Enzyme Engineering

2. Biosynthesis and Production of Enzymes

2.1 Screening of Enzymes

2.2 Biosynthesis of Enzymes

2.3 Production of Enzymes

2.1 Screening of Enzymes

*** Screening?**

Searching for new or suitable microbial enzymes in nature

Industrial Biotechnology

Rapid growing field

-By 2010 biocatalysis will be used in production of 60% of fine chemical (McKinsey analysis)

Applications

-Pharmaceuticals

-Food ingredients(sweetener, vitamins)

-Feed additives and other agrochemicals

-Polymers

-Biofuel

Needed:

Novel enzymes and pathways

Discovery of Novel Enzyme

Screening environmental samples by enrichment cultures

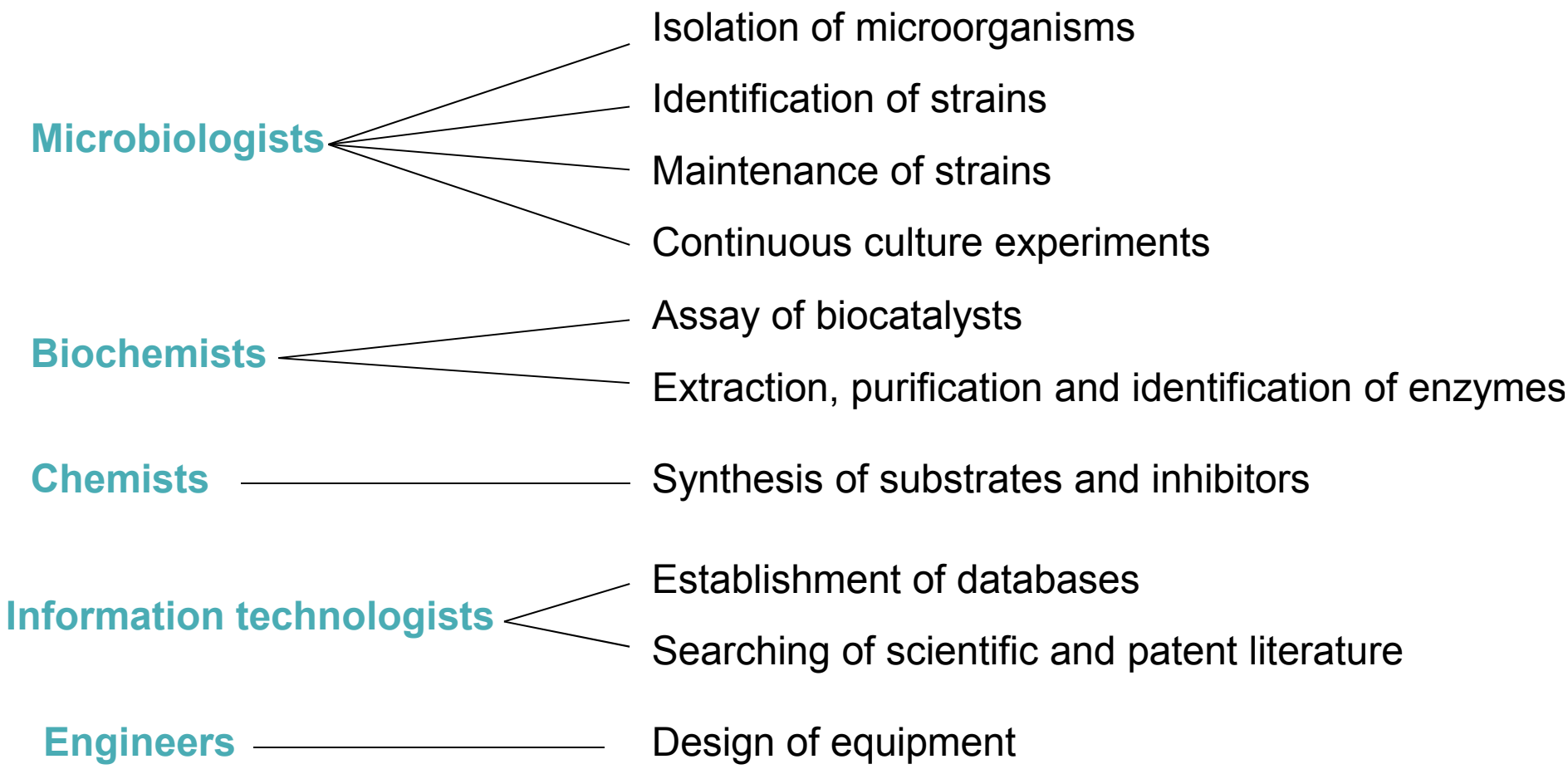
Metagenome approach

Sequence-based discovery

Still to be discovered:

Enzymes involved in the biosynthesis or catabolism of approximately 40 naturally occurring chemical functional groups are still not known

Screening for Novel Enzymes



Screening for Novel Enzymes

Practical approaches to screening

Fogarty and Kelley specified the following general criteria for screening for a microorganisms for the production of an enzyme

(1) Extensive screening must be undertaken to select the most suitable organisms

(2) The organism must produce the enzyme in good yield in a relatively short time and, ideally, in submerged culture

(3) The organism must grow and produce the enzyme on inexpensive readily available nutrients;

Screening for Novel Enzymes

(4) The organism should be easily removed from the fermentation liquor

(5) The enzyme should preferably be produced extracellularly and readily isolated from the fermentation liquor

(6) The organism should be non-pathogenic and unrelated phylogenically to a pathogen

(7) Ideally, the organism should not produce toxins or other biologically active materials

(8) The organism should be genetically stable and not susceptible to bacteriophages

Screening for Novel Enzymes

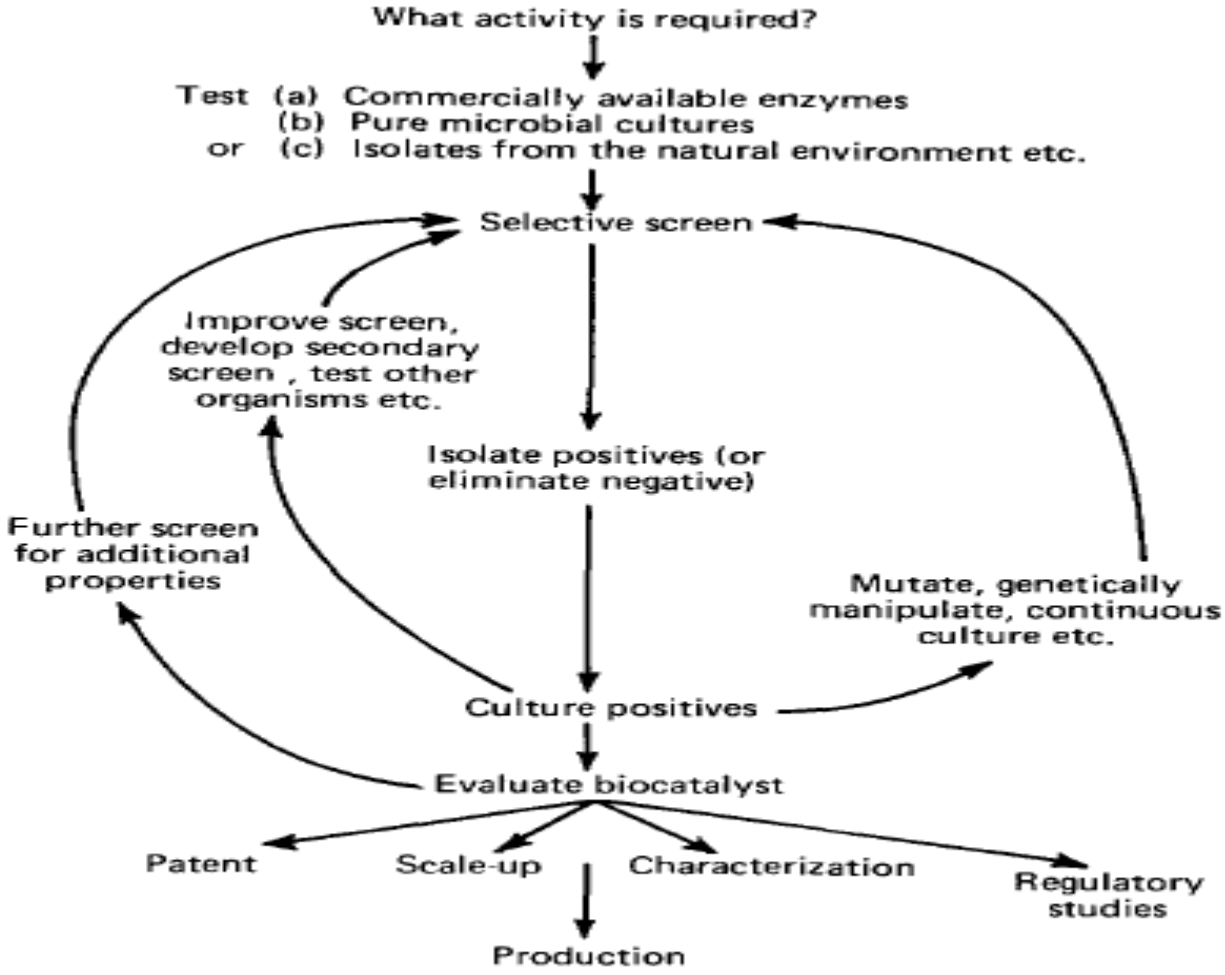


Figure 2 Flow diagram of important screening operations

Enzyme Microbial Technology. 1987, vol 9, issue 4, 194-213

Screening for Novel Enzymes

New techniques for producing novel biocatalysts

A number of techniques are being developed which promise to enable us to obtain new enzymes. These include the following

(1) Genetic engineering techniques such as protein engineering

(2) Combining chemical and enzyme catalysts

(3) Trying to discover new unnatural substrates for existing enzymes

(4) Exploiting the use of different reaction conditions, such as changes in metal ion or solvent

(5) The de novo generation of new activities by renaturing and then crosslinking a protein in the presence of a competitive inhibitor to the required enzyme activity

Screening for Novel Enzymes

Screening of microorganisms for novel enzymes

Gene probe technology	Metagenomics
-Cultivating -Hybridization technique -Detection of presents and location of specific genes in organisms -Evaluate distribution of a gene among different species -Taxonomic markers -Search for novel enzymes in microbial isolates Limitation : microbial strain can just be screened if cultivation possible	-Non-cultivating -Screening of uncultivated microorganisms -Fluorescence in situ hybridization(FISH) -16S rRNA clone library 16S r RNA clone library used to identify uncultivated microorganisms

Screening for Novel Enzymes

Extremophiles are microbes that can live and reproduce in harsh environments

The enzymes of an extremophile are adapted to function optimally at extreme condition

Microorganisms	Growth Condition	Enzymes and other biomolecules
Thermophiles	50-110℃	Amylases, lipase, xylanases, etc
Mesophiles	20-50 ℃	Almost all the enzymes
Psychrophiles	0−20 ℃	Proteases, dehydrogenase, etc
Alkaliphiles	pH>9	Cellulases, proteases, etc
Halophiles	3-20% salts	Compatible solutes, membranes

2.2 Biosynthesis of Enzymes

Enzyme Biosynthesis

Biosynthesis – Constitutive / Inducible

Induction

Catabolic enzymes are usually induced by the addition of a substrate

Repression

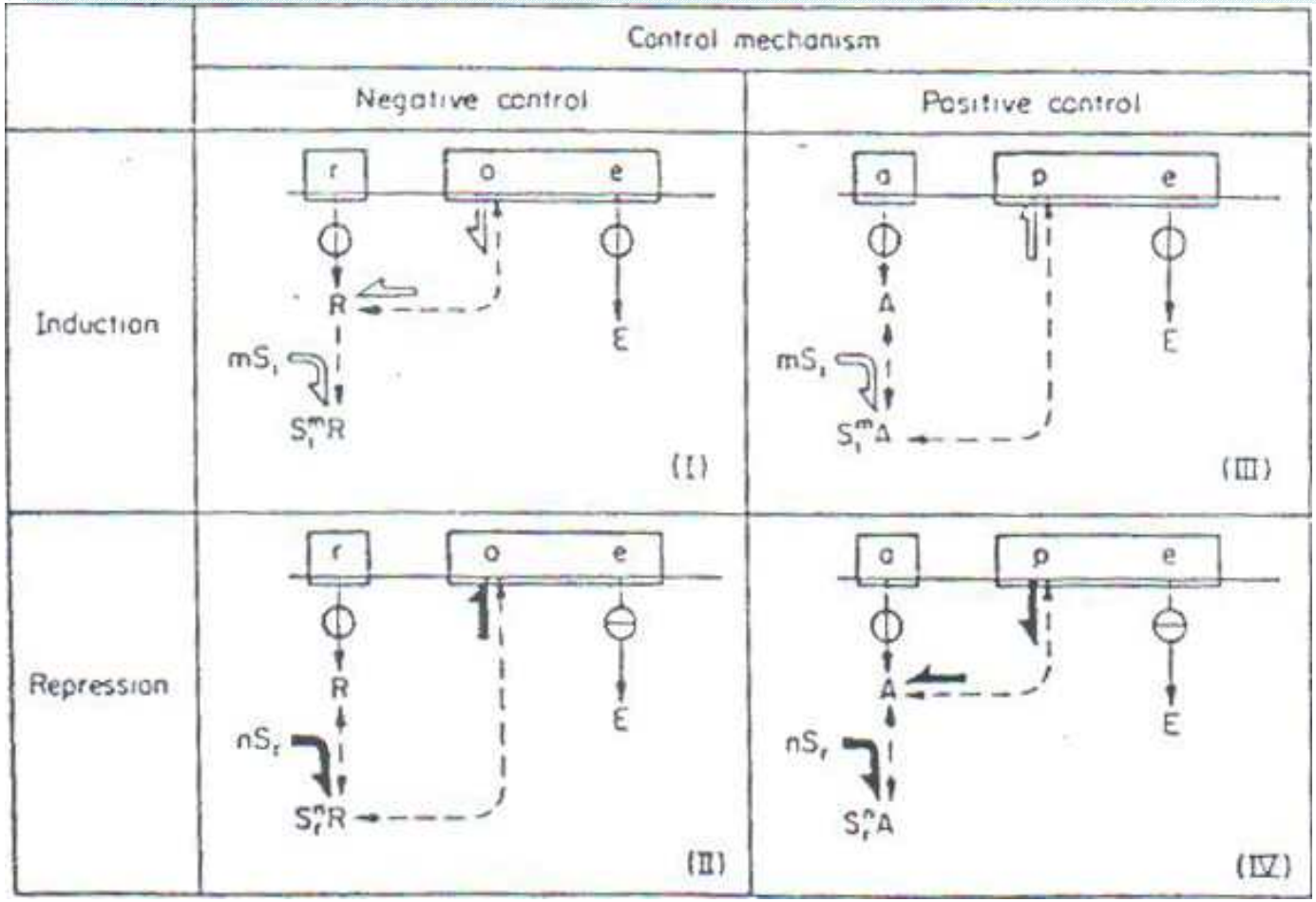
Enzymes are repressed by the presence of intermediates or end-products of the enzyme reaction

The formation of a specific enzyme depends on

(i) Initiation of mRNA polymerization (transcription)

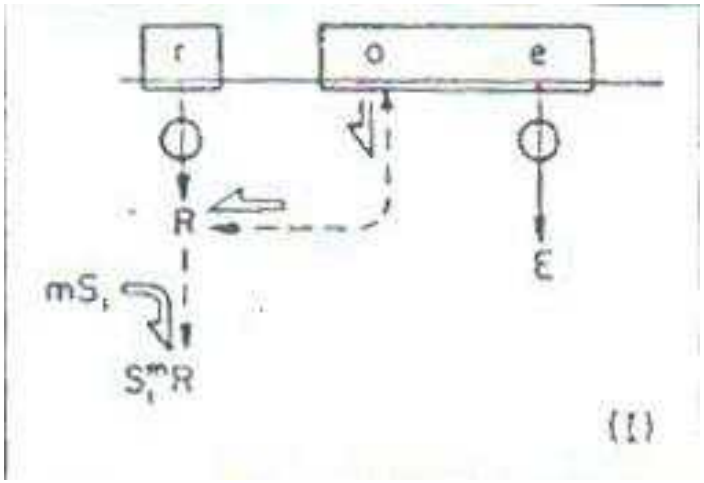
(ii) Polymerization of a protein (translation)

Regulatory Mechanisms

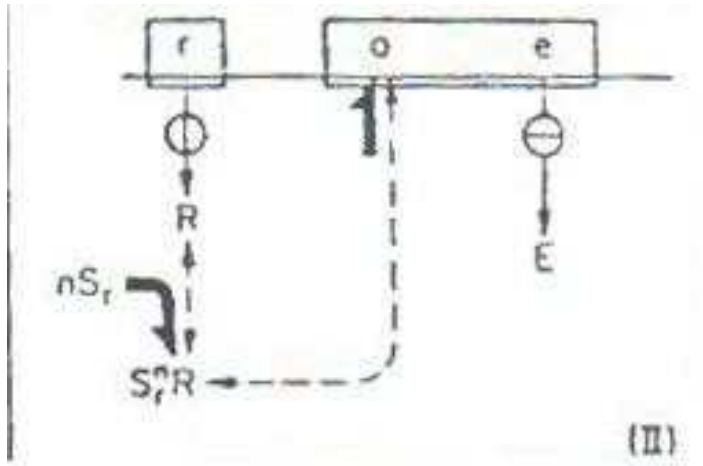


Induction and repression of enzymes in microbial culture,
Kiyoshi toda, J.CHEM TECH BIOTECHNOL 1981, 31, 775-790

Negative Control



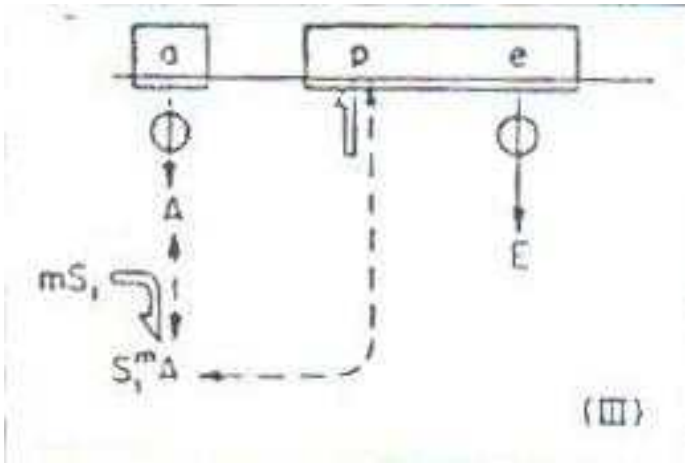
(Negative induction)



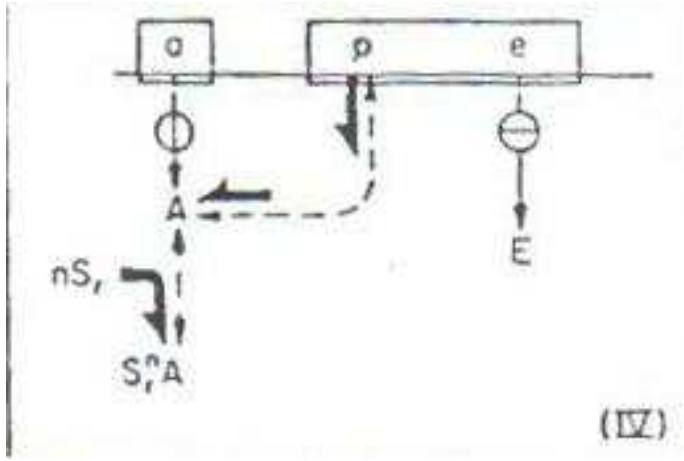
(Negative repression)

The repressor (or its precursor) molecule attaches to an operator region (o) in the DNA molecule by itself (R) or after forming a complex(S_r-R) with an effector(S_r). The binding obstructs the formation of mRNA resulting in a reduction in the synthesis of the enzyme(E). This type of enzyme regulation if designated as the negative control of enzyme synthesis

Positive Control



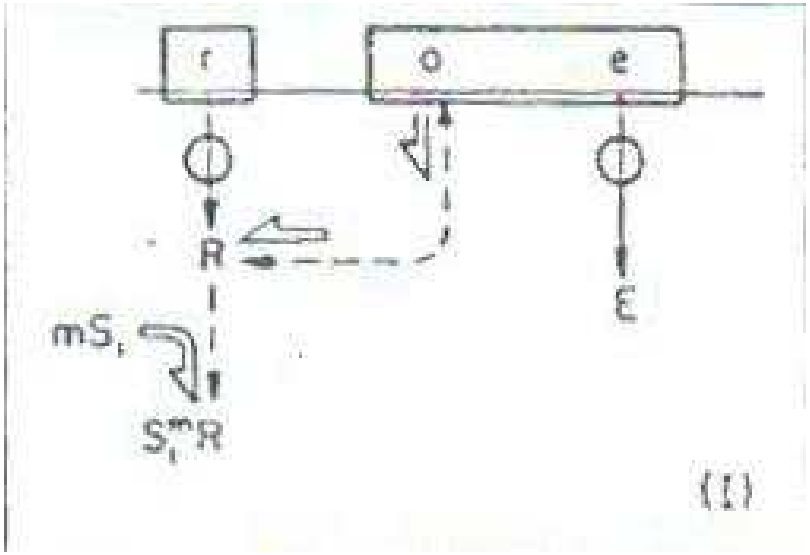
Positive Induction



Positive repression

The activator molecule(A) by itself or after forming a complex(S_i-A) with an effector(S_i) binds on a promotor region(p) in the DNA molecule. The binding enables RNA polymerase to synthesize m RNA which carries the genetic codon(e) of a specific protein structure to the place where peptide polymerization of the enzyme(E) can occur. This type of enzyme regulation is called positive control since the activator stimulates protein synthesis

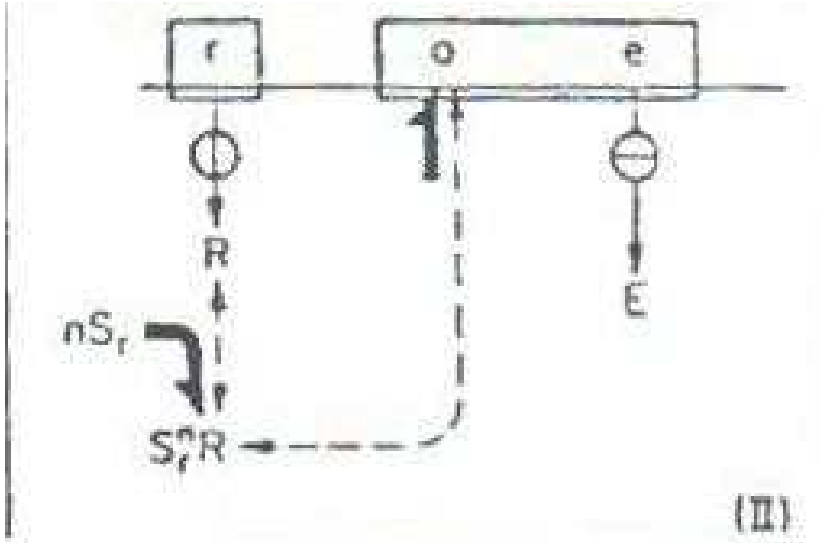
Negative Induction



B-Galactosidase system

Enzyme regulation represented by type I is exemplified by the β-galactosidase system. Four(m=4) molecules of isopropyl thiogalactoside(Si) bind with a molecule of lactose repressor(R) with a binding coefficient of 2×10^6 mol/dm^{-3}, thus derepressing the operator region to allow synthesis of β-galactosidase(E)

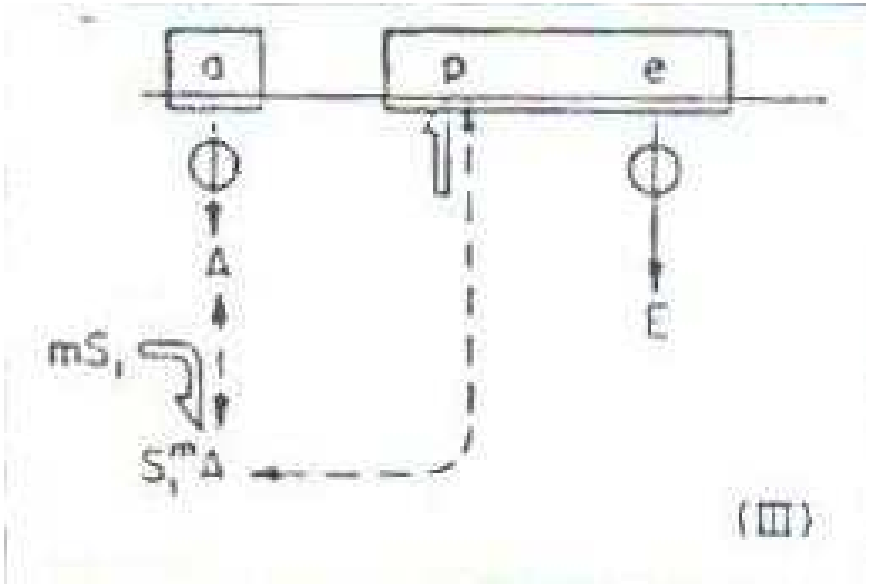
Negative Repression



Histidine system

The histidine system is an example of type II, where histidine(Sr) binds with an operator regions in the DNA molecule after forming a complex with the apo-repressor molecule, thus decreasing the synthesis of the histidine anabolising enzyme system. The effector molecule, causing enzyme repression by binding with apo-repressor, is usually known as the co-repressor and is generally a reaction product of the enzyme. Any unnecessarily excessive production of enzyme is controlled by such a mechanism.

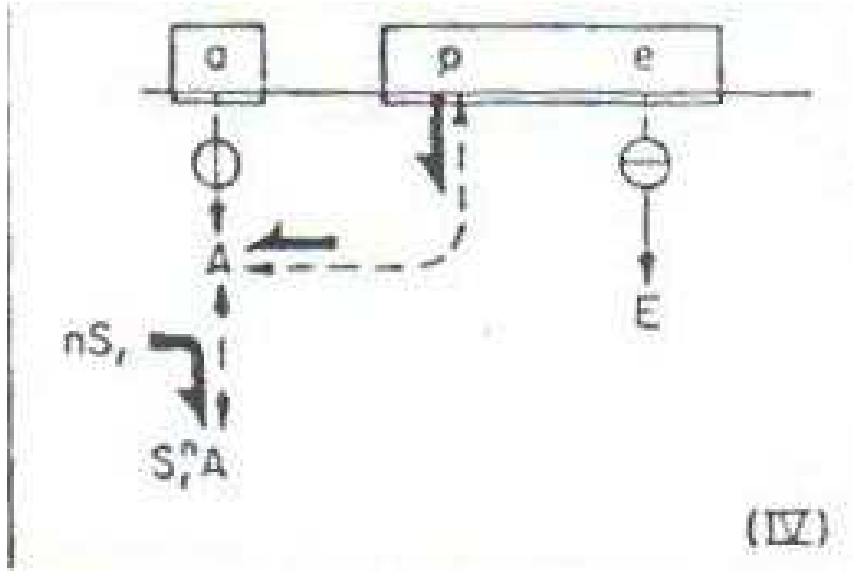
Positive Induction



Arabinose system

The arabinose system is represented by type Ⅲ. A precursor of the activator(A) which, it is suggested is a repressor molecule itself, binds with L-arabinose(S_i) to become a genuine activator(S_i^m-A). This complex acts on a promotor gene(p) for promoting the synthesis of the arabinose-catabolising enzyme system(E) including L-arabinose isomerase, L-ribulose kinase and L-ribulose 5-phosphate 4-epimerase. Studies of the arabinose system reveal that the derepression represented by type Ⅰ also occurs concurrently.

Positive repression



Alkaline phosphatase system

Alkaline phosphatase is regulated by the mechanism shown by type Ⅳ. Inorganic phosphate is an anti-activator, which gives a repressive effect in the positive control of enzyme synthesis. Functionally, it resembles the co-repressor in negative control as represented by type Ⅱ.

Mathematical Models

Negative Control

$$R + mS \leftrightarrow RS^m \qquad K_1 = \frac{[RS^m]}{[R][S]^m} \quad \cdots \quad (1)$$

$$O + R \leftrightarrow OR \qquad K_2 = \frac{[OR]}{[O][R]} \quad \cdots \quad (2)$$

R: Repressor
S: Inducer
O: Operator

$$[R_t] = [R] + [RS^m] \quad \cdots \quad (3)$$

$$[O_t] = [O] + [OR] \quad \cdots \quad (4)$$

$$K_1 = \frac{[RS^m]}{[R][S]^m}$$

$$\Rightarrow K_1[S]^m = \frac{[RS^m]}{[R]} = \frac{[R_t]-[R]}{[R]} = \frac{[R_t]}{[R]} - 1 = \frac{[R_t]K_2[O]}{[OR]} - 1 \text{ by eqn (2), (3)}$$

$$\Rightarrow 1 + K_1[S]^m = \frac{[R_t]K_2[O]}{[OR]} = \frac{[R_t]K_2[O]}{[O_t]-[O]} = \frac{K_2[R_t]}{\frac{[O_t]}{[O]} - 1} \text{ by eqn (4)}$$

$$\Rightarrow \frac{[O_t]}{[O]} = 1 + \frac{K_2[R_t]}{1 + K_1[S]^m}$$

$$\Rightarrow Q = \frac{[O]}{[O_t]} = \frac{1}{1 + \frac{K_2[R_t]}{1 + K_1[S]^m}} = \frac{1 + K_1[S]^m}{1 + K_1[S]^m + K_2[R_t]}$$

S_1 concentration ↑
$\Rightarrow Q_1 \fallingdotseq 1.0$

S_1 concentration → 0
$\Rightarrow Q_1 = 1/(1+K_1[S]^m) \fallingdotseq 0$

2.3 Production of Enzymes

Industrial Enzyme Production

Microbial enzymes are produced by both surface and submerged fermentation processes.

Surface culture	Submerged culture
Requires much space for trays	Uses compact closed fermentors
Requires much labor	Requires minimum of labor
Little power requirement	Needs considerable power for air compressors and agitators
Minimum control necessary	Requires careful control
Recovery involves extraction with aqueous solution, filtration or centrifugation, and perhaps evaporation and/or precipitation	Recovery involves filtration or centrifugation, and perhaps evaporation and/or precipitation

Medium Preparation

Composition of medium for bacterial amylase production

Ground soybean meal	1.85%
Amber BYF(autolyzed brewers yeast fraction, Amber Laboratories)	1.50
Distillers' dried solubles	0.76
N.Z Amine(enzymatic casein hydrolyzate, Sheffield Chemical Co.)	0.65
Lactose	4.75
$MgSO_4 \cdot 7H_2O$	0.04
Hodag KG-1 antifoam (Hodag Chemical Corp.)	0.05
Water	90.40

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