Lactose Operon

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lactose operon

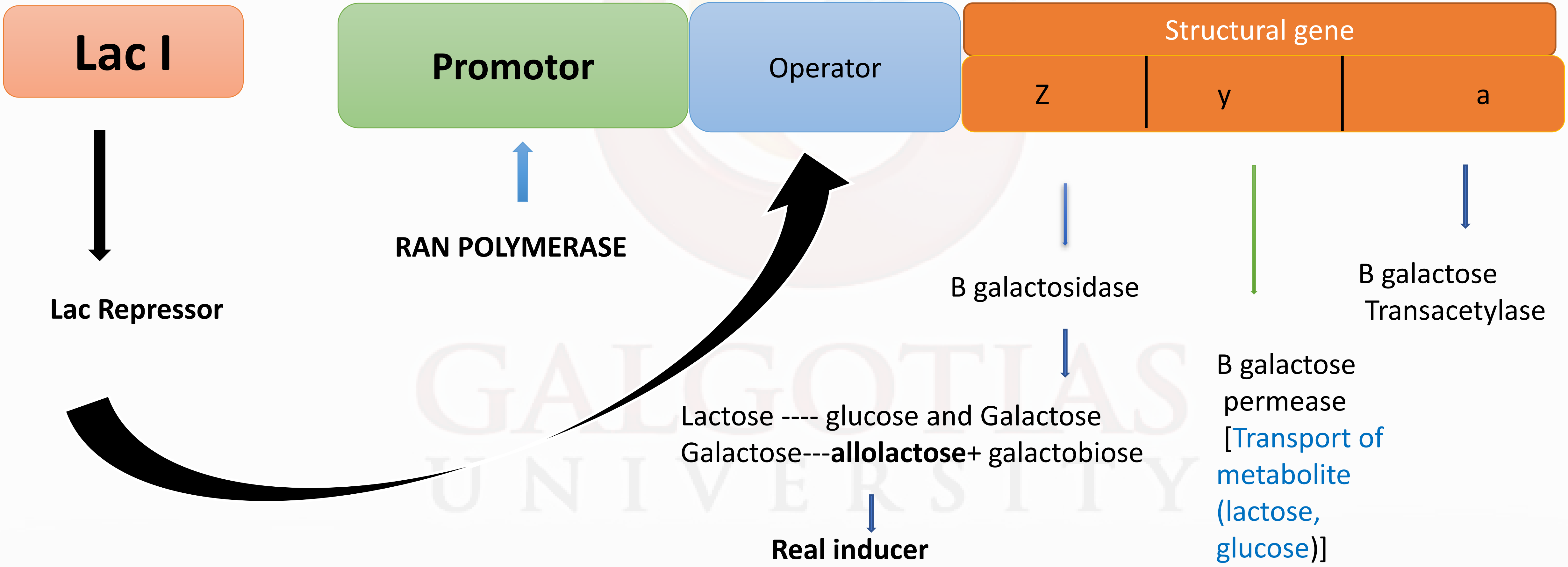
- The ***lactose* operon** (lac operon) is an operon required for the transport and metabolism of lactose in *E.coli* and many other enteric bacteria.

- Although glucose is the preferred carbon source for most bacteria, the *lac* operon allows for the effective digestion of lactose when glucose is not available through the activity of beta-galactosidase.

- This lactose metabolism system was used by François Jacob and Jacques Monod to determine how a biological cell knows which enzyme to synthesize.

- Their work on the lac operon won them the Nobel Prize in Physiology in 1965.

Lactose Operon model



Lactose operon

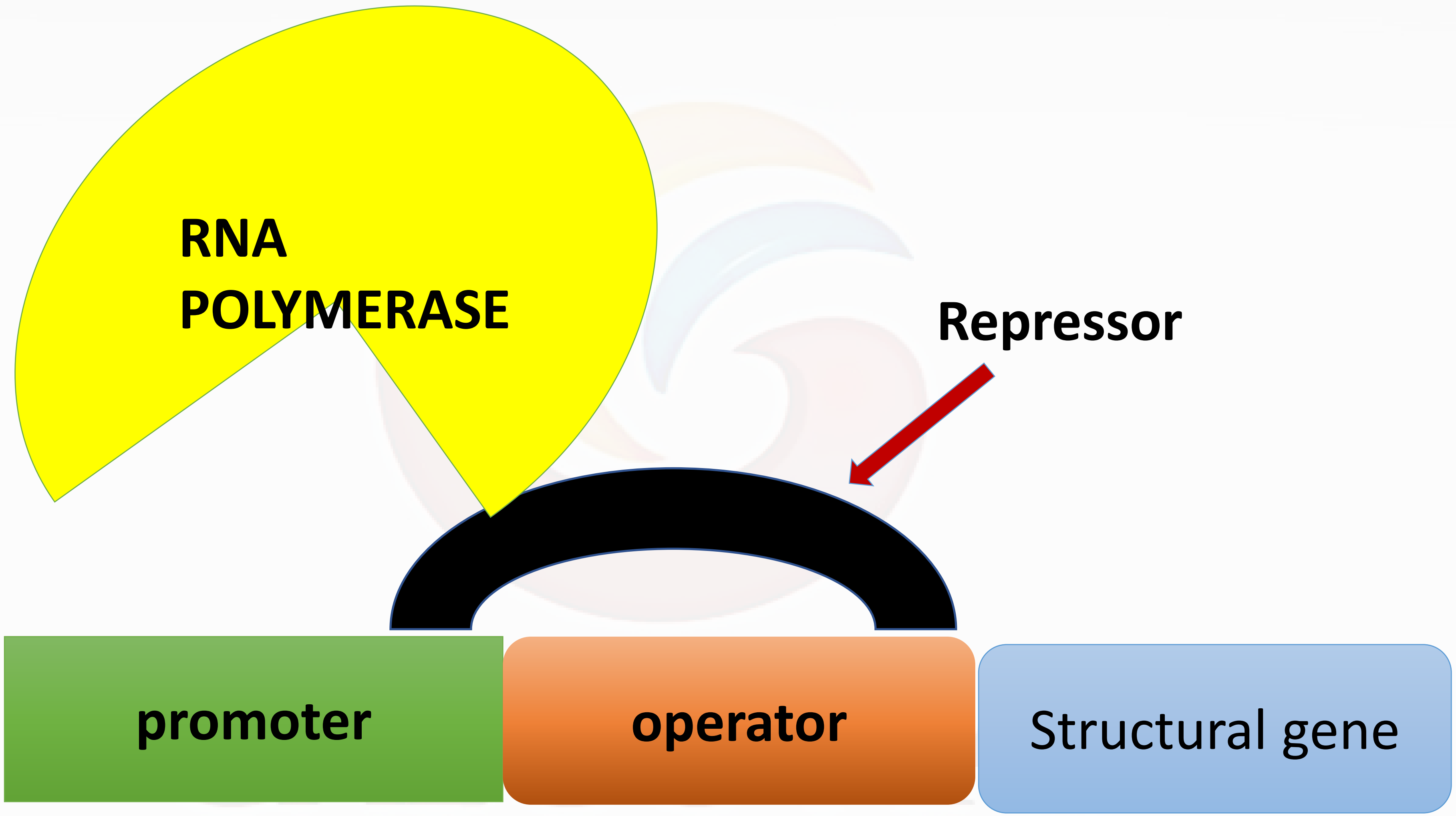
- The lactose utilization system of *Escherichia coli* is encoded by the *lac* operon, which consists of a regulatory promoter-operator region and three structural genes *lacZYA* (Mueller-Hill B et al., 1996).

- Whereas the gene products of *lacY* and *lacZ* are involved in uptake and cleavage (metabolism) of lactose (*lacY* encodes lactose permease, *lacZ* encodes β-galactosidase; the function of the transacetylase LacA is less clear (Chapman & Hall et al., 1996)

- If present, lactose is actively transported into the cell by lactose permease (Mueller-Hill B et al., 1996).

- Intracellular lactose is metabolized by LacZ in two distinct reactions: The disaccharide is either cleaved into the monosaccharides glucose and galactose, which are used for cell growth, or it is converted into its isomer allolactose, which represents the natural inducer of the *lac* operon (Chapman & Hall et al., 1996).

- Through sequestration of the LacI repressor, allolactose prevents binding of the repressor to the operator sites.



Ray Diagram showing Repressor binding on operator with small part of promoter inhibit binding RNA polymerase to promoter

- C AMP+ CAP------bind RNA polymerase----------activate transcription of structural gene

- Lactose------- activate transcription of structural gene

(**Allolactose** bind repressor and promote to separate from operator)

- Glucose----Inactivate transcription of structural gene

- No lactose-----repressor bind operator----Inactivate structural gene
- Isopropyl-β-D-thiogalactopyranoside (IPTG) is frequently used as an inducer of the *lac* operon for physiological work

- The proteins are not produced by the bacterium when lactose is unavailable as a carbon source.

- The *lac* genes are organized into an operon; that is, they are oriented in the same direction immediately adjacent on the chromosome and are co-transcribed into a single polycistronic mRNA molecule.

- (**Polycistronic mRNA** is a **mRNA** that encodes several proteins and is characteristic of many bacterial and chloroplast mRNAs.

- Polycistronic mRNAs consist of a leader sequence which precedes the first gene. The gene is followed by an intercistronic region and then another gene (Chapman & Hall et al., 1996)

- **The first control mechanism is the regulatory response to lactose,**

- **Condition1. If lactose is missing from the growth medium**, the repressor binds very tightly to a short DNA sequence just downstream of the promoter near the beginning of *lacZ* called the *lac operator*. The repressor binding to the operator interferes with binding of RNAP to the promoter, and therefore mRNA encoding LacZ and LacY is only made at very low levels.

- **Condition2.When cells are grown in the presence of lactose**, however, a lactose metabolite called allolactose, made from lactose by the product of the *lacZ* gene, binds to the repressor, causing an allosteric shift. Thus altered, the repressor is unable to bind to the operator, allowing RNAP to transcribe the *lac* genes and thereby leading to higher levels of the encoded proteins.

The second control mechanism is a response to glucose

1. The catabolite activator protein (CAP) homodimer to greatly increase production of β-galactosidase in the absence of glucose.

2. Cyclic adenosine monophosphate (cAMP) is a signal molecule whose prevalence is inversely proportional to that of glucose.

3. It binds to the CAP, which in turn allows the CAP to bind to the CAP binding site (a 16 bp DNA sequence upstream of the promoter) which assists the RNAP in binding to the DNA.

4. In the absence of glucose, the cAMP concentration is high and binding of CAP-cAMP to the DNA significantly increases the production of β-galactosidase, enabling the cell to hydrolyze lactose and release galactose and glucose.

- **If both glucose and lactose are present, the transport of glucose blocks the transport of the inducer of the *lac* operon?**

- Glucose is transported into the cell by the PEP-dependent phosphotransferase system.

- Transport of glucose is accompanied by its phosphorylation by $EIIB^{Glc}$,

  draining the phosphate group from the other PTS proteins, including $EIIA^{Glc}$.

Transport of lactose is accompanied by its phosphorylation by $EIIA^{Glc}$

- The unphosphorylated form of $EIIA^{Glc}$ binds to the *lac* permease and prevents it from bringing lactose into the cell.

- Therefore, if both glucose and lactose are present, the transport of glucose blocks the transport of the inducer of the *lac* operon.

Thanks