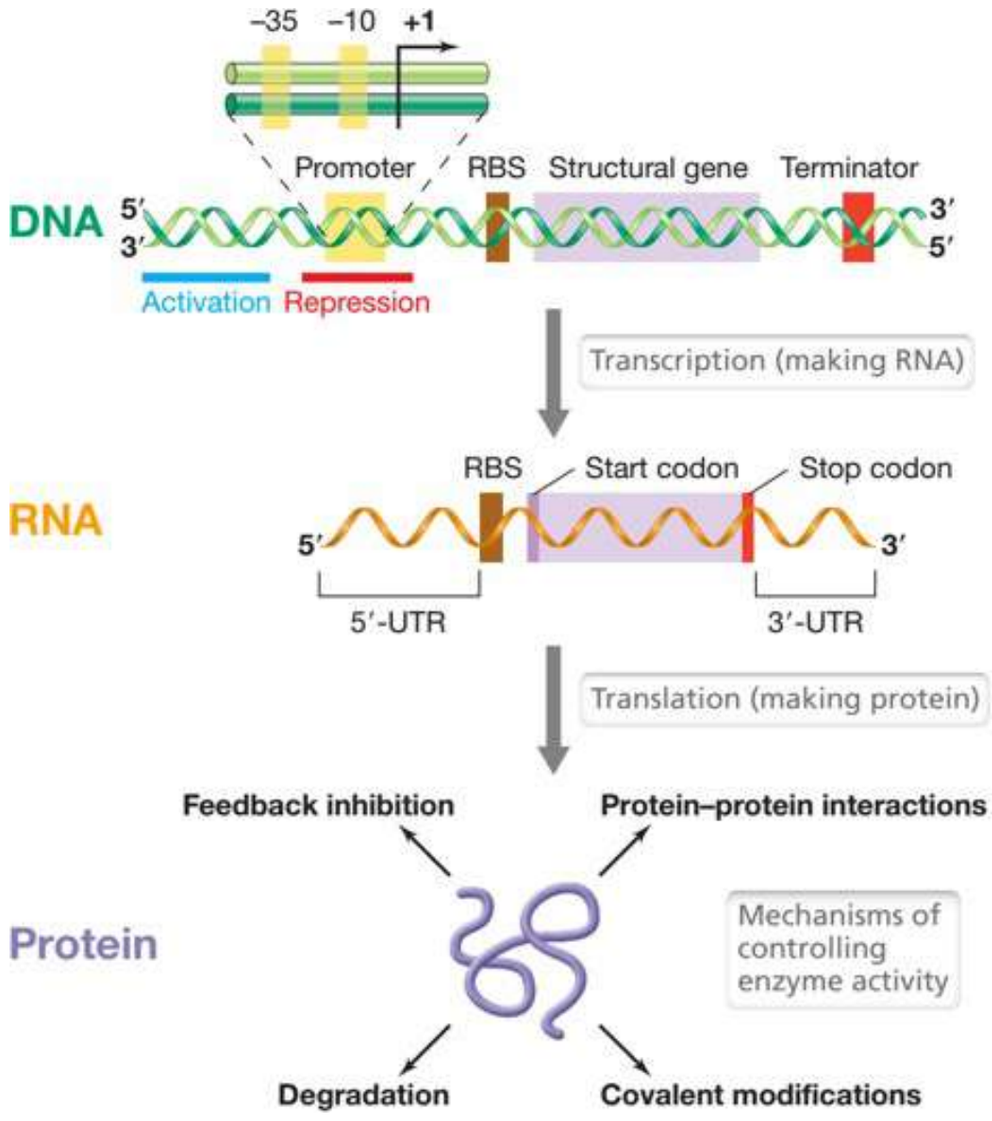
RNA-Based Regulation, I

- RNA can regulate gene expression at the level of transcription & of translation
- RNA molecules **that are not translated to give proteins are known as noncoding RNA (ncRNA)**: **rRNA, tRNA, RNA present in the signal recognition particle** that catalyzes some types of protein secretion
- Small RNAs (sRNAs) that range from **40–400 nucleotides long** and regulate gene expression are **widely distributed**
- **sRNA binds to other RNAs or to small molecules**—> control of gene expression



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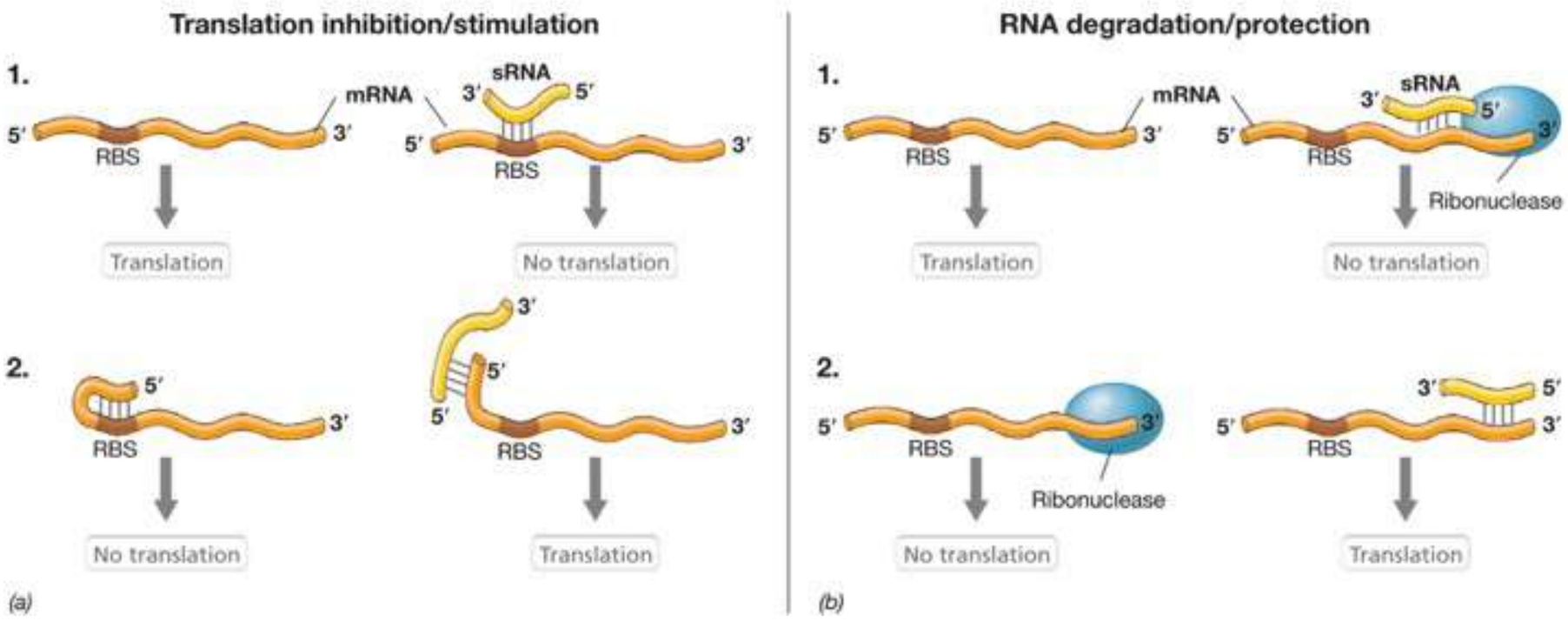
RNA-Based Regulation, II

- Small RNAs (sRNAs) exert their **effects by base-pairing** directly to other RNA molecules, usually mRNAs, which have regions of complementary sequence
- **Binding** immediately **modulates** rate of target mRNA translation b/c ribosome cannot translate double-stranded RNA
- **sRNAs** provide additional mechanism to **regulate protein synthesis** once its corresponding **mRNA** has **already** been **transcribed**
- **sRNA interaction** affect **mRNA stability** —> binding of sRNA to its target can: either 3. increase or 4. decrease degradation of the transcript by bacterial ribonucleases —> modulating protein expression



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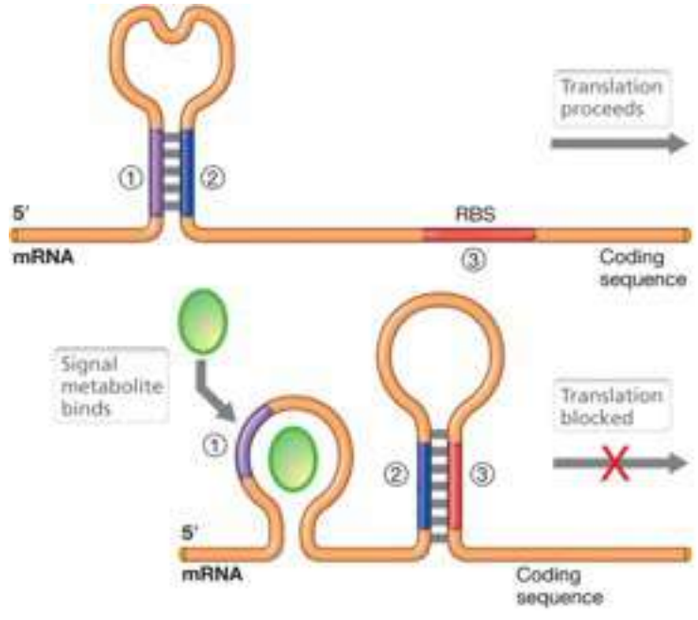
Riboswitches

- **RNA** can specifically recognize and **bind other molecules** e.g. low-molecular-weight metabolites
- Binding due to RNA folding into a specific **3D structure** that recognizes target molecule
- **Catalytically active RNAs are called ribozymes**
- **Riboswitches: RNA molecules resemble repressors and activators in binding small metabolites and regulating gene expression**



- In **riboswitch** (no regulatory protein exert control) after synthesized mRNA control translation —> **metabolite binds directly to mRNA**
- Riboswitch **mRNAs** contain regions **upstream of their coding** sequences that can fold into specific 3D structures that bind small molecules: **recognition domains, "switch"** exist as 2 alternative secondary structures, one with the small molecule bound and the other without
- Riboswitches control synthesis of enzymes in **biosynthetic pathways**
- **Primitive mechanism of metabolic control:** RNA life forms could have controlled other RNAs synthesis

Riboswitches are intergrated in the in a specific pathway

TABLE 6.3 **Riboswitches in biosynthetic pathways of *Escherichia coli***

Type	*Example of biosynthetic pathway*
Vitamins	Cobalamin (B_{12}), tetrahydrofolate (folic acid), thiamine
Amino acids	Glutamine, glycine, lysine, methionine
Nitrogen bases of nucleic acids	Adenine, guanine (purine bases)
Others	Flavin mononucleotide (FMN), *S*-adenosylmethionine (SAM), glucosamine 6-phosphate (peptidoglycan precursor), cyclic di-GMP (biofilm signaling molecule)

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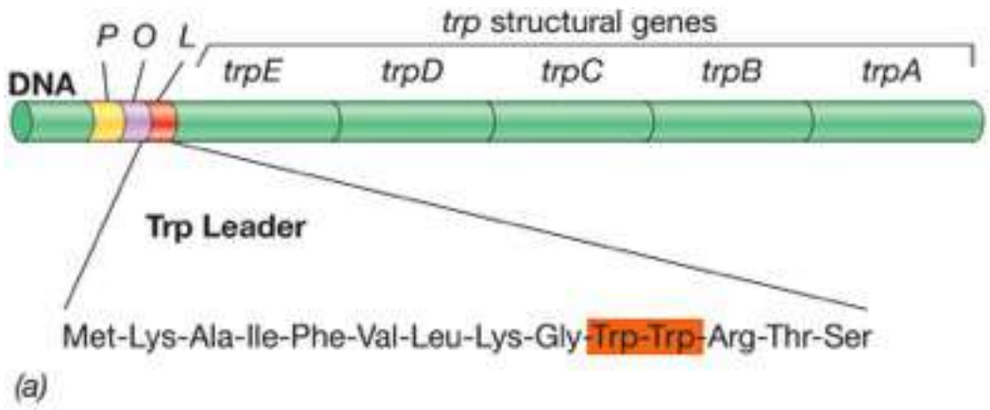
Attenuation

- Attenuation is a form of **transcriptional control —> prematurely terminating mRNA synthesis**
- Control is exerted after the initiation of transcription but before its completion
- **Number of completed transcripts** from an operon is **reduced**, even though the **number of initiated transcripts is not**
- First part of mRNA to be made, peptide **leader**, can fold into **2 alternative secondary** structures: one structure allows continued synthesis vs other secondary structure causes premature termination
- **mRNA folding** depends either on events **at the ribosome or on the activity of regulatory proteins**
- **In attenuation control: transcription rate is influenced by translation rate**

Tryptophan operon

- Trp operon contains structural genes for five proteins of **Trp biosynthetic pathway** plus, promoter (P), operator (O) and regulatory sequences at the beginning of the operon: **leader sequence (L) encoding for short leader peptide**
- Transcription of the entire trp operon is under **negative control**
- **Leader peptide sequence contains tandem trp codons near its terminus and functions as an attenuator**

- If **Trp >>** many charged Trp-tRNAs—> **leader peptide** is **synthesized** —> **termination** of transcription
- If **Trp << T**rp-rich leader peptide is not synthesized —> the rest of the **operon is transcribed**
- <u>Transcription and translation are simultaneous processes</u>
- Transcription is **attenuated b/c mRNA folds into a unique stem–loop that inhibits RNA polymerase**
- Stem–loop structure forms b/c two stretches of nucleotides near each other are complementary—> bases pair



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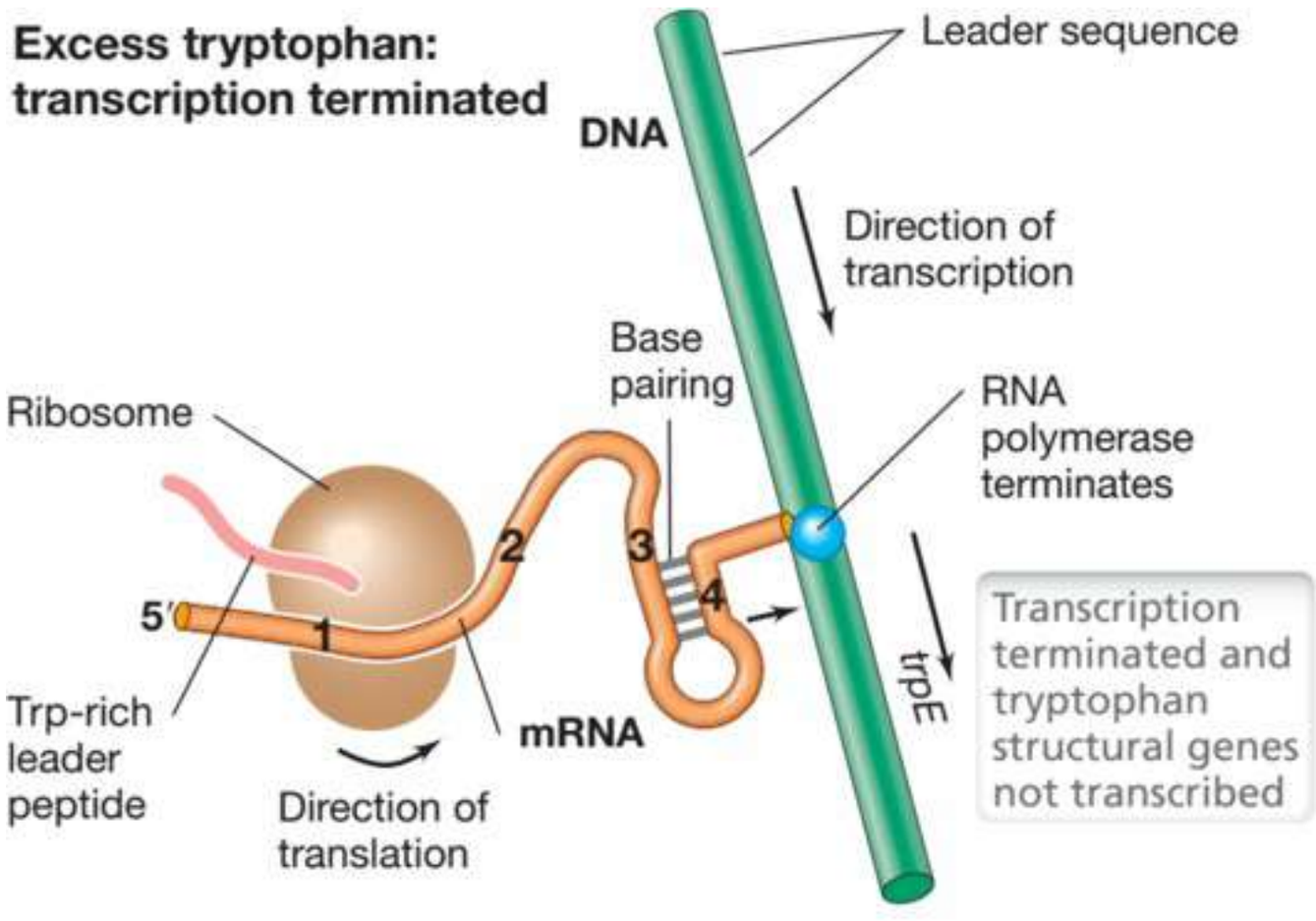
Concentration-Time coupling, I

- Trp is >>
- Ribosome **translates the leader** sequence (1, 2) —> stop codon
- Remainder of the leader sequence then forms a stem–loop on mRNA (3:4)
- **Transcription —> termination**



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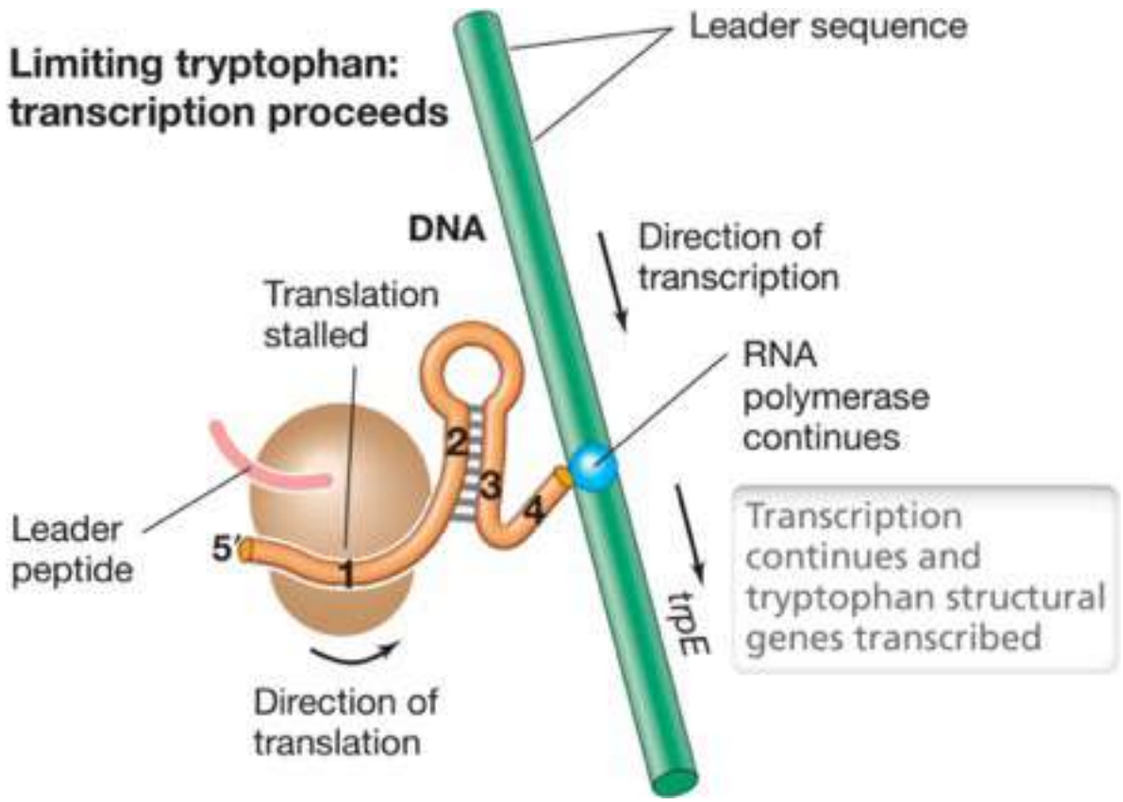
Concentration-Time coupling, II

- Trp is limiting, <<<
- During leader transcription, ribosome **pauses** at a trp codon because of a shortage of charged tryptophan tRNAs
- The presence of the **stalled ribosome** at this position allows a stem–loop to form (2:3) that differs from the terminator stem–loop
- **2:3 stem– loop prevents formation of terminator 3:4 stem–loop**
- **RNA polymerase to move past the termination site and begin transcription of trp structural genes**



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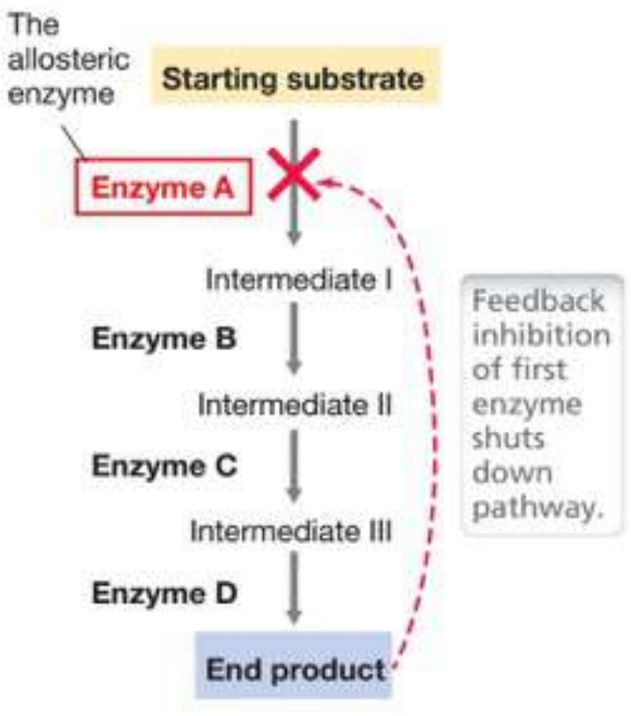
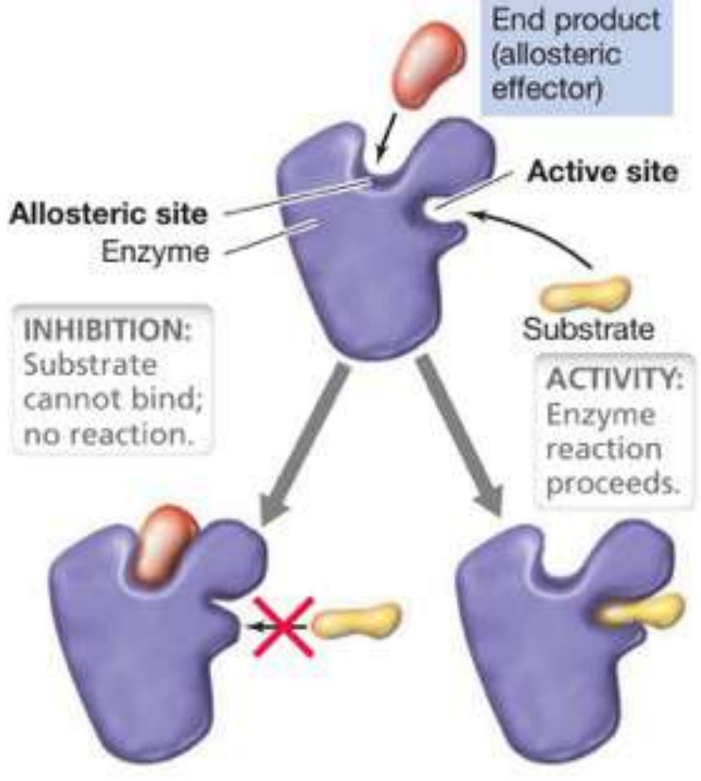
Enzyme Regulation

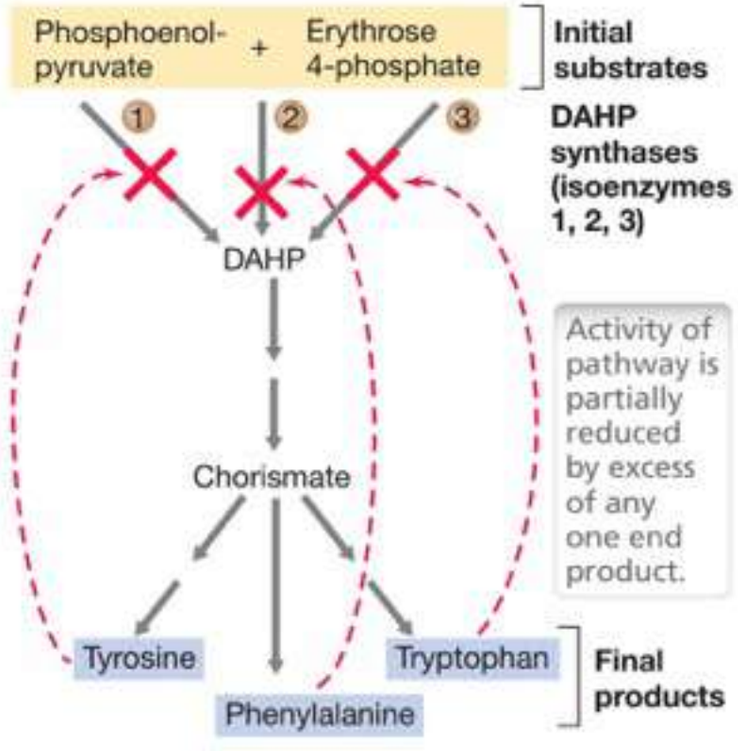
- Cellular mechanisms control enzyme activity already present in the cell through processes such as feedback inhibition and post-translational regulation
- **Feedback Inhibition**: **temporarily shuts off the reactions in an entire biosynthetic pathway** b/c excess of the end product of the pathway inhibits activity of an early (typically the first) enzyme of the pathway
- **Isoenzymes are different proteins** that **catalyze the same reaction** but are subject to **different regulatory controls**



(a) **Feedback inhibition**



(b) **Allosteric inhibition**



(c) **Isoenzyme inhibition**

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