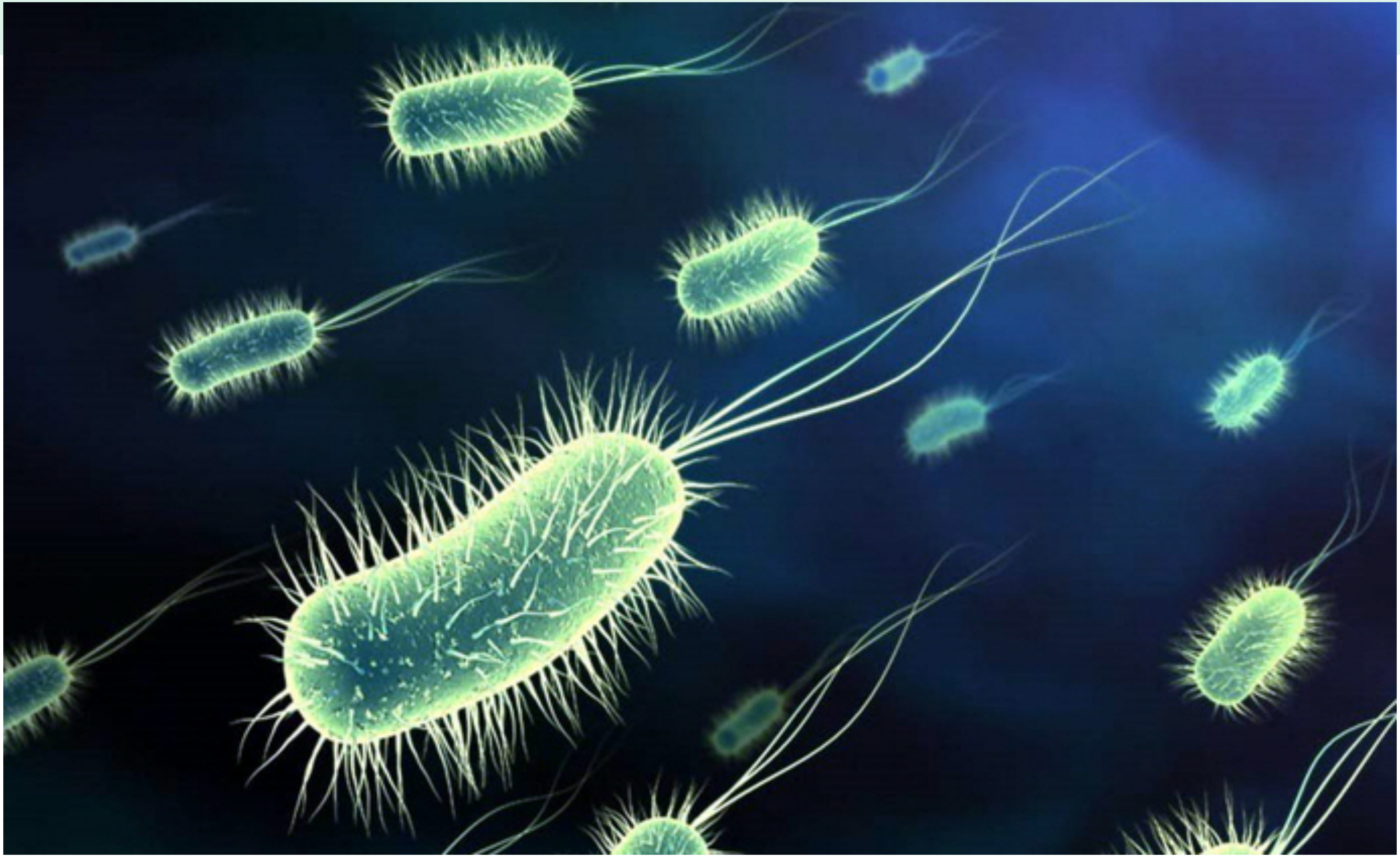
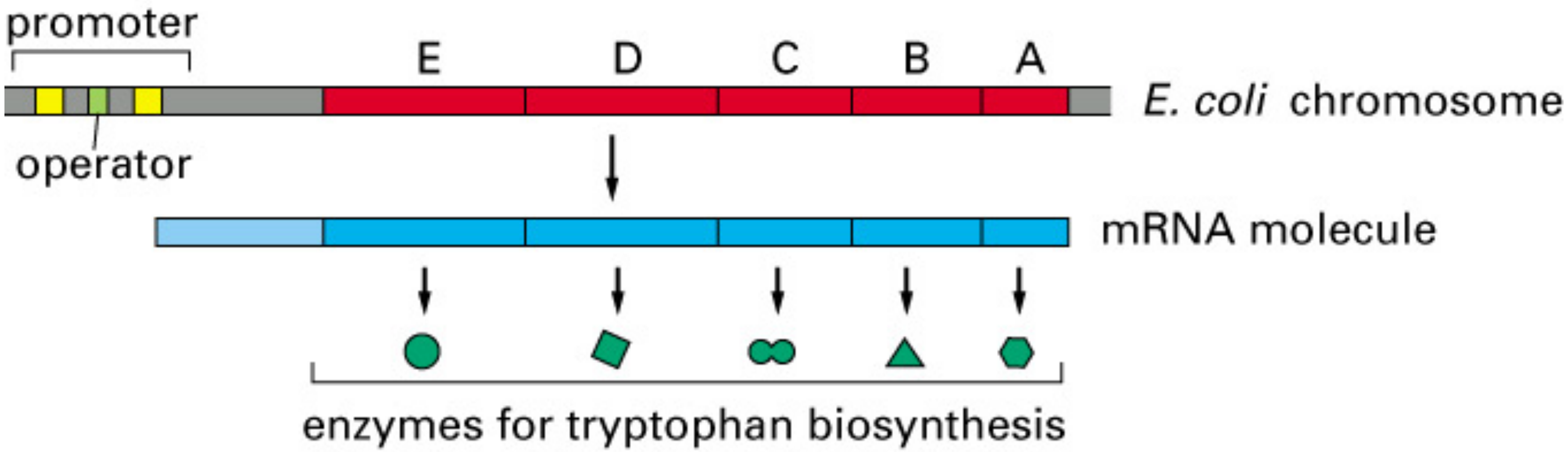
Controllo dell'espressione genica: procarioti



Regolazione dell’espressione nei procarioti

1. Operon



2. Regulation mostly on the transcriptional level.

POSITIVE: energy saving, regulation of only “one”mRNA molecule to activate a parthway,
Coupling transcription/translation, polycistronic mRNAs, regulation of entire pathway.

NEGATIVE: gradual regulation is difficult, regulation is limited to a low number of signals, post-translational regulation accelerates response

I principi della regolazione trascrizionale

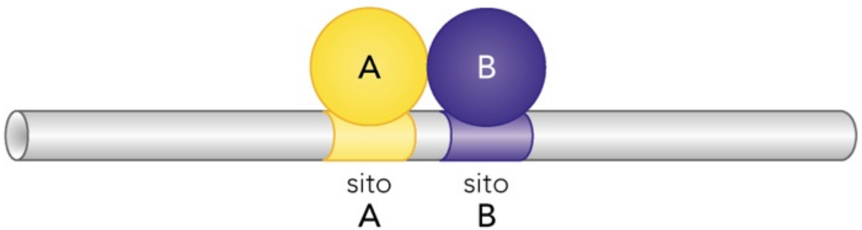
1. In bacteria, **activators and repressors of transcription are typically activated by interacting with small molecules**

2. **Regulators of transcription act by controlling the access of RNA Pol at the promoter** → transition closed/open complex, structural change of promotor, mRNA expression + translation

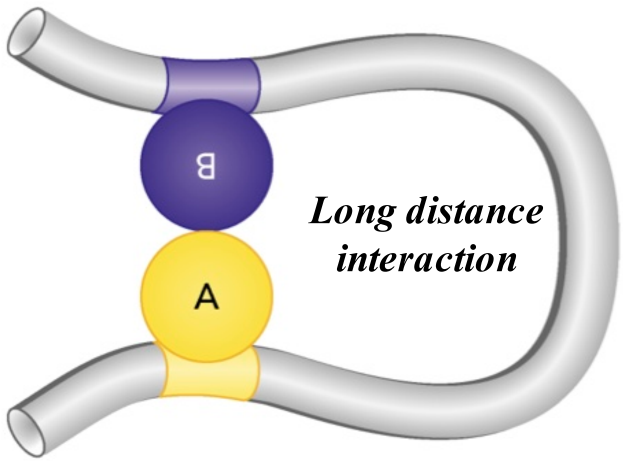
3. **REPRESSORS**: bind to the **OPERATOR**

4. **ACTIVATORS**:
→binds in vicinity to promoter and helps **RNA Polymerase recruitment**
→act in an **allosteric** manner to induce the transition from the closed to open complex

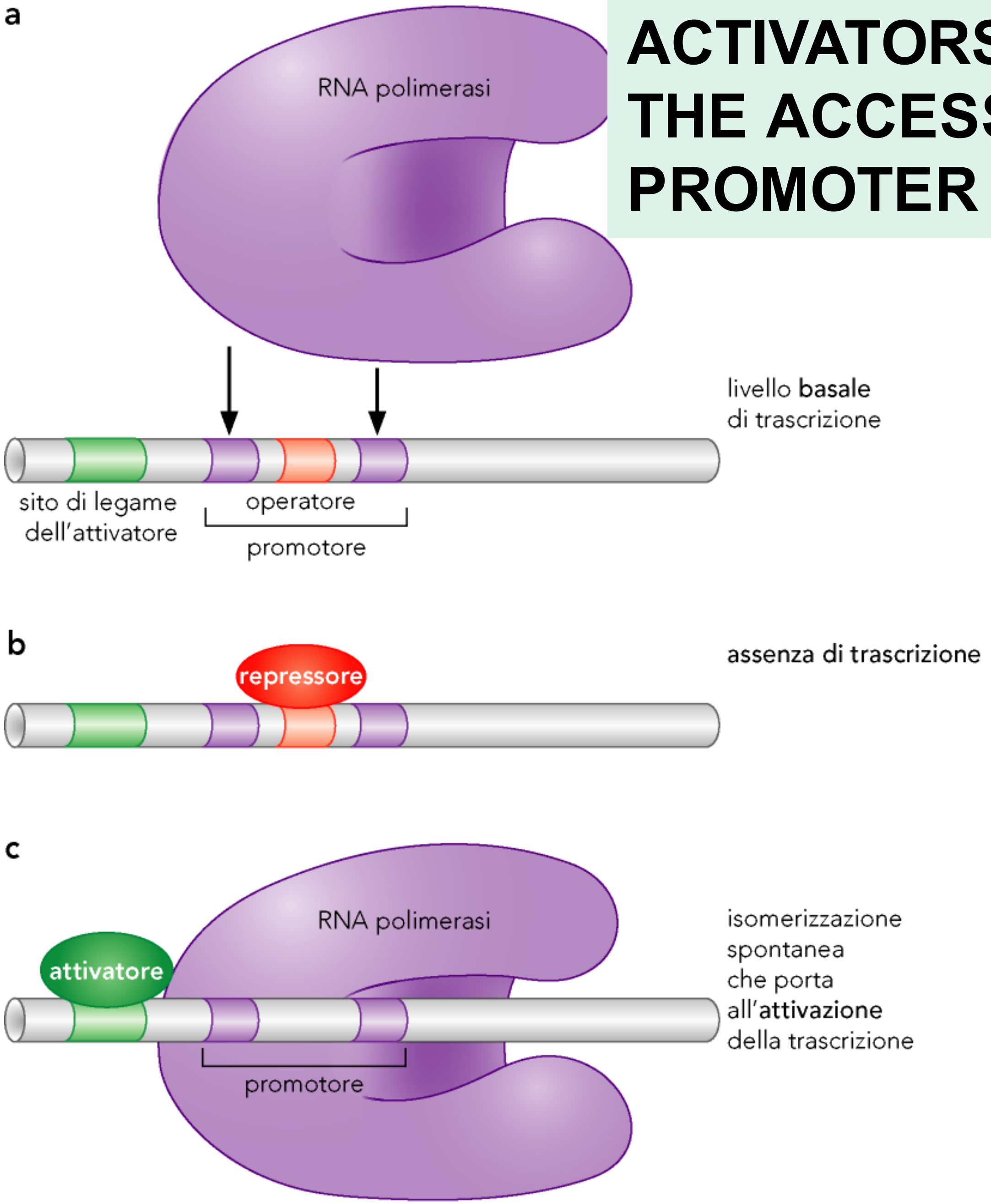
a



short distance interaction



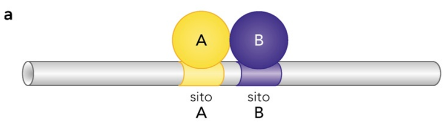
ACTIVATORS/REPRESSORS REGULATE THE ACCESS OF RNA Pol TO PROMOTER



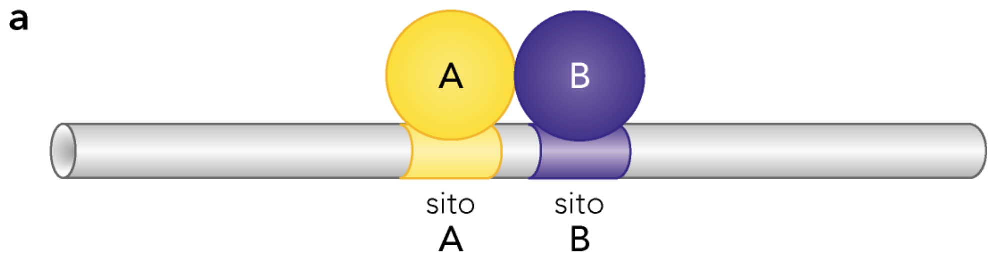
Basal level of transcription: determined by interaction of sigma factor and sigma factor binding site (sequence dependent)

Repressor:
Occupies operator close to Promoter. RNA Pol can not access promoter.

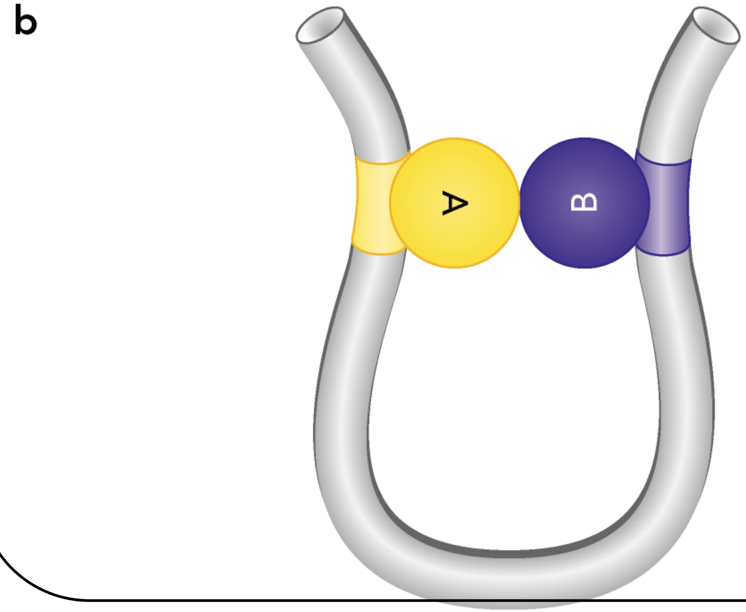
Activator:
Occupies binding site close to promoter and recruits RNA Pol (reclutamento o cooperative binidng



ACTIVATORS/REPRESSORS REGULATE THE ACCESS OF RNA Pol TO PROMOTER

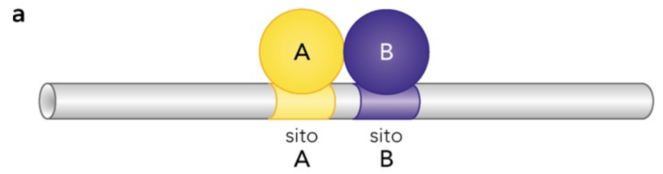


Short distance interaction

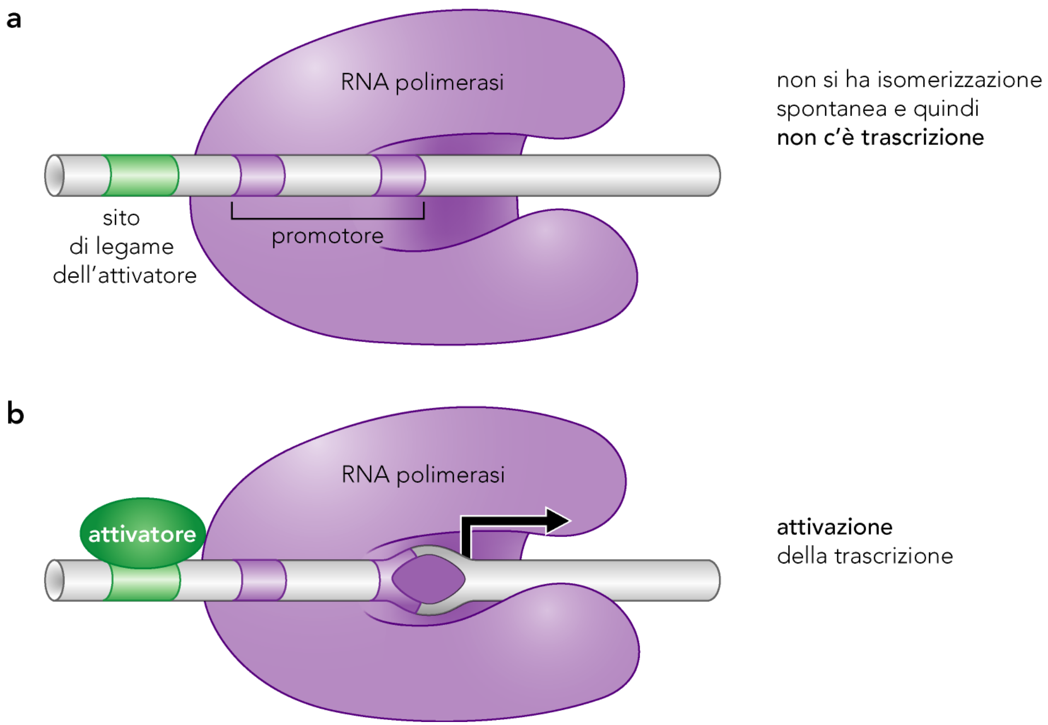


Long distance
Interaction between
Two factors (for example:
PolII and activator)

ACTIVATORS/REPRESSORS REGULATE THE INITIATION OF TRANSCRIPTION



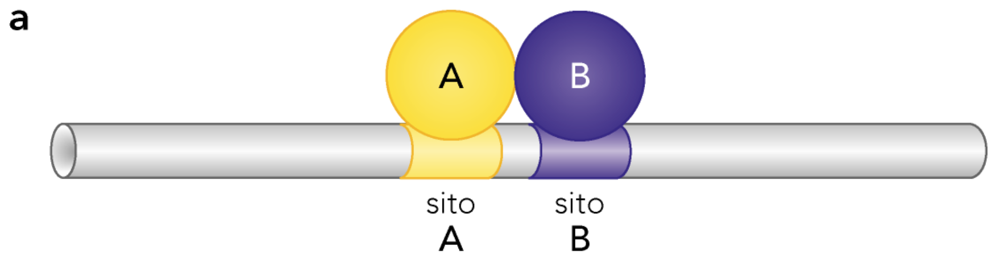
SHORT DISTANCE INTERACTION



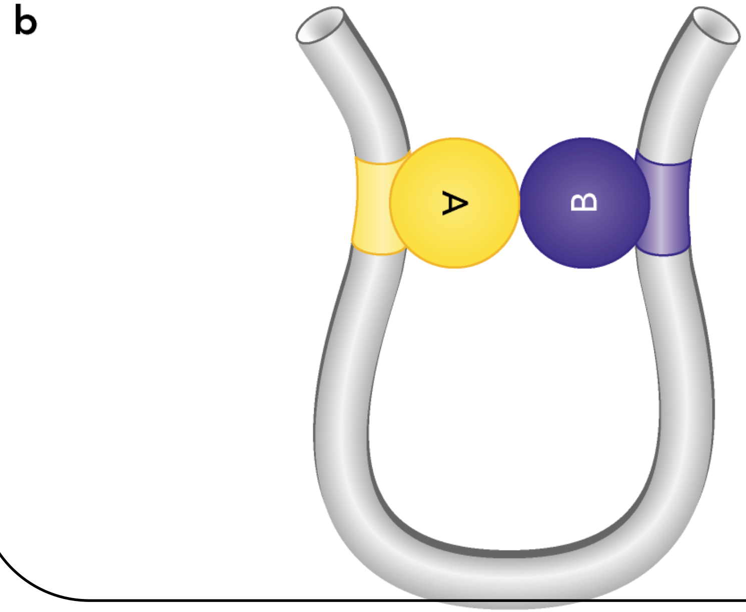
Activator:

Allosteric activation: Pol occupies promoter without efficiently forming an open complex.(low, basal expression). Binding of activator induces efficient conformational change that leads to the transition from closed to open complex

ACTIVATORS/REPRESSORS REGULATE THE ACCESS OF RNA Pol TO PROMOTER



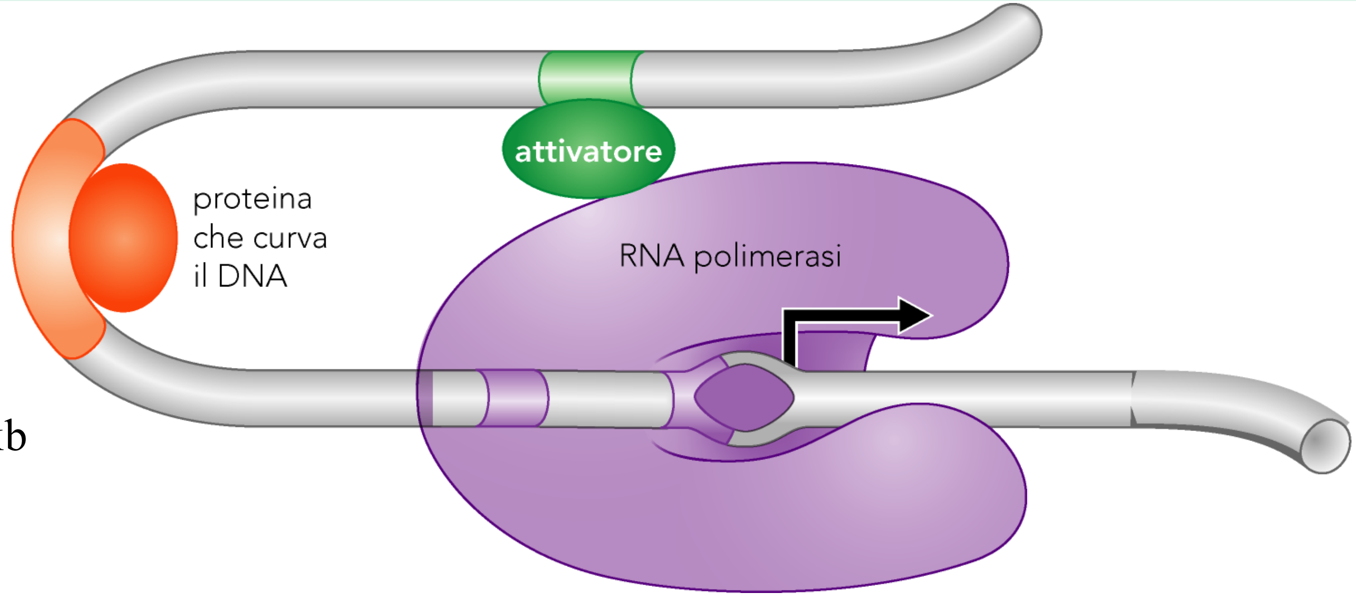
Short distance interaction



Long distance
Interaction between
Two factors (for example:
PolII and activator)

Architectural proteins allow the formation of looped DNA

Ansa:
fino al 3kb

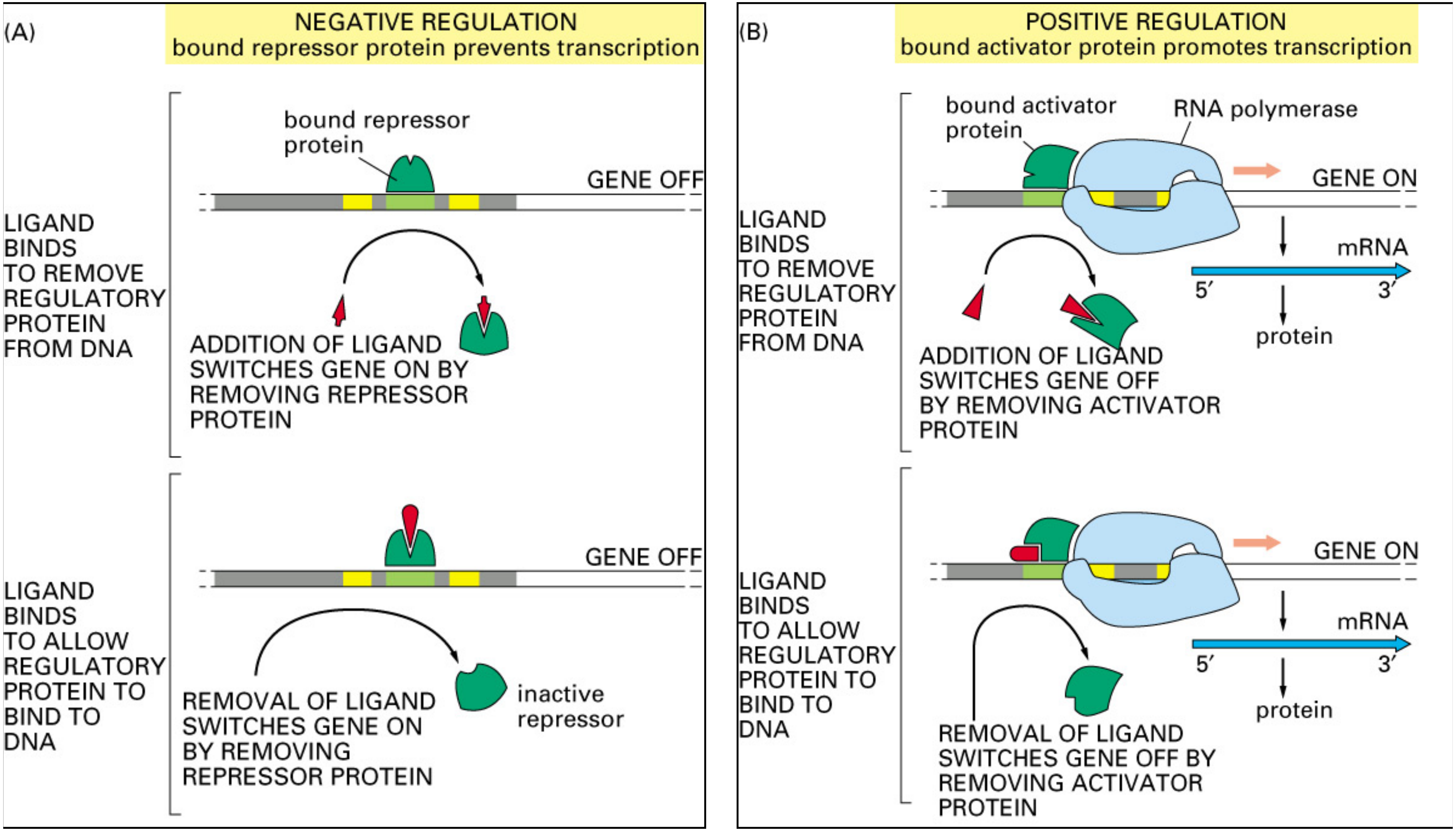


→ Promoter and activator site are separated

→ Architectonical protein bends DNA

→ Activator close to promoter/RNA Pol

Activity of repressors are controlled by small ligands



Hallmark models for gene regulation in procaryotes

1. The Lactose Operon – Lac Operon (Pol recruitment)

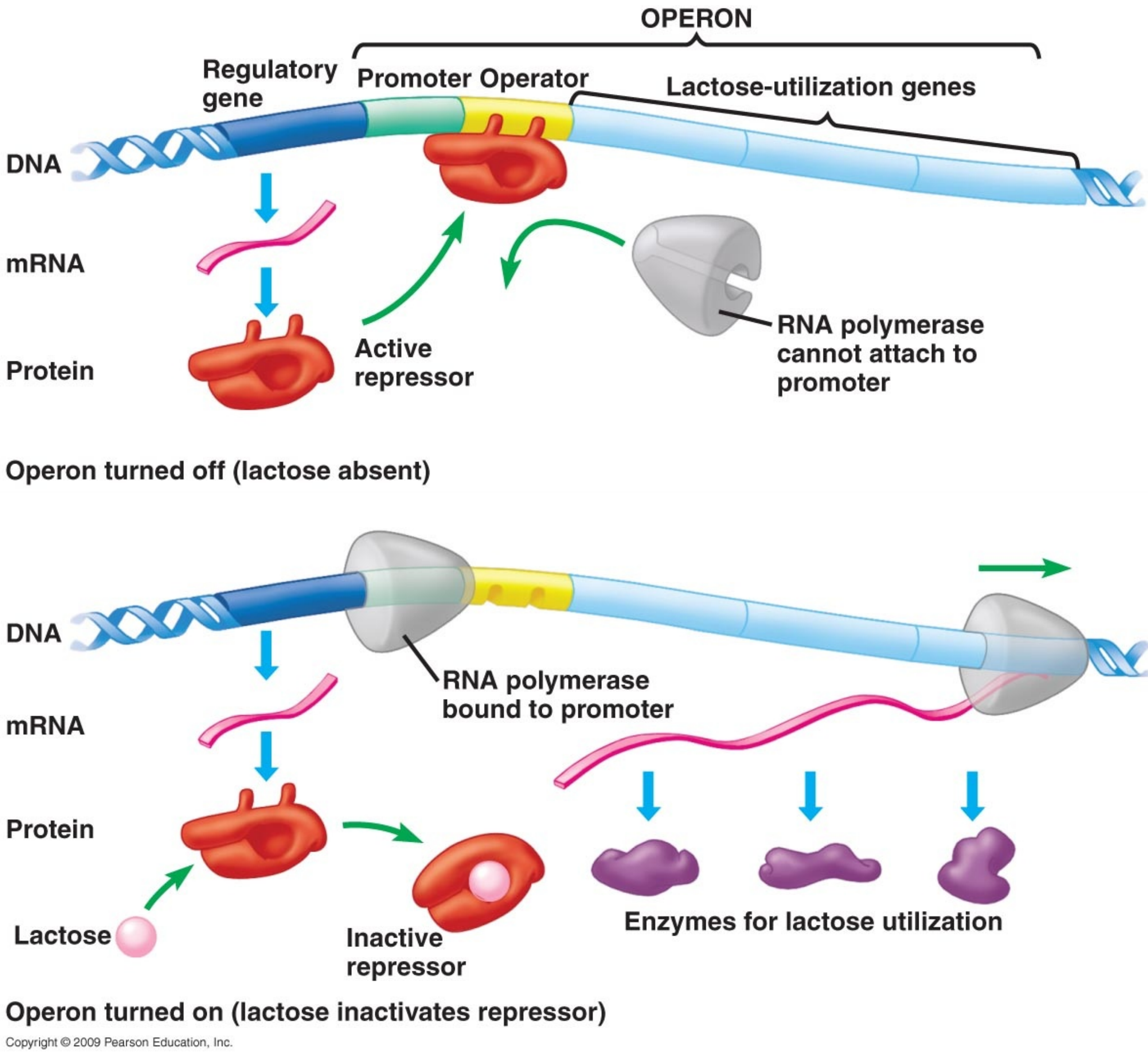
2. The tryptophane operon (attenuation)

3. The mercury resistance operon (allosteric activation)

4. Anti-activation/Antiattivazione (araBAD operon) Allosteric mechansim

5. Lambda Phage
--different levels of regulation—
Lytic and lysigenic life cycle

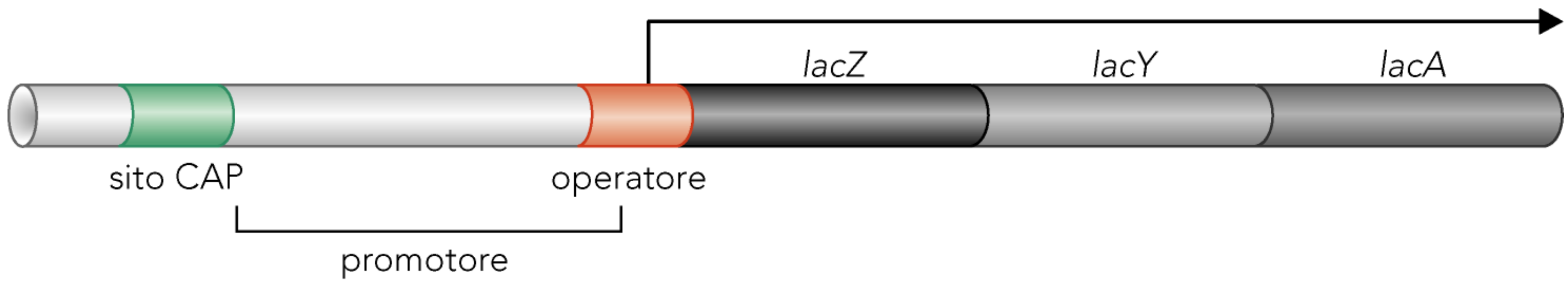
1. The Lactose Operon:



+ GLUCOSE
- LACTOSE

-GLUCOSE
+ LACTOSE

Lac – operon



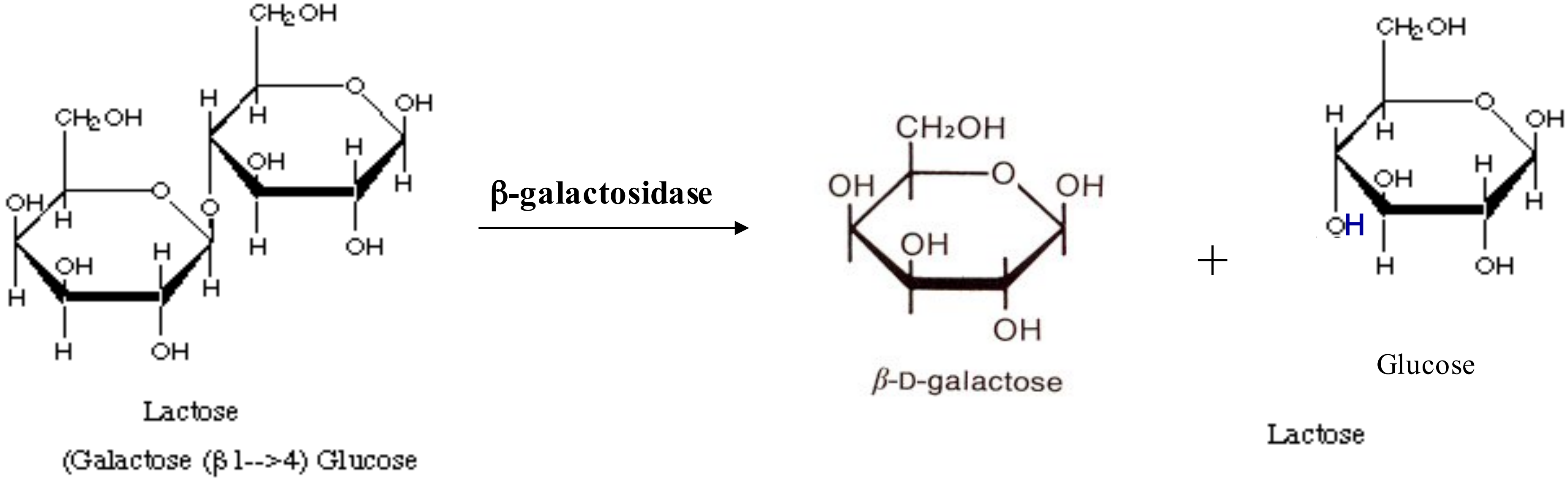
Lac – Operon:

3 genes encoding a ploycistronic mRNA that is translated into 3 proteins:
→ lacZ: beta galactosidase: cleaves lactose
→ lacY: lactose permease: transmembrane protein; imports lactose
→ lacA: thiogalactoside transacetylase – eliminates toxic thiogalactoside that are also imported by lacY

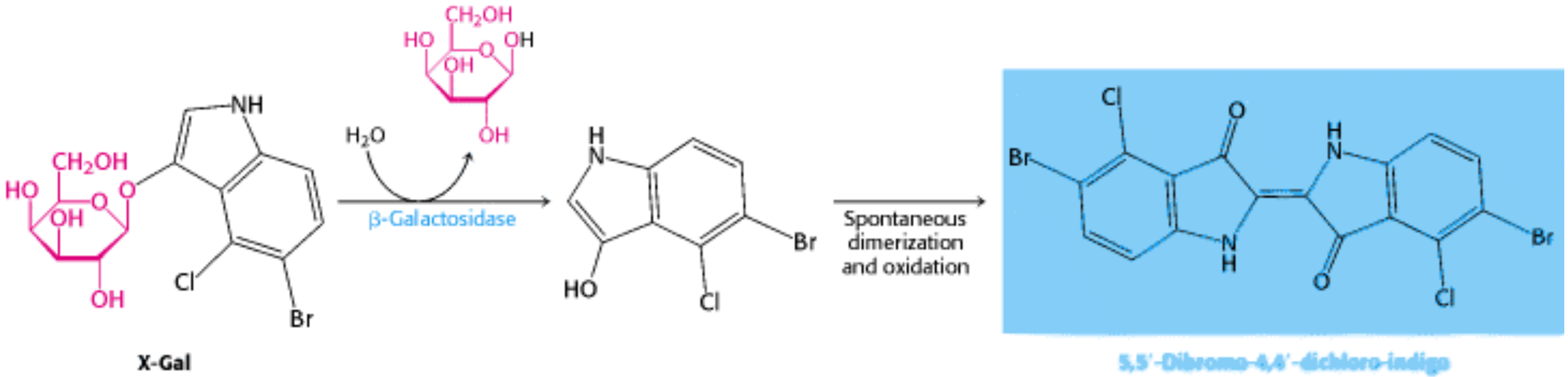
Lac Operon Repressor:
LacI: gene **located in vicnity to the Lac operon** that encodes the repressor for the lac-operon

Promoter:
Consists of core-promoter, operator and CAP site (**catabolite activator proteins binding site**)
CAP binds to CAP site when high cAMP levles (=low enery status!!)

Chemistry



How to measure beta-galactodsidase levels

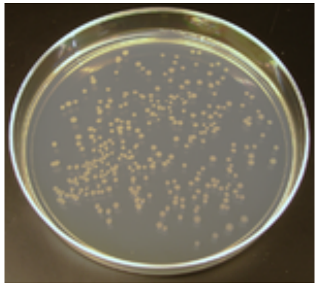


Following the β-Galactosidase Reaction. The galactoside substrate X-Gal produces a colored product on cleavage by β-galactosidase. The appearance of this colored product provides a convenient means for monitoring the amount of the enzyme both in vitro and in vivo.

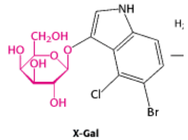
Combining chemistry with genetics

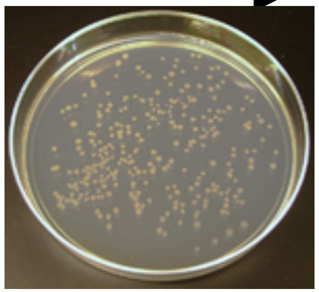
E.coli grows on petri-dishes " with agar ,containing sources of energy

glucosio	lattosio
+	+
+	−
−	+

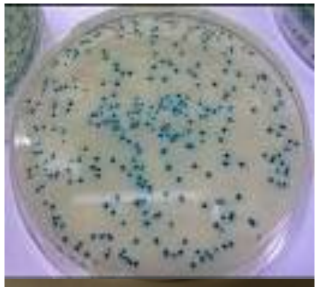


low beta-galactosidase activity Lac operon "OFF"

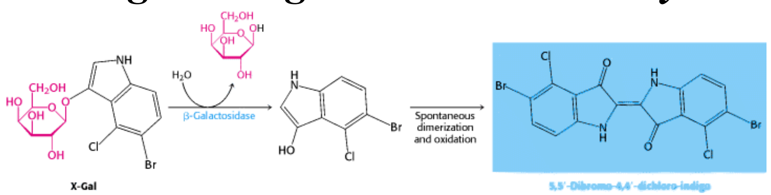


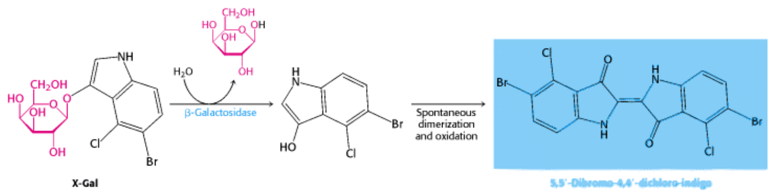


no beta-galactosidase activity Lac operon "OFF"



high beta-galactosidase activity = Lac operon "ON"

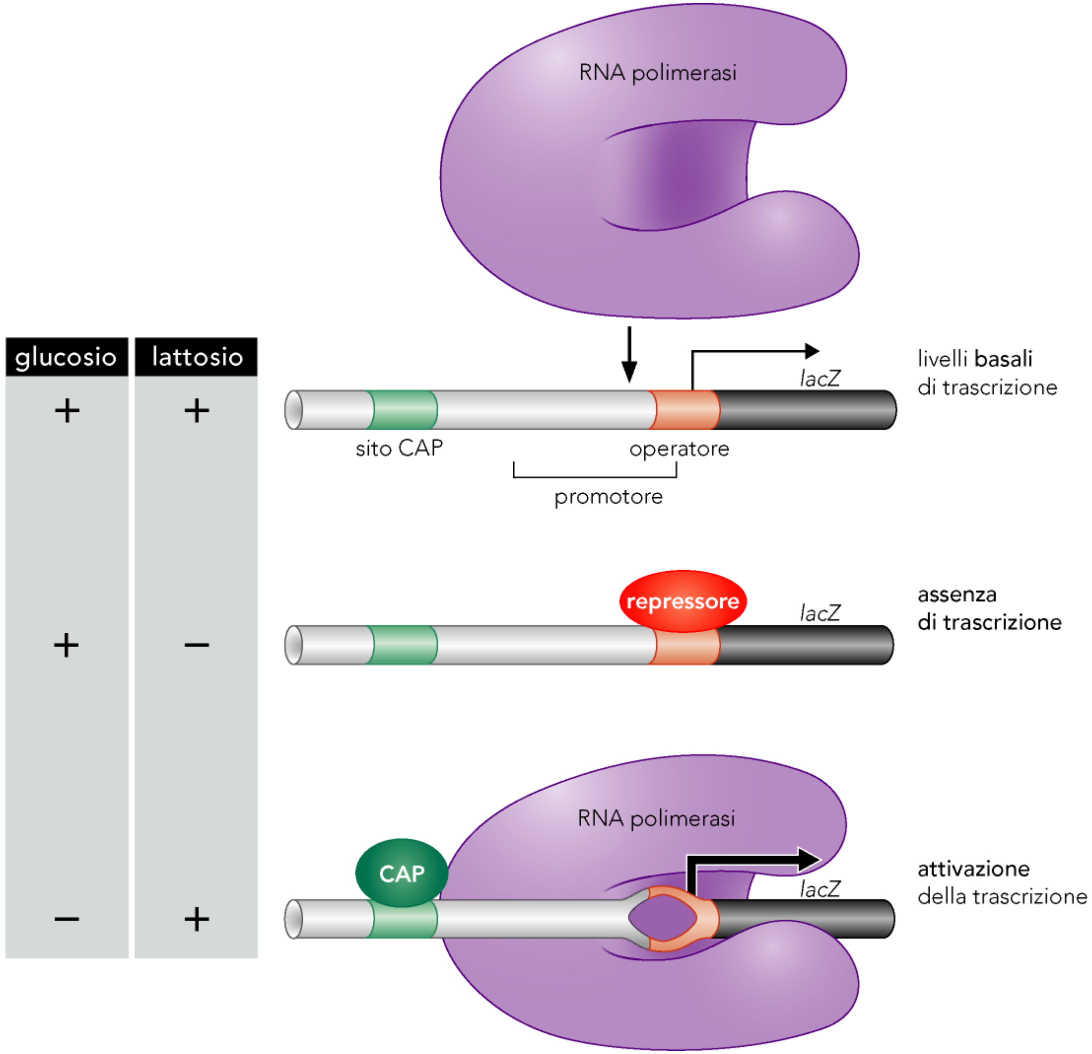




Plates are "painted" with X-Gal (transparent)

+X-Gal

Regulation of the lac operon



Lac promoter has inefficient -35 box and lacks UP element: requires additional protein – CAP - to activate transcription

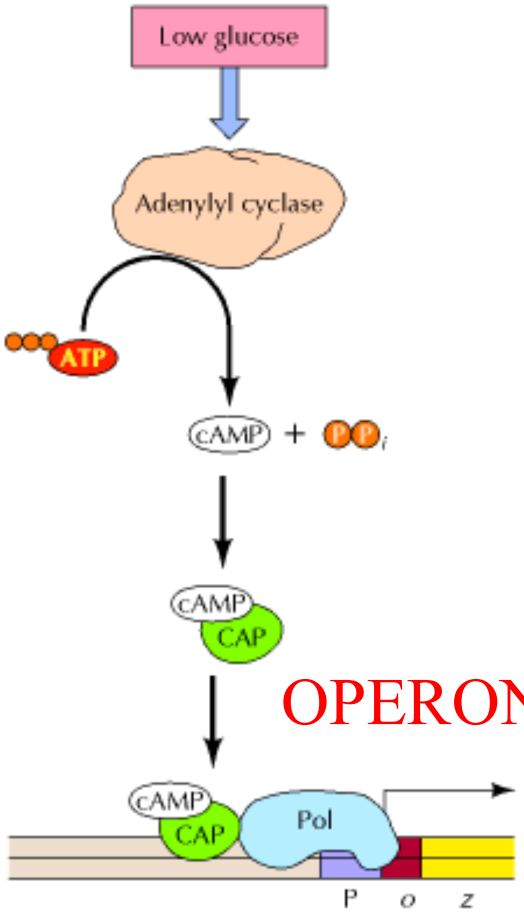
Glucose is preferred source of energy; low lac-operon transcription

No lactose: lac repressor binds to operator and represses transcription

No glucose: CAP brings RNA Pol to Promoter → activation of lacZ, lacY and lacA Expression

Regulation CAP and repressor proteins by cAMP and allolactose

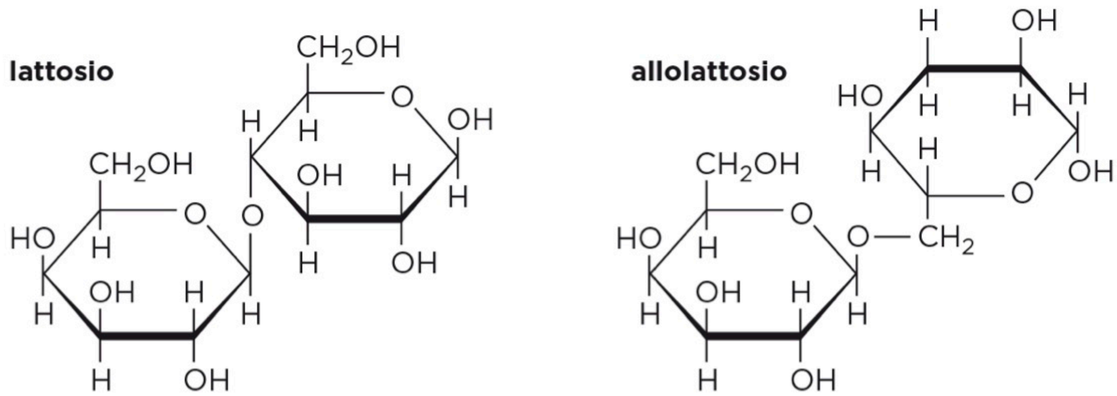
CAP - cAMP



OPERON:ON

Positive control of the *lac* operon by low glucose
Low levels of glucose activate adenylyl cyclase, which converts ATP to cyclic AMP (cAMP). Cyclic AMP then binds to the catabolite activator protein (CAP) and stimulates its binding to regulatory sequences of various operons concerned with the metabolism of alternative sugars, such as lactose..

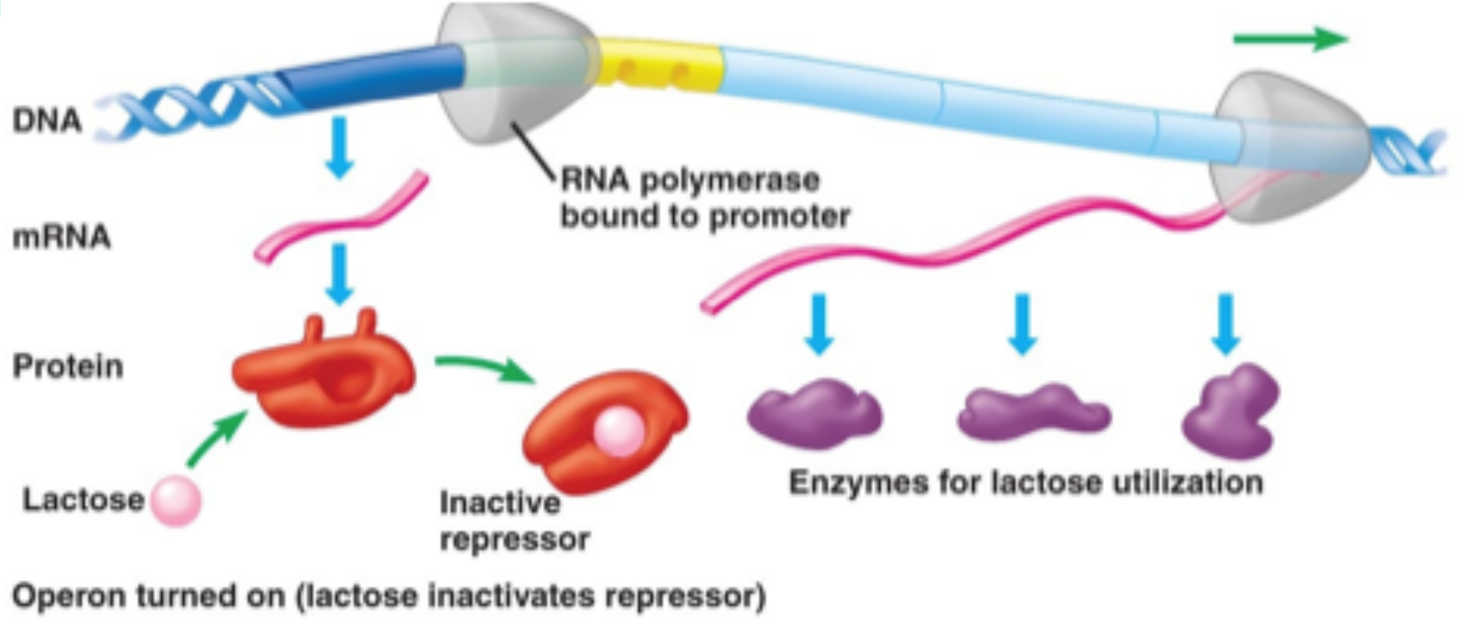
Repressor - allolactose

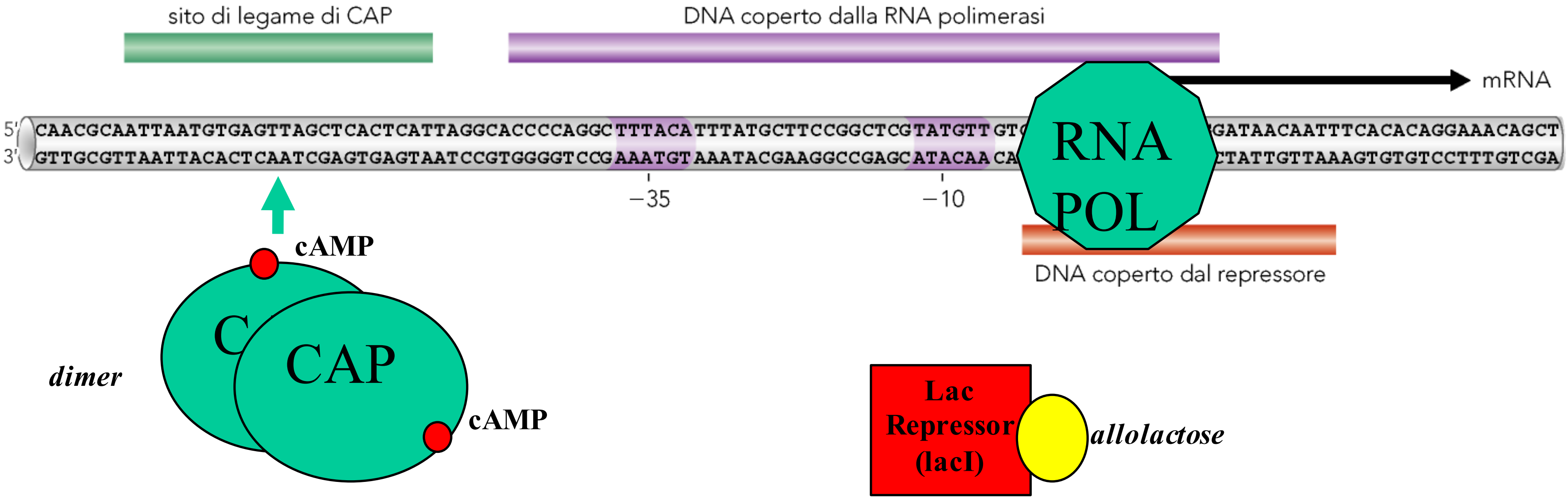


OPERON:ON

Negative control by the *lac repressor inhibited by allolactose*
actose is converted to allolactose upon entty of lactose into bacterium. Allolactose binds the lac-repressor. Allolactose-repressor cannot bind operon.

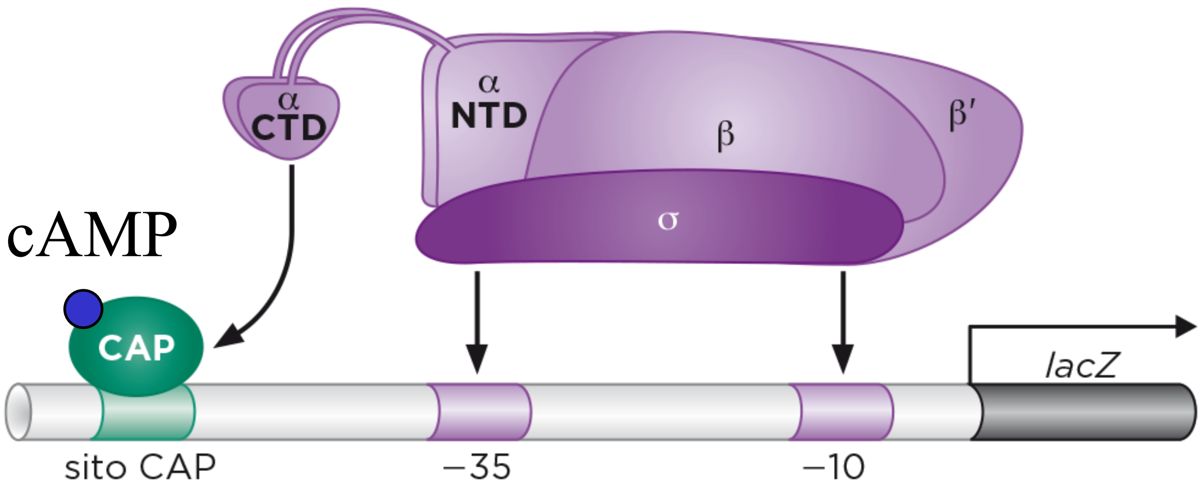
Regulation of the lac operon: PRESENCE OF LACTOSE LOW GLUCOSE



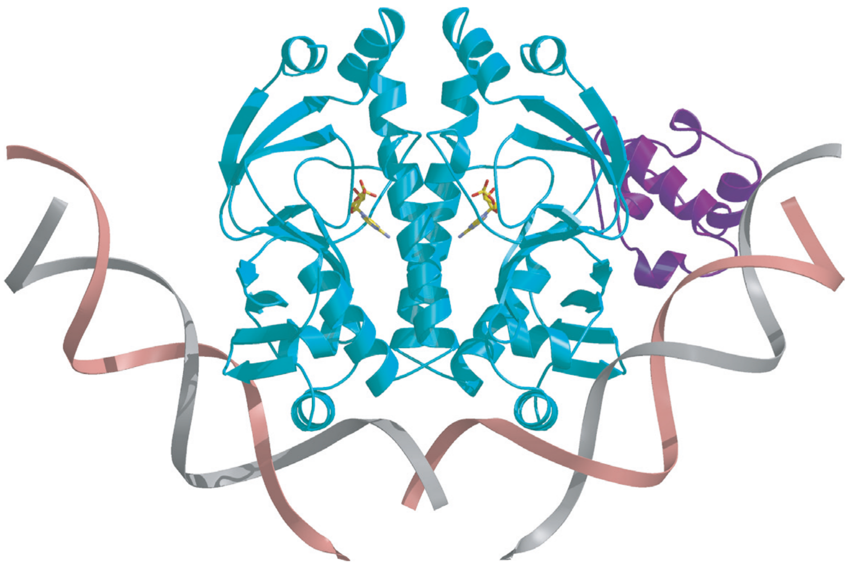


Note: CAP regola vari altri geni, tra cui cui quelli dell’operone Galattosio.

Regulation of the lac operon: PRESENCE OF LACTOSE - LOW GLUCOSE

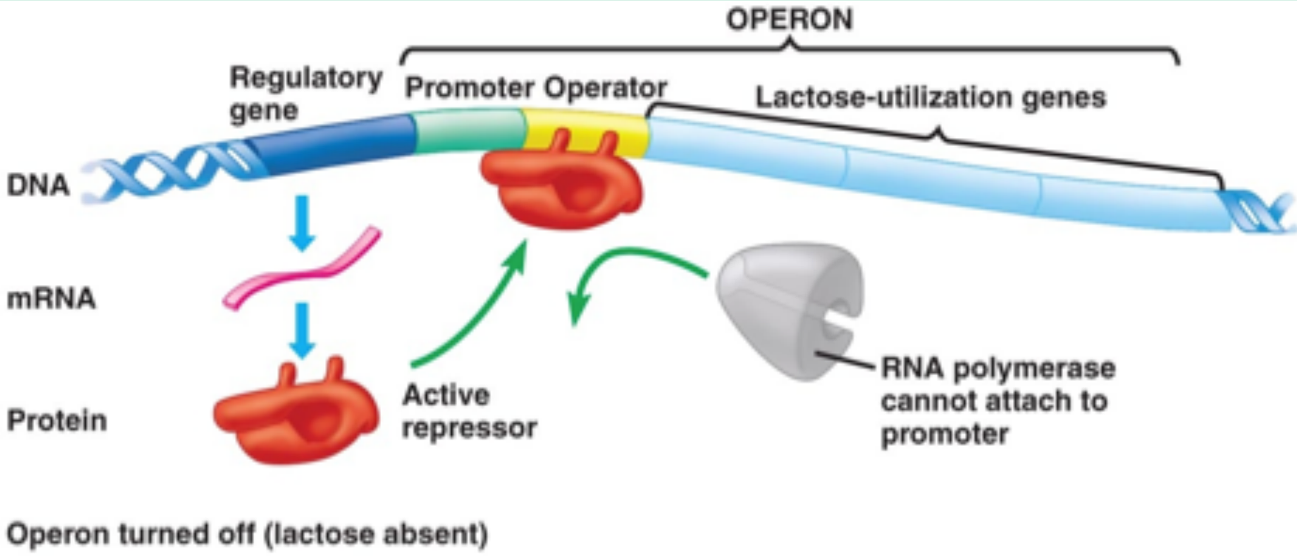


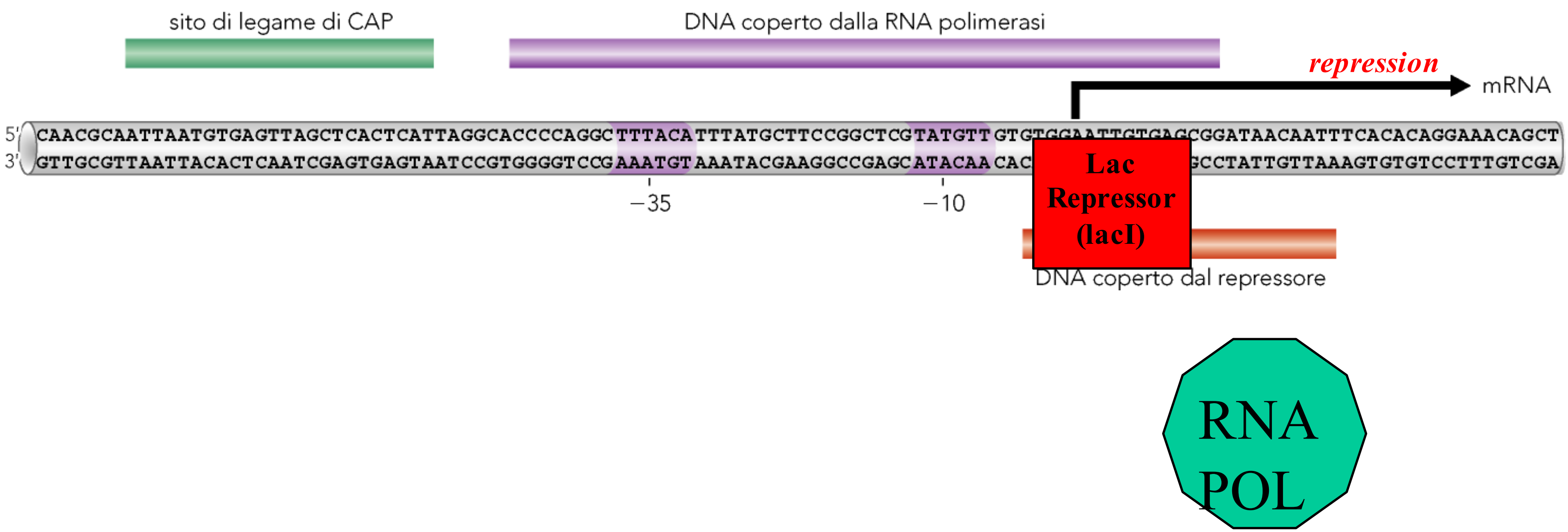
alphaCTD interacts specifically interacts with CAP
→Required for efficient Pol recruitment



CAP dimer+cAMP With alpha CTD; DNA bended

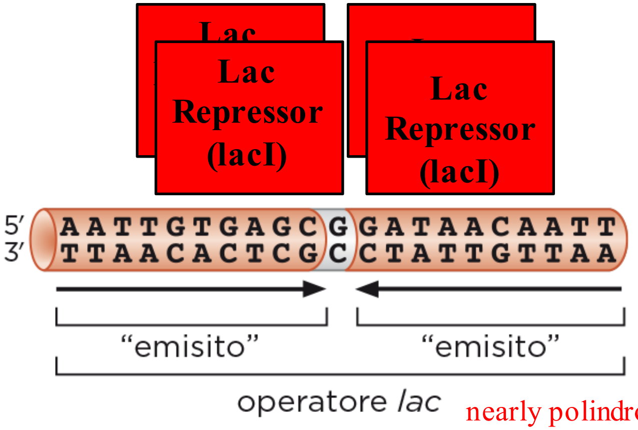
Regulation of the lac operon: ABSENCE OF LACTOSE PRESENCE OF GLUCOSE



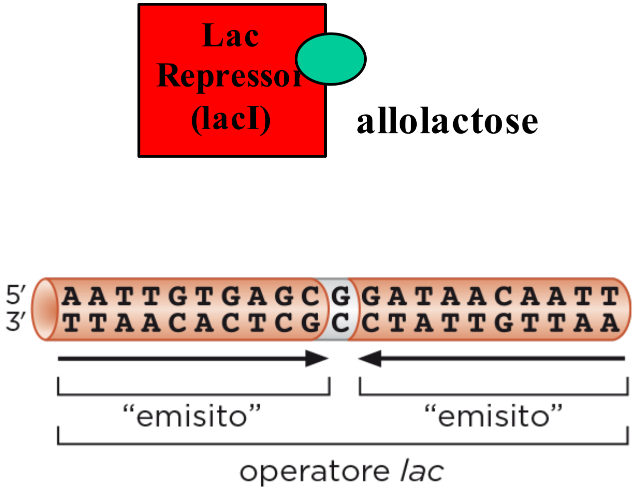


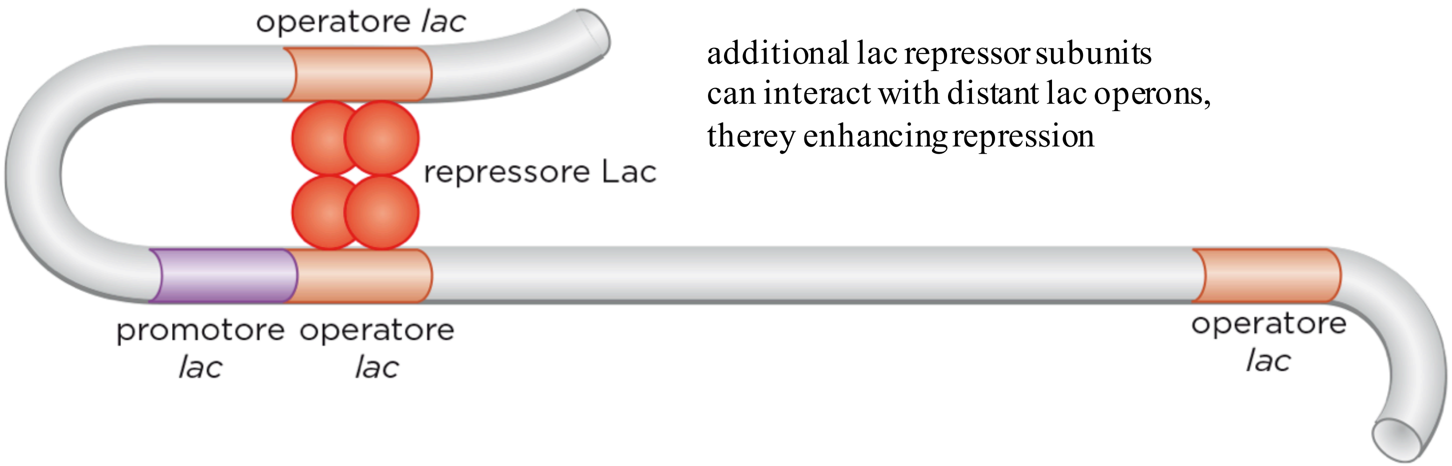
Regulation of the lac operon: ABSENCE OF LACTOSE PRESENCE OF GLUCOSE

2 sub-units bind DNA
2 sub-units do not have contact to operator



nearly polindromic





additional lac repressor subunits can interact with distant lac operons, therey enhancing repression

Duplice controllo dell'operon di lac

cAMP activator	Allolactose repressor
Low "OFF"	High "OFF"
Low "OFF"	Low "ON"
High "ON"	Low "ON"
High "ON"	High "OFF"

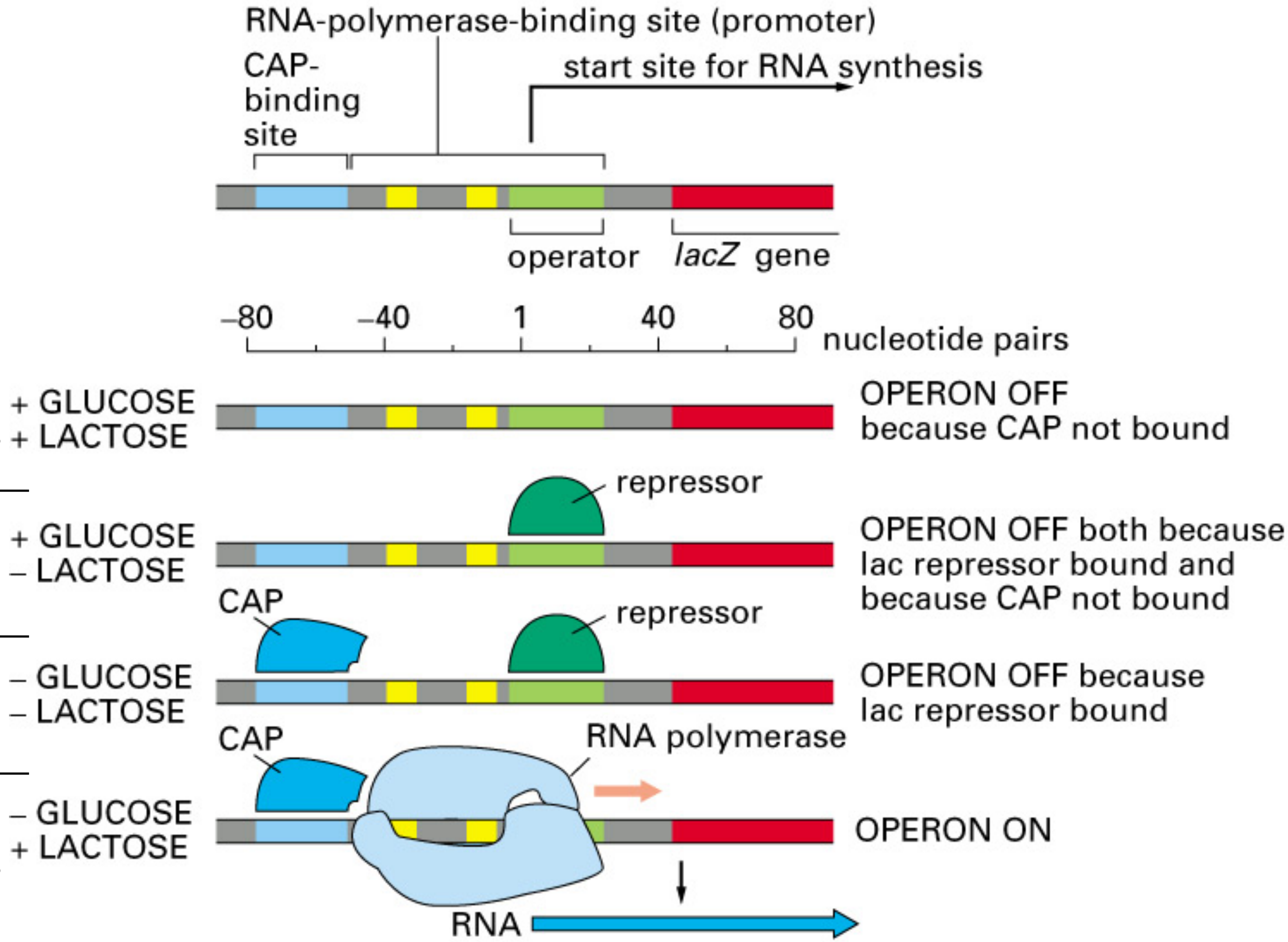
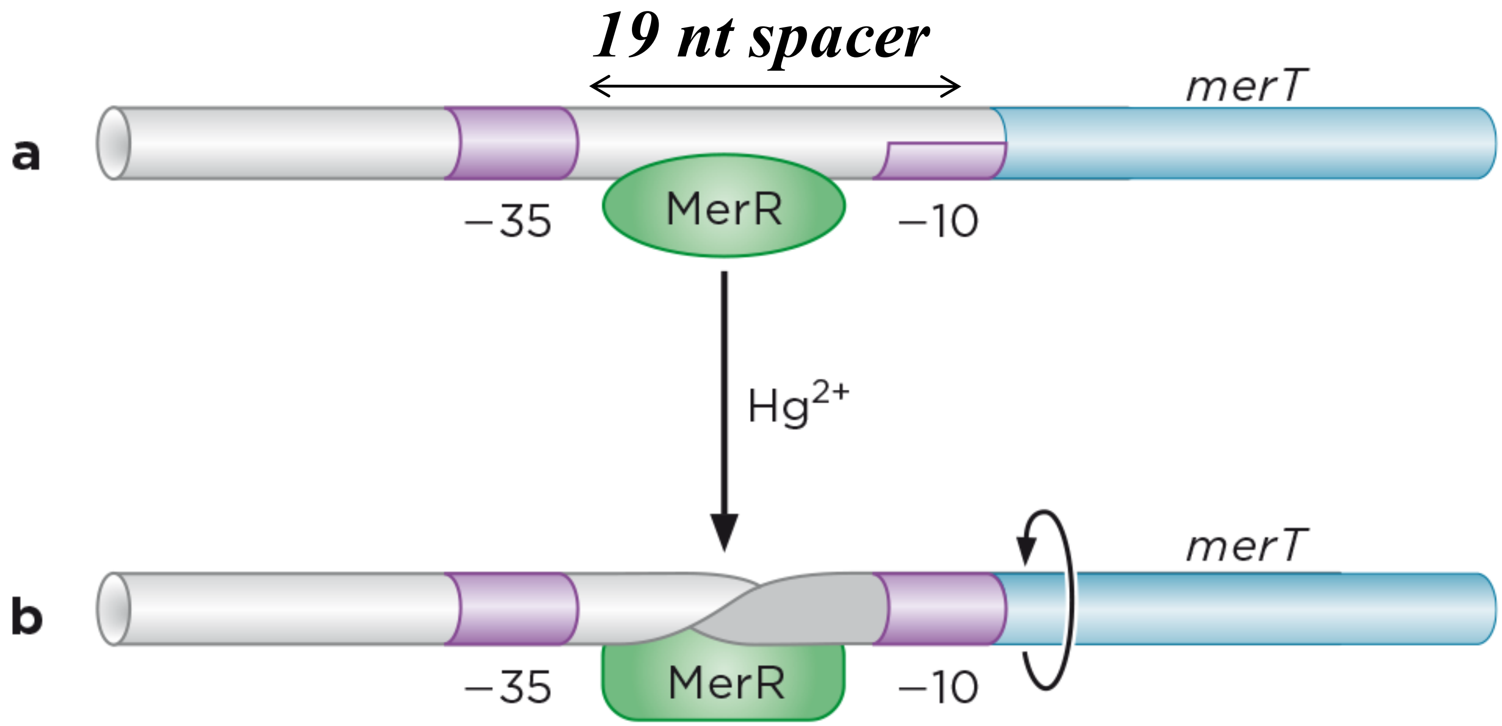


Figure 7–38. Molecular Biology of the Cell, 4th Edition.

3. Allosteric activation of MerR

MerR: Bind to the operator when mercurio is absent

MerR + Hg^{2+}: activates the **Mer operon** to mediate resistance to mercurio/mercury (converted into less toxic form)



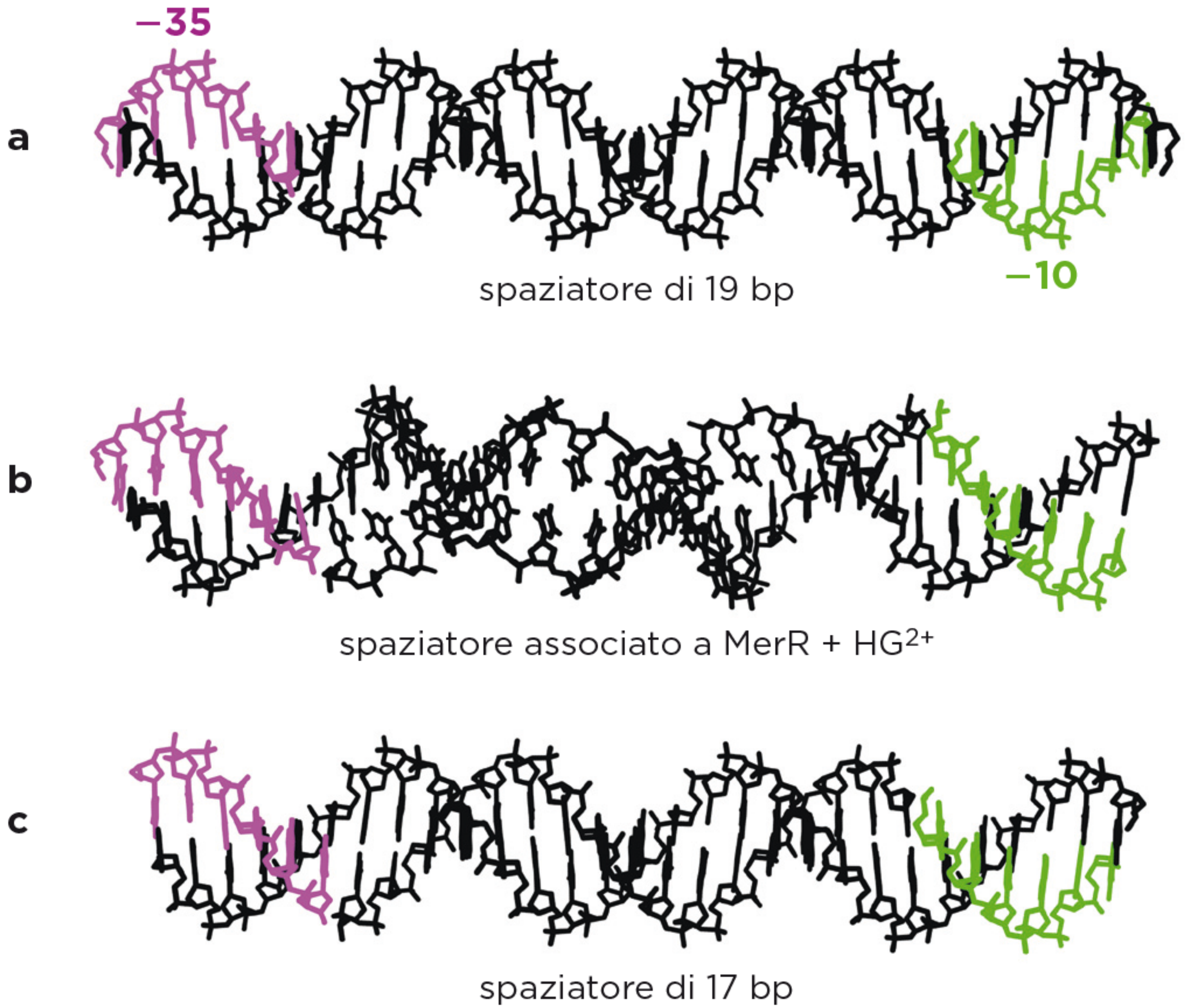
Mercuric ions are transported outside the cell by a series of transporter proteins. This mechanism involves the binding of Hg^{2+} by a pair of cysteine residues on the MerP protein located in the periplasm. Hg^{2+} is then transferred to a pair of cysteine residues on MerT, a cytoplasmic membrane protein, and finally to a cysteine pair at the active site of MerA (mercuric reductase). Next, Hg^{2+} is reduced to Hg^0 in an NADPH-dependent reaction. The non-toxic Hg^0 is then released into the cytoplasm and volatilizes from the cell.

Allosteric activation of MerR

MerR bound to promoter region. +19bp spacer renders Promoter inefficient. RNA pol can bind, but initiation is not efficient!! -10 box -35 box not positioned well.

MerR bound to Hg2+ causes conformational change of MerR. Twisting of DNA to mimic an ideal +17bp spacer
Activation of Trp Sigma factor efficiently binds promoter boxes

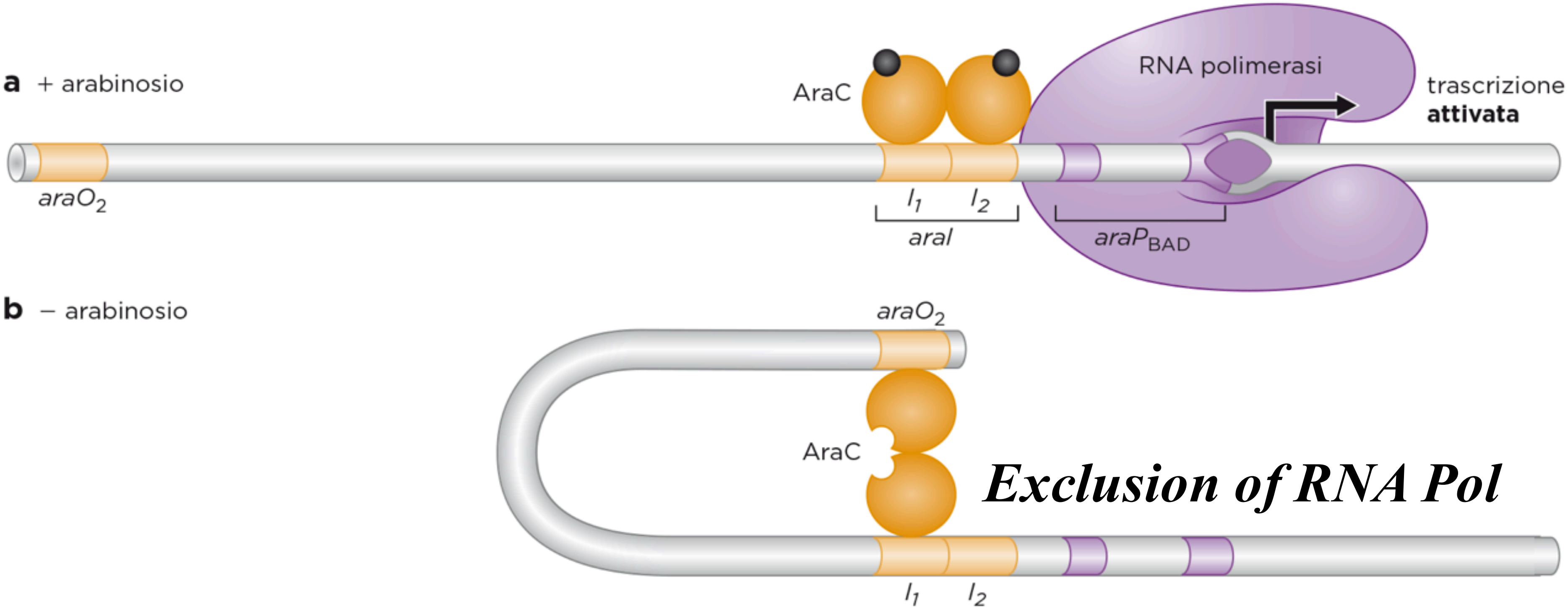
Normal promoter; -10 and -35 boxes faces to RNA Pol/sigma



Allosteric activation: interaction with metabolite causes activation of protein or enzyme

4. Anti-activation/Anti-attivazione represses the Ara Operon

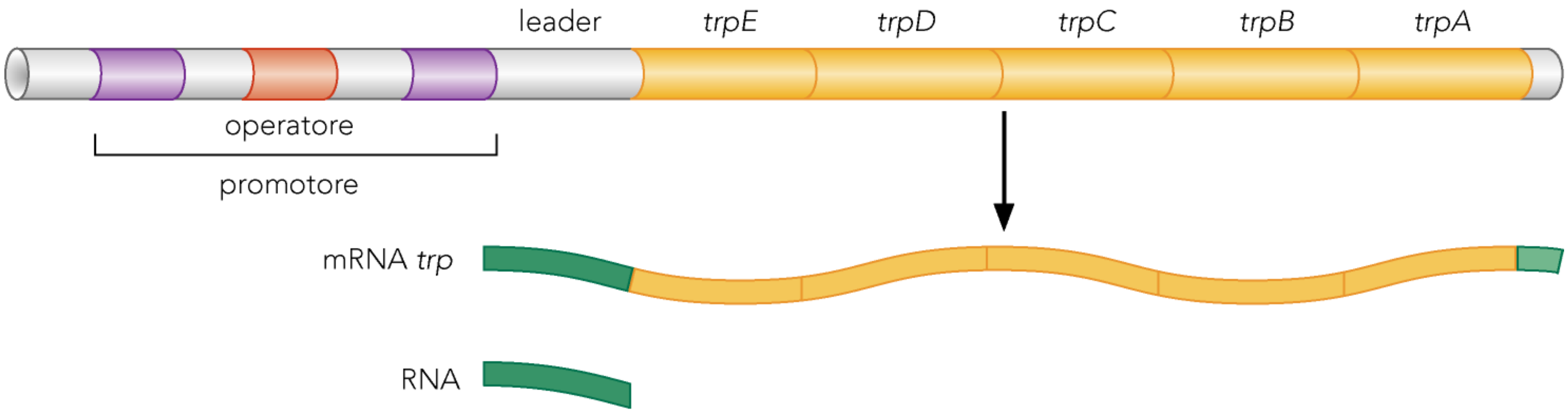
araBAD Operon: metabolism of arabinose; ON when arabinose is present and glucose is absent



Allosteric mechanism

Usage in laboratry: inducible gene expression system

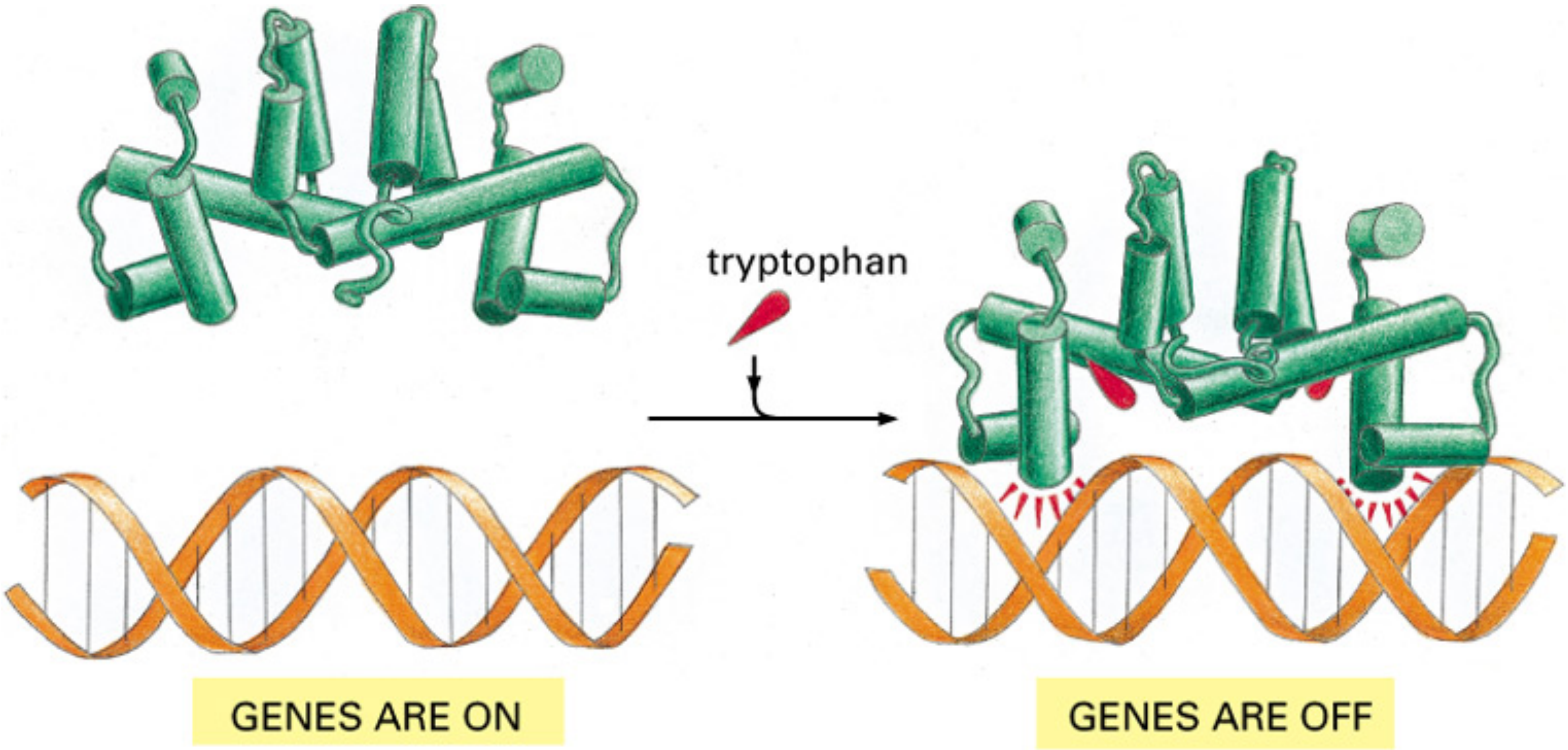
2. The tryptophane operon



Tryptophane operon encodes a policistronic mRNA that gives rise to 5 proteins for the synthesis of tryptophane (triptofano)

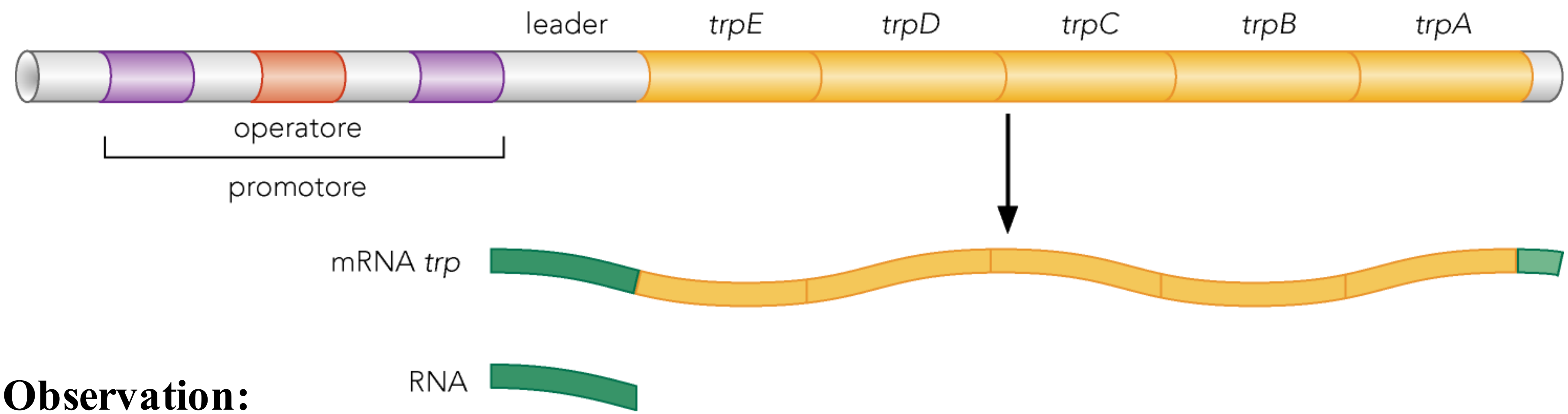
Operon is regulated by 2 main repressive mechanisms:
→Repression: repressor-tryptophane complex binds to operator
→ Attenuation: premature termination of transcript; acts on transcription and translation

Tryptophane binds to repressor



High tryptophane levels → complex formation → repressor binds to operator → Exclusion of RNA pol from promoter of Trp Operon

La attenuazione

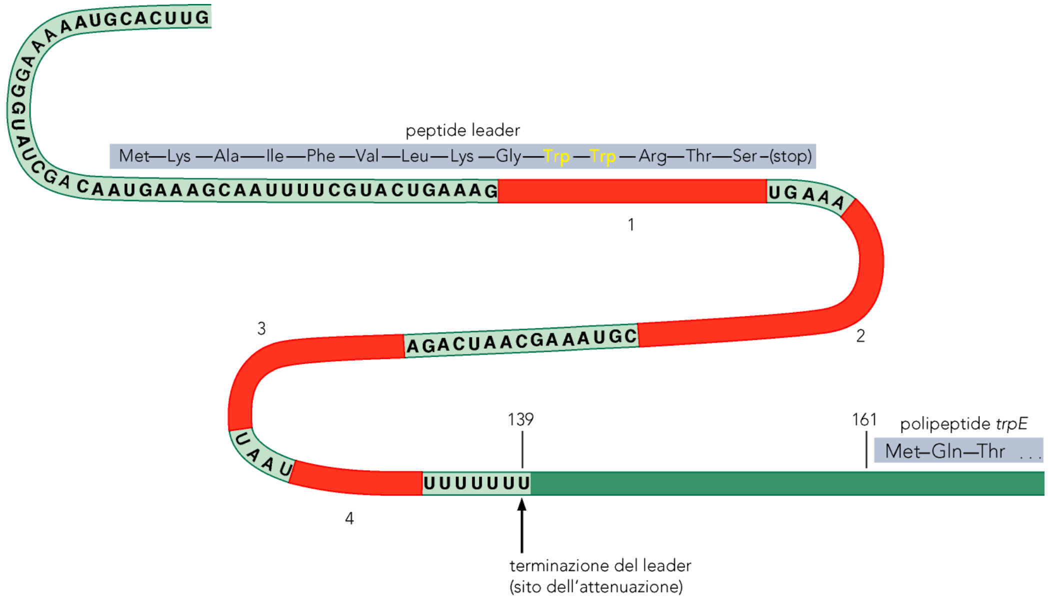


Observation:

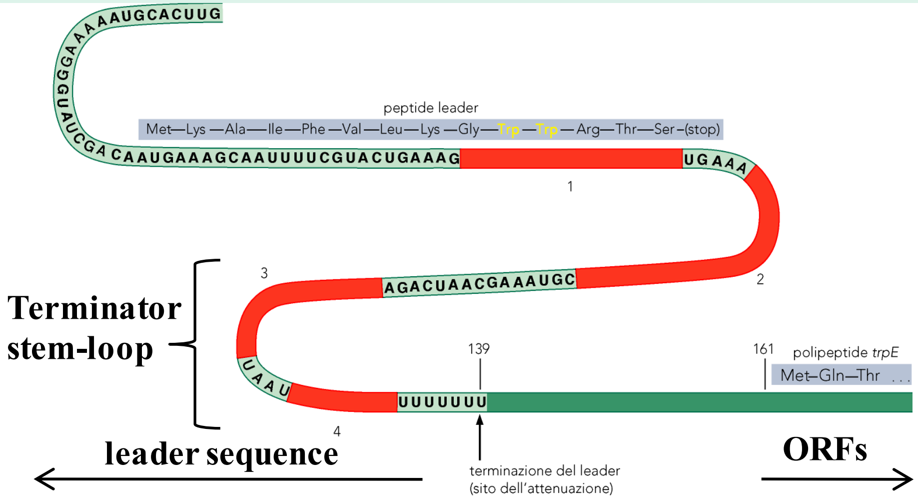
Under high Trp levels:
only short mRNA detectable→ Termination of transcription upstream of trpE

Under low Trp levels:
expression of full-length Trp

Deletion experiments: deletions in defined location inside the mRNA result in the usage of the normal transcription temination signal under high Trp growht conditions
MECHANISM???



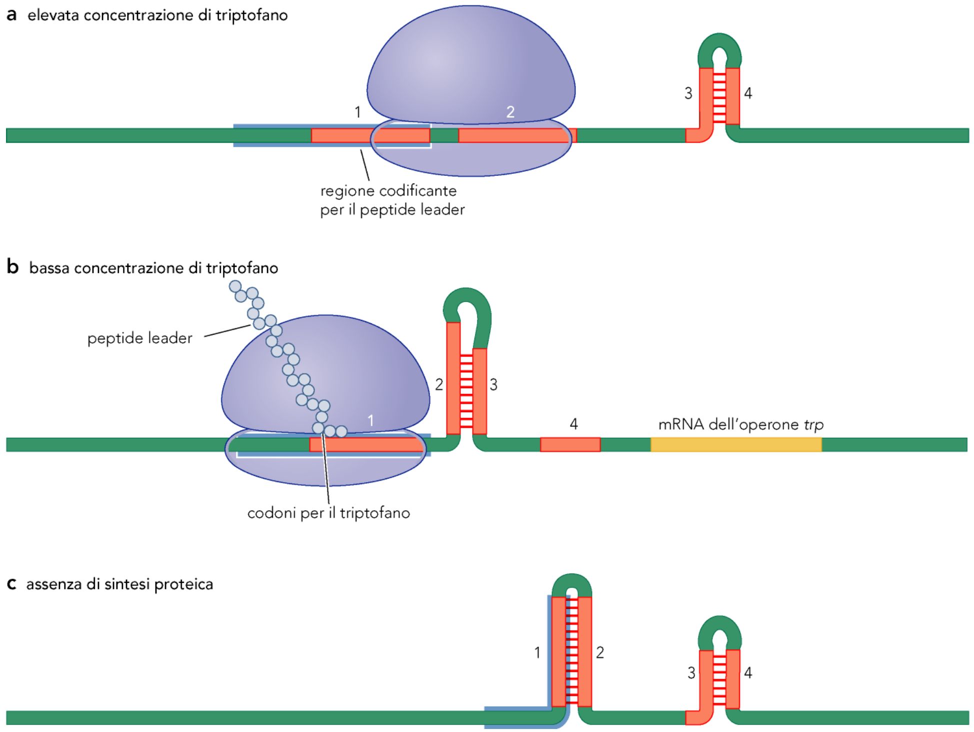
La attenuazione



sequence 1 + 2 can form stem loop

sequence 2 + 3 can form stem loop

sequence 3 + 4 can form stem loop



High Trp:
TrpTrp codons in leader are easily translated by ribosome. Fast passage of ribosome prevents seq 1+2 stem loop. This Allows the formation **of seq3+4 terminator stem loop** in the 3'region of the leader sequence. TERMINATION of transcription upstream of ORFs (**Rho independent**)

Low Trp:
TrpTrp codons in leader are not efficiently translated. Ribosome is slowed down, therefore allowing the formation of seq2+3 stem loop. In this manner seq3+4 stem terminator loop cannot fold. Transcription runs until 3'end of Trp operon mRNA.

Absence of protein synthesis:
No usage of Trp leader by Ribosome. Stem loop seq 1+2 and stem loop Seq3+4 form. Termination of transcription at stem loop Seq 3+4

Attenuation via leader peptides in Thr, Phe, His operons

(A)
Thr Operon

Met - Lys - Arg - Ile - Ser - Thr - Thr - Ile - Thr - Thr - Thr - Ile - Thr - Ile - Thr - Thr -

5′ AUG AAA CGC AUU AGC ACC ACC AUU ACC ACC ACC AUC ACC AUU ACC ACA 3′

(B)
Phe Operon

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

5′ AUG AAA CAC AUA CCG UUU UUC UUC GCA UUC UUU UUU ACC UUC CCC UGA 3′

(C)
His Operon

Met - Thr - Arg - Val - Gln - Phe - Lys - His - His - His - His - His - His - His - Pro - Asp -

5′ AUG ACA CGC GUU CAA UUU AAA CAC CAC CAU CAU CAC CAU CAU CCU GAC 3′

Figure 31-35
Biochemistry, Sixth Edition
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Thr: Threonine
Phe_ Phenylalanine
His: Histidine

Hallmark models for gene regulation in procaryotes

1. The Lactose Operon – Lac Operon (Pol recruitment)

2. The tryptophane operon (attenuation)

3. The mercury resistance operon (allosteric activation)

4. Anti-activation/Antiattivazione (araBAD operon) Allosteric mechansim

5. Lambda Phage
--different levels of regualtion—
Lytic and lysigenic life cycle