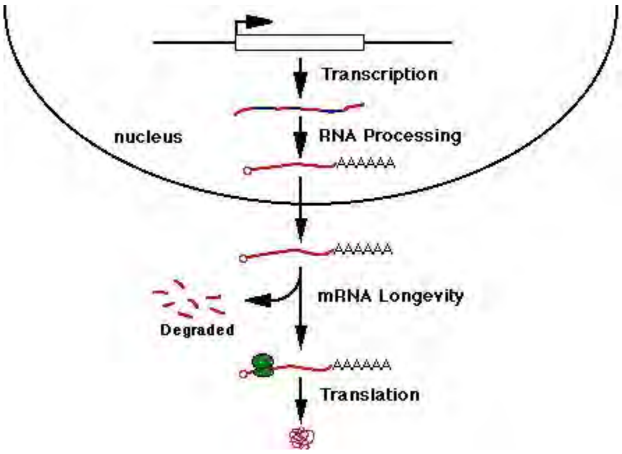
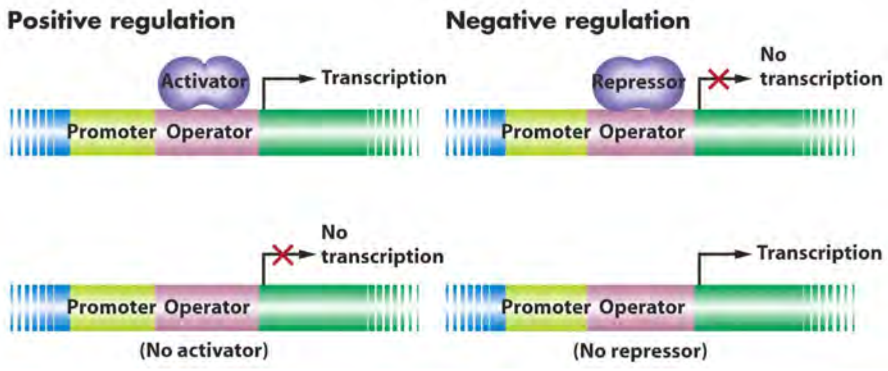
Genregulatie



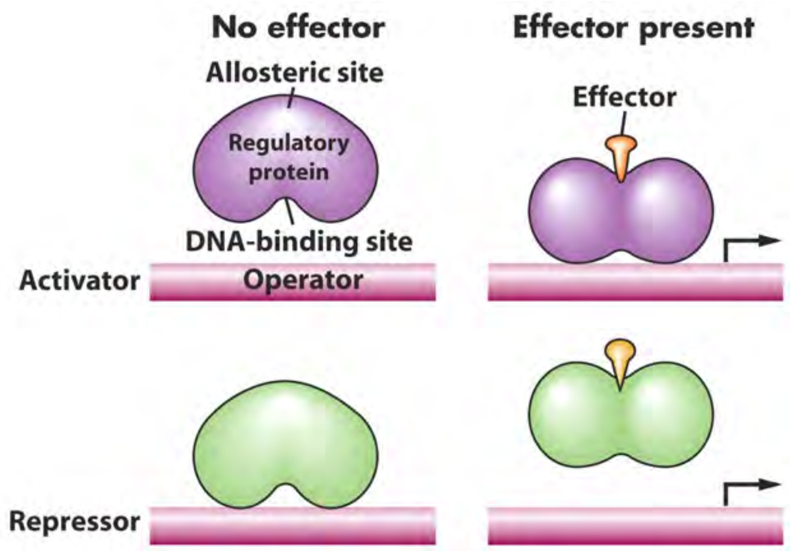
Expressieprofiel – kwantiteit – transcriptiefactoren – domeinen

Prokaryote genregulatie: de basis



Bacterien als nutritionele opportunisten

Activators en repressors komen voor in verschillende conformaties……



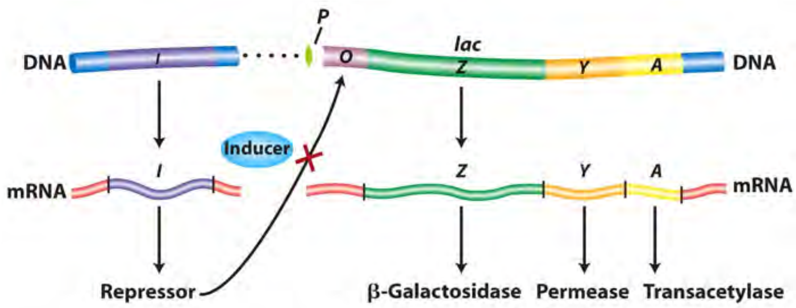
Conformationele veranderingen

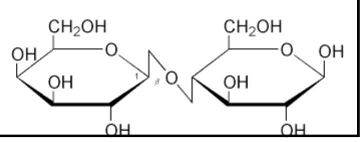
Overzicht:

1) Het *lac* operon
2) Het *ara* operon
3) Het *trp* operon
4) Faag λ cuclus
5) Sporulatie

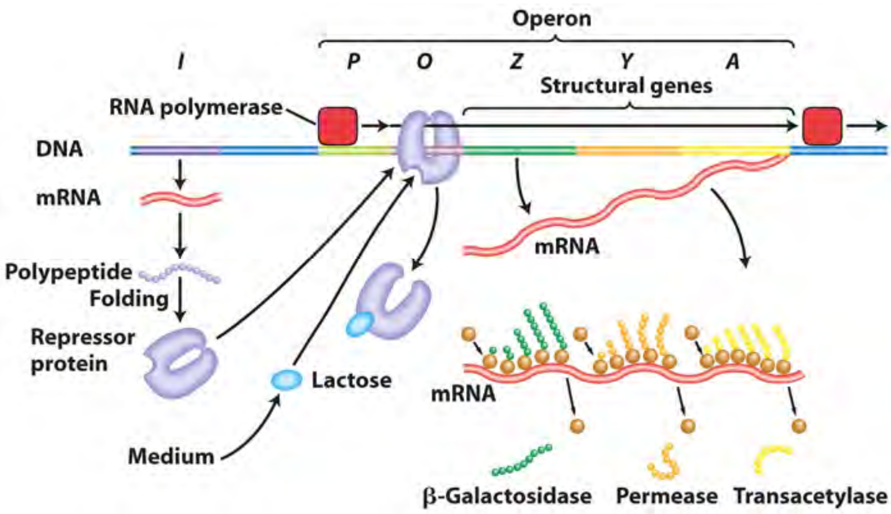
Regulatie van het *lac* operon:

Deel 1: inductie van het *lac* operon, negatieve controle door lacI





Inductie van het *Lac* operon



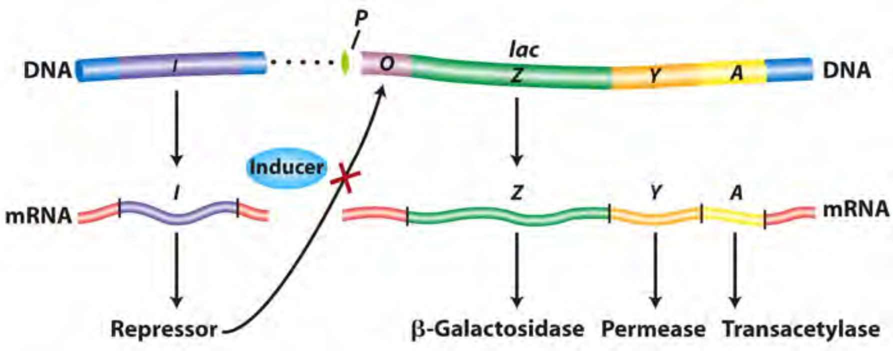
De ontdekking van regulatie van het lac operon

Genregulatie:

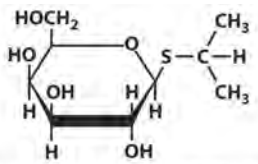
1. Een biochemisch systeem dat toelaat mRNA niveaus en/of eiwitactiviteiten te meten

2. Gecontroleerde condities voor metingen

3. Mutanten waarbij genexpressie veranderd is

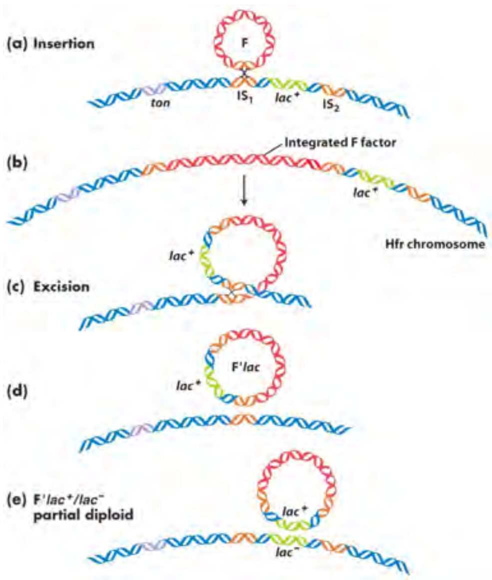


IPTG: de chemische inducer
1000 maal inductie na 3 minuten



Isopropyl-β-D-thiogalactoside (IPTG)

Partieel diploïden aan de hand van F'factors

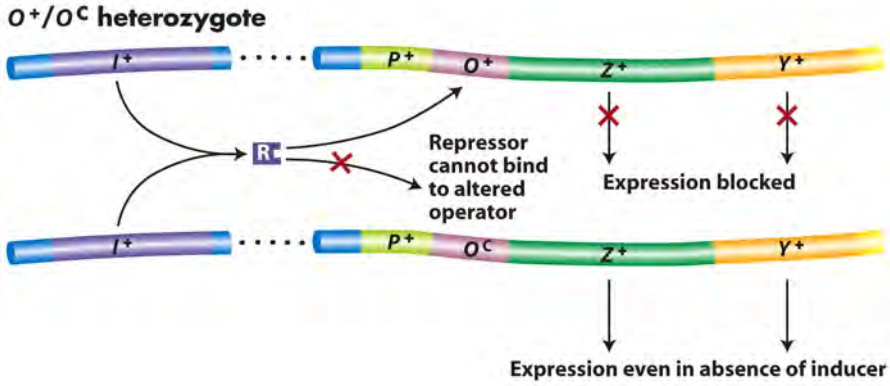


De O^c mutant: mutatie in de regulatie van het lac operon

TABLE 10-1 Synthesis of β-Galactosidase and Permease in Haploid and Heterozygous Diploid Operator Mutants

		β-Galactosidase (Z)		*Permease (Y)*		
Strain	Genotype	Noninduced	Induced	Noninduced	Induced	Conclusion
1	$O^+Z^+Y^+$	−	+	−	+	Wild type is inducible
2	$O^+Z^+Y^+/F'O^+Z^-Y^+$	−	+	−	+	Z^+ is dominant to Z^-
3	$O^CZ^+Y^+$	+	+	+	+	O^C is constitutive
4	$O^+Z^-Y^+/F'O^CZ^+Y^-$	+	+	−	+	Operator is cis-acting

Note: Bacteria were grown in glycerol (no glucose present) with and without the inducer IPTG. The

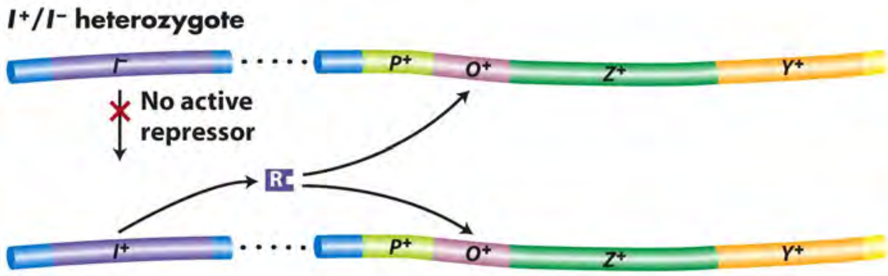


I^- mutatie, mutatie in de regulatie van het lac operon

TABLE 10-2 Synthesis of β-Galactosidase and Permease in Haploid and Heterozygous Diploid Strains Carrying I^+ and I^-

		β-Galactosidase (Z)		*Permease* (Y)		
Strain	Genotype	Noninduced	Induced	Noninduced	Induced	Conclusion
1	$I^+Z^+Y^+$	−	+	−	+	I^+ is inducible
2	$I^-Z^+Y^+$	+	+	+	+	I^- is constitutive
3	$I^+Z^+Y^+/F'I^-Z^+Y^+$	−	+	−	+	I^+ is dominant to I^-
4	$I^-Z^-Y^+/F'I^+Z^+Y^-$	−	+	−	+	I^+ is trans-acting

Note: Bacteria were grown in glycerol (no glucose present) and induced with IPTG. The presence of the maximal level of the enzyme is indicated by a plus sign; the absence or very low level of an enzyme is indicated by a minus sign. (All strains are O^+.)

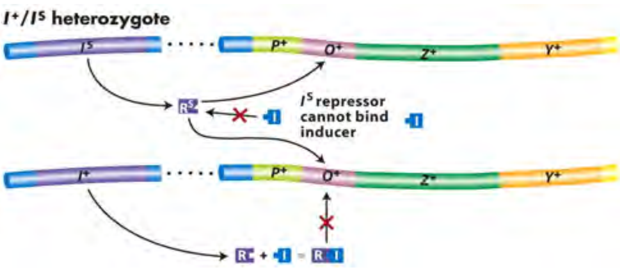


Genetische evidentie voor het allosterisch domein van I repressor

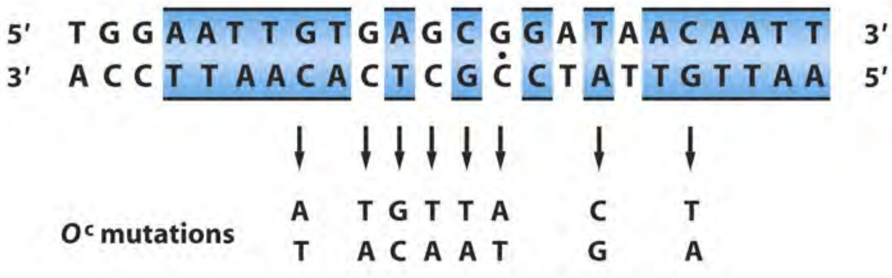
TABLE 10-3 Synthesis of β-Galactosidase and Permease by the Wild Type and by Strains Carrying Different Alleles of the *I* Gene

		β-Galactosidase (Z)		Permease (Y)		
Strain	Genotype	Noninduced	Induced	Noninduced	Induced	Conclusion
1	$I^+Z^+Y^-$	–	+	–	+	I^+ is inducible
2	$I^SZ^+Y^+$	–	–	–	–	I^S is always repressed
3	$I^SZ^+Y^+/F'I^+Z^+Y^+$	–	–	–	–	I^S is dominant to I^+

Note: Bacteria were grown in glycerol (no glucose present) with and without the inducer IPTG. Presence of the indicated enzyme is represented by +; absence or low levels, by –.



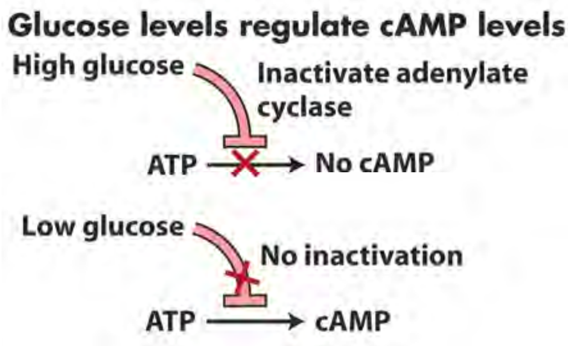
De operator sequentie werd achterhaald door DNAse protectie-experimenten

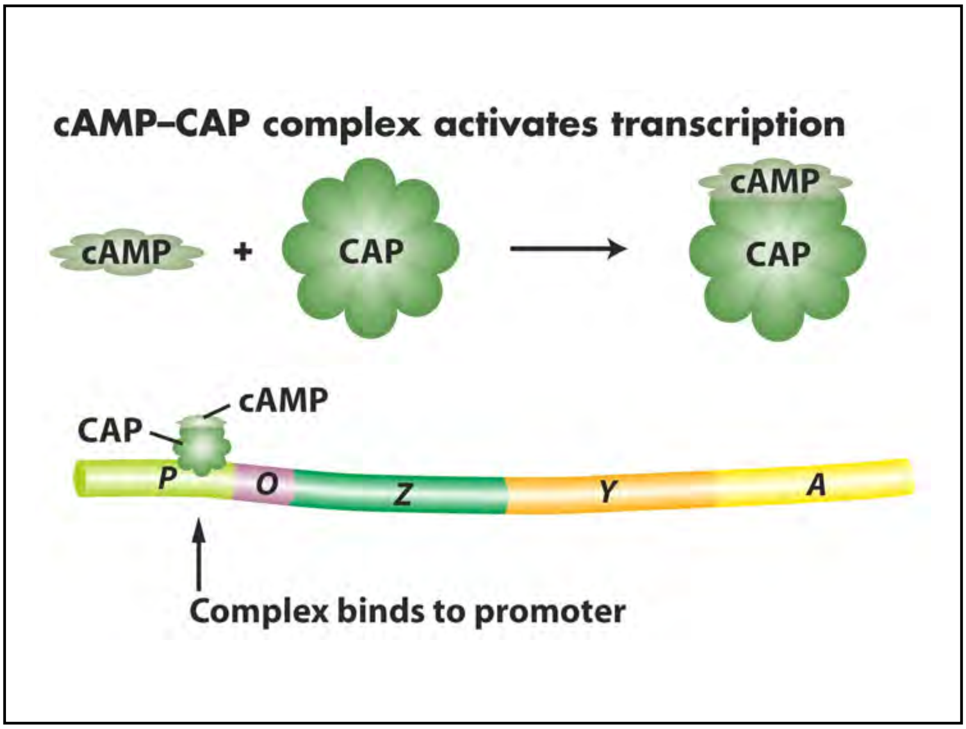


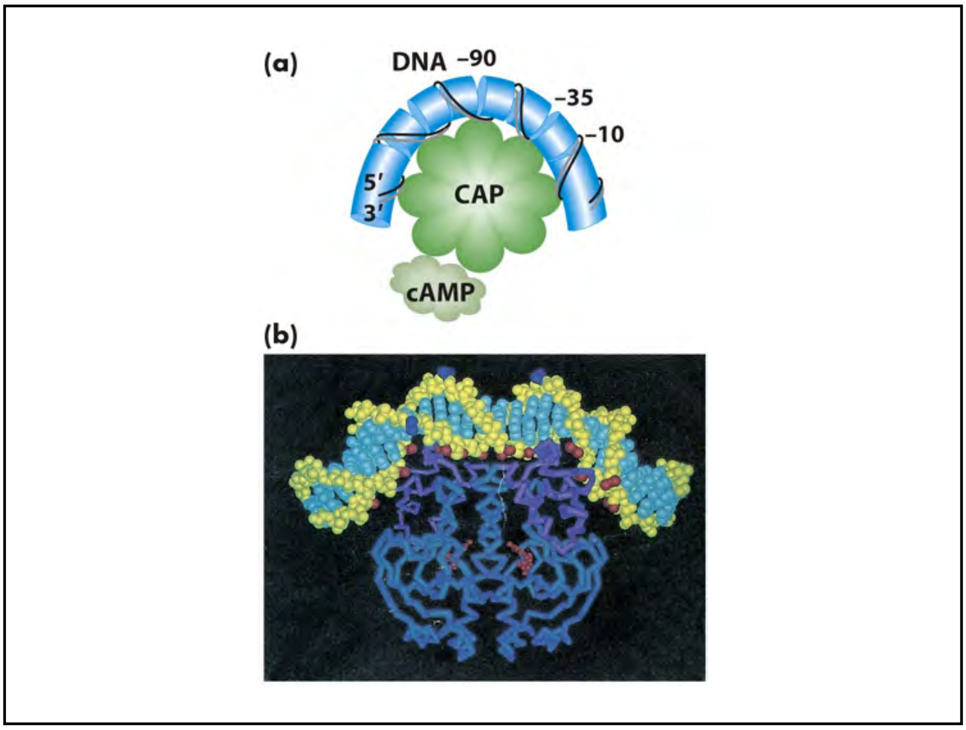
17-25 nucleotiden
Net voor de structurele genen
Wordt ook overgeschreven
Tweevoudige symmetrie

Regulatie van het *lac* operon:

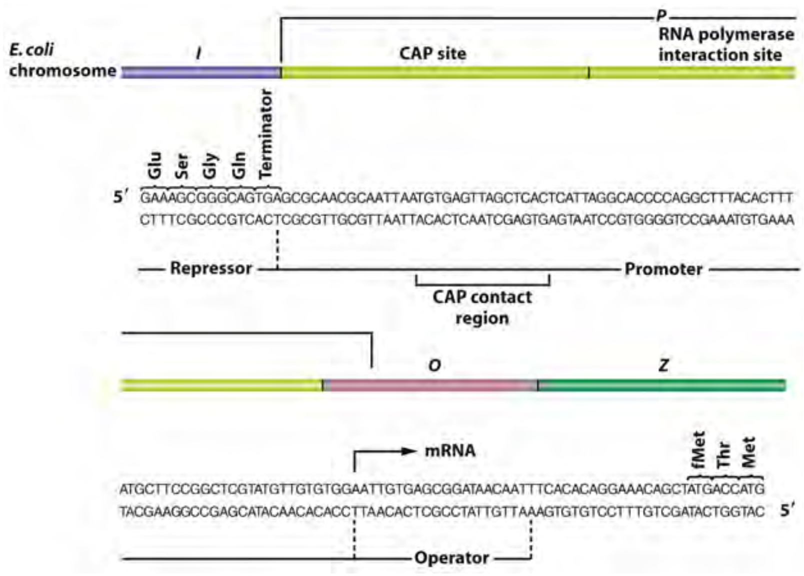
Deel 2: katabolische repressie, invloed van glucose

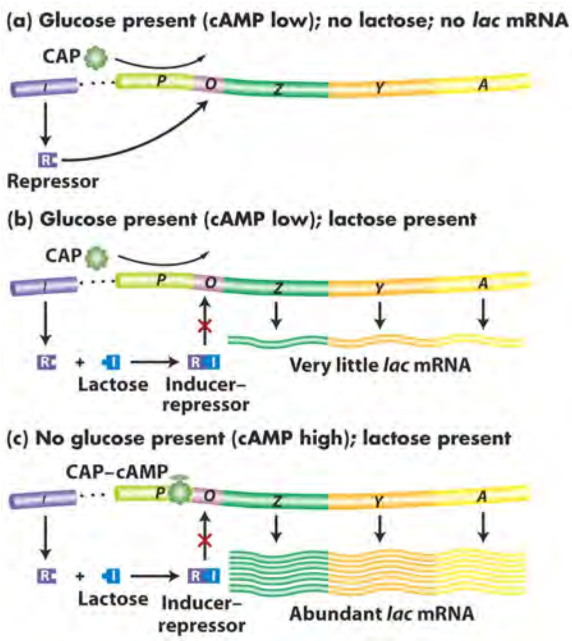



cAMP–CAP complex activates transcription
cAMP
+
CAP
cAMP
CAP
CAP
cAMP
P
O
Z
Y
A
Complex binds to promoter


(a)
DNA
–90
–35
–10
5′
3′
CAP
cAMP
(b)

Samengevat:

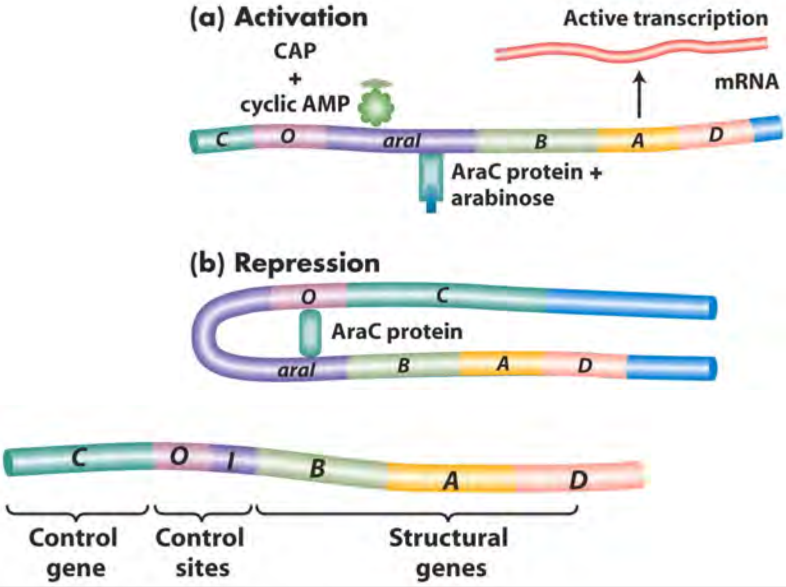

E. coli chromosome
I
CAP site
P
RNA polymerase interaction site
Glu
Ser
Gly
Gln
Terminator
5′ GAAAGCGGGCAGTGAGCGCAACGCAATTAATGTGAGTTAGCTCACTCATTAGGCACCCCAGGCTTTACACTTT
CTTTCGCCCGTCACTCGCGTTGCGTTAATTACACTCAATCGAGTGAGTAATCCGTGGGGTCCGAAATGTGAAA
Repressor
Promoter
CAP contact region
O
Z
mRNA
fMet
Thr
Met
ATGCTTCCGGCTCGTATGTTGTGTGGAATTGTGAGCGGATAACAATTTCACACAGGAAACAGCTATGACCATG
TACGAAGGCCGAGCATACAACACACCTTAACACTCGCCTATTGTTAAAGTGTGTCCTTTGTCGATACTGGTAC 5′
Operator


(a) Glucose present (cAMP low); no lactose; no lac mRNA
CAP
P
O
Z
Y
A
Repressor
(b) Glucose present (cAMP low); lactose present
CAP
Lactose
Inducer–repressor
Very little lac mRNA
(c) No glucose present (cAMP high); lactose present
CAP–cAMP
Lactose
Inducer–repressor
Abundant lac mRNA

Overzicht:

1) Het *lac* operon
2) Het *ara* operon
3) Het *trp* operon
4) Faag λ cuclus
5) Sporulatie

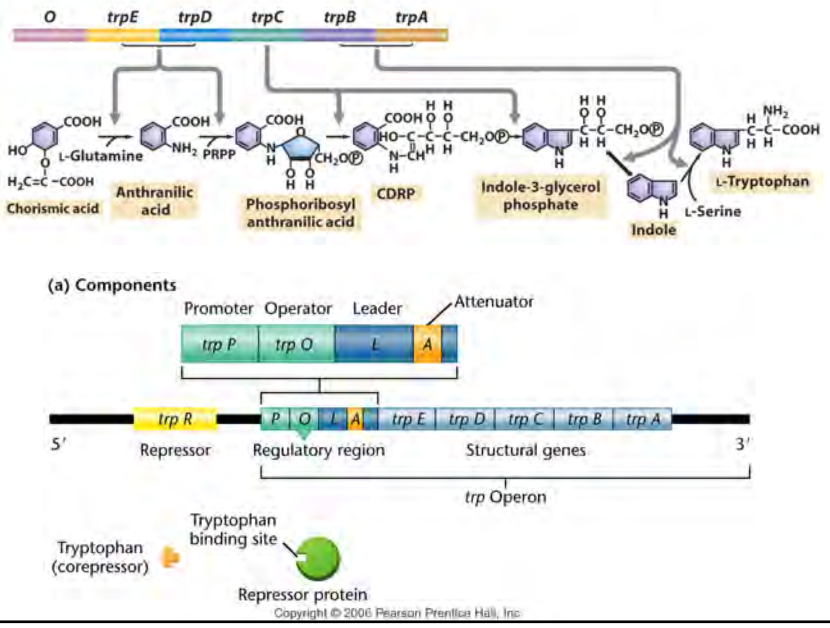
Het arabinose operon: positieve en negatieve controle door dezelfde regulator



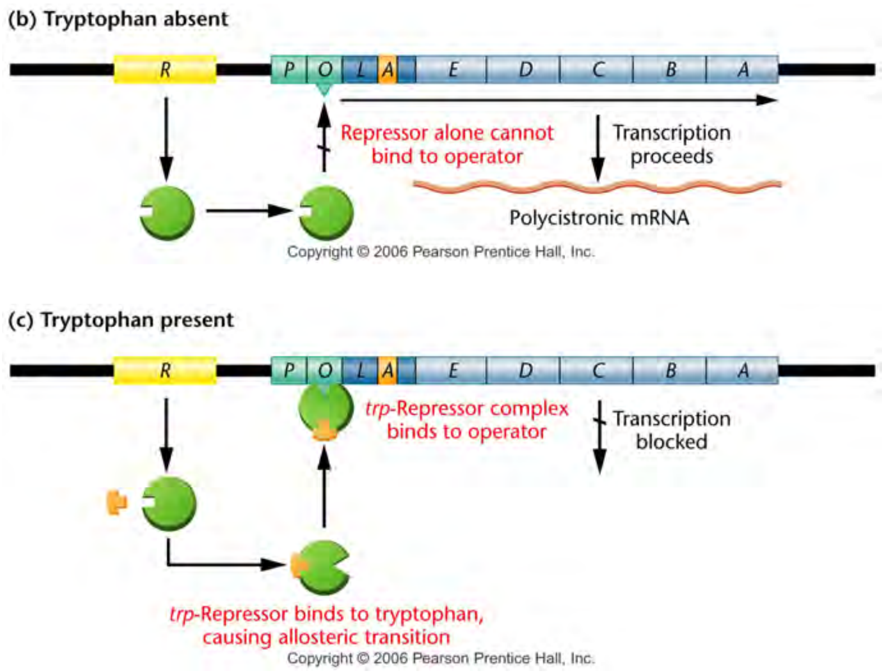
Overzicht:

1) Het *lac* operon
2) Het *ara* operon
3) Het *trp* operon
4) Faag λ cuclus
5) Sporulatie

Het tryptofaan (*trp*) operon: een represseerbaar systeem



Attenuatie



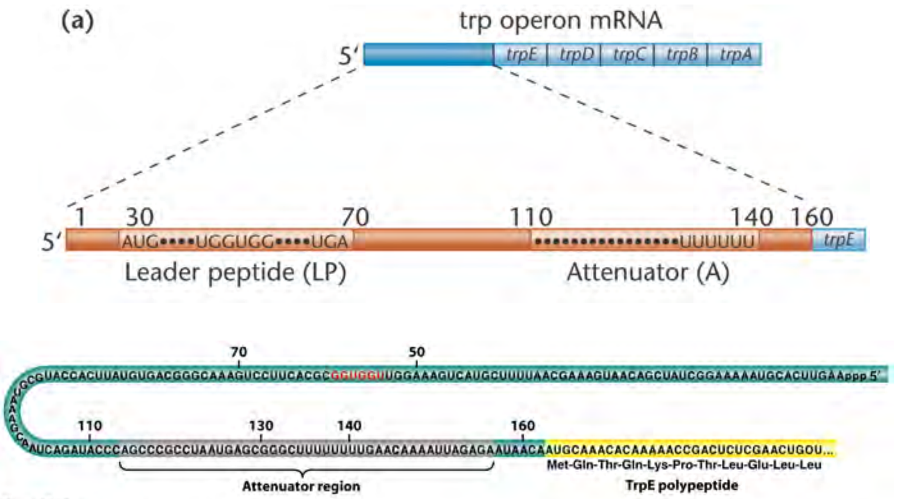


Figure 10-22
Introduction to Genetic Analysis, Ninth Edition

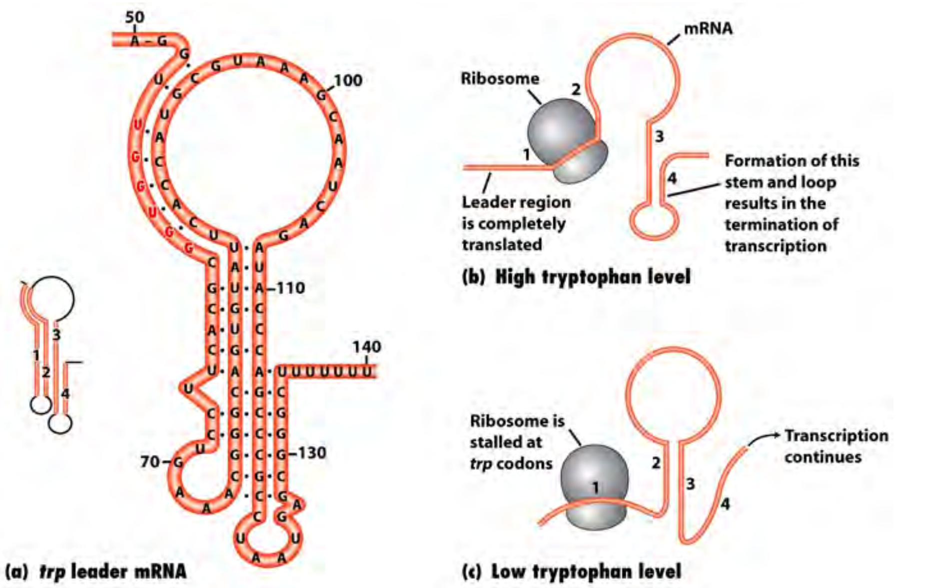


Figure 10-23
Introduction to Genetic Analysis, Ninth Edition

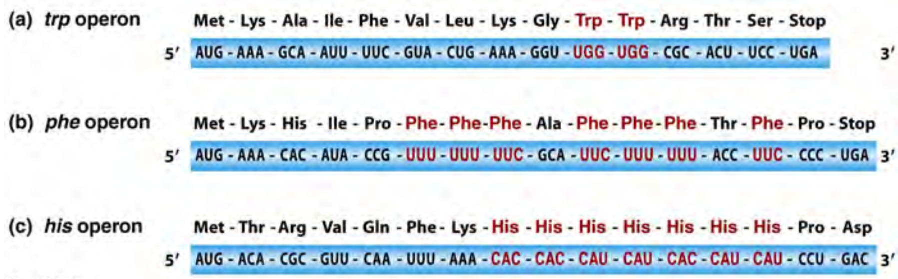


Figure 10-24
Introduction to Genetic Analysis, Ninth Edition

Overzicht:

1) Het *lac* operon
2) Het *ara* operon
3) Het *trp* operon
4) Faag λ cuclus
5) Sporulatie

Getemperde faag lambda als voorbeeld van een complexe genetische switch by keuze lytische en lysogene cyclus

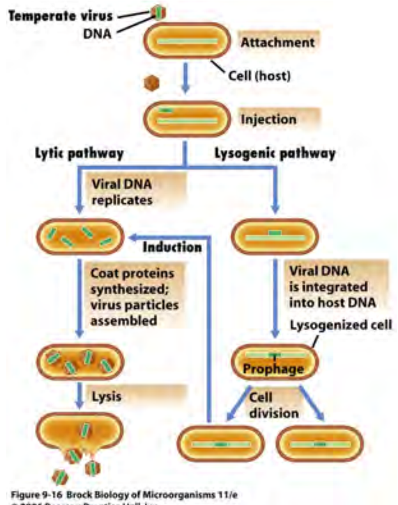


Figure 9-16 Brock Biology of Microorganisms 11/e
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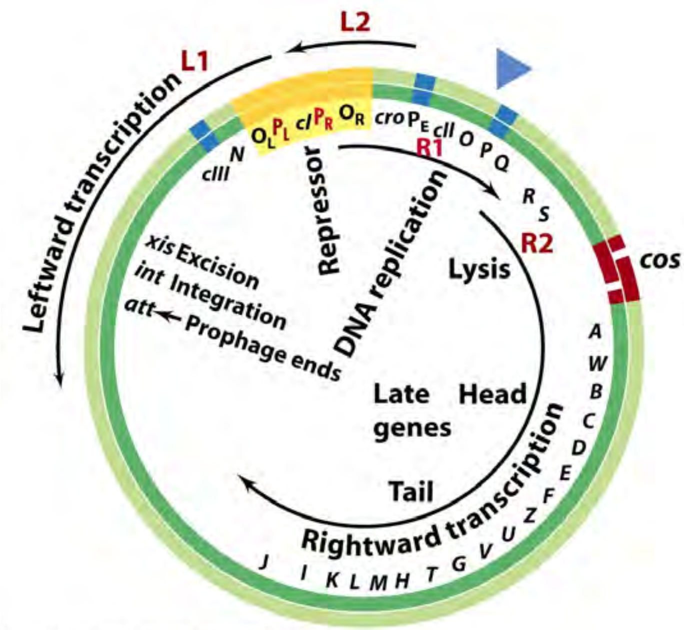


Figure 9-18b Brock Biology of Microorganisms 11/e

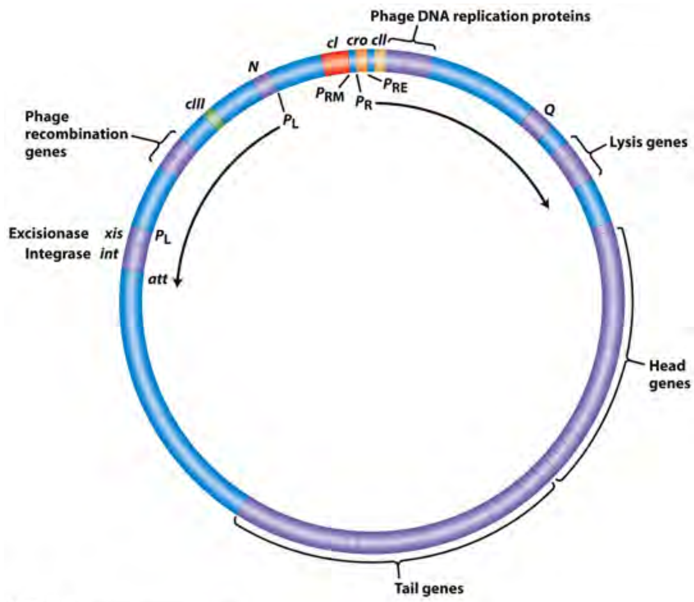


Figure 10-26
Introduction to Genetic Analysis, Ninth Edition

De “default pathway”: de lytische cyclus

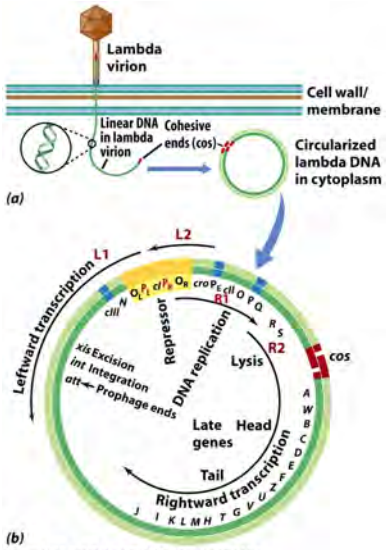


Figure 9-18 Brock Biology of Microorganisms 11/e

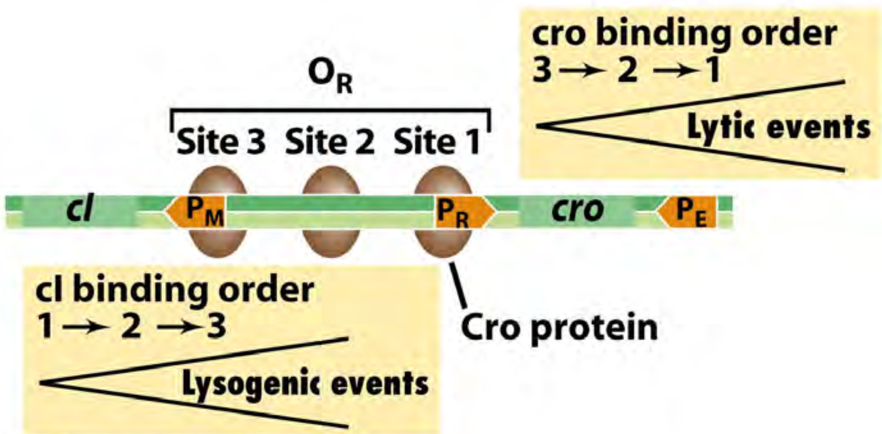
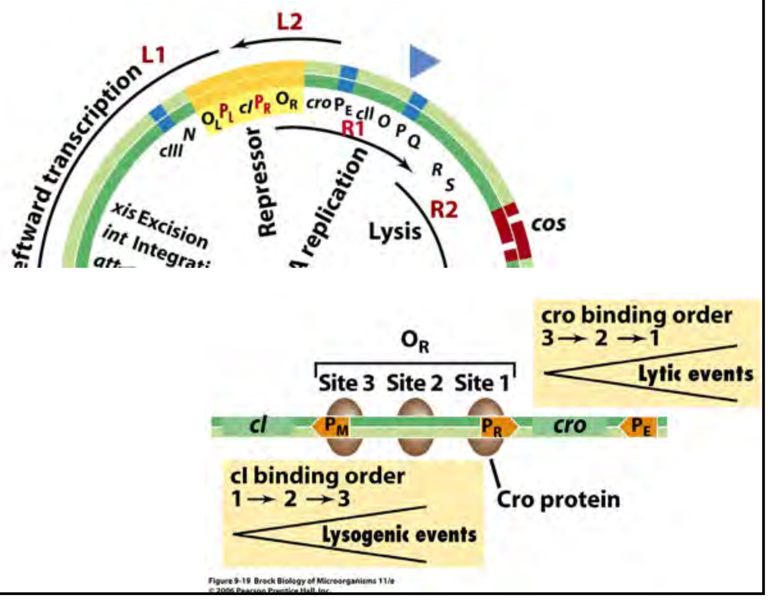
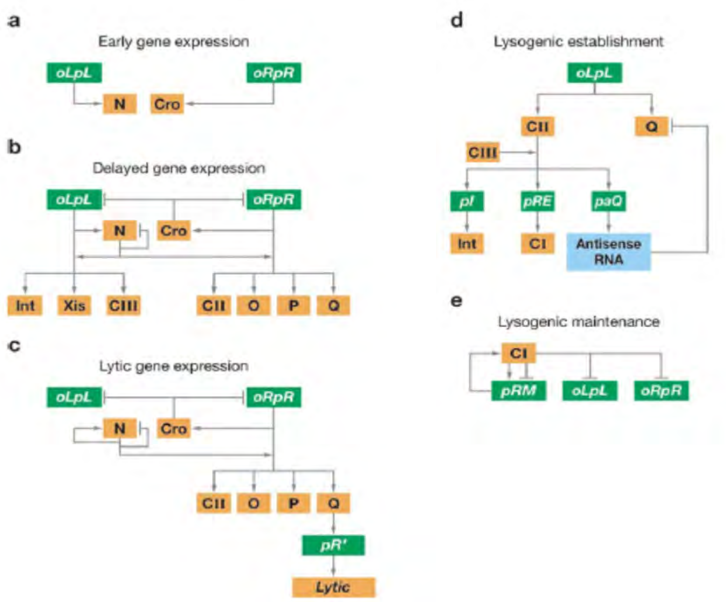


Figure 9-19 Brock Biology of Microorganisms 11/e

Doorlopen van de lysogene cyclus





Overzicht:

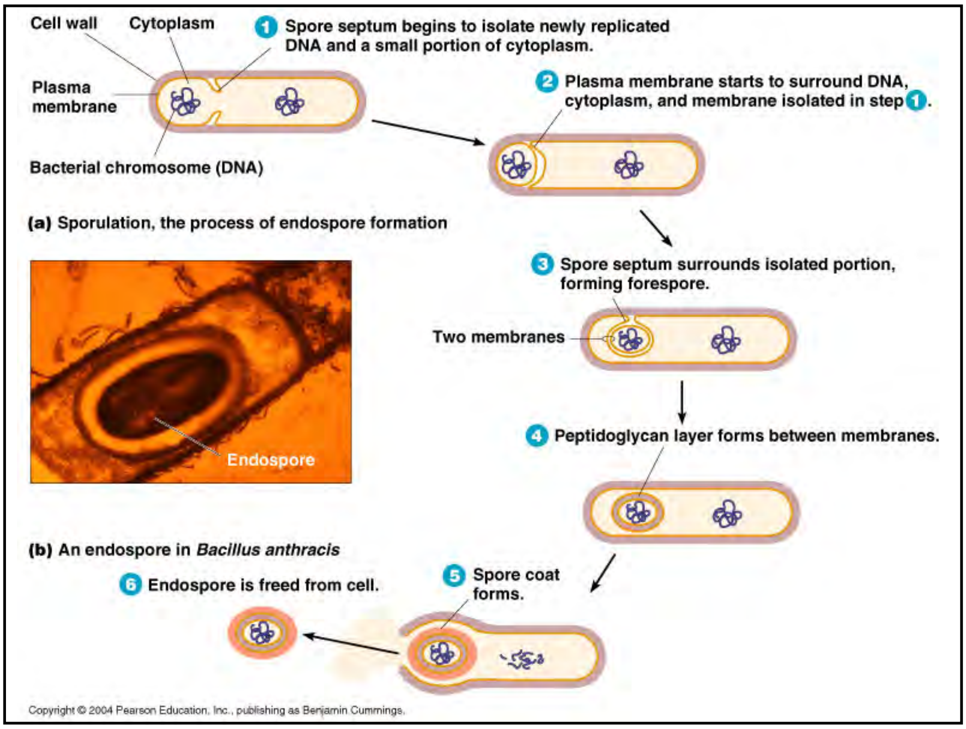
1) Het *lac* operon
2) Het *ara* operon
3) Het *trp* operon
4) Faag λ cuclus
5) Sporulatie

Endospore vorming bij *Bacillus subtilis*

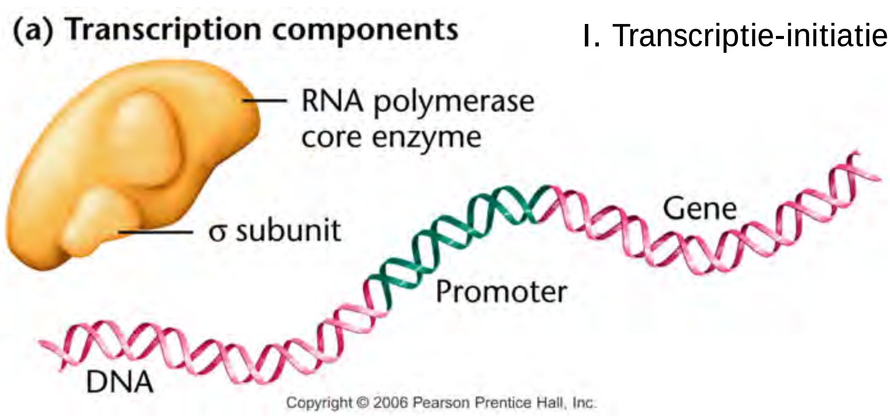
Hoe sigmafactoren differentiatie controleren

Bacteriële differentiatie

De metamorfose van een vegetatieve cel tot een rustende cel, resistent tegen hitte, uitdroging, straling…



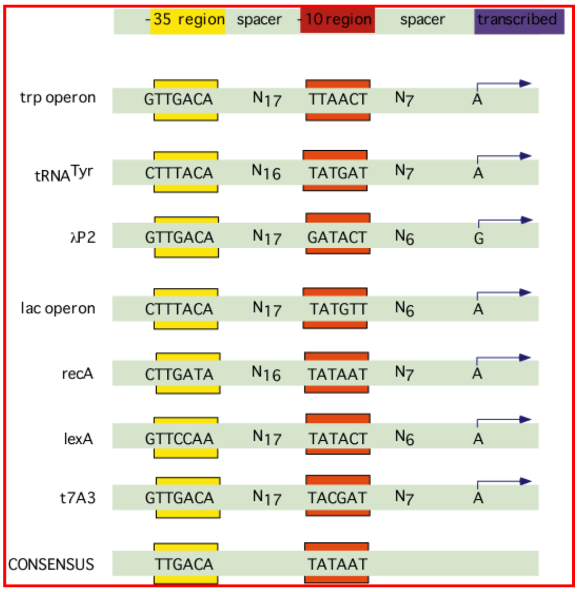
I. Transcriptie-initiatie



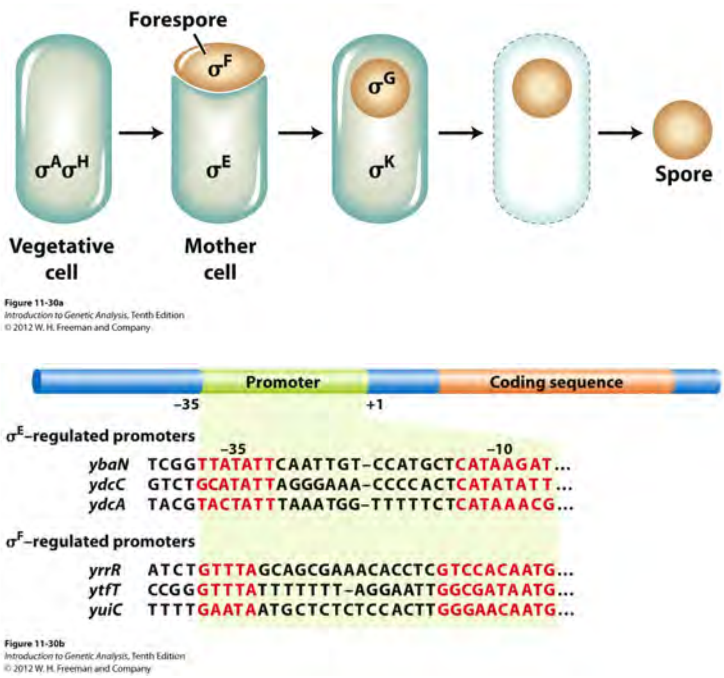
De sigma factoren herkennen specifieke sequenties in de promoter

Cis element

Transacting factor



Sequenties die herkend worden door σ70



Criss-cross regulatie