Autogenous Regulation of Gene Expression

The mechanism by which a protein directly controls expression of its own structural gene is described.

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A large number of observations over the past few years have provided evidence for an aspect of regulation of protein synthesis that has not, up to now, been reviewed. The essence of this regulatory mechanism is that a protein specified by a given structural gene is itself a regulatory element which modulates expression of that very gene. Thus, the protein regulates the rate at which additional copies of that same protein are synthesized as well as the rate of synthesis of any other protein encoded in the same operon. Whether the gene (or operon) is under positive or negative control, whether it is inducible or repressible, whether or not it is responsive to catabolite repression, and whether control is exerted at the level of transcription or translation are not relevant to the essential nature of the mechanism. As was suggested by Jacob and Monod (*1*), a regulatory macromolecule appears to be involved in controlling the expression of all systems regulated at the genetic level in microorganisms. The regulatory mechanism under discussion here, which I shall call autogenous regulation, requires only the added stipulation that this regulatory molecule is specified by the nucleotide sequence of one of the structural genes of the operon that it regulates.

It is sometimes difficult to determine whether or not a regulatory mechanism is autogenous. One of the problems is that close linkage of a regulatory gene to the operon which its product regulates is not sufficient evidence that the regulatory gene is part of that operon. For example, the gene that specifies the repressor of the lactose operon of *Escherichia coli* is in juxtaposition to the lactose operon but is not under control of the same promoter and operator. Another problem is that a protein specified by a structural gene of an operon may affect expression of the operon only indirectly. If the effect is through some direct interaction of the protein at the level of translation, the mechanism can still be said to be autogenous. But if the effect is due to a metabolic activity of the protein, the mechanism is not autogenous. For example, any mutation in the gene for an enzyme of a metabolic pathway that limits the activity of the enzyme sufficiently, may alter expression of the operon by causing an alteration in the intracellular concentration of a coeffector for the operon, such as a substrate, intermediate, or end product of the pathway. In the case of microorganisms, it should be possible to determine whether a protein exerts a direct effect on expression of an operon, or an indirect effect; in mammalian cells this is more difficult to do.

The basic idea of autogenous regulation as a general regulatory mechanism is not entirely new. One form of this mechanism was mentioned as early as 1964 by Maas and McFall (*2*) who suggested that the first and allosteric enzyme of a metabolic pathway may play a role in regulating expression of the operon in which its structural gene resides. Primarily on theoretical grounds, Vogel (*2a*), Gruber and Campagne (*3*), Englander and Page (*4*), Cline and Bock (*5*), and Koshland and Kirtley (*6*) suggested similar regulatory mechanisms, involving control at the level of translation exerted by the nascent polypeptide chain. Over the past decade a large number of studies have indicated that many systems in prokaryotic and eukaryotic organisms may be autogenously regulated. But these studies have appeared sporadically and have remained, up to now, disconnected. The purpose of this review is to gather together these separate strands, to weave them into a single fabric, and to begin the task of attempting to discern what patterns emerge.

I will summarize the findings from a number of autogenously regulated systems, beginning with a detailed discussion of the system for histidine biosynthesis. The more general aspects of autogenous regulation and the special regulatory capabilities it confers upon metabolic systems will be discussed at the end of this article.

Autogenous Regulation in Bacteria

The system for histidine biosynthesis. I first became interested in the question of whether autogenous regulation may be of general significance during studies on regulation of the histidine operon of *Salmonella typhimurium*. Studying the kinetics of repression of the enzymes for histidine biosynthesis, I and my co-workers noted that there was a distinctive pattern in which the intracellular concentrations of the enzymes began to decline when histidine was added to a culture of derepressed cells (*7*). This pattern showed that the decline of enzyme concentration occurred in a temporal sequence that corresponded with the positional sequence of genes in the histidine operon. However, this pattern was altered whenever the allosteric site of the hexameric enzyme encoded in the first structural gene of the operon was chemically blocked, as it is in the presence of the histidine analog, 2-thiazolalanine, or was structurally damaged, as it is in feedback insensitive mutants (*8*). Indeed, under certain conditions, repression of the histidine operon was prevented altogether by such alterations in the first gene product, and it was found that this regulatory defect was not due to loss of enzymic activity but to loss of some other function of the enzyme (*9*). These findings led us to consider the possibility that this enzyme, which catalyzes the first step in the pathway for histidine biosynthesis (*10*), plays a direct role in the repression process.

It has been known for some time that aminoacylated histidine transfer ribonu-

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cleic acid (histidyl-tRNA) is required for repression of the histidine operon (*11–13*). It was generally assumed that this compound was a corepressor which fulfilled its function as a complex, the repressor, formed by interaction with a regulatory protein, the aporepressor (*14*). But despite intensive efforts to identify an aporepressor by genetic means, no such protein has been found; Ames and Hartman and their colleagues (*12, 13*) have demonstrated that all constitutive strains in which the mutation lies outside the histidine operon display impaired synthesis, maturation, or aminoacylation of histidine tRNA. An interesting feature of this work is that the procedure that was used for selecting regulatory mutants (*13*) excluded any mutant that could not grow in the absence of histidine—that is, any mutant in which one of the enzymes for histidine biosynthesis was severely damaged.

Recently, by an entirely different selection procedure, Rothman-Denes and Martin (*15*) have isolated a salmonella mutant in which a regulatory defect is the result of a single mutation in the first gene of the histidine operon. Since then, we have been able to isolate a number of mutants of salmonella in which a mutation in the first gene of the operon renders the first enzyme of the pathway insensitive to (feedback) inhibition by histidine and also renders the operon constitutive (*16*). Patthy and Dénes (*17*) had already reported studies on a mutant of *E. coli* that produces a feedback resistant first enzyme and has a faulty repression mechanism; they assume both defects to be due to a single mutation in the first gene of the histidine operon. Because of these regulatory effects of the first enzyme for histidine biosynthesis, and because histidyl-tRNA is required for repression, we considered the possibility that regulation of the histidine operon involves an interaction between the two molecules.

The idea that regulation of the histidine operon requires an interaction between histidyl-tRNA and the first enzyme for histidine biosynthesis leads to several important predictions. First, a group of mutants should be identifiable in which mutation of the first gene leads to constitutive expression of the operon. This prediction is only partly satisfied by isolation of the mutants described above, since all mutants of this type are only partially constitutive (*15–17*). Second, mutations in the first gene that affect regulation of the histidine operon should be *trans* recessive—that is, in a merodiploid strain the first gene of the operon of one genote should affect expression of the operon of the other genote, with the wild-type gene dominant to the mutant allele. A *cis/trans* test of the effect of regulatory mutations in the first gene has shown that these mutations are indeed *trans* recessive, as predicted (*16*). This indicates that the first enzyme exerts its effect on the repression process not by acting locally, at its site of synthesis, but by acting as a freely diffusible molecule. Third, it should be possible to find conditions under which the enzyme would interact with histidyl-tRNA in vitro. Experiments designed to test this prediction have shown that the enzyme has a very high affinity for tRNA in vitro, that it binds histidine tRNA in preference to any other species of tRNA, and that it binds the aminoacylated form of histidine tRNA in preference to deacylated $tRNA^{His}$ (*18*). Fourth, since the wild-type enzyme binds histidyl-tRNA specifically, then the enzyme from feedback insensitive mutants, which display the regulatory defects described above, should show altered binding to histidyl-tRNA. Recently, the enzyme was purified from a feedback insensitive strain and, as predicted, was found to be unable to bind histidyl-tRNA (*19*). This finding suggests that the results of the binding experiments in vitro are a reflection of regulation in vivo.

If the first enzyme for histidine biosynthesis acts as a regulatory protein that exerts its effect at the genetic level, then the enzyme should not only interact with histidyl-tRNA, but should also interact directly with some regulatory element in the DNA of the histidine operon. In a study designed to test such an interaction, the enzyme was incubated with the DNA of a defective ϕ80 transducing bacteriophage (*20*) that carries the histidine operon. The enzyme was found to bind to this ϕ80d*his* DNA, and the binding was not inhibited by DNA from the wild-type ϕ80 phage (*21*). Also unable to compete for the specific binding was the DNA from a phage carrying the histidine operon with a mutation in its operator gene (*21*). Thus, the enzyme binds specifically to the bacterial DNA carried in the genome of ϕ80d*his*, apparently at a site in or near the control region of the histidine operon.

To test the functional equivalent of this binding, a system was developed for transcribing ϕ80d*his* (*22*) DNA in vitro (*23*). First, it was shown that a species of RNA is synthesized in vitro from ϕ80d*his* DNA which was not synthesized when wild type (ϕ80) phage DNA was used as template (*23a*). We could show that hybridization of this RNA to the R (right-hand) strand of the phage DNA, which is the "sense" strand for the histidine operon (*23b*), was inhibited by histidine operon messenger RNA (mRNA) that had been synthesized in vivo. This R strand-specific RNA made in vitro was found to sediment in sucrose gradients at 38*S*, very close to the known sedimentation constant of histidine operon mRNA (*24*). Thus, it has been concluded that the 38*S* RNA synthesized in vitro from the R strand of the phage DNA is, in fact, mRNA for the histidine operon. When transcription of ϕ80d*his* DNA was carried out in vitro in the presence of the first enzyme for histidine biosynthesis, a dramatic change was noted. Although most species of RNA synthesized from the DNA were identical, the histidine operon mRNA was no longer made. This finding shows that the enzyme influences transcription of the histidine operon in vitro, acting as a negative control element (*23*).

Thus, the conclusion that the histidine system is autogenously regulated is based on many observations which indicate that the enzyme encoded in the first structural gene of the operon plays a role in regulating expression of the operon. Certain chemical and structural modifications of the enzyme in vivo lead to an inability of the operon to be repressed normally; the enzymic activity of the enzyme is not required for the enzyme to perform its regulatory function; the enzyme exerts its regulatory function in the *trans* position, with the wild-type gene dominant to the mutant allele; the enzyme binds histidyl-tRNA specifically and with high affinity; certain mutations in the first structural gene of the histidine operon result in (low level) constitutive expression of the operon; the enzyme binds specifically to some nucleotide sequence in the regulatory region of the histidine operon; and the enzyme blocks transcription of the histidine operon specifically in a purified system for transcribing the DNA of ϕ80d*his* in vitro. Further work on systems for the synthesis of histidine operon mRNA and the histidine enzymes in vitro, now in progress in several laboratories, will be required before all of the details of the repression mechanism are fully understood.

The histidine system illustrates several features of autogenous regulation that are to be found in other biosynthetic systems as well. First, it is the first structural gene of the operon that specifies the regulatory protein; second, the regulatory protein has an additional function—as an enzyme; and third, the regulatory protein is the enzyme that catalyzes the first step of the biosynthetic pathway and is allosteric, responding to feedback inhibition by the end product of the pathway.

Other systems for amino acid biosynthesis. The regulatory system for the five enzymes that participate in the biosynthesis of isoleucine and valine (the *ilv* system) is in certain respects similar to that for the enzymes in the histidine system. The genes that control the synthesis of the five enzymes are located together in a small segment of the chromosome [for a recent review, see (*25*)]. This *ilv* segment is organized into more than one operon, but regulation of the whole region is coordinated. The first structural gene of the *ilvADE* operon specifies the structure of threonine deaminase (threonine dehydratase, E.C. 4.2.1.16), the enzyme catalyzing the first step of the pathway for isoleucine biosynthesis. This allosteric enzyme is composed of four identical subunits (*26*), is specifically inhibited by the end product of the metabolic pathway, isoleucine (*27*), and, in vitro, binds specifically to all three of the relevant species of aminoacylated tRNA, leucine, isoleucine, and valine (*28, 29*). Recent work on this system has shown that threonine deaminase plays a role in regulation of the whole *ilv* region; certain mutations in the *ilvA* gene result in abnormal regulation of all of the *ilv* genes (*28, 30, 31*). The *ilv* system appears to be even more complex in its regulation than does the histidine system. For example, there are three operons in the *ilv* gene cluster, not just one, and the system is subject to multivalent repression, involving all three of the amino acids, leucine, isoleucine, and valine (*27, 32*). Perhaps studies on systems in vitro for transcription of the *ilv* genes and for synthesis of the *ilv* enzymes will provide evidence that will help to elucidate the details of the regulatory mechanism.

The first two examples I have given for autogenous regulation, *his* and *ilv*, are similar in several ways. Both are systems for amino acid biosynthesis, both are repressible systems, and in both systems the first structural gene of the operon specifies the structure of a multisubunit allosteric enzyme that catalyzes the first step of the metabolic pathway, is inhibited by the end product of the pathway, and binds the relevant species of aminoacylated tRNA. An obvious question that arises from these two examples is whether or not other systems for amino acid biosynthesis are also subject to autogenous regulation. It is interesting that the pattern of a first structural gene that specifies the first and feedback sensitive enzyme is also found in the systems for leucine (*33*) and tryptophan (*34*) biosynthesis; involvement of the relevant species of tRNA in regulation is also found in the biosynthetic system for leucine (*35*) and perhaps for others as well. But these characteristics are not sufficient proof of autogenous regulation; they only indicate that it may be worthwhile to look more deeply into the possibility of an autogenous regulatory mechanism.

About 8 years ago, Somerville and Yanofsky (*36*) proposed that the tryptophan operon of *E. coli* is autogenously regulated. They suggested that anthranilate synthetase, the first, and feedback sensitive, enzyme involved in tryptophan biosynthesis, the structure of which is specified by the first gene of the tryptophan operon, plays a role in regulating expression of the whole operon. This suggestion was based on the finding that in certain mutants the first enzyme of the tryptophan pathway was either inactive or feedback resistant, and in addition the tryptophan operon was less sensitive to repression or derepression. However, in these studies, the strains were not proved to contain single mutations involving only the first structural gene (*37*). The recent finding of Jackson and Yanofsky (*38*) that the tryptophan operon of a mutant in which almost the entire first structural gene has been deleted is still under normal repression control has been taken as evidence against the idea that the product of this gene plays a direct role in repression of the operon. In the case of the tryptophan system, an unlinked genetic locus has been identified as the structural gene for the aporepressor of the tryptophan operon (*39*). There is still the possibility that the first enzyme plays some other role in regulating expression of the tryptophan operon, though such a role, if it exists at all, would be likely to be an ancillary one.

Leisinger *et al.* (*40*) have suggested that acetylornithine aminotransferase (E.C. 2.6.1.11), one of the enzymes for arginine biosynthesis, plays a role in repression of the arginine enzymes in *E. coli*. This suggestion was based on the finding that the enzyme can be dramatically altered in vivo by treatment of cells with magnesium ions when the *arg* genes are repressed, but no such alteration occurs when the *arg* genes are derepressed. Presumably, the susceptibility of the enzyme to alteration depends upon whether or not it is bound in some sort of regulatory complex (*40*).

Other autogenously regulated systems. The studies of Magasanik and his colleagues (*41–45*) have provided an understanding of the system for utilization of histidine in bacterial cells, the most clearly defined example of an autogenously controlled system. The pathway consists of four enzymes, each catalyzing one of the steps in the degradation of histidine to glutamic acid, ammonia, and formamide. The genes that specify the structures of these enzymes are contiguous in a small region of the chromosome known as the *hut* (histidine-utilizing) genes (*41*). They are organized into two operons that are regulated in a coordinated fashion, and are inducible in salmonella by the first intermediate of the metabolic pathway, urocanate. The finding that signifies the autogenous manner in which the system is regulated is that the repressor of both *hut* operons is specified by one of the structural genes of one of the *hut* operons (*42, 44, 45*). Thus, induction of the system results not only in an increase in the intracellular concentrations of the enzymes for histidine degradation, but also in an increase in the intracellular concentration of the *hut* repressor (*45*). So far, no other function for this repressor protein has been identified.

Garen and Otsuji (*46*), studying regulation of alkaline phosphatase (E.C. 3.1.3.1) synthesis in *E. coli*, identified and purified a protein which, though it is not the repressor itself, is required for repression of alkaline phosphatase. Synthesis of this protein, specified by the *R2a* gene, was found to be regulated together with alkaline phosphatase, both proteins being repressed or derepressed under the same genetic and physiological conditions. Thus, although this regulatory protein is not *the* repressor, it does appear to be a protein that plays a role in regulating expression of its own gene as well as the alkaline phosphatase gene. However, before deciding whether the system is autogenously regulated, one would have to show definitively that the *R2a* protein

does not exert its effect enzymically, catalyzing formation or destruction of an inducer or corepressor.

Another example of an enzyme that directly regulates expression of its own structural gene is the dihydrofolate reductase of *Diplococcus pneumoniae*. Sirotnak and his co-workers (*47, 48*) have studied a group of mutants in each of which a single mutation in the structural gene for the enzyme causes not only a structural change in the enzyme but also a greatly increased intracellular concentration of the enzyme. They have shown convincingly that the increase in concentration of enzyme protein, measured as immunologically cross-reacting material (*48*), is caused by an increase in its rate of synthesis, not by any change in its stability or its rate of degradation. Thus, dihydrofolate reductase appears to act not only as an enzyme, but also as regulator of its own structural gene.

Studies by McFall and her colleagues (*49–52*) on regulation of the D-serine deaminase operon of *E. coli* served as the basis for what was probably the first proposal for autogenous regulation (*2*). This operon, which contains at least one known structural gene, the gene specifying the catabolic enzyme, D-serine deaminase (D-serine dehydratase, E.C. 4.2.1.13) is subject to induction by D-serine. Like several other catabolic systems, it is also regulated by catabolite repression through the system involving adenosine 3′,5′-monophosphate (cyclic AMP) and its binding protein (*52*). McFall has been able to show that there is a classical operator gene, in which mutations are *cis* dominant and lead to constitutive expression of the operon (*50*). In addition, she has shown that the operon contains another gene in which mutations result in constitutive expression of the operand, but mutations in this gene are not *cis* dominant (*51*). Because of this, and because of several other features of such mutations (*50, 52*), McFall and Bloom (*52*) have suggested that this gene may actually be the structural gene for D-serine deaminase itself or may be another structural gene of the operon, the product of which is not yet identified. In any case, the available data are consistent with the conclusion that this gene is a structural gene that resides within the D-serine deaminase operon and specifies a protein that has a regulatory function, autogenously controlling expression of the D-serine deaminase operon.

Another example of autogenous regulation may be that of the adenylosuccinase (adenylosuccinate-AMP lyase, E.C. 4.3.2.2) of salmonella. Berberich and Gots (*53*) concluded from their genetic and biochemical studies that this enzyme plays a role in regulating expression of its own structural gene [*purB* (purine)]. They were able to isolate a mutant with a lesion in the *purB* locus that displayed three defects: the adenylosuccinase activity was only 20 percent of that of the wild type; the stability of the enzyme differed from that of the wild-type enzyme; and, as determined immunologically, the *purB* gene was dramatically repressed (*53*). No metabolic consequence of the mutation was apparent that would explain the last finding as being the result of an indirect effect of the enzyme. Thus, it is possible that the protein specified by the *purB* locus may normally function not only as the enzyme, adenylosuccinase, but also as a positive regulatory protein that facilitates expression of the *purB* gene.

The studies of Henning and coworkers (*54*) clearly indicate autogenous regulation of the pyruvate dehydrogenase system of *E. coli*. The pyruvate dehydrogenase complex consists of three enzymes (pyruvate dehydrogenase, E.C. 1.2.4.1; dihydrolipoamide transacetylase, E.C. 2.3.1.12; and dihydrolipoamide dehydrogenase, E.C. 1.6.4.3) which together catalyze conversion of pyruvate, nicotinamide adenine dinucleotide (NAD), and coenzyme A to acetyl-CoA, carbon dioxide, and NADH (*55*). The genes that specify the structures of at least two, perhaps all three, of these enzymes are located together on the bacterial chromosome and function as a single operon, the *ace* (acetate) operon, which is inducible by pyruvate (*56, 57*). Henning suggested that the protein specified by the first gene of the *ace* operon functions both as an enzyme and as a regulatory protein for the operon. The strongest among several lines of evidence on which this suggestion was based was the finding that strains that contain a nonsense mutation of the first gene, and in which the second gene product is still produced, produce the second gene product at a low constitutive level; furthermore, expression of the second gene becomes normally responsive to regulation again when a nonsense suppressor is introduced into the strain (*58*). As in the case of the histidine system, the regulatory function of the first gene product acts in the *trans* position and the wild-type first gene is dominant to the mutant allele (*58*). Also as in the histidine system, the regulatory properties of the enzyme are independent of the catalytic function of the enzyme (*57*).

Autogenous Regulation in Bacteriophages

A clear example of autogenous regulation is the positive effect of the repressor of bacteriophage lambda on expression of its own structural gene, *c*I. Kourilsky *et al.* (*59*) and Heinemann and Spiegelman (*60*) have presented evidence that such a regulatory mechanism operates under conditions in which the lysogenic state is being maintained. There are two modes of synthesis for the lambda repressor—the establishment mode, in which the lysogenic state becomes established, and the maintenance mode, in which the lysogenic state is maintained (*61*). The two modes differ with respect to the size of the transcriptional unit in which the *c*I gene is included. The smaller of these units is synthesized in the maintenance mode; the larger, in the establishment mode. It is in the maintenance mode that the repressor acts as a positive regulator of its own structural gene (*59, 60*).

Russel (*62*) has provided evidence for autogenous regulation of the DNA polymerase specified by gene 43 of the bacteriophage, T4. She has shown that in all amber mutants and many of the temperature-sensitive mutants tested, the rate of synthesis of defective enzyme, identified as a radioactively labeled band on sodium dodecyl sulfate acrylamide gels, is greatly increased. Thus, it appears that the wild-type enzyme normally acts to inhibit expression of its own structural gene. In similar studies, Gold *et al.* (*63*) and Krisch (*64*) have shown that the protein encoded in the T4 gene 32 plays the role of a negative control element in regulating expression of its own structural gene.

Autogenous Regulation in Fungi

Cove and Pateman (*65*) have proposed that in *Aspergillus nidulans*, nitrate reductase acts not only as an enzyme but also as a regulatory protein that controls the rate of nitrate reductase biosynthesis as well as the rate of synthesis of nitrite reductase and hydroxylamine reductase. Certain muta-

tions in the structural gene for nitrate reductase result in constitutive synthesis of all three enzymes (*66*). Cove and Pateman have shown that this effect is not due to accumulation of an internal inducer. An interesting feature of the nitrate reductase system is that nitrate reductase does not appear to be *the* repressor; another gene, the *nir* gene, has been identified that specifies a regulatory protein (*67*). Cove and Pateman (*65*) have concluded that this regulatory protein by itself is an activator; it is converted to a repressor by free nitrate reductase, but cannot be so converted by nitrate reductase when the enzyme is bound to nitrate. Thus, the regulatory role of nitrate reductase appears to be one in which the enzyme is able to determine whether another regulatory protein will cause activation or repression of a group of genes, depending upon whether or not the enzyme is bound to nitrate. Since one of the genes controlled by this system is the structural gene for nitrate reductase itself, the system can be said to be autogenously regulated.

Messenguy and Fink (*68*) have studied regulation of the system for histidine biosynthesis in *Saccharomyces cerevisiae* and have found that in this organism, as in bacteria, the first enzyme for histidine biosynthesis plays a role in regulating expression of the histidine genes. They have isolated a large number of mutants in each of which a single mutation in the structural gene for the first enzyme not only causes a defect in the catalytic or feedback properties of the enzyme but also prevents the repression mechanism from responding normally to changes in the intracellular concentration of histidine. They showed that the regulatory defect in these mutants did not result from any metabolic alteration. It is interesting to note that in *S. cerevisiae* most of the *his* genes are scattered among several chromosomes, not organized into a single operon (*69*); yet the autogenous feature of their regulation is maintained.

Magee and his colleagues (*70, 71*) have studied the isoleucine-valine system in *S. cerevisiae*. Their conclusions have been similar to those reached by workers studying the *ilv* system in bacteria. The first enzyme for isoleucine biosynthesis, threonine deaminase, appears to be involved in regulating expression of its own structural gene as well as expression of the other *ilv* genes (*70*).

Dorfman (*72*) has suggested that in *S. cerevisiae* the enzyme, adenylosuccinate synthetase (E.C. 6.3.4.4), plays both an enzymic and a regulatory role. He has isolated mutants in which the structural gene for this protein is altered, causing a loss of enzymic activity, and regulation of purine biosynthetic activity is partially constitutive. Metabolic studies by Dorfman and his colleagues (*73*) indicate that the regulatory defect is not due to the altered enzymic activity of the mutant enzyme, but to the altered ability of the mutant enzyme to fulfill its function in regulating the rate of adenylosuccinate synthetase formation. The regulatory mechanism thus appears to be truly autogenous.

Autogenous Regulation in Mammalian Cells

Recent studies by Stevens and Williamson (*74*) suggest that the biosynthesis of mouse myeloma protein is autogenously regulated. Myeloma protein, like all immunoglobulins, is composed of two identical heavy polypeptide chains and two identical light polypeptide chains. Stevens and Williamson (*74*) found that the protein binds specifically to heavy-chain mRNA, preventing further translation of this message. Thus, it appears that at least one immunoglobulin—myeloma protein—acts autogenously at the level of translation, regulating the rate at which additional copies of one of its component parts, the heavy polypeptide chain, is synthesized.

Yoshida (*75*) has reported the identification of a variant of glucose-6-phosphate dehydrogenase (E.C. 1.1.1.49) in a human being, that is associated with overproduction of the enzyme. In this case, a single amino acid substitution in the protein was found. To explain his finding, Yoshida (*75*) considered the possibility of some form of autogenous regulation, in which the enzyme affects function of its own structural gene by acting either at the level of transcription or at the level of translation, but he could not rule out the possibility that the mutant enzyme might affect gene function in some more indirect (metabolic) way. Similar findings, in which alterations in the structures of mammalian proteins are associated with overproduction of the mutant proteins, have been reported for human pseudocholinesterase (*76*) and the human hemoglobins Hijiyama (*77*), Hikari (*78*), and J (*79*).

Discussion

I have described autogenous regulation and have given several examples of autogenously regulated systems in phage, in bacteria, in fungi, and in mammalian cells. Undoubtedly there are other examples that have been unjustly excluded and many more that have yet to be discovered. If autogenous regulation is to be a useful concept in the general area of biological regulatory mechanisms, it must be used with some restraint. Many systems of control may appear to be autogenous in character before much is known about them; for example, a protein that catalyzes formation of its own inducer may appear to be an autogenous regulator until the biochemistry of the system has been fully understood. Minimal requirements for labeling a regulatory mechanism "autogenous" include demonstration that the regulatory protein influences expression of its own structural gene, not merely expression of structural genes closely linked to its own; that the regulatory function of the regulatory protein is not dependent upon any catalytic or other function this protein may have; and that the regulatory protein is not involved in the transport or metabolism of an inducer or corepressor. Ideally, experimental data should be obtained from systems in vitro, in which cell extracts are used for the synthesis of specific mRNA or protein, and in which the effects of addition of purified regulatory protein can be assessed. A number of examples in the literature were not included in this article because a metabolic basis for the findings had not been ruled out. Even so, it is not unlikely that among the examples included here one or more may turn out not to be autogenously regulated.

It is to be hoped that as more autogenously regulated systems are studied, new features of the phenomenon will be appreciated, adding to our understanding of this mechanism. It is too early to decide whether autogenous regulation was among the first regulatory mechanisms to evolve, and too early also to extrapolate from the examples at hand to give an indication of how widespread the phenomenon may be.

It has become clear, partly through studies on systems in vitro in which cell extracts are used for synthesis of enzymes directed by specific DNA, that a multiplicity of controls may influence the expression of genes (*80*). For ex-

ample, Zubay and his colleagues (*81*) have recently shown that, aside from the effects of inducer, repressor, and cyclic AMP and its binding protein, β-galactosidase synthesis directed by λd*lac* DNA in vitro is stimulated by guanosine tetraphosphate (*81*). Zubay (*82*) has also obtained evidence for the existence of a protein that stimulates transcription of the tryptophan operon in vitro and has suggested that it might be a regulatory protein for all operons of biosynthetic systems. If any one of the proteins that influence expression of a gene is autogenously regulated, then regulation of the entire system is autogenous.

The association between first gene and first enzyme, which we have noted in systems for the biosynthesis of a number of amino acids, is a striking one. It appears that this association is not fortuitous, but arose and was preserved during the evolutionary process because it confers upon the organism some selective advantage in the continual tests for survival.

It seems appropriate to ask what selective advantage autogenous regulation may have aside from simplicity of design and aside from maintaining all of the genetic elements of a system together in one place on the chromosome, protecting them from being separated by recombination. One of the possible answers is, as suggested by Hagen and Magasanik (*45*), that autogenous regulation provides the cell with a buffered control system. In the case of a negatively controlled system, such as the *hut* system, the organism responds to changes in the environment not only by synthesizing the enzymes at an appropriate new rate but also by altering the rate of synthesis of the regulatory protein that tends to oppose the response. Such a mechanism results in the ability of an organism to avoid extreme changes in gene expression that might otherwise occur as the organism meets new environmental conditions.

Autogenous regulation can be a conservative mechanism, as suggested for the D-serine deaminase system. Studies on this system indicate that the regulatory mechanism allows expression of the operon to be closely geared to protection of the organism from the toxic compound, D-serine. When D-serine is present in the culture medium, it enters the cell and binds to the repressor of the D-serine deaminase operon—D-serine deaminase itself or another protein encoded in the same operon. This leaves the operator of the operon free, facilitating more rapid synthesis of the catabolic enzyme, D-serine deaminase, which results in a rapid destruction of the toxic D-serine. But if induction of the operon also leads to an increase in the rate of synthesis of its repressor, then as soon as the intracellular concentration of repressor exceeds that which is necessary to bind the intracellular D-serine, the free repressor stops further expression of the operon. Thus, the cell manages to survive the toxic compound in the most economical way: it produces only a sufficient amount of the enzyme required to destroy D-serine, but no more; once the amount of free D-serine has been reduced by enzymic degradation or by becoming bound to repressor, further synthesis of the catabolic enzyme is halted.

Thomas (*83*) and, more recently, Kourilsky and Gros (*84*) have discussed various combinations of simple regulatory mechanisms. These combinations produce complex regulatory systems which the latter authors refer to as open circuits and closed regulatory loops. The closed regulatory loop is a system controlled by two regulatory proteins, each of which controls the rate of synthesis of the other. Such a loop has many of the properties of an autogenously regulated system. Up to now, I have discussed autogenously regulated systems in which a single regulatory protein directly controls the rate of transcription of its own structural gene. As Kourilsky and Gros (*84*) have observed, the closed regulatory loop involving two regulatory proteins is theoretically equivalent to this. As a matter of fact, the case of a protein which controls expression of its own structural gene is the simplest case of the closed regulatory loop.

Kourilsky and Gros (*84*) have discussed closed regulatory loops in terms of the various possible combinations of effector proteins—repressors and activators. They suggest that a combination of two activators results in mutual amplification of expression of the two genes. A combination of one repressor and one activator results in what they call a burst of gene expression, meaning that it results in transient expression of the regulated gene or genes. A combination of two repressors results in what they call a reciprocal switch, meaning that by this mechanism expression of a gene, or group of genes, may be inhibited very severely in extent and duration. In fact, Eisen *et al.* (*85*), discussing an example of such a loop in bacteriophage lambda, viewed this last mechanism as a model for differentiation in its simplest form.

It is important to recognize that the final effect on gene expression that results from each type of closed regulatory loop depends upon whether the loop functions in an isolated manner. The moment a loop is perturbed, the prediction of how gene expression will be affected will depend partly upon the nature of the perturbation. For example, in the closed regulatory loop formed by a repressor that represses further repressor formation, an inducer that inactivates the repressor would impose an additional set of parameters on the regulatory system. Furthermore, expression of autogenously regulated genes will also depend upon the affinities and intracellular concentrations of the various interacting participants in the regulatory system, such as repressor, operator, inducer, activator, and corepressor. For example, severe and prolonged inhibition of gene expression will occur in a negatively controlled autogenous regulatory system only if the affinity of repressor for operator is very high.

Sompayrac and Maaløe (*86*) have pointed out that an autogenously regulated repressible system in its pure form is able to provide a cell with a constant concentration of the protein or proteins specified by the regulated operon, independent of the growth rate of the cell and independent of cell size. They have proposed, on theoretical grounds, that such a system would be well suited for control of DNA replication. Recently, Hayward *et al.* (*87*) have demonstrated that the rate at which RNA polymerase is synthesized in *E. coli* is subject to specific regulation. It is tempting to speculate that in this case, too, the mechanism of the regulation is autogenous.

Summary

A new term, autogenous regulation, is used to describe a phenomenon that is not a new discovery but rather is newly appreciated as a mechanism common to a number of systems in both prokaryotic and eukaryotic organisms. In this mechanism the product of a structural gene regulates expression of the operon in which that structural gene resides. In many (perhaps all) cases, the regulatory gene product has several functions, since it may act not only as a regulatory protein but also as an enzyme, structural protein, or antibody,

for example. In a few cases, this protein is the multimeric allosteric enzyme that catalyzes the first step of a metabolic pathway, gearing together the two most important mechanisms for controlling the biosynthesis of metabolites in bacterial cells—feedback inhibition and repression. Autogenous regulation may provide a mechanism for amplification of gene expression (*84*); for severe and prolonged inactivation of gene expression (*85*); for buffering the response of structural genes to changes in the environment (*45, 52*); and for maintaining a constant intracellular concentration of a protein, independent of cell size or growth rate (*86*). Thus, autogenous regulation provides the cell with means for accomplishing a number of different regulatory tasks, each suited to better satisfying the needs of the organism for its survival.

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