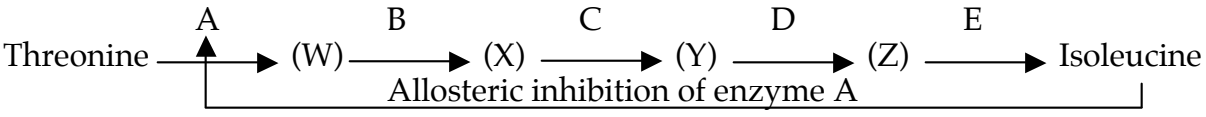
Control of Metabolic Processes

As described earlier, the metabolic processes occurring within living organisms (glycolysis, respiration, photophosphorylation, etc.), are dependent upon the enzymes present within cells, and these are determined by the genes present, or the information carried within DNA molecules. Whether or not a specific cell is using one or another metabolic process is determined by regulatory mechanisms functioning at various levels. In this section, two mechanisms for controlling metabolism will be described, one functioning at the enzyme level, and the other functioning at the gene level.

Feedback inhibition:

Most enzymes are proteins (though some are RNA molecules), so are composed of multiple amino acids connected together in long chains. They typically have **tertiary structure** (are 3-dimensional), and many are **quaternary** (are composed of multiple polypeptide chains). As described earlier, enzymes are specific in their action, and bind with their substrate or reactant molecules through regions on their surfaces called reactive sites or binding sites. Enzymes can also be inhibited, i.e., the catalytic activity of enzymes can be blocked through either **competitive** or **allosteric** inhibition.

Feedback inhibition is a regulatory mechanism involving the **allosteric inhibition** of one or more enzymes (usually the first or second) involved a common metabolic pathway. The inhibitor is usually the **end-product** of the pathway, so this mechanism can also be called **end-product inhibition**. In bacteria the biosynthesis of isoleucine (an amino acid) involves threonine (also an amino acid) as a substrate and a metabolic pathway with five steps catalyzed by five different enzymes. The enzymes involved are represented by the letters A-E, and the metabolic intermediates by the letters W-Z, in the diagram below.



When isoleucine begins to accumulate within the cytoplasm, it acts as an **allosteric inhibitor** of the first enzyme in the pathway (enzyme A), and effectively shuts down isoleucine synthesis. Because the end-product of the pathway acts to reduce its own production, this is an example of **negative feedback**. Feedback or end-product inhibition occurs within both eukaryotic and prokaryotic cells and allows organisms to control metabolic processes relatively quickly (exerts rapid control). This mechanism is also reversible, because when the concentration of end-product is decreased, the first enzyme is no longer inhibited. Though used extensively, this mechanism is not efficient in terms of energy conservation, because when metabolic pathways are inhibited, enzymes are inactive. This means the cell had to expend considerable energy in the formation of m-RNA molecules and polypeptides to establish a metabolic pathway no longer being used. A more efficient means of controlling metabolism can be exerted at the gene level.

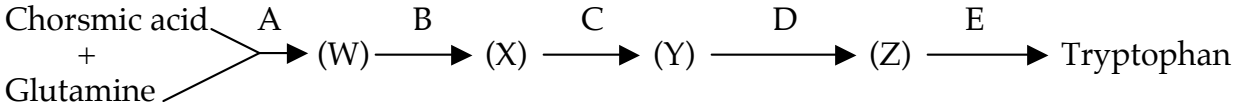
Genetic control:

In prokaryotic cells, the genes coding for enzymes involved in a common metabolic pathways are often arranged together within specific regions of the chromosome called **operons**. An **operon** is a segment of DNA (a nucleotide sequence) containing a series of structural genes and the control elements regulating the transcription of those genes. The "control elements" typically include a **promoter site** or sequence and a region known as the **operator site** (an attenuator site may also be present, but will not be included here). Recall that promoter sites are nucleotide sequences recognized by the **sigma factors** of **RNA polymerase**. Promoters interacting with sigma factors determine where transcription will begin and in which direction it will proceed along DNA molecules. The operator site functions like an "on-off" switch, and is influenced by DNA-binding proteins called **repressers**. When a represser protein is bound to the operator, transcription is blocked (repressed), but if the represser is not bound, transcription is allowed to proceed.

There are many different metabolic pathways controlled by operons in different types of bacteria, but two such systems found within **E. coli** cells are commonly used as examples of genetic control mechanisms.

Tryptophan biosynthesis – Control involving a repressible operon:

The operon controlling **tryptophan biosynthesis** in *E. coli* is commonly used as an example of a **repressible operon**, i.e., one in which transcription is usually occurring, but can be repressed or "turned off". **Tryptophan** is an amino acid synthesized from glutamine and chorismic acid by means of a metabolic pathway involving five enzymes, as diagrammed below.



In this pathway, enzymes are designated by the letters "A-E", and metabolic intermediates formed within the pathway are designated by the letters "W-Z". The genes coding for the enzymes above are arranged together within the **tryptophan biosynthesis operon**. This operon includes a promoter site, an operator site and an attenuator site, but only the first two control elements will be described here.

Promoter	Operator	Attenua	Gene A	Gene B	Gene C	Gene D	Gene E

The **promoter site** is the nucleotide sequence **sigma factor** binds with to start transcription. The **operator site** is the nucleotide sequence the **represser protein** binds with to block or **repress transcription**. In this case, the gene coding for the represser protein is not part of the operon, but is located in a different region of the chromosome. The represser protein associated with the tryptophan biosynthesis operon is **inactive alone**, so cannot bind with the operator site. Under most circumstances this operon is "on", i.e., transcription is allowed to proceed. While the operon is active, transcription results in the formation of m-RNA molecules that travel to the ribosome and code for the production of enzymes A-E. The biosynthesis is then allowed to proceed and tryptophan levels within the cell increase.

Tryptophan serves as a **corepresser**, i.e., a compound that can bind with the inactive represser protein and activate it (much like coenzymes and cofactors activate enzymes). The tryptophan-represser complex can then bind with the operator site and block transcription of the genes present within the operon.

This mechanism is very efficient in terms of energy conservation because when tryptophan concentrations become high, the cell will not just inhibit the enzymes involved in tryptophan synthesis, it will stop making the m-RNA molecules necessary to form the polypeptides needed to run the tryptophan biosynthesis pathway.

Lactose utilization – Control involving an inducible operon:

Lactose utilization in *E. coli* is controlled by an **inducible operon**, i.e., one in which transcription is usually repressed or "off", but can be **induced** or "turned on". This operon (often referred to as the ***lac* operon**) includes three structural genes, a promoter and an operator. A regulatory gene near the lac operon promoter site codes for a represser protein that is **active alone**. The represser protein is **constitutive**, so is always being made; consequently, under most circumstances the structural genes within the operon are not being transcribed. Note – When *E. coli* cells are living within the intestines of adult cows, there is no lactose available, so making enzymes to catabolize it would be a waste of energy. The *E coli* cells conserve energy by repressing the transcription of the *lac* operon genes.

Promoter	*lac*I	Promoter	Operator	*lac*Z	*lac*Y	*lac*A

In this diagram, the *lac*I gene is the regulatory gene coding for the represser protein, promoter sites are nucleotide sequences where sigma factors bind to begin transcription, the operator site is where the active represser protein binds to block (repress) transcription and the structural genes *lac*Z, *lac*Y and *lac*A code for enzymes associated with lactose utilization. Since the represser is constitutive, and active alone, transcription of the three structural genes is usually being repressed, but not entirely. Why not?

The structural genes within the *lac* operon code for enzymes. Two of these, *lac*Y and *lac*Z encode enzymes directly involved in lactose utilization, i.e., **β-galactoside permease** (an enzyme allowing lactose to enter the cell) and **β-galactosidase** (an enzyme that breaks lactose into glucose and galactose), respectively. The third gene, *lac*A, codes for the enzyme **thiogalactoside transacetylase**. This enzyme catalyzes a chemical reaction converting lactose into **allolactose**. Allolactose is significant, because it serves as the *inducer* for the lac operon. When allolactose is abundant, it binds with the represser protein and **inactivates** it, i.e., changes its configuration so it can no longer bind with the operator site. With the represser removed, transcription is allowed to proceed.

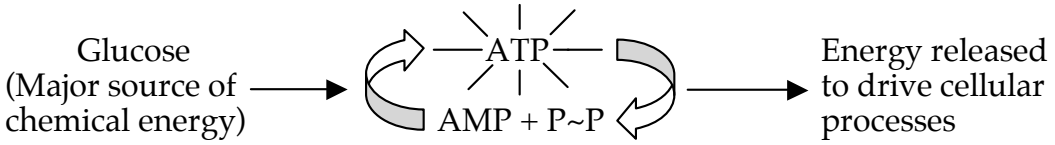
At this point, one might well ask the following question. If the enzymes encoded by the genes of the *lac* operon are not being made, lactose cannot enter the cell and allolactose cannot be formed; therefore, how can this operon ever be induced? Apparently the operon is "off" like a leaky faucet, some transcription occurs even when the operon is being repressed. This is because although the represser protein binds tightly to the operator site, it only **interferes** with RNA polymerase, and doesn't completely block transcription.

Although inducers (e.g., allolactose) partially control the transcription of **inducible genes**, **inducible operons** are also regulated by a mechanism called **catabolite repression**. This mechanism involves **cyclic-AMP** as a regulatory molecule. Note – The *lac* operon is not the only inducible operon in *E. coli* cells; other operons have similar control mechanisms.

Catabolite repression:

Catabolite repression is a mechanism allowing bacteria such as *E. coli* to utilize constitutive enzymes in favor of inducible ones. This mechanism adds a second layer of control and improves the efficiency of inducible operons, e.g., the "leaky" systems as described above.

A **catabolite** is any substance a cell can catabolize (break down) to release energy. Glucose is a common catabolite, and as described earlier can be broken down by various metabolic pathways. Since *E. coli* cells are **facultatively anaerobic**, they can use either fermentation or respiratory pathways to catabolize glucose. In either case, when glucose is available, **ATP** is being made, and energy is available to drive cellular processes. The flow of energy through *E. coli* cells is **vastly simplified** in the diagram presented below.



In this diagram, the covalent bonds within glucose molecules are being broken through catabolic processes (recall fermentation and cellular respiration), and the energy released is being used to convert AMP + P~P into ATP. Since ATP is the energy currency of the cell, it is constantly being broken down and the energy released is used to drive cellular processes, e.g., active transport, flagellar motion, replication, transcription, etc. The catabolism of ATP yields AMP + P~P. This diagram is **not accurate**, because as was explained earlier, ATP is formed from ADP + Pi (adenosine diphosphate and inorganic phosphate); however, the diagram is accurate to this extent: **When ATP levels increase, c-AMP levels decrease.** When associated with glucose catabolism, this occurs because the transport of glucose into cells inhibits the activity of **adenylate cyclase**, the enzyme responsible for converting ATP into cyclic-AMP.

In its cyclic form, 3'-5'-cyclic adenosine monophosphate (**cyclic-AMP**), AMP serves as a **regulatory molecule,** i.e., as a "second messenger" or a molecule involved in **signal transduction**. Cyclic-AMP can bind with a protein called **catabolite activating protein** (CAP) also called cyclic-AMP receptor protein (CRP), to form a complex that can interact with DNA. The cyclic-AMP-CAP complex binds to DNA at a site very near the promoter site on the *lac* operon and makes it easier for the sigma factor of RNA polymerase to bind, i.e., it **enhances the promotor site** (makes it more attractive to sigma factor). When cyclic-AMP levels are high, the c-AMP-CAP complex is bound to DNA and the structural genes of the *lac* operon are transcribed (transcription is allowed to occur), assuming lactose is available and some of it has been converted into allolactose. When the lac operon promoter site is not "enhanced", sigma factor is only weakly attracted to it.

So, if *E. coli* cells are placed into a TSI slant, which of the three sugars present will they use first, and why? Although the *lac* operon is "leaky" in terms of the control exerted upon it by the represser protein, very little transcription of the *lac* operon structural genes will occur as long as glucose is available to the *E. coli* cells. The enzymes involved in glucose catabolism are **constitutive**, and not under control of a represser. The *E. coli* will use glucose first, and only when glucose is no longer available, will they use lactose.