

METABOLIC PATHWAYS FOR THE BIOSYNTHESIS OF INDUSTRIAL MICROBIOLOGY PRODUCTS

In order to be able to manipulate microorganisms to produce maximally materials of economic importance to humans, but at minimal costs, it is important that the physiology of the organisms be understood as much as is possible.

A yeast cell will divide and produce CO₂ under aerobic conditions if offered a solution of glucose and ammonium salts. The increase in cell number resulting from the growth and the bubbling of CO₂ are only external evidence of a vast number of chemical reactions going on within the cell. The yeast cell on absorbing the glucose has to produce various proteins which will form enzymes necessary to catalyze the various reactions concerned with the manufacture of proteins, carbohydrates, lipids, and other components of the cell as well as vitamins which will form coenzymes. A vast array of enzymes are produced as the glucose and ammonium initially supplied are converted from one compound into another or metabolized. *The series of chemical reactions involved in converting a chemical (or a metabolite) in the organism into a final product is known as a metabolic pathway.* When the reactions lead to the formation of a more complex substance, that particular form of metabolism is known as *anabolism* and the pathway an anabolic pathway. When the series of reactions lead to less complex compounds the metabolism is described as *catabolism*. The compounds involved in a metabolic pathway are called *intermediates* and the final product is known as the end-product (see Fig. 1).

Catabolic reactions have been mostly studied with glucose. Four pathways of glucose breakdown to pyruvic acid (or glycolysis) are currently recognized. They will be discussed later. Catabolic reactions often furnish energy in the form of ATP and other high energy compounds, which are used for biosynthetic reactions. A second function of catabolic reactions is to provide the carbon skeleton for biosynthesis. Anabolic reactions lead to the formation of larger molecules some of which are constituents of the cell.

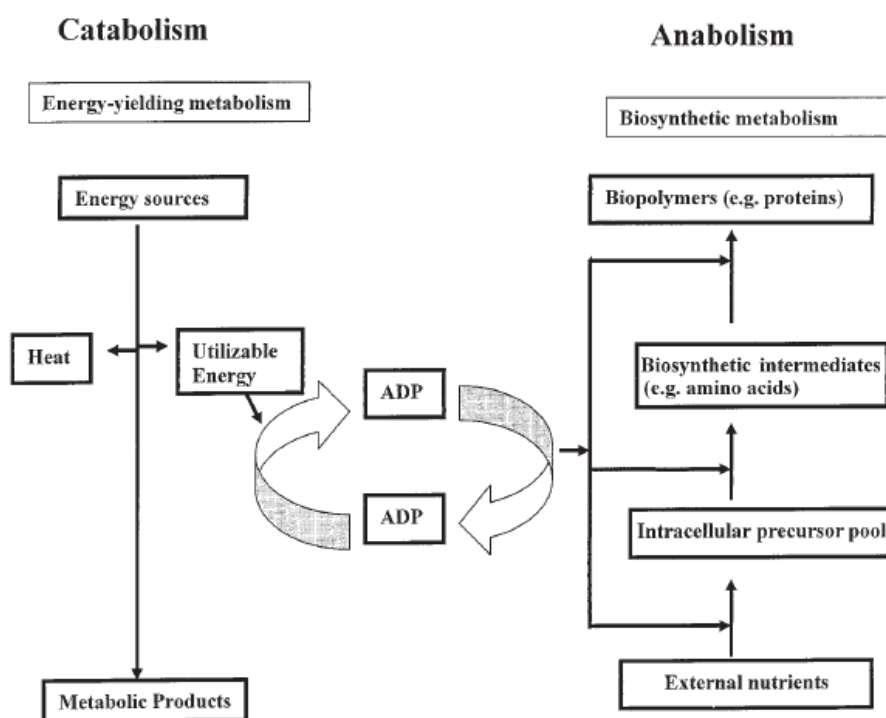


Fig. 1 Metabolism: Relationship between Anabolism and Catabolism in a Cell

Although anabolism and catabolism are distinct phenomena some pathways have elements of both kinds. Metabolic intermediates which are derived from catabolism and which are also available for anabolism are known as amphibolic intermediates. Methods for the study of metabolic pathways are well reviewed in texts on microbial physiology and will therefore not be discussed here.

INDUSTRIAL MICROBIOLOGICAL PRODUCTS AS PRIMARY AND SECONDARY METABOLITES

Products of industrial microorganisms may be divided into two broad groups, those which result from primary metabolism and others which derive from secondary metabolism. The line between the two is not always clear cut, but the distinction is useful in discussing industrial products.

1 Products of Primary Metabolism

Primary metabolism is the inter-related group of reactions within a microorganism which are associated with growth and the maintenance of life. Primary metabolism is essentially the same in all

living things and is concerned with the release of energy, and the synthesis of important macromolecules such as proteins, nucleic acids and other cell constituents. When primary metabolism is stopped the organism dies. Products of primary metabolism are associated with growth and their maximum production occurs in the logarithmic phase of growth in a batch culture. Primary catabolic products include ethanol, lactic acid, and butanol while anabolic products include amino-acids, enzymes and nucleic acids. Single-cell proteins and yeasts would also be regarded as primary products (Table 1)

Table 1 Some industrial products resulting from primary metabolism

<i>Anabolic Products</i>	<i>Catabolic Products</i>
1. Enzymes	1. Ethanol and ethanol-containing products, e.g. wines
2. Amino acids	2. Butanol
3. Vitamins	3. Acetone
4. Polysaccharides	4. Lactic acid
5. Yeast cells	5. Acetic acid (vinegar)
6. Single cell protein	
7. Nucleic acids	
8. Citric acid	

2 Products of Secondary Metabolism

In contrast to primary metabolism which is associated with the growth of the cell and the continued existence of the organism, secondary metabolism, which was first observed in higher plants, has the following characteristics. Secondary metabolism has no *apparent* function in the organism. The organism continues to exist if secondary metabolism is blocked by a suitable biochemical means. On the other hand it would die if primary metabolism were stopped. Secondary metabolites are produced in response to a restriction in nutrients. They are therefore produced after the growth phase, at the end of the logarithmic phase of growth and in the stationary phase (in a batch culture). They can be more precisely controlled in a continuous culture. Secondary metabolism appears to be restricted to some species of plants and microorganisms (and in a few cases to animals). The products of secondary metabolism also appear to be characteristic of the species. Both of these observations could, however, be due to the inadequacy of current methods of recognizing secondary metabolites. Secondary metabolites usually have 'bizarre' and unusual chemical structures and several closely related metabolites may be produced by the same organism in wild-type strains. This latter observation indicates the existence of a variety of alternate and closely-related pathways. The ability to produce a particular secondary metabolite, especially in industrially important strains is easily lost. This phenomenon is known as strain degeneration. Owing to the ease of the loss of the ability to synthesize secondary metabolites, particularly when treated with acridine dyes, exposure to high temperature or other treatments known to induce plasmid loss secondary metabolite production is believed to be controlled by plasmids (at least in some cases) rather than by the organism's chromosomes. A confirmation of the possible role of plasmids in the control of secondary metabolites is shown in the case of leupetin, in which the loss of the metabolite following irradiation can be reversed by conjugation with a producing parent. The factors which trigger secondary metabolism, the inducers, also trigger morphological changes (morphogenesis) in the organism.

Inducers of Secondary Metabolites

Autoinducers include the γ -butyrolactones (butanolides) of the actinomycetes, the Nacylhomoserine lactones (HSLs) of Gramnegative bacteria, the oligopeptides of Grampositive bacteria, and B-factor [3'-(1-butylphosphoryl)adenosine] of rifamycin production in *Amycolatopsis mediterranea*. They function in development, sporulation, light emission, virulence, production of antibiotics, pigments and cyanide, plasmiddriven conjugation and competence for genetic transformation. Of great importance in actinomycete fermentations is the inducing effect of endogenous γ -butyrolactones, e.g. A-factor (2-S-isocapryloyl-3R-hydroxymethyl- γ -butyrolactone). A-factor induces both morphological and chemical differentiation in *Streptomyces griseus* and *Streptomyces bikiniensis*, bringing on formation of aerial mycelia, conidia, streptomycin synthases and streptomycin. Conidia can actually form on agar without

A-factor but aerial mycelia cannot. The spores form on branches morphologically similar to aerial hyphae but they do not emerge from the colony surface. In *S. griseus*, A-factor is produced just prior to streptomycin production and disappears before streptomycin is at its maximum level. It induces at least 10 proteins at the transcriptional level. One of these is streptomycin 6-phosphotransferase, an enzyme which functions both in streptomycin biosynthesis and in resistance. In an A-factor deficient mutant, there is a failure of transcription of the entire streptomycin gene cluster. Many other actinomycetes produce A-factor, or related γ -butyrolactones, which differ in the length of the side-chain. In those strains which produce antibiotics other than streptomycin, the γ -butyrolactones induce formation of the particular antibiotics that are produced, as well as morphological differentiation.

Secondary metabolic products of microorganism are of immense importance to humans. Microbial secondary metabolites include antibiotics, pigments, toxins, effectors of ecological competition and symbiosis, pheromones, enzyme inhibitors, immunomodulating agents, receptor antagonists and agonists, pesticides, antitumor agents and growth promoters of animals and plants, including gibberellic acid, antitumor agents, alkaloids such as ergometrine, a wide variety of other drugs, toxins and useful materials such as the plant growth substance, gibberellic acid (Table 2). They have a major effect on the health, nutrition, and economics of our society. They often have unusual structures and their formation is regulated by nutrients, growth rate, feedback

control, enzyme inactivation, and enzyme induction. Regulation is influenced by unique low molecular mass compounds, transfer RNA, sigma factors, and gene products formed during post-exponential development. The syntheses of secondary metabolism are often coded for by clustered genes on chromosomal DNA and infrequently on plasmid DNA.

Unlike primary metabolism, the pathways of secondary metabolism are still not understood to a great degree. Secondary metabolism is brought on by exhaustion of a nutrient, biosynthesis or addition of an inducer, and/or by a growth rate decrease. These events generate signals which effect a cascade of regulatory events resulting in chemical differentiation (secondary metabolism) and morphological differentiation (morphogenesis). The signal is often a low molecular weight inducer which acts by negative control, i.e. by binding to and inactivating a regulatory protein (repressor protein/receptor protein) which normally prevents secondary metabolism and morphogenesis during rapid growth and nutrient sufficiency. Thousands of secondary metabolites of widely different chemical groups and physiological effects on humans have been found. Nevertheless a disproportionately high interest is usually paid to antibiotics, although this appears to be changing. It would appear that the vast potential utility of microbial secondary metabolites is yet to be realized and that many may not even have been discovered. Part of this 'lopsided' interest may be due to the method of screening, which has largely sought antibiotics.

Table 2 Some industrial products of microbial secondary metabolism

Product	Organism	Use/Importance
Antibiotics		
Penicillin	<i>Penicillium chrysogenum</i>	Clinical use
Streptomycin	<i>Streptomyces griseus</i>	Clinical use
Anti-tumor Agents		
Actinomycin	<i>Streptomyces antibioticus</i>	Clinical use
Bleomycin	<i>Streptomyces verticulus</i>	Clinical use
Toxins		
Aflatoxin	<i>Aspergillus flavus</i>	Food toxin
Amanitine	<i>Amanita</i> sp	Food toxin
Alkaloids		
Ergot alkaloids	<i>Claviceps purpurea</i>	Pharmaceutical
Miscellaneous		
Gibberellic acid	<i>Gibberella fujikuroi</i>	Plant growth hormone
Kojic acid	<i>Aspergillus flavus</i>	Food flavor
Muscarine	<i>Clitocybe rivalosa</i>	Pharmaceutical
Patulin	<i>Penicillium urticae</i>	Anti-microbial agent

TROPHOPHASE-IDIOPHASE RELATIONSHIPS IN THE PRODUCTION OF SECONDARY PRODUCTS

From studies on *Penicillium urticae* the terms trophophase and idiophase were introduced to distinguish the two phases in the growth of organisms producing secondary metabolites. The trophophase (Greek, tropho = nutrient) is the feeding phase during which primary metabolism occurs. In a batch culture this would be in the logarithmic phase of the growth curve. Following the trophophase is the idio-phase (Greek, idio = peculiar) during which secondary metabolites peculiar to, or characteristic of, a given organism are synthesized. Secondary synthesis occurs in the late logarithmic, and in the stationary, phase. It has been suggested that secondary metabolites be described as 'idiolites' to distinguish them from primary metabolites.

ROLE OF SECONDARY METABOLITES IN THE PHYSIOLOGY OF ORGANISMS PRODUCING THEM

Since many industrial microbiological products result from secondary metabolism, workers have sought to explain the role of secondary metabolites in the survival of the organism. Due to the importance of antibiotics as clinical tools, the focus of many workers has been on antibiotics. This discussion while including antibiotics will attempt to embrace the whole area of secondary metabolites. Some earlier hypotheses for the existence of secondary metabolism are apparently no longer considered acceptable by workers in the field. These include the hypotheses that secondary metabolites are food-storage materials, that they are waste products of the metabolism of the cell and that they are breakdown products from macro-molecules. The theories in currency are discussed below; even then none of these can be said to be water tight. The rationale for examining them is that a better understanding of the organism's physiology will help towards manipulating it more rationally for maximum productivity.

The competition hypothesis: In this theory which refers to antibiotics specifically, secondary metabolites (antibiotics) enable the producing organism to withstand competition for food from other soil organisms. In support of this hypothesis is the fact that antibiotic production can be demonstrated in sterile and non-sterile soil, which may or may not have been supplemented with organic materials. As further support for this theory, it is claimed that the wide distribution of β -lactamases among microorganisms is to help these organisms detoxify the β -lactam antibiotics. The obvious limitation of this theory is that it is restricted to antibiotics and that many antibiotics exist outside Beta-lactams.

The maintenance hypothesis: Secondary metabolism usually occurs with the exhaustion of a vital nutrient such as glucose. It is therefore claimed that the selective advantage of secondary metabolism is that it serves to maintain mechanisms essential to cell multiplication in operative order when that cell multiplication is no longer possible. Thus by forming secondary enzymes, the enzymes of primary metabolism which produce precursors for secondary metabolism therefore, the enzymes of primary metabolism would be destroyed. In this hypothesis therefore, the secondary metabolite itself is not important; what is important is the pathway of producing it.

The unbalanced growth hypothesis: Similar to the maintenance theory, this hypothesis states that control mechanisms in some organisms are too weak to prevent the over synthesis of some primary metabolites. These primary metabolites are converted into secondary metabolites that are excreted from the cell. If they are not so converted they would lead to the death of the organism.

The detoxification hypothesis: This hypothesis states that molecules accumulated in the cell are detoxified to yield antibiotics. This is consistent with the observation that the penicillin precursor penicillanic acid is more toxic to *Penicillium chrysogenum* than benzyl penicillin. Nevertheless not many toxic precursors of antibiotics have been observed.

The regulatory hypothesis: Secondary metabolite production is known to be associated with morphological differentiation in producing organisms. In the fungus *Neurospora crassa*, carotenoids are produced during sporulation. In *Cephalosporium acremonium*, cephalosporin C is produced during the idiophase when arthrospores are produced. Numerous examples of the release of secondary metabolites with some morphological differentiation have been observed in fungi. One of the most intriguing relationships between differentiation and secondary metabolite production, is that between the production of peptide antibiotics by *Bacillus* spp. and spore formation. Both spore formation and antibiotic production are suppressed by glucose; non-spore

forming mutants of bacilli also do not produce antibiotics, while reversion to spore formation is accompanied by antibiotic formation has been observed in actinomycetes. Many roles have been assigned to antibiotics in spore formers but the most clearly demonstrated has been the essential nature of gramicidin in sporulation of *Bacillus* spp. The absence of the antibiotic leads to partial deficiencies in the formation of enzymes involved in spore formation, resulting in abnormally heat-sensitive spores. Peptide antibiotics therefore suppress the vegetative genes allowing proper development of the spores. In this theory therefore the production of secondary metabolites is necessary to regulate some morphological changes in the organism. It could of course be that some external mechanism triggers off secondary metabolite

production as well as the morphological change.

The hypothesis of secondary metabolism as the expression of evolutionary reactions: Zahner has put forth a most exciting role for secondary metabolism. To appreciate the hypothesis, it is important to bear in mind that both primary and secondary metabolism are controlled by genes carried by the organism. Any genes not required are lost. According to this hypothesis, secondary metabolism is a clearing house or a mixed bag of biochemical reactions, undergoing tests for possible incorporation into the cell's armory of primary reactions. Any reaction in the mixed bag which favorably affects any one of the primary processes, thereby fitting the organism better to survive in its environment, becomes incorporated as part of primary metabolism. According to this hypothesis, the antibiotic properties of some secondary metabolites are incidental and not a design to protect the microorganisms. This hypothesis is attractive because it implies that secondary metabolism must occur in all microorganisms since evolution is a continuing process. If that is the case, then the current range of secondary metabolites is limited only by techniques sensitive enough to detect them. That this is a possibility is shown by the increase in the number of antibiotics alone, since new methods were recently introduced in the processes used in screening for them. If therefore adequate methods of detection are devised it is possible that more secondary metabolites of use for humans could be found.

PATHWAYS FOR THE SYNTHESIS OF PRIMARY AND SECONDARY METABOLITES OF INDUSTRIAL IMPORTANCE

The main source of carbon and energy in industrial media is carbohydrates. In recent times hydrocarbons have been used. The catabolism of these compounds will be discussed briefly because they supply the carbon skeletons for the synthesis of primary as well as for secondary metabolites. The inter-relationship between the pathways of primary and the secondary metabolism will also be discussed briefly.

1 Catabolism of Carbohydrates

Four pathways for the catabolism of carbohydrates up to pyruvic acid are known. All four pathways exist in bacteria, actinomycetes and fungi, including yeasts. The four pathways are the Embden-Meyerhof-Parnas, the Pentose Phosphate Pathways, the Entner Duodoroff pathway and the Phosphoketolase. Although these pathways are for the breakdown of glucose. Other carbohydrates easily fit into the cycles.

The Embden-Meyerhof-Parnas (EMP Pathways): The net effect of this pathway is to reduce glucose (C₆) to pyruvate (C₃) (Fig. 2). The system can operate under both aerobic and anaerobic conditions. Under aerobic conditions it usually functions with the tricarboxylic acid cycle which can oxidize pyruvate to CO₂ and H₂O.

Under anaerobic conditions, pyruvate is fermented to a wide range of fermentation products, many of which are of industrial importance (Fig. 3).

The pentose Phosphate Pathway (PP): This is also known as the Hexose Monophosphate Pathway (HMP) or the phosphogluconate pathway. While the EMP pathway provides pyruvate, a C₃ compound, as its end product, there is no end product in the PP pathway. Instead it provides a pool of triose (C₃) pentose (C₅), hexose (C₆) and heptose (C₇) phosphates. The primary purpose of the PP pathway, however, appears to be to generate energy in the form of NADP⁺ for biosynthetic and other purposes and pentose phosphates for nucleotide synthesis (Fig. 4)

The Entner-Duodoroff Pathway (ED): The pathway is restricted to a few bacteria especially *Pseudomonas*, but it is also carried out by some fungi. It is used by some organisms in the anaerobic breakdown of glucose and by others only in gluconate metabolism (Fig. 5)

The Phosphoketolase Pathway: In some bacteria glucose fermentation yields lactic acid, ethanol and CO₂. Pentoses are also fermented to lactic acid and acetic acid. An example is *Leuconostoc mesenteroides* (Fig. 6).

Pathways used by microorganisms

The two major pathways used by microorganisms for carbohydrate metabolism are the **EMP** and the **PP** pathways. Microorganisms differ in respect of their use of the two pathways. Thus *Saccharomyces cerevisiae* under aerobic conditions uses mainly the **EMP** pathway; under anaerobic conditions only about 30% of glucose is catabolized by this pathway. In *Penicillium chrysogenum*, however, about 66% of the glucose is utilized via the PP pathway. The **PP** pathway is also used by *Acetobacter*, the acetic acid bacteria. Homofermentative bacteria utilize the EMP pathway for glucose breakdown. The **ED** pathway is especially used by *Pseudomonas*.

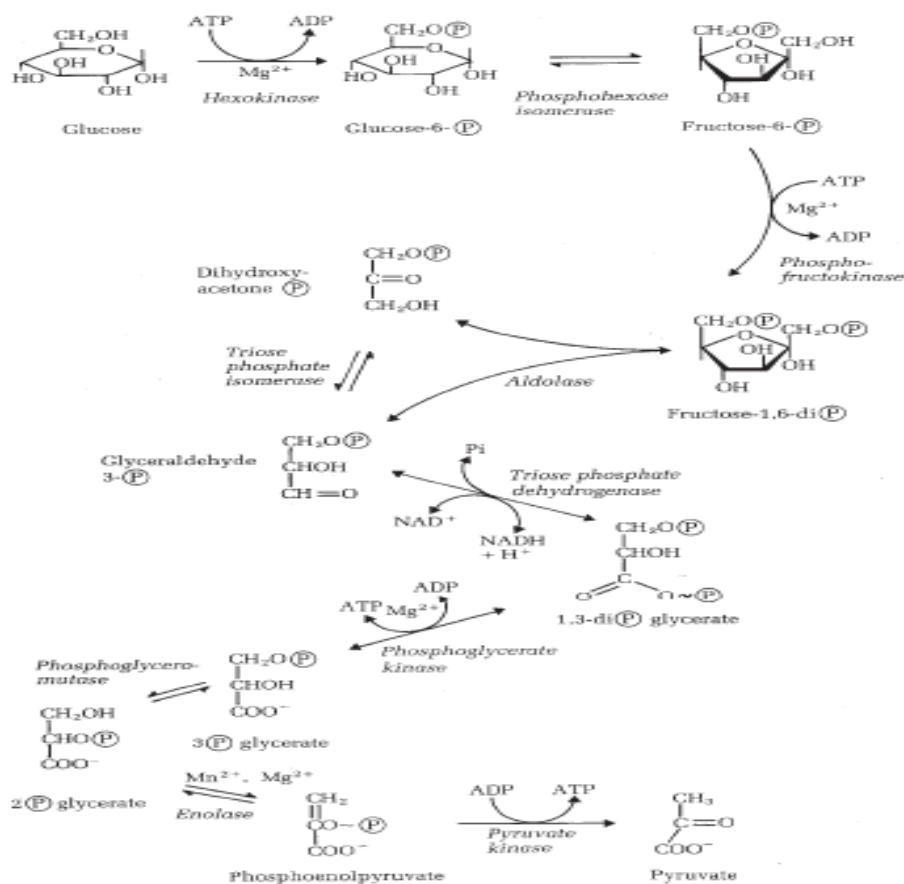


Fig. 5.2 The Embden-Meyerhof - Parnas Pathway

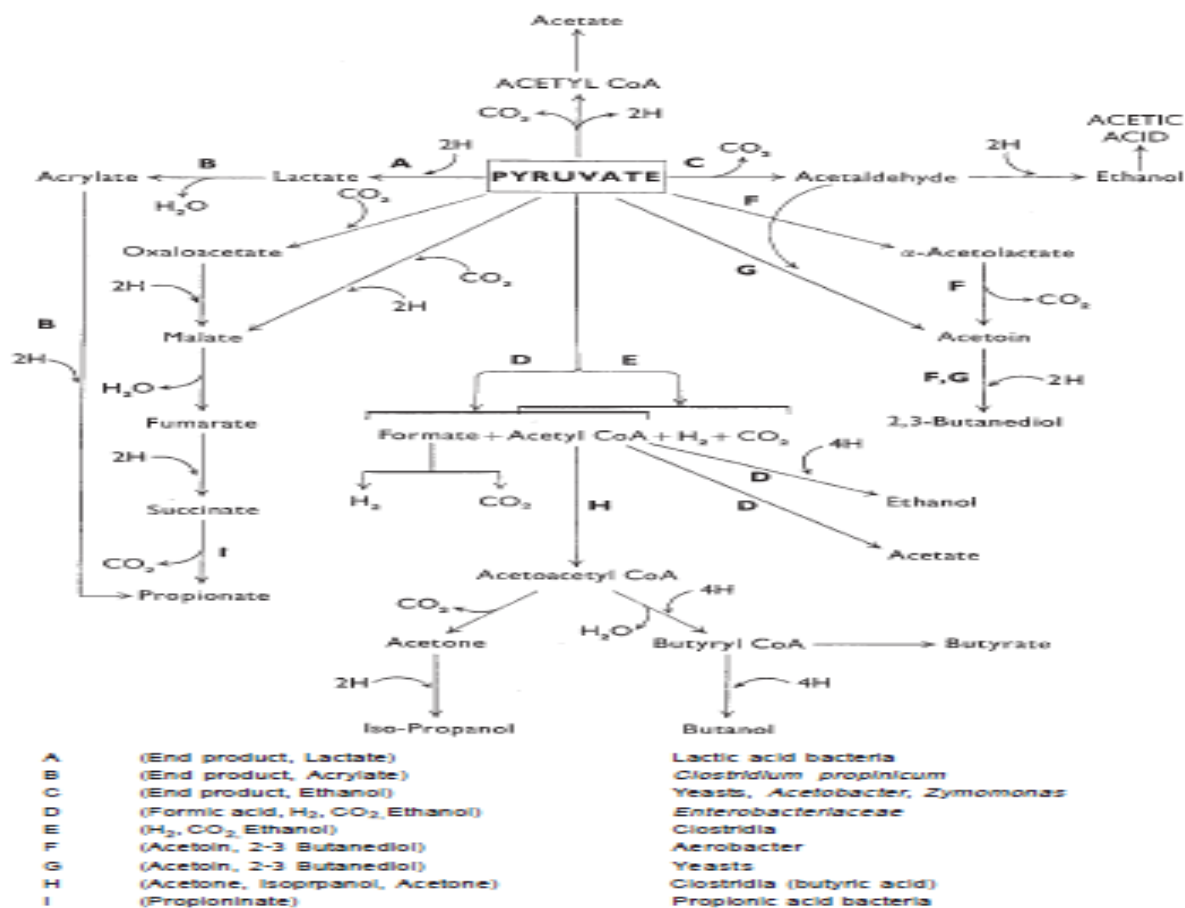


Fig. 5.3 Products of the Fermentation of Pyruvate by Different Microorganisms

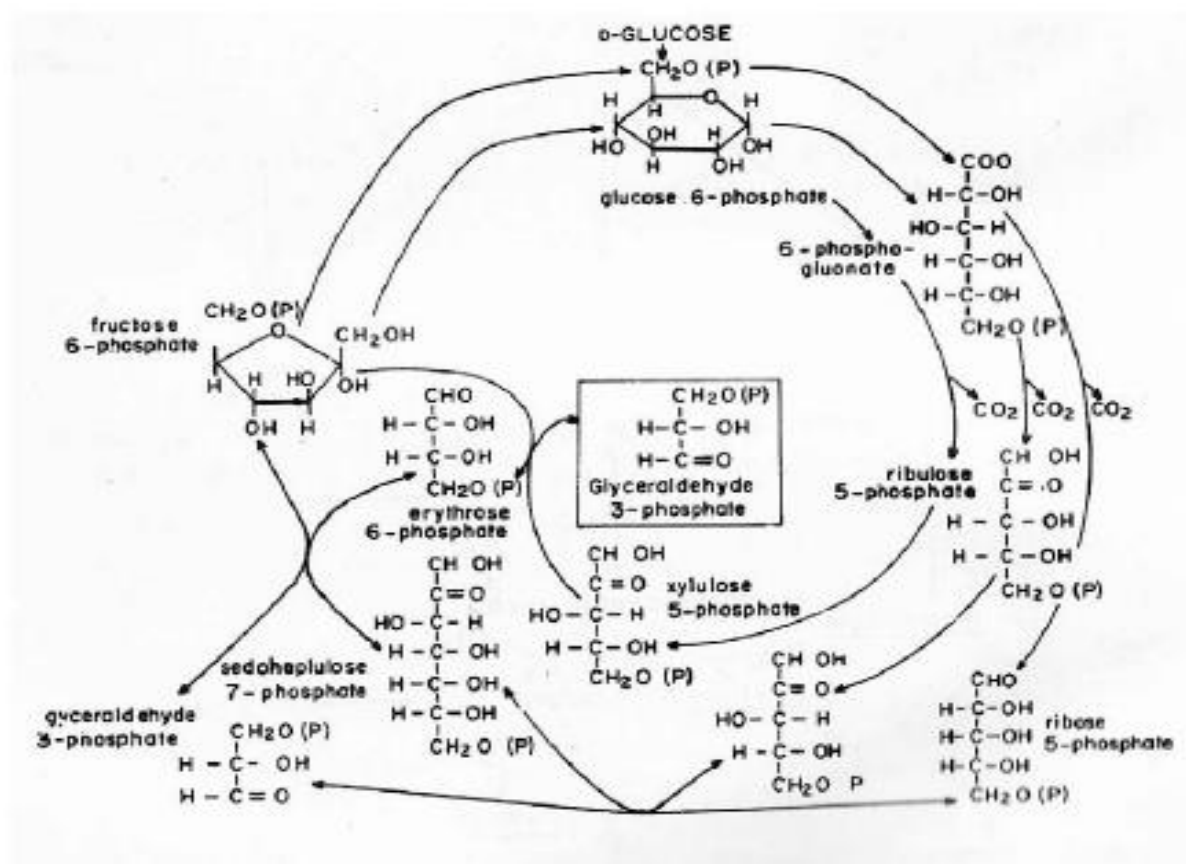


Fig. 5.4 The Pentose Phosphate Pathway

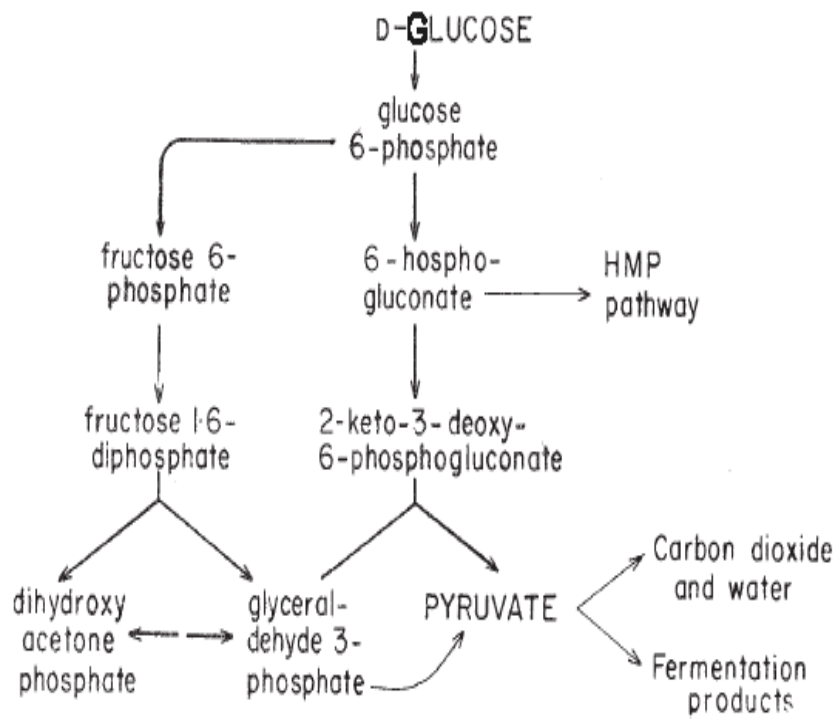


Fig. 5.5 The Enter-Doudoroff Pathway

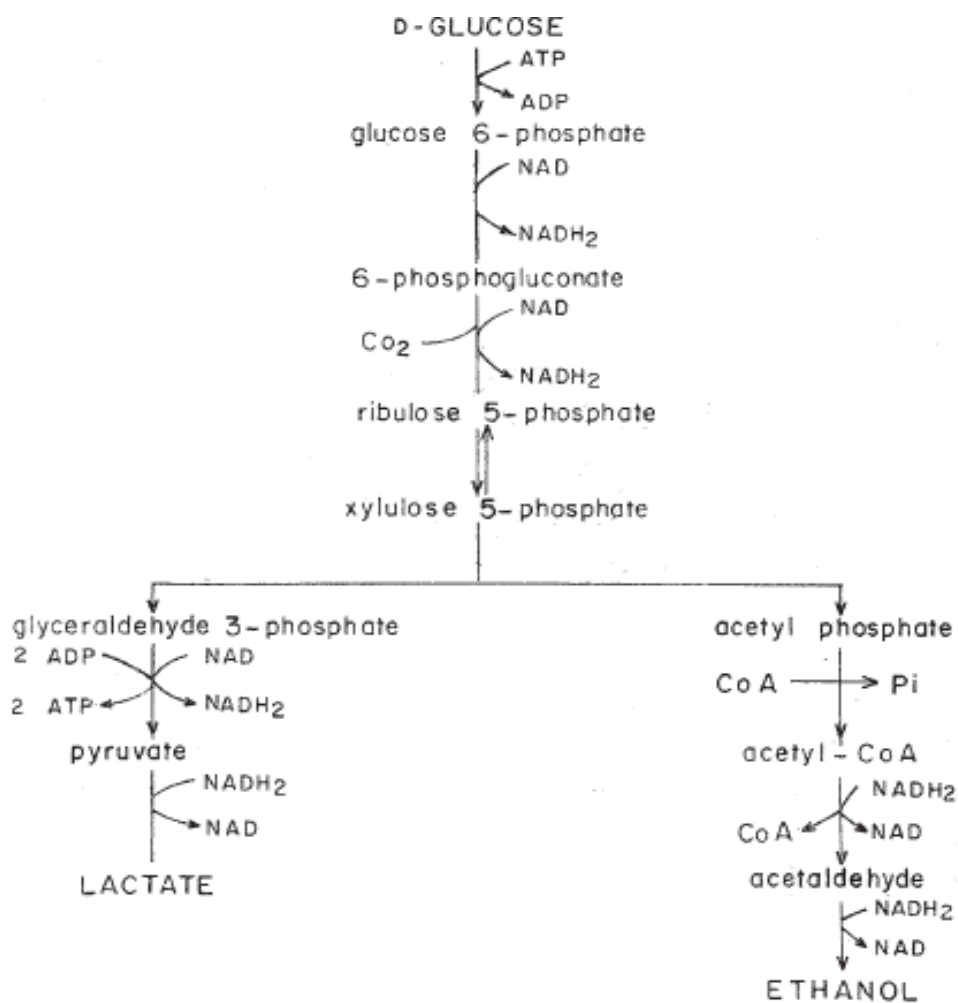


Fig. 5.6 The Phosphoketolase Pathway

Overproduction of metabolites of industrial microorganisms

The complexity of the activities which go on within a cell was mentioned we discussed the metabolism of a yeast cell introduced into an aqueous solution of glucose and ammonium salts. The yeast cell must first permit the entry into itself of the glucose and ammonium salts. Under suitable environmental conditions such as pH and temperature it will grow by budding within about half an hour. For these buds to occur, hundreds of activities will have gone on within the cell. New proteins to be incorporated into enzymes and other structures will have been synthesized; nucleic acids for the chromosomes and carbohydrates for the cell walls will all have been synthesized. Hundreds of different enzymes will have participated in these synthetic activities. The organism must synthesize each of the compounds at the right time and in the appropriate quantities. If along side ammonium salts, amino acids were supplied, the yeast cells would stop absorbing the ammonium salt and instead utilize the supplied 'readymade' substrate.

A few yeasts can utilize starch. If our yeast belonged to this group and was supplied nothing but starch and ammonium salts, it would secrete extracellular enzyme(s) to breakdown the starch to sugars. These sugars would then be absorbed and would be used with ammonium salts, for the synthetic activities we described earlier. Clearly therefore, while the organism's genetic apparatus determines in broad terms the organism's overall synthetic potentialities, what is actually synthesized depends on what is available in the environment. Most importantly, the organism is not only able to 'decide' when to manufacture and secrete certain enzymes to enable it to utilize materials in the environment, but it is able to decide to stop the synthesis of certain compounds if they are supplied to it. These sensing mechanisms for the switching on and off of the synthetic processes enable the organism to avoid the overproduction of any particular compound. If it did not have these regulatory mechanisms it would waste energy and resources (which are usually scarce in natural environments) in making materials it did not require.

An efficient or 'stringent' organism which does not waste its resources in producing materials it does not require will survive well in natural environments where competition is intense. Such an organism while surviving well in nature would not, however, be of much use as an industrial organism. The industrial microbiologist or biotechnologist prefers, and indeed, seeks, the wasteful, inefficient and 'relaxed' organism whose regulatory mechanisms are so poor that it will overproduce the particular metabolite sought. Knowledge of these regulatory mechanisms and biosynthetic pathways is essential, therefore, to enable the industrial microbiologist to derange and disorganize them so that the organism will overproduce desired materials.

Then will follow a discussion of methods by which the microbiologist consciously deranges these two mechanisms to enable overproduction. Regulatory methods and ways of disorganizing microorganisms for the overproduction of metabolites are far better understood in primary metabolites than they are in secondary metabolites. Indeed for some time it was thought that secondary metabolites did not need to be regulated since the microorganisms had no apparent need for them. They are currently better understood and it is now known that they are also regulated. In the discussions that follow, primary metabolites will first be considered. Only a minimum of examples will be given in respect of regulatory mechanisms of primary metabolites. Textbooks on microbial physiology may be consulted for the details.

MECHANISMS ENABLING MICROORGANISMS TO AVOID OVERPRODUCTION OF PRIMARY METABOLIC PRODUCTS THROUGH ENZYME REGULATION

Some of the regulatory mechanisms enabling organisms to avoid over-production are given in Table 1. Each of these will be discussed briefly.

Table 1 Regulatory mechanisms in microorganisms

1. Substrate Induction
2. Catabolite Regulation
 - 2.1 Repression
 - 2.2 Inhibition
3. Feedback Regulation
 - 3.1 Repression
 - 3.2 Inhibition
 - 3.3 Modifications used in branched pathways
 - 3.3.1 Concerted (multivalent) feedback regulation
 - 3.3.2 Cooperative feedback inhibition
 - 3.3.3 Cumulative feedback regulation
 - 3.3.4 Compensatory feedback regulation
 - 3.3.5 Sequential feedback regulation
 - 3.3.6 Isoenzyme feedback regulation
4. Amino acid Regulation of RNA synthesis
5. Energy Charge Regulation
6. Permeability Control

Substrate Induction

Some enzymes are produced by microorganisms only when the substrate on which they act is available in the medium. Such enzymes are known as *inducible* enzymes. Analogues of the substrate may act as the inducer. When an inducer is present in the medium a number of different inducible enzymes may sometimes be synthesized by the organism. This happens when the pathway for the metabolism of the compound is based on sequential induction. In this situation the organism is induced to produce an enzyme by the presence of a substrate. The intermediate resulting from the action of this enzyme on the substrate induces the production of another enzyme and so on until metabolism is accomplished. The other group of enzymes is produced whether or not the substrate on which they act, are present. These enzymes are known as *constitutive*.

Enzyme induction enables the organism to respond rapidly, sometimes within seconds, to the presence of a suitable substrate, so that unwanted enzymes are not manufactured.

Molecular basis for enzyme induction: The molecular mechanism for the rapid response of an organism to the presence of an inducer in the medium relates to protein synthesis since enzymes are protein in nature. Two models exist for explaining on a molecular basis the expression of genes in protein synthesis: one is a negative control and the other positive. The negative control of Jacob and Monod first published in 1961 is the better known and more widely accepted of the two and will be described first.

The Jacob-Monod Model of the (negative) control of protein synthesis

In this scheme (Fig. 6.1) the synthesis of polypeptides and hence enzymes protein is regulated by a group of genes known as the operon and which occupies a section of the chromosomal DNA. Each operon controls the synthesis of a particular protein. An operon includes a regulator gene (R) which codes for a repressor protein. The repressor can bind to the operator gene (O) which controls the activity of the neighboring structural genes (S). The production of the enzymes which catalyze the transcription of the message on the DNA into mRNA (namely, RNA polymerase) is controlled by the promoter gene (P). If the repressor protein is combined with the operator gene (O) then the movement of RNA polymerase is blocked and RNA complementary to the DNA in the structural genes (S) cannot be made. Consequently no polypeptide and no enzyme will be made. In the absence of the attachment of the repressor to the operator gene, RNA polymerase from the promoter can move to, and transcribe the structural genes, S. Inducible enzymes are made when an inducer is added. Inducers inactivate or remove the repressor protein thus leaving the way clear for protein synthesis. Constitutive enzymes

occur where the regulator gene (R) does not function, produces an inactive repressor, or produces a repressor to which the operator cannot bind. Often more than one structural gene may be controlled by a given operator.

Mutations can occur in the regulator (R) and operator (O) genes thus altering the nature of the repressor or making it impossible for an existing repressor to bind onto the operator. Such a mutation is called constitutive and it eliminates the need for an inducer.

The structural genes of inducible enzymes are usually repressed because of the attachment of the repressor to the operator.

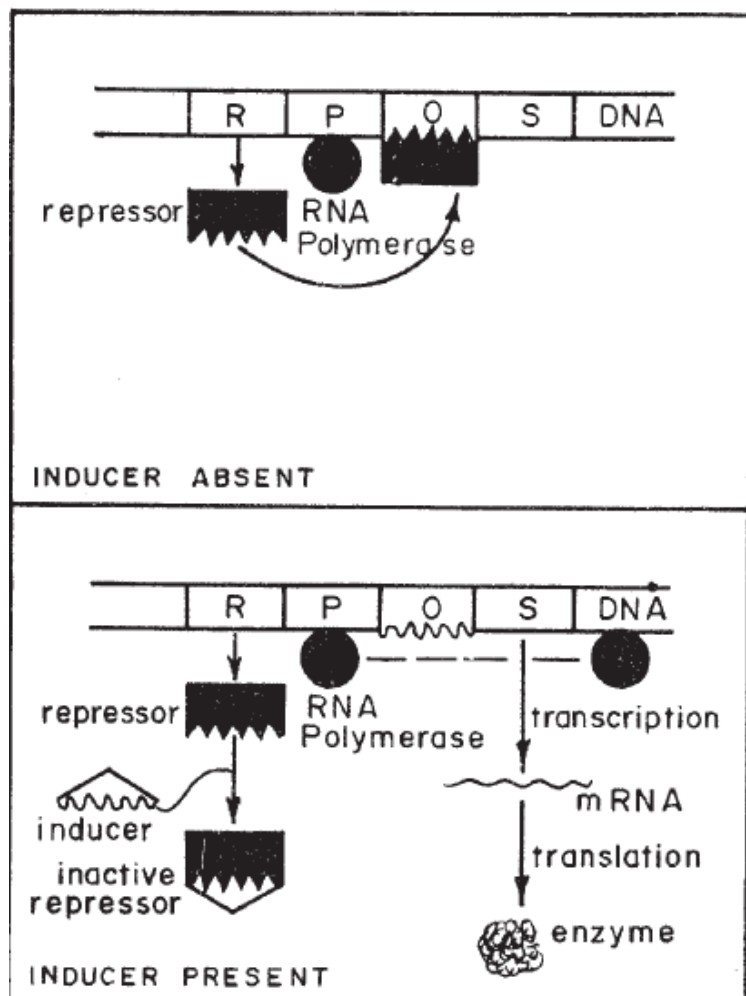


Fig. 6.1 Diagram Illustrating Negative Control of Protein Synthesis According to the Jacob and Monod Model

During induction the repressor is no longer a hindrance, hence induction is also known as de-repression. In the model of Jacob and Monod gene expression can only occur when the operator gene is free. (i.e., in the absence of the attachment of the repressor protein the operator gene O. For this reason the control is said to be negative.

Positive control of protein synthesis

Positive control of protein synthesis has been less well studied but has been established in at least one system, namely the *ara* operon, which is responsible for L-arabinose utilization in *E. coli*. In this system the product of one gene (*ara C*) is a protein which combines with the inducer arabinose to form an activator molecule which in turn initiates action at the operon. In the scheme as shown in Fig. 6.2, 'C' protein combines with arabinose to produce an arabinose – 'C' protein complex which binds to the Promoter P and initiates the synthesis of the various enzymes (isomerase, kinase, epimerase) which convert L-arabinose to D-xylulose-5-phosphate, a form in which it can be utilized in the Pentose Phosphate pathway. Positive control of protein synthesis also operates during catabolite repression (see below).

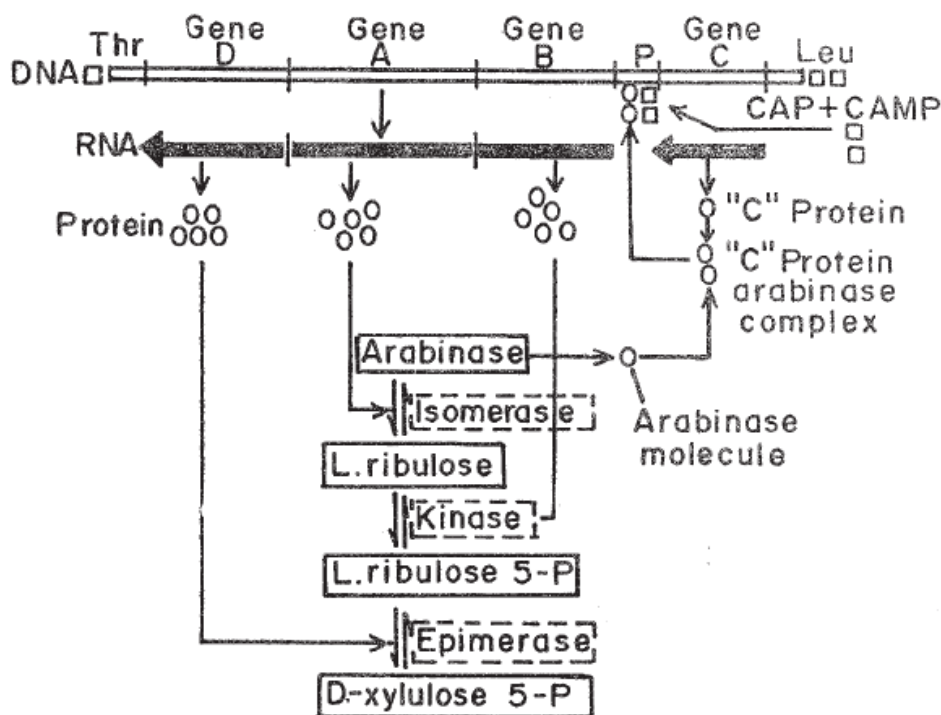


Fig. 6.2 Diagram Illustrating Positive Control of Protein Synthesis

The presence of carbon compounds other than inducers may also have important effects on protein synthesis. If two carbon sources are available to an organism, the organism will utilize the one which supports growth more rapidly, during which period enzymes needed for the utilization of the less available carbon source are repressed and therefore will not be synthesized. As this was first observed when glucose and lactose were supplied to *E. coli*, it is often called the 'glucose effect', since glucose is the more available of the two sugars and lactose utilization is suppressed as long as glucose is available. It soon became known that the effect was not directly a glucose effect but was due to some catabolite. The term *catabolite repression* was therefore adopted as more appropriate. It must be borne in mind that other carbon sources can cause repression (see later) and that sometimes it is glucose which is repressed. The active catabolite involved in catabolite repression has been found to be a nucleotide cyclic 3'5'-adenosine monophosphate (cAMP), (Fig. 6.3). In general, less c-AMP accumulates in the cell during growth on carbon compounds supporting rapid growth of the organism, vice versa.

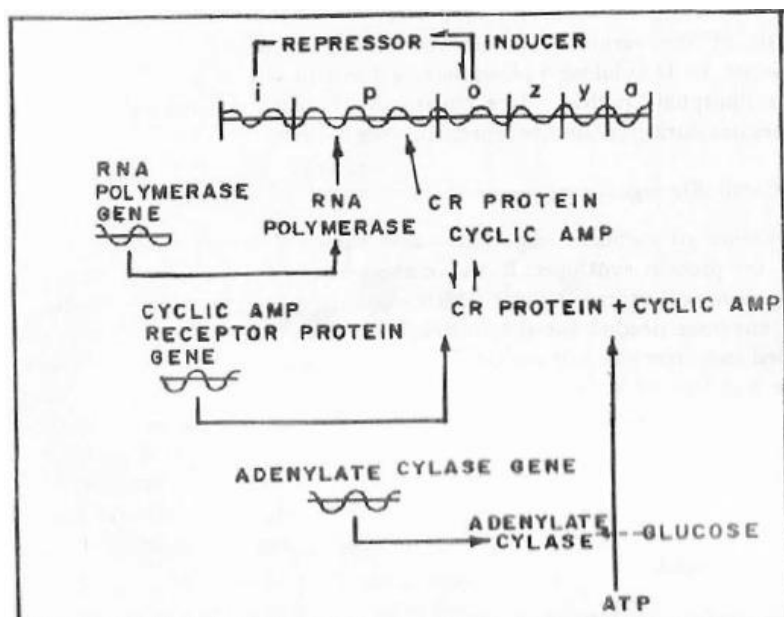


Fig. 6.3 Action of Cyclic Amp on the Lac Operon

During the rapid growth that occurs on glucose, the intracellular concentration of cyclic AMP is low. C-AMP stimulates the synthesis of a large number of enzymes and is necessary for the synthesis of the mRNA for all the inducible enzymes in *E.coli*. When it is low as a result of growth on a favorable source the enzymes which need to be induced for the utilization of the less available substrate are not synthesized. Unlike the negative control of Jacob and Monod, c-Amp exerts a positive control.

Another model explains the specific action in catabolite repression of glucose. In this model an increased concentration of c-AMP is a signal for energy starvation. When such a signal is given, c-Amp binds to an intracellular protein, c-AMP-receptor protein (CRP) for which it has high affinity. The binding of this complex to the promoter site of an operon stimulates the initiation of operon transcription by RNA polymerase (Fig. 6.3).

The presence of glucose or a derivative of glucose inhibits adenylate cyclase the enzyme which converts ATP to c-AMP. Transcription by susceptible operons is inhibited as a result. In short, therefore, catabolite repression is reversed by c-AMP. In recent times, for instance, it has been shown that c-AMP and CRP are not the only mediators of catabolite repression. It has been suggested that while catabolite repression in enterobacteria at least is exerted by the catabolite(s) of a rapidly utilized glucose source it is regulated in a two-fold manner: positive control by c-AMP and a negative control by a catabolite modulation factor (CMF) which can interfere with the operation of operons sensitive to catabolite repression. In *Bacillus* c-AMP has not been observed, but an analogue of c-AMP is probably involved.

Feedback Regulation

Feedback or end-product regulations control exerted by the end-product of a metabolic pathway, hence its name. Feedback regulations are important in the control over anabolic or biosynthetic enzymes whereas enzymes involved in catabolism are usually controlled by induction and catabolite regulation. Two main types of feedback regulation exist: feedback inhibition and feedback repression. Both of them help adjust the rate of the production of pathway end products to the rate at which macromolecules are synthesized (see Fig. 6.4).

Feedback inhibition

In feedback inhibition the final product of metabolic pathway inhibits the action of earlier enzymes (usually the first) of that sequence. The inhibitor and the substrate need not resemble each other, hence the inhibition is often called *allosteric* in contrast with the *isosteric* inhibition where the inhibitor and substrate have the same molecular conformation. Feedback inhibition can be explained on an enzymic level by the structure of the enzyme molecule. Such enzymes have two type of protein sub-units. The binding site on the sub-unit binds to the substrate while the site on the other sub-unit binds to the feedback inhibitor. When the inhibitor binds to the enzyme the shape of the enzymes is changed and for this reason, it is no longer able to bind on the substrate. The situation is known as the allosteric effect.

Feedback Repression

Whereas feedback inhibition results in the reduction of the activity of an already synthesized enzyme, feedback repression deals with a reduction in the rate of synthesis of the enzymes. In enzymes that are affected by feedback repression the regulator gene (R) is said to produce a protein aporepressor which is inactive until it is attached to corepressor, which is the end-product of the biosynthetic pathway. The activated repressor protein then interacts with the operator gene (O) and prevents transcription of the structural genes (S) on to mRNA. A derivative of the end-product may also bring about feedback repression. It is particularly active in stopping the over production of vitamins, which are required only in small amounts (see Fig. 6.1). While feedback inhibition acts rapidly, sometimes within seconds, in preventing the wastage of carbon and energy in manufacturing an already available catabolite, feedback repression acts more slowly both in its introduction and in its removal. About two generations are required for the specific activity of the repressed enzymes to rise to its maximum level when the repressing metabolite is removed; about the same number of generations are also required for the enzyme to be repressed when a competitive metabolite is introduced.