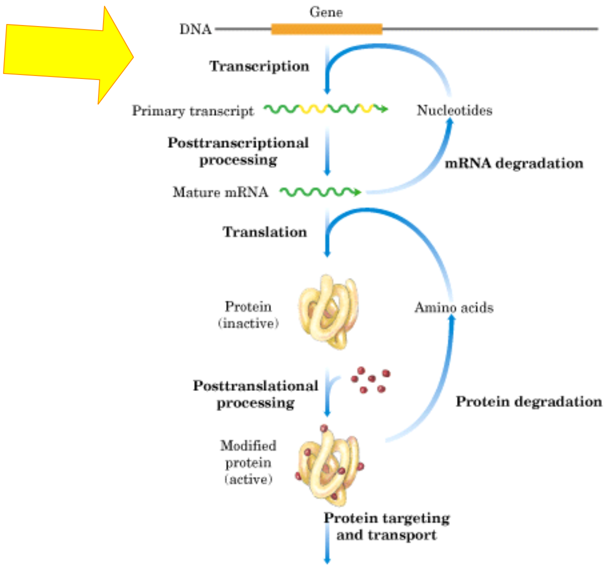
regulación de la transcripción

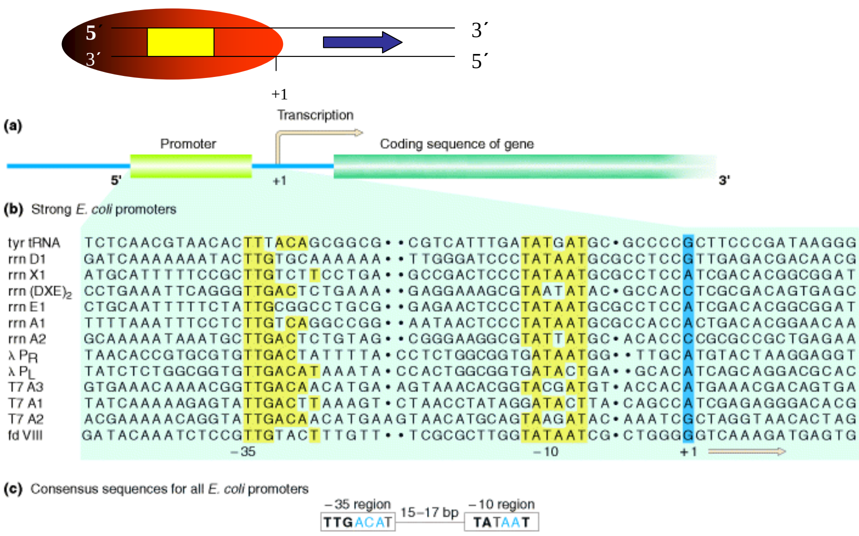
Dr. Víctor Romanowski, 2009



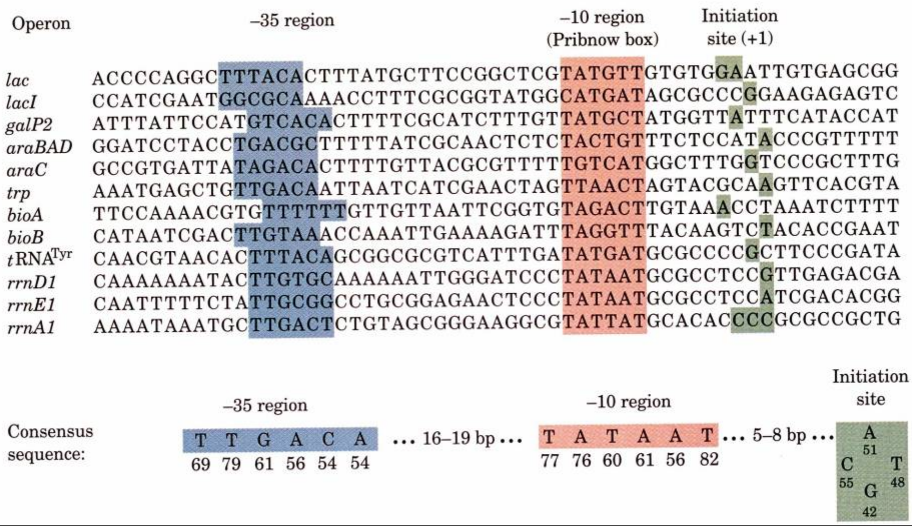
regulación de la transcripción en procariotas

Promotores

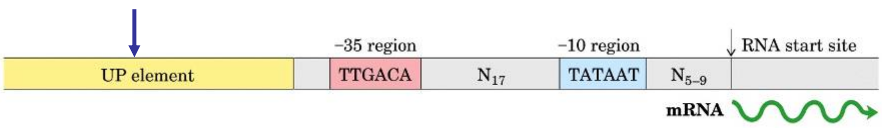
Secuencias de DNA que posicionan a la RNA polimerasa (RNAP) en el sitio de iniciación



La RNAP reconoce dos secuencias principales en los promotores bacterianos



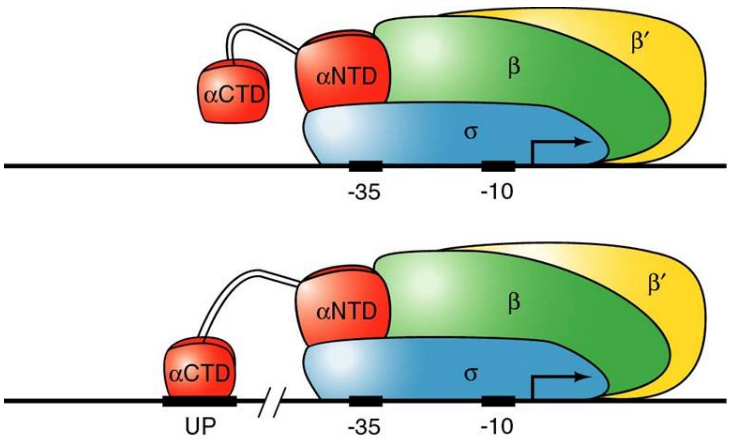
Some very highly expressed bacterial promoters contain a third RNAP recognition sequence



-35 and -10 regions are bound by the **σ** subunit of RNAP.

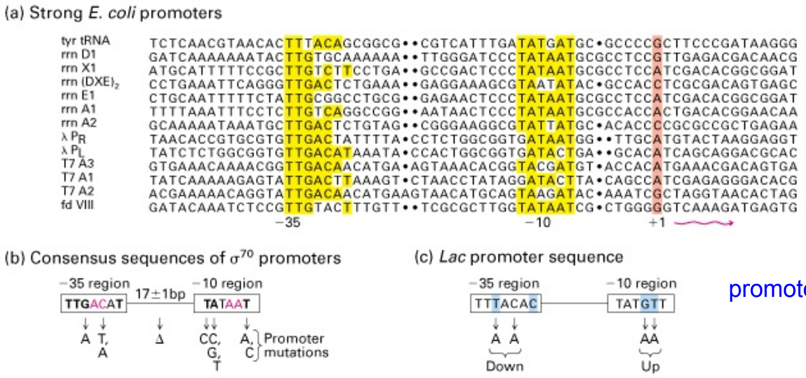
UP element, located between positions -40 and -60, is bound by the **α** subunit of RNAP.

EL dominio C terminal (CTD) de la **subunidad alfa de la RNA** polimerasa **interactúa** con el **elemento UP** del promotor



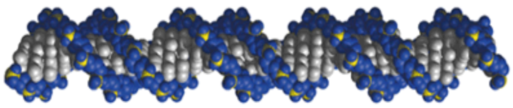
"fuerza del promotor"

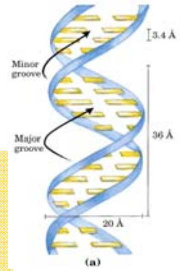
Las diferencias de las secuencias de los promotores de *E. coli* afectan la frecuencia de iniciación de la transcripción



promotor débil

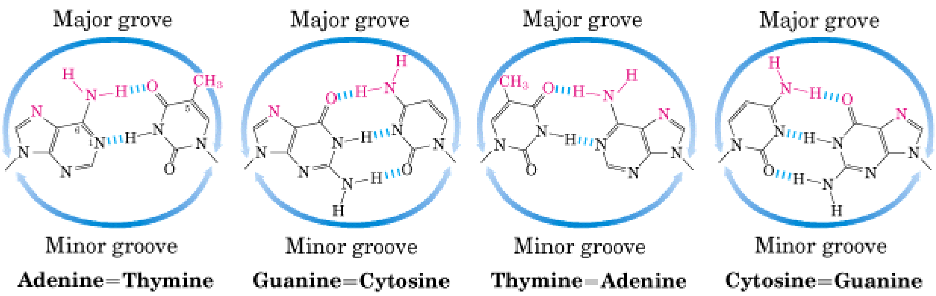
efectos de algunas mutaciones

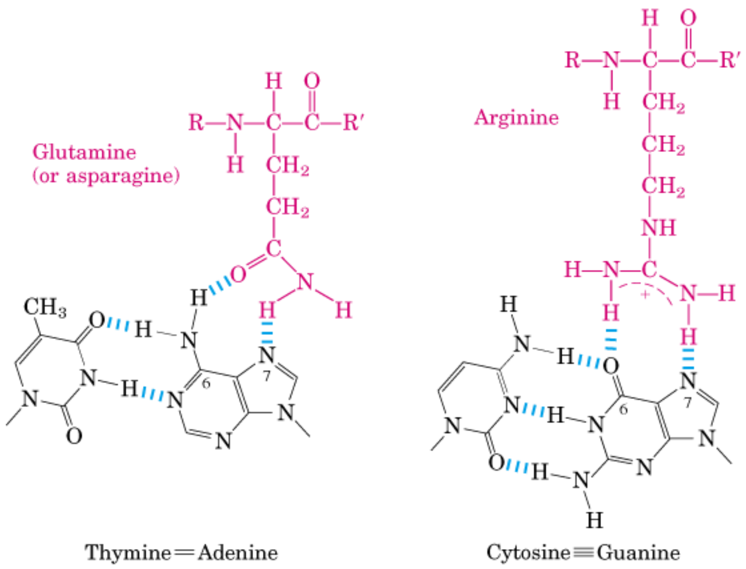




Regulación de la transcripción de genes:

interacción DNA (surco mayor) - proteínas





Regulación de la transcripción en procariotas

- No todos los genes se expresan igual
- El nivel de expresión es regulado por señales ambientales
- La transcripción es regulada por elementos en el DNA y proteínas que interactúan con ellos
- Típicamente se regula la etapa de iniciación

Three types of transcriptional regulatory proteins

1. Sigma factors (positive control)
   Alter specificity of RNAP for a given promoter or set of promoters

2. Repressors (negative control)
   Impede access of RNAP to promoter

3. Activators (positive control)
   Enhance RNAP-promoter interaction

Tipos de proteínas que regulan la transcripción

1. Factores Sigma (control positivo)
   Cambian la especificidad de la RNApol por los promotores
2. Represores (control negativo)
   Impide el acceso de la RNApol al promotor
3. Activadores (control positivo)
   Favorece la interacción RNApol-promotor

The σ *subunit* contributes to specific initiation

It decreases the affinity of RNA polymerase for *general regions* of DNA by a factor of 10^4

Enables *RNA polymerase to recognize promoter sites*

this helix has been implicated in recognizing the TATAAT sequence of the -10 region

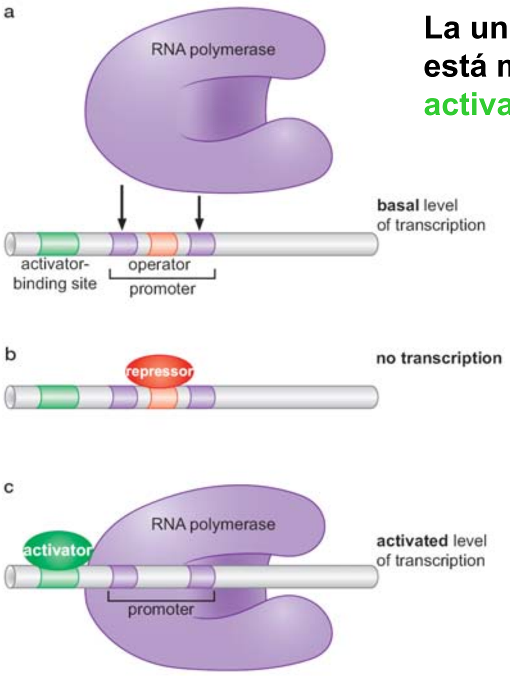
the promoter site is encountered by a ***random walk in one dimension*** *rather than in three dimensions*.

The **σ** subunit is released when the nascent RNA chain reaches nine or ten nucleotides in length. After its release, it can assist initiation by another core enzyme.

Thus, the **σ** *subunit acts catalytically*.

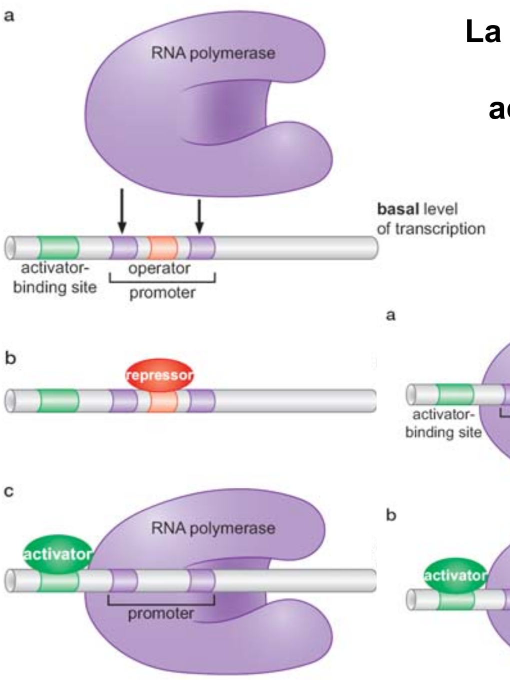
σ^{70}	**Most genes**	TTGACAT	TATAAT
σ^{32}	**Genes induced by heat shock**	TCTCNCCCTTGAA	CCCCATNTA
σ^{28}	**Genes for motility and chemotaxis**	CTAAA	CCGATAT
σ^{38}	**Genes for stationary phase and stress response**	?	?
σ^{54}	**Genes for nitrogen metabolism and other functions**	CTGGNA	TTGCA

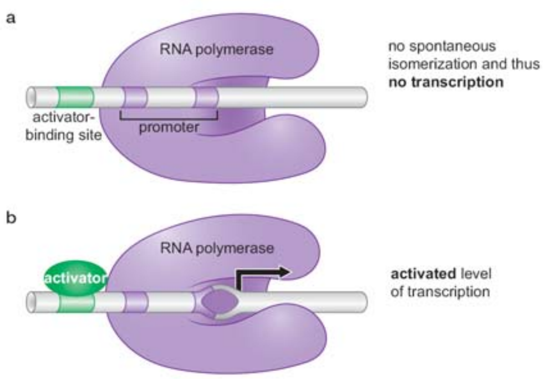
La unión de la RNAP está modulada por proteínas activadoras y represoras



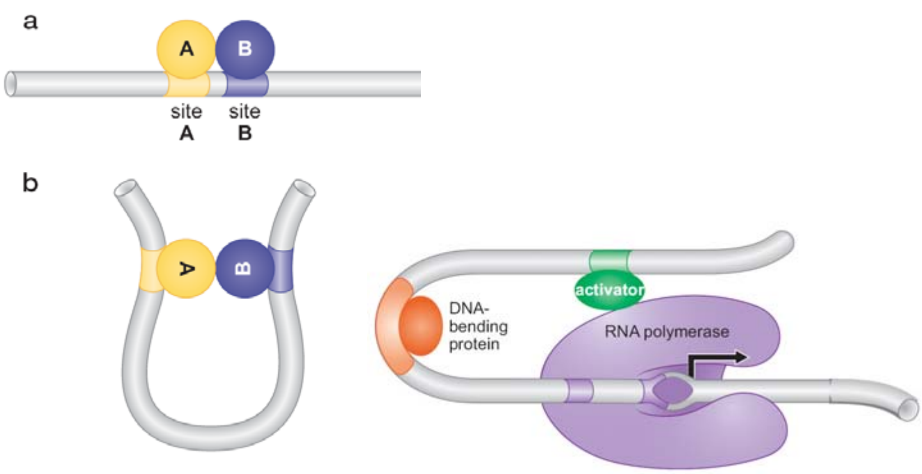
Reclutamiento
Cambio alostérico

La unión está modulada por proteínas activadoras y represoras

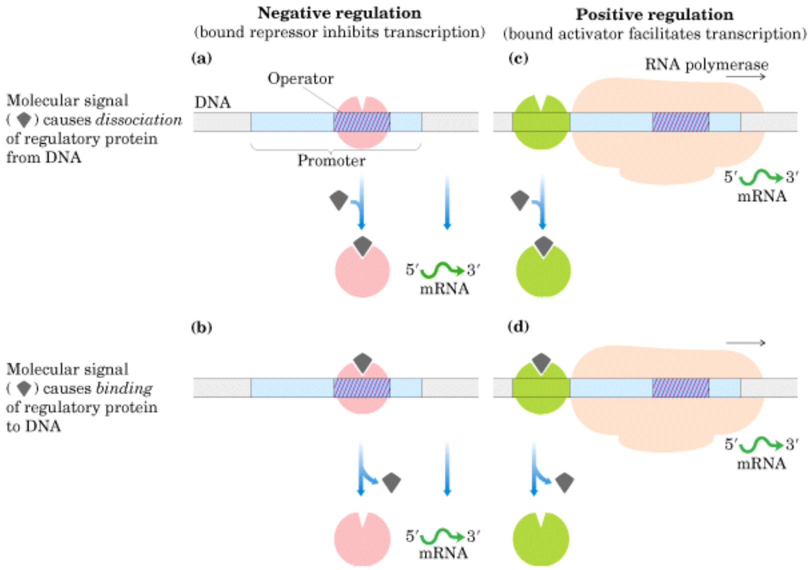




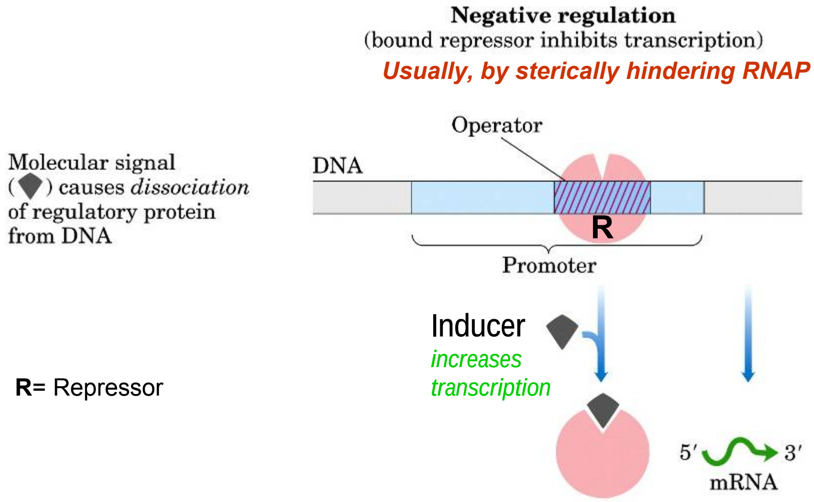
Acción a distancia



Regulación negativa y positiva

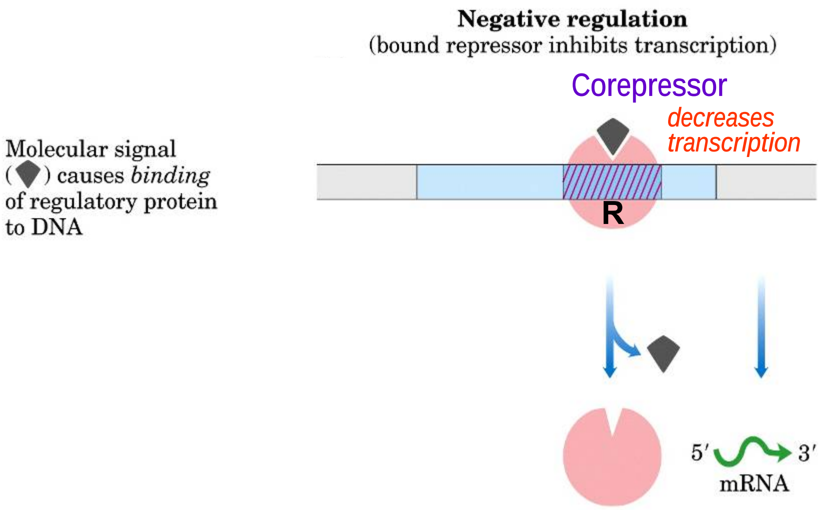


Role of repressors at inducible promoters



*

Role of repressors at repressible promoters



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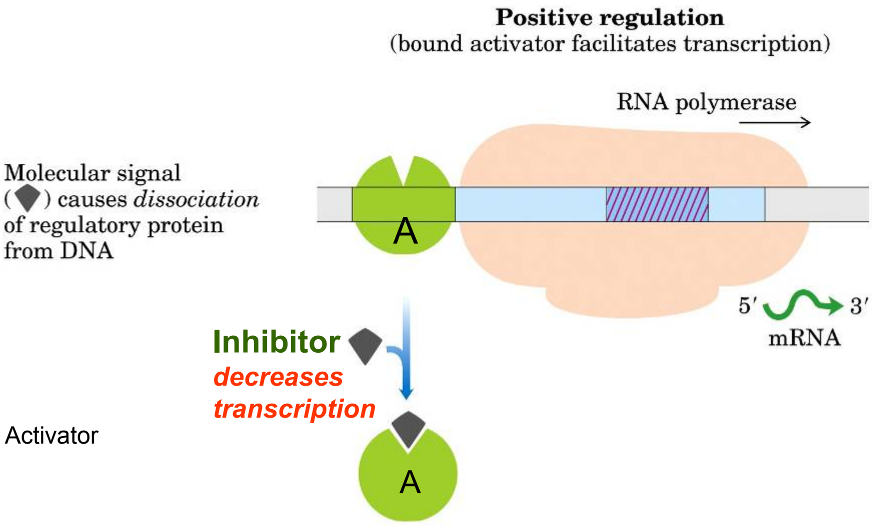
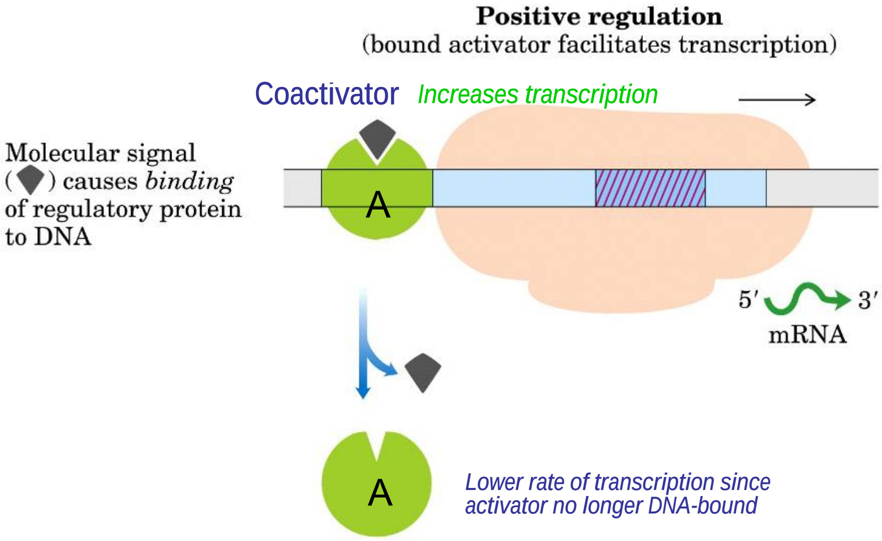
Role of activators at promoters subject to inhibition



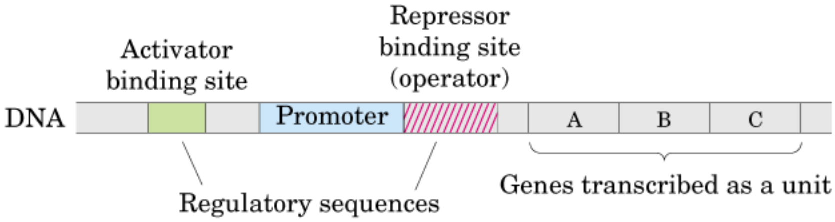
A= Activator

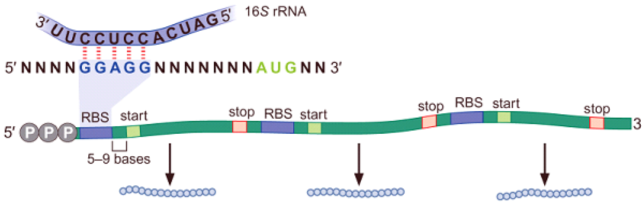
Role of Activators at Promoters Subject to Coactivation

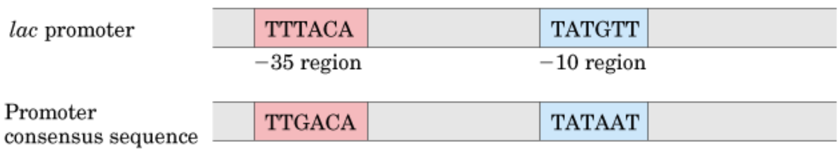


*

operon

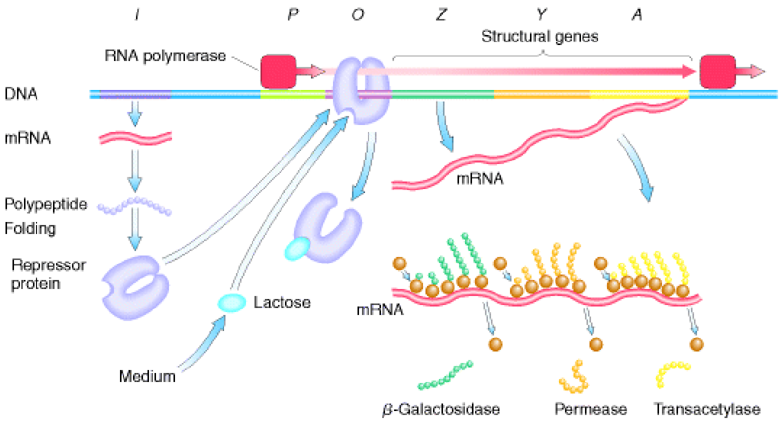

Repressor
binding site
(operator)
Activator
binding site
DNA
Promoter
A
B
C
Regulatory sequences
Genes transcribed as a unit


3' UUCCUCCACUAG 5'
16S rRNA
5' NNNNGGAGGNNNNNNNAUGNN 3'
RBS
start
stop
RBS
start
stop
RBS
start
stop
5'
P P P
3
5–9 bases

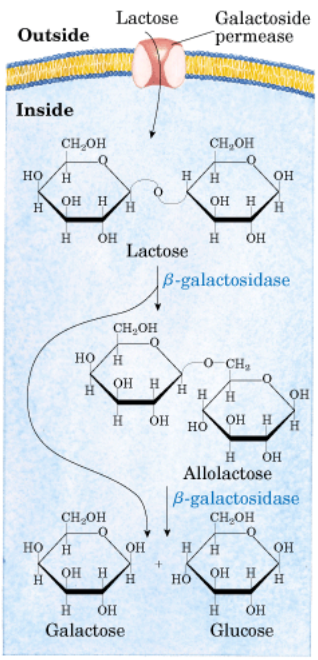

lac promoter
TTTACA
TATGTT
−35 region
−10 region
Promoter
consensus sequence
TTGACA
TATAAT

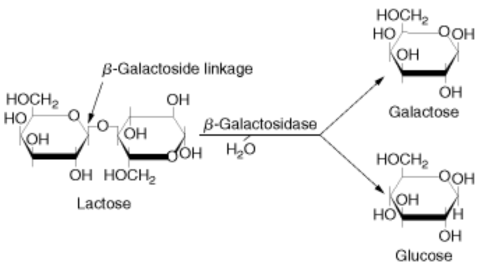
(b)

Lac operon

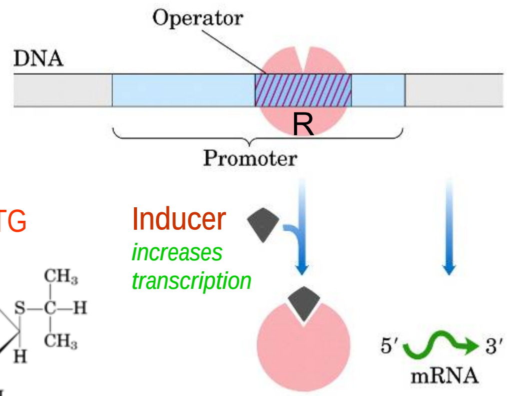


- *lacZ* codes for the enzyme β-galactosidase, tetramer of-500 kD. The enzyme breaks a β-galactoside into its component sugars.
- *lacY* codes for the β-galactoside permease, a 30 kD membrane-bound protein This transports β-galactosides into the cell.
- *lac A* codes for β-galactoside transacetylase, an enzyme that transfers an acetyl group from acetyl-CoA to β-galactosides.



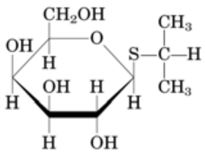


Binding of inducer to repressor lowers its affinity to operator



R= Repressor= **Lac I**

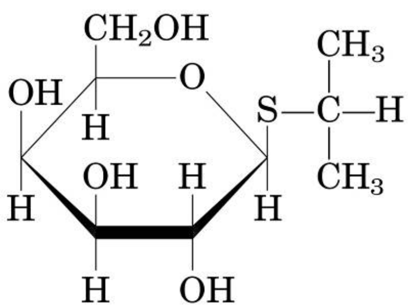
Inducer: *allo*-lactose, IPTG



Isopropylthiogalactoside (IPTG)

*

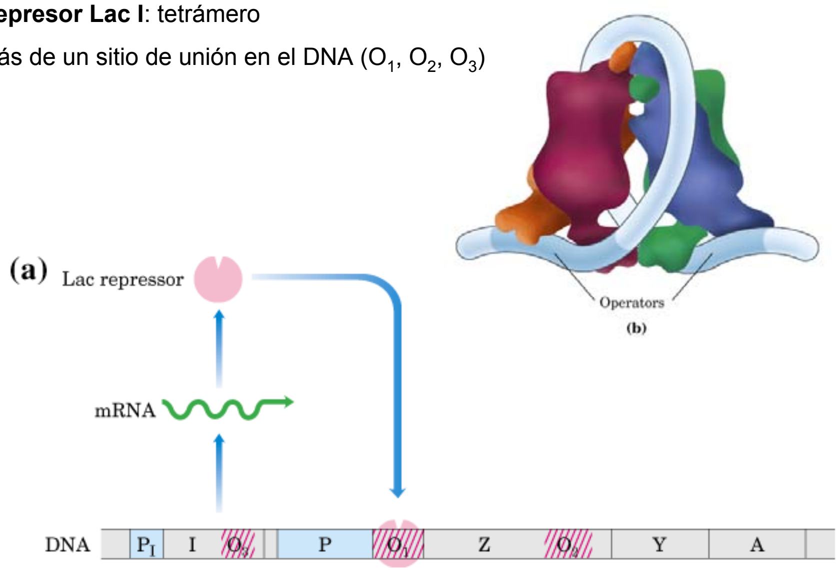
IPTG is a non-metabolizable inducer of the *lac* operon



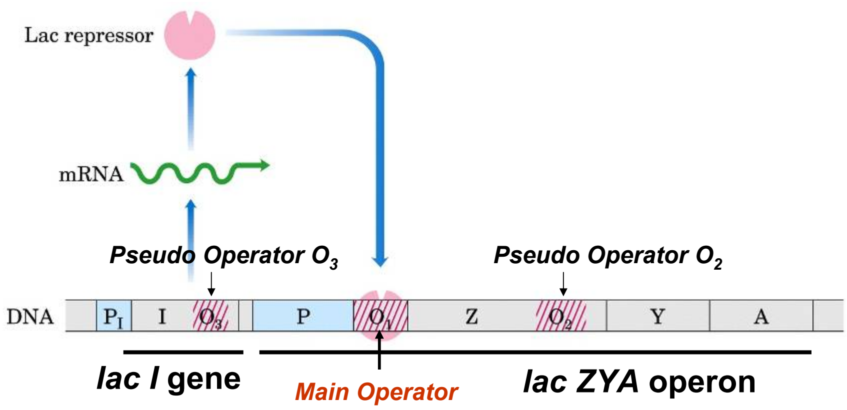
Isopropylthiogalactoside
(IPTG)

Represor Lac I: tetrámero

Más de un sitio de unión en el DNA (O_1, O_2, O_3)



Lac repressor binds cooperatively to two DNA elements: the main Operator O_1 and a pseudo Operator (O_2 or O_3)



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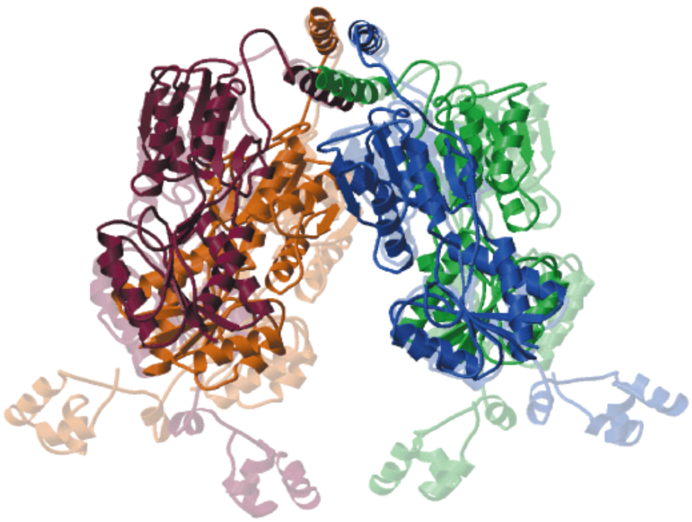
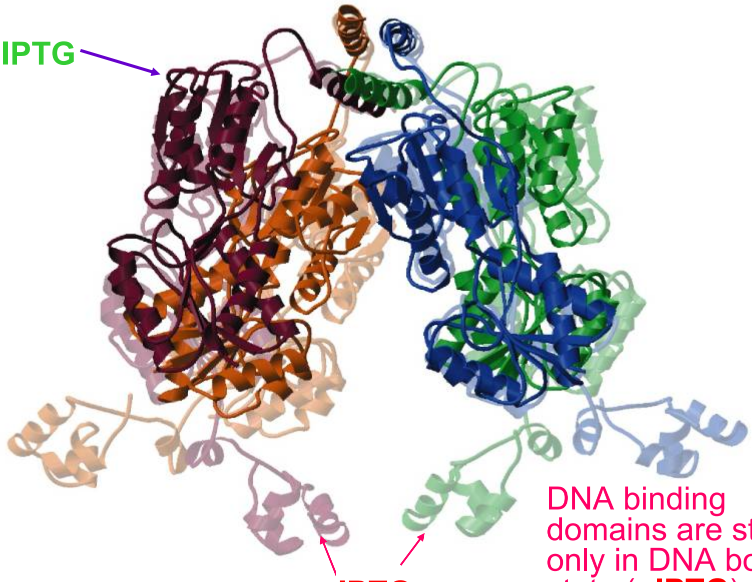


(d)

En colores pálidos: estructura
con afinidad por las secuencias O

IPTG induces a dramatic **conformational change** in **Lac repressor**, product of the *lac* I gene



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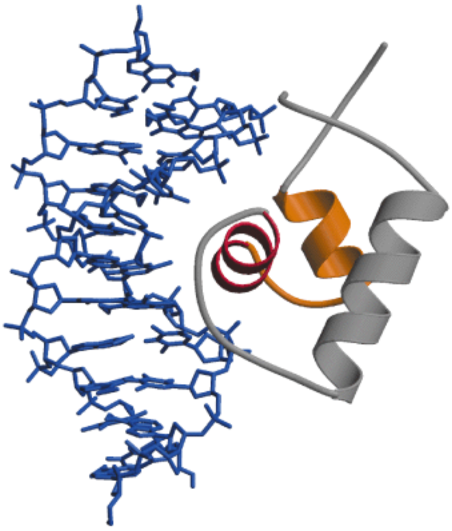
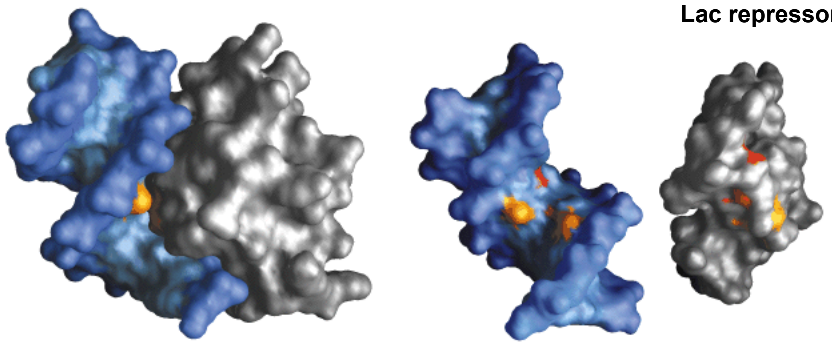
Helix-loop-helix

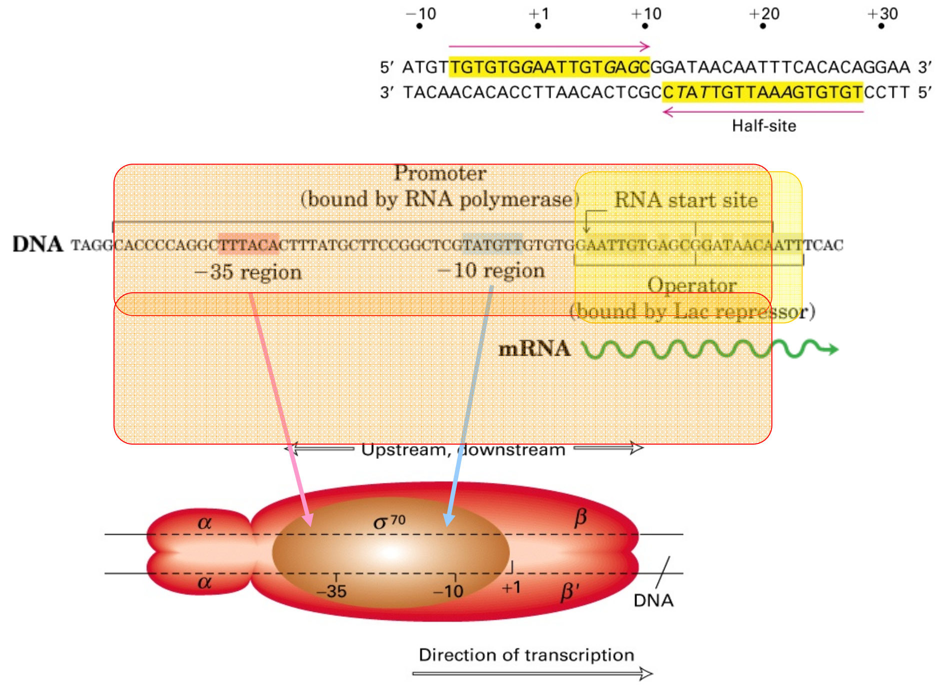
Lac repressor



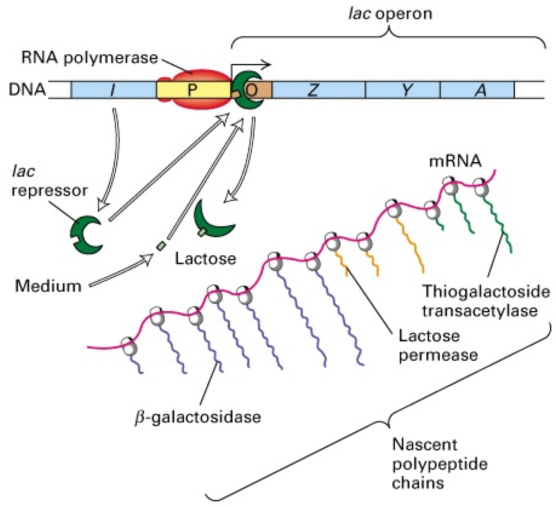
(a)



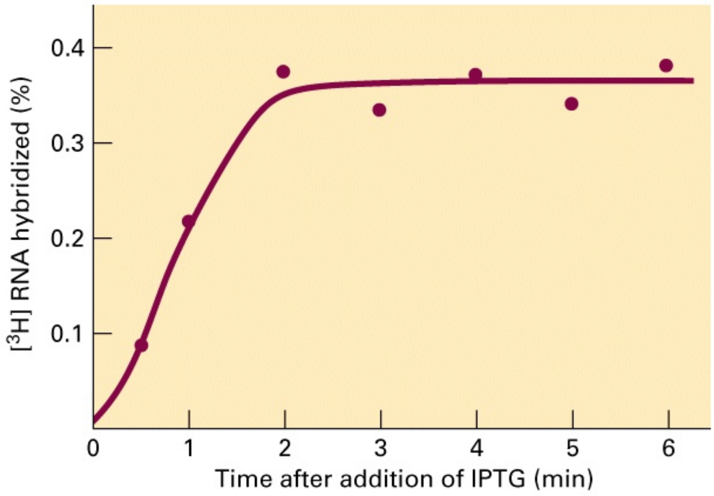
(c) (d)



Control de genes bacterianos: modelo de Jacob-Monod



Biochemical experiments confirm that induction of the *lac* operon leads to an increased synthesis of *lac* mRNA



Some mutants affecting lactose metabolism

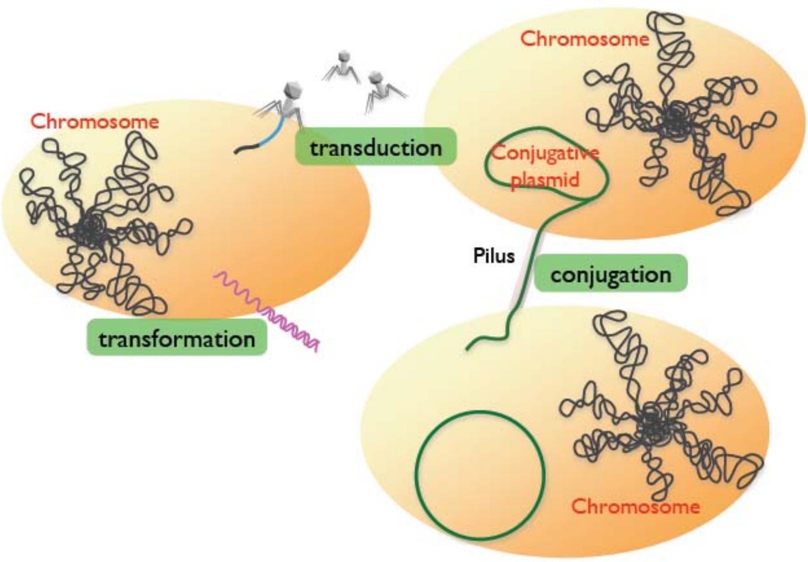
Mutant	**Effect on Repressor**	**Resulting Phenotype**
O^c (operator constitutive)	Repressor cannot bind operator.	The lac operon is always expressed, even in the absence of lactose.
I$^-$ (inhibitor minus)	Repressor cannot bind operator.	The lac operon is always expressed, even in the absence of lactose.
I^s (super repressor)	Repressor cannot bind lactose; thus, it cannot be released from the operator site.	The lac operon is never expressed, even in presence of lactose.

Genetic exchange in bacteria

- Transformation
- Conjugation
- Transduction

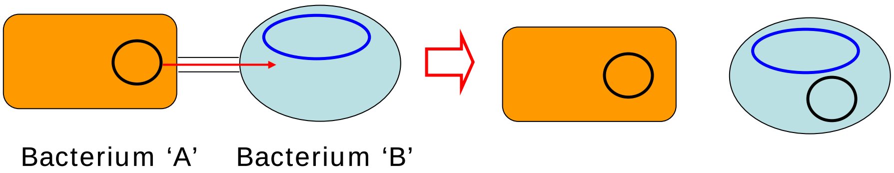
- Recombination (within one bacterium; foreign DNA can recombine)

Intercambio genético entre bacterias

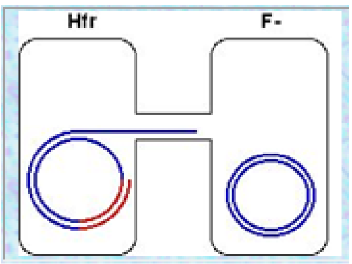


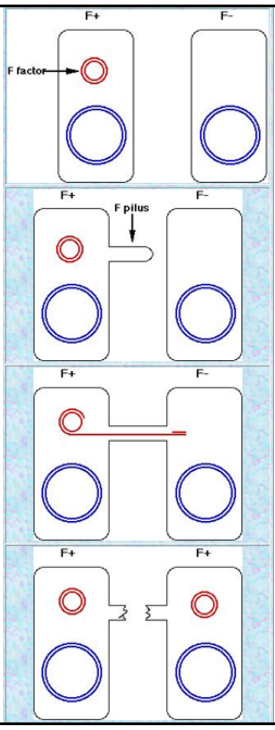
Conjugation – 'bacterial sex'

- Method of gene transfer from one bacterium to another

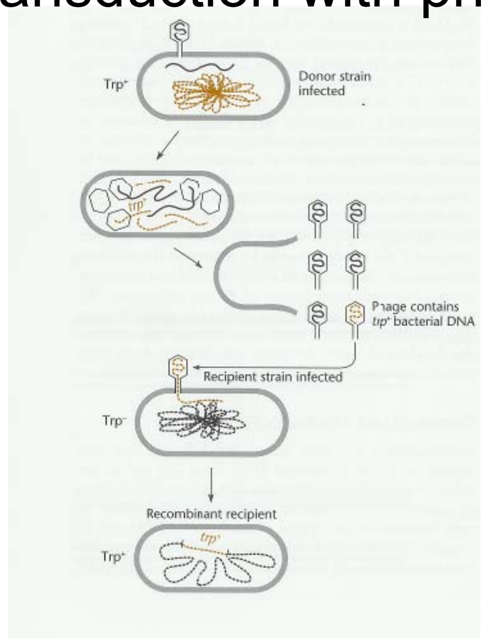


Conjugación

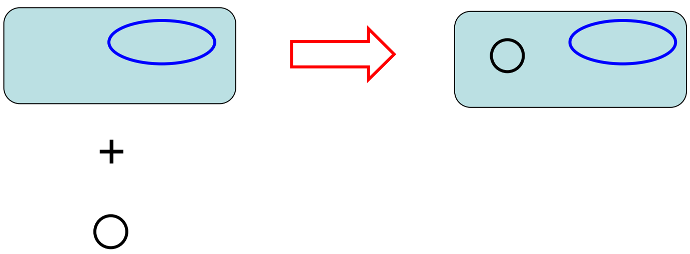




Transduction with phage

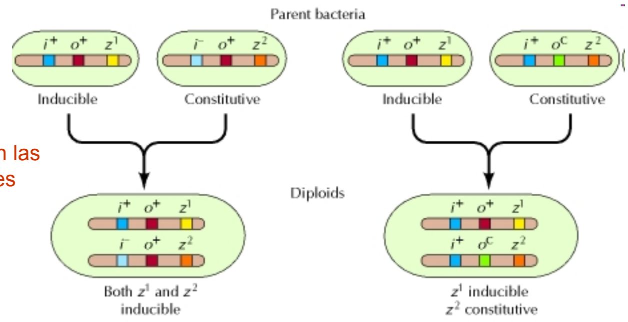


Transformation



bacteria picks up a piece of DNA

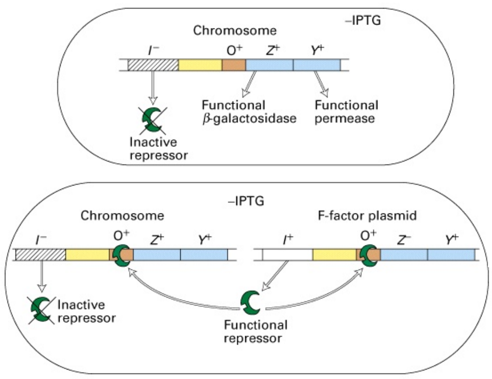
Experimental evidence for cis- and trans acting DNA sequences



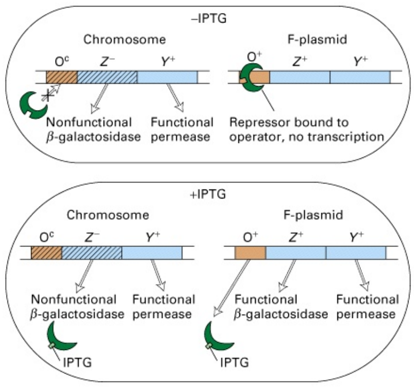
OJO: solamente se representan las porciones del genoma relevantes

Regulation of *β*-galactosidase in diploid *E. coli*
The mating of two bacterial strains results in diploid cells that contain genes from both parents. In these examples, it is assumed that the genes encoding β-galactosidase (the *z* genes) can be distinguished on the basis of structural gene mutations, designated z^1 and z^2. In an i^+/i^- diploid (left), both structural genes are inducible; therefore, i^+ is dominant over *i-* and affects expression of *z* genes on both chromosomes. In contrast, in an o^c/o^+ diploid (right), the *z* gene linked to *oc* is constitutively expressed, whereas that linked to o^+ is inducible. Therefore, *o* affects expression of only the adjacent *z* gene on the same chromosome.

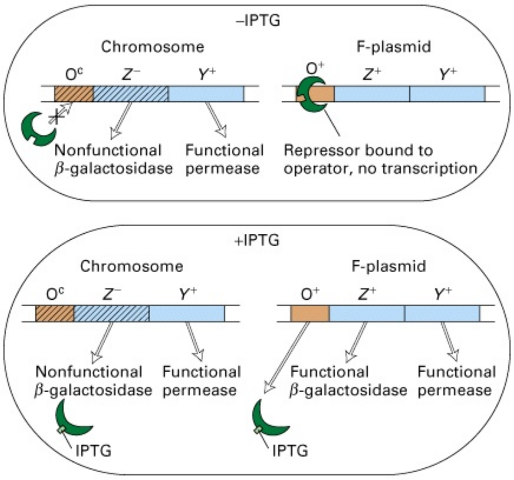
Experimental evidence for *trans*-acting genes/proteins



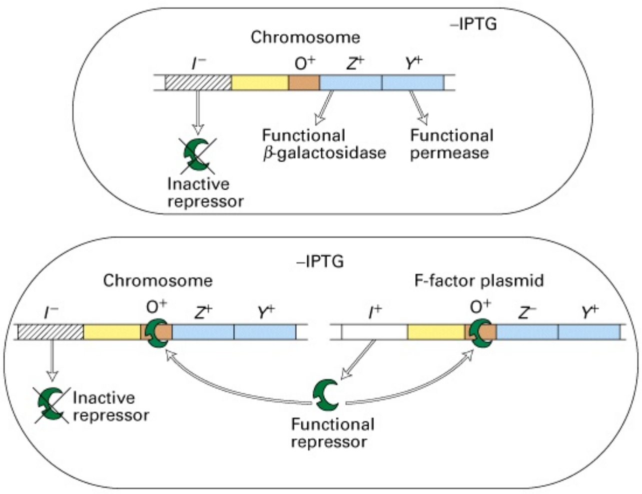
Experimental evidence for *cis*-acting DNA sequences



Experimental evidence for cis-acting DNA sequences



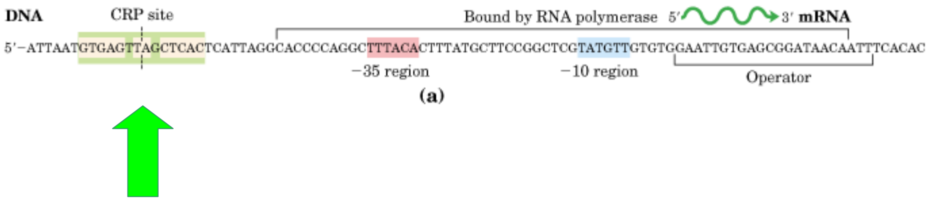
Experimental evidence for trans-acting genes/proteins



Control positivo de la transcripción del operon *lac*

cAMP-CAP (cAMP-CRP)

Transcripción de mRNA-lac sólo cuando no hay glucosa



catabolite activator protein (CAP)

cAMP receptor protein (CRP)

The lac control region contains three critical *cis*-acting sites

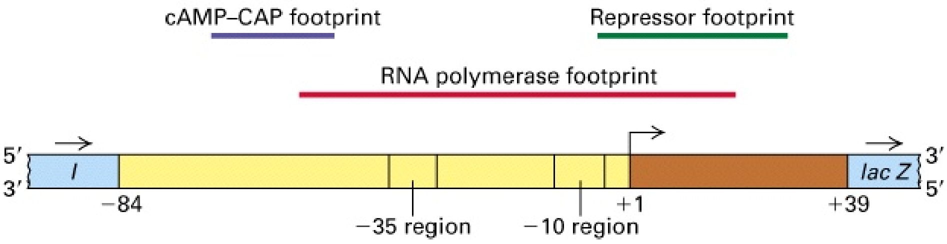
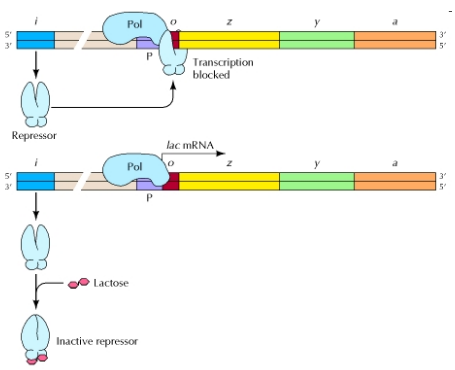
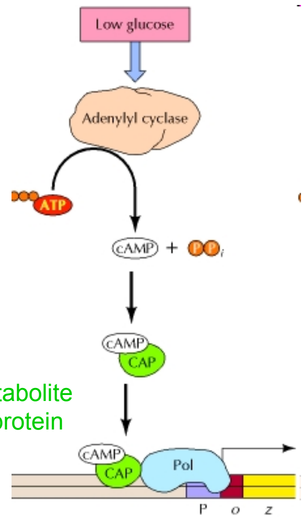


Figure 10-9

Positive control of the *lac* operon is exerted by cAMP-CAP

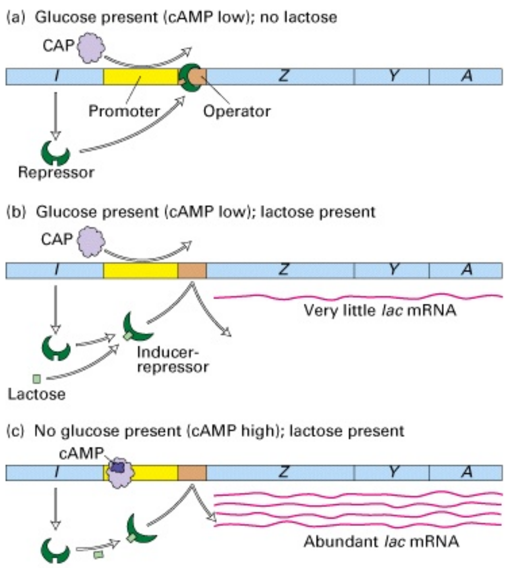


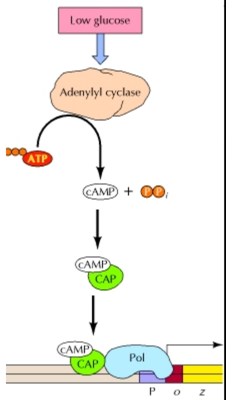


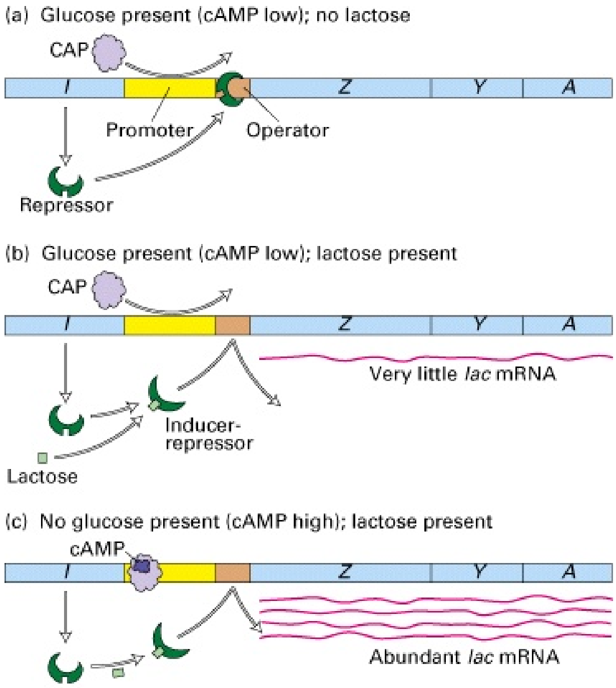
CAP = catabolite activator protein

Positive control of the *lac* operon is exerted by cAMP-CAP

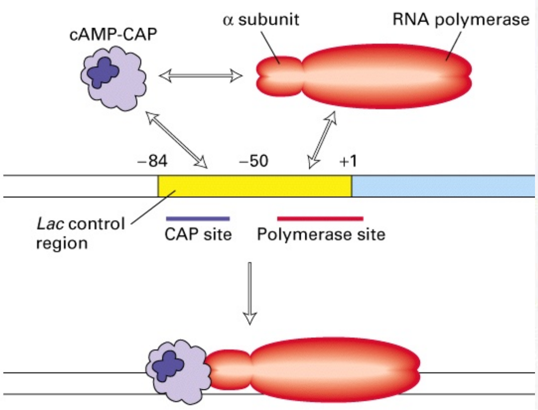
CAP = catabolite activator protein







Cooperative binding of cAMP-CAP and RNA polymerase to the *lac* contol region activates transcription



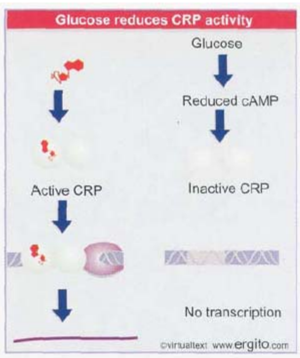
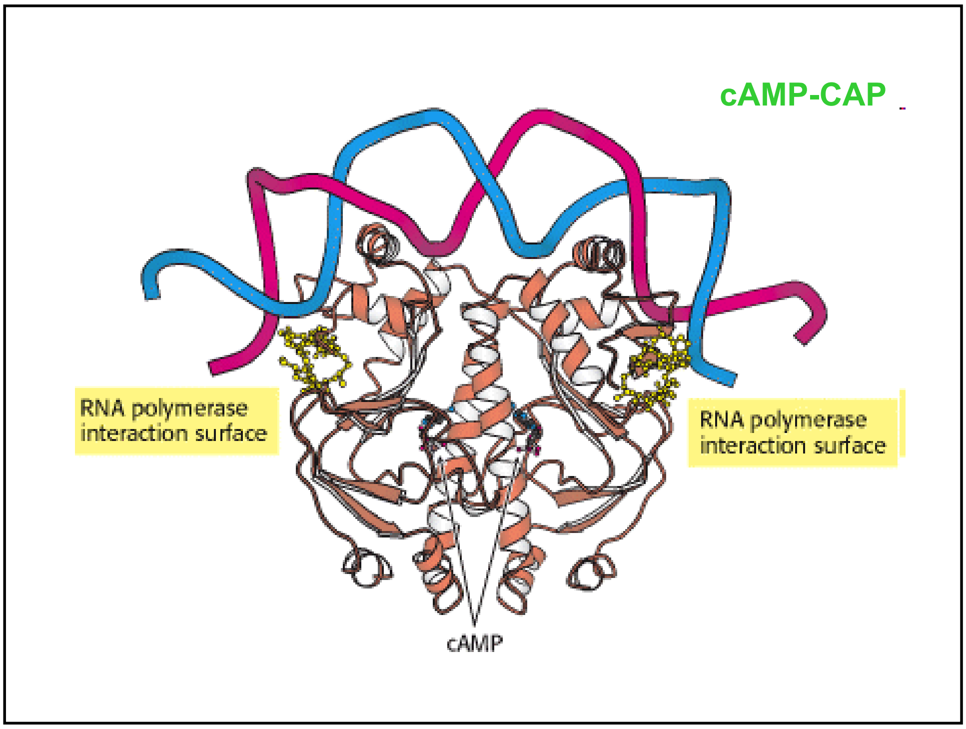
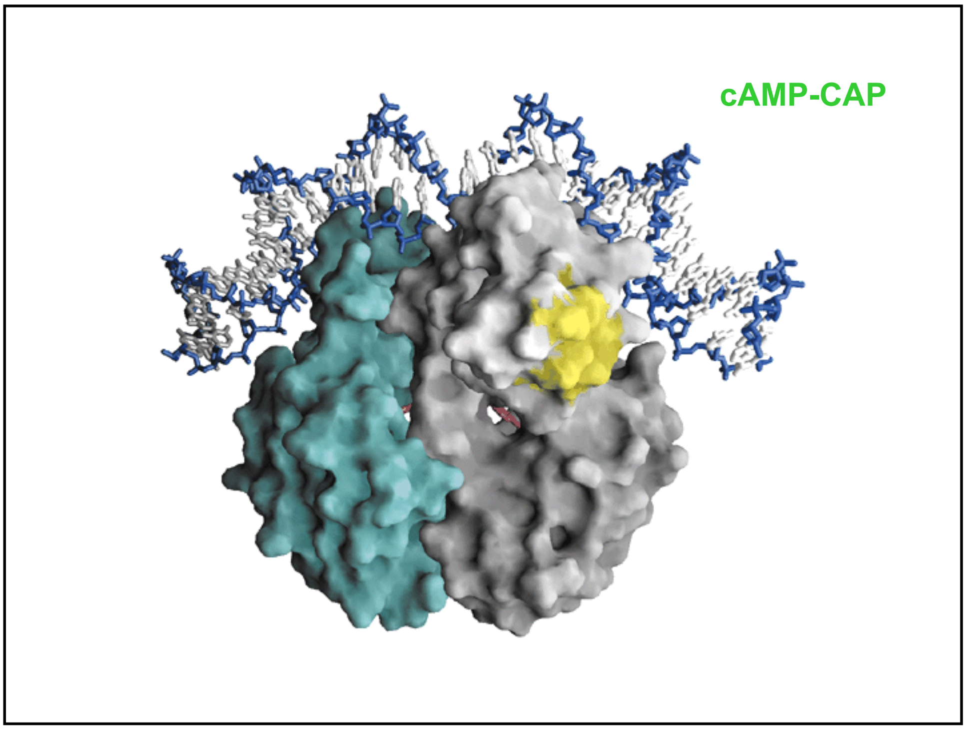


Figure 11.6 By reducing the level of cyclic AMP, glucose inhibits the transcription of operons that require CRP activity.

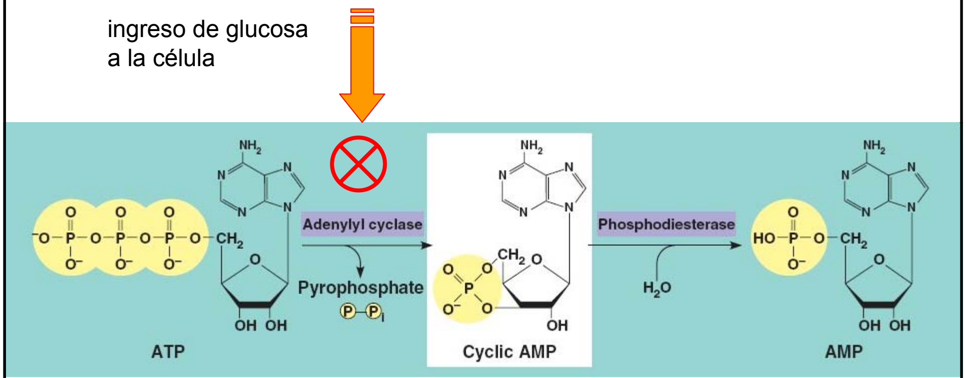

cAMP-CAP
RNA polymerase interaction surface
RNA polymerase interaction surface
cAMP


cAMP-CAP

Represión catabólica

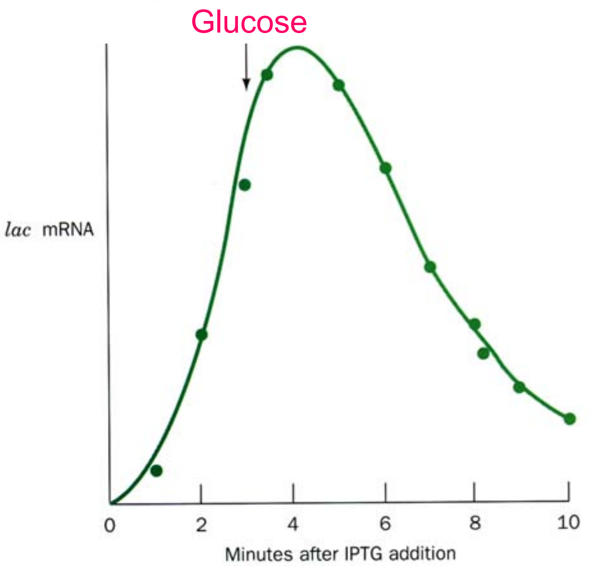
Es el resultado de la disminución del la concentración intracelular de cAMP

ingreso de glucosa
a la célula



*

Catabolite Repression: rapid but not immediate



*

Glucose does not affect the abundance of *lac* mRNA immediately because:

1. synthesis of cAMP must cease
2. intracellular cAMP levels must drop (actively transported out of cell)
3. cAMP must dissociate from CAP
4. CAP must release from lac promoter DNA
5. recruitment of RNAP, and concomitant transcription initiation, must decline
6. pre-synthesized *lac* mRNA must be degraded by intracellular RNases (responsible for the short half life of virtually all bacterial mRNAs)

Catabolite Repression

IS **NOT** AN EXAMPLE OF
NEGATIVE CONTROL

Rather, catabolite repression occurs since the **stimulatory effect of CAP is lost** (for it is **unable to bind DNA**).

CATABOLITE REPRESSION
RESULTS FROM A
LOSS OF POSITIVE CONTROL