Transcription

The Players:

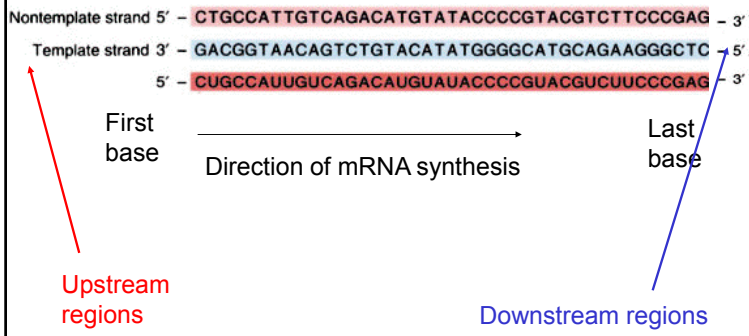
RNA polymerase Synthesizes 5' to 3'

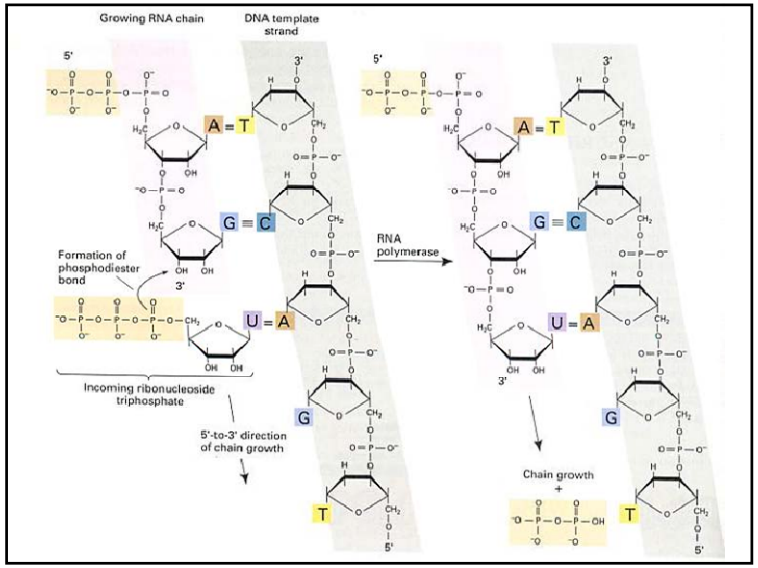
uses NTPs (ribose) not dNTP (deoxyribose)

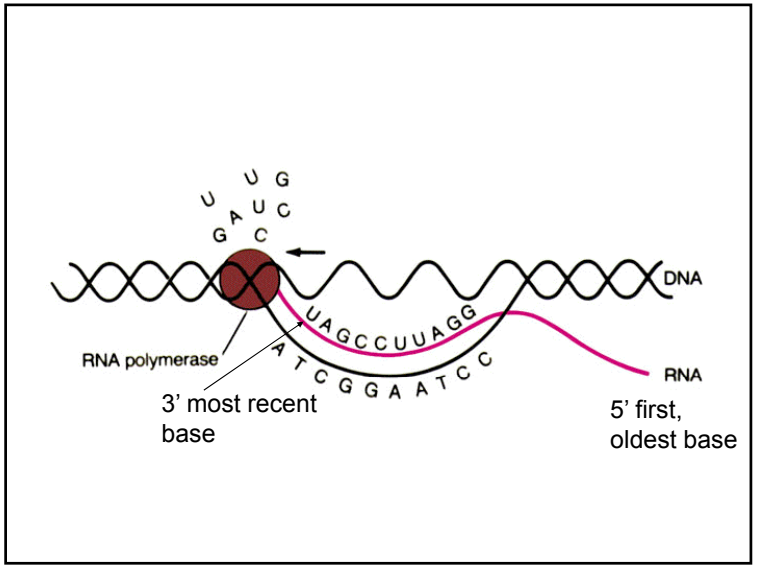
uses UTP and not TTP

does not need primer, Why?

DNA template (Promoter)







Which of the following mutations within a gene would be the worst?

A) Insert a single base

B) Change the 3rd base of a codon.

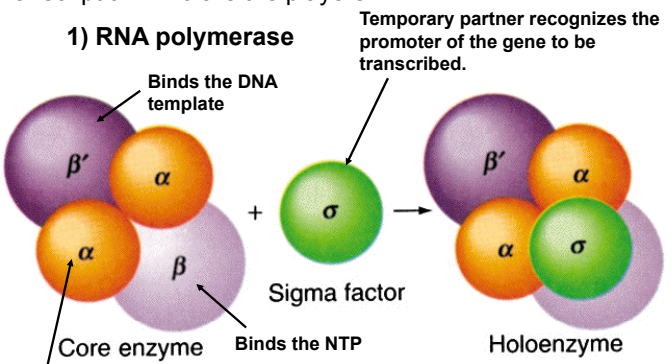
C) Change the 2nd base of a codon

D) Delete three bases

E) Delete one base and insert a different base in the next codon

Transcription: who are the players?

1) RNA polymerase



First mechanism for gene regulation.

Different σ subunits recognize different promoters with different efficiencies!

2) Promoters recognized by σ subunit of the RNA polymerase. We are viewing the non-template (coding) strand.

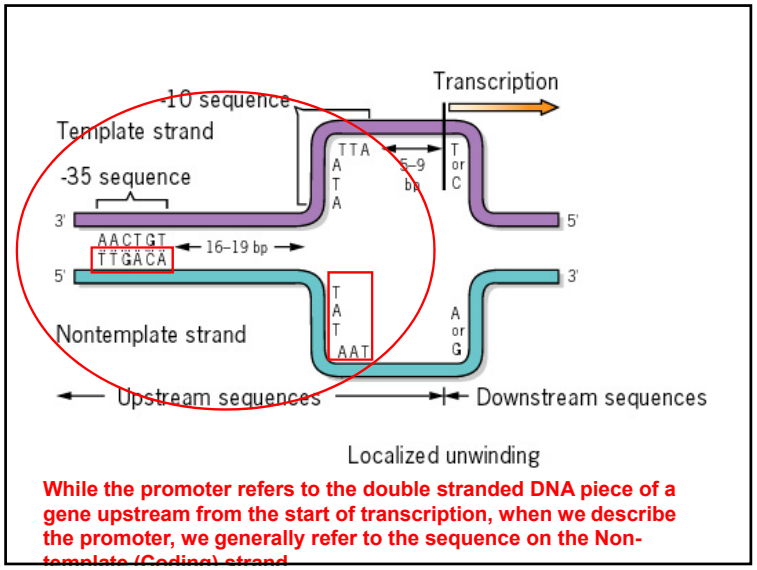
Second mechanism to control gene expression.

Different promoters have different affinities for σ subunits. Some are strong promoters and others are weak!

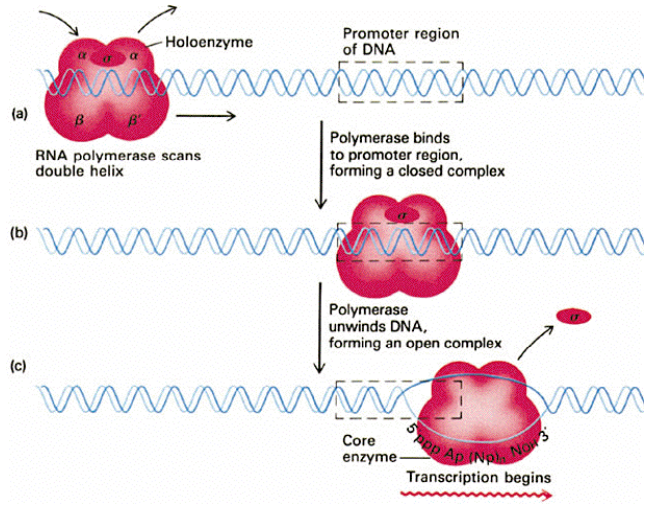
Transcription begins here at +1, often a purine (A or G)

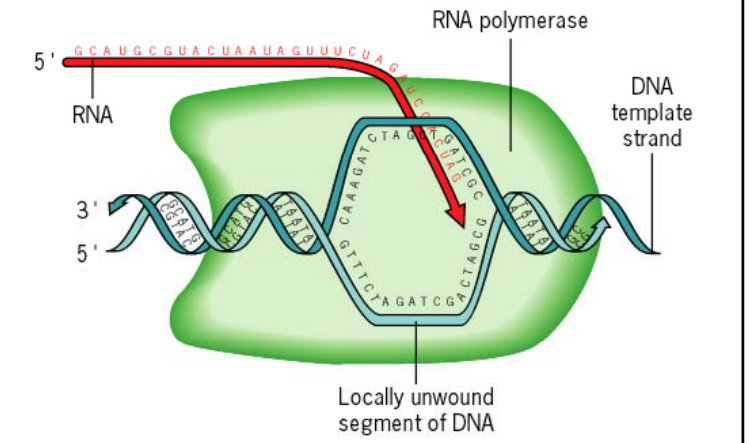
Sigma factors recognize different promoters with different efficiencies!! Mechanism for controlling gene expression.

Gene	Factor	-35 sequence	Separation	-10 sequence
rpoD	σ^{70}	TTGACA	16-18 bp	TATAAT
rpoH	σ^{32}	CCCTTGAA	13-15 bp	CCCGATNT
rpoN	σ^{54}	CTGGNA	6 bp	TTGCA
fliA	σ^{28}	CTAAA	15 bp	GCCGATAA
sigH	σ^{H}	AGGANPuPu	11-12 bp	GCTGAATCA

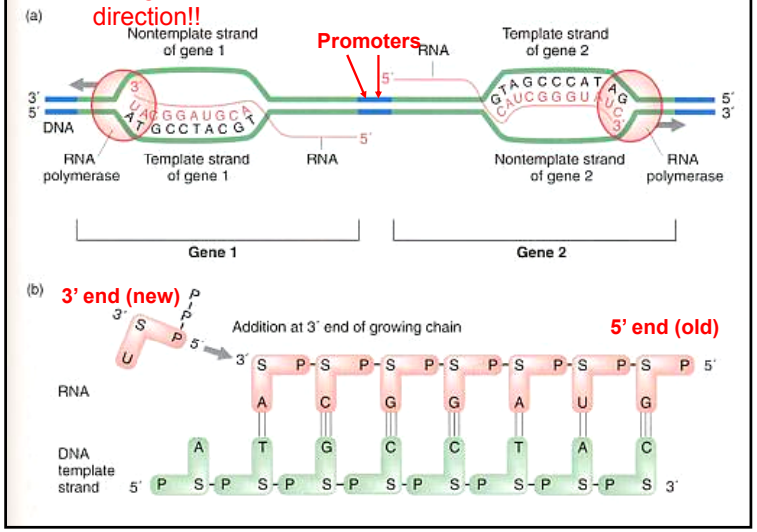


While the promoter refers to the double stranded DNA piece of a gene upstream from the start of transcription, when we describe the promoter, we generally refer to the sequence on the Non-template (Coding) strand





The genes can be orientated in either direction!!

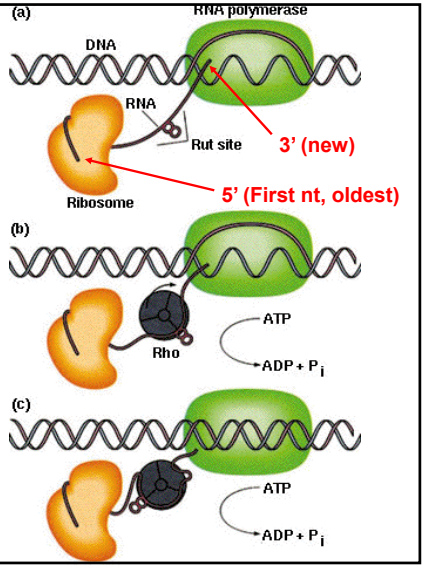


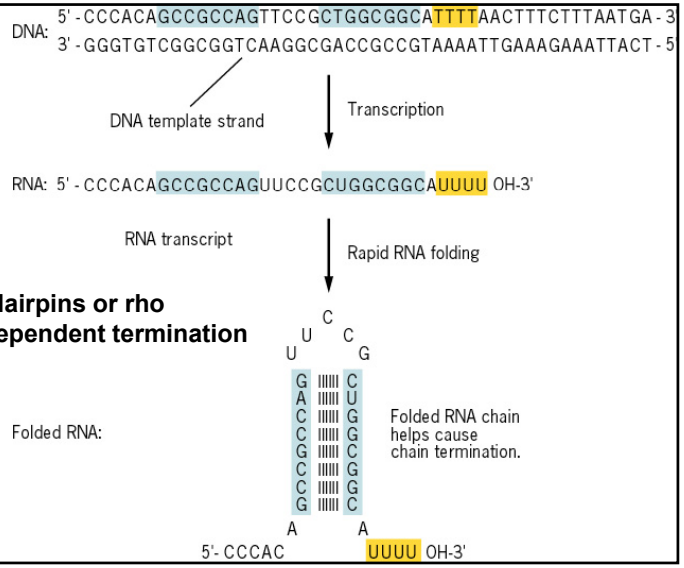
When and how does transcription stop?

At least two mechanisms:

1) **Rho dependent termination**

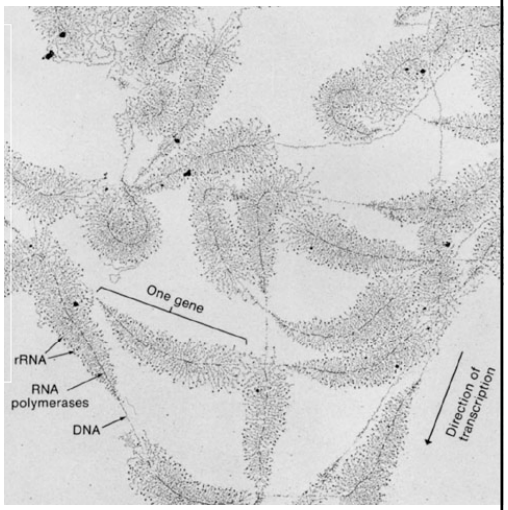
Rho protein recognizes and binds to the 40-60 nt rut site (rho utilization site) of the mRNA

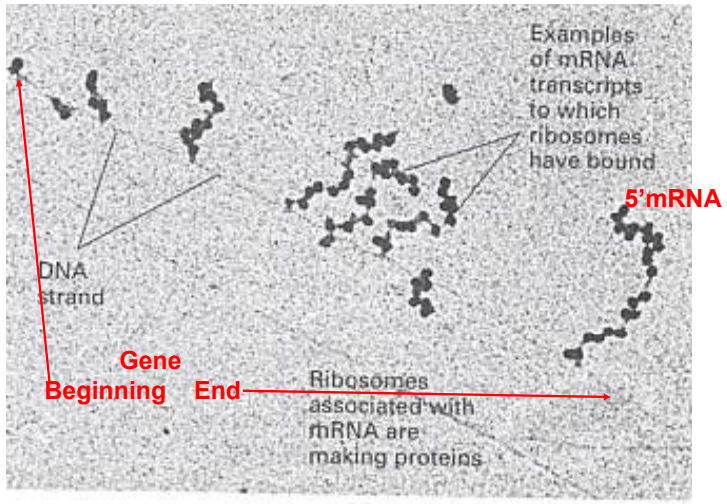




An important feature of prokaryotic transcription and translation.

Translation can occur before transcription has completed because the processes are not compartmentalized. (Less control of gene expression here than in Eukaryotes)





How are genes regulation in Bacteria? Are all genes turned on at all times?

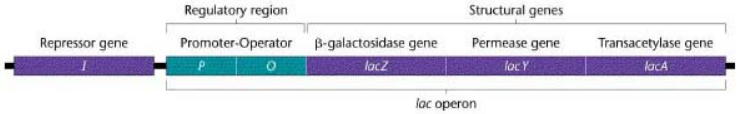
1) Different σ subunits recognize different promoters.

2) Different promoters; some are efficient others not.

3) Termination sequences vary and can influence transcription rates.

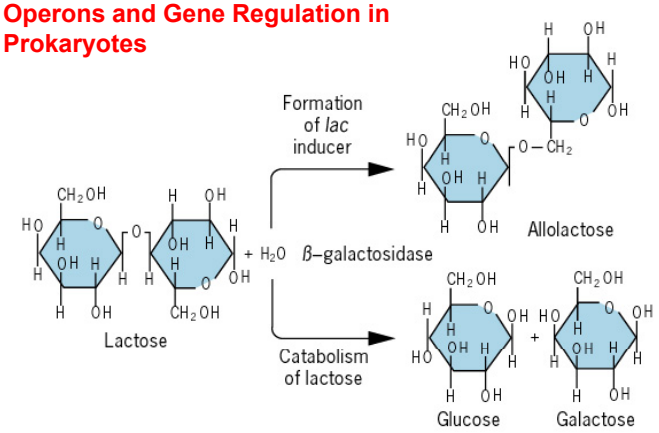
4) RNA degradation

5) Multiple genes (polycistronic) with related functions are often clustered together in bacteria and under the control of a single promoter.



6) Also there are mechanisms to slow down or block transcription by RNA polymerase.

Operons and Gene Regulation in Prokaryotes



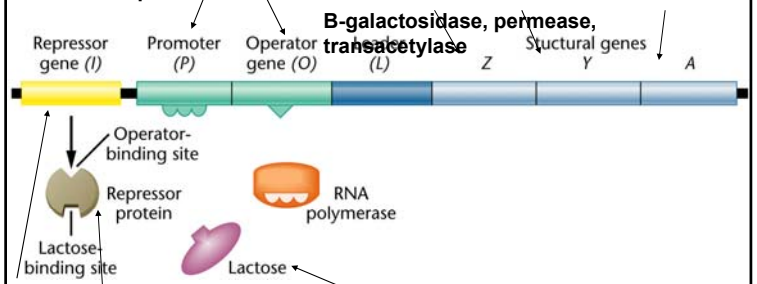
Two physiologically important reactions catalyzed by β-galactosidase.

Basic Components of the Lac Operon (Jacob and Monod 1960)

Promoter and Operator, are regulatory regions; they do not code for proteins

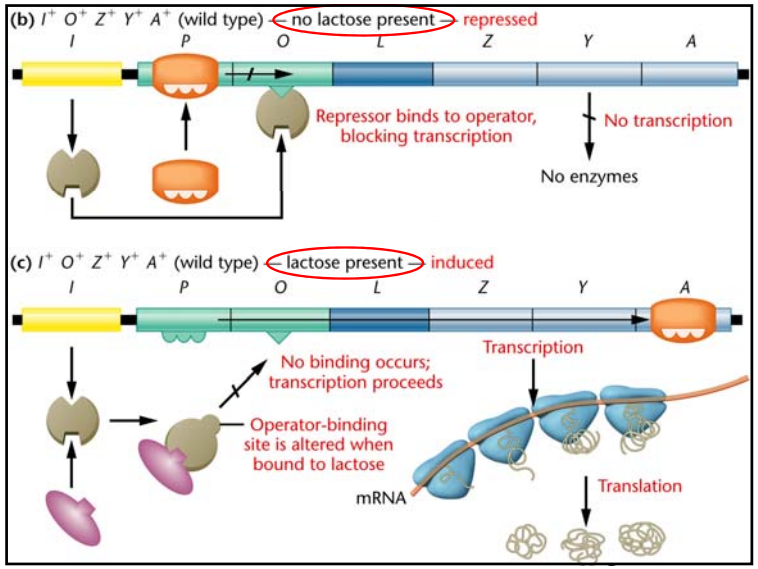
Three structural genes that make enzymes

B-galactosidase, permease, transacetylase



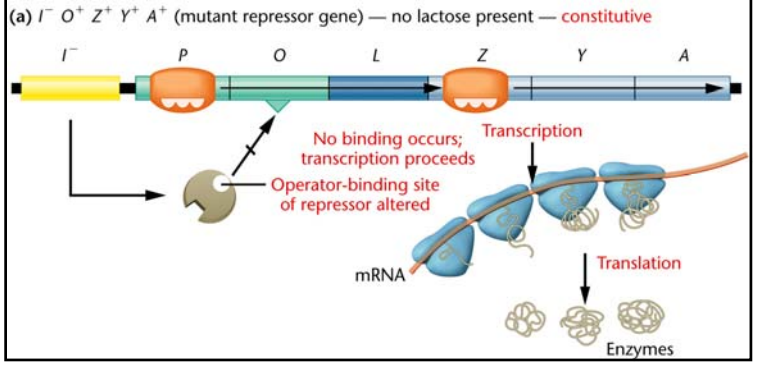
***Lac I* gene codes for repressor**

Lactose/allolactose sugar, kicks the system into gear!!

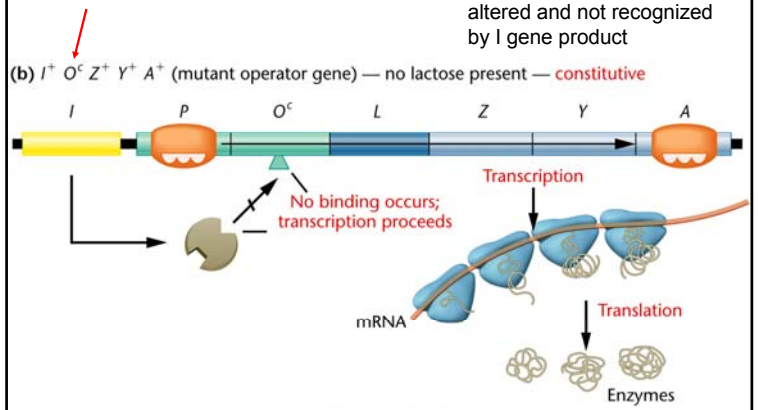


Mutants were discovered: No lactose, but operon was on and B gal made!! How would this work?

I^- = I gene produces a defective product or non-functional



O^c = O region sequence is altered and not recognized by I gene product

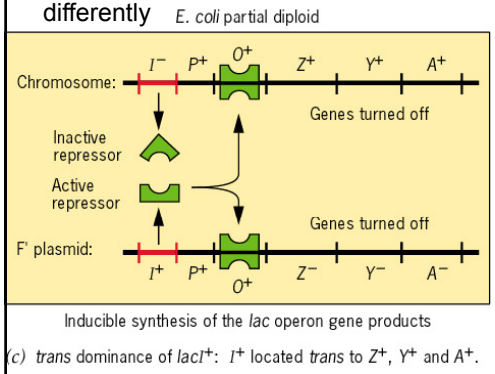


These are called constitutive mutations. How do you think they found out that these were different genes??

Essentially, with "Complementation Tests"

Examine the function of constitutive mutants with F' plasmid and partial diploids in bacteria.

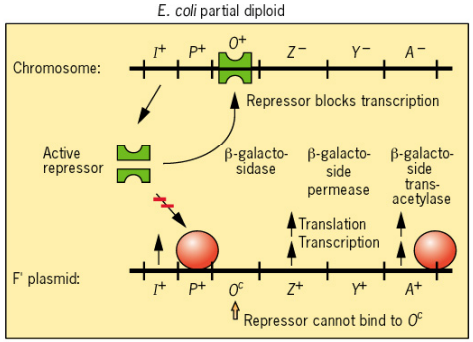
Mutants mapped to different places and they behaved differently



Inducible synthesis of the *lac* operon gene products

(c) *trans* dominance of *lacI*$^+$: I^+ located *trans* to Z^+, Y^+ and A^+.

Add a functional I+ elsewhere and control of the Operon is restored!!



Inducer absent; functional *lac* operon gene products are synthesized

(*b*) Constitutive synthesis of the *lac* operon gene products in an F′ $I^+ P^+ O^c Z^+ Y^+ A^+ / I^+ P^+ O^+ Z^- Y^- A^-$ bacterium.

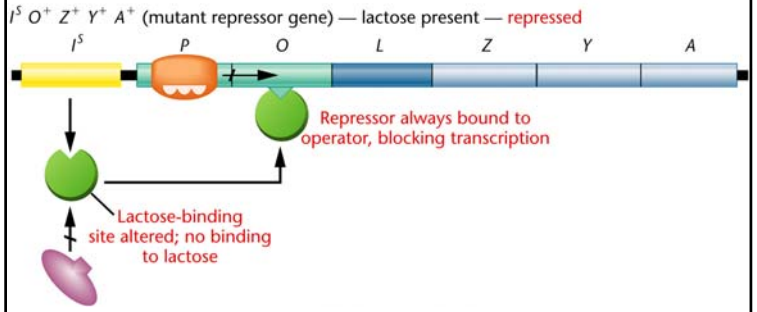
Add a functional O+ elsewhere and control NOT restored, still always ON!!

Fig 23.8 The operator acts only in the *cis* configuration.

Other Mutants Discovered:

Repressor protein (I gene) has two active sites, one to bind to the Operator and one to bind to lactose. What would a mutation affecting the latter site do?

Permanently repress the operator, always turned off: I^s "super-repressed"



The repressor protein has two active sites. If a mutation (**X**) occurs in the LacI gene as shown which changes the protein as shown, what will be the result?

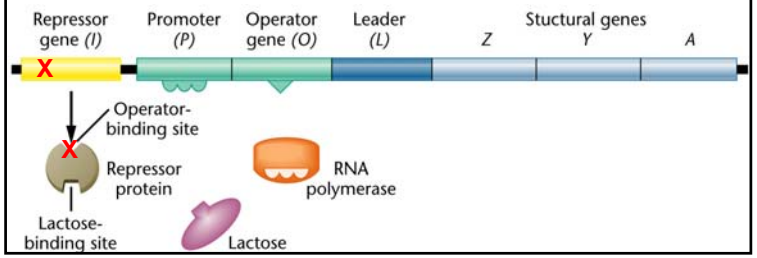
A) β-galactosidase will be made when lactose is present

B) β-galactosidase will be made when lactose is absent

C) β-galactosidase will never be made

D) β-galactosidase will be made when galactose is present

E) β-galactosidase will be constantly made

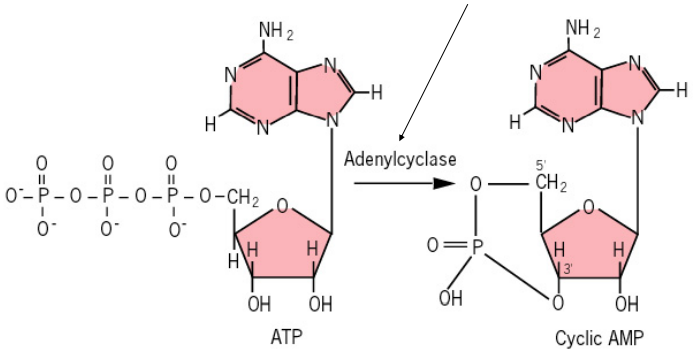


So Far…

We see a control system that nicely turns on genes only when they are needed to metabolize a particular product (lactose).

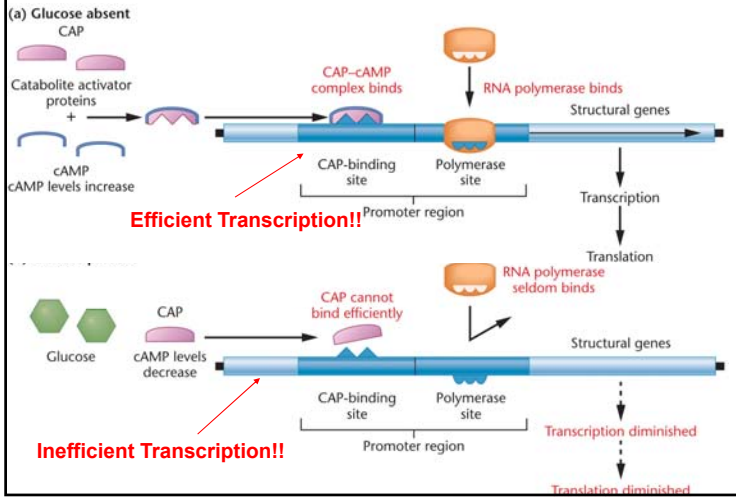
What about control when lactose is present, but the product (glucose) is not needed.

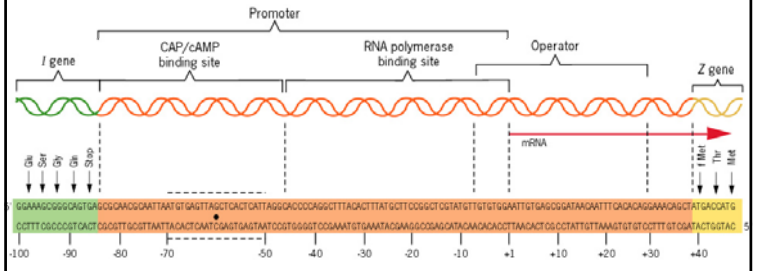
Glucose inhibits this enzyme



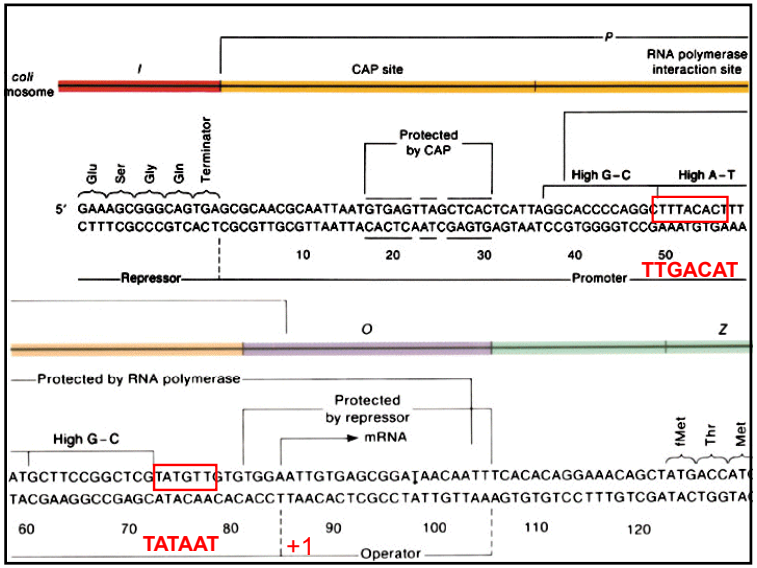
The adenylcyclase-catalyzed synthesis of cyclic AMP (cAMP) from ATP.

Catabolic Repression of the lac Operon, even tighter regulation of the operon. Glucose is easier to use, so if present, why use lactose?





Organization of the promoter-operator region of the *lac* operon.



A B C D E

Where is the repressor protein encoded?

Where does it bind?