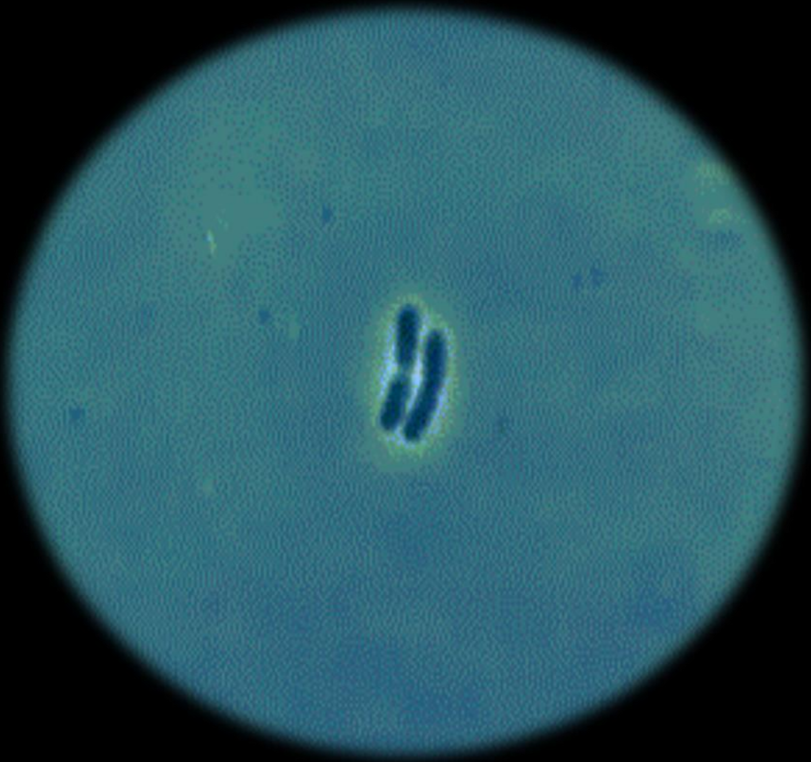
Microbiologie 2



Microbiologie 2

Allereerst

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- sije@pl.hanze.nl
- D0.109

Microbiologie 2

Inhoud

- Regulatie van operons
- Bouw en het functioneren van virussen
- Interactie tussen mensen en micro-organismen
- Werking van antibiotica

Microbiologie 2

Leeruitkomsten (1/2)

- Uitleggen hoe bacteriën het metabolisme (enzymactiviteit) op verschillende niveaus (DNA, RNA, eiwit) kunnen reguleren.
- De bouw van verschillende virussen beschrijven en de replicatiemechanismen van verschillende virussen uitleggen.
- Uitleggen hoe virussen de gastheer kunnen infecteren en wat het effect van een infectie op de gastheer is.

Microbiologie 2

Leeruitkomsten (2/2)

- Uitleggen hoe micro-organismen bestreden kunnen worden d.m.v. anti-microbiële middelen en hoe micro-organismen resistenties tegen deze middelen kunnen ontwikkelen.
- Uitleggen welke bacteriën normaal op/in het menselijk lichaam voorkomen, hoe pathogene bacteriën infecties kunnen veroorzaken en welk schade ze kunnen veroorzaken (toxiciteit).

Microbiologie 2

Literatuur

Brock Biology of Microorganisms, Madigan et al., 15th ed.

H6 Microbial Regulatory systems [niet: 6.5]
H7 Molecular Biology of Microbial Growth [alleen 7.10]
H8 Viruses and Their Replication
H10 Viral Genomics, Diversity and Ecology [niet: 10.3 t/m 10.5, 10.10]
H24 Microbial Symbioses with Humans [alleen 24.1, 24.2 en 24.5]
H25 Microbial Infections and Pathogenesis
H28 Clininal Microbiology and Immunology [alleen 28.10 t/m 28.12]

Microbiologie 2

Literatuur

Brock Biology of Microorganisms, Madigan et al., 16th ed.

H7: Microbial Regulatory systems [NIET: 7.4 (archaea); Stringent response; General Stress Response; Pho-regulon; Inactivation of Sigma factors]

H8: Molecular Biology of Microbial Growth [ALLEEN 8.11]

H5: Viruses and Their Replication

H9: Genetics of Bacteria and Archaea [ALLEEN 9.12 CRISPR]

H11: Viral Genomics, Diversity and Ecology [NIET: 11.3; 11.5; bacteriophage MS2; 11.10, 11.14]

H24: Microbial Symbioses with Humans [ALLEEN 24.1, 24.2 en 24.5]

H25: Microbial Infections and Pathogenesis [NIET: 25.4]

H28: Immune disorders and Antimicrobial Therapy [ALLEEN 28. t/m 28.7]

Microbiologie 2

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Brock Biology of Microorganisms, Madigan et al., 16th ed.

H7: Microbial Regulatory systems [NIET: 7.4; Stringent response; General Stress Response; Pho-regulon; Inactivation of Sigma factors]

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H9: Genetics of Bacteria and Archaea [ALLEEN 9.12 CRISPR]

H11: Viral Genomics, Diversity and Ecology [NIET: 11.3; 11.5; bacteriophage MS2; 11.10, 11.14]

H24: Microbial Symbioses with Humans [ALLEEN 24.1, 24.2 en 24.5]

H25: Microbial Infections and Pathogenesis [NIET: 25.4]

H28: Immune disorders and Antimicrobial Therapy [ALLEEN 28. t/m 28.7]

PowerPoint® Lecture Presentations

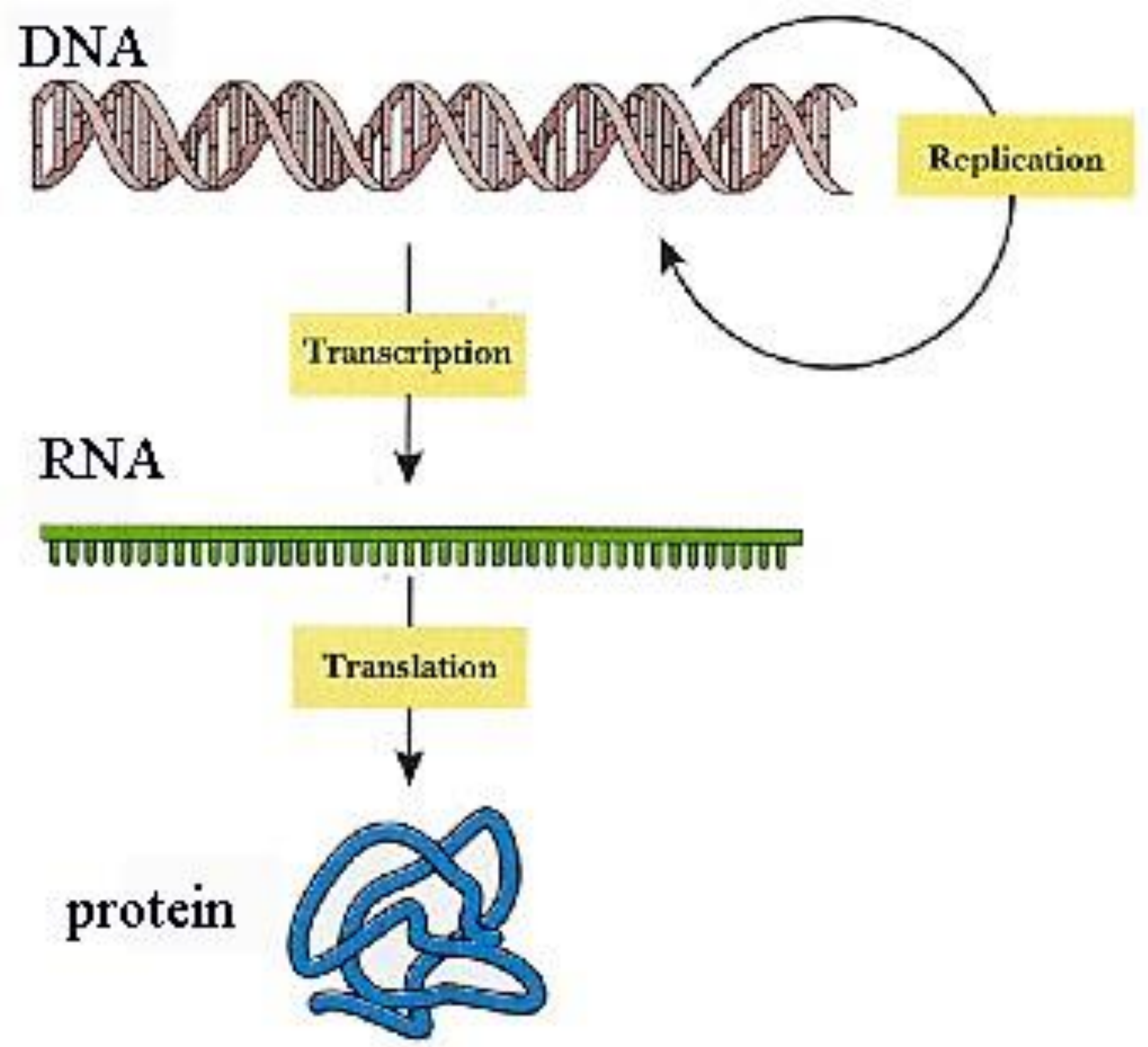
CHAPTER 7

Microbial Regulatory Systems

I. DNA-Binding Proteins and Transcriptional Regulation

- 7.1 DNA-Binding Proteins
- 7.2 Transcription Factors and Effectors
- 7.3 Repression and Activation
- Niet 7.4 Transcriptional Controls in *Archaea*
- 7.8 The *lac*-operon

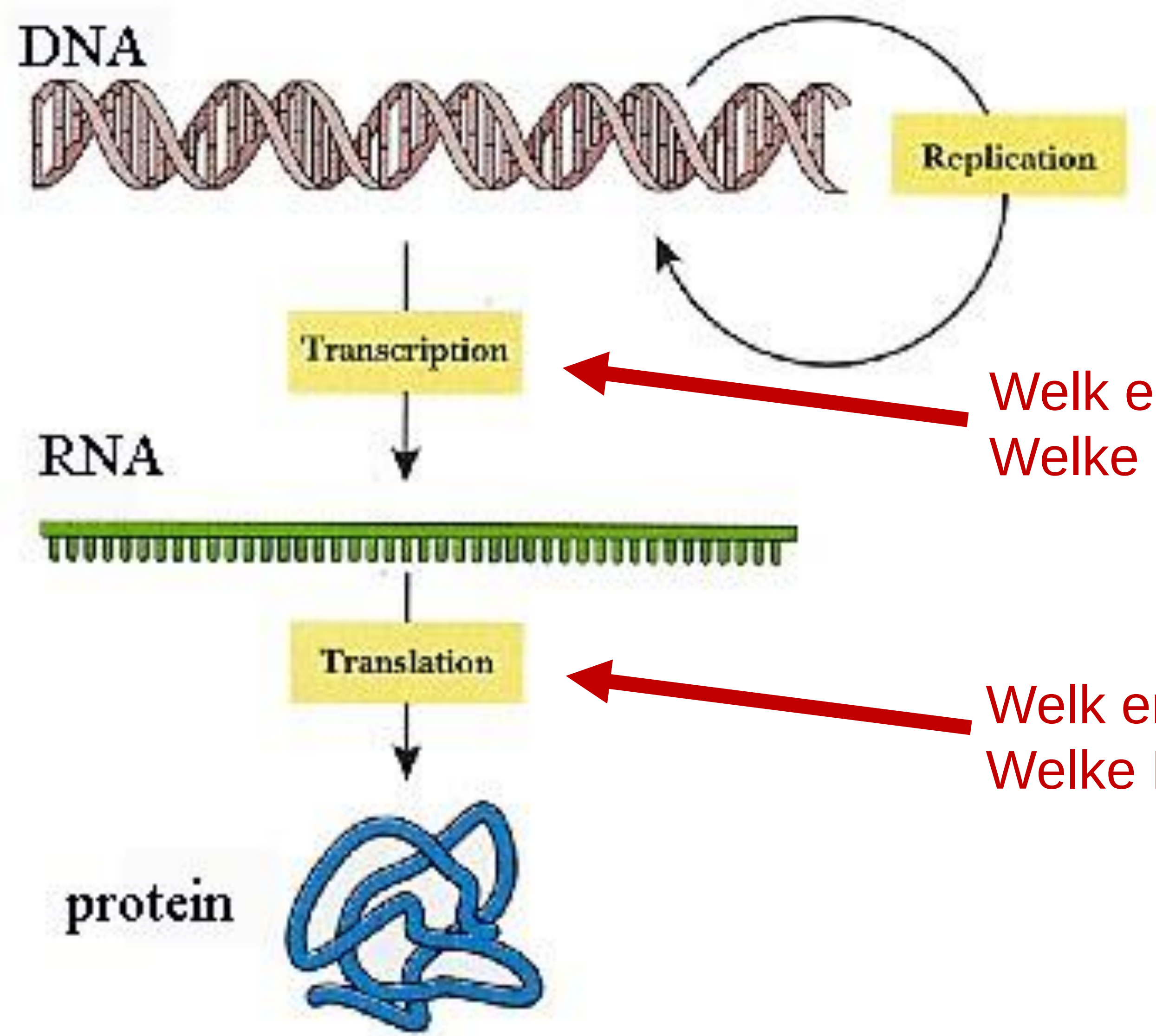
Centrale dogma



Genexpressie:

Alles wat nodig is om van gen tot functioneel eiwit te komen.

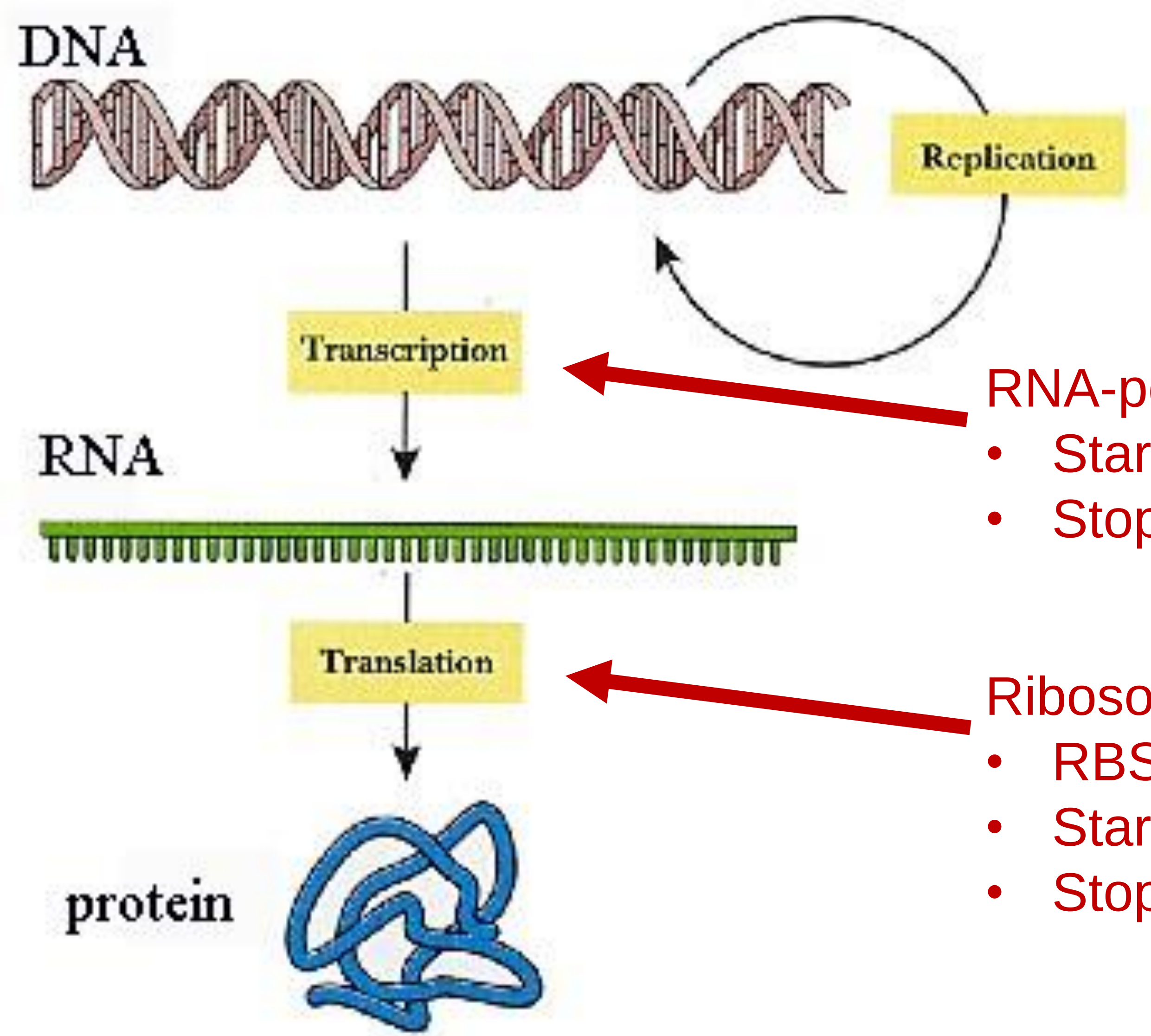
Centrale dogma



Welk enzym hierbij betrokken?
Welke DNA-info is nodig?

Welk enzym hierbij betrokken?
Welke DNA-info is nodig?

Centrale dogma



RNA-polymerase
- Start: Promoter (TATA-box)
- Stop: Terminator

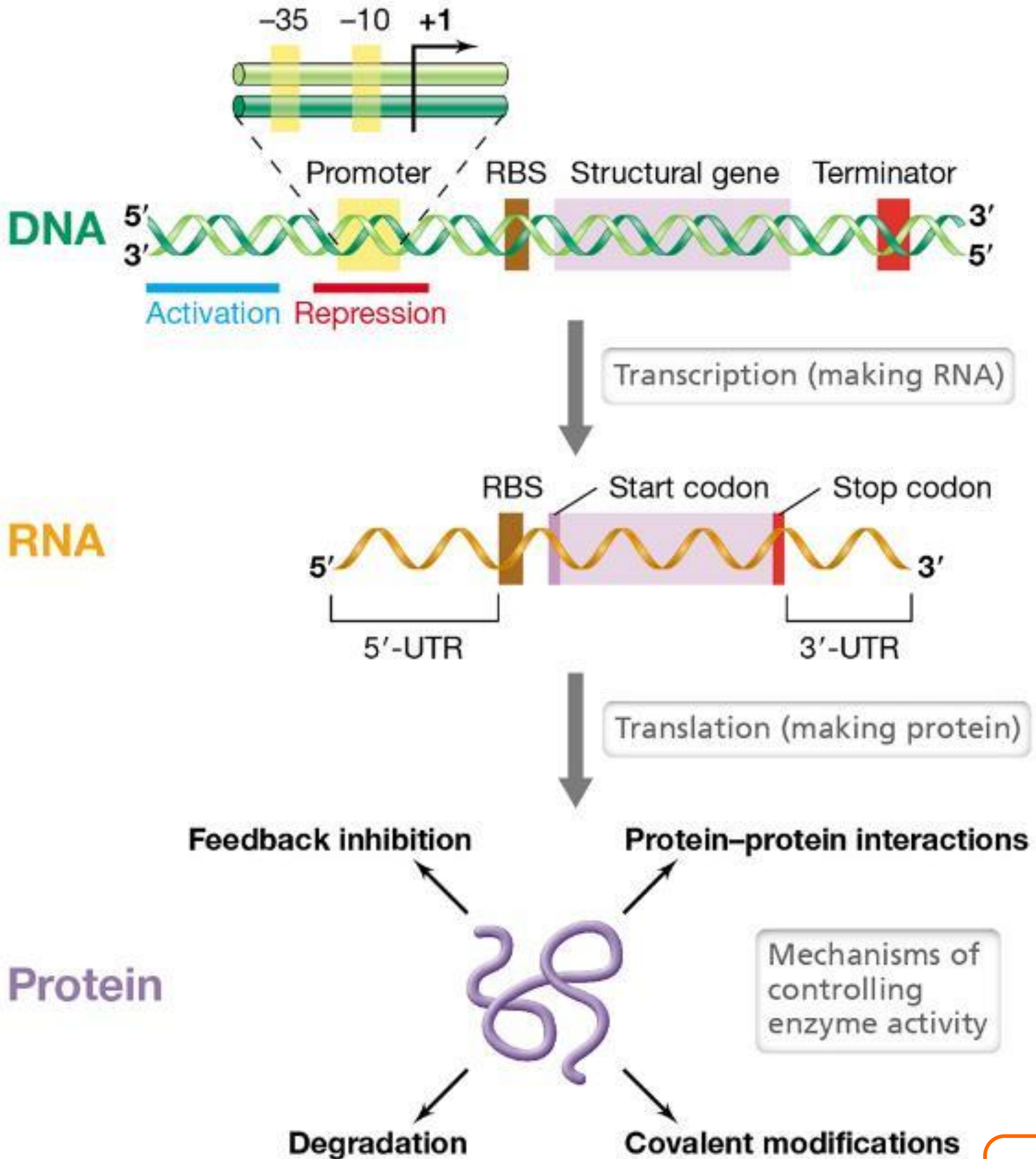
Ribosoom
- RBS
- Start-codon
- Stop-codon

Wat is een gen?

- Start- en stop-codon in hetzelfde frame (= ORF (Open Reading Frame)).
- Ribosome Binding Site (RBS), promoter, terminator

NB: in prokaryoten (in Eukaryoten iets anders…)

- Promoter => DNA sequence dat door eiwitten herkend wordt.
- Vereiste => DNA bindende eiwitten



–35
–10
+1
Promoter
RBS
Structural gene
Terminator
DNA
5′
3′
3′
5′
Activation
Repression
Transcription (making RNA)
RBS
Start codon
Stop codon
RNA
5′
3′
5′-UTR
3′-UTR
Translation (making protein)
Feedback inhibition
Protein–protein interactions
Protein
Mechanisms of controlling enzyme activity
Degradation
Covalent modifications

Figure 7.1

7.1 DNA-Binding Proteins

- Specific binding sites for regulatory proteins: often *inverted repeats*
- Homodimeric proteins: proteins composed of two identical polypeptides
- Each polypeptide has a domain (region with specific structure and function) that binds to one inverted repeat. (Figure 7.2)

Hier:

- Homodimeer
- Bindt in grote groef

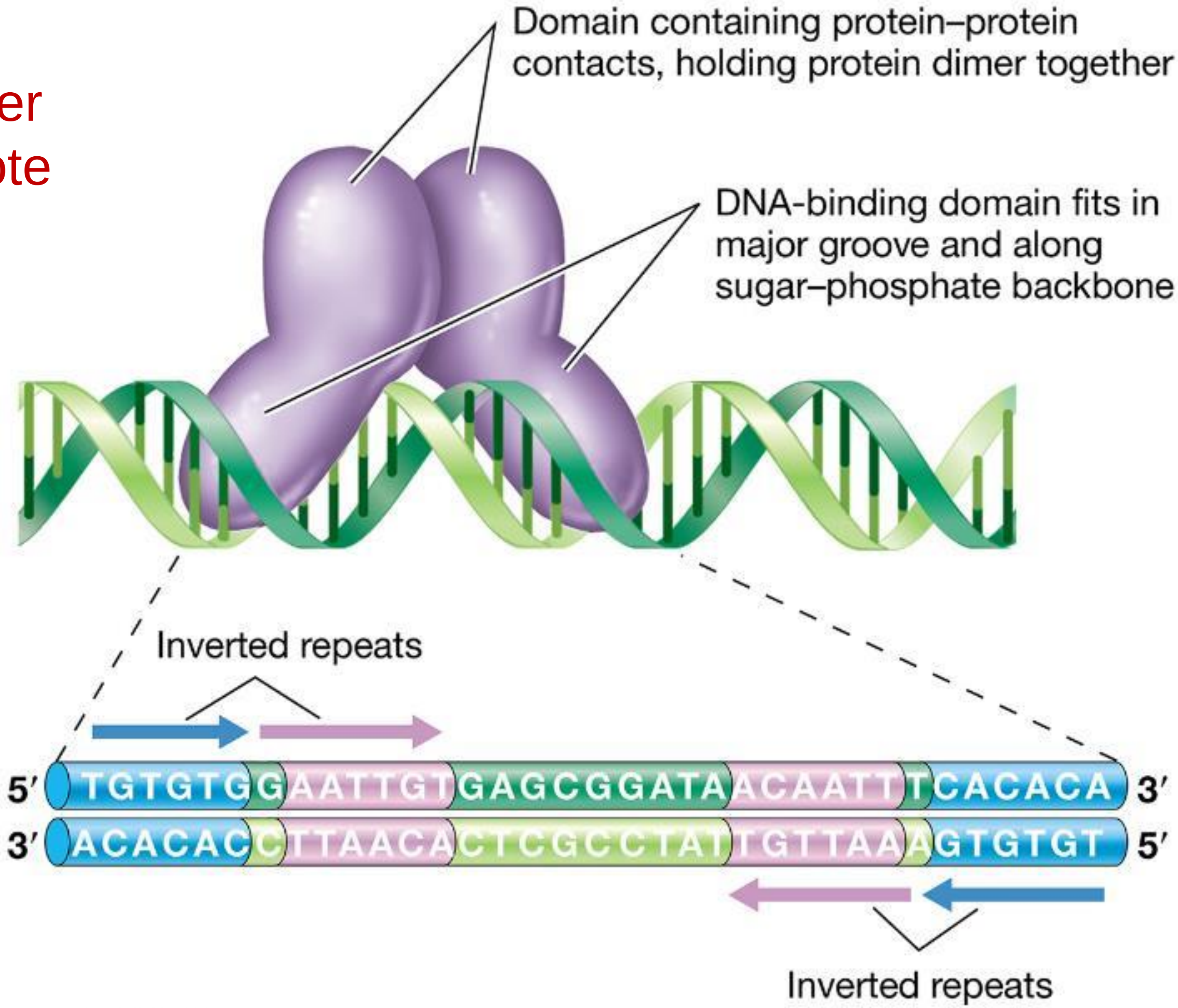
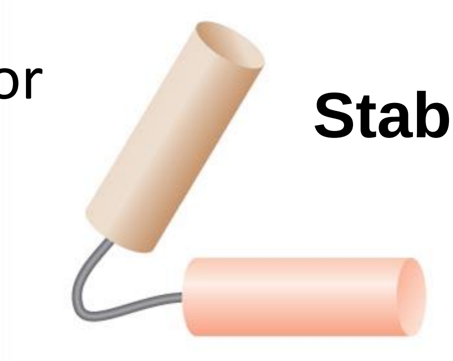


Figure 7.2

Helix-turn-helix

- α-helix-turn-α-helix
- eerste helix herkent DNA
- tweede helix stabiliseert
- turn vaak glycine (waarom?)
- Vb: trp repressor en lac repressor



Veel voorkomend motief bij DNA bindende eiwitten:
Helix-turn-helix

Voorbeelden:
lac en *trp* repressors

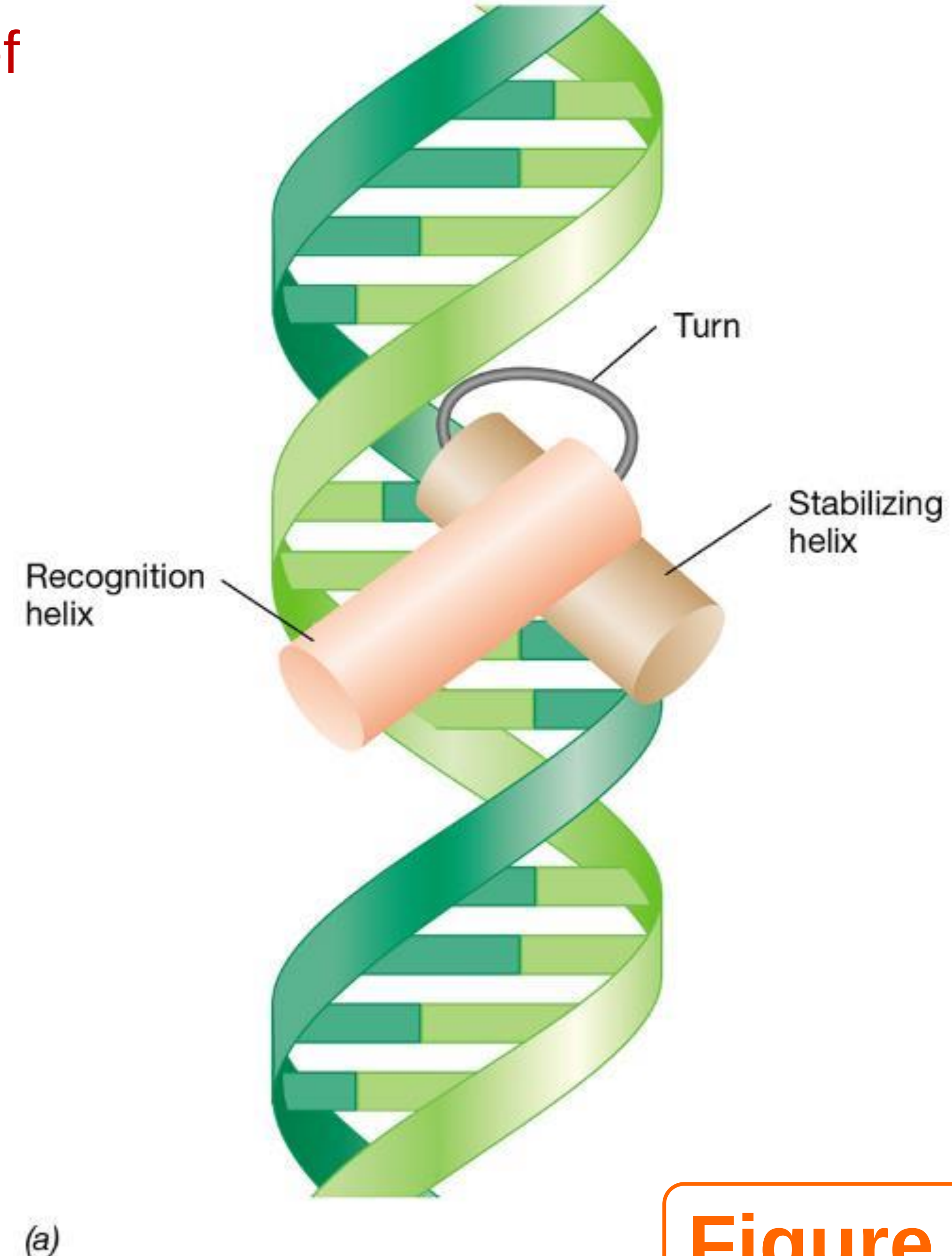
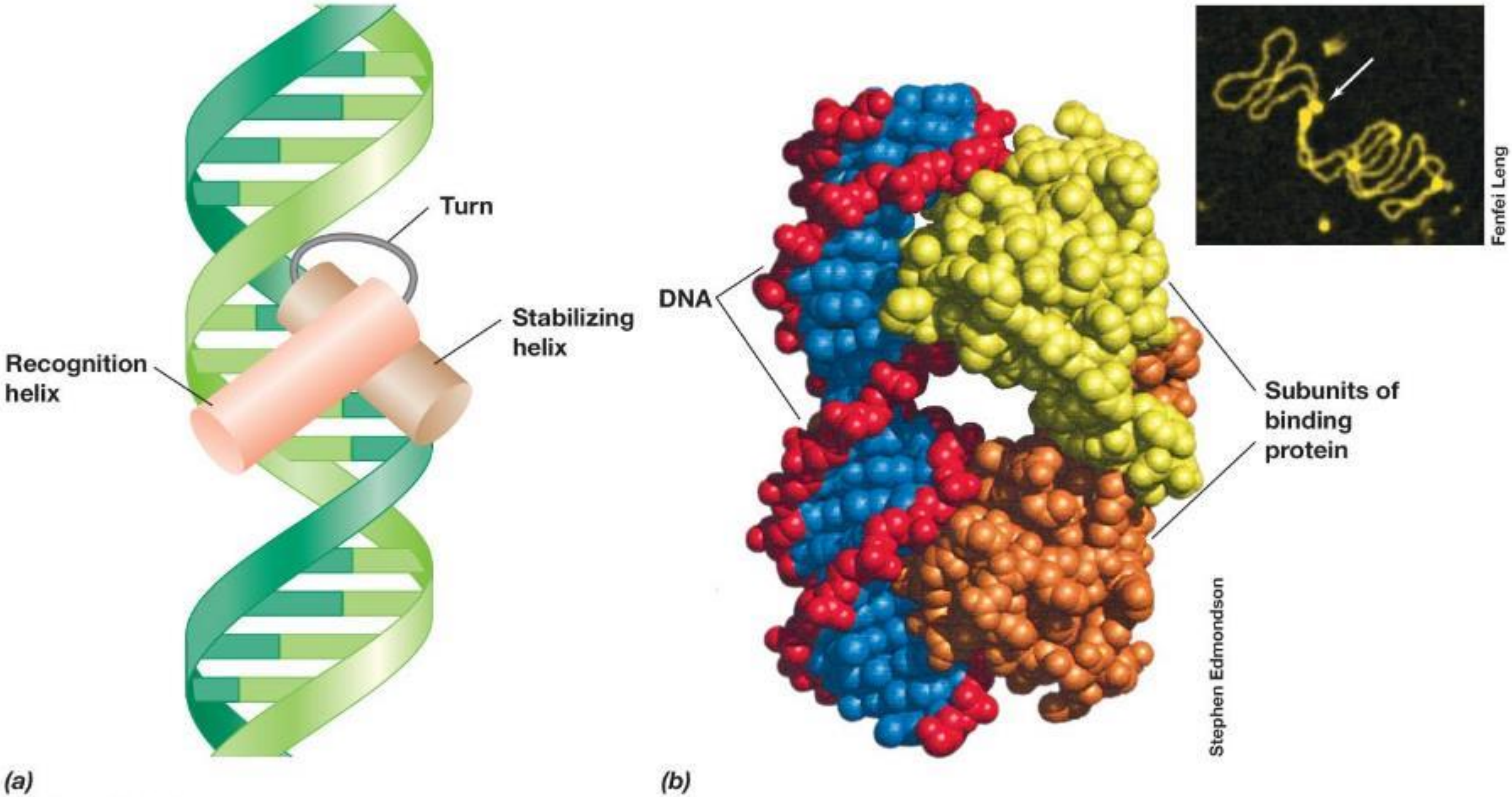


Figure 7.3a

Helix-turn-helix

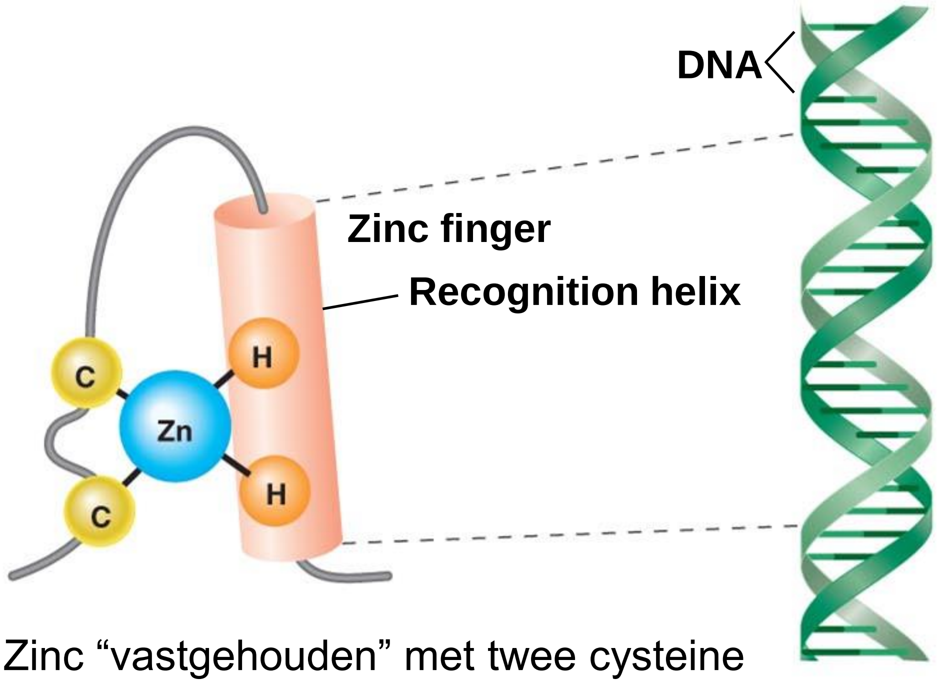


7.1 DNA-Binding Proteins

- 2 Andere typen DNA-bindende eiwitten
  - *zinc finger*
    - eukaryotic regulatory protein structure that binds a zinc ion
  - *leucine zipper*
    - contains regularly spaced leucine residues
    - function to hold two recognition helices in the correct orientation

Zinc finger

Zink ‘vastgehouden’ met twee cysteines
‘Finger’ is een alfa helix
Meestal meerdere zinc fingers aanwezig in 1 eiwit

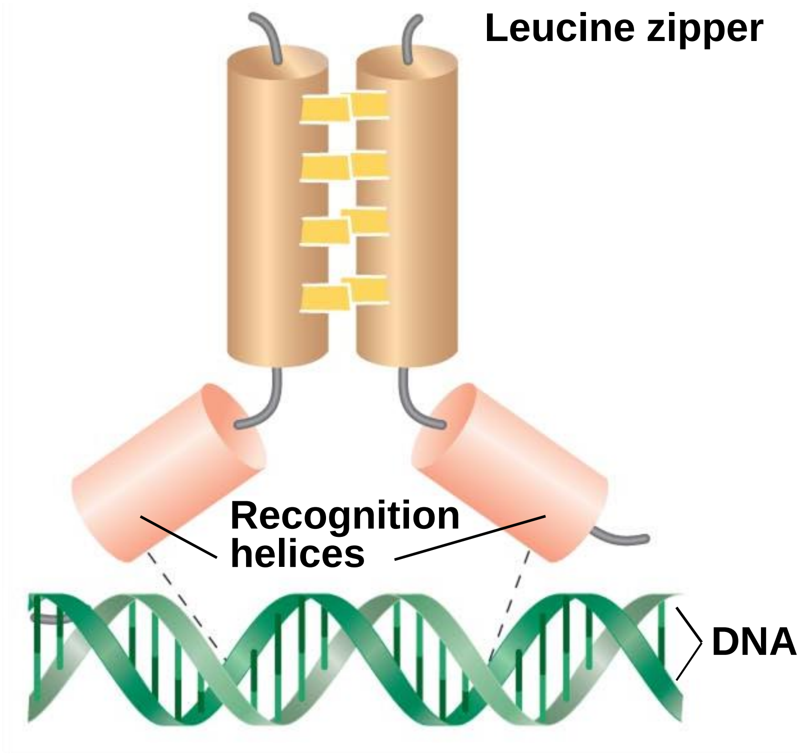


Zinc “vastgehouden” met twee cysteine

https://www.youtube.com/watch?v=WyU2v7HT6bw

Leucine zipper

- Leucine zipper
  - Leucine residues spaced every seven amino acids
  - “zipper” is structural and does not bind DNA



https://www.youtube.com/watch?v=2-qFLfVymnw

7.1 DNA-Binding Proteins

- Multiple outcomes after DNA binding are possible.

1. Catalyze (*e.g.*, transcription by RNA polymerase)
2. Repression (*negative regulation*)
3. Activation (*positive regulation*)

7.3 Repression and Activation

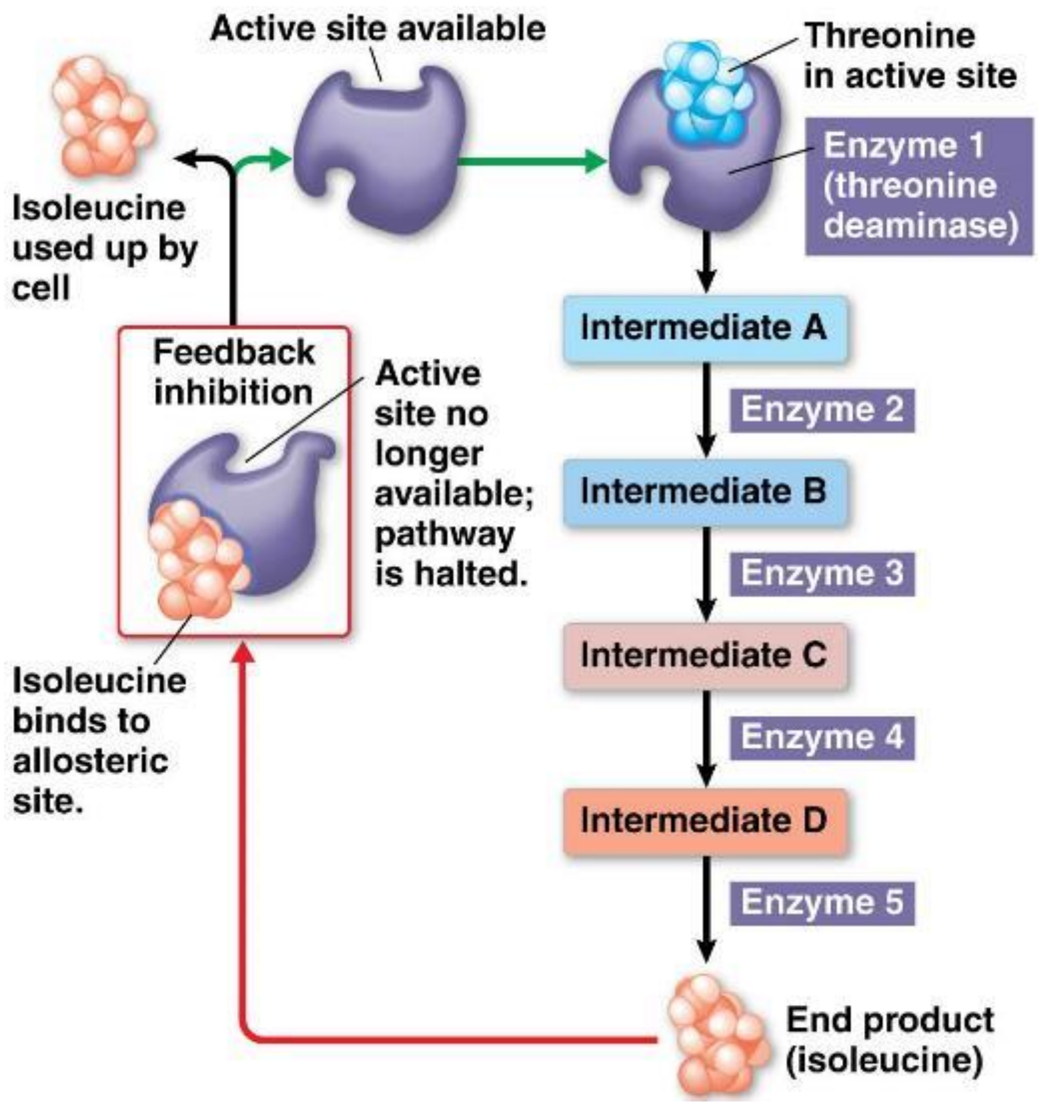
Negative control: a regulatory mechanism that stops transcription

- repression: preventing the synthesis of an enzyme in response to sufficient amounts of a product (Figure 7.4)

Voorbeeld negatieve feedback:

Het product remt z'n eigen aanmaak!

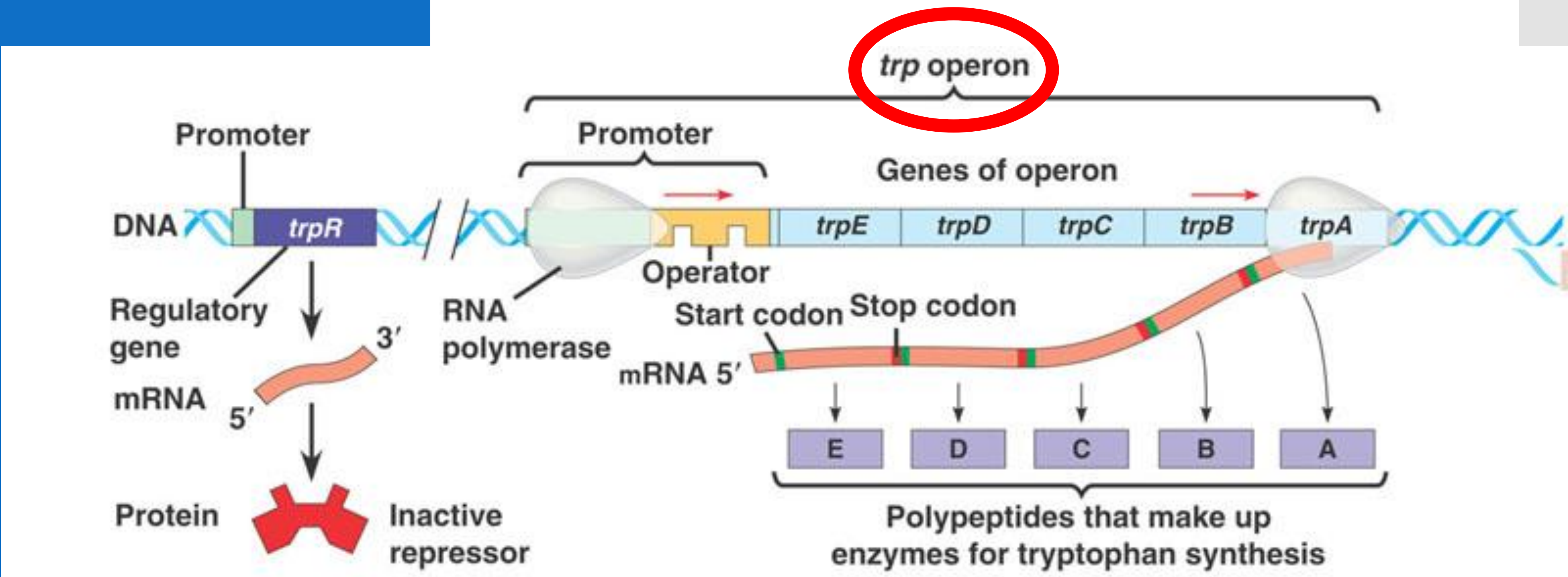
NB: Dit is op enzymatisch niveau



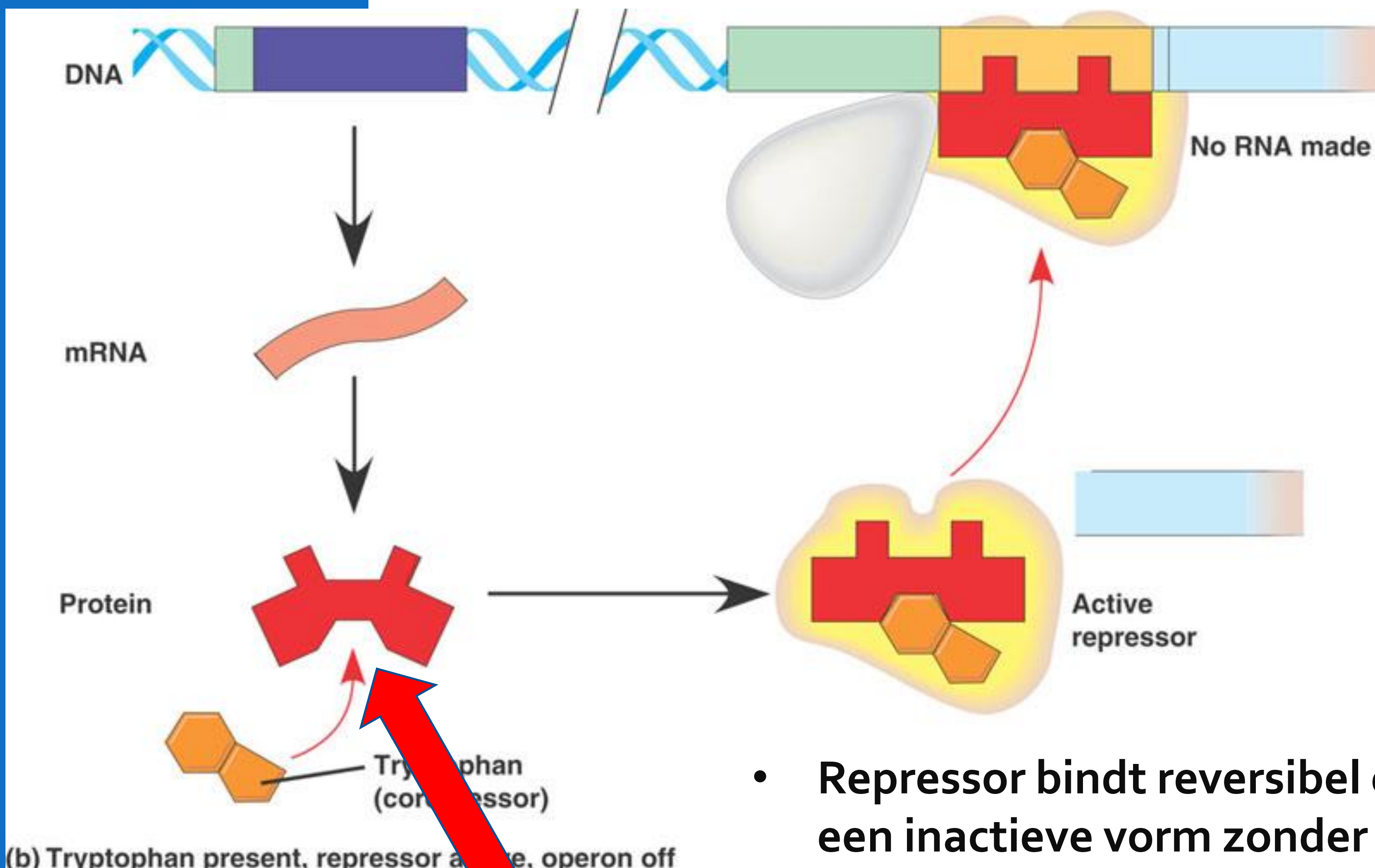
Bio2

Bio3!

Operon



(a) Tryptophan absent, repressor inactive, operon on



- Repressor bindt reversibel en is in een inactieve vorm zonder trp
- Trp is hier een corepressor

Allosterische zijde

Ander voorbeeld negatieve feedback op genexpressieniveau: Arginineproductie

<u>Het product remt z'n eigen aanmaak!</u>

Hier door productie enzymen te voorkomen.

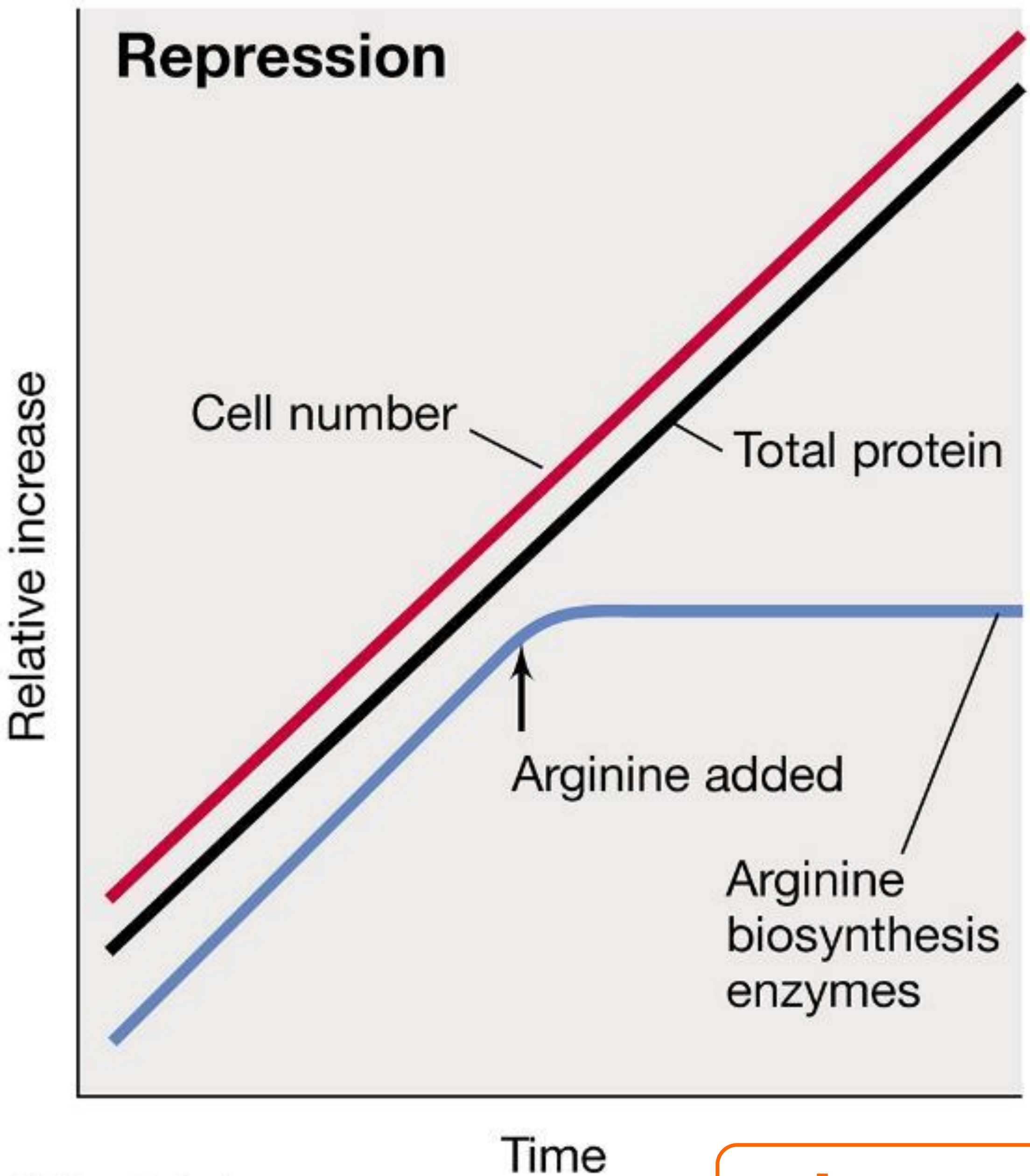
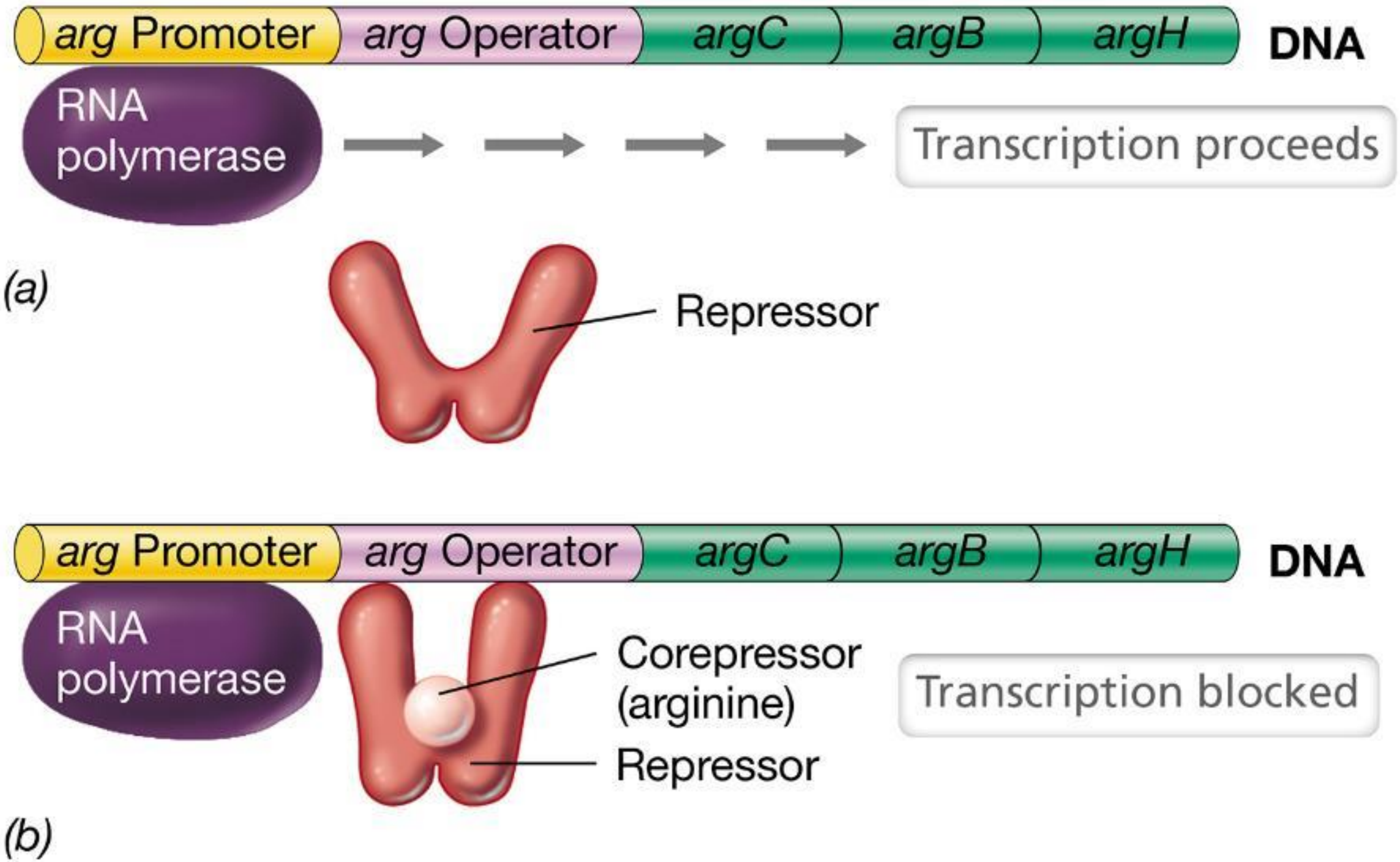


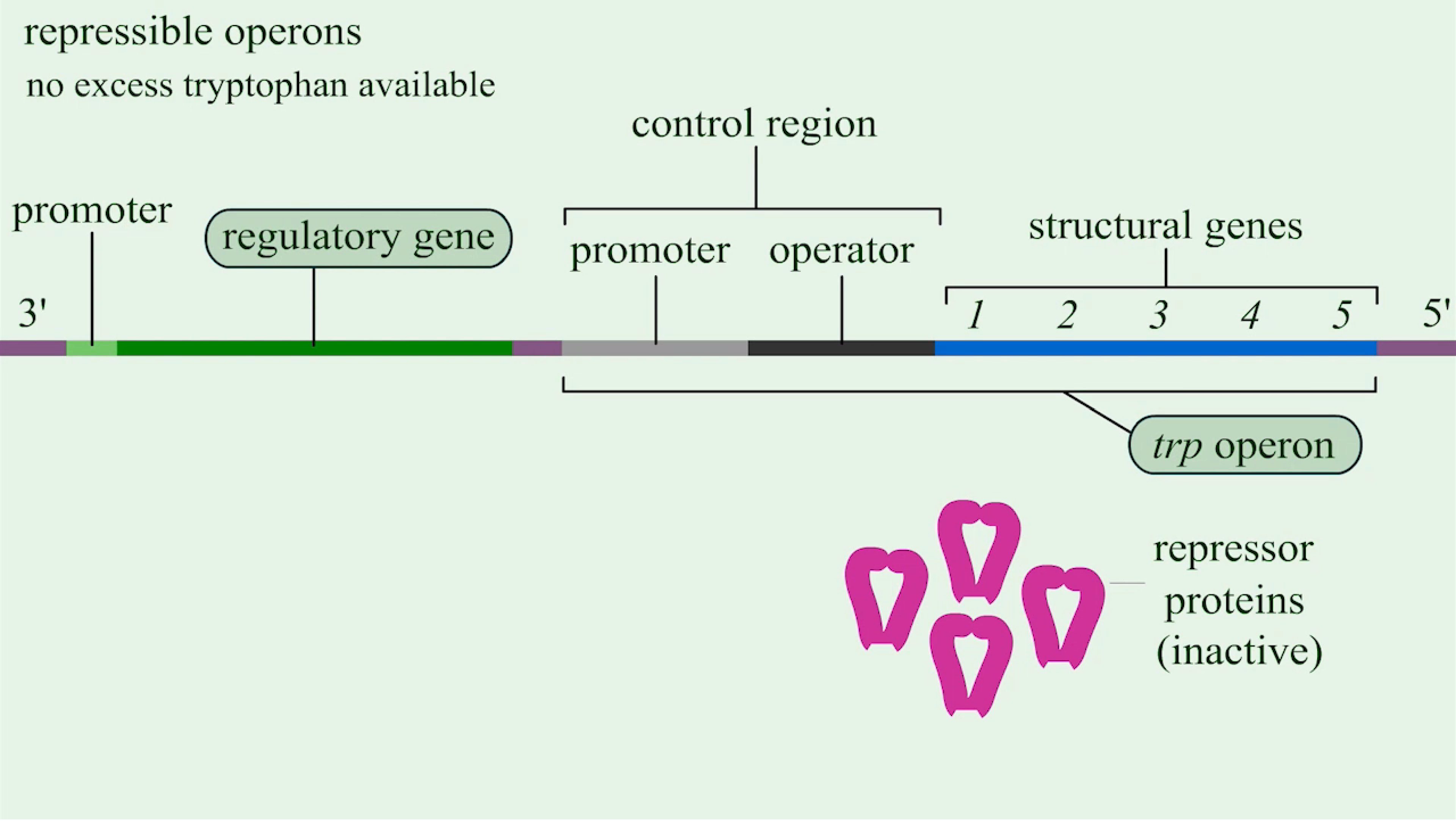
Figure 7.5



arg Promoter
arg Operator
argC
argB
argH
DNA
RNA polymerase
Transcription proceeds
(a)
Repressor
arg Promoter
arg Operator
argC
argB
argH
DNA
RNA polymerase
Corepressor (arginine)
Repressor
Transcription blocked
(b)

Figure 7.5

Operons: Repression



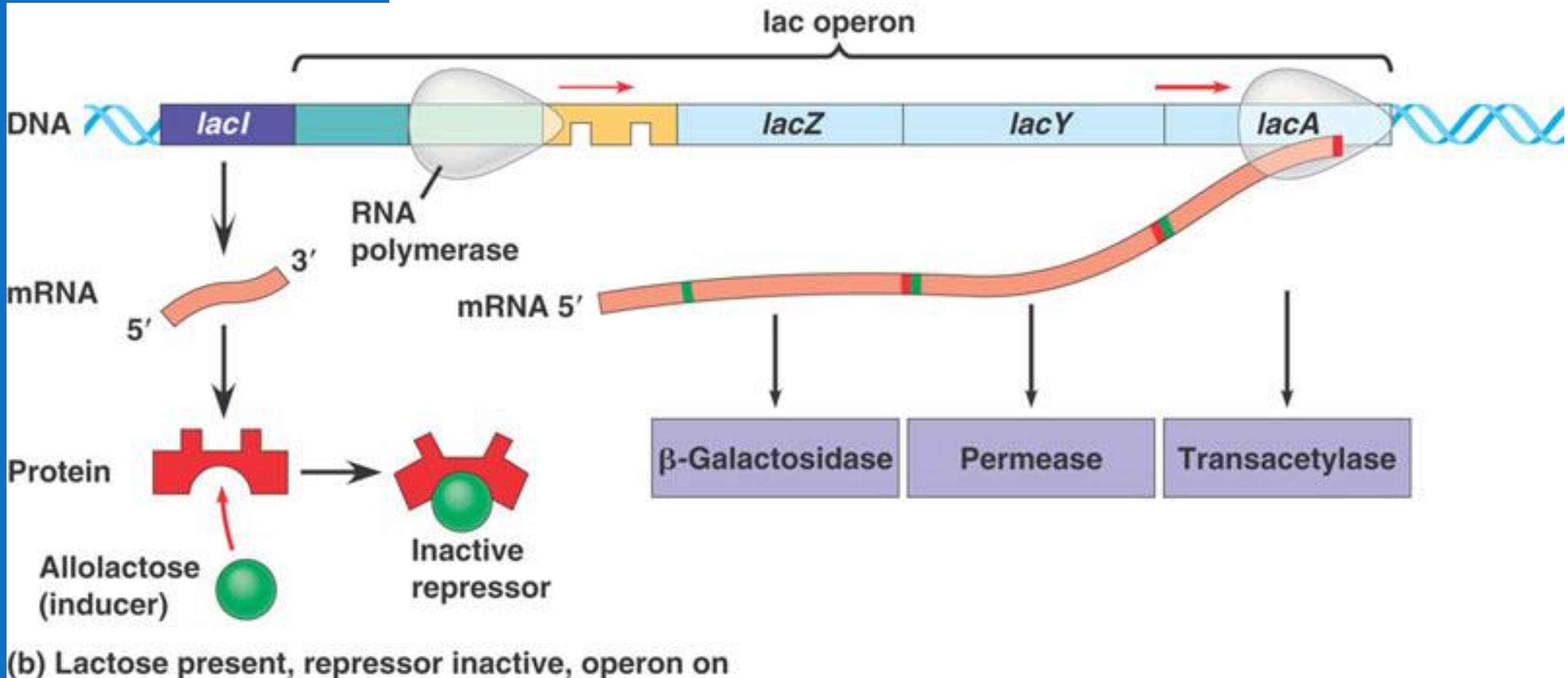
7.3 Repression and Activation

Induction: production of an enzyme in response to presence of substrate (Figure 7.6)

- typically affects catabolic enzymes (*e.g.*, *lac* operon)
- ensures enzymes are synthesized only when needed

Inductie: expressie mogelijk maken

Bio3!



- **Alleen *lac* enzymen als lactose aanwezig is.**
- **Lactose is hier een <u>inducer</u>**

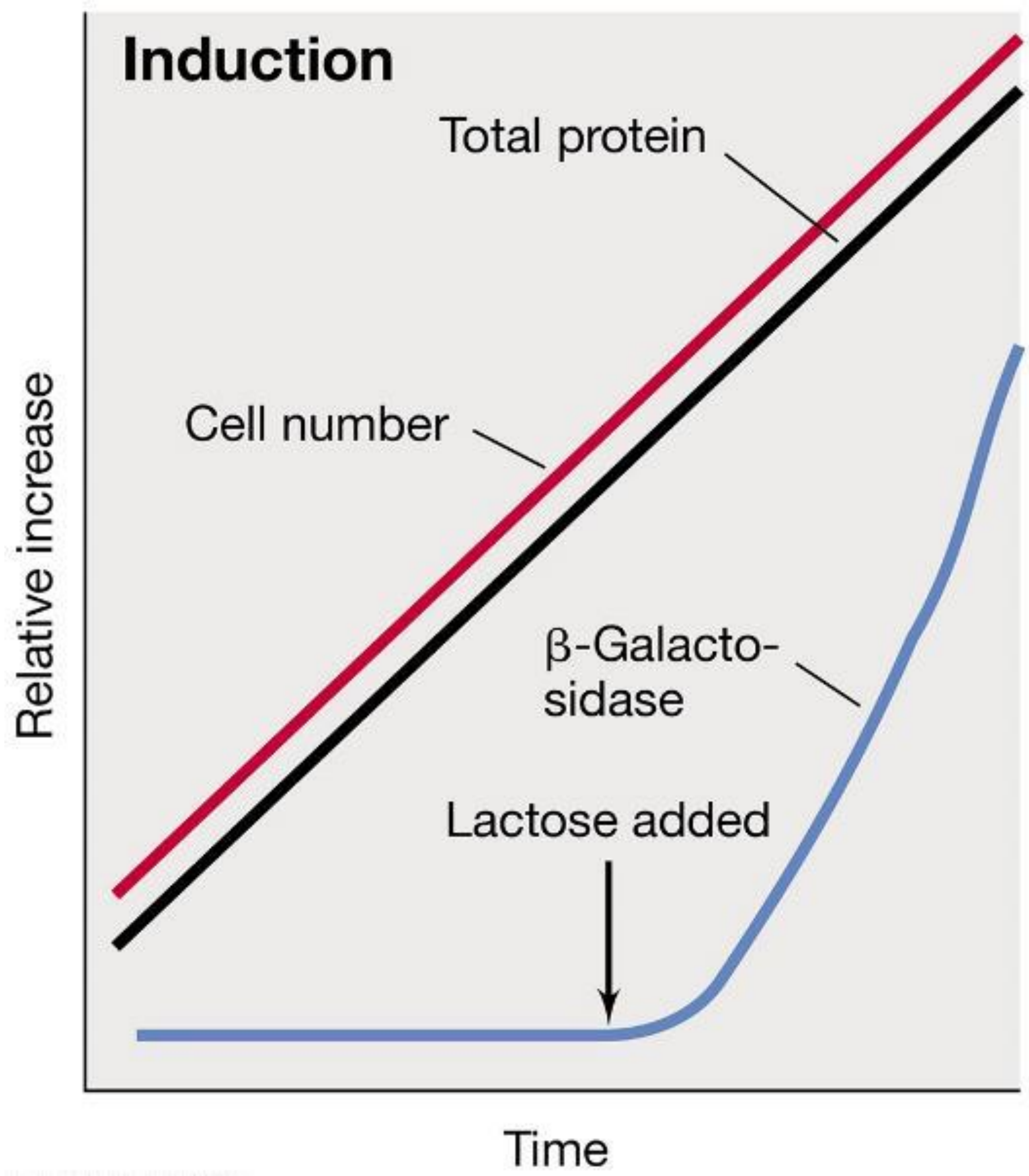

Induction
Total protein
Cell number
β-Galacto-
sidase
Lactose added
Relative increase
Time

Figure 7.6

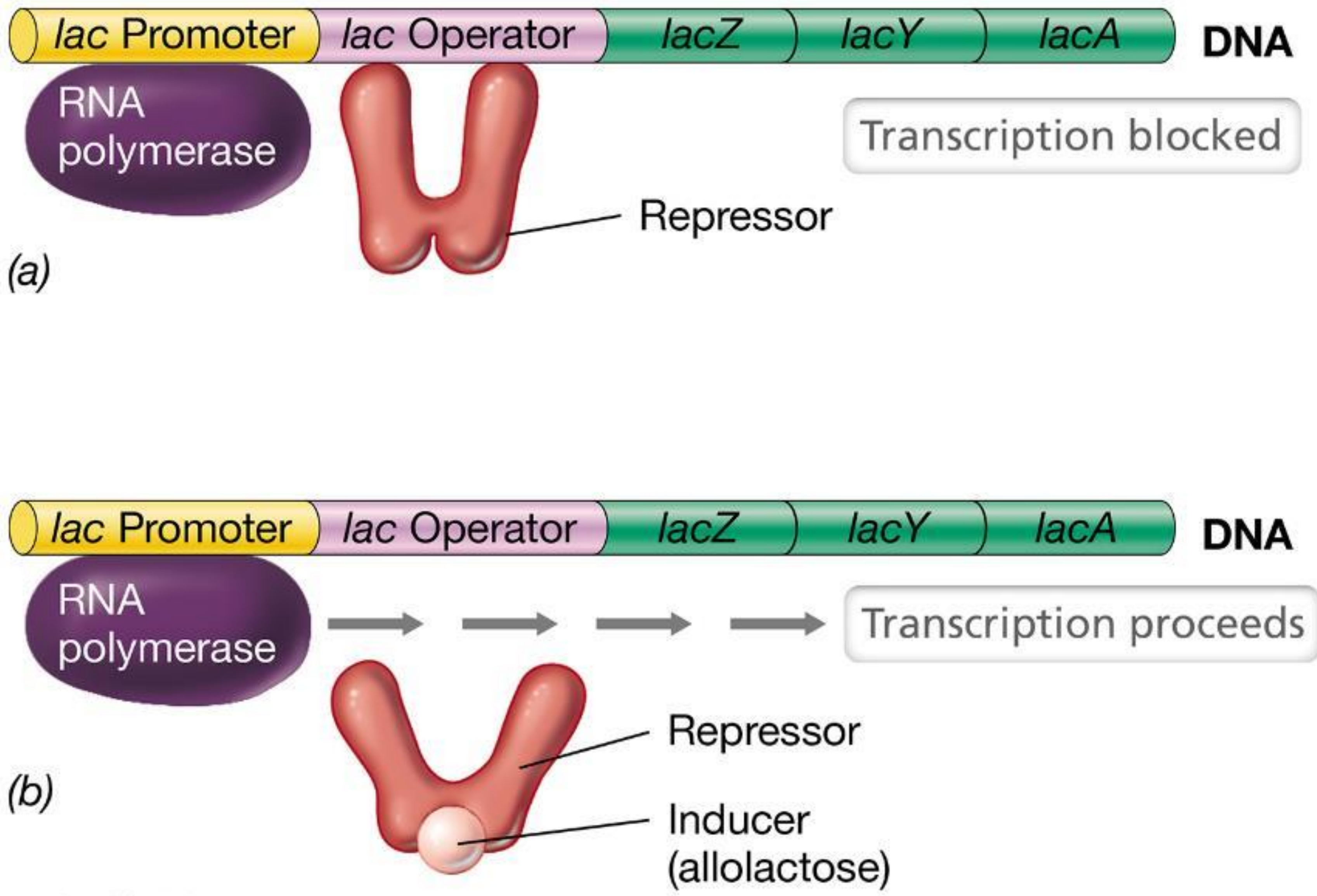
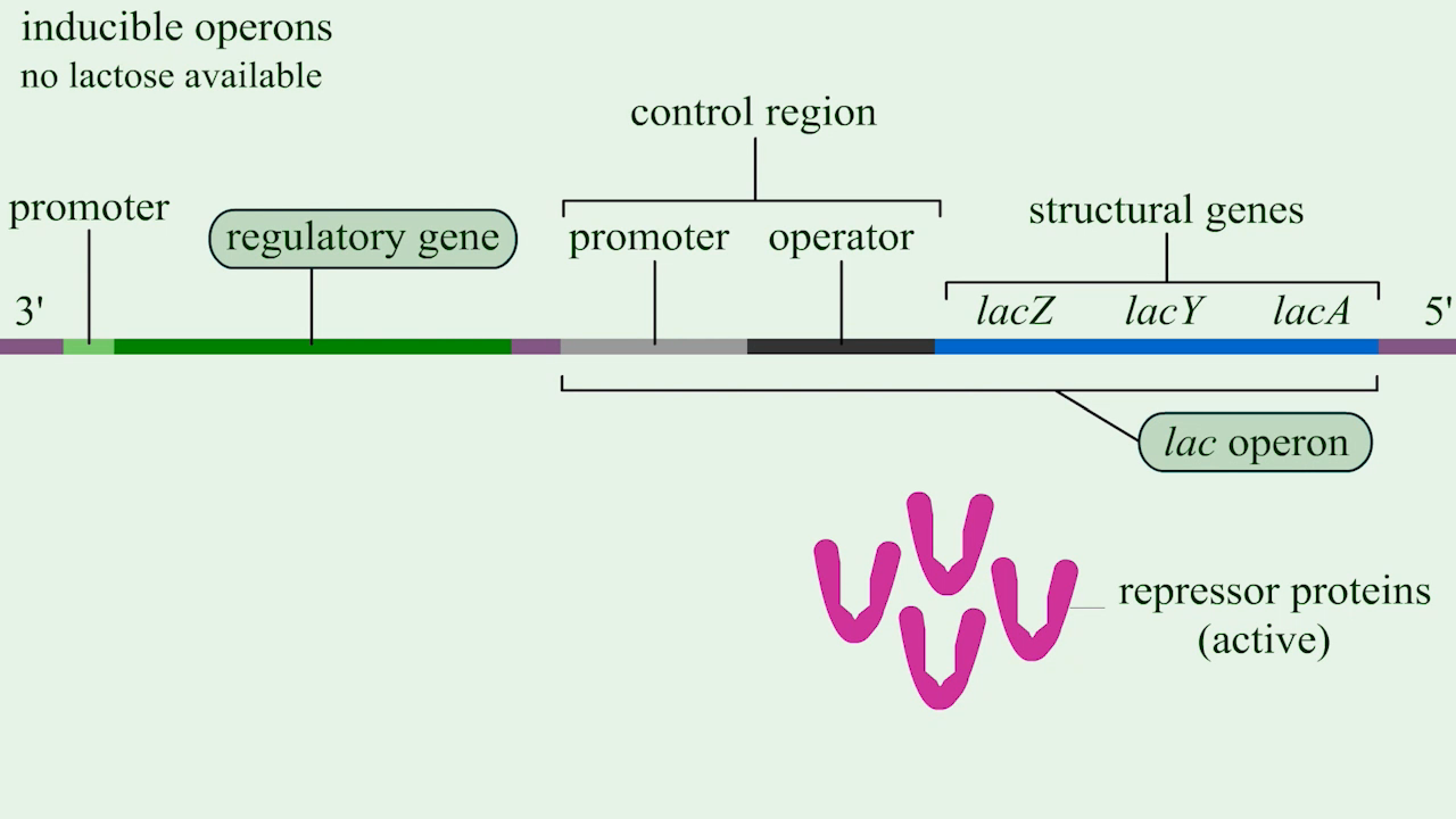

lac Promoter
lac Operator
lacZ
lacY
lacA
DNA
RNA polymerase
Transcription blocked
Repressor
(a)
lac Promoter
lac Operator
lacZ
lacY
lacA
DNA
RNA polymerase
Transcription proceeds
Repressor
Inducer (allolactose)
(b)

Figure 7.6

Operons: Induction



7.3 Repression and Activation

Effectors: collective term for inducers and repressors

- typically small molecules
- can be structural analogs of substrates/products, (*e.g.*, isopropylthiogalactoside [IPTG], allolactose)

7.3 Repression and Activation

- Positive control: regulator protein activates the binding of RNA polymerase to DNA
- Maltose catabolism in *E. coli* (Figure 7.8)
  - Maltose activator protein cannot bind to DNA unless it first binds maltose (inducer).
- Activator proteins bind specifically to *activator-binding site* (certain DNA sequence that is <u>not called an operator</u>).

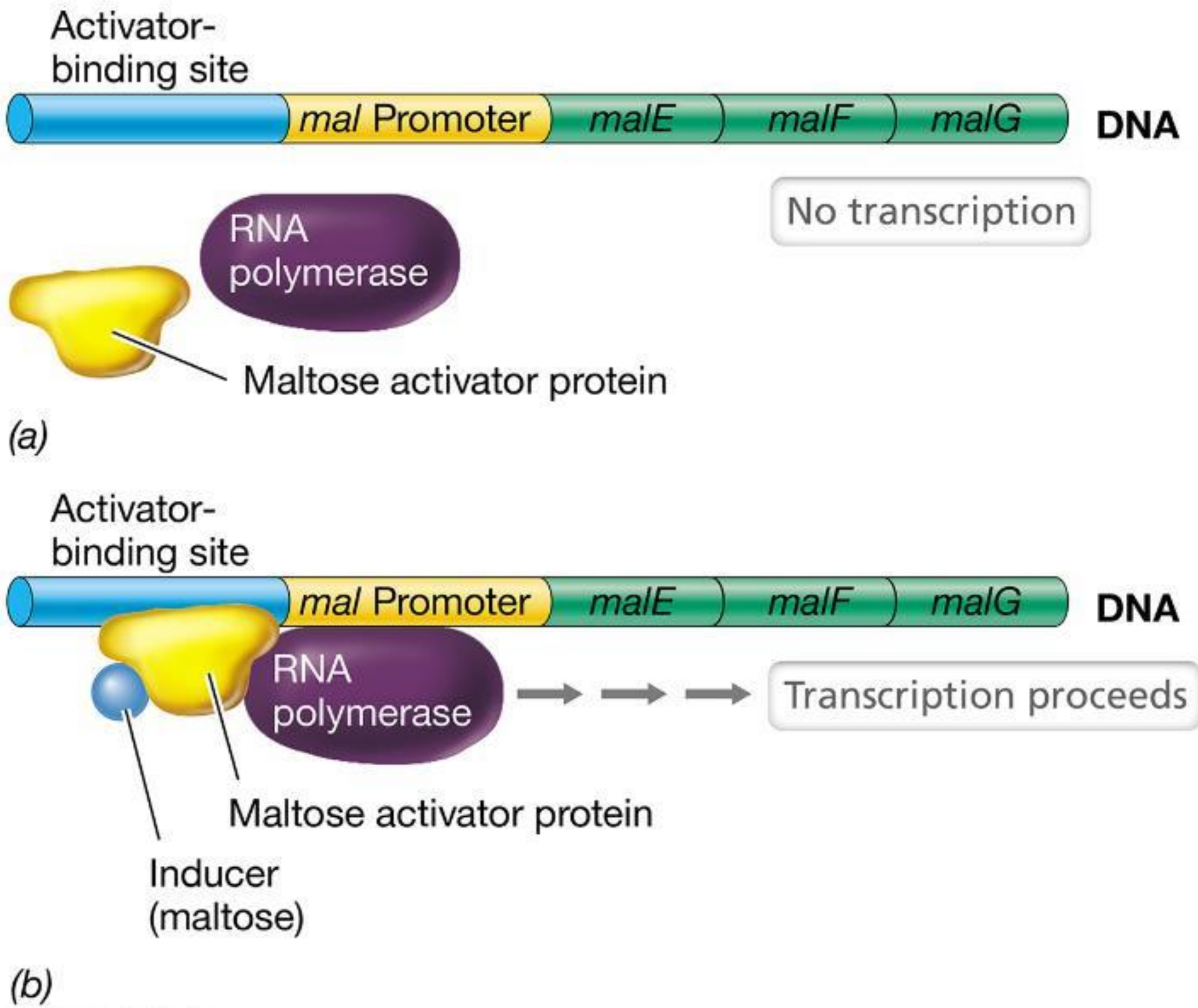

Activator-
binding site
mal Promoter
malE
malF
malG
DNA
No transcription
RNA
polymerase
Maltose activator protein
(a)
Activator-
binding site
mal Promoter
malE
malF
malG
DNA
RNA
polymerase
Transcription proceeds
Maltose activator protein
Inducer
(maltose)
(b)

Figure 7.9

7.3 Repression and Activation

- Promoters of positively controlled operons only weakly bind RNA polymerase.
- Activator protein helps RNA polymerase recognize promoter.
  - may bend DNA structure (Figure 7.7)
  - may interact directly with RNA polymerase (Figure 7.9)
- Many operons have multiple types of control.

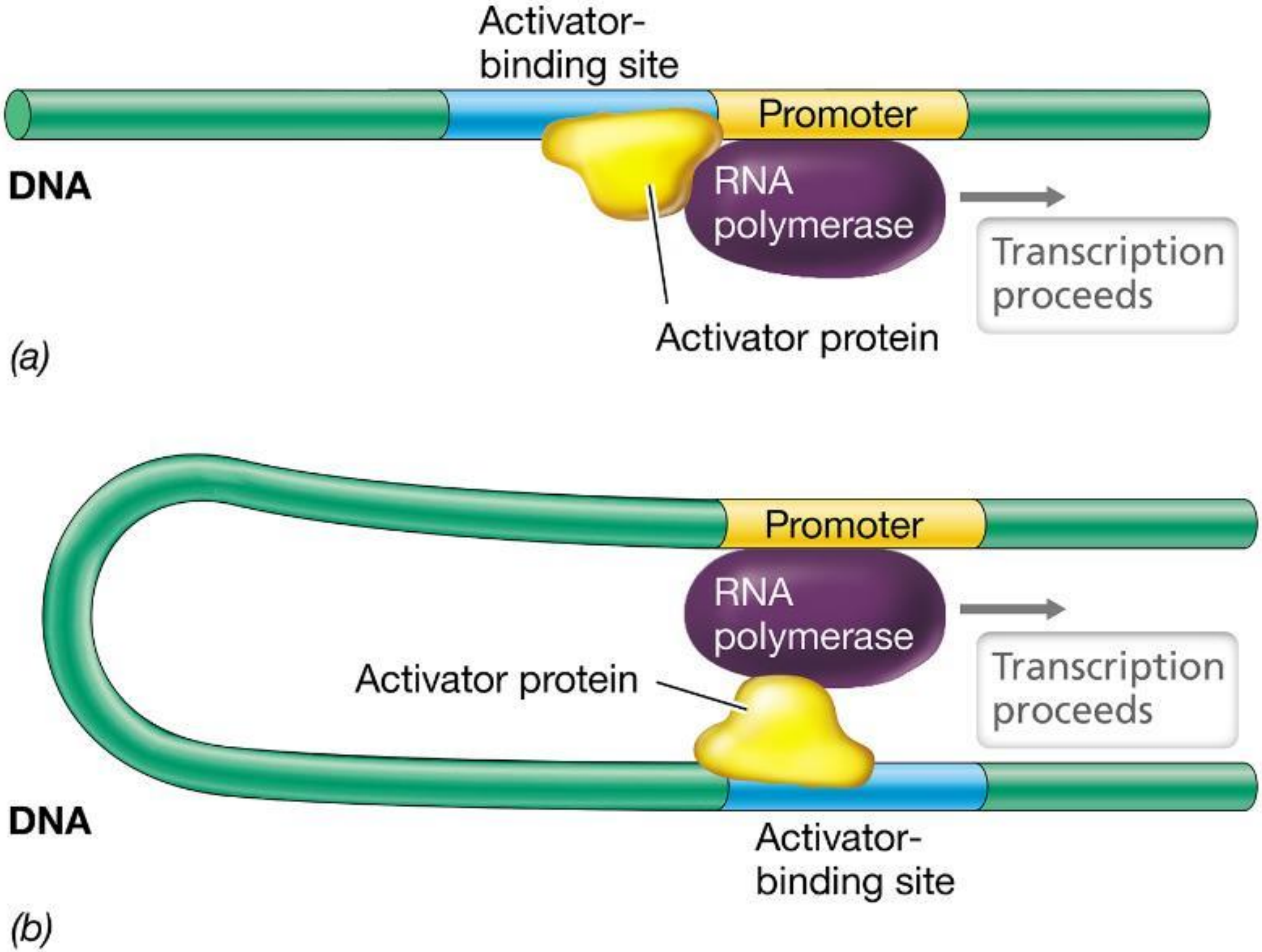
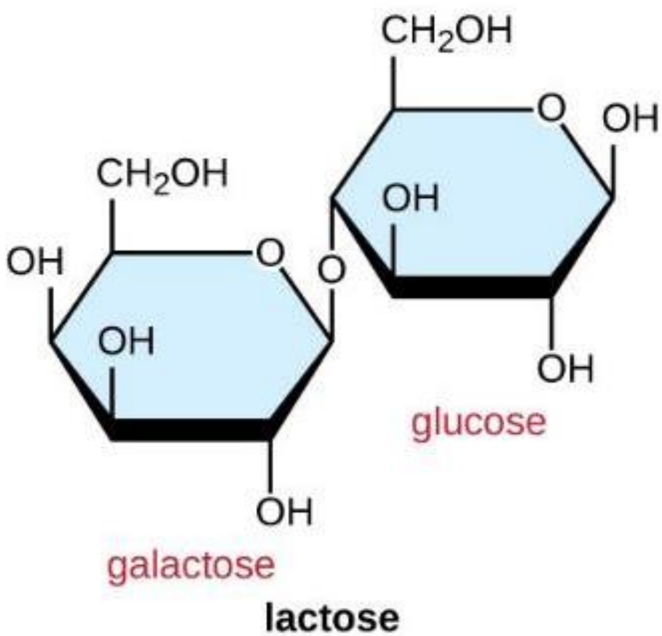

Activator-
binding site
Promoter
DNA
RNA
polymerase
Transcription
proceeds
Activator protein
(a)
Promoter
RNA
polymerase
Transcription
proceeds
Activator protein
DNA
Activator-
binding site
(b)

Figure 7.7

Positieve regulatie

Flashback Bio3: *lac*-operon

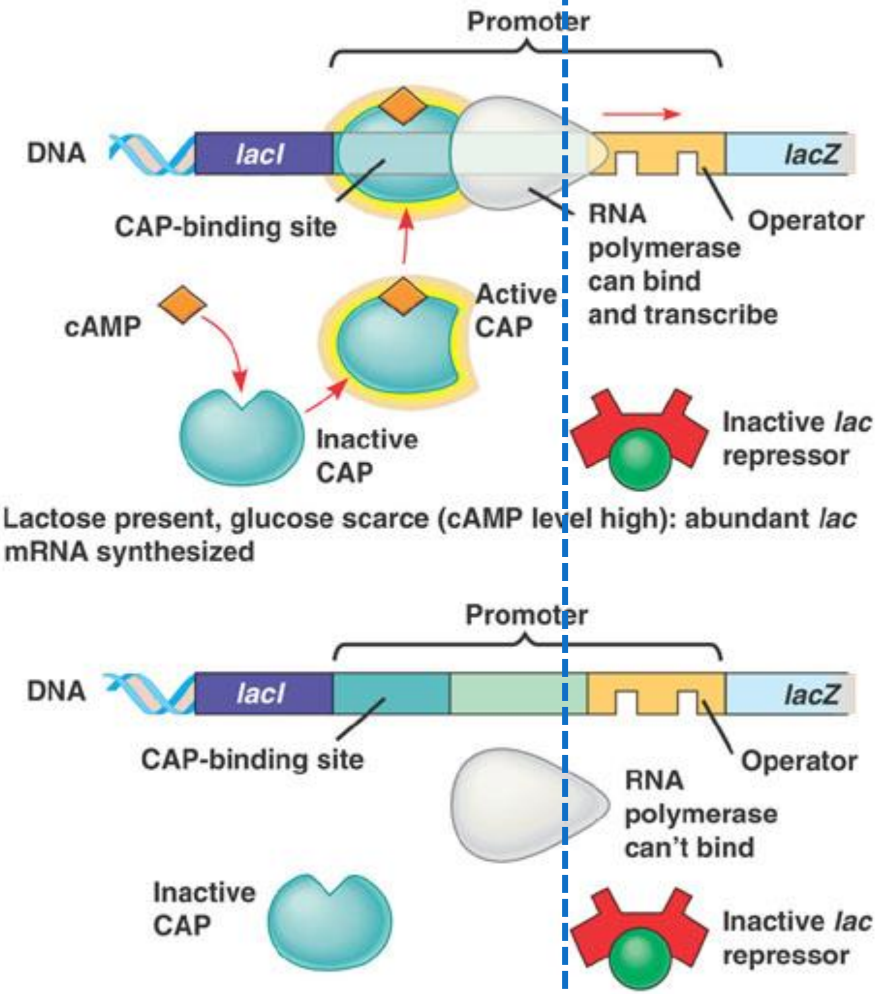
NB: Voedselvoorkeur!



Positieve regulatie

Cataboliet-repressie:

Als glucose laag is hoog cAMP => breakdown van lactose



(a) Lactose present, glucose scarce (cAMP level high): abundant *lac* mRNA synthesized

(b) Lactose present, glucose present (cAMP level low): little *lac* mRNA synthesized

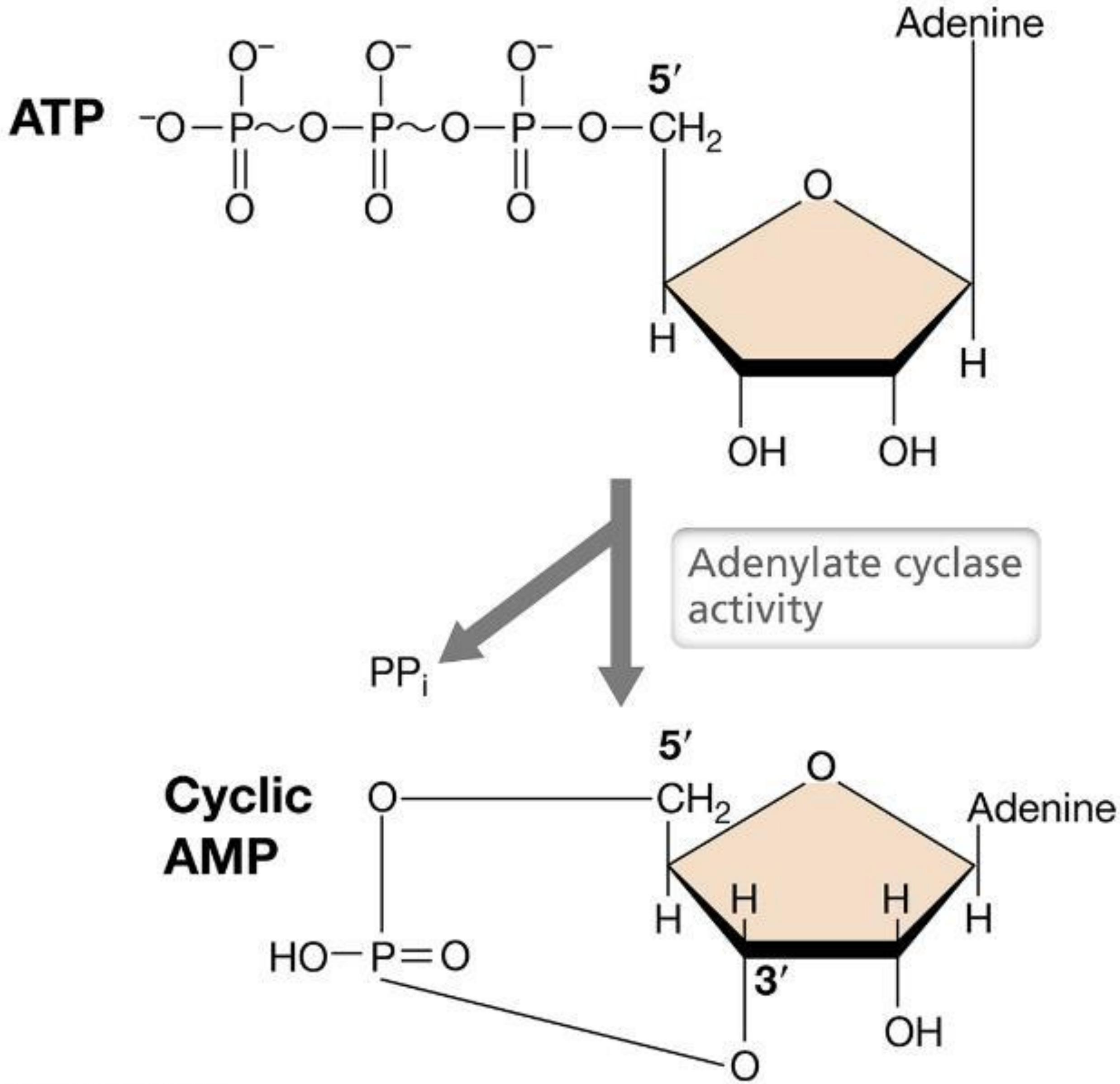
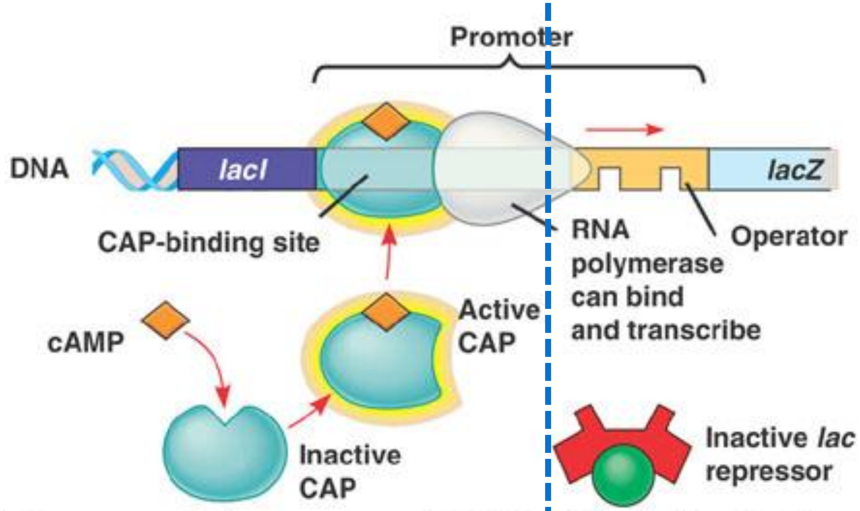

ATP
Adenine
5′
$^-O-P\sim O-P\sim O-P-O-CH_2$
O
H
H
OH
OH
Adenylate cyclase activity
PP_i
Cyclic AMP
5′
CH_2
O
Adenine
H
H
H
H
HO—P=O
3′
OH
O

Figure 7.22

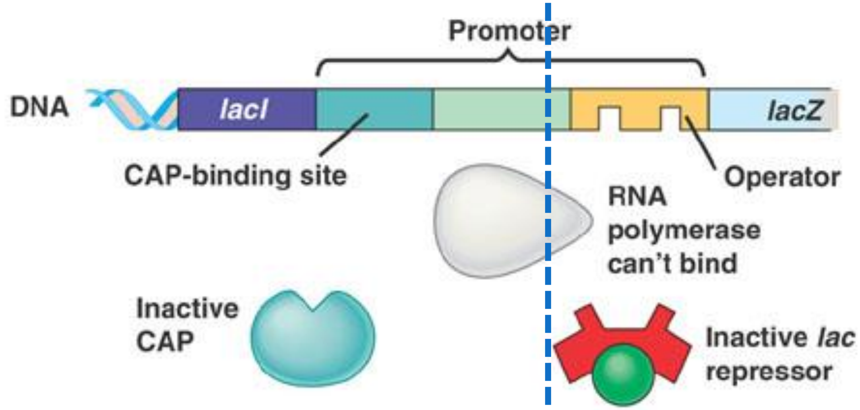
Positieve regulatie

NB: CAP = CRP

https://en.wikipedia.org/wiki/Catabolite_activator_protein



(a) Lactose present, glucose scarce (cAMP level high): abundant *lac* mRNA synthesized



(b) Lactose present, glucose present (cAMP level low): little *lac* mRNA synthesized

7.3 Repression and Activation

- Cyclic AMP and cyclic AMP receptor protein
  - In catabolite repression, transcription is controlled by the *cyclic AMP receptor protein* (*CRP*), an activator protein, and is a form of positive control.
  - CRP binds to DNA only if it has bound *cyclic adenosine monophosphate* (*cyclic AMP* or *cAMP*). (Figure 6.13)
    - regulatory nucleotide derived from a nucleic acid precursor (ATP)

7.3 Repression and Activation

For *lac* genes to be transcribed (Figure 7.14):

- Cyclic AMP level must be high enough for CRP protein to bind to CRP-binding site.
- Lactose or another inducer must be present to prevent lactose repressor (LacI) binding.

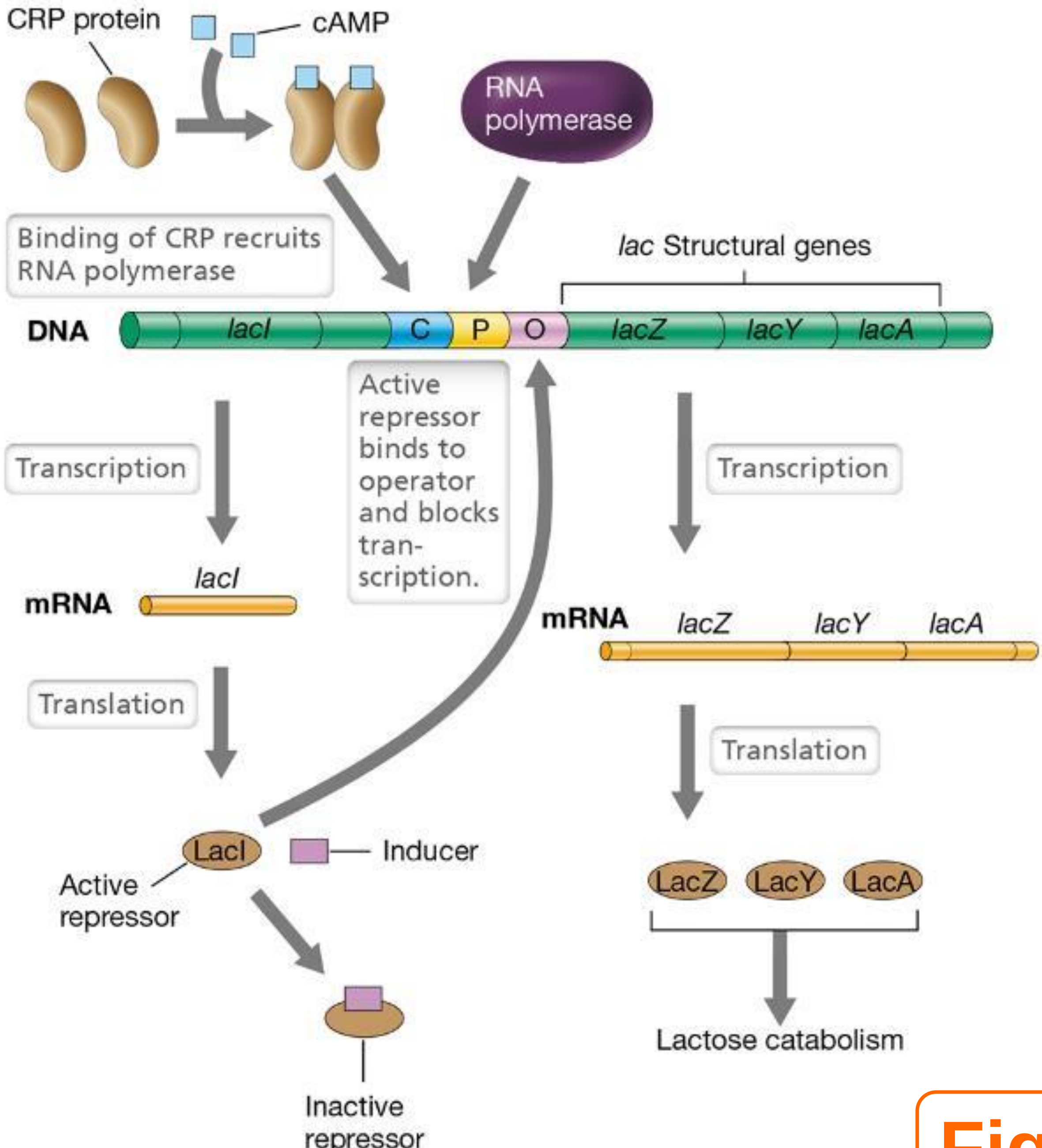

CRP protein
cAMP
RNA polymerase
Binding of CRP recruits RNA polymerase
lac Structural genes
DNA
lacI
C
P
O
lacZ
lacY
lacA
Active repressor binds to operator and blocks tran-scription.
Transcription
Transcription
lacI
mRNA
mRNA
lacZ
lacY
lacA
Translation
Translation
LacI
Inducer
Active repressor
LacZ
LacY
LacA
Lactose catabolism
Inactive repressor

Figure 7.20

7.3 Repression and Activation

- Genes for maltose are spread out over the chromosome in several operons. (Figure 7.11)
  - Each operon has an activator-binding site.
  - Multiple operons controlled by the same regulatory protein are called a regulon.
- Regulons also exist for negatively controlled systems (*e.g.*, arginine regulon).

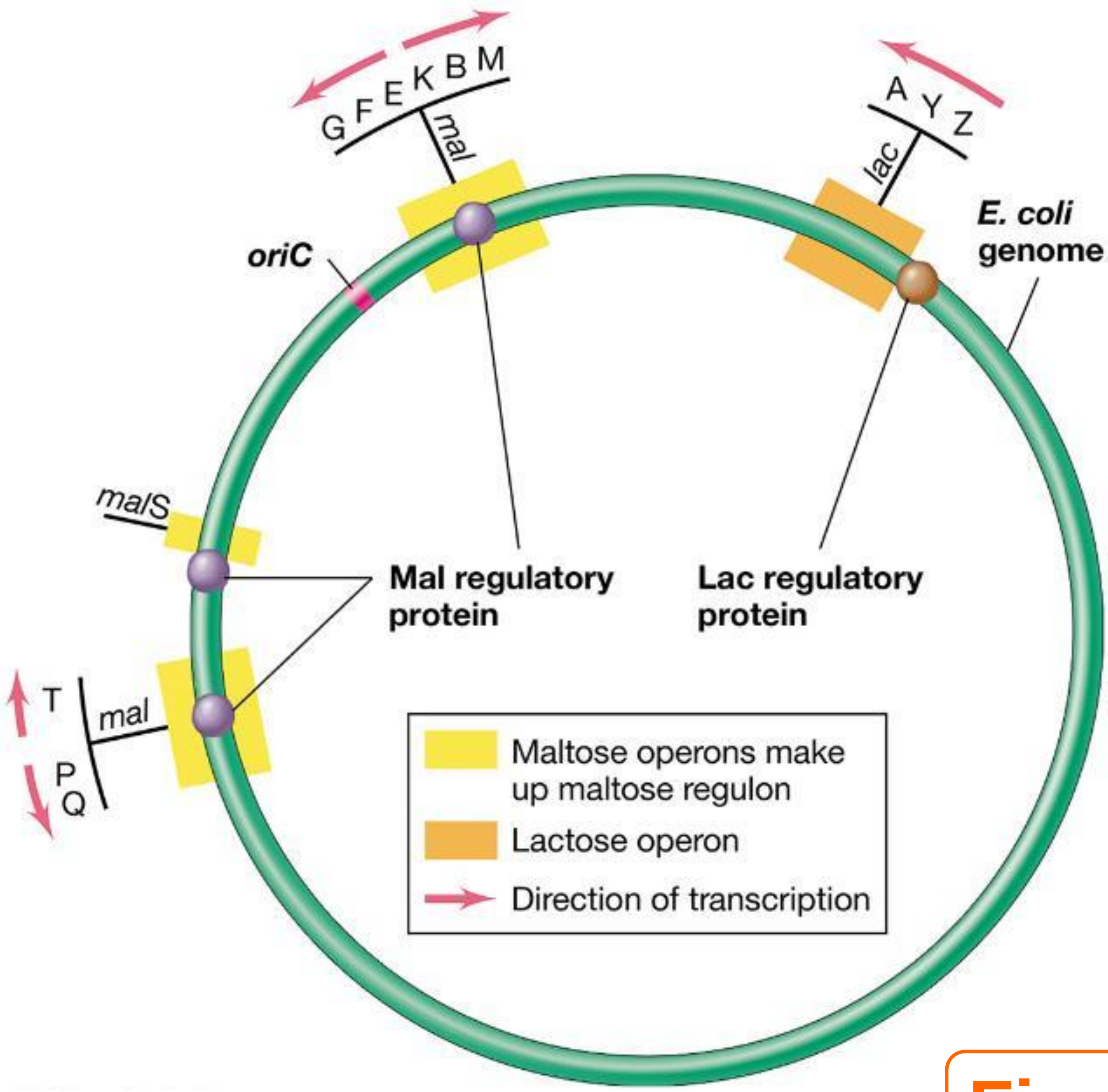

G F E K B M
mal
A Y Z
lac
E. coli
genome
oriC
malS
Mal regulatory
protein
Lac regulatory
protein
T
mal
P
Q
Maltose operons make
up maltose regulon
Lactose operon
Direction of transcription

Figure 7.10

7.3 Repression and Activation

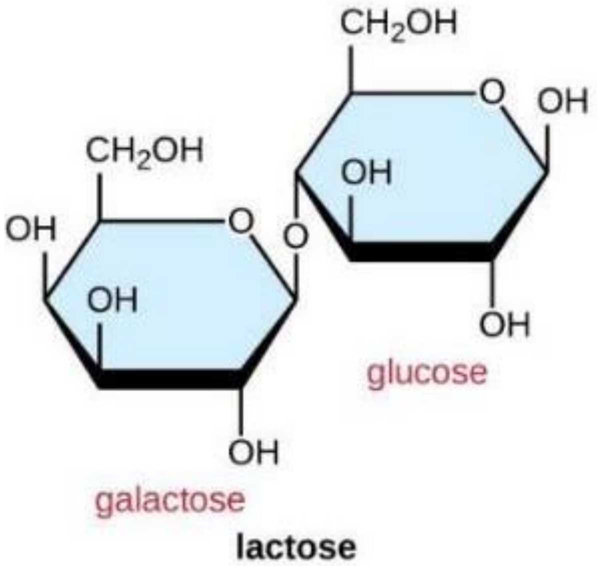
- *Global control systems:* regulate expression of many different genes simultaneously (*e.g.,* lactose operon and maltose regulon)
- Catabolite repression is an example of global control.
  - controls use of carbon sources if more than one present
  - Synthesis of unrelated catabolic enzymes (*e.g.,* lactose operon and maltose regulon) is repressed if glucose is present in growth medium.
  - also called “glucose effect”
  - ensures that the "best" carbon and energy source is used first

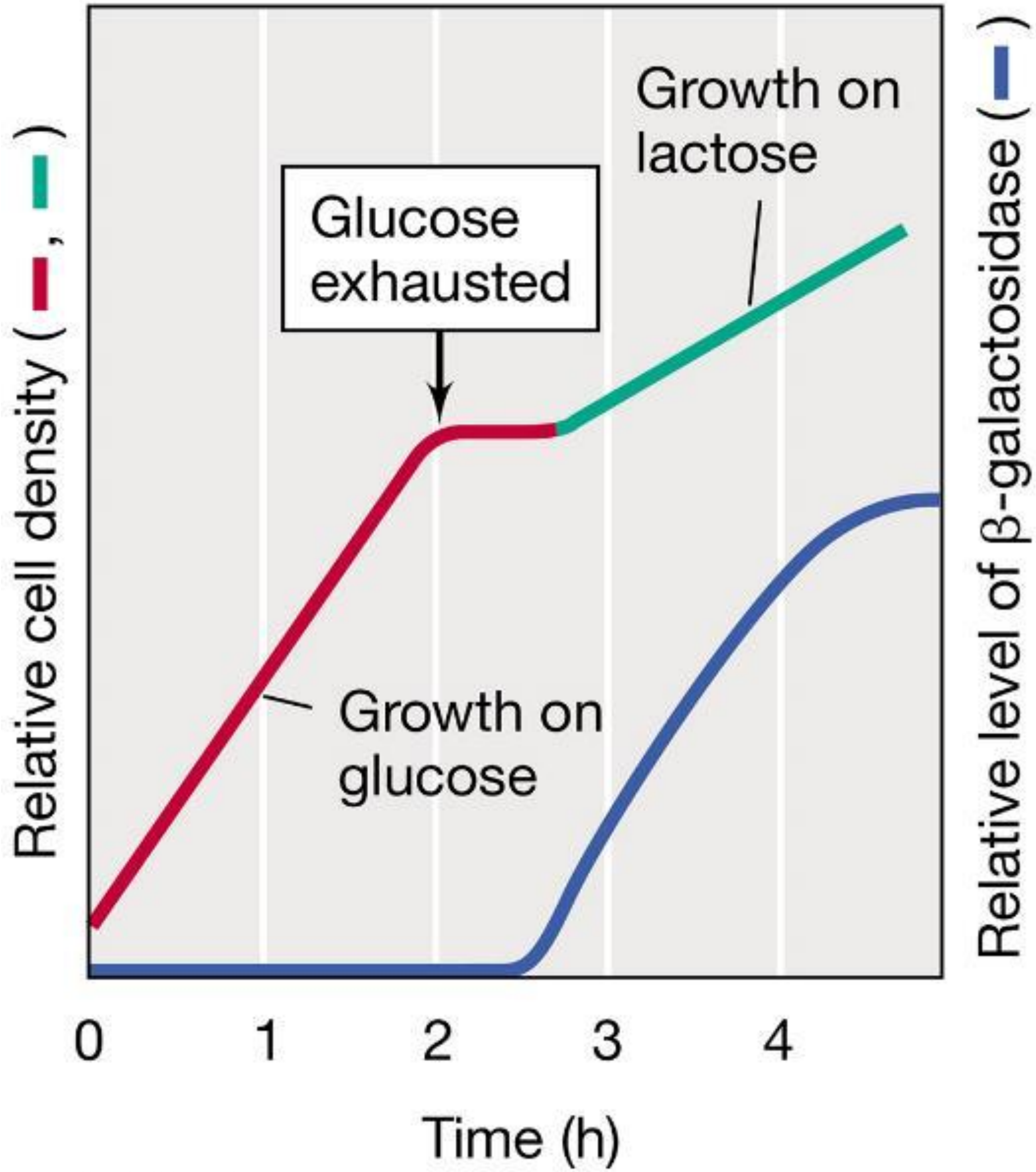
7.4 Global Control and the *lac* Operon

- *Diauxic growth:* two exponential growth phases if two energy sources available (Figure 6.12)
  - better energy source consumed first, growth stops
  - After lag, growth resumes with second energy source.

Flashback Bio3: *lac*-operon

NB: Voedselvoorkeur!





Glucose exhausted
Growth on lactose
Growth on glucose
Relative cell density (,)
Relative level of β-galactosidase ( )
0
1
2
3
4
Time (h)

Figure 7.21

EINDE LES 1

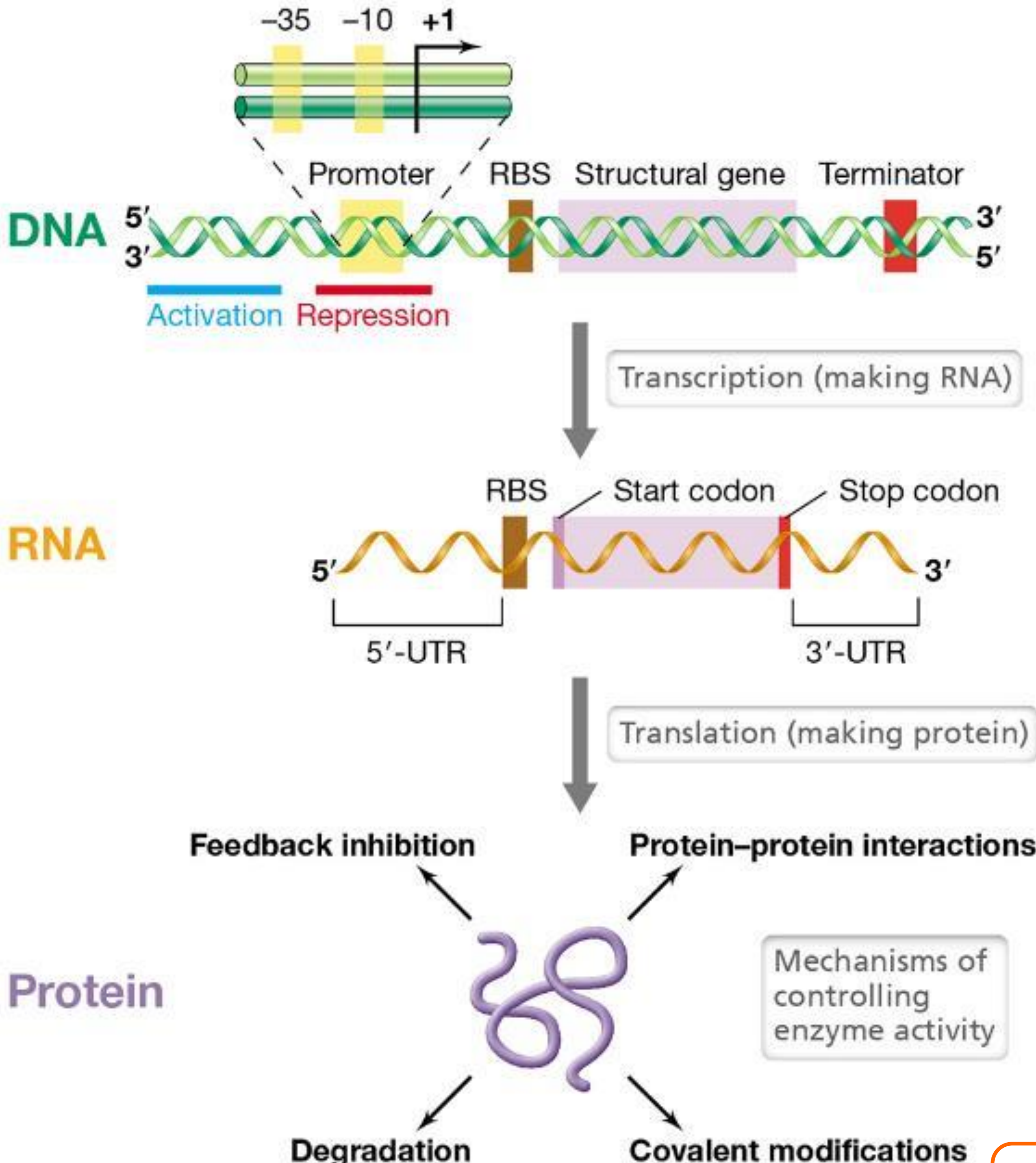
Microbiologie 2: Les 2

II. Sensing and Signal Transduction

Dictyostelium chemotaxis to cAMP

https://www.youtube.com/watch?v=lFx73Wq2QSQ

Microbiologie 2: Les 1 Flashback!



–35
–10
+1
Promoter
RBS
Structural gene
Terminator
DNA
5′
3′
3′
5′
Activation
Repression
Transcription (making RNA)
RBS
Start codon
Stop codon
RNA
5′
3′
5′-UTR
3′-UTR
Translation (making protein)
Feedback inhibition
Protein–protein interactions
Protein
Mechanisms of controlling enzyme activity
Degradation
Covalent modifications

Figure 7.1

Hier:

- Homodimeer
- Bindt in grote groef

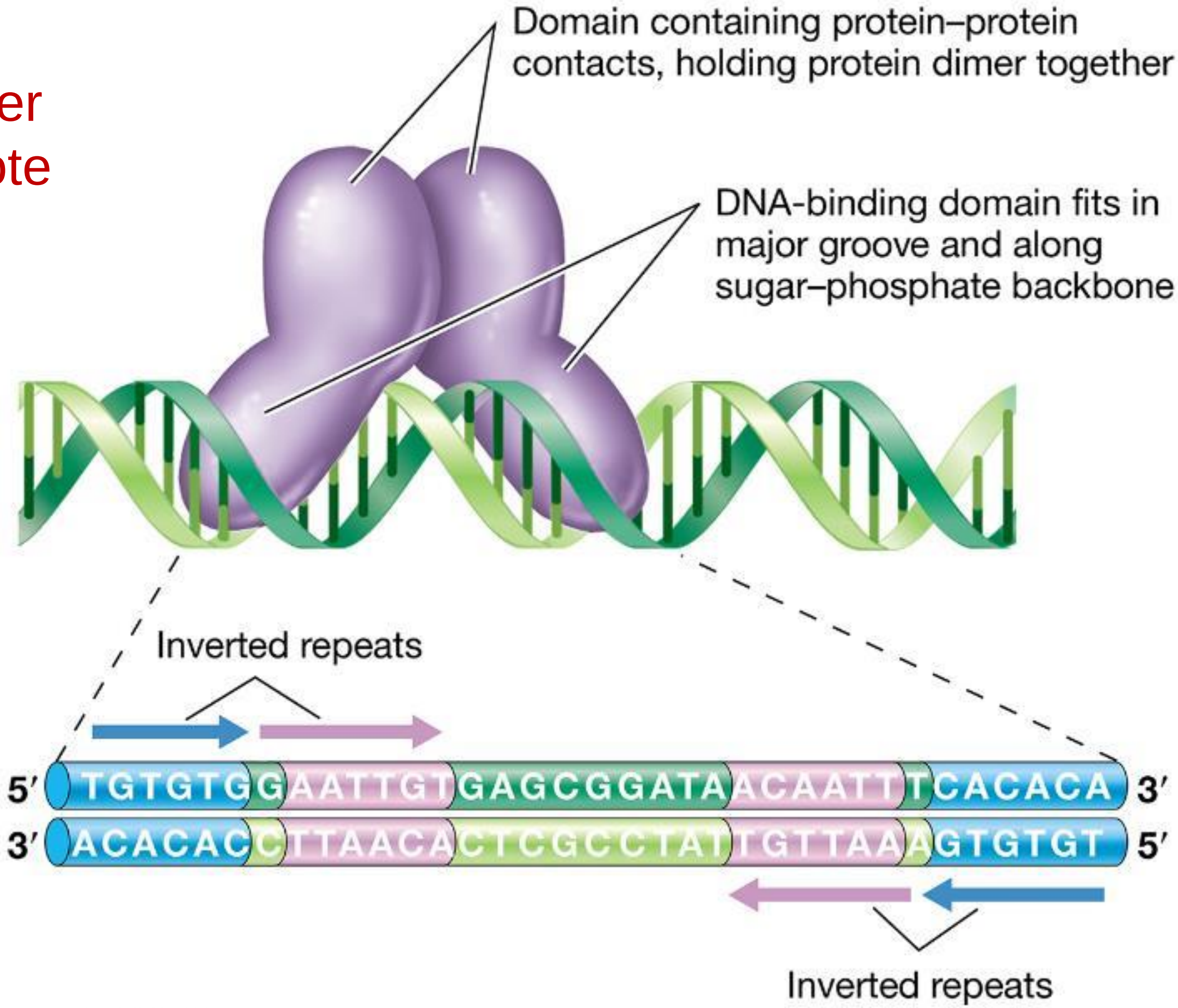


Figure 7.2

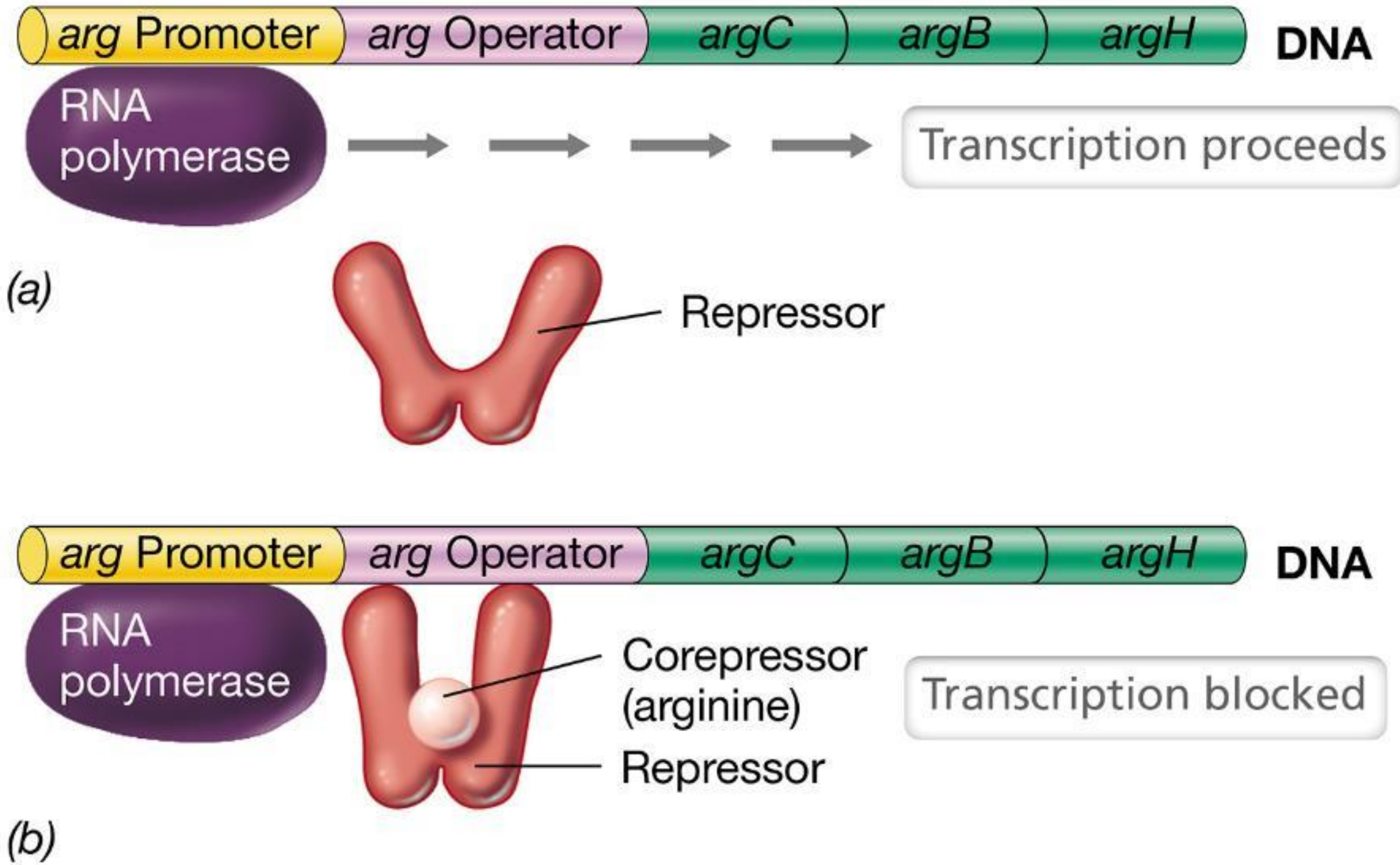

arg Promoter
arg Operator
argC
argB
argH
DNA
RNA polymerase
Transcription proceeds
(a)
Repressor
arg Promoter
arg Operator
argC
argB
argH
DNA
RNA polymerase
Corepressor (arginine)
Repressor
Transcription blocked
(b)

Figure 7.6

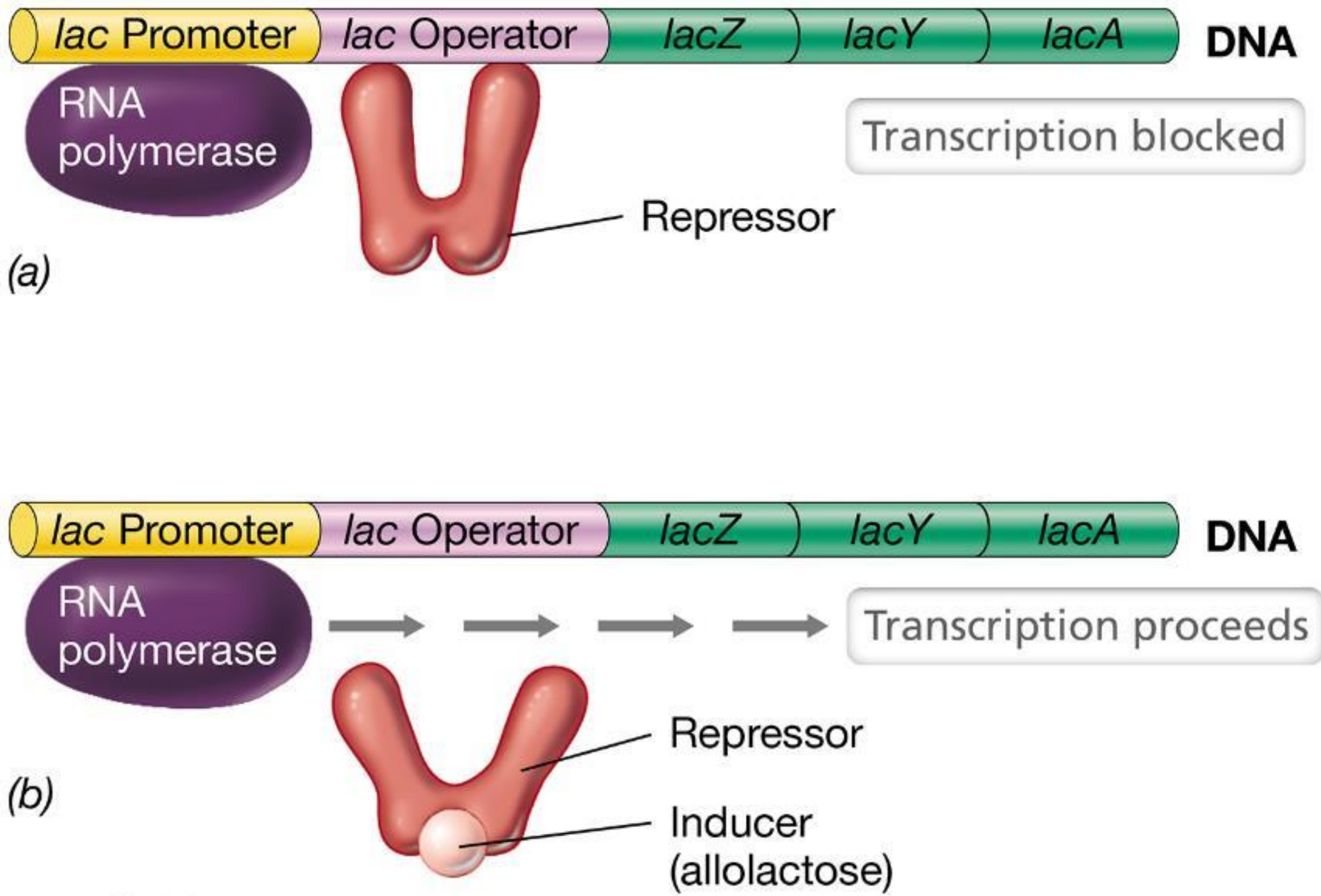

lac Promoter
lac Operator
lacZ
lacY
lacA
DNA
RNA polymerase
Transcription blocked
Repressor
(a)
lac Promoter
lac Operator
lacZ
lacY
lacA
DNA
RNA polymerase
Transcription proceeds
Repressor
Inducer (allolactose)
(b)

Figure 7.7

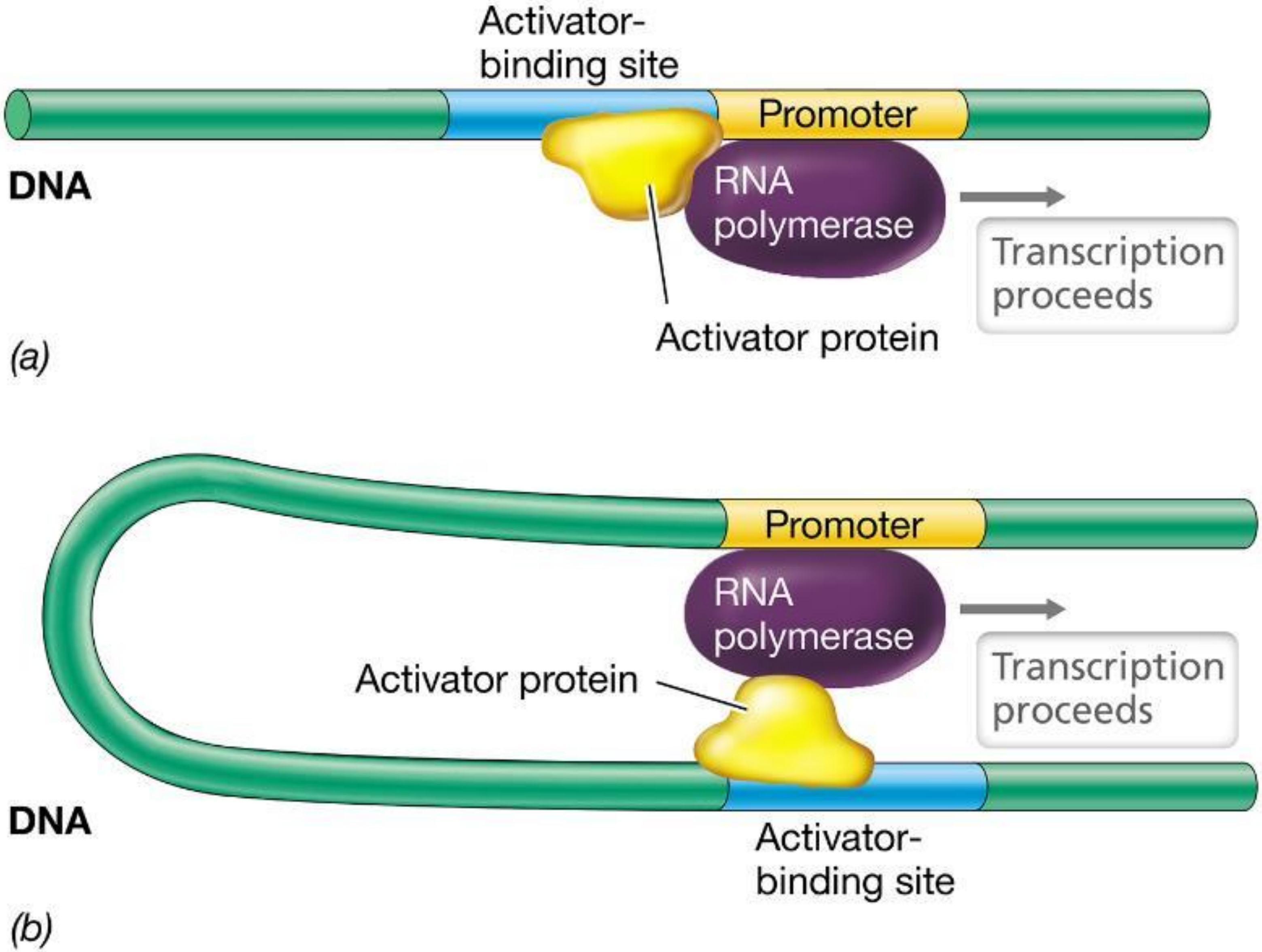

Activator-
binding site
Promoter
DNA
RNA
polymerase
Transcription
proceeds
Activator protein
(a)
Promoter
RNA
polymerase
Transcription
proceeds
Activator protein
DNA
Activator-
binding site
(b)

Figure 7.10

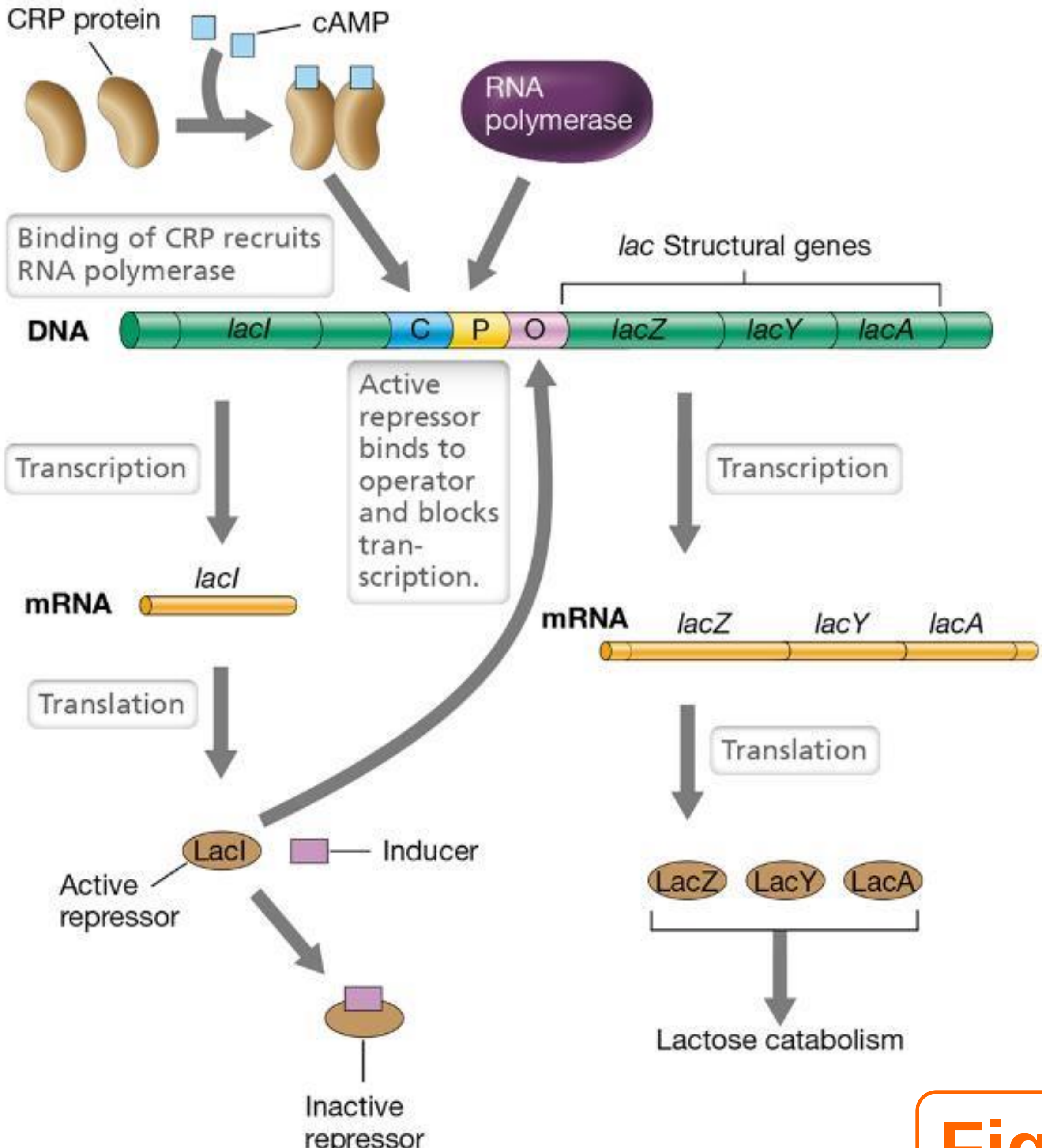

CRP protein
cAMP
RNA polymerase
Binding of CRP recruits RNA polymerase
lac Structural genes
DNA
lacI
C
P
O
lacZ
lacY
lacA
Transcription
Active repressor binds to operator and blocks tran-scription.
Transcription
lacI
mRNA
mRNA
lacZ
lacY
lacA
Translation
Translation
LacI
Inducer
Active repressor
LacZ
LacY
LacA
Lactose catabolism
Inactive repressor

Figure 7.14

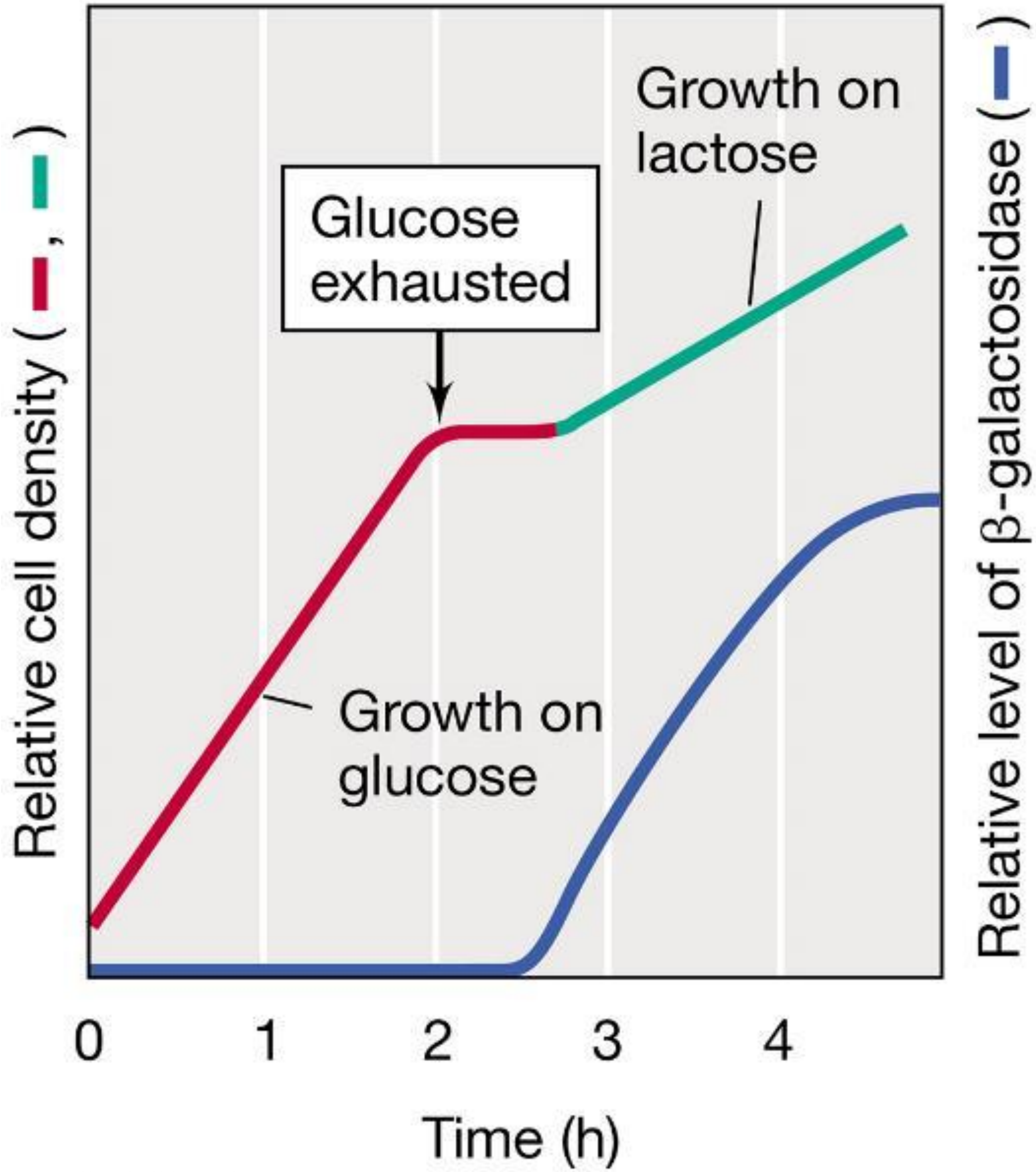

Relative cell density (,)
Relative level of β-galactosidase ( )
Glucose exhausted
Growth on lactose
Growth on glucose
0
1
2
3
4
Time (h)

Figure 7.21

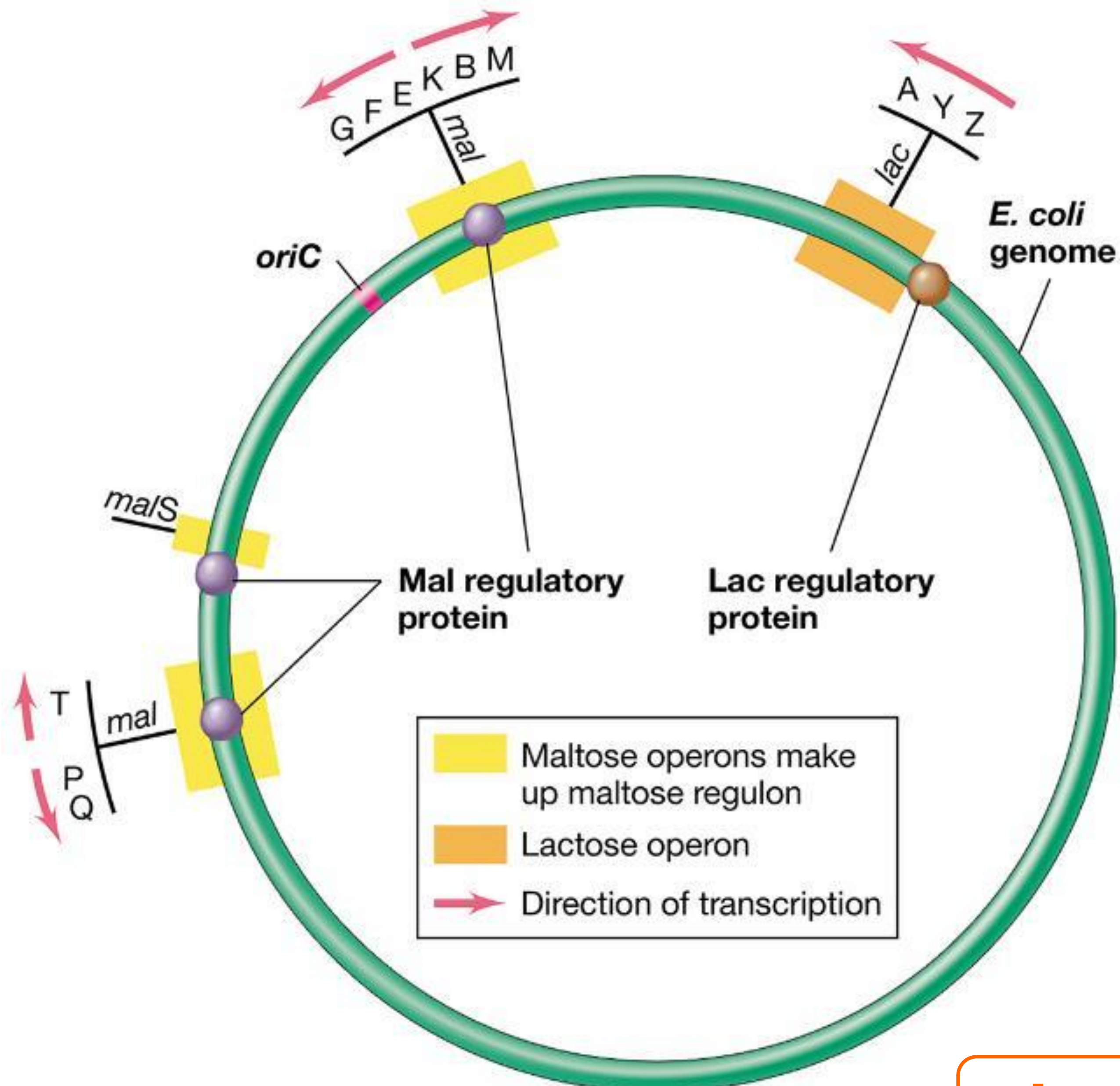

G F E K B M
mal
A Y Z
lac
oriC
E. coli genome
malS
T
mal
P
Q
Mal regulatory protein
Lac regulatory protein
Maltose operons make up maltose regulon
Lactose operon
Direction of transcription

Figure 7.11

II. Sensing and Signal Transduction

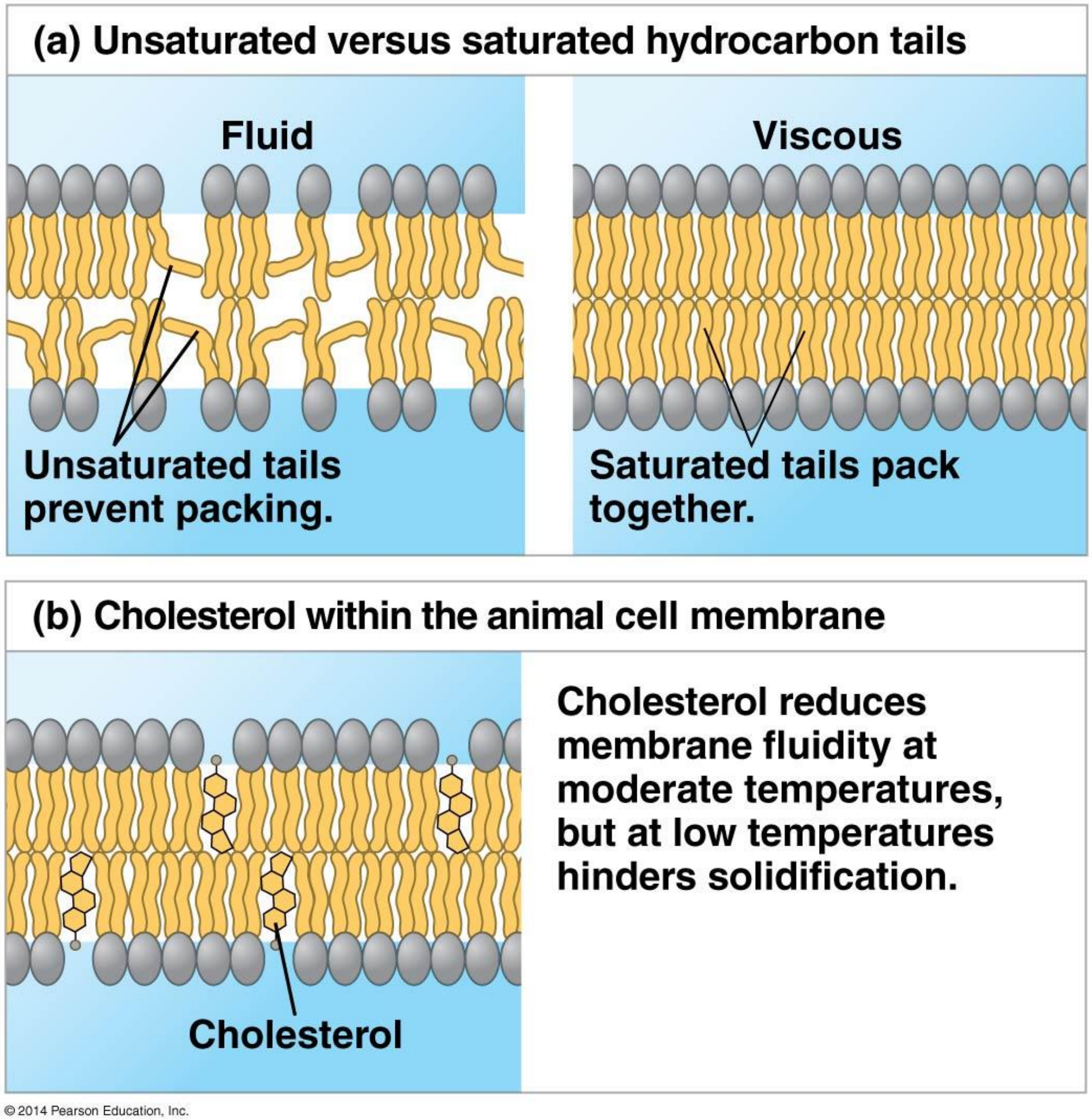
Dictyostelium chemotaxis to cAMP

https://www.youtube.com/watch?v=lFx73Wq2QSQ

II. Sensing and Signal Transduction

- 7.5 Two-Component Regulatory Systems
- 7.6 Regulation of Chemotaxis
- 7.7 Cell-to-Cell Signalling (Quorum sensing)

Celmembraan



- Alleen kleine, <u>apolaire</u> moleculen kunnen passeren (bv steroïd hormonen) of geïmporteerd (bv. suikers).
- Kan het signaal niet door het membraan => o.h.a. two-component regulatory systems

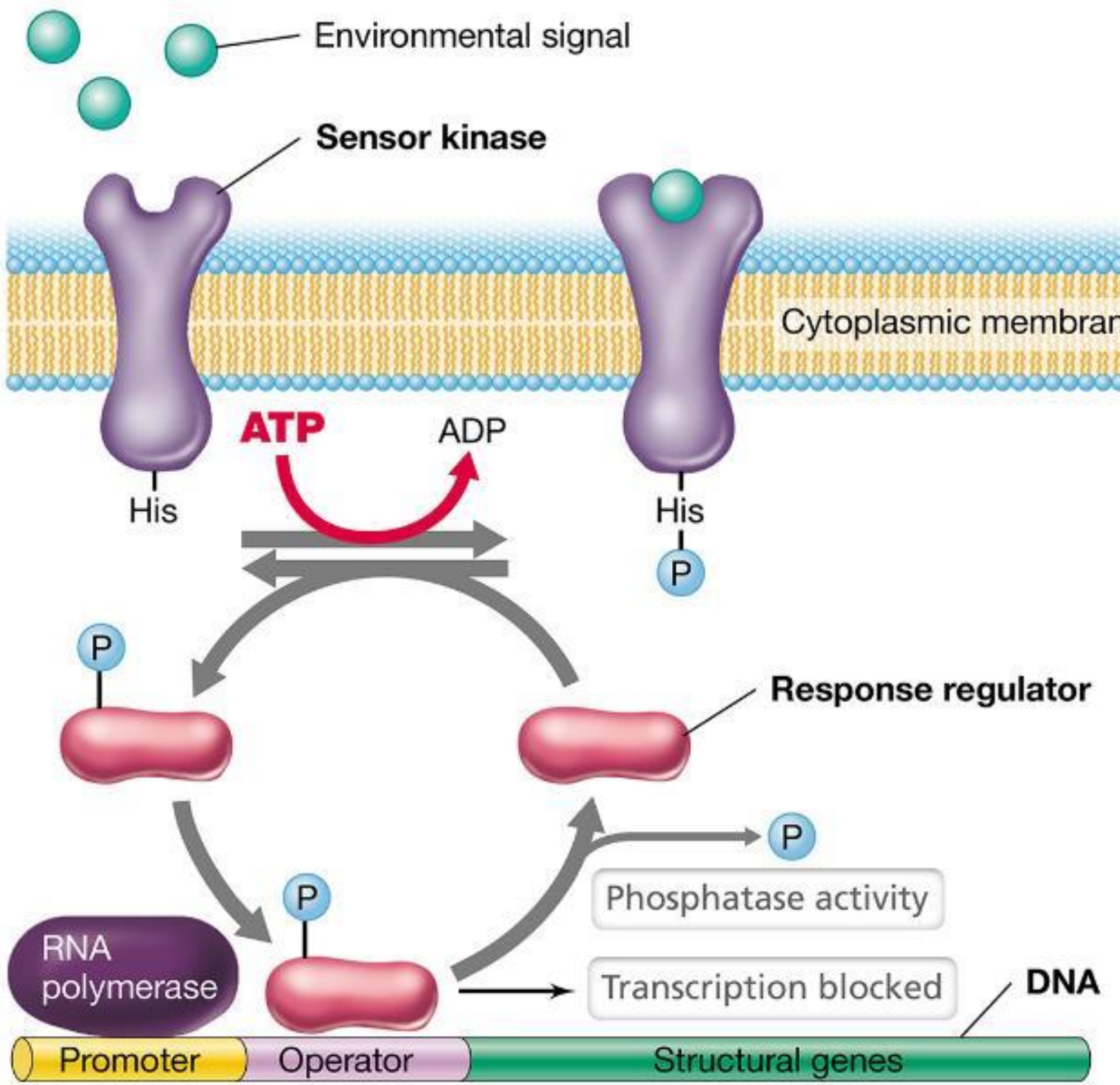

Environmental signal
Sensor kinase
Cytoplasmic membrane
ATP
ADP
His
His
P
P
Response regulator
P
P
Phosphatase activity
RNA polymerase
Transcription blocked
DNA
Promoter
Operator
Structural genes

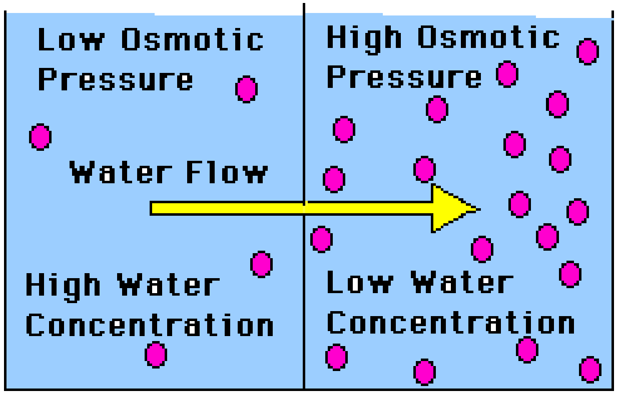
Figure 7.13

7.5 Two-Component Regulatory Systems

- Two-component regulatory systems (Figure 7.17)
  - made up of two different proteins
    - sensor kinase (in cytoplasmic membrane): detects environmental signal and autophosphorylates
    - response regulator (in cytoplasm): DNA-binding protein that regulates transcription
  - also has feedback loop
    - terminates signal
    - uses *phosphatase* that removes phosphate from response regulator

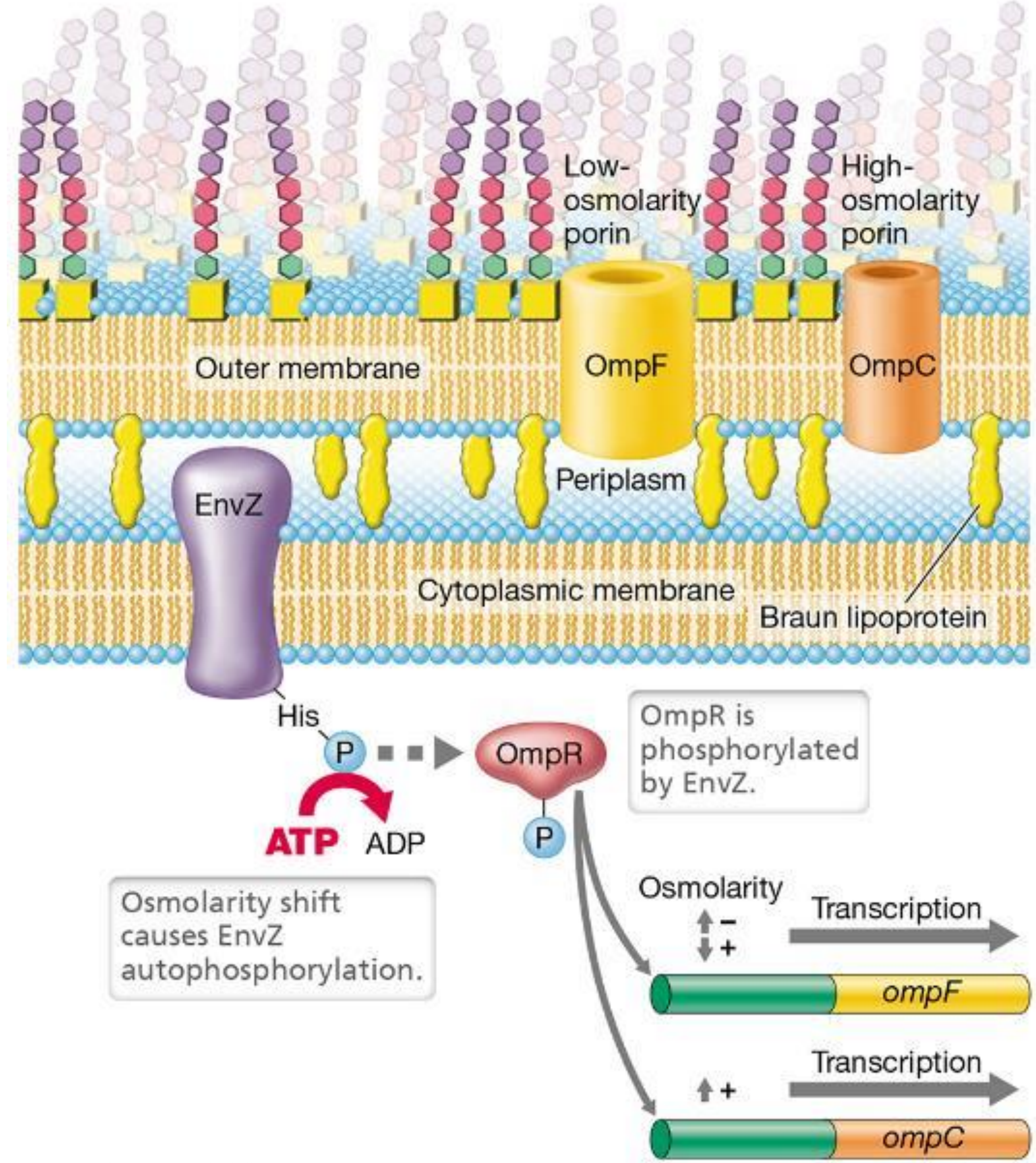
7.5 Two-Component Regulatory Systems

- Almost 50 different two-component systems in *E. coli* (Table 7.1)
  - examples include phosphate assimilation, nitrogen metabolism, and osmotic pressure response
  - example: OmpC and OmpF (Figure 6.18)
- Some signal transduction systems have multiple regulatory elements.
  - example: Ntr and Nar



…OmpF has a larger pore diameter (1.12 nm) than OmpC (1.08 nm) which results in a 10-fold faster diffusion rate that provides a selective advantage at low osmolarity to rapidly scavenge scarce nutrients.

https://en.wikipedia.org/wiki/EnvZ/OmpR_two-component_system



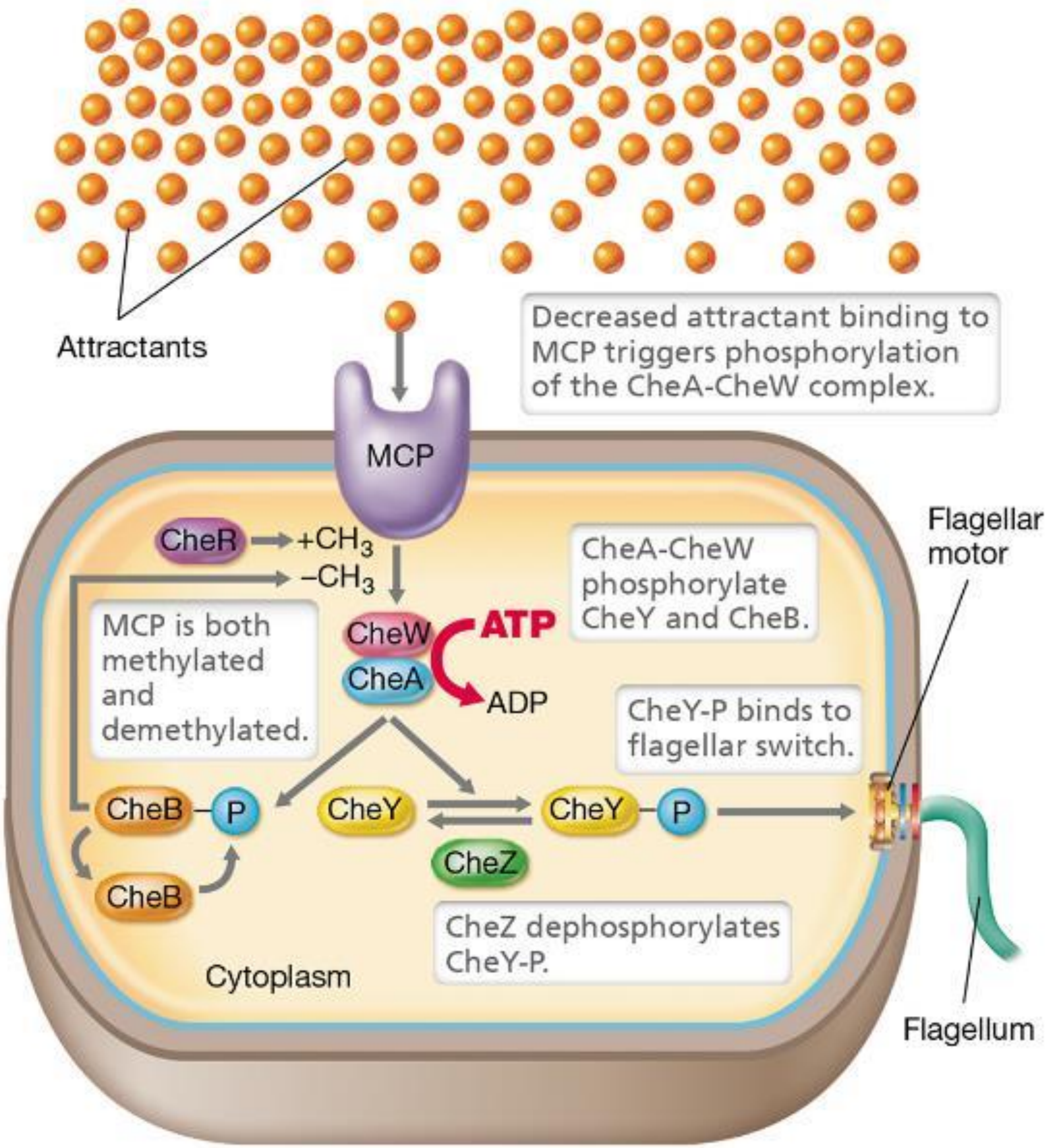
- **High osmolarity = high OmpR-P**
- **Concentratie OmpR-P is crucial voor expressie-effect.**

Figure 7.14

7.7 Regulation of Chemotaxis

- Modified two-component system used in *chemotaxis* to
  - sense temporal changes in attractants or repellents
  - regulate flagellar rotation
  - thus regulate activity of *preexisting proteins* instead of modifying transcription of genes

I.h.g.van een attractant



- CheY-P causes tumbling
- **Minder signaal = meer CheY-P** (en CheB-P)
- Weinig CH_3 => Sensitief voor attractant
- Weinig attractant = veel tumbling = weinig CH_3 = sensitief voor attractant
- Gevolg: constante hoeveelheid attractant = veel tumbeling. Alleen weer zwemmen bij voelen hogere conc. attractant.

Figure 7.17

7.6 Regulation of Chemotaxis

- *Adaptation:* Stop responding and reset
  - *feedback loop*
    - allows the system to reset itself to continue to sense the presence of a signal
    - relies on response regulator CheB
    - involves modification of MCPs: methylation stops response to attractants and increases response to repellants

https://www.youtube.com/watch?v=HVIa440b8uM

7.6 Regulation of Chemotaxis

- Other taxes
  - Che proteins also play a role.
  - *phototaxis:* movement toward light
    - Light sensor replaces MCPs.
  - *aerotaxis:* movement toward oxygen
    - Redox protein monitors oxygen level.

7.7 Quorum Sensing

Hawaiian bobtail squid

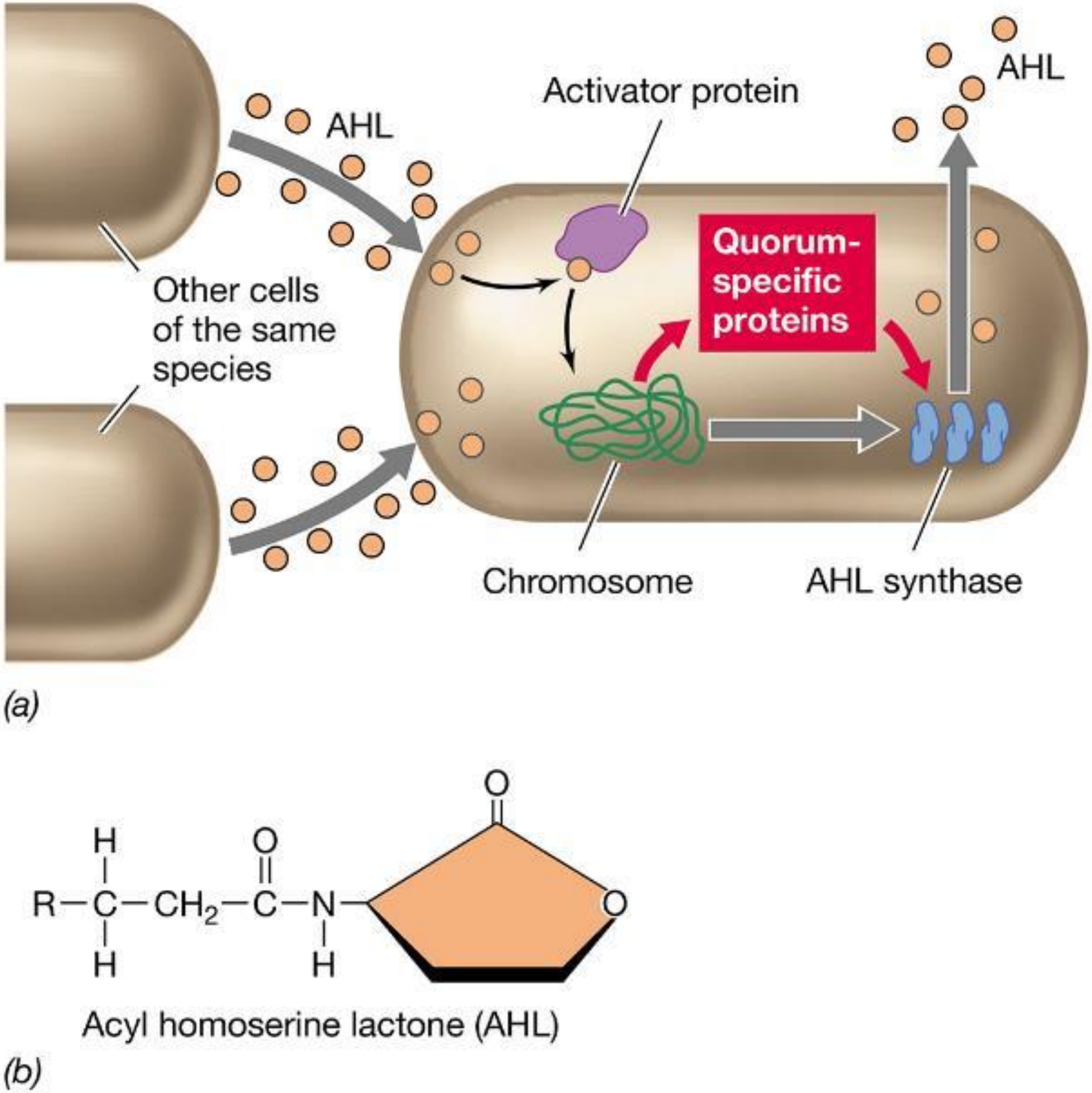
Light organ => *Aliivibrio fischeri* => bioluminescent bacterium

7.7 Quorum Sensing

- Prokaryotes can respond to the presence of other cells of the same species.
- Quorum sensing: mechanism by which *Bacteria* and some *Archaea* assess their population density.
  - ensures that a sufficient number of cells are present before initiating a response that, to be effective, requires a certain cell density (*e.g.*, toxin production by pathogenic bacterium)

7.7 Quorum Sensing

- Each species of bacterium produces a specific autoinducer signaling molecule. (Figure 7.18)
  - diffuses freely across the cell envelope
  - reaches high concentrations inside cell only if many cells are nearby and making the same autoinducer
  - binds to specific activator protein or sensor kinase, triggering transcription of specific genes

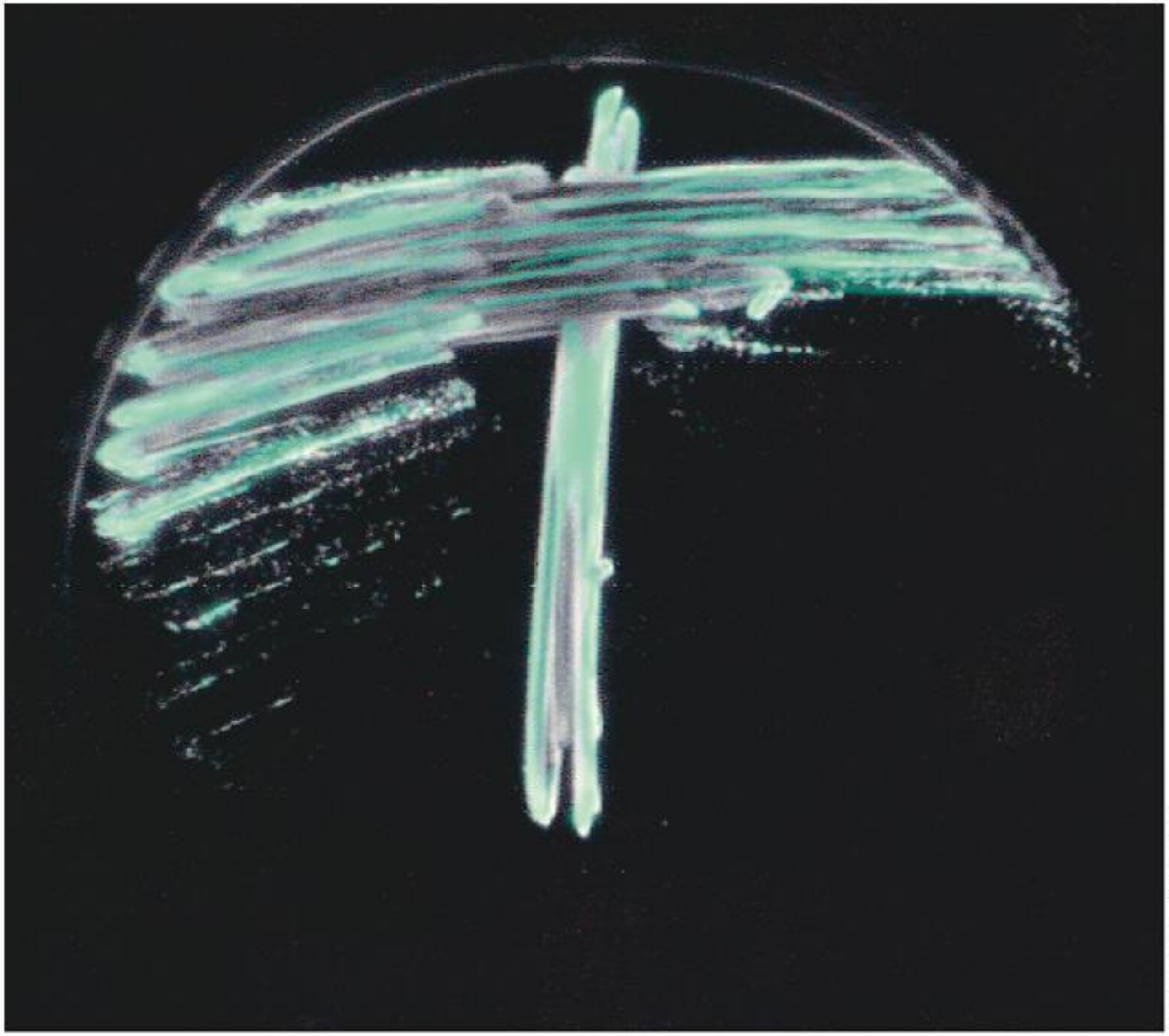


AHL
Activator protein
AHL
Other cells
of the same
species
Quorum-
specific
proteins
Chromosome
AHL synthase
(a)
O
H
O
R–C–CH2–C–N–
O
H
H
Acyl homoserine lactone (AHL)
(b)

Figure 7.18

7.7 Quorum Sensing

- Several different classes of autoinducers
  - *acyl homoserine lactone* (AHL): first to be identified
  - autoinducer 2 (AI-2): a common autoinducer among many gram-negative species
  - short peptides used as autoinducers by gram-positive species
- Quorum sensing first discovered as mechanism regulating light production in bacteria including *Aliivibrio fischeri* (Figure 7.19)
  - *Lux* operon encodes bioluminescence.
- Also occurs in microbial eukaryotes (*e.g., Saccharomyces cerevisiae* and *Candida*)

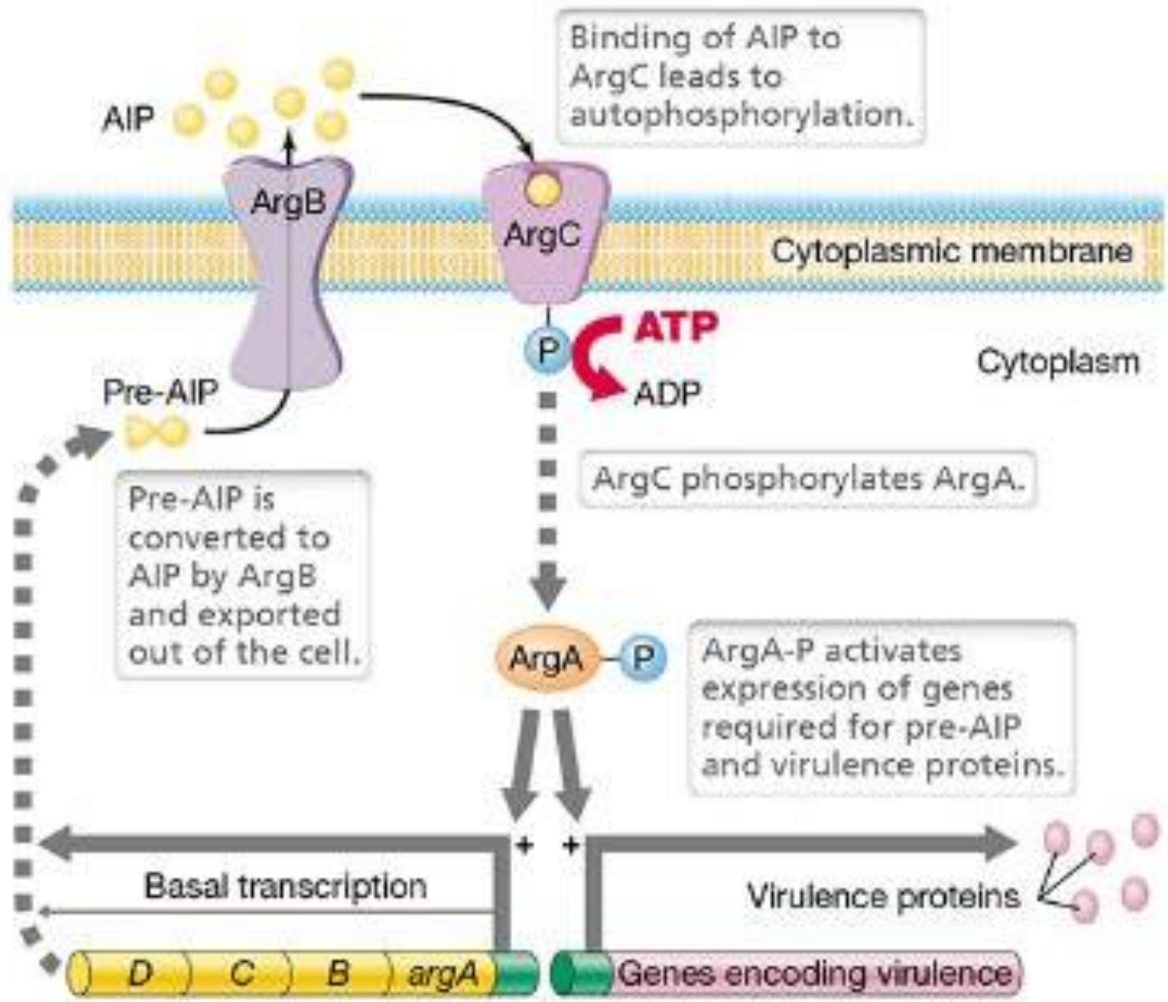


Timothy C. Johnston

Figure 7.19

7.8 Quorum Sensing

- Virulence factors
  - example: *Staphylococcus aureus*
    - secretes small peptides that damage host cells or alter host's immune system
    - under control of *autoinducing peptide* (AIP)
      - activates several proteins that lead to production of virulence proteins (Figure 7.20*b*)



(b) **Virulence factor production in *Staphylococcus aureus***

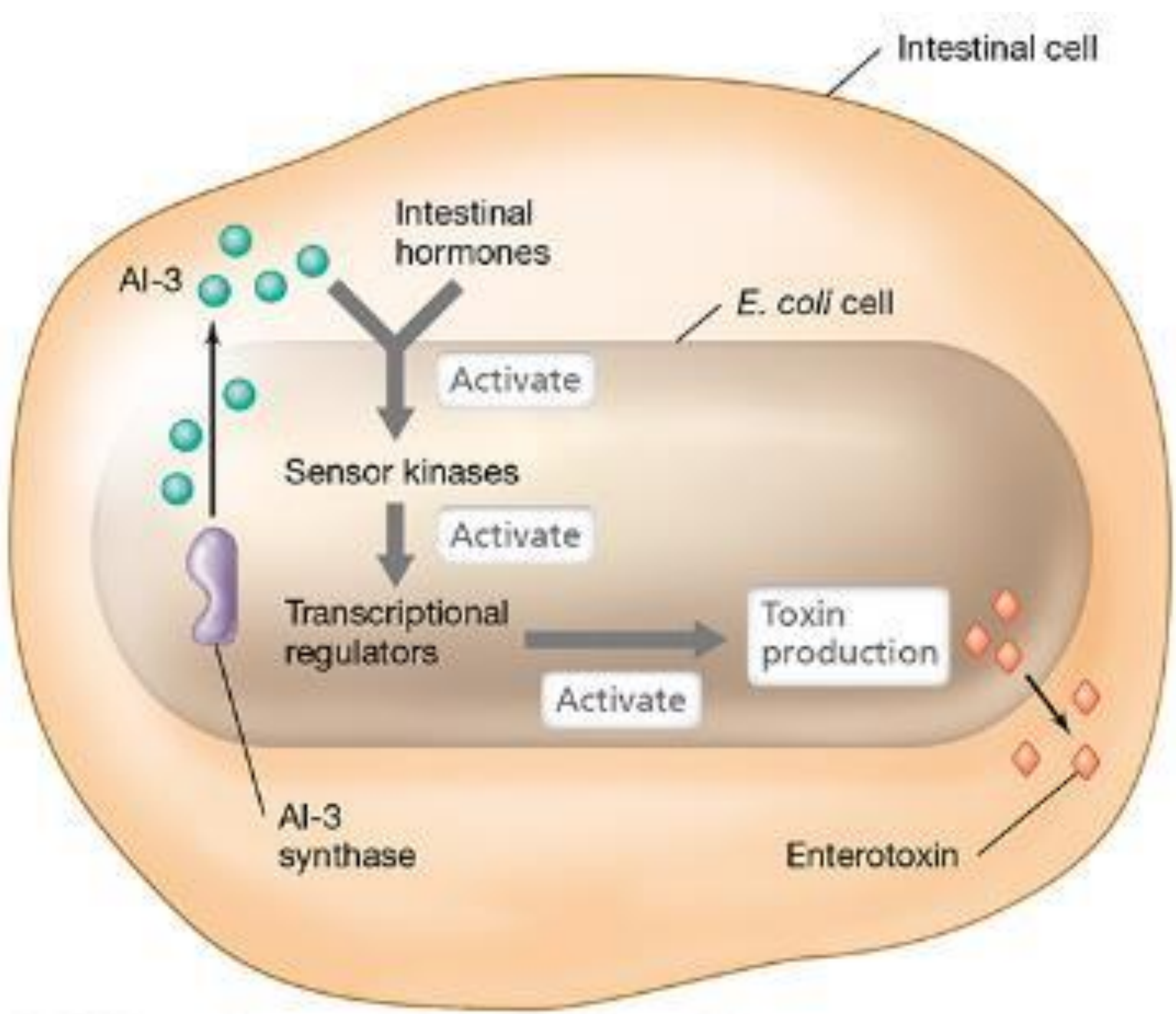
Figure 7.20

7.7 Quorum Sensing

- Quorum-sensing disruptors are potential drugs for dispersing biofilms and preventing virulence gene expression.

7.7 Quorum Sensing

- Virulence factors
  - example: *Escherichia coli* O157:H7 (Figure 7.20)
    - shiga toxin–producing strain
    - produces AHL AI-3 that induces virulence genes
    - Epinephrine plus norepinephrine plus AI-3 bind to sensor molecules in plasma membrane.
      - activates motility, toxin secretion, and production of lesion-forming proteins



(a) **Virulence factor production in Shiga toxin-producing *Escherichia coli***

Figure 7.20

EINDE LES 2

Microbiologie 2: Les 3

II. Sensing and Signal Transduction

- Nog vragen over de vorige les?

7.11 The Heat Shock Response

- Heat shock response
  - heat shock proteins: counteract damage of denatured proteins and help cell recover from temperature stress
    - very ancient proteins
    - induced by heat, exposure to ethanol or ultraviolet (UV) radiation
  - largely controlled by alternative sigma factor RpoH (Figure 6.26)

Sigma-factor => onderdeel RNA-polymerase

(dus anders dan een activator zoals Crp [zie *lac*-operon])

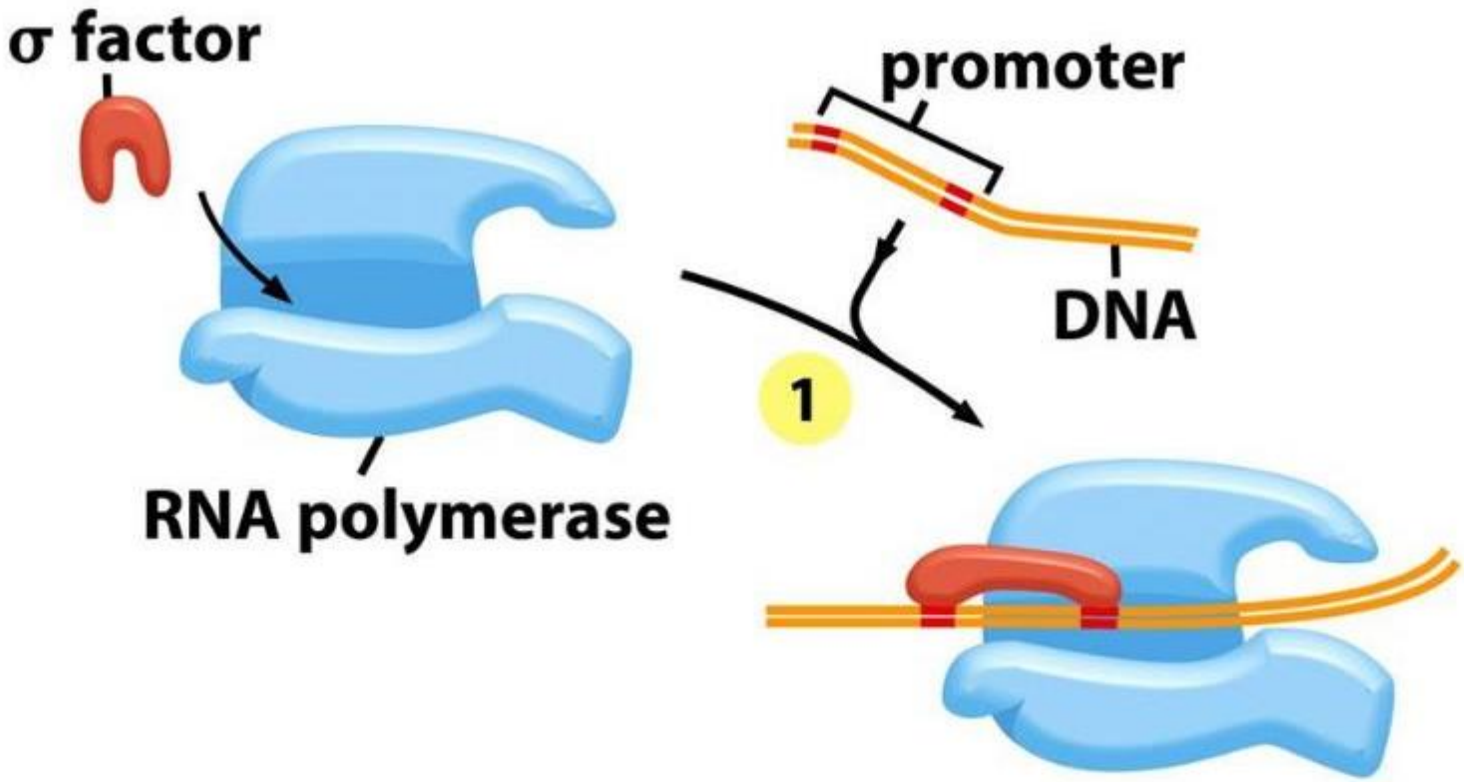


Figure 6-11 (part 1 of 7) *Molecular Biology of the Cell* (© Garland Science 2008)

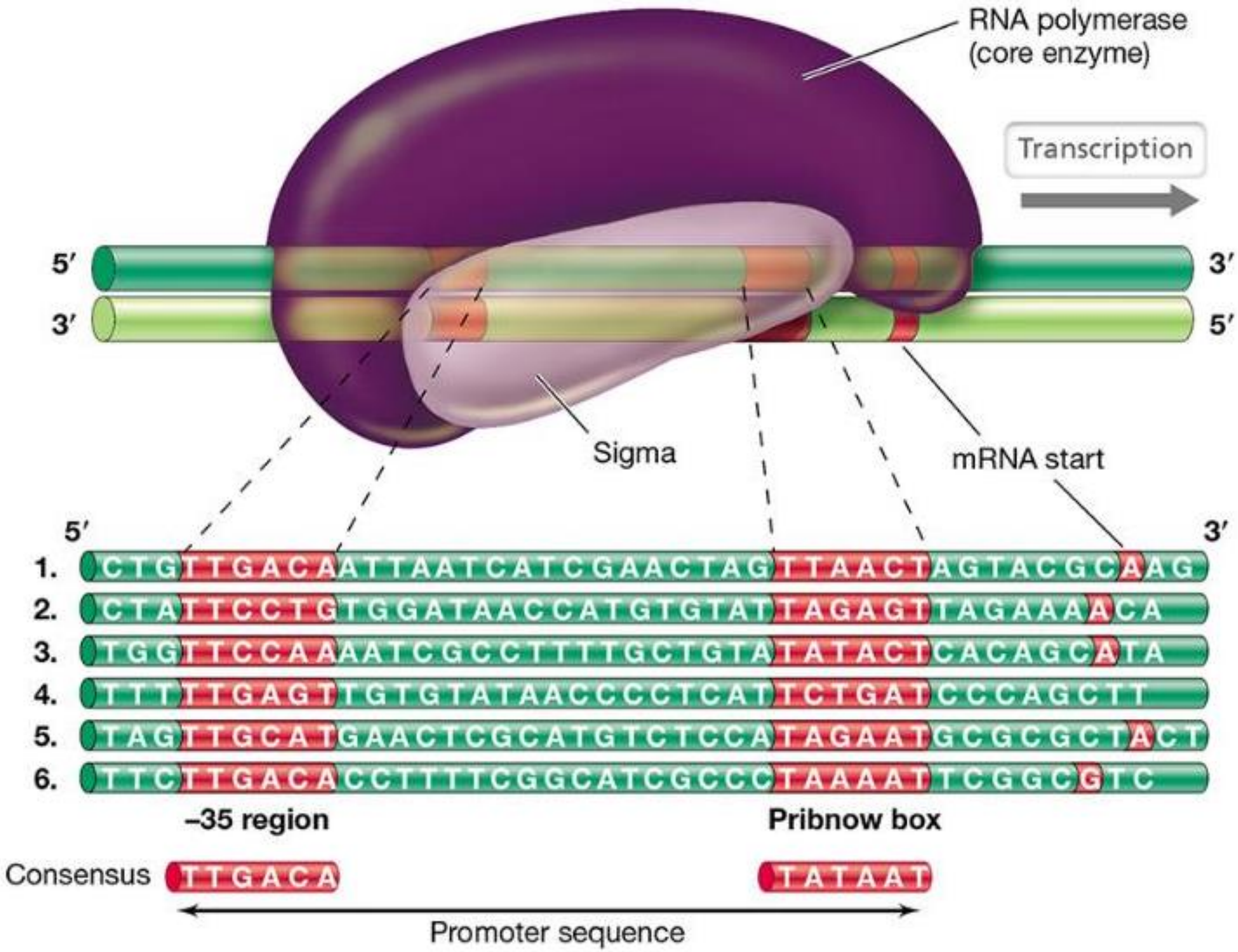
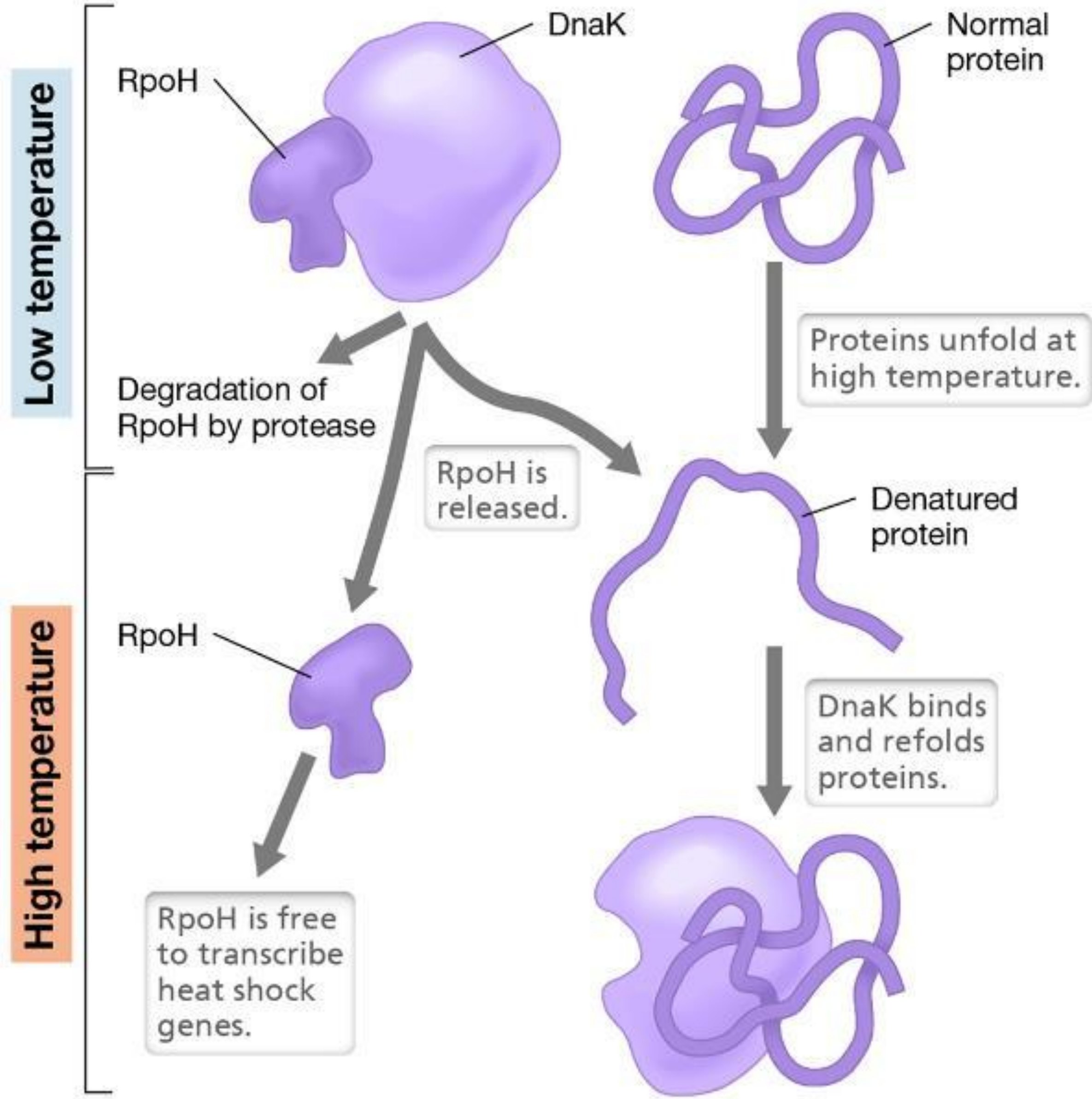

RNA polymerase
(core enzyme)
Transcription
5′
3′
3′
5′
Sigma
mRNA start
5′
3′
1. CTGTTGACAATTAATCATCGAACTAGTTAACTAGTACGCAAG
2. CTATTCCTGTGGATAACCATGTGTATTAGAGTTAGAAAACA
3. TGGTTCCAAAATCGCCTTTTGCTGTATATACTCACAGCATA
4. TTTTTGAGTTGTGTATAACCCCTCATTCTGATCCCAGCTT
5. TAGTTGCATGAACTCGCATGTCTCCATAGAATGCGCGCTACT
6. TTCTTGACACCTTTTCGGCATCGCCCTAAAATTCGGCGTC
–35 region
Pribnow box
Consensus
TTGACA
TATAAT
Promoter sequence

Figure ….

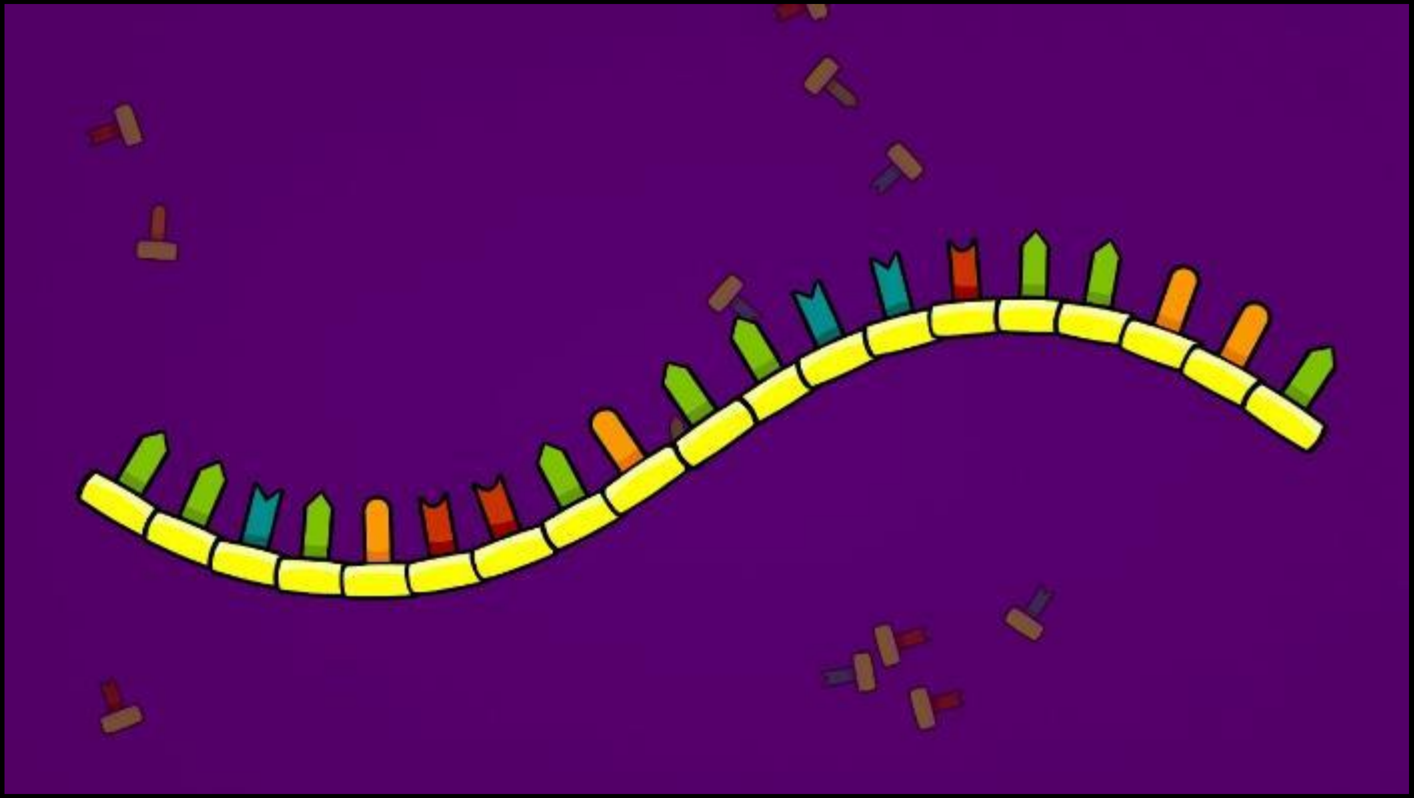


Low temperature
High temperature
RpoH
DnaK
Normal
protein
Proteins unfold at
high temperature.
Degradation of
RpoH by protease
RpoH is
released.
Denatured
protein
RpoH
DnaK binds
and refolds
proteins.
RpoH is free
to transcribe
heat shock
genes.

Figure 7.27

Microbiologie 2: Les 3

III. RNA-Based Regulation



III. RNA-Based Regulation

Voorgaande voorbeelden sturen allemaal gen-expressie (wel/niet transcriptie). Wat als het mRNA al gevormd is, maar ‘je’ hebt dat eiwit niet meer nodig?

III. RNA-Based Regulation

- 7.12 Regulatory RNAs

7.12 Regulatory RNAs

- Noncoding RNA (ncRNA): RNA that is not translated to protein
  - *small RNAs* (sRNAs): 40–400 nucleotides that regulate gene expression in prokaryotes and eukaryotes

7.12 Regulatory RNAs

- sRNAs can bind to complementary (m)RNA sequences
    - block a ribosome-binding site (RBS), decreasing expression
    - open up a blocked RBS, increasing expression
    - increase degradation of mRNA, preventing synthesis
    - decrease degradation of mRNA, increasing synthesis

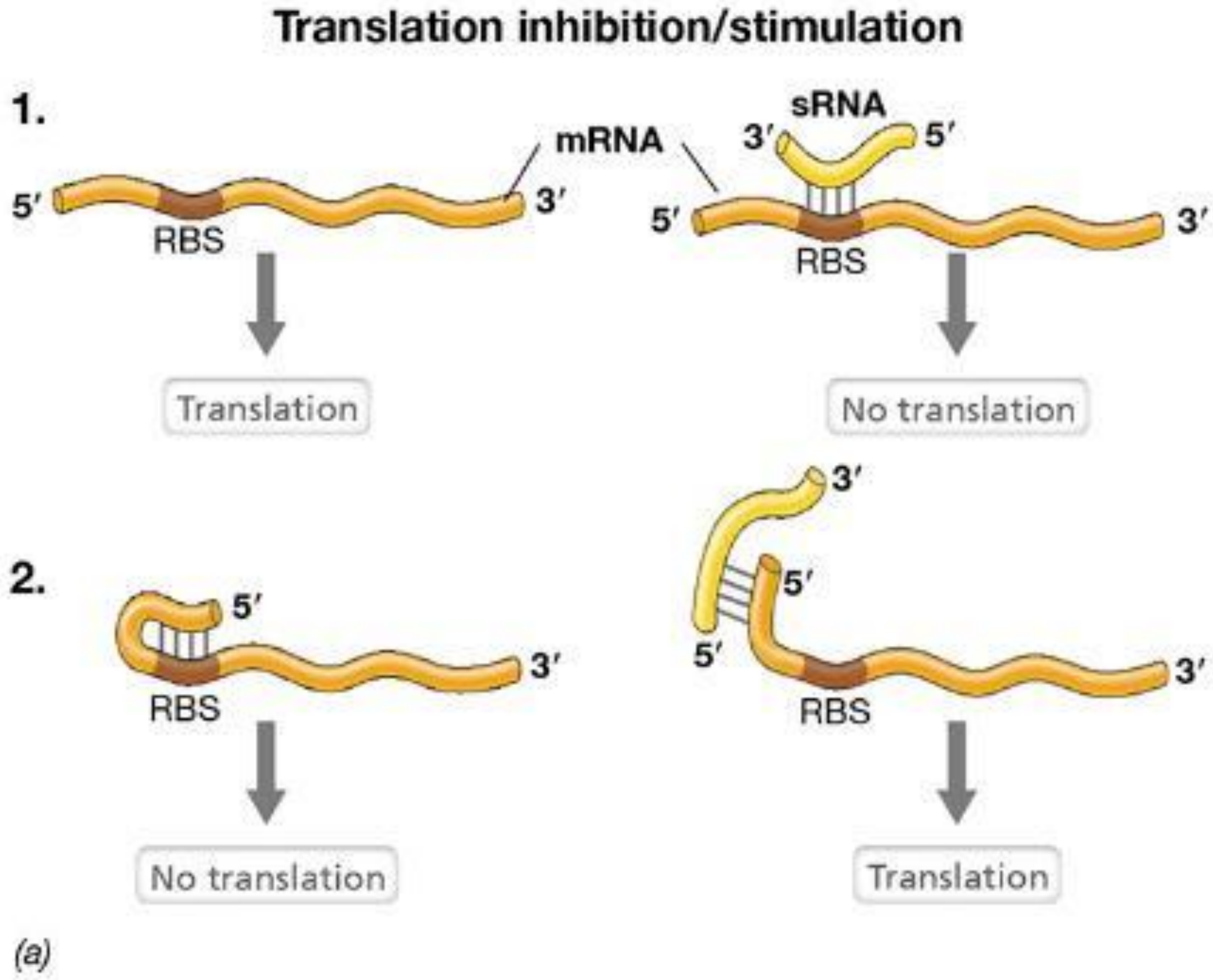

Translation inhibition/stimulation
1.
mRNA
sRNA
5′
3′
RBS
Translation
No translation
2.
No translation
Translation
(a)

Figure 7.28

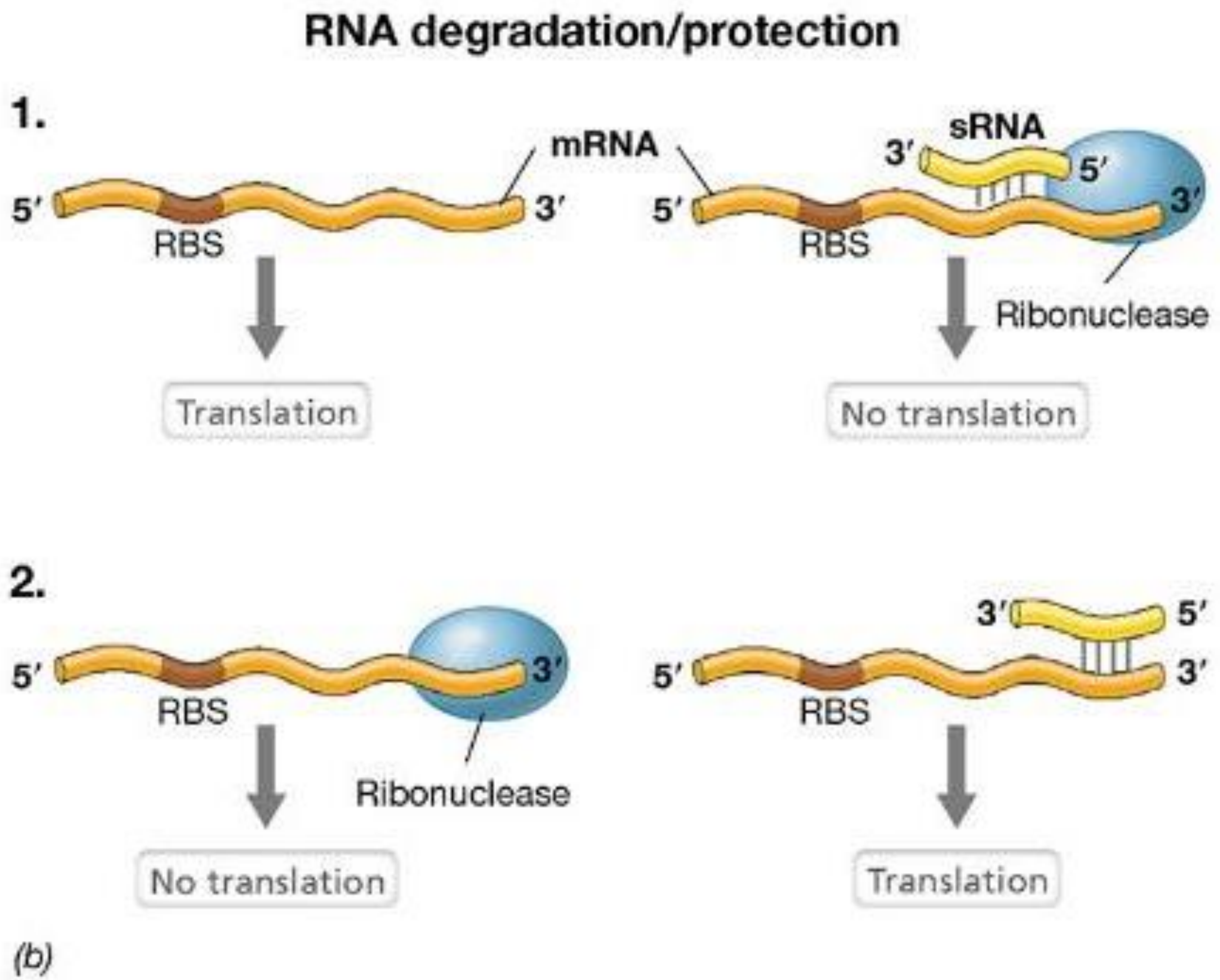

RNA degradation/protection
1.
mRNA
5′
3′
RBS
Translation
sRNA
3′
5′
5′
3′
RBS
Ribonuclease
No translation
2.
5′
3′
RBS
Ribonuclease
No translation
3′
5′
5′
3′
RBS
Translation
(b)

Figure 7.28

7.12 Regulatory RNAs

- example: SgrS (een sRNA) in *E. coli* expressed to avoid accumulation of glucose 6-phosphate (G6P) (Figure 7.29)

- *ptsG* encodes a glucose transporter. Eenmaal in de cel wordt glucose gefosforyleerd tot G6P.

Weinig G6P

G6P stress

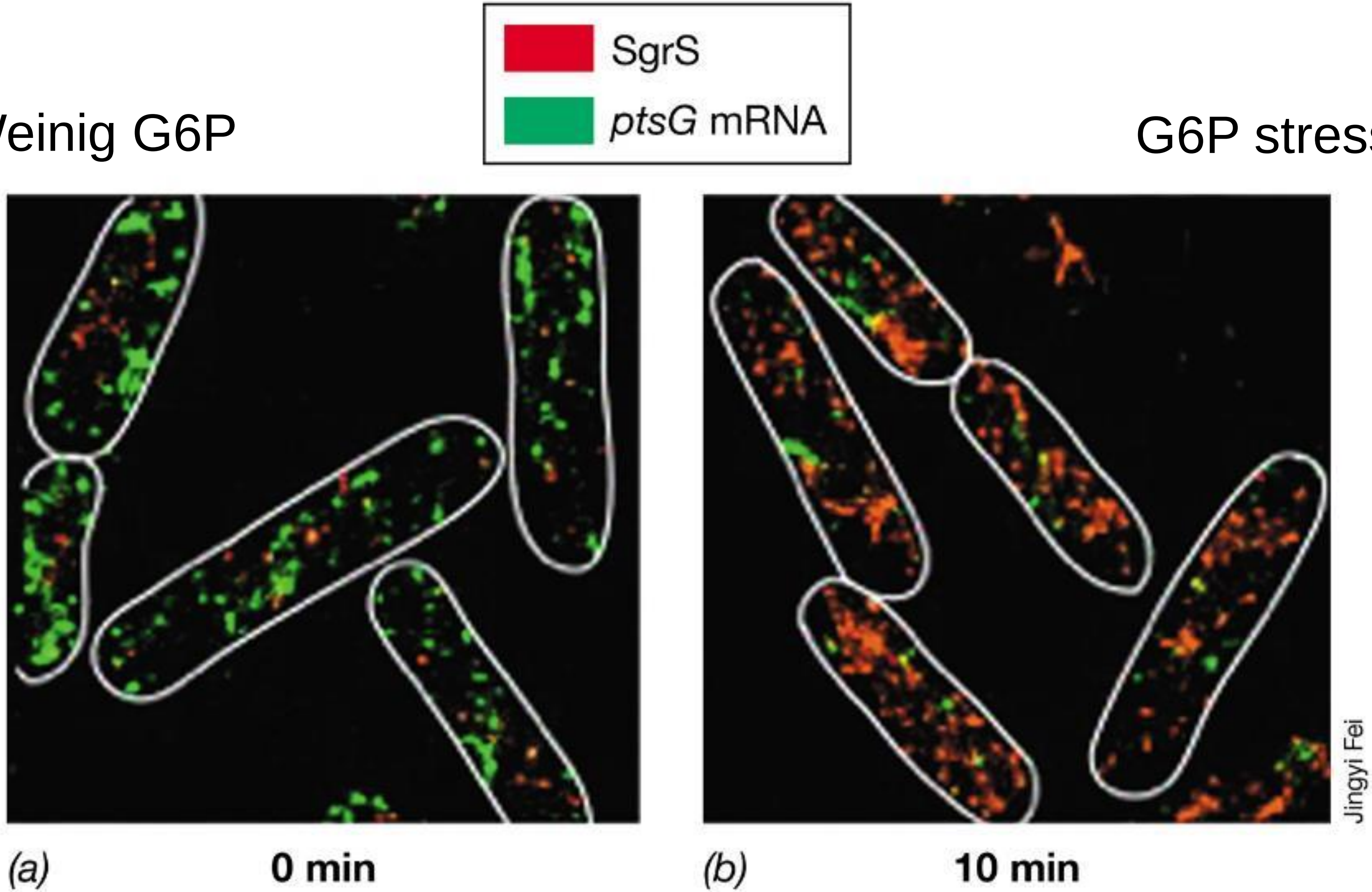


Figure 7.29

7.12 Regulatory RNAs

- Types of small RNA
  - *Trans-sRNAs* (*e.g.*, RyhB and SgrS) are encoded in the intergenic region.
  - limited complementarity to target molecule, may only base-pair with 5–11 nucleotides
  - Binding of trans-sRNA to targets depends on Hfq, a small protein that binds to both RNA molecules to facilitate interaction. (Figure 7.30)
    - Hfq is an *RNA chaperone.*

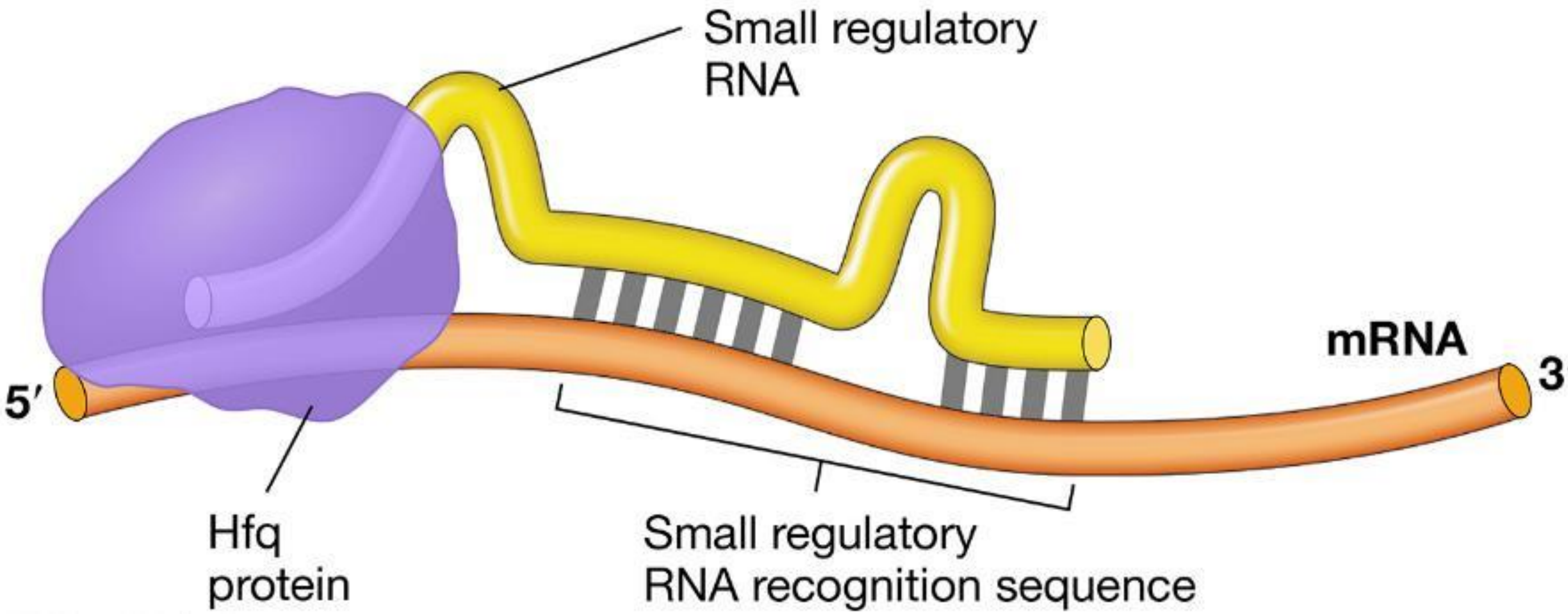
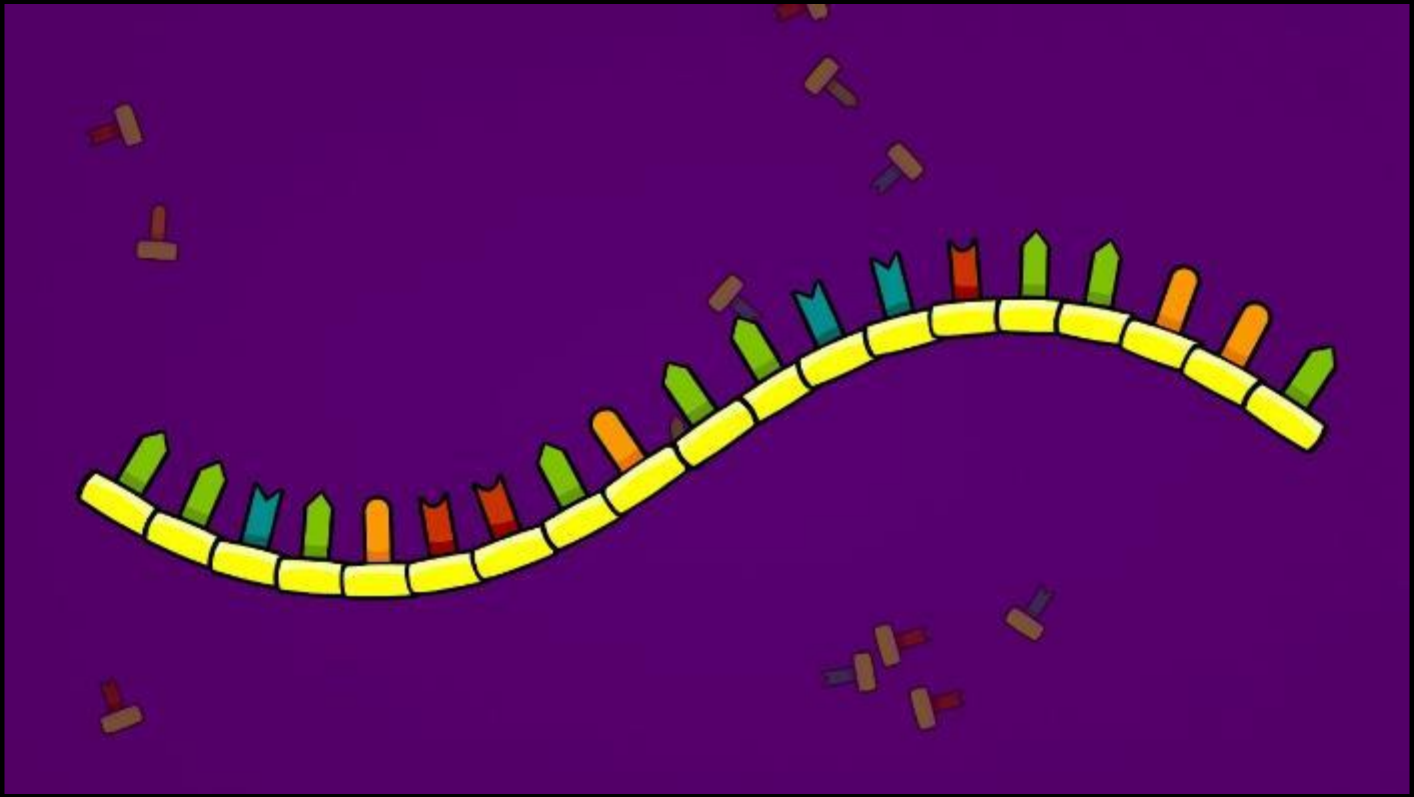

Small regulatory
RNA
mRNA
5′
3′
Hfq
protein
Small regulatory
RNA recognition sequence

Figure 7.30

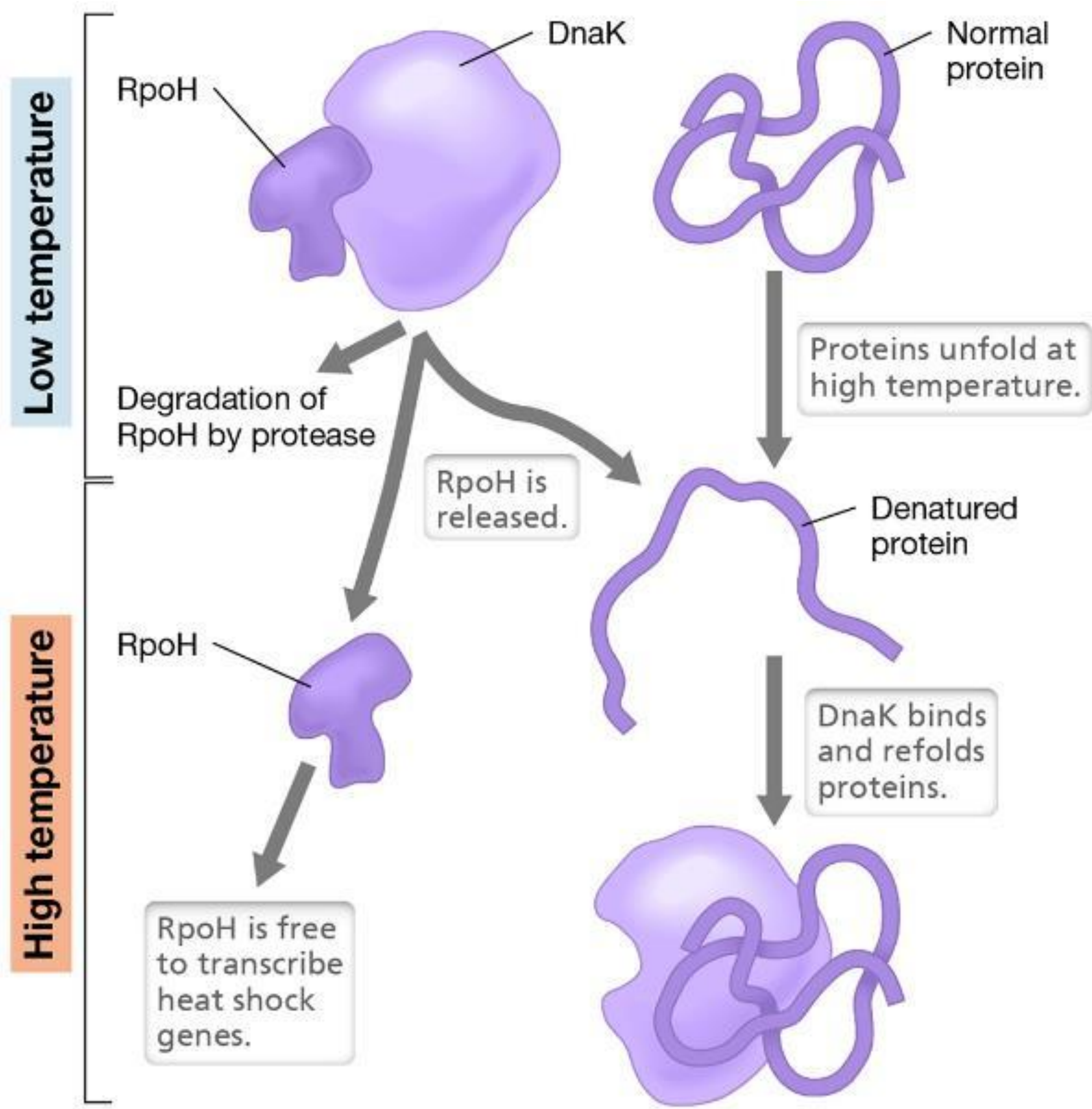
EINDE LES 3

Microbiologie 2: Les 4

III. RNA-Based Regulation



Microbiologie 2: Les 3 Flashback!



Heat shock response

- Sensing denatured proteins
- Alternative sigma factor RpoH

Figure 7.27

7.12 Regulatory RNAs

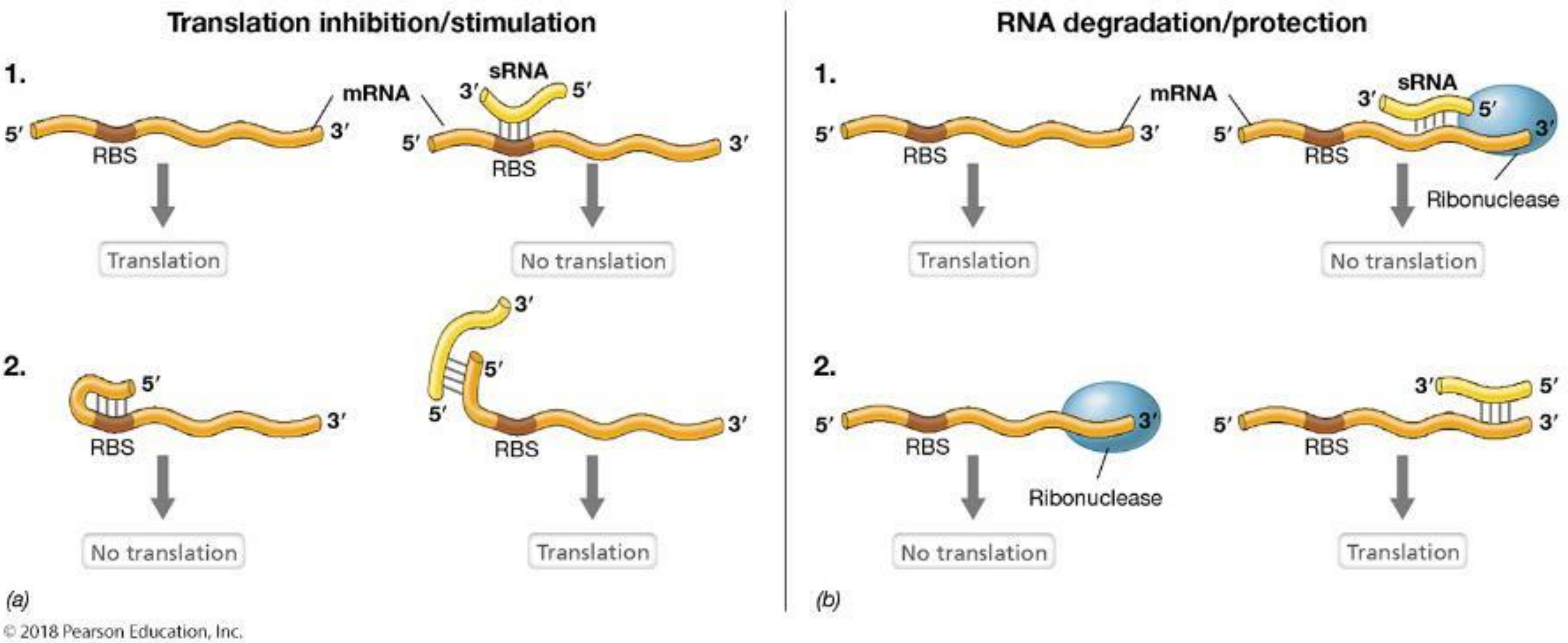


Figure 7.28

III. RNA-Based Regulation

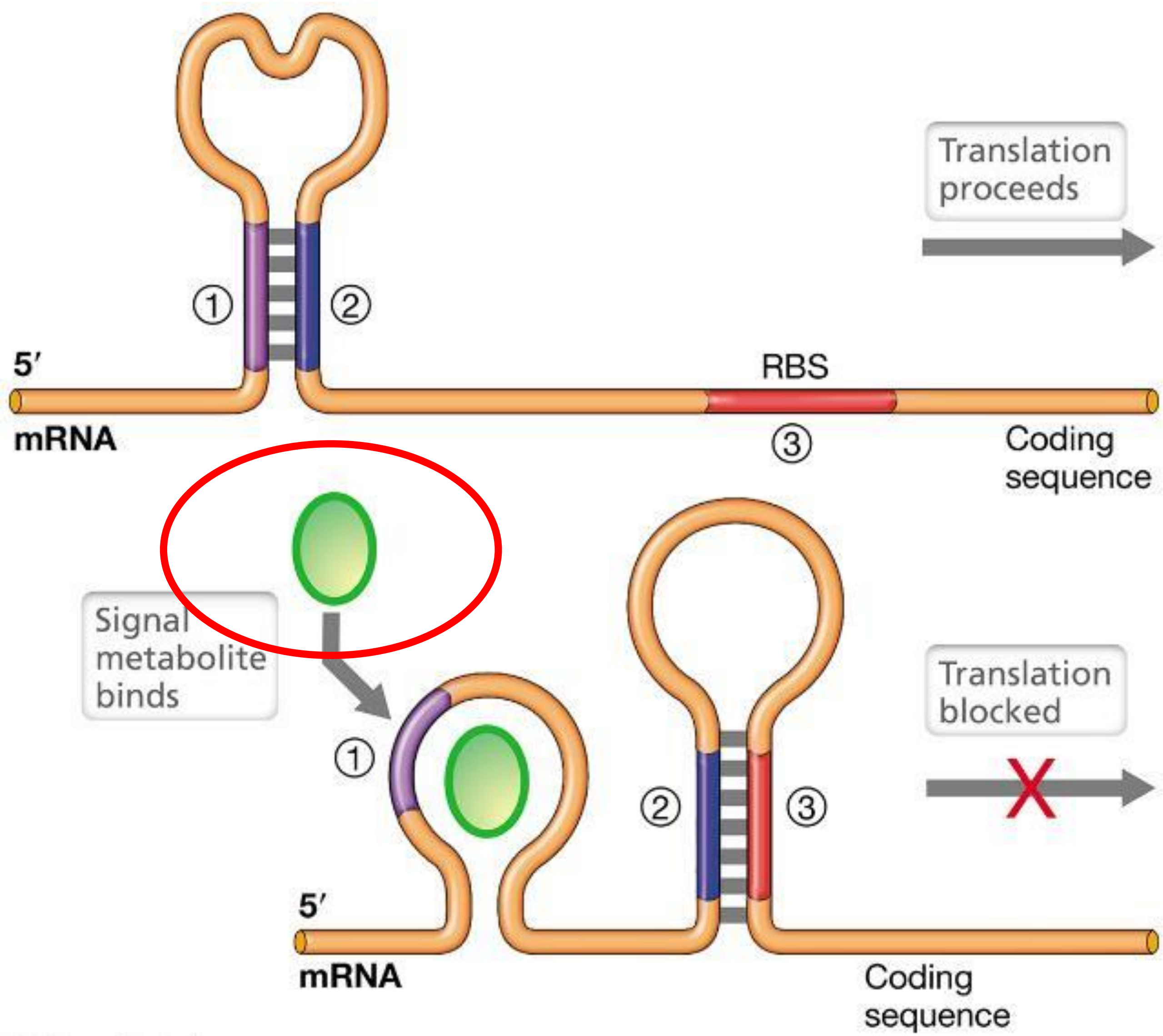
- 7.12 Regulatory RNAs
- 7.13 Riboswitches
- 7.14 Attenuation

Vandaag!
Verder met regulatie *nadat* de promoter ‘geactiveerd’ is.

7.13 Riboswitches

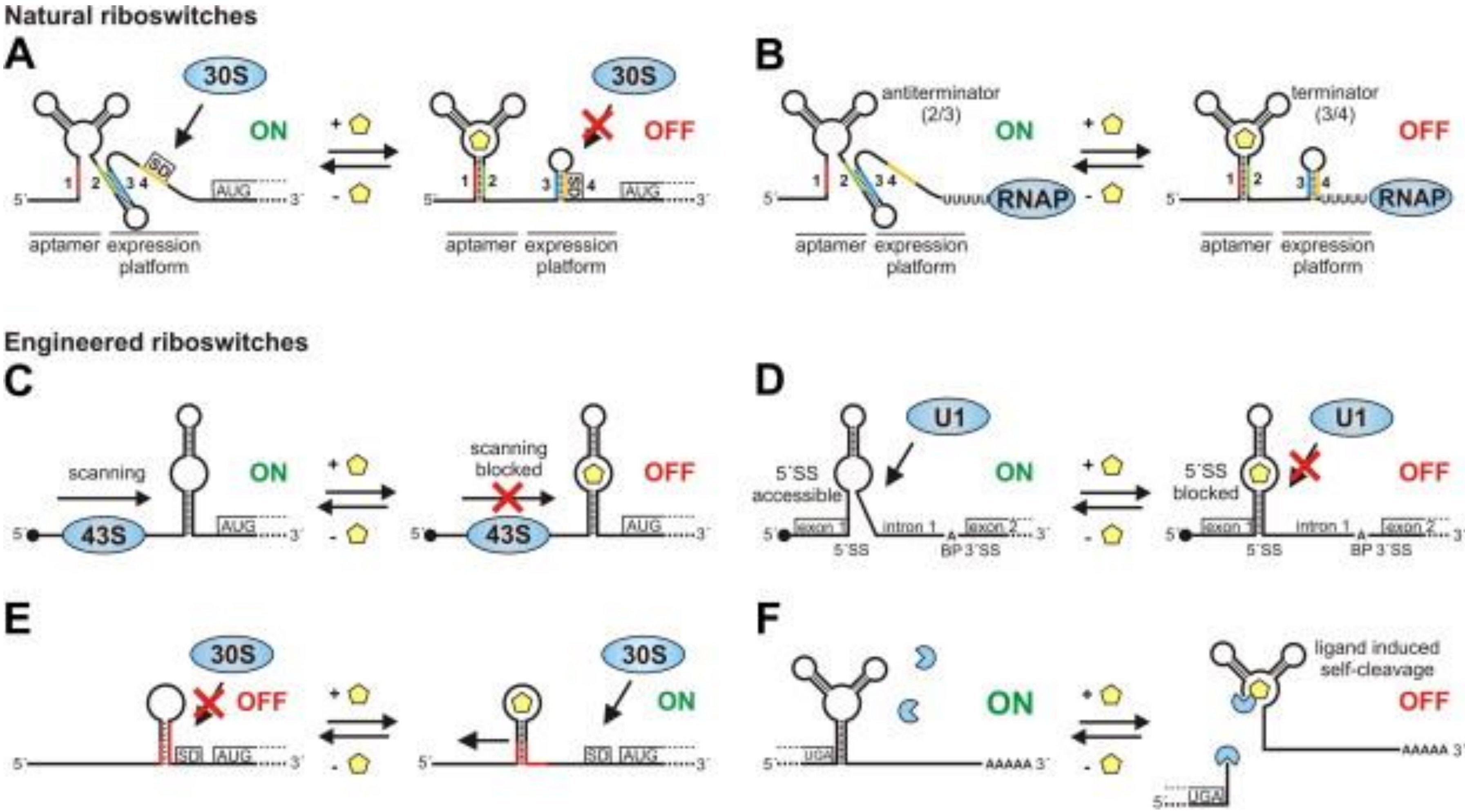
- *Riboswitches:* 5’ part of RNA molecules activate/repress <u>translation</u>

- Mechanisms of riboswitches
  - Metabolite binds directly to mRNA.
  - small molecule binding domain at 5′ end of mRNA
    - two alternative structures, one with small molecule bound and other without (Figure 7.31)



Translation proceeds
1
2
5′
RBS
mRNA
3
Coding sequence
Signal metabolite binds
Translation blocked
1
2
3
5′
mRNA
Coding sequence

Figure 7.31



https://www.researchgate.net/publication/227341862_Engineered_riboswitches_Expanding_researchers%27_toolbox_with_synthetic_RNA_regulators

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)

Table 7.3

7.13 Riboswitches

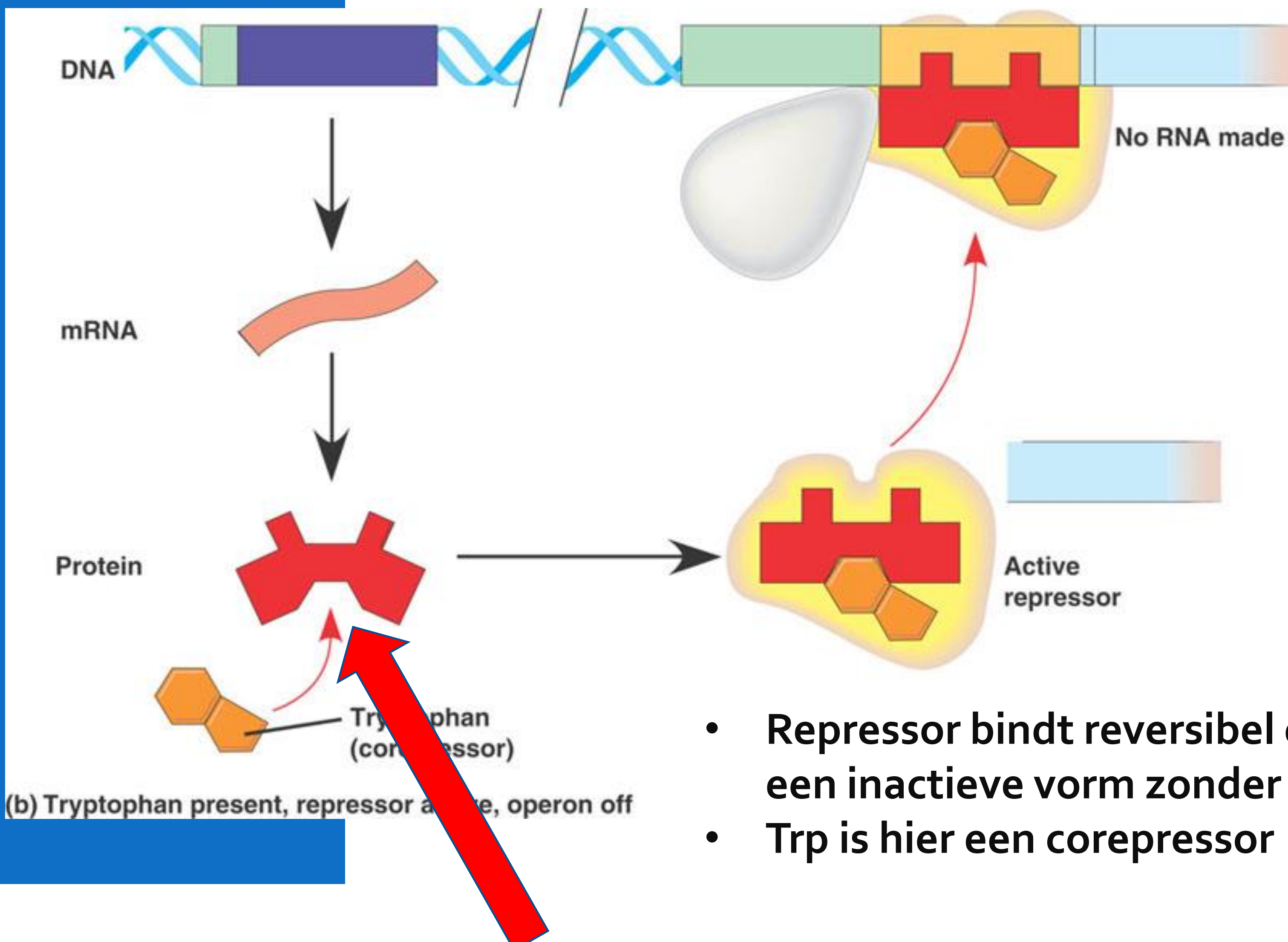
- Riboswitches and evolution
  - believed to be remnants of RNA world (before cells, DNA, and protein were present)
  - found in some bacteria, fungi, and plants

7.14 Attenuation

- Premature termination of mRNA synthesis
  - *Leader* (first part of mRNA structure) can fold into two alternative secondary structures either allowing synthesis or causing premature termination.
  - not found in eukaryotes because transcription and translation are separated (organelles)

7.14 Attenuation

- Tryptophan operon (Figure 7.33)
  - contains structural genes for five proteins + promoter and regulatory sequences
  - more than one type of regulation
  - *Leader sequence* encodes *leader peptide.*



Allosterische zijde

- Repressor bindt reversibel en is in een inactieve vorm zonder trp
- Trp is hier een corepressor

7.14 Attenuation

- Mechanism (Figure 7.33)
  - new mRNA folds into a stem-loop that inhibits <u>RNA polymerase</u>

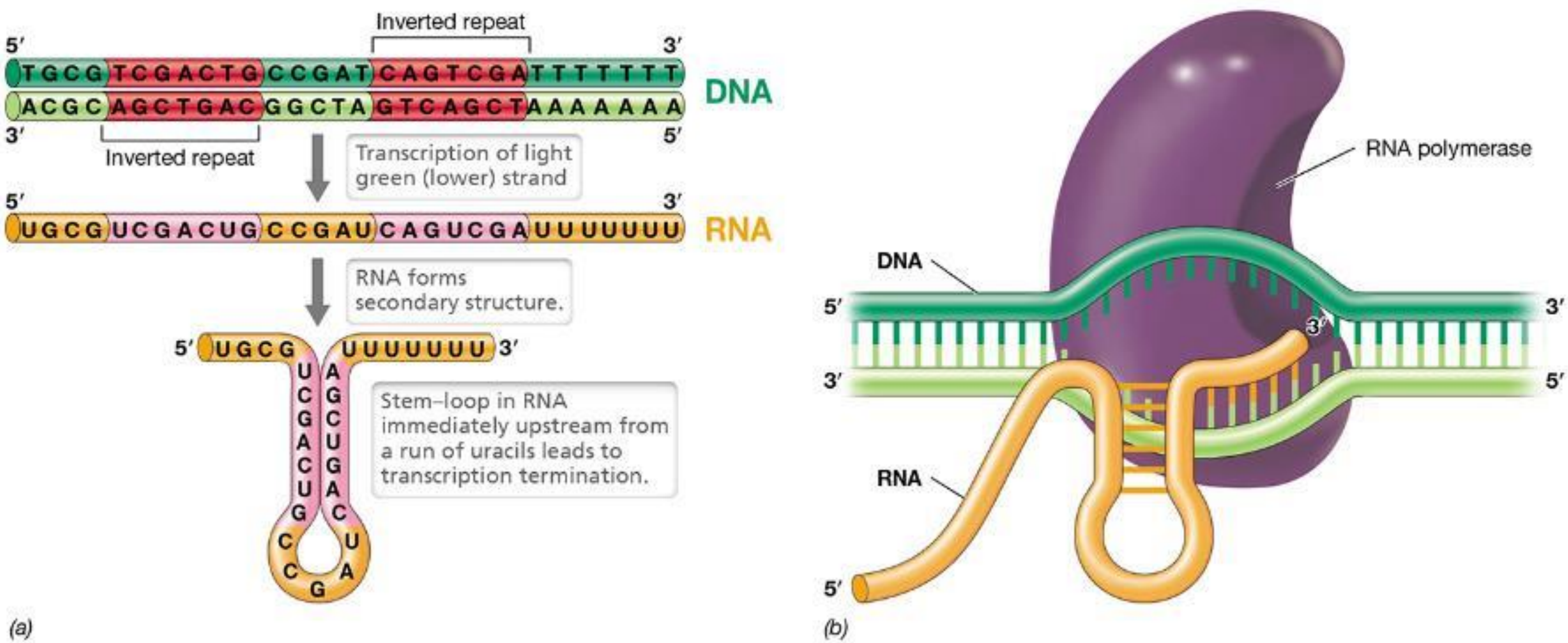
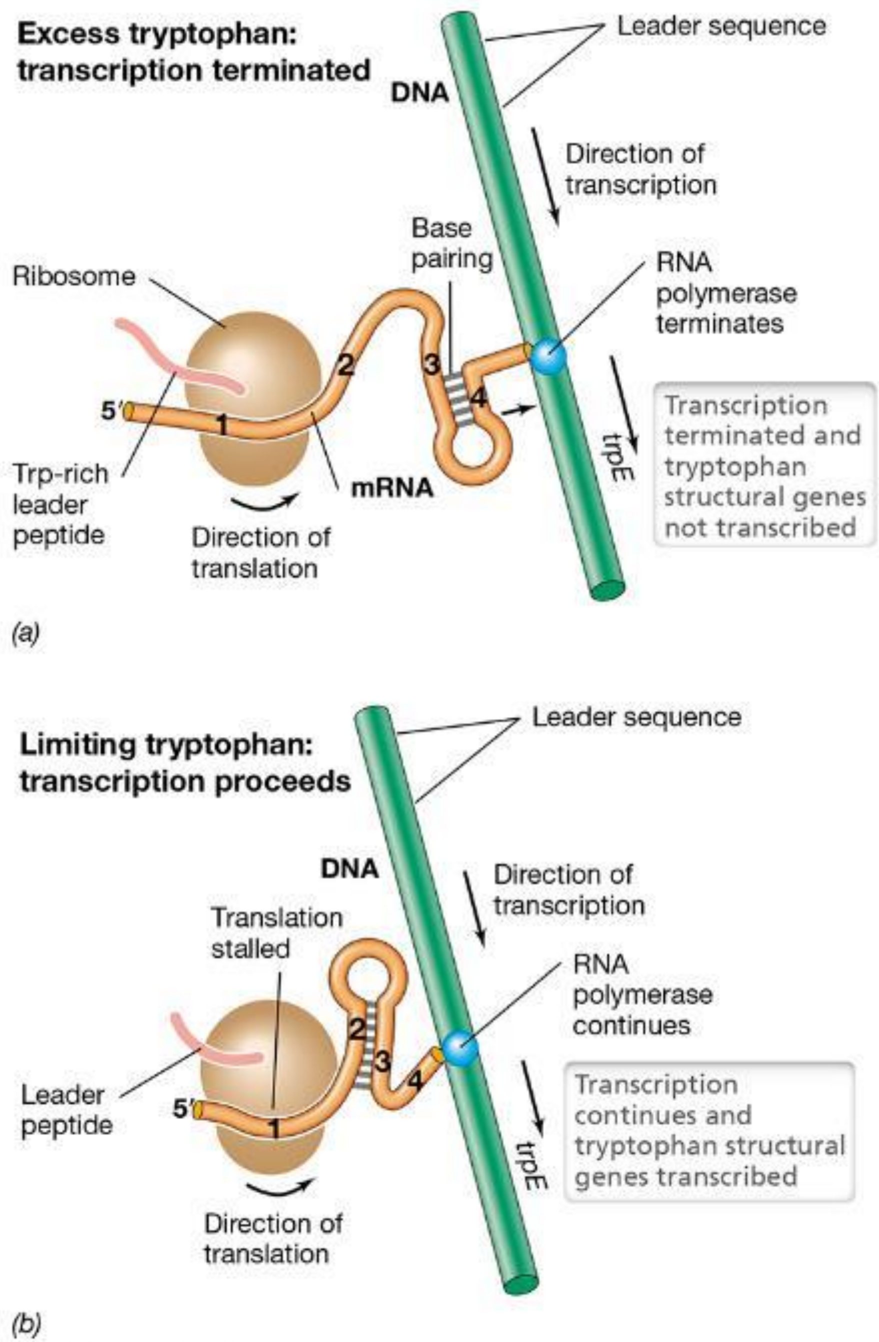
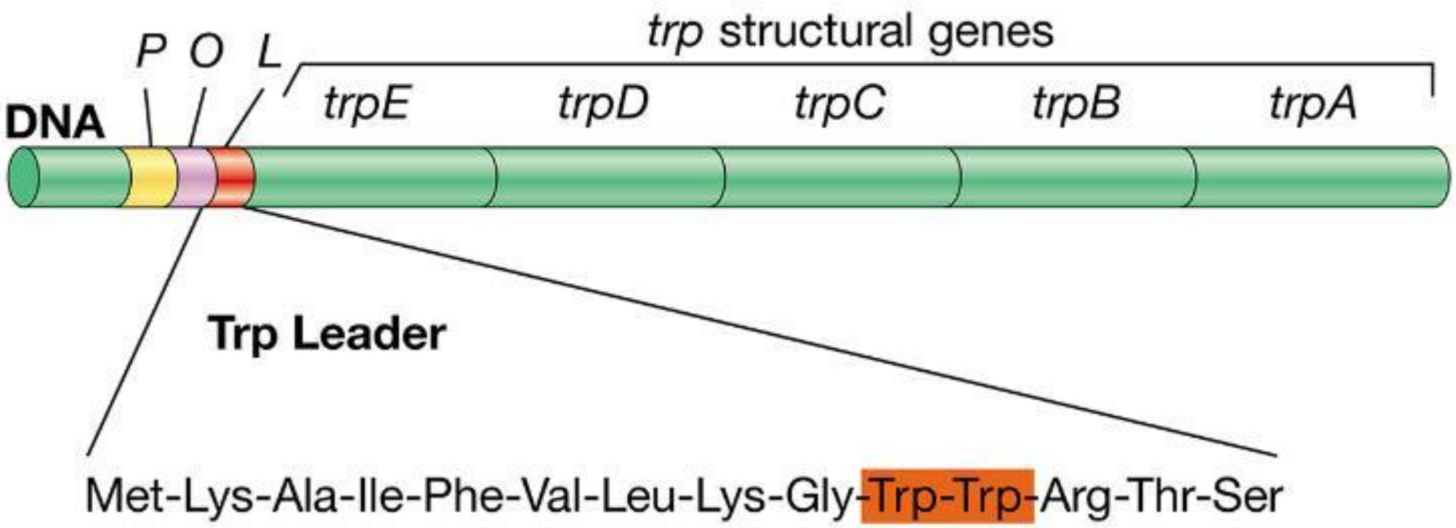

Inverted repeat
5′
3′
TGCGTCGACTGCCGATCAGTCGATTTTTTTT
ACGCAGCTGACGGCTAGTCAGCTAAAAAAAA
3′
5′
DNA
Inverted repeat
Transcription of light green (lower) strand
5′
3′
UGCGUCGACUGCCGAUCAGUCGAUUUUUUU
RNA
RNA forms secondary structure.
5′ UGCG
UUUUUUU 3′
UCGACUUG
AGCUGAC
CCGAU
Stem–loop in RNA immediately upstream from a run of uracils leads to transcription termination.
(a)
RNA polymerase
DNA
5′
3′
3′
5′
3′
RNA
5′
(b)

Figure 6.23



Excess tryptophan: transcription terminated
DNA
Leader sequence
Direction of transcription
Base pairing
RNA polymerase terminates
Ribosome
2
3
4
5′
1
Transcription terminated and tryptophan structural genes not transcribed
trpE
Trp-rich leader peptide
mRNA
Direction of translation
(a)
Limiting tryptophan: transcription proceeds
Leader sequence
DNA
Direction of transcription
Translation stalled
RNA polymerase continues
Leader peptide
5′
1
2
3
4
Transcription continues and tryptophan structural genes transcribed
trpE
Direction of translation
(b)

Figure 7.33



(a)

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

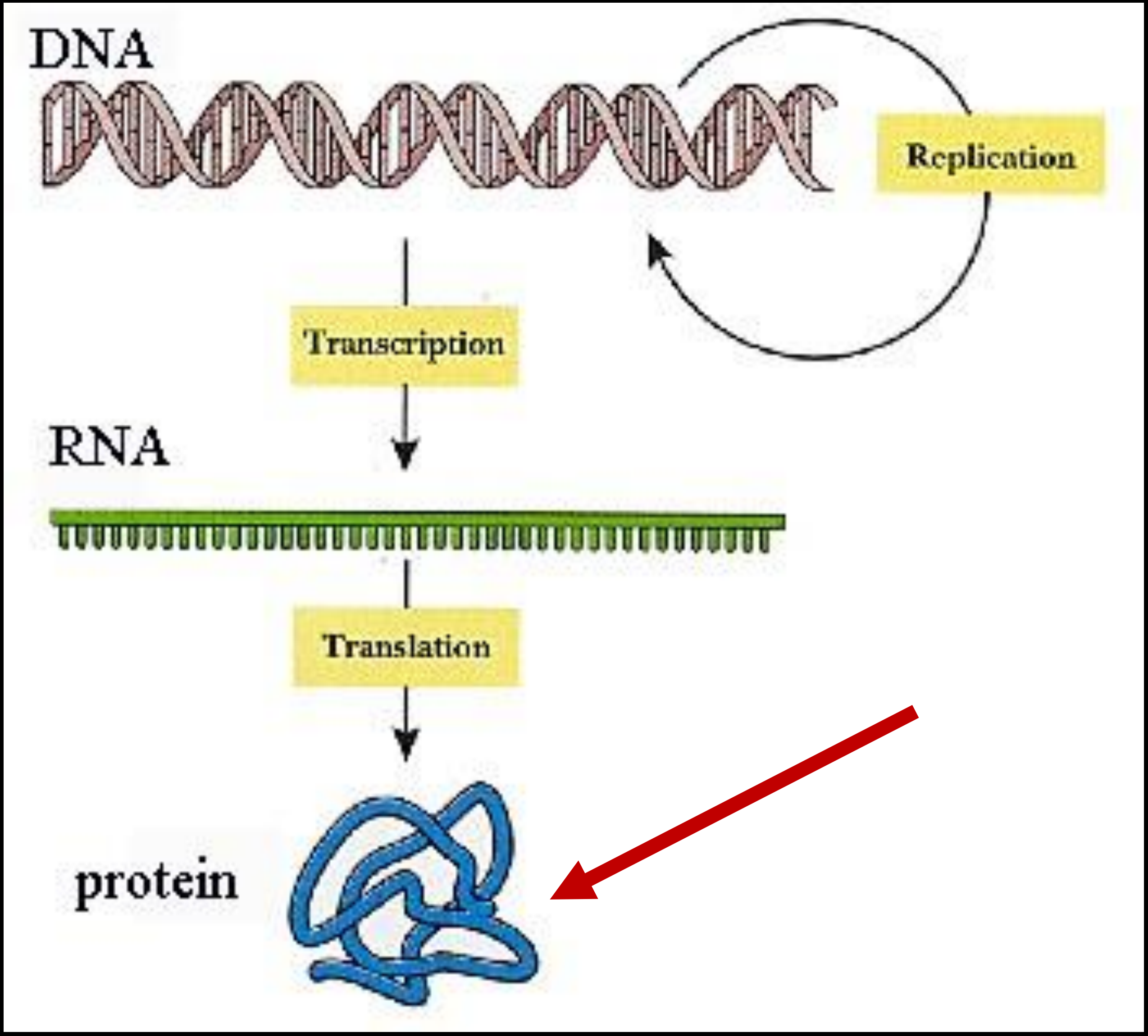
Phenylalanine Met-Lys-His-Ile-Pro-Phe-Phe-Phe-Ala-Phe-Phe-Phe-Thr-Phe-Pro

(b)

Figure 7.32

Microbiologie 2: Les 4

IV. Regulation of Enzymes and Other Proteins



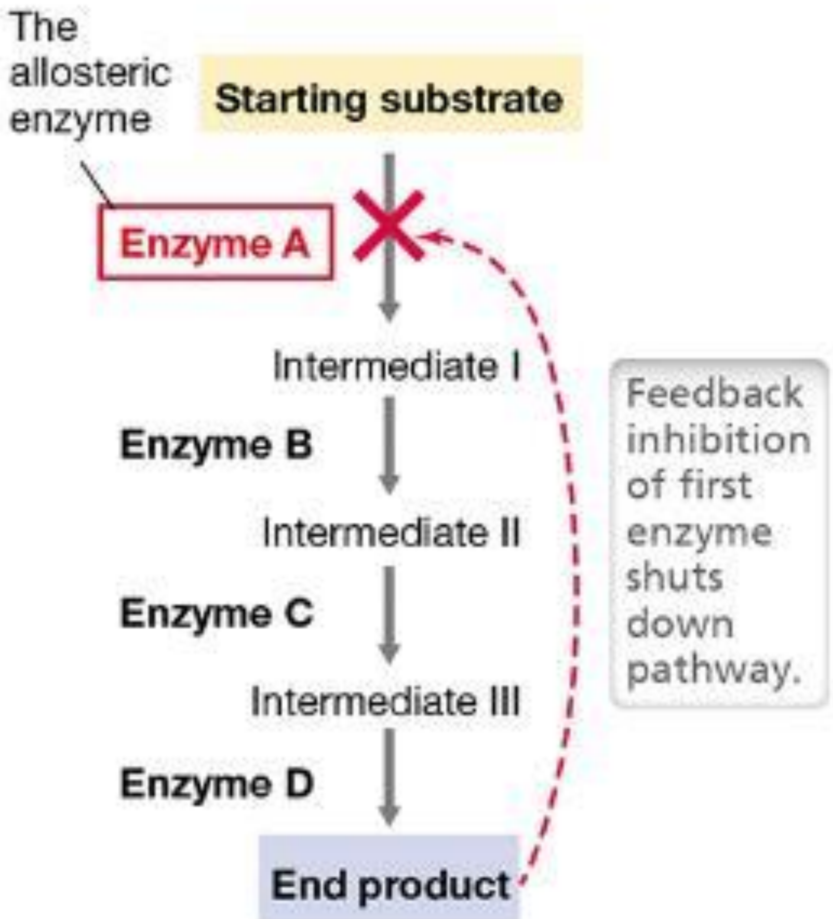
IV. Regulation of Enzymes and Other Proteins

- 7.15 Feedback Inhibition
- 7.16 Post-Translational Regulation

Oftewel: hoe te reguleren als je enzym al gemaakt is?

7.15 Feedback Inhibition

- Temporarily turning off a biosynthetic pathway (Figure 7.34*a*)
  - End product of the pathway inhibiting its production.
  - reversible reaction
  - Inhibited enzyme is an allosteric enzyme.
    - two binding sites: active (substrate-binding) and allosteric (end product binds)
    - Binding at *allosteric site* changes conformation, preventing substrate binding.

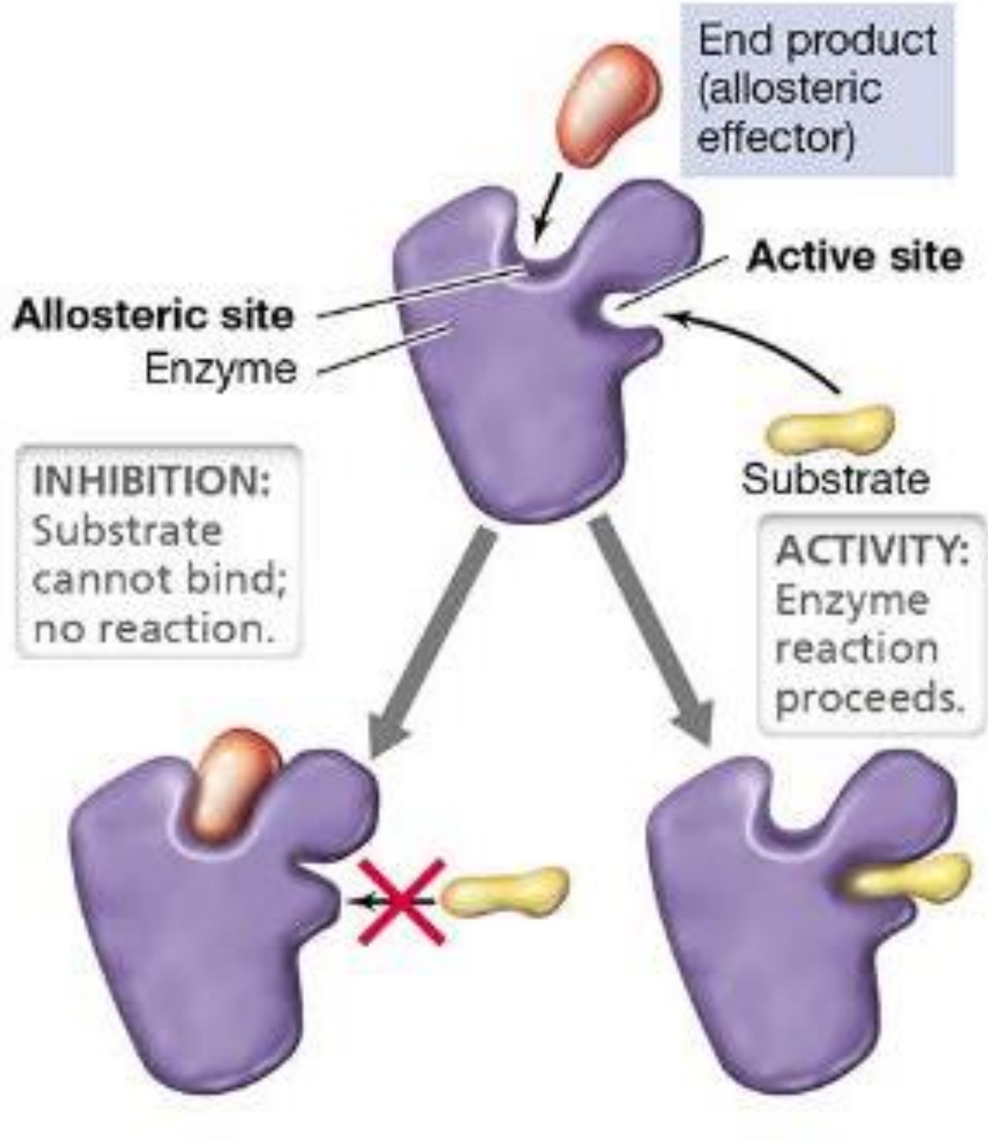


(a) **Feedback inhibition**

Figure 7.34

7.15 Feedback Inhibition

- Temporarily turning off a biosynthetic pathway (Figure 7.34*a*)
  - End product of the pathway inhibiting its production.
  - reversible reaction
  - Inhibited enzyme is an allosteric enzyme.
    - two binding sites: active (substrate-binding) and allosteric (end product binds)
    - Binding at *allosteric site* changes conformation, preventing substrate binding.



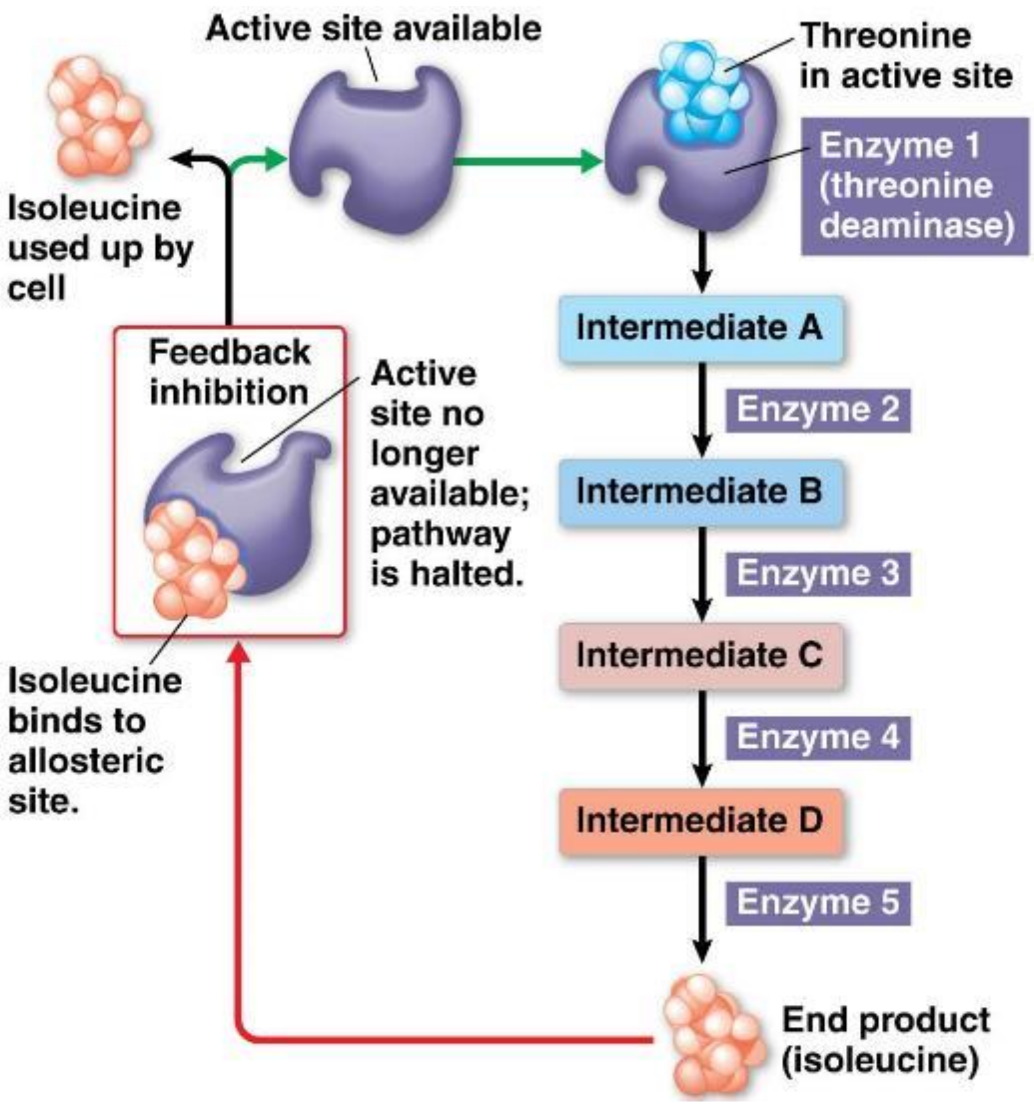
(b) **Allosteric inhibition**

Figure 7.34

Voorbeeld negatieve feedback:

<u>Het product remt z'n eigen aanmaak!</u>

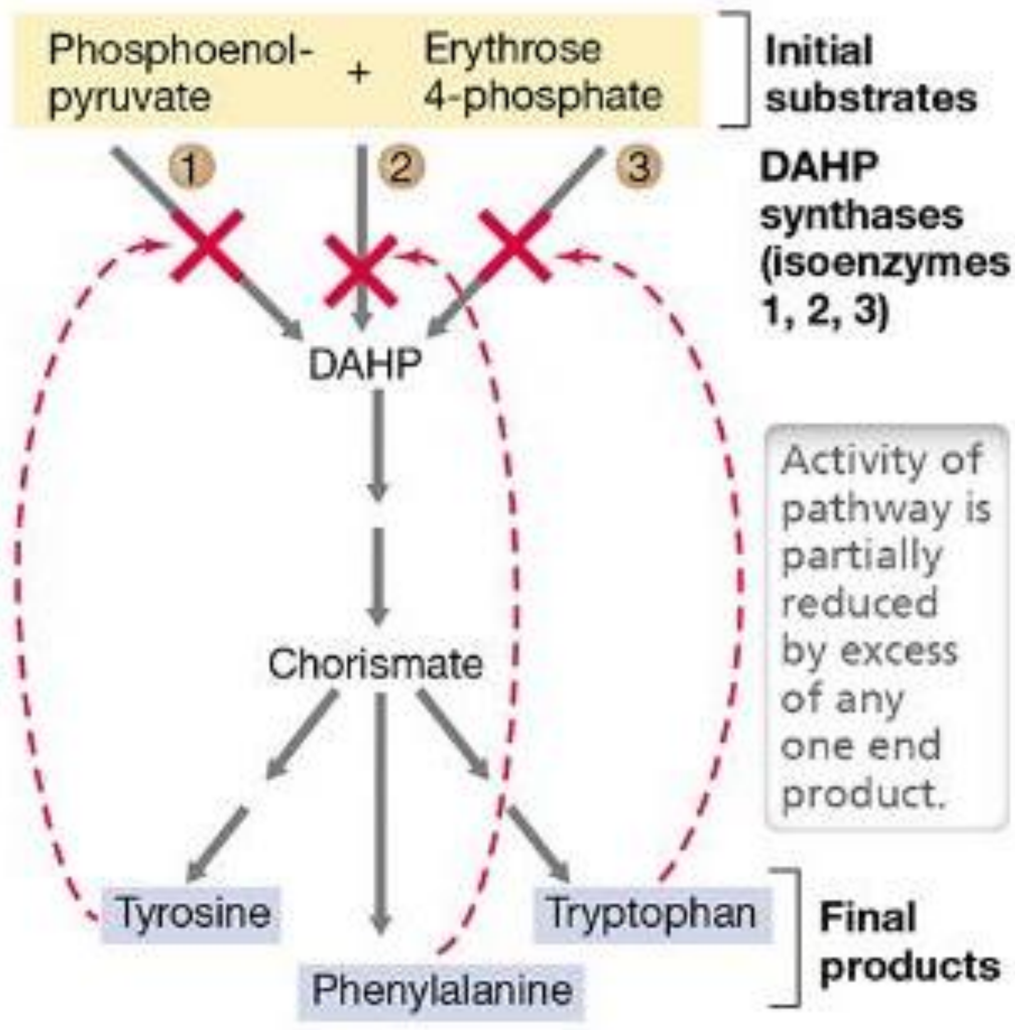
NB: Dit is op enzymatisch niveau



Bio2

7.15 Feedback Inhibition

- Some pathways controlled by feedback inhibition use *isoenzymes:* different enzymes that catalyze the same reaction but are subject to different regulatory controls.
  - example: DAHP synthase for aromatic amino acids



(c) **Isoenzyme inhibition**

Isozymes are enzymes that differ in amino acid sequence but catalyze the same chemical reaction. These enzymes usually display different kinetic parameters, or different regulatory properties.

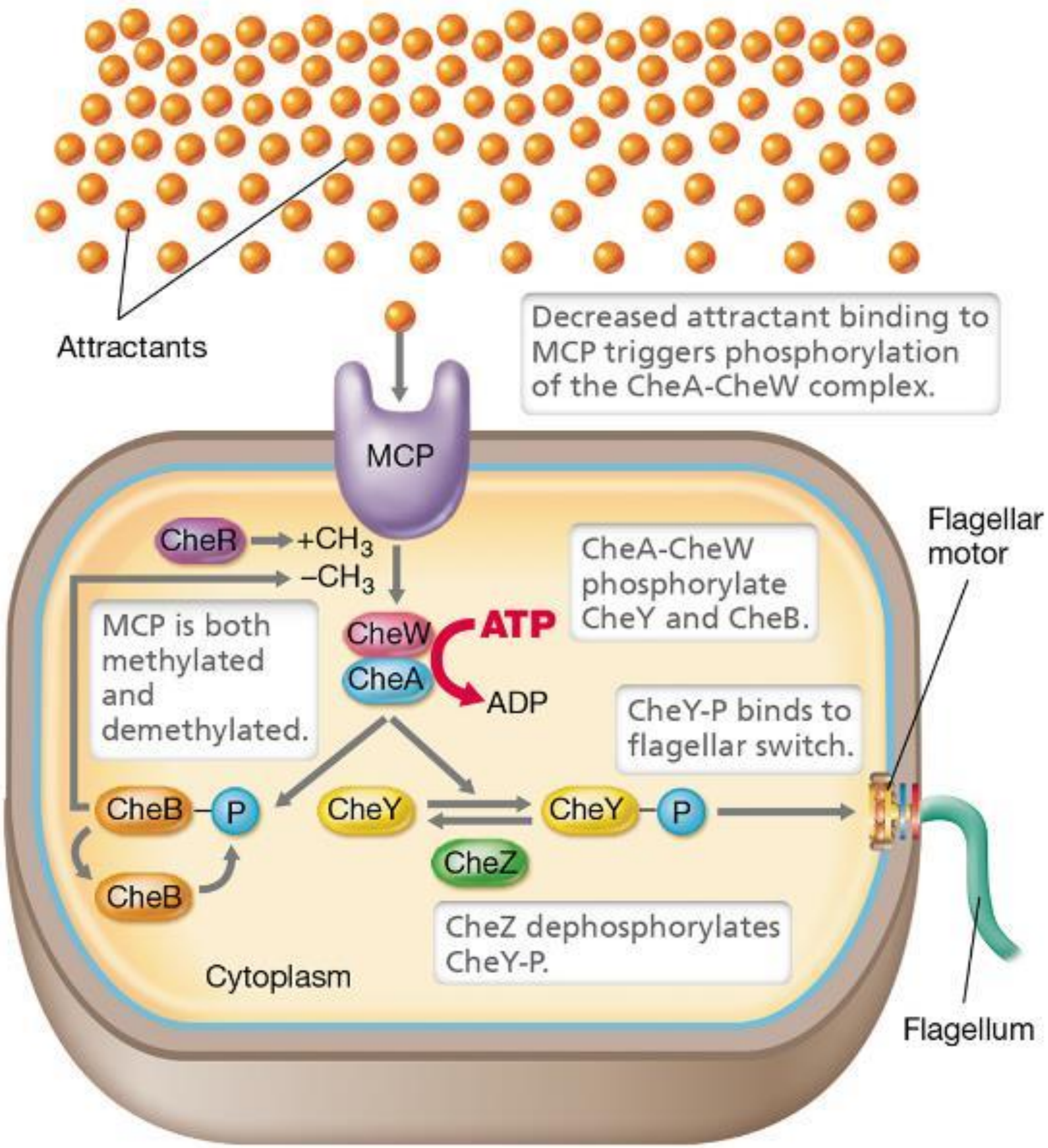
Wiki

Figure 7.34

7.16 Post-Translational Regulation

- Biosynthetic enzymes can also be regulated by covalent modifications.
  - Regulation involves a small molecule attached to or removed from the protein that affects activity.
  - Common modifiers include adenosine monophosphate (AMP), adenosine diphosphate (ADP), inorganic phosphate (PO_4^{3-}), and methyl groups (CH_3).

I.h.g. van een attractant



- CheY-P causes tumbling
- **Minder signaal = meer CheY-P** (en CheB-P)
- Weinig CH_3 => Sensitief voor attractant
- Weinig attractant = veel tumbling = weinig CH_3 = sensitief voor attractant
- Gevolg: constante hoeveelheid attractant = veel tumbeling. Alleen weer zwemmen bij voelen hogere conc. attractant.

Figure 7.17

7.16 Post-Translational Regulation

- Regulation of PII signal transduction proteins
  - regulate nitrogen metabolism
  - Modifications like *uridylylation* (addition of UMP, uridine monophosphate), *adenylylation* (addition of AMP), and *phosphorylation* affect activity.
  - Uridylylation by GlnD affects ammonia assimilation. (Figure 6.34)

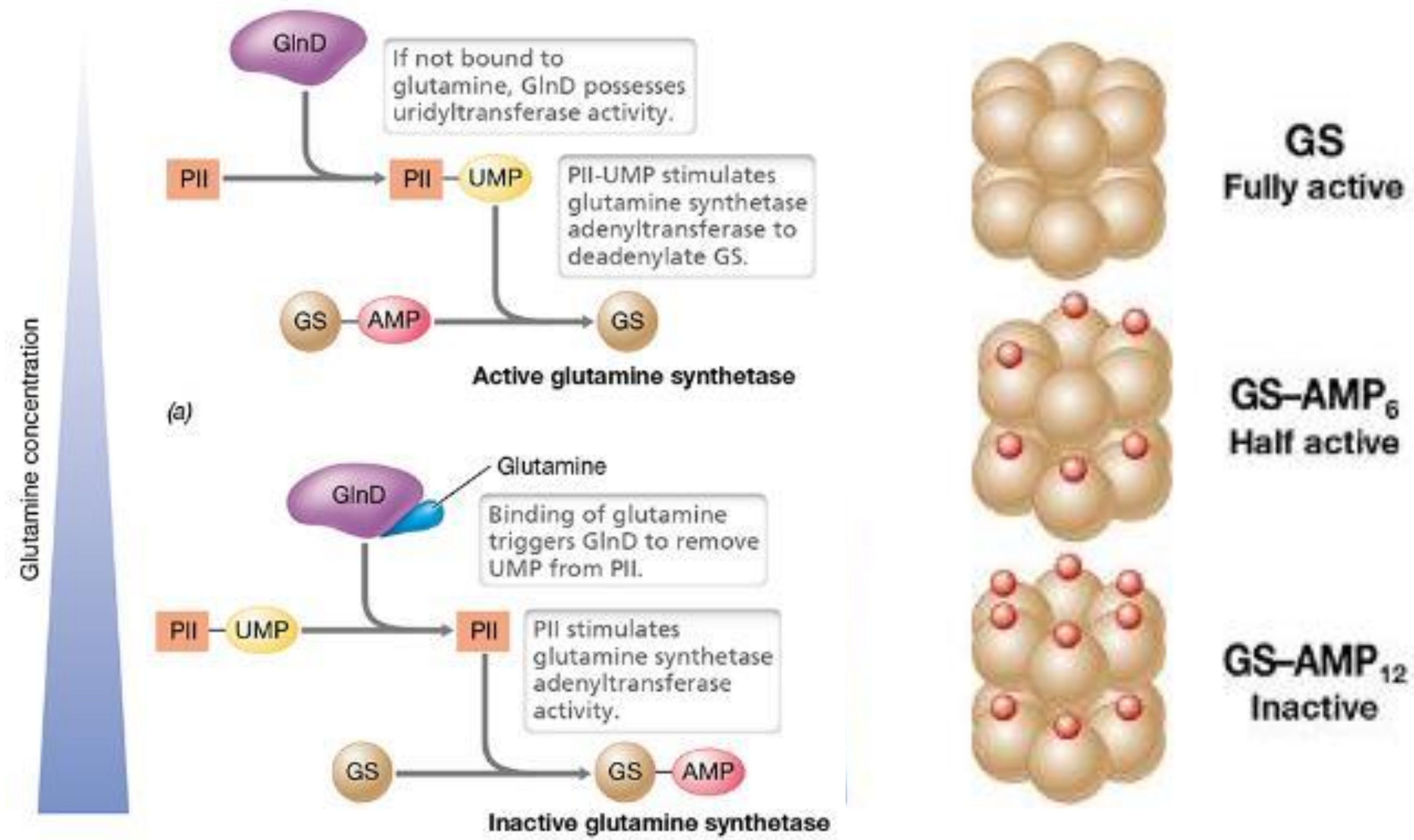

Glutamine concentration
GlnD
If not bound to glutamine, GlnD possesses uridyltransferase activity.
PII
PII
UMP
PII-UMP stimulates glutamine synthetase adenyltransferase to deadenylate GS.
GS
AMP
GS
Active glutamine synthetase
(a)
GlnD
Glutamine
Binding of glutamine triggers GlnD to remove UMP from PII.
PII
UMP
PII
PII stimulates glutamine synthetase adenyltransferase activity.
GS
GS
AMP
Inactive glutamine synthetase
GS
Fully active
GS–AMP6
Half active
GS–AMP12
Inactive

Figure 7.35

EINDE LES 4, EINDE H7