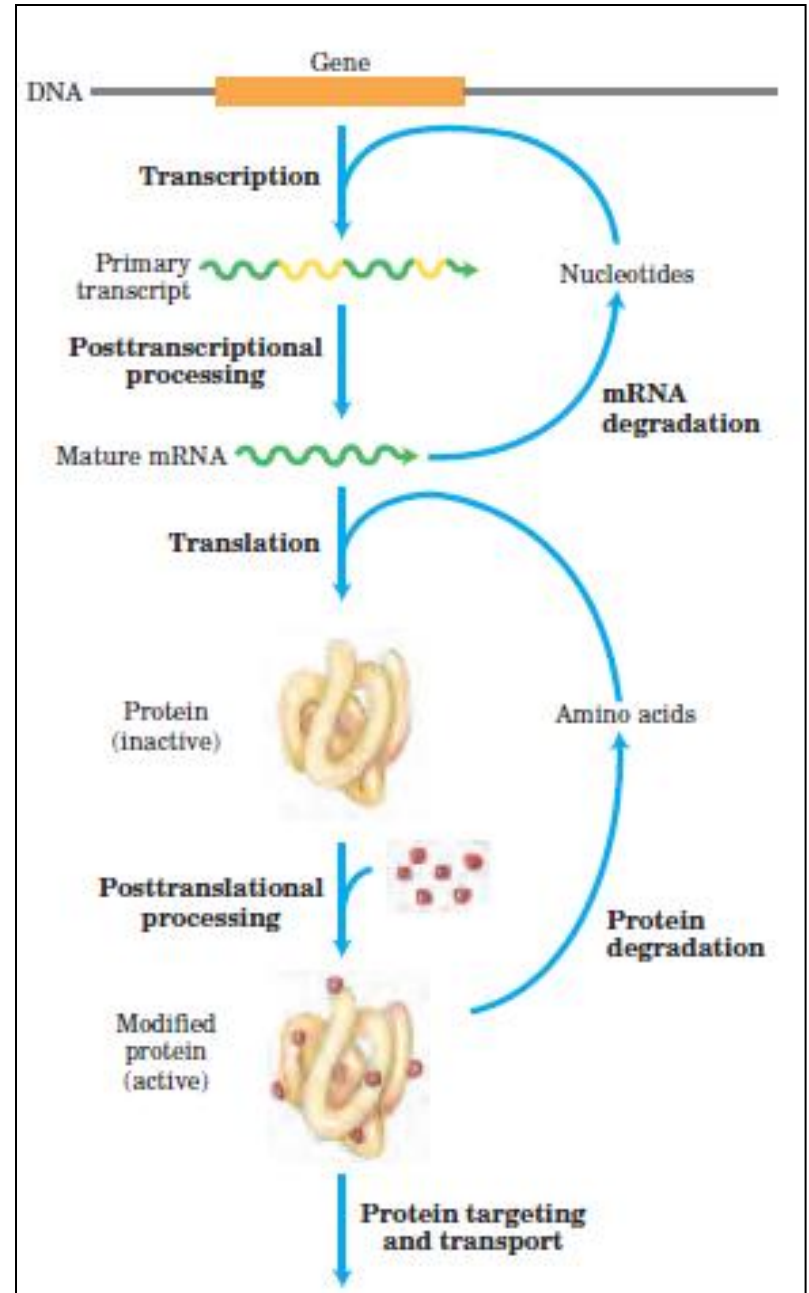


GENE EXPRESSION

Gene expression

- A process by which information from a gene is used to synthesize a functional gene product (protein)
- Requirement for some gene products change over time and depends on the need of cell
- E.g. when DNA is heavily damaged, cell need many DNA repair enzymes
- There are 7 processes that affect the production of protein and these processes has several potential points of regulations



Principle of gene regulation

- Genes products that are required at all times are (e.g enzyme of metabolic pathway) expressed at constant level in the cell – **housekeeping genes**
- For other gene products, cellular rise to fall in response to **molecular signals** – **regulated gene expression**
- Gene products that increase in conc of particular inducer is refer to **inducible genes** , the process is induction (e.g high level of DNA damage induce production expression of DNA repair enzymes)
- Gene products that decrease the in conc of particular repressor – **repressible genes**. The process is called repression

Two types of gene expression

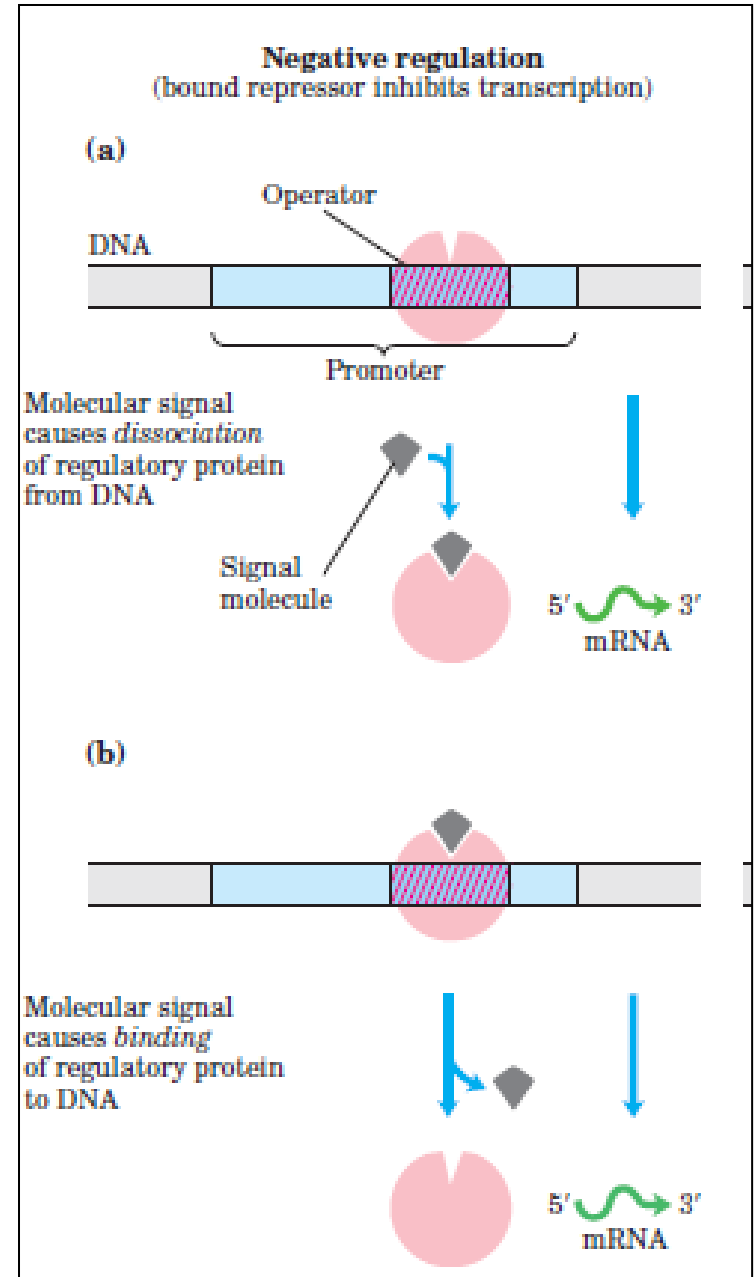
- 1) when the expression of a gene is increased due to the presence of specific regulatory element – **positive regulation**
 - 2) When the expression of genetic information is decreased due to the presence of specific regulatory element – **negative regulator**
- # a double –ve has the +ve effect. An effector that inhibits the fx of a –ve regulatory appears lead to positive regulation

Transcription regulation

- Depending on how RNAP interact with promoter
- 3 types of proteins involve:
 - **Specificity factors** – alter the specificity of RNAP for a give promoters
 - **Repressor** – bind to operators near the promoter- prevent access of RNAP to the promoter or movement along DNA after binding (-ve regulation)
 - **Activator** – bind to enhancer DNA sites (near/distant from the promoter) - enhance the RNAP-promoter interaction – little transcription occurs in the absence of the activator

-ve regulation

- a) Repressor (**pink**) binds to the operator in the absence of the molecular signals, the external signal causes dissociation of the repressor to allow transcription
- b) Repressor binds in the presence of the signal, when the signal is removed, transcription continues



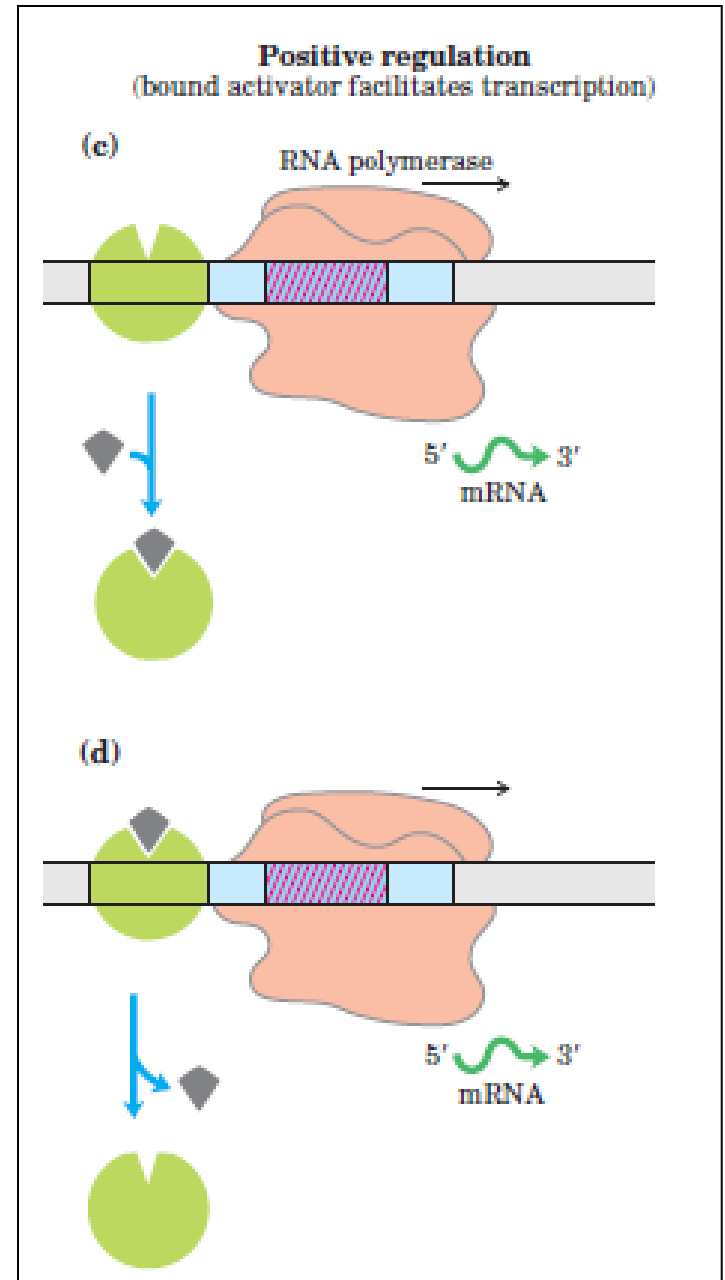
+Ve regulation

c) **Activator (green) binds in the absence of the molecular signal and transcription proceeds.**

When the signal is added, the activator dissociates and transcription is inhibited

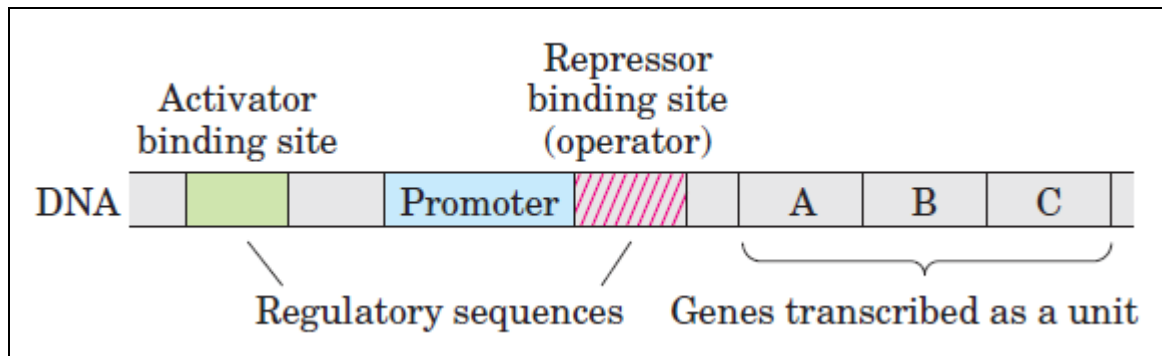
d) **Activator binds in the presence of the signal – transcription proceeds**

when the signal is removed, the activator dissociate and transcription is inhibited



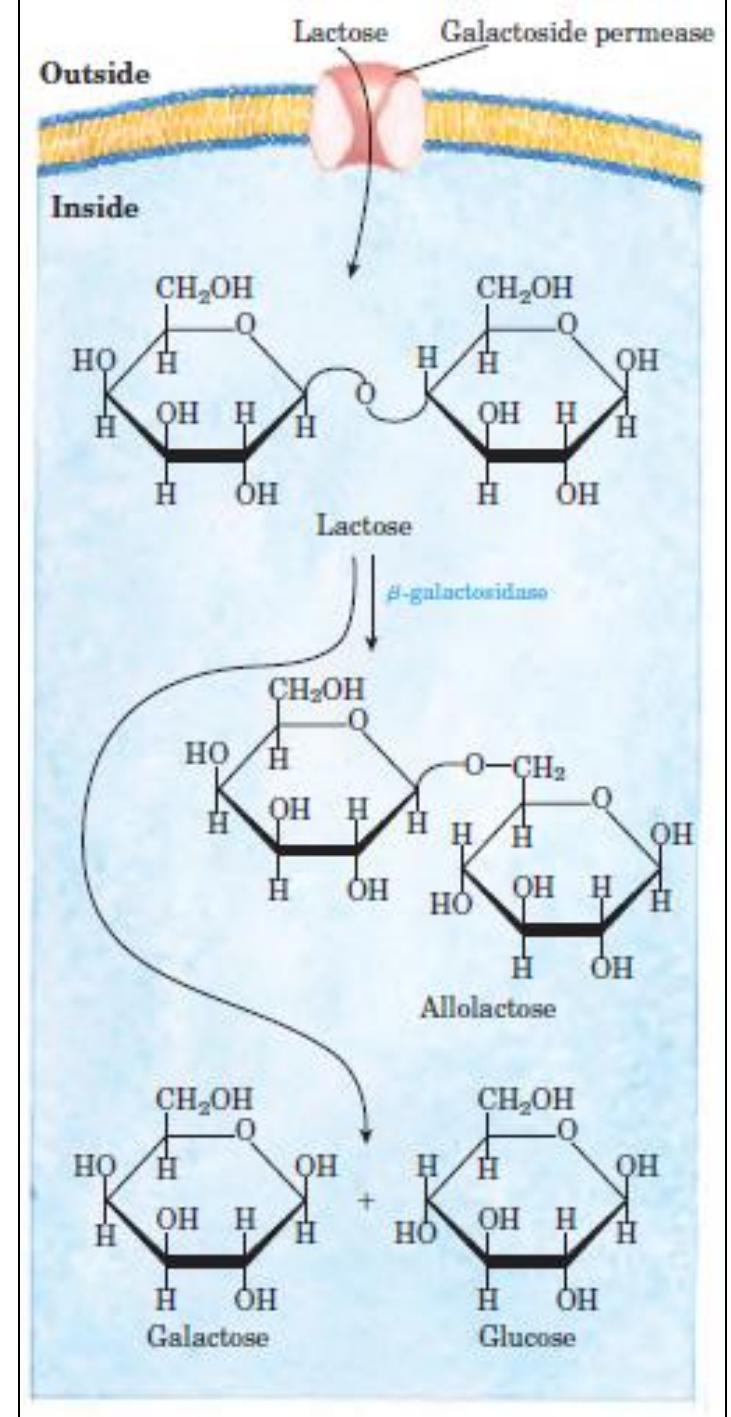
Gene expression in prokaryote

- Bacteria have a simple and general mech for gene regulation
- Genes encoding products that participate in a set of related processes – clustered on a chromosome and transcribed together
- Many prok mRNAs are polycistronic – multiple genes on a single transcript (gene cluster) and one promoter initiate expression of these cluster of genes
- Gene cluster and a promoter+additional sequences that fx together in regulation - **OPERON**



Gene expression in prokaryote

- The first well defined studies – lactose metabolism in E.Coli
- Lactose metab involve formation of:
 - a. **β -galactosidase** (cleaves lactose to galactose and glucose)
 - b. **galactoside permease** (transports lactose into the cell)
 - c. **thiogalactoside transacetylase** (modify toxic galactosides to facilitate removal from cell)
- When E.coli is presented with lactose or lactose analogs – the expression of β -galactosidase, galactoside permease and thiogalactoside transacetylase **increased 100 fold – 1000 fold**

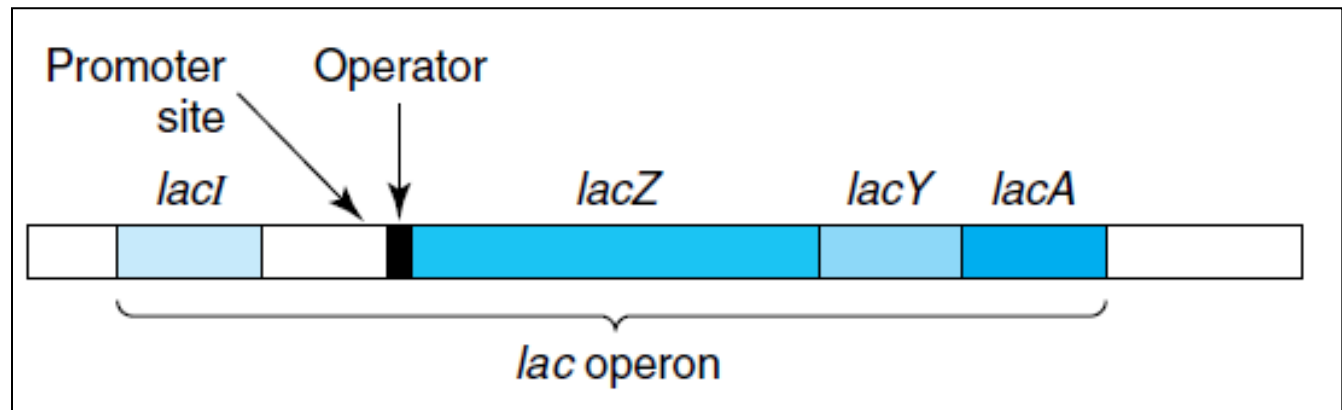
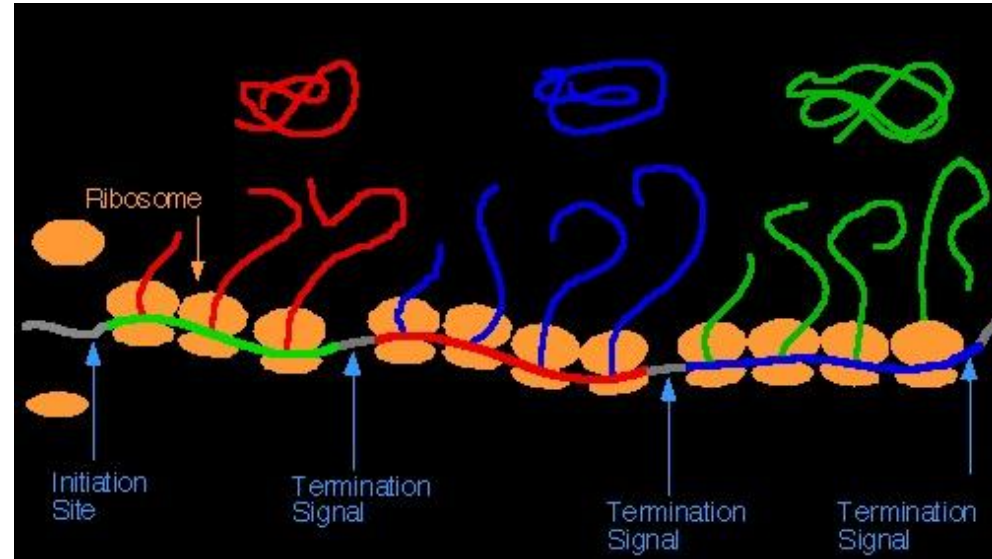


Lac Operon

- In truth, operation **regulation is rarely so simple**
- A bacterium's environment is too complex for its genes to be controlled by one signal
- Therefore few mechanism/signal needed to control one gene expression

Lac Operon

- The lactose (*lac*) operon → β -galactosidase (Z), galactoside permease (Y) and thiogalactoside transacetylase (A)
- Each of these linked genes is transcribed into one large mRNA molecule that contains multiple AUG and UAA codons for each cistron .
- Each prot is translated separately



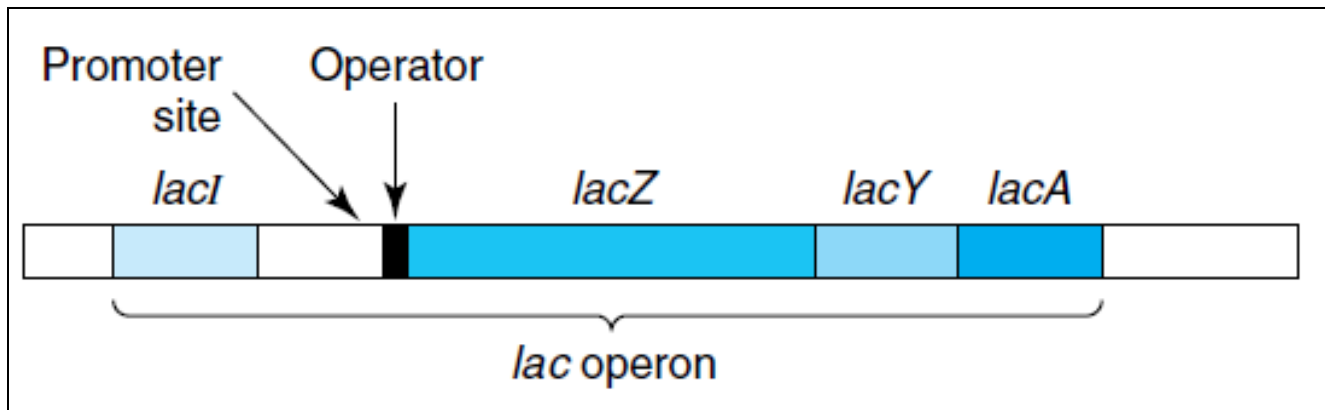
What happen to the expression of the lac operon when only lactose is present?

What happen to the expression of the lac operon when both glucose and lactose are present?

when only lactose is present..

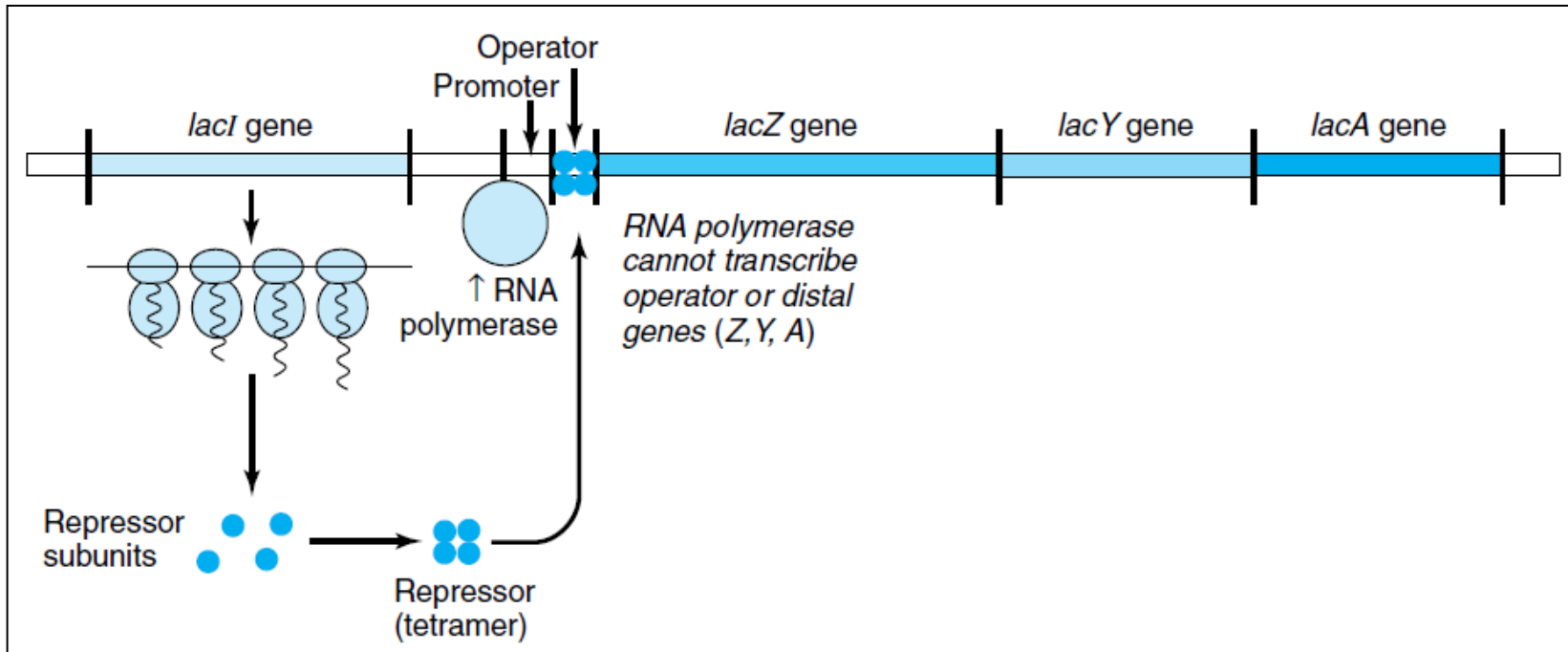
Two mechanisms are involved:

1. dissociation of lac repressor
2. role of catabolite gene activator protein (CAP)



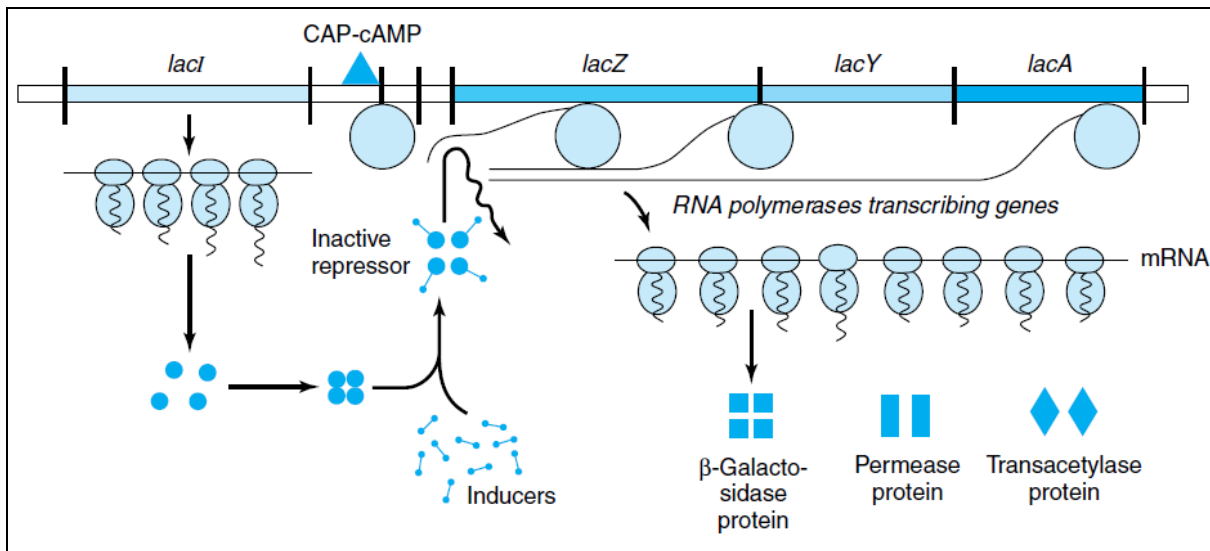
Absence of lactose

- *LacI* gene is expressed at a constant rate – producing **LacI repressor** (4su)- high affinity to operator
- **LacI repressor** bind to operator locus – prevents transcription of the operator locus and *lacZ*, *lacY* and *lacA* genes
- Thus, LacI repressor- **negative regulator**; in its presence, expression of *lacZ*, *lacY* and *lacA* is prevented



Presence of lactose

- Lactose- fx as **inducer**
- Small amounts of lactose are able to enter the cell even in the absence of permease
- **LacI** repressor have a high affinity for the inducer
- Binding of inducer to a repressor molecule attached to the operator locus- induces conformational change in the structure of the repressor-cause it dissociate from the DNA (now its affinity to DNA is 10^3 lower)
- RNAP that attached to the promoter will begin transcription process
- Thus, an inducer **derepresses the lac operon**

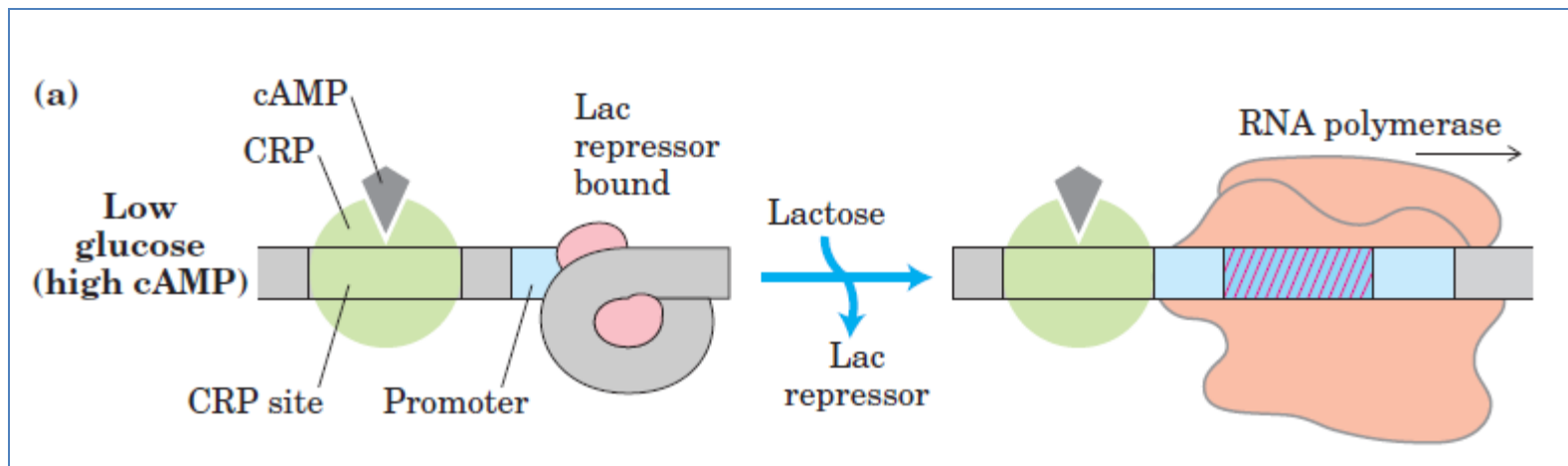


However, despite the presence of lactose...

- Despite the presence of lactose, transcription still progress poorly
- Need additional factor to enhance the transcription --- **catabolite gene activator**

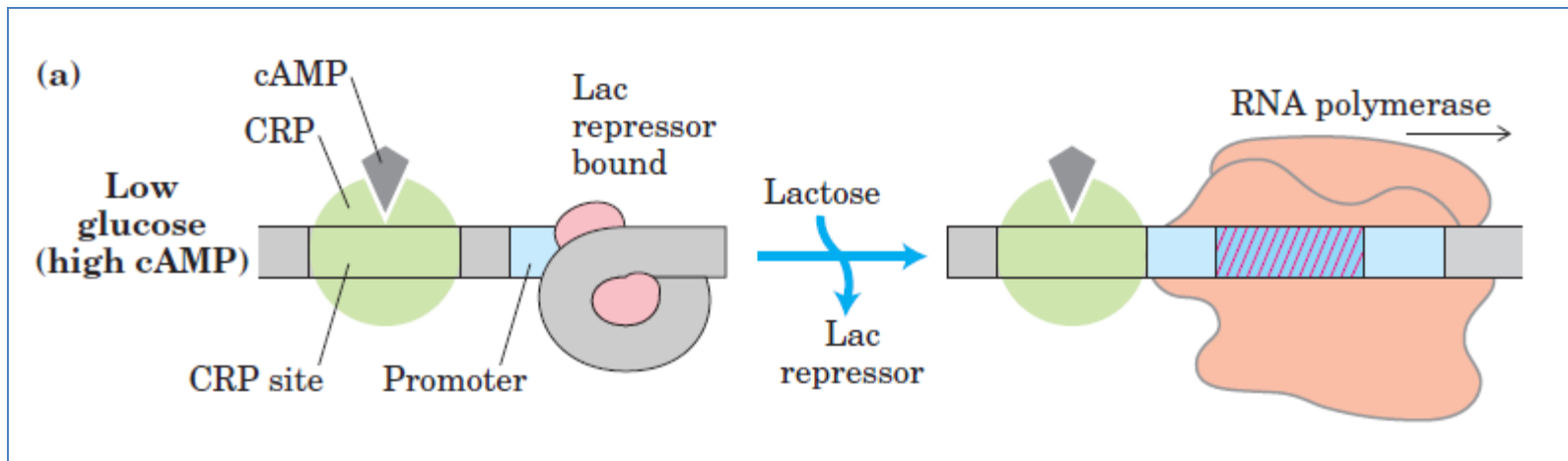
Role of Catabolite Gene Activator Protein (CAP)

- **Catabolite gene activator** protein is a dimer and acts as a positive regulator of many catabolic operons like the Lac Operon
- Attachment of RNAP to the promoter site requires the presence of CAP bound to cAMP
- Absence of glucose in the cell activates adenylate cyclase which catalyzes the synthesis of cAMP from ATP. cAMP will form CAP-cAMP complex
- This complex binds to promoter site immediately. This stimulates the initiation of transcription of the lac operon structural genes in the absence of repressors



Summary

- CAP-cAMP has little effect on the lac operon when the lac repressor is blocking transcription
- Dissociation of the repressor from the lac operator has little effect on transcription of lac operon
- **Both factor (CAP-cAMP) and dissociation of lac repressor work together**
- The open complex of RNAP and the promoter does not form readily unless CAP-cAMP is present

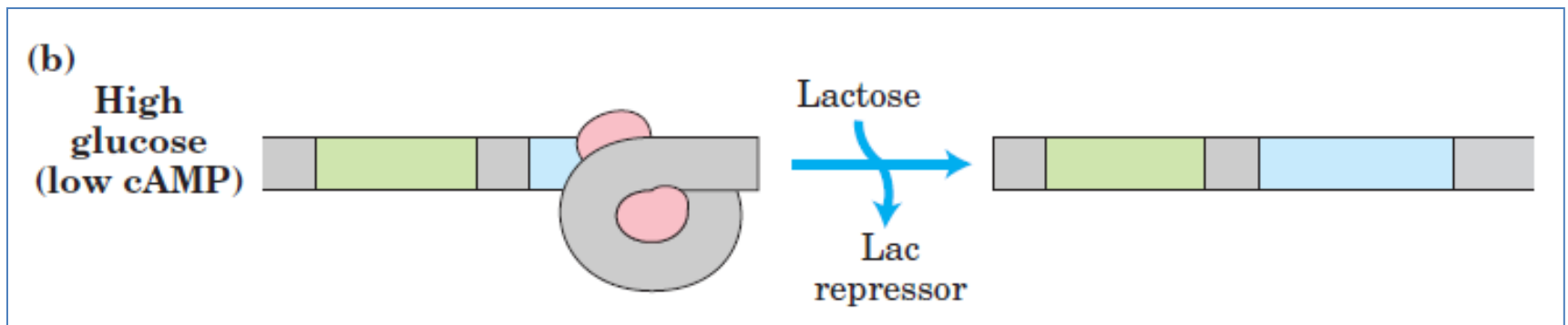


When both glucose and lactose are present..

- When E.coli is exposed to both lactose and glucose as sources of carbon – **it will first metabolize the glucose** and then temporarily stop growing until the genes of the lac operon become induced to metabolize lactose
- Glucose are **more preferred energy source**, can be metabolized directly in glycolysis
- Other sugars can also be used for energy, but need extra steps and enzymes
- Expressing the genes for proteins that metabolize sugars such as lactose is wasteful when glucose is abundant

When both glucose and lactose are present..

- Catabolite repression occur – restrict expression of genes required to metabolized lactose or other sugars when glucose is present
- In the presence of glucose, the synthesis of cAMP is inhibited
- The effect of glucose on CAP is mediated by the cAMP interaction.
- CAP binds to the promoter only when it is complexed with cAMP
- As (cAMP is declined) CAP binding to DNA declines, thereby decreasing the expression of the lac operon



Gratuitous inducers

- Isopropyl thiogalactoside (IPTG), a structural analogue of lactose can induce the lac operon by inactivating repressor molecules
- IPTG cannot be hydrolyzed by β -galactosidase

