# COVENANT UNIVERSITY
# NIGERIA

*TUTORIAL KIT*
*OMEGA SEMESTER*

## PROGRAMME: BIOCHEMISTRY

**COURSE: BCH 423**

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# BCH 423: Metabolic Regulations

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1. Identify the physicochemical factors involve in regulating enzyme catalyzed reaction.
    a. **Substrate availability:**
    b. **Cofactor availability**
    c. **Product Removal**

2. List the types of substrate availability involvement in enzyme regulation?
    a. **Limitation by total substrate availability,**
    b. **Physical unavalability of substrate and,**
    c. **Chemical unavalability of substrate.**

3. Suggest the two main factors that must be met for a cofactor to relevant in enzyme regulation reactions.
    a. **The concentration of the cofactor involve must be reduce to very low level.**
    b. **The cofactor is specific for the pathway in question (i.e. not required by other pathways).**
4. The regulation of fatty acids oxidation may occur via variation in concentration of ……without necessarily affecting other metabolic pathways or processes.
   **Carnithine**
5. Carnithine can only penetrate the mitochondria membrane in the form of ……
   **Fatty acyl carnithine**
6. Identify the condition(s) that must be met for product removal to be relevant in the regulation of an enzyme catalised reaction.
   **The reaction of a pathway must be all at or close to equilibrium.**

7. With respect to the regulation of the conversion of pyruvate to lactate, increase ……. via the muscle will consequently increase the rate of …… removal and also increase the rate of conversion of pyruvate to lactate.

**Blood flow; Product 'lactate'**

8. What are the fators that regulate the rate of an enzyme catalise reaction under the law of mass action.
   **a.) Concentration (of Substrate, product, and cofactors).**
   **b.) pH.**
9. State the three conditions that can be approach that can be approach for the regulation of enzyme catalised reaction under the law of mass action.
    a. **Equilibrium condition**
    b. **Non equilibrium condition**
    c. **Steady state condition**
10. What is meant by 'regulatory enzyme'.
    **A regulatory enzyme is an enzyme in a biochemical pathway which, through its responses to the presence of certain other biomolecules, regulates the pathway's activity.**
11. Discuss the mode of operation of an inducible operon

**Ara Operon**

This operon is responsible for the breakdown of arabinose molecules in the cell.
**Arabinose is first converted to ribulose by arabinose isomerase**, the product of ***araA*gene**, then **phosphorylated by ribulokinase**, the product of ***araB*gene** and finally converted to **xylulose-5-phosphate via ribulose-5-phosphate epimerase**, the product of ***araD*gene**.

The last product enters the pentose phosphate pathway and yields reducing power or provides precursor metabolites for glycolysis.

These 3 structural genes have a single promoter, namely ***pBAD***and are regulated by the product of ***araC*gene**, designated as **AraC**.
If arabinose is present:

• **arabinose binds to AraC protein**

• **AraC-arabinose binds to the *araI* site** (the inducer site)

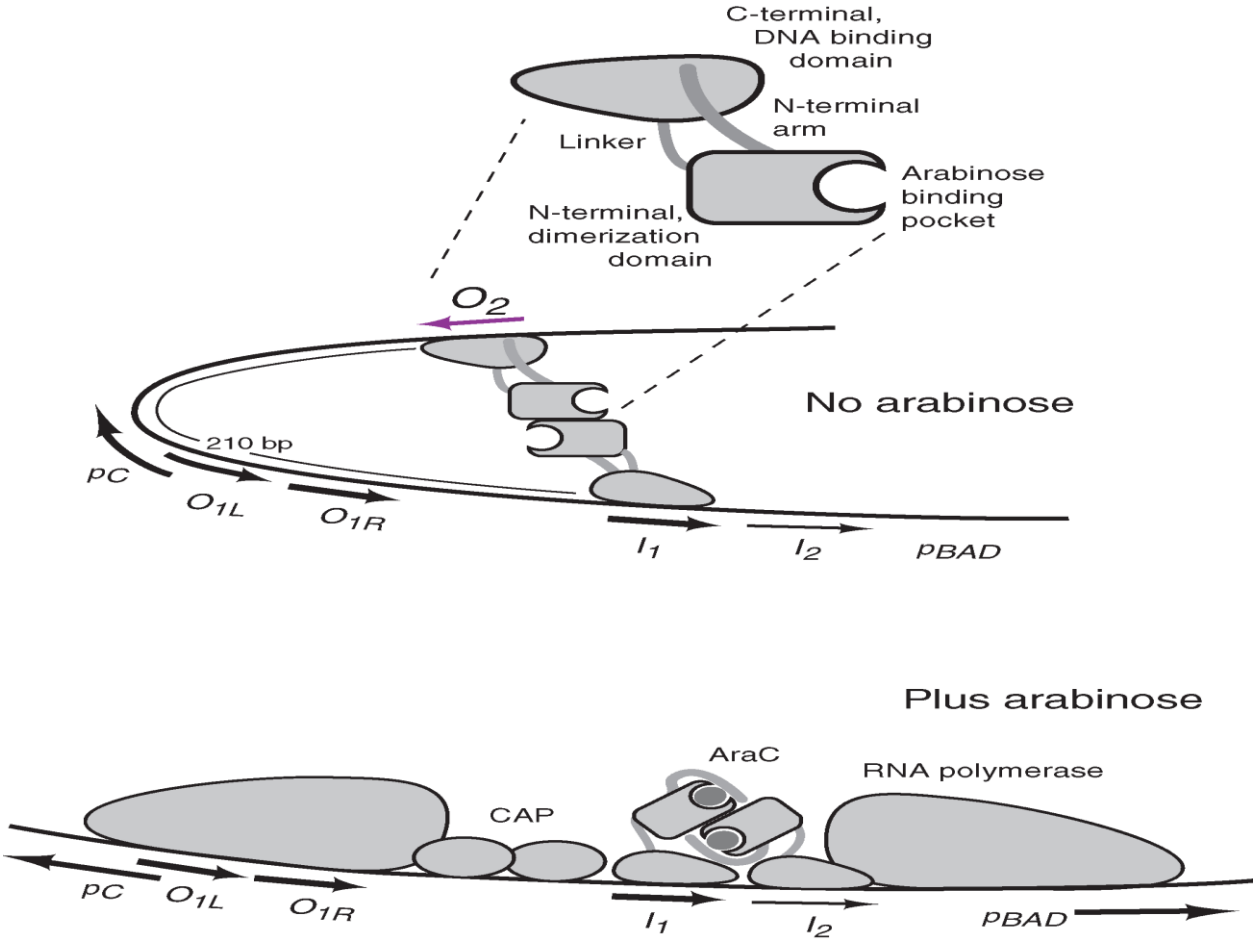
and glucose is absent:

• **CAP-cAMP binds to the CAP site**

• **RNA polymerase binds ara BAD promoter**

• **Genes for arabinose enzymes B, A, and D are**

**transcribed, translated, arabinose is metabolized**




12. **What are the regulatory patterns of the lac operon**

**GLUCOSE ONLY**

If glucose is present, [cAMP] is low, doesn't bind to CAP which can't bind to promoter and doesn't allow transcription.**Glucose prevents transcription by pulling the CAP activator off.**

**LACTOSE ONLY**

A small amount of the lactose gets into the cell, and affects regulation of the operon: The lac repressor is still synthesized. The repressor can bind to lactose. When it does so, the repressor undergoes a **conformational change** (change of shape). Molecules that change shape when they bind to another molecule are called allosteric molecules. When it undergoes the conformational change, the lac repressor is unable to bind to the operator region. RNA polymerase is therefore not blocked, and is able to transcribe the genes of the operon. The enzymes encoded by those genes will be produced, the lac permease will transport more lactose into the cell, and beta-galactosidase will cleave the lactose into glucose and galactose, which can then be further metabolized by other enzymes, producing energy for the cell. Lactose, therefore, is able to **induce** the synthesis of the enzymes necessary for its metabolism (by preventing the action of the repressor). As such, lactose is the inducer of the lac operon. The bottom line for the lac operon, then, is that when lactose is absent, lactose-metabolizing enzymes are not produced, and when lactose is present, those enzymes are produced.

**BOTH GLUCOSE AND LACTOSE**

If both glucose and lactose are around, the bacterium wants to turn off lactose metabolism in favour of glucose metabolism. cAMP level diminishes.

**NONE**

The lac operon is repressed because there is no inducer**.**

**13. Discuss the transcriptional regulation of cholesterol biosynthesis**

**TRANSCRIPTIONAL REGULATION**

Activation of transcriptional control occurs through the regulated cleavage of the membrane-bound transcription factor sterol regulated element binding protein, SREBP. Transcriptional control requires the presence of an octamer sequence in the gene termed the sterol regulatory element, SRE-1. It has been shown that SREBP is the transcription factor that binds to SRE-1 elements. Full-length SREBPs have several domains and are embedded in the membrane of the endoplasmic reticulum (ER). The N-terminal domain contains a transcription factor motifof the basic helix-loop-helix (bHLH) type that is exposed to the cytoplasmic side of the ER. There are 2 transmembrane spanning domains followed by a large C-terminal domain also exposed to the cytosolic side. The C-terminal domain (CTD) interacts with a protein called SREBP cleavage-activating protein (SCAP). SCAP is a large protein also found in the ER membrane and contains at least 8 transmembrane spans. The C-terminal portion, which extends into the cytosol, has been shown to interact with the C-terminal domain of SREBP. The regulation of SREBP activity is further controlled within the ER by the interaction of SCAP with insulin regulated protein (Insig). When sterols are scarce, SCAP does not interact with Insig. Under these conditions the SREBP-SCAP complex migrates to the Golgi where SREBP is subjected to proteolysis. The cleavage of SREBP is carried out by 2 distinct enzymes. The regulated cleavage occurs in the lumenal loop between the 2 transmembrane domains. This cleavage is catalyzed by site-1 protease, S1P. The function of SCAP is to positively stimulate S1P-mediated cleavage of SREBP. The second cleavage, catalyzed by site-2 protease, S2P, occurs in the first transmembrane span, leading to release of active SREBP. In order for S2P to act on SREBP, site-1 must already have been cleaved. The result of the S2P cleavage is the release of the N-terminal bHLH motif into the cytosol. The bHLH domain then migrates to the nucleus where it will dimerize and form complexes with transcriptional coactivators leading to the activation of genes containing the SRE motif. To control the level of SREBP-mediated transcription, the soluble bHLH domain is itself subject to rapid proteolysis.

**14. How is the catabolite control protein A relevant in metabolic regulation**

CcpA is a member of the LacI/GalR family of transcription factors. It is a master regulator in gram positive bacteria. It shows a weak affinity for its cognate operator site known as CATABOLITE RESPONSIVE ELEMENT (*cre*). Therefore, CcpA needs activation for effective binding to its operator. CcpA is allosterically regulated principally by a protein-protein interaction.CcpA is activated by interaction with the phosphorylated form of Hpr (S. *xylosus*) and Crh (B. *subtilis*).The phosphorylated proteins are abundant in response to the abundance of glucose and fructose 1,6-bisphosphate in the cell.Small molecules such as G6P and FBP are also known allosteric effectors that fine-tune CcpA. CcpA regulated genes include genes expressing the utilization of Lactose,Xylose, Maltose.*Cre*sites serving as the operator for CcpA are found in their promoter sequence.

15. Discuss the operation of catabolite repressor / activator (CRA) protein
16. Write briefly on the regulation of purine nucleotide biosynthesis
17. How is the synthesis of DNA from RNA regulated
18. Discuss exhaustively the ara operon
19. Explain explicitly a complication that can arise from pyrimidine nucleotide biosynthesis
20. What is leschnyhansyndrom