FACULTY OF ENGINEERING & TECHNOLOGY

DEPARTMENT OF BIOTECHNOLOGY

Feedback Inhibition

•**Feedback inhibition** (or end product inhibition) is a mechanism for the **inhibition of preformed enzymes** that is seen primarily in the regulation of whole biosynthetic pathways, e.g. pathways involved in the synthesis of the amino acids.

•Such pathways usually involve many enzymatic steps, and the final (end) product is many steps removed from the starting substrate. By this mechanism, the final product is able to feed back to the first step in the pathway and to regulate its own biosynthesis.

•In feedback inhibition, the end product of a biosynthetic pathway inhibits the activity of the first enzyme that is unique to the pathway, thus controlling production of the end product. The first enzyme in the pathway is an allosteric enzyme. Its allosteric site will bind to the end product (e.g. amino acid) of the pathway which alters its active site so that it cannot mediate the enzymatic reaction which initiates the pathway. Other enzymes in the pathway remain active, but they do not see their substrates.

•The pathway is shut down as long as adequate amounts of the end product are present. If the end product is used up or disappears, the inhibition is relieved, the enzyme regains its activity, and the organism can resume synthesis of the end product.

•Thus, if a *E. coli* bacterium swims out of a glucose minimal medium into milk or some other medium rich in growth factors, the bacterium can stop synthesizing any of the essential metabolites that are made available directly from the new environment.

•One of the most intensely studied bacterial pathways is the pathway of tryptophan biosynthesis (Figure 1)

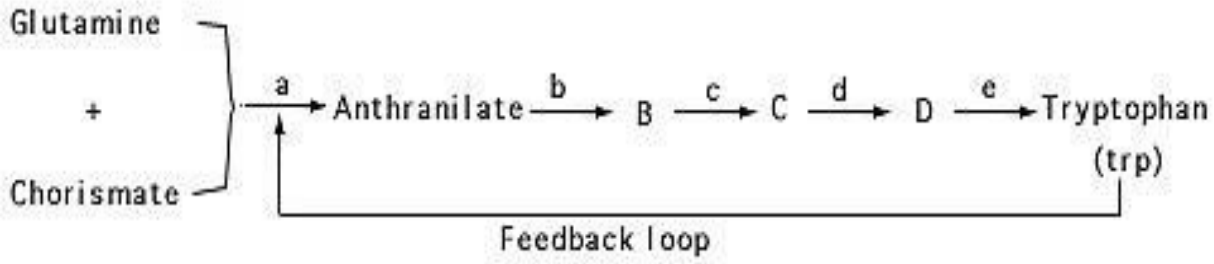

Figure: The pathway of tryptophan biosynthesis in *E. coli*. The pathway is regulated by the process of feedback inhibition. Tryptophan (trp), the end product of the pathway, is the effector molecule that binds to the allosteric site of Enzyme a, the first enzyme in the pathway. When trp is bound to the enzyme the catalytic (active) site of Enzyme a is altered so that it is unable to react with its substrates and the synthesis of anthranilate is inhibited.

The pathway of tryptophan biosynthesis is regulated by feed back inhibition. Tryptophan is the effector molecule for allosteric enzyme a. When the end product of the pathway (tryptophan) attaches to enzyme **a**, the enzyme is inactive and can no longer join glutamine and chorismic acid into anthranilate.

If tryptophan is disjoined from the enzyme the pathway is resumed, and tryptophan synthesis will continue. Tryptophan biosynthesis is also regulated at a genetic level by the processes of enzyme repression (below) and attenuation.

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In the case of feedback inhibition (above), the signal molecule, tryptophan, is a negative effector of Enzyme **a** in the pathway of tryptophan biosynthesis, because when it binds to Enzyme **a**, it inactivates the enzyme.

In enzyme repression (below) tryptophan is a signal molecule that acts as a positive effector of the trp repressor protein because when it binds to the repressor it activates the protein, so that it binds to the trp DNA.

If a metabolic pathway branches, leading to the synthesis of two amino acids, each end product (amino acid) can control its own synthesis without affecting the other (Figure).

For example, the amino acids proline and arginine are both synthesized from glutamic acid. Each amino acid can regulate the first enzyme unique to its own synthesis without affecting the other, so that a surplus of arginine will not shut off the synthesis of proline and vice versa.

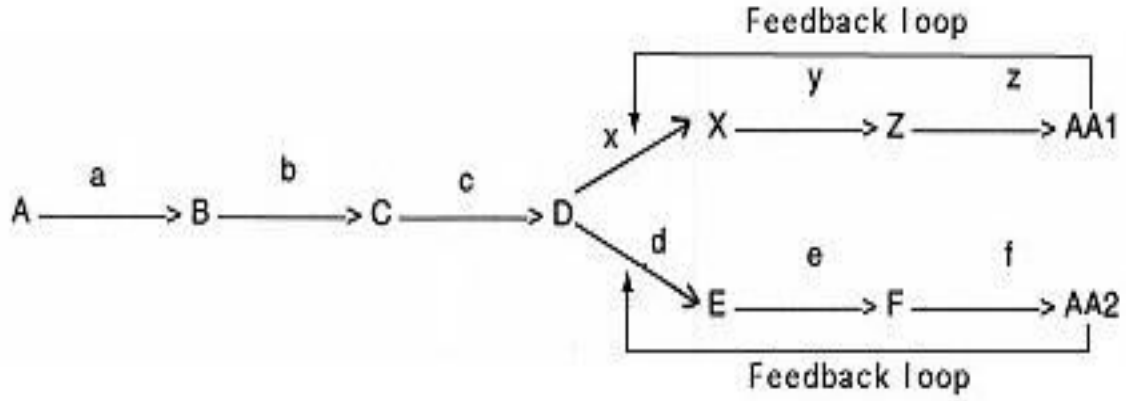

Figure. Generalized scheme for regulation of a branched metabolic pathway by the process of feedback inhibition.

Enzyme Repression

•Enzyme repression is a form of negative control (down-regulation) of bacterial transcription. This process, along with that of enzyme induction, is called negative control because a regulatory protein brings about inhibition of mRNA synthesis which leads to decreased synthesis of enzymes.

•Although feedback inhibition shuts off synthesis of the end product of a pathway, it still allows some waste of energy and carbon if the cell continued to manufacture enzymes for which it has no use.

•It is the process of enzyme repression that prevents the synthesis of the enzymes concerned with the synthesis of that particular end product. In the case of the pathway of tryptophan biosynthesis , the end product of the pathway, tryptophan, serves as an effector molecule that can shutdown the synthesis of the Enzymes a, b, c, d, and e that are concerned with tryptophan biosynthesis. This results in saving of many molecules of ATP which must be expended during protein synthesis, and it conserves amino acid precursors for synthesis of other proteins.

•The process is slower to act than is feedback inhibition (which acts immediately) because pre-existing enzymes have to be diluted out as a result of cell division before its effects are seen.

•The genes for tryptophan biosynthesis in *Escherichia coli* are organized on the bacterial chromosome in the **tryptophan operon (trp operon)**.

•An **operon** is a cluster of genes that are controlled by the same elements and which are coordinately transcribed and translated. The trp operon consists of a Promoter (P) region, an Operator (O) region, an Attenuator (A) region, and the five structural genes for the enzymes involved in tryptophan biosynthesis (Trp A-E)

•The components of the trp operon and its control elements are described in Figure

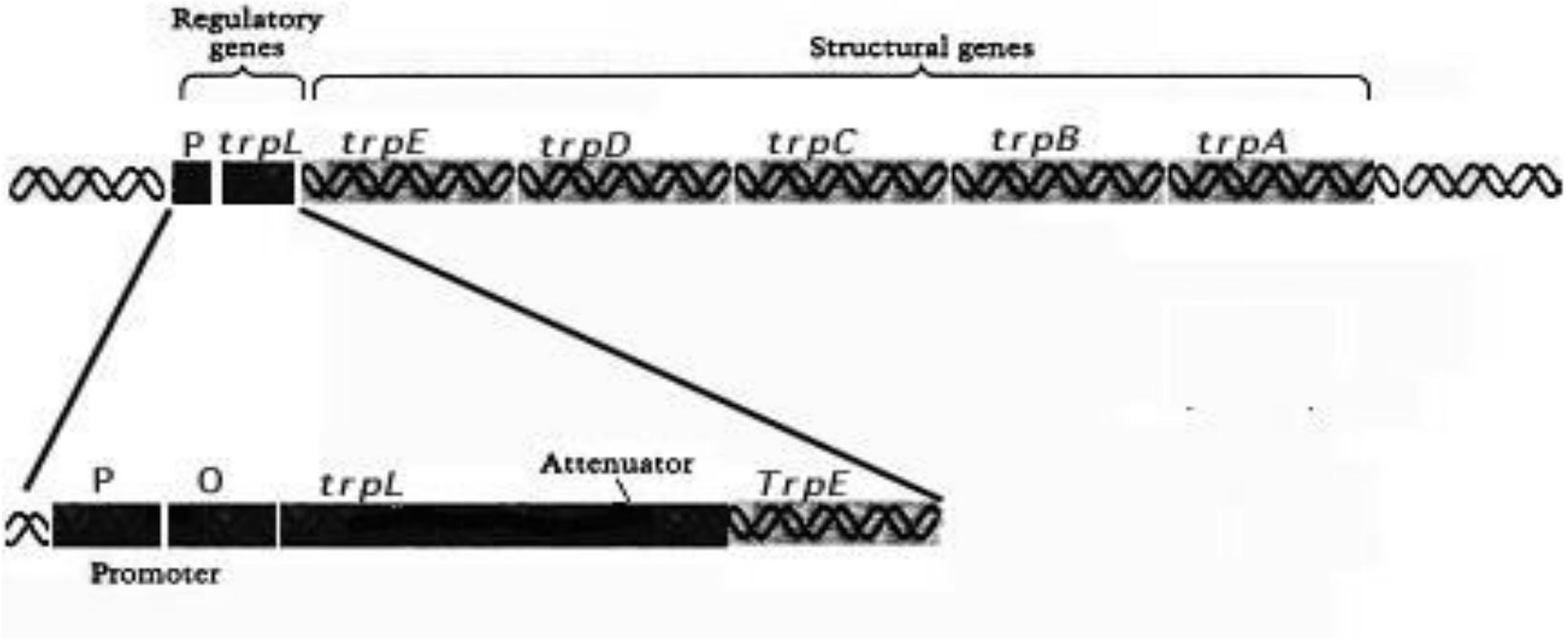

Figure . Genetic organization of the Trp operon and its control elements

R = Regulatory gene that encodes for the trp Repressor protein that is concerned with regulating the synthesis of the 5 gene products. An active repressor binds to a specific nucleotide sequence in the operator region and thereby blocks binding of RNAp to the promoter to initiate transcription.

O = Operator specific nucleotide sequence on DNA to which an active Repressor binds.

P = Promoter specific nucleotide sequence on DNA to which RNA polymerase binds to initiate transcription. If the repressor protein binds to the operator, RNAp is prevented from binding with the promoter and initiating transcription. Therefore, none of the enzymes concerned with tryptophan biosynthesis are synthesized.

A = Attenuator DNA sequence which lies between the operator and the structural genes for trp biosynthesis. The attenuator is a barrier that RNA polymerase must traverse if it is to transcribe the genes for tryptophan biosynthesis. In the presence of trp, most RNAp molecules fall off the DNA before transcribing the trp genes. In the absence of trp, RNAp is able to traverse the attenuator region to successfully transcribe the trp genes.

Trp A, B, C, D, E = Structural genes for enzymes involved in tryptophan biosynthesis.
Trp = tryptophan end product of the biosynthetic pathway. When combined with the repressor protein the Repressor is active. Trp is called a corepressor.

•The trp operon is regulated by a regulatory gene (Trp L) associated with the trp promoter. The product of the Trp L gene is the trp Repressor, an allosteric protein which is regulated by tryptophan. The Repressor is produced constitutively in small amounts in an inactive form.

•When the Repressor combines with tryptophan it becomes activated and binds to the DNA of the trp operon in such a way that it blocks the transcription of the structural genes for tryptophan.

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•Thus, in the presence of tryptophan, transcription of the genes for tryptophan biosynthesis are repressed (tryptophan is not produced), while in the absence of tryptophan, the genes for tryptophan biosynthesis can be transcribed (tryptophan is produced).

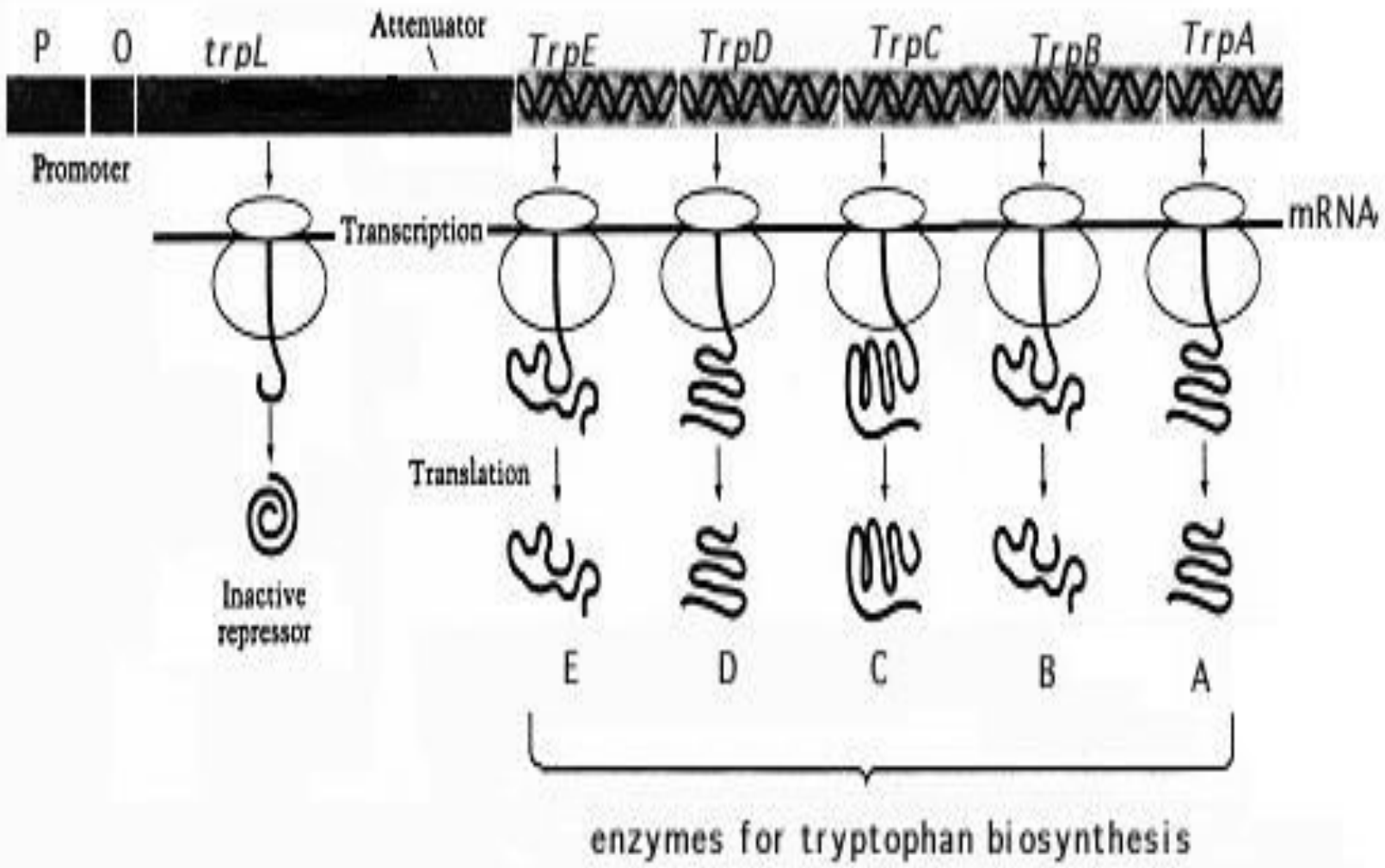

Figure. Derepression of the trp operon. In the absence of trp the inactive repressor cannot bind to the operator to block transcription. The cell must synthesize the amino acid.

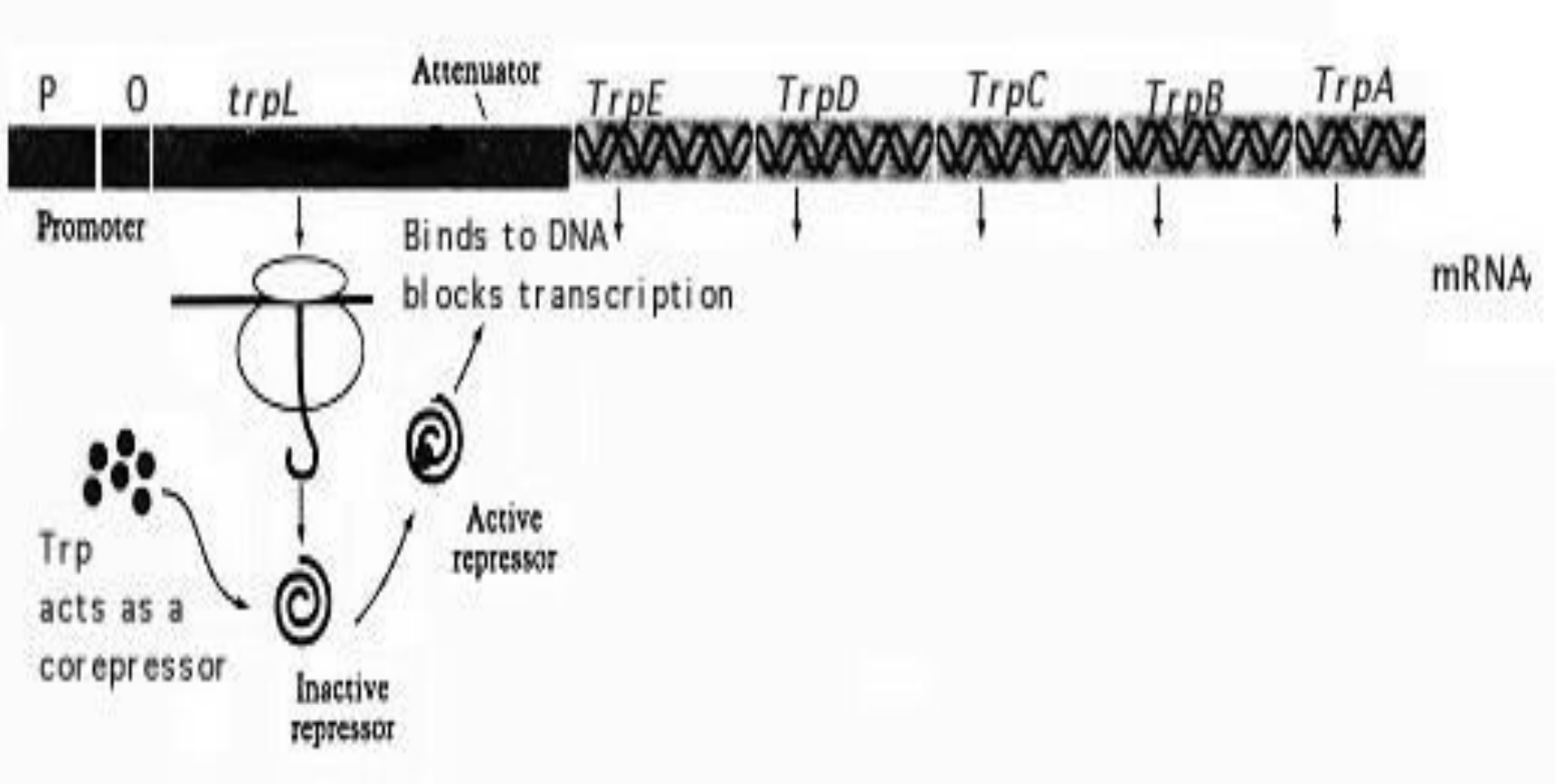

Figure . Repression of the trp operon. In the presence of tryptophan the trp operon is repressed because trp activates the repressor. Transcription is blocked because the active repressor binds to the DNA and prevents binding of RNA polymerase.