Intracellular Regulatory Mechanisms

Regulation in multicellular forms may be an elaboration upon the pattern evolved in microorganisms.

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A characteristic property of living organisms is that nearly all their activities are highly ordered. This implies the existence of efficient regulatory mechanisms. In as highly developed an organism as man this regulation must be exerted at many levels of complexity. At the level of greatest complexity there are activities apparently controlled by decision-making processes in the central nervous system which, in turn, may evoke responses that are more clearly automatic, involving reflex arcs and hormonal secretions. At present, probably none of these more complex regulatory devices can be interpreted on the molecular level. More success has been achieved in the study of regulatory mechanisms which operate in single cells—that is, mechanisms which originate in and are concerned with the function of a single cell. While most such studies have been made with single-cellular forms, such as bacteria, more and more are being made with cells of multicellular plants and animals. It would be expected, however, that where the structures involved in the activity are essentially the same, the regulation of the activity would involve a common mechanism (*1*).

Regulation of Enzyme Activity

The simplest pattern of control over a cellular activity is that which affects the operation of a sequence of enzymic steps. For example, one of the earliest sequences to be analyzed was the series of five enzymes required to convert threonine to isoleucine (*2*).

$$\text{Threonine} \xrightarrow{1} \alpha\text{-ketobutyrate} \xrightarrow{2} \alpha\text{-acetohydroxybutyrate} \xrightarrow{3} \alpha,\beta\text{-dihydroxy-}\beta\text{-methylvalerate} \xrightarrow{4} \alpha\text{-keto-}\beta\text{-methylvalerate} \xrightarrow{5} \text{isoleucine.}$$

This sequence is but one of many which enable bacterial cells to convert a simple carbon source, such as acetic acid or a sugar, to all the complex components of cell material. If preformed isoleucine is added to the growth medium, the synthesis of isoleucine is immediately and almost completely quenched and the exogenous isoleucine is preferentially used. The mechanism by which this quenching is achieved is as follows. The first enzyme in the sequence is extremely sensitive to isoleucine, the endproduct of the sequence. The presence of isoleucine in excess blocks the first step, and thus effectively prevents excessive or unnecessary function in the entire sequence of enzymes.

This pattern of self-regulation was first revealed in the glycolytic pathway by Dische (*3*), who, in 1940, discovered that the phosphorylation of glucose in erythrocytes was prevented by the addition of phosphoglycerate. The pattern has been subsequently found in a number of biosyntheses. The endproduct-sensitive enzyme which has been studied most thoroughly is that which catalyzes the first specific step in the pathway to the pyrimidine bases. Gerhart and Pardee (*4*) have examined the interaction of this enzyme, aspartate transcarbamylase, with its endproduct inhibitor, cytidine triphosphate (CTP). These studies have revealed the kind of mechanism that will probably be found to underlie the interaction of other endproduct-sensitive enzymes with their inhibitors.

Mechanism of Endproduct Inhibition

The inhibition of aspartate transcarbamylase was of considerable early interest because of the dissimilarity between the structure of CTP and that of the substrate (aspartate) with which it competed. It would be difficult to visualize a site with an affinity for either CTP or aspartate, as would be possible with classical examples of competitive inhibition (see Fig. 1).

An alternative explanation that the inhibitor and substrate combine with the enzyme at two sites was suggested by the demonstration that treatment of the enzyme with heat, with mercuric ions, or with urea abolished the endproduct sensitivity of the enzyme without destroying its catalytic activity. The two sites, though physically separate, were nevertheless functionally linked. It was also observed that the loss of endproduct sensitivity through heat treatment was accompanied by a dissociation of the enzyme into four subunits.

The kinetic behavior of the subunits was quite different from that of the native, CTP-sensitive enzyme, suggesting that in the native enzyme there are interactions between the subunits which decrease the catalytic activity. It is thought that aspartate overcomes these interactions much as combination with oxygen overcomes the interactions between the heme groups in hemoglobin. However, CTP, the inhibitor, enhances them. Although alterations in the state of aggregation of the subunits may not account for the varying degrees of catalytic activity that are exhibited by the enzyme as the amounts of adenosine triphosphate (ATP), CTP, and substrates are varied, the binding of the CTP appears to involve an extensive conformational change which produces an "unfavorable" configuration of the enzyme surface at the catalytic site.

The idea that the binding of a small molecule induces extensive conformational changes in a protein is not new. Koshland (*5*) has emphasized the role of a substrate in inducing a conformational change in the structure of an enzyme—a change that is essential for the occurrence of catalysis. Earlier, Wyman (*6*) proposed that conformational changes accounted for the influ-

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ence that the combination of oxygen with one of the heme groups of hemoglobin has on the reactivity of the remaining heme groups. Monod *et al.* (*7*) have introduced the term *allosteric proteins*, to include the class of enzymes in which combination with a small molecule "effector" (such as CTP in the case of aspartate transcarbamylase) leads to a change in configuration such that the catalytic activity is altered. The word *allosteric* appears to have been employed originally to describe those inhibitors that were sterically unrelated to the substrate of the enzyme which they inhibited, in contrast to the "isosteric" inhibitors classically associated with competitive antagonisms of enzyme action. The concept of allosteric interaction has, however, been broadened to include any change in activity of an enzyme which is brought about by the selective binding of a molecule at a site on the enzyme that is distinct from the substrate site. Although the terminology was proposed before endproduct inhibition of aspartate transcarbamylase had been shown to involve subunit interaction, it has been extended to include the conformational changes that are exhibited by hemoglobin upon combination with oxygen. Monod *et al.* (*7*) have pointed out that an allosteric enzyme serves as a chemical transducer that allows interaction between compounds that might otherwise not have been able to interact, and that it thus may provide a link between the compounds.

While aspartate transcarbamylase has provided the prime example of an enzyme sensitive to endproduct, several other systems have also been studied. One of these is ribosyl-5-phosphate-ATP-pyrophosphorylase, the enzyme catalyzing the first step in histidine biosynthesis (*8*). The enzyme has been highly purified, and inhibition by the endproduct (histidine) has been analyzed by Martin (*9*). Like aspartate transcarbamylase, this enzyme loses endproduct sensitivity as a result of several procedures, including exposure to mercuric ions. It appears, however, that loss of sensitivity does not result in failure to bind histidine but only in a loss of the *effect* of the binding. Evidence was obtained that this enzyme, too, is composed of several subunits, and is actually a much larger molecule than any of the other enzymes in the histidine biosynthetic pathway that have been studied.

Studies on another endproduct-sensitive enzyme, threonine deaminase, have been hampered by the fact that the enzyme has thus far been only partially purified. However, its kinetic properties are strikingly similar to those of purified aspartate transcarbamylase, and the changes in the properties of threonine deaminase that result upon exposure to endproduct, mercuric ions, and urea are best interpreted as being due to conformational changes induced in a molecule composed of interacting subunits (*10*).

One could compile at this time a fairly long list of enzymes which catalyze reactions recognized as initial steps of sequences leading to specific endproducts and which are inhibited by these endproducts. In addition, several enzymes have been recognized which require small-molecule activating compounds that do not appear to take part in the reaction (*7, 11*). It appears quite likely that combination of the enzyme with these activating compounds is essential for conversion of the protein to a catalytically active state. While in some cases, such as the dependence of the pyruvate carboxylation reaction on acetyl coenzyme A (*12*), the physiological role of the interactions are somewhat subtle, they provide an illustration of the range of allosteric interactions envisaged by Monod *et al.* (*7*).

Although endproduct inhibition has appeared to involve an allosteric transition in each case in which the investigation has proceeded far enough, it would be possible for a perfectly adequate endproduct inhibition to involve a classical steric interaction. For example, oxaloacetate is a powerful inhibitor of succinic dehydrogenase (*13*). Regardless of whether, in this rather simple example, endproduct inhibition does or does not play a regulatory [feedback (*14*)] role, it is conceivable that the inhibition by oxaloacetate is isosteric rather than allosteric. At the same time it should be emphasized that allosteric transitions include a class of interactions far larger than the class of rather special cases of endproduct inhibition considered and described here. In turn, allosteric transitions are but special cases of induced conformational changes which may be an essential feature of all interactions between small molecules and proteins, as discussed by Koshland (*5*).

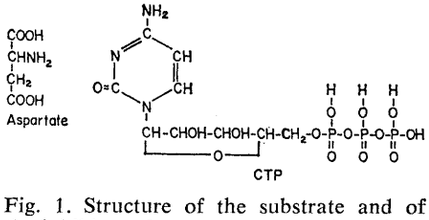

Fig. 1. Structure of the substrate and of the inhibitor of aspartate transcarbamylase.

Regulation of Enzyme Synthesis

A regulatory mechanism complementary to that already described is one which affects not the *activities* of enzymes but their *formation*. That there are mechanisms controlling the appearance of enzymes must be concluded from the fact that a single fertilized mammalian egg cell gives rise to so complex a structure as an adult mammal with a multitude of different cell types with differing enzymic composition. Although the mechanism of differentiation is as yet far too complex to be interpreted in molecular terms, it has been possible to study, as a model system, the factors affecting the formation of a particular enzyme [such as tryptophan pyrrolase (*15*)] in a particular animal tissue (such as the liver). Here again, however, such studies have been more successful in bacteria.

That results with bacteria have been especially significant is due to the fact that, for numerous pathways in bacteria, the responsible enzymes are formed only when they are of use. Thus, if the product of a biosynthetic pathway is supplied from without, the entire sequence of enzymes responsible for its biosynthesis will be virtually absent (*16*). In other words, there has been a specific repression of enzyme synthesis. Similarly, a number of enzymes normally responsible for the dissimilation of energy sources appear only when the relevant energy source is present; such enzymes are termed "inducible." For example, β-galactosidase, which is responsible for the cleavage of lactose into its component hexoses, is by far the best studied of the inducible enzymes in bacteria. Typically, for organisms such as *Escherichia coli*, the enzyme is found only in cells grown in a medium containing lactose. Particularly interesting is the further observation that the lactose is not used as long as the medium also contains glucose, a sugar which is more readily utilized; induction of β-galactosidase occurs only after the glucose has been consumed.

The analysis of these two seemingly reciprocal phenomena controlling the synthesis of specific enzymes has in-

volved both biochemical and genetic techniques. The genetic approach was particularly important, since the genetic information which specifies the structure of an enzyme is distinct from that which specifies the amount of the enzyme formed under various conditions. Thus, mutants have been isolated which form a perfectly normal enzyme but possess an altered mechanism for regulating its formation. Fine-structure genetic mapping of the mutational sites in such mutants and determination of the biochemical consequences of these mutations have yielded information that led to the proposal of a number of reasonable models. The one which appears to account for the greatest number of facts, and to ignore the fewest, is that proposed by Jacob and Monod (*7, 17*).

This model has the advantage of being sufficiently explicit to suggest further experiments, yet not so rigid as to resist modification when the newer facts warrant it. (As a matter of fact, the model has undergone such an evolution, as the result of several subtle modifications, that a reviewer hardly knows whether to refer to the first bold model, which was in itself a considerable achievement, or to a more sophisticated version which may until now have been disseminated only by way of the "preprint" route. Nevertheless, at any given time the contemporary version of the Jacob-Monod model has seldom been outdated by more than one experimental fact.) While the model has been derived mainly from findings of Jacob and Monod themselves, and of their collaborators, on the induction of β-galactosidase, studies in other laboratories on repression in the pathways to synthesis of histidine (*18*) and arginine (*19*) and on the rapidly labeled ("informational") fraction of RNA (*20*) have been of considerable importance in the development of the model.

The model in its present form takes into account several recognized properties of the protein-forming system. These include the following.

1) The instructions for the synthesis of a specific protein are transmitted from mother to daughter cell by replication of the corresponding regions of DNA.

2) The information in a region of DNA is transcribed into RNA when RNA polymerase, beginning at one end (designated the promotor), produces a single-stranded RNA molecule (messenger RNA) that is "complementary" to *one* of the DNA strands.

3) The region of DNA transcribed into a single message may contain instructions for the synthesis of several proteins.

4) The translation of the message occurs on ribosomes through the action of soluble enzymes which polymerize amino acid residues attached to specific adapter molecules. Each adapter molecule (sRNA) contains a site for the recognition of the nucleotide triplet code word corresponding to the amino acid it carries. A single message may be translated several times to yield more than one copy of the polypeptide or polypeptides it specifies.

5) Under certain conditions, a specific region of DNA cannot be transcribed (this is called repression). Repression obtains if a specific cytoplasmic factor, itself the ultimate product of a specific (repressor) gene and presumably a protein, interacts with a site (operator) adjacent to the promotor in that DNA region. This factor may require activation by a small molecule (for example, a derivative of the endproduct of a biosynthetic sequence). On the other hand, the factor may be prevented from interaction with the DNA (this is called induction) when certain small molecules are present (for example, the substrate of an enzyme coded by the region). The repressor would thus appear to be another example of an allosteric protein subject to stimulation by a repressor or to inactivation by an inducer.

6) Mutations can affect transcription by modifying the repressor or the operator or by destroying the promotor for the DNA region in question.

Space does not permit even a summary of the evidence that has been marshaled by Jacob and Monod in support of their model. Because of the astute deductions and logic that were employed in its development, any or all of the original essays are recommended reading.

Omitted from the foregoing outline is mention of the interaction whereby the induction of an inducible enzyme is prevented when glucose or some other "preferred" carbon source is present. This phenomenon has long been known for many inducible, degradative enzymes and has been called the "glucose effect." Recently Magasanik and Mandelstam (*21*) have independently shown that if certain biosynthetic activities, such as protein synthesis, are made to proceed slowly, through limitation of a growth factor, enzymes normally repressible when glucose is a carbon source can also be repressed by succinate or acetate. It is appropriate, therefore, to employ the more general term *catabolite repression* or *metabolite repression* used by these workers and to consider the glucose effect a special case. McFall and Mandelstam (*22*) have attributed metabolite repression to products derived from the action of these enzymes. For example, they have provided evidence that the metabolite repressor for β-galactosidase is galactose, which is not only a product of β-galactosidase action but also, as a component of the cell wall, a normal metabolite in *Escherichia coli*.

It should be emphasized that catabolite repression has not yet been accounted for in terms of the Jacob-Monod model. Experiments with yeast have indicated that glucose interferes with the formation of β-glucosidase (a maltose-splitting enzyme) by preventing its release from ribosomes (*23*). On the other hand, in *E. coli* strains in which β-galactosidase is inducible, catabolite repression is accompanied by an interference with the formation of the specific messenger (*24*). The specific repressor gene (*i*), however, is not required for catabolite repression; this is indicated by experiments performed with a constitutive strain in which the repressor gene was deleted (*25*). Thus, catabolite repression involves a second system of regulation. However, it has not yet been shown whether, in the absence of the *i* gene, the catabolite repressor exerts its effect upon transcription (as it does in strains in which the enzyme is inducible) or interferes in some specific way with the translation mechanism (*26*). The answer to this question must await the development of more sensitive methods for the quantitative measurement of specific messengers.

Coordination of Control Mechanisms

It is clear from the foregoing discussion that, by decreasing the level of enzyme in a biosynthetic pathway, repression alone could prevent overproduction of the product. The cell's response to addition of the product to the medium would, however, be considerably delayed while existing enzyme levels were lowered by dilution among the daughter cells. Furthermore, when growth had ceased, functioning of the

existing enzymes could continue, provided sufficient carbon and energy were available. Inhibition by endproduct, on the other hand, would cause cessation of metabolite synthesis at the end of the growth period and, moreover, at any time that the endproduct of the pathway became available, endogenous function could be immediately stopped. However, examination of mutants lacking control over a pathway by endproduct inhibition or repression has revealed that the overproduction of metabolites is influenced by both processes. Thus, isoleucine, which is formed by the sequence of five enzymes shown in Fig. 1, is overproduced by cells in which the sensitivity of threonine deminase to endproduct has been lost, as well as in cells which have lost the ability to repress the formation of the five enzymes (*27*). It would thus appear that inhibition by endproduct has been evolved in such cells only insofar as it is needed for controlling the output of a pathway when the enzymes in that pathway are partially repressed, as most enzymes appear to be in cells growing "normally" in a medium containing a single carbon and energy source. This view is supported also by the finding of Moyed (*28*) that an organism could escape the inhibitory effect of a histidine analogue which inhibited the same early step that histidine itself inhibits. This escape was achieved by virtue of the derepression of all the enzymes in the histidine pathway, which resulted from interference with endogenous histidine formation. In this case the inhibitor exerted a "false feedback" sufficiently effective to prevent growth when the level of enzyme was low but not when the level of enzyme was at its maximum.

There is a distinct and obvious advantage, from the viewpoint of cell economy, in the kind of rigid control that is exerted on metabolic pathways by endproduct inhibition and repression. However, any enzyme that is thus suited (and limited) to a single function cannot play a versatile role in the cell. Yet, in some cases a given chemical transformation serves more than one function. There are two common situations in which this might occur. One is the case in which the same reaction has a biosynthetic and a catabolic role (*29*). The other is the case of the multifunctional enzymes, such as occur in pathways that branch (*30*) or in parallel pathways (for example, those required for both isoleucine and valine biosynthesis) (*2*). The more commonly found pattern of control is that of multiple enzymes (each rigidly suited to its physiological role). An alternative is multifunctional enzymes which are repressed only if all the ultimate products of the branching pathway are present (multivalent repression) (*31*).

Control of Nucleic Acid Synthesis

Before the extent to which the results with bacterial systems can be extrapolated to multicellular organisms is appraised, two other postulated regulatory circuits should be mentioned. Clearly, the messengers for biosynthetic and catabolic systems do not constitute all the species of RNA formed in the cell. The other two obvious classes are sRNA (the amino acid adapter) and the RNA of the ribosomes. On the basis of the observation that (in "normal" cells) net RNA synthesis ceases whenever a cell is starved for an amino acid, it has been postulated that RNA synthesis is blocked by any uncharged sRNA (that is, sRNA which is not combined with an amino acid) (*32, 33*).

Some support for this hypothesis has recently been obtained in the finding that sRNA molecules can combine with RNA polymerase to prevent DNA-dependent RNA synthesis, but that they are much more effective as inhibitors when they are uncharged (*34*). However, it would seem that when a cell is deprived of an amino acid it is the synthesis of sRNA and ribosomal RNA that is blocked, since during that time there is a turnover of messenger RNA (*35*). Furthermore, like repression of enzyme synthesis, the amino acid linked repression of RNA synthesis is gene-controlled and can be lost by mutation (*32*). In view of the evidence that there are numerous genes for ribosomal RNA (*36*), this genetic locus must have a pleiotropic effect. An alternative view is that it may be responsible for a cytoplasmic repressor which is activated by an uncharged sRNA molecule.

Recently a mechanism has been proposed for the control of the relication of DNA itself (*37*). According to the hypothesis, each genophore (*38*) in the bacterial cell (that is, the "main genophore" and any plasmids or other self-reproducing DNA units that may be present) contains a gene which gives rise to a cytoplasmic factor called the initiator. An initiator initiates the replication of only its homologous DNA unit. Each replicating unit is termed a "replicon." It was further proposed that replication of the (circular) genophore begins at a point, the replicator, intimately connected with a structure on the inner surface of the cell. When one round of replication has been completed, the two daughter genophores remain fixed to a common point. A second round of replication cannot occur until the growth of cell material, and particularly of the cell surface in that region, has separated the two genophores. The replicator of the genophore thus corresponds to the operator of an operon, while the initiator is analogous to the repressor and could correspond to an enzyme which prepares the DNA to become a template for DNA polymerase.

Although both the initiator and the repressor are regarded as cytoplasmic factors, the two are thought to act in a reciprocal manner. Thus, the repressor prevents a gene function (transcription) which would proceed in its absence, whereas the initiator stimulates a gene function (replication) which would not occur in its absence. The repressor provides an "off" mechanism, the initiator provides an "on" mechanism. This view is supported by the finding that certain bacterial mutants are unable to grow at 37°C, as they cannot initiate replication of their DNA at that temperature (*39*). However, replication, once initiated at a lower temperature, can be completed at 37°C. The heat-sensitive element in these cells thus appears to be the initiator, its heat-sensitivity indicating further that it is protein in nature. Other experiments have demonstrated that this genetic lesion interfering with DNA synthesis is recessive, in contrast to the dominance of repression. Additional evidence supporting the model is given in a recent paper by Jacob *et al.* (*40*).

Regulation in Multicellular Forms

In multicellular organisms, homeostatic mechanisms have been selected to integrate the collective functioning of the many cell types which constitute the whole organism. Thus, optimal expression of a certain function in a specialized cell may depend, not on the cell's own needs, but on the requirement imposed by the other cells

and tissues of the body. Regulation of such a function requires the intercellular controls provided by humoral and nervous mechanisms. It is therefore pertinent to ask whether the regulatory mechanisms described, which were evolved as intracellular controls in bacteria, are functional also in multicellular plants and animals.

An answer to this question has been obtained for those pathways which function within a given cell primarily for the benefit of that cell alone. In particular, it has been found that the pathways which lead to formation of the purines and pyrimidines are controlled at the same early steps in animals and plants as in bacteria (*41*). Although the nucleotide to which the endproduct-sensitive enzyme responds may not be the same in all cases, the physiological principle is the same, since the various nucleotides are interconvertible and any one of them might serve as the "indicator" of pool size.

Also deserving of special mention is one example of metabolic regulation that has for some time been known to occur in animal tissue, as well as in microorganisms. This is the Pasteur effect, which is the inhibition of glycolysis exhibited by cells in response to a shift from anaerobic to aerobic conditions. In the past, the Pasteur effect has been attributed to a variety of causes, ranging from an oxidation of critical sulfhydryl groups to a competition for key intermediates (*42*). Explanations based on such competition have been extended to take into account the contributory effect of the compartmentation which, in mammalian cells, separates the enzymes of the glycolytic pathway from those of the citric acid cycle and respiratory chain. However, observations made on animal tissue have recently provided a basis for a model of feedback control in which glucose dissimilation is impeded when aerobic metabolism provides large amounts of ATP. It had been known that glucose-6-phosphate is an inhibitor of hexokinase (*43*). More recently, it was observed that one of the irreversible steps in glycolysis, that catalyzed by phosphofructokinase, is inhibited by ATP (*44*). Here again, the evolution of a rapidly acting control mechanism has been achieved through the development of a mechanism by which the presence of an ample supply of the ultimate product can be recognized (*45*). Whether this interaction is related to the very early observation of Dische (*3*) has not been ascertained.

For the existence of a control over enzyme synthesis in vascular plants and in animals corresponding to that so well documented in bacteria, the evidence is less clear. Several fairly well defined systems have been studied, and the physiological conditions which affect synthesis of the enzymes have been elucidated (*46*). However, it has not been possible to subject these findings to the kind of genetic analysis which was so essential to the development of the Jacob-Monod model. Furthermore, since the degree of organization within the cells of animals and plants is considerably more complex than that in bacteria, it might be expected that the regulatory mechanisms might also involve different and more complex interrelationships. For example, the existence of chromosomes in which a considerable portion of the DNA is at any one time complexed with histones—complexes which are nonfunctional at the level of transcription (*47*)—offers a mechanism by which a large segment of the genome could be rendered physiologically inert. Even so, it is quite conceivable that, once a segment of DNA is liberated from the histone, as in the lampbrush chromosome (*48*), specific gene-operator-repressor systems might also function.

This parameter of regulation has been demonstrated in the effect of the insect hormone ecdysone, which causes the transient appearance of chromosome puffs in two specific regions on a chromosome in conjunction with the induction of pupal molting (*49*). While the genetic material thus liberated may still have been controlled by some sort of repressor system, none of it could have been transcribed (irrespective of the presence or absence of inducers or repressors at the time) until the hormone intervened. This example demonstrates not only that the combination of DNA and histones can be specifically regulated but also that at least part of the differentiation process may be accompanied by the appearance or disappearance of gene activities by a means other than somatic mutation.

Undoubtedly, with the evolution of other structural differences between cells of bacteria and cells of multicellular forms, other means of regulation have evolved. The segregation of numerous activities to specialized compartments within the cell (for example, respiration in the mitochondria) has probably provided some degree of regulation appropriate to the greater size of such cells, but has probably made necessary other control devices not required in bacteria. The integration of metabolism, cell growth, and cell division must involve numerous feedback circuits which in principle may be as simple as the circuit controlling the regulation of pyrimidine biosynthesis. Furthermore, with recognition of the versatility of "allosteric transitions," one can readily visualize a general pattern by which one activity in the cell may be performed in complete harmony with another.

Summary

The study of metabolic regulation in microorganisms has revealed several simple but efficient regulatory circuits. In one, the operation of an entire sequence of enzymes is controlled by the activity of the initial enzyme which contains a specific inhibitor site. When this site is combined with the endproduct of the sequence, the catalytic site is rendered inactive. In another, the formation of an entire sequence of enzymes is controlled by means of a cytoplasmic mediator which blocks the transcription of the genetic message (repression) when activated by the endproduct, or which allows the transcription (induction) when activated by the substrate of the first enzyme in the sequence. Additional circuits have been proposed for the regulation of RNA and DNA synthesis. The same regulatory devices could account, in part, for intracellular metabolic control in more complex animal and plant forms. However, superimposed upon these simple control circuits will be found others which take advantage of the greater degree of organization in these cells and of the possibilities for regulating gene function that are provided by the chromosomes. The pattern of proteins with special control sites, such as have evolved in the relatively simple controls found in bacteria, may also be found essential for intercellular controls involving nervous and humoral mechanisms.

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