Jñānabha, Sect. A, Vol. 2, July 1972.

GENE REGULATION IN HIGHER ORGANISMS

By

H. K. SRIVASTAVA

(Professor of Agricultural Botany, Institute of Advanced Studies, Meerut University, Meerut.)

(Received on July 3, 1973)

1. INTRODUCTION :

It is generally considered that the genetic information in most of the complete cells of a complex metazoan organism is identical with that of every other cell. Thus the tremendous diversity of cell phenotypes found in an organism must be derived from; (a) cells expressing only a limited amount of its full genetic potential and (b) cells expressing different portions of their genome. Cell differentiation is based almost certainly on the regulation of gene activity, so that for each state of differentiation a certain set of genes is active in transcription (transfer of genetic information from DNA to RNA) while other genes remain inactive (Britten and Davidson, 1969). It has been established in bacteria and viruses that DNA is the primary genetic material and that genetic information is expressed through an intermediate messanger RNA, which acts as direct template for protein synthesis (Watson, 1969). A bacterial gene is "active" only when its corresponding messenger RNA is produced. Therefore, regulation of gene function depends on controlling the synthesis of specific messenger RNA's. Important regulatory processes occur at all levels of biological organisation. The genes controlling lactose metabolism in bacteriophage and *Escherichia coli* are activated by a specific "Inducer" that combines with the repressor, causing the latter to detach from the DNA and permitting the messenger RNA to be synthesized (Jacob and Monod, 1961; Ptashne, 1967). The formation of messenger RNA for the group of genes controlling lactose metabolism is further subjected to inhibition by the attachment of specific protein repressors to specific regulatory genes on the chromosome.

The elegance of these ideas and the clarity with which they have subsequently been verified in prokaryotes have led their widespread acceptance as an explanation for gene regulation in higher organisms. This acceptance has been bolstered by the demonstration that the fundamental mechanism of information flow (DNA——————→ RNA ——————→ Protein) in higher organism is virtually identical with that in micro-organism. Thus, in both cases, DNA is the primary genetic material, genetic information is expressed by transcription into RNA, and the codes assigning specific RNA triplets to specific amino acids of proteins are essentially indentical (Marshall *et al.*, 1967). However, certain features of the structure and function of the genetic apparatus of eukaryotic cells are very different from their bacterial counterparts. These differences raise the possibility that the mechanism which regulate gene expression in the two cases may also be significantly different. Some of the expremental evidences relating to these features are :

1. Change in state of differentiation in higher organism is often mediated by simple, external signals, as, for example, in the action of hormones or embryonic induction agents (Tomkins *et al* 1969; Tomkins and Martin, 1970).

2. A given state of differentiation tends to require the integrated activation of a very large number of noncontiguous genes (Epstein and Beckwith, 1968).

3. There exists a significant class of genomic sequences which are transcribed into RNA in the nuclei of higher organisms but appear to be absent from cytoplasmic RNA's or only a small fraction of these ever reaches the cytoplasm.

4. The genome present in higher organism is extremely large, compared to that in prokaryotes.

5. This genome differs strikingly from the bacterial genome due to the presence of a large fraction of repetitive nuclotide sequences which are scattered throughout the genome (Britten and Davidson, 1971).

6. Furthermore, these repetitive sequences are transcribed in differentiated cells according to cell type-specific patterns.

7. The cells of higher organisms also appear to use more complex mechanisms for processing nascent polypeptides than bacteria do. For example,

the initial product of translation of the poliovirus RNA seems to be a single, long polypeptide chain that is cleaved into the smaller viral components (Jacobson and Baltimore, 1968).

The extent of current interest in regulatory process is documented by the many articles and reviews on this subject. Recent work on protein synthesis and the genetic code has demonstrated the main outline of the pathway by which geneic information is translated in to the amino acid sequences of proteins. In contrast, the mechanisms which regulate gene expression and function are much less understood in higher organisms. It is, therefore, proposed to discuss current aspects of gene regulation in eukaryotes with particular regard to the operon. The reader's attention is also directed to other recent articles on regulation (Epstein and Beckwith 1968), Clever, 1968, Martin, 1969, Lewin, 1970).

II. THE OPERON CONCEPT :

One of the useful concepts in the study of biological regulation has been the concept of the operon, which was formulated in bacteria in three different kinds; 1). The clustering on the chromosome of genes that determine the enzymes of a biochemical pathway 2). Coordinate repression and depression of the enzymes controlled by these clusters 3). Specific regulatory mutations which affect the expression of the cluster of genes The crystalization and clear exposition of the operon theory were presented by Jacob and Monod (1961). The operon has also been reviewed by Ames and Martin (1964). Generally aceepted characteristics of the bacterial operon are :—

A. CONTROL OF A GENE CLUSTER

An operon consists of a cluster of genes that are activated or inactivated as a unit

B. INDUCTION AND REPRESSION

Certain specific small molecules (inducers or Co-repressors) that are substrates or products of the metabolic pathway-the enzymes of which are coded by the genes of the operon-can cause a rapid increase (induction) or decrease (repression) in the rate of synthesis of all the enzymes of the operon.

Jacob an Monod both of the Pasteur Institute in Paris proposed in 1961 an ingenious model to explain induction and repression. According to

them there are, in general, two types of genes, the structural genes (SG) which determine the amino acid sequence in enzyme proteins and the regulator gene (RG) which produces a cytoplasmic protein called repressor (R). Operator (o) is considered to be the initiation point at a certain part of DNA adjacent to structural genes. The operator and structural gene unit is called an operon. The RG functions by forming a protein molecule called as repressors. Repressors by themselves are assumed to be nonfunctional. However, when a repressor attaches itself to an inducer usually a substrate molecule, the repressor is assumed to become inactivated and thus connot attach itself to the operator and hence does not block the messenger-RNA transcription. In a repressible system. the repressor attaches itself to a specific corepressor, usually a specific metabolite, and is assumed to become activated and attaches itself to its specific operator gene, thereby blocking the transcription of specific messenger RNA.

C. COORDINATE CONTROL

The ratio of the amount of an enzyme to that of any other enzyme of the same operon is constant for given growth conditions, regardless of the extent of repression or induction (Ames and Garry, 1959).

D. POLYGENIC MESSAGE

The operon is the chromosomal unit of transcription for the formation of a melecule of messenger RNA of the same length as the operon. Experiments have shown that transcription of DNA into RNA begins at the operator end of an operon (Alpers and Tomkins, 1966) and that translation of messenger RNA into protein also begins at the operator end of the message (Berberich, 1966). Since it is known that polypeptide chains are assembled sequentially from amino-to carboxyl terminal ends (Maughton and Zintzis, 1962), it appears that genes are oriented in sequential order so that the operon end of a cistron codes from the aminoterminal end of the corresponding protein molecule.

E. POLARITY

The polygenic message is translated unidirectionally by ribosomes that starts at the operator end. Certain mutations in the genes of an operon, besides, causing a loss of function of the affected gene, also cause polarity, a relative decrease in the amount of all enzymes specified by the genes located

on the side of the mutation distal to the operator (Jacob and Monod, 1961). Polarity results from the occurrence of either "nonsense" mutations-the polypeptide-terminating triplets UAG or UAA (Yanofsky and Ito, 1966) or frame shift mutations (Banks, 1971) which give rise to nonsense triplets as a result of the shift in the reading frame during the translation of messenger RNA.

F. REGULATOR MUTATIONS

For most operons so far examined, several classes of mutations have been discovered which activate the operon in the absence of the low molecular weight inducer or co-repressor, usually required. These mutations are of two types: (i) The operator constitutive types (O), which maps at the starting end of the operon and affects only contiguous genes on the same chromosome; and (ii) the regulator gene type (e.g.i. in the lac operon) which lies on a different part of the chromosome and is recessive to the wild type. Because of these dominance relation-ships, it is generally concluded that the regulator genes produce a substance, the repressor, which inhibits operon function. The operator is to be the site of action of the repressor.

G. THE APO-REPRESSOR

The apo-repressor is a protein since it is able to exist in active or inactive forms as the results of its interaction with a small molecule co-inducer. This is most readily explained as an allosteric effect, the protein possessing a binding site for the operator which is affected by the binding of the co-inducer at a second, allosteric site. This can also most easily account for the altered behaviour of i gene regulatory mutants-is mutants for instance, are those which have lost this second, Co-inducer, bindiug site. Constitutive regulator mutants of both the alkaline phosphatase and lac systems of *E. Coli.* can be suppressed by an external nonsense condon suppressor; this phenomenon is known to be exerted at the level of translation into protein. If the apo-repressor protein interacts with DNA to prevent transcription, rather than with RNA to prevent translation, it should bind *in vitro* to DNA containing its operator, but not to DNA lacking the recognition site.

H. THE PROMOTOR

When Jacob and Monod proposed their theory of the operon in 1961, they suggested that a small segment of DNA, called the operator, located at one end of a series of genes, might control their function. This means that the

oprator may possess the distinct properties of both comprising the recognition site of the apo-repressor and of initiating the transcription (or possibly translation) of the RNA. This would be the site where RNA polymerase might start transcribing the genes of the operon into a polygenic messanger RNA and where the repressor protein which controls the structural genes might act to stop transcription. However, recent experiments have revealed that the operator is not the site for the initiation of transcription, but that some separate locus must bear responsibility for this (Lewin, 1970). Later, more mutants of the lactose operon of *E. coli* were identified to divide this region into two parts; the operator at which repressor protein binds and the promotor at which RNA polymerase apparently binds and starts transcription. A third site has recently been added, for the promotor itself may comprise an RNA polymerase binding site and a region where the cyclc AMP system acts to switch on inducible operons. All these sites are adjacent, however, in an order which suggest simple model for the control of gene expression. The binding of apo-repressor to the operator at a site located between the promotor and the structurrl genes suggests that it blocks the progress of RNA polymerase into the operon, and thus prevents it from transcribing the structural genes (Arditti *et al*, 1968). It is difficult to investigase *in vivo* the interactions which take place between apo-repressor and operator, and between RNA polymerase and promotor, and this system should assist determination of the precise mode of action of the apo-repressor. Since this segment of DNA contains only a single promotor, it should also make possible further investigations into the action of the sigma factor and the initiation of transcription by RNA polymerase.

I. SIGMA FACTOR

Sigma factor is known to be a positive control element in the development of bacteriophase T_4, promoting transcription of a specific class of T_4 genes. The ability of *E. coli* RNA polymerase to transcribe native DNA depends on the presence of a protein factor (sigma factor) which is normally found in association with the enzyme. The sigma factor acts at the very first step in initation, namely the specific binding of the enzyme to the promotor site to form a "preinitiation complex" (Bantz and Bantz, 1970).

The operon concept as developed in bacteria is not easily adapted to higher organisms. In higher organisms, however, the uuits of transcription and translation are not always of the same length. It is not clear as to which aspects of the complex regulatory mechanisms the term "operon" should refer.

Transcriptional regulation in animal cells occurs at the level of the activation or inactivation of entire chromosomes, large chromosomal segments, and possibly smaller units. Besides, translational control at the level of messenger RNA, transfer RNA, ribosomal function, and protein synthesis also occurs. Selection of messengers, perhaps by their stabilization, could be regulated because more of the genome is transcribed than actually functions as messenger RNA. Biochemical arguments for the existence of operons in animal cells have been based on co-ordinate rises and falls in enzyme levels but this type of evidence in not very significant. Despite the difficulty of transferring the idea of an operon, it is important to evaluate the role of gene clusters in higher organisms. In the operon, genetic linkage doviously allows functionally related genes to be activated by a common mechanism. Several examples of operon-like behaviour have been discovered in eukaryotes. The 3 genes governing the enzymes of the galactose pathway in yeast are clustered and controlled together by several unlike regulatory elements (Douglas and Hawthorne, 1966). Three genes of the histidine biosynthetic pathway in yeast are clustered, as are 5 genes of the aromatic pathways in Neurospora (Giles, 1965).

III. REPETITIVE DNA SEQUENCES AND GENETIC COMPLEXITY:—

Experiments in recent years have demonstrated that the genome of higher organisms contains a large amount of order which is manifested as repeated nuclectide sequences in the DNA. The possible implications of repetitive DNA sequences for the mechanism of control of genetic activity in higher organism have been considered (Britten and Davidson, 1969). Repetitive DNA sequences involved in regulation have also been invoked by Georgiev (1969), who has suggested that messenger RNA is transcribed together with adjacent repetitive sequences. Large populations of repeated DNA sequences appear to be present universally in the genomes of eukaryotes sequences above the fungi. Evidence indicates that new families of repeated DNA requences have been incorporated throughout evolution (Rice, 1971). repeated sequence relationship have been detected between organisms whose ancestors diverged hundreds of millions of years ago. For example, 5 percent sequence homology exists between the repetitive DNA's of fish and primates, and 10 percent between the repetitive DNA's of birds and primates. Other lines of evidence also indicate that the incorporation of new families of repetitive sequences can occur suddenly on an evolutionary time scale. Such sudden replicative events are termed "Saltatory replications" (Britten and Kohne, 1968). Although the precise mechanism of

saltatory replications is not known, the following processes seem necessary (Britten and Davidson, 1971). (1) Many copies are made of DNA sequences and appear in the germ cells of certain individuals. (2). The copies are some how integrated into the genome so that they are duplicated. (3) Over a sufficient period of time they are disseminated throughout the population and its evolutionary descendants. Dissemination could result from their association with a favourable genetic element or simply because of their multiplicity. (4) Individual sequences become scattered among many chromosomes and is transcribed intimately along the length of the DNA of the genome. (5) The growth of the family of repeated DNA is eventually terminated or controlled. Subsequently, individual members of the sequence families would diverge from each other through base substitutions. Also, the length of the recognisable related regions is presumably reduced by the events of rearrangement which led to their interspersion throughout the genome.

While this may be an adequate summary of the broad outlines of the history of repeated DNA families, there is subtle or no information on function and the resulting selection pressures of repeated DNA sequences in higher organisms. Britten and Davidson (1971) also commented on possible mechanism of saltatory replication, although there is again no useful experimental evidence. Four classes of events present themselves as possibilities:

(i) Erratic bchaviour of a DNA polymerase perhaps caught in a closed short loop without adequate termination controls; (2) Geometric growth of a series of short duplicated sequence due to unequal crossing over; (3) the excessive replication of some nuclear element analogous to the episomes of bacteria (not yet observed in higher organisms); (4) the integration into the genome of many copies of a viral genome or viral-borne sequence.

IV. THE MODEL OF GENEREGULATION:

An elegant model of gene regulation in ukaryotes was first advanced by Britten and Davidson (I969). Five elements are used in the model. Genes which specify cellular products such as enzymes (i.e., are regulated rather than regulatory in nature) are termed "Producer" genes. A set of producer genes whose products carry out a closely related set of functions is termed a "battery". An example of a battery would be the producer genes coding for the group of liver enzymes required for purine synthesis, which might well be activated simultaneously. The activity of the producer genes of a battery is

controlled through the interaction of particular diffusible regulatory molecules with a DNA sequence termed the "receptor" gene contigous to each producer gene of the battery. Therefore, the genes of a battery may be located at a distance from each other, or even occur on different chromosomes. The diffusible regulatory molecules of the model are derived from "integrator" genes. These regulatory molecules may ba the RNA transcribed from the integrator sequences or they may be proteins derived by translation of such RNA molecules. In either case the RNA transcribed from the integrator genes is reffered as "activator" RNA, since it bears information to be used for gene activation.

There are integrator genes whlch are transcribed in response to substances arising elsewhere. such as those hormones which affect gene activity. These substances are postulated to interact with a "sensor" structure adjacent to the integrator genes and thereby initiate transcription. Since such substances in goneral do not have an affinity for particular DNA sequence, the sensor structures must include macromolecules serving as specific binding sites.

GENE

The gene is the basic unit of life, capable of self reproduction which is the prime criterion. The word "gene" remains into use since long time though most geneticists do not like using this word any more, but its concept has entirely been changing during the last two or three decades. The classical concept of a gene assumed it to be a anitany particle by criteria involving each of the three kinds of observations: (i) a gene is a unit of chromosomal structure not subdivisible by chromosomal breakage or crossing over, (ii) A gene is a unit of physiological function or expression, and (iii) a gene is a unit of mutation. The smallest gene element that is interchangeable (but not divisable) by genetic recombination is termed as "Recon". The "mution" is defined as the smallest gene element that, when altered, can give rise to a mutant form of the organism. The "cistron" is considered to be a genetic unit of function sub-divisable into ultimate units of recombination or recon. However, the more recent difinition of the gene considers it to be a region of the genome with a narrowly definable or elementary function. It need not contain information for specifying the primary structure of a protein.

PRODUCER GENE

A region of the genome transcribed to yield a template RNA molecule or other species of RNA molecules (except those engaged directly in genomic regulation) is known as producer gene. This term is used in a manner analogous to that in which the term "structural gene" had been used in the context of certain bacterial regulation systems. Products of the producer gene include all RNA's other than those exclusively performing genomic regulation by recognition of a specific sequence. Among producer genes, for example are the genes on which the messenger RNA template for a hemoglobin subunit is synthesized, and also the genes on which transfer RNA molecules are synthesized.

RECEPTOR GENE

A DNA sequence linked to a producer gene which causes transcription of the producer gene to occur when a sequence spicific complex is formed between the receptor sequence and an RNA molecule (called an activator RNA). A receptor complex may include the DNA, histones, polymerases, and so forth. This model is concerned primarily with interrelations among the DNA sequences present in the genome.

ACTIVATOR RNA

The RNA molecules which form a sequence-specific complex with the receptor genes linked to producer genes. The complex suggested here is between native double stranded DNA and a single-stranded RNA molecule. The role proposed for activator RNA could well be carried out by protein molecules coded by these RNA's without charging the formal structure of the model.

INTEGRATOR GENES

The function of this gene is to synthesize an activator-RNA. The term integrator is intended to emphasize the role of these genes in leading, by way of their activator KNA's, to the co-ordinated activity of a number of producer genes. A set of linked integrator genes is activated together in response to a specific initiating event, resulting in the concerted activity of a number of producer genes not sharing a given receptor gene sequence.

SENSOR GENE

A gene sequence serving as a binding site for agents which induce the occurrence of specific patterns of activity in the genome is called sensor gene. Binding of these inducing agents is a sequence specific phenomenon dependent on the sensor gene sequence, and it results in the activation of the integrator gene or genes linked to the sensor gene. Such agents include, for example, hormones and other molecules active in intercellular relations as well as in intracellular control. Most of them will not bind to sensor gene DNA, and an intermediary structure such as a specific protein molecule will be required. This structure must complex with the inducing agent and must bind to the sensor gene DNA in a sequence specific way.

BATTERY OF GENES

These are the set of producer genes whose products carry out a closely related set of functions. A particular cell state will usually require the operation of many batteries. Batteries are vital to gene function and gene regulation.

An important function for repeated DNA sequences in higher organisms has been emphsized by this model. It appears that the existence of gene control functions in the cell (which can hardly be denied) logically requires the existence of repeated DNA sequence in the genome. The only way in which this implication could be totally avoided would be, to propose that all gene regulation operates through a kind of "falling domino" process in which each gene activates or represses a single other gene (Britten and Davidson, 1969). If, on the other hand, one regulatory gene is assumed to affect more than one regulated gene, then regions of sequence similarity which provide the molecular basis for the recognition process are implied among the regulated genes. This argument in itself suggests that some posts of the transcription from unrepeted sequences which is experimentally observed is likely to be involved in cellular control process.

NON-REPETITIVE DNA AND GENETIC COMPLEXITY :—

The term "complexity" is useful to express the amount of diverse DNA sequence in a given preparation and it is defined as the number of nuclotide pairs present in a single set of all the diverse sequences. In other words, the complexity is equal to the genome size as long as repeated sequences are absent. If repeated sequences are present an abvious extension is to count all of the

nucleotide pairs in a single copy of each of the repeated sequences. This is an apparently simple definition. However, the repeated sequences are rarely identical to each other. A very wide range of degrees of similarity or difference occurs among the members of most sets of related sequences. No direct evidence is available to show that the relationships among the members of the set of sequences are actually used be the organism, although there are theoritical reasons (Hood *et al.*, 1970; Britten and Davidson, 1969) for believing that they may be. In the cases of salmon (Britten and Kohne, 1968), Wheat (Bendich and McCarthy, 1970) and the urodele *Necturus* (Strauss, 1971), more then 80 percent of the genome appears to be repetitive at the customary experimental criteria. However, other conditions particularly less stringent criteria, may supply unexpected insight into the history and patterns of occurrence of repeated DNA (Britten and Davidson. 1971).

The most direct approach to wards an assessment of the function of non-repeated DNA is through measurements of transcription of RNA from these sequence fractions. It is known that repeated sequences are transcribed into RNA, although it is not known how many of the individual member sequences of a set are transcribed. The complexity of the DNA which is observed to be transcribed yields a lower limit for the functional complexity of the genes expressed in any given circumstances. For newborn mice (Gelderman and Rake, 1971) it appears that more than 12 percent of the none-repetitive sequences in the genomes are transcribed, and thus the functional complexity of the mouse genome at this stage is at least 4×10^8 nucleotide pairs. This very large number is not yet interpretable, since if all of this RNA were messenger it would code for nearly a million different hemoglobin-sized proteins. Other measurements (Devidson and Hough, 1971) indicate that the RNA stored in the occytes of *Xenopus* represents at least 1.2 percent of the non-repetitive DNA. The complexity of this RNA is such that it could code for 40,000 different hemoglobin:sized proteins. Thus, it appears that a significant portion of the potential information content of these genomes is actually expressed. It is important to note that in the *Xenopus* occyte, many more copies are present of repetitive sequence transcripts than of non-repetitive sequence transcripts. This observation suggests a functional distinction between the repetitive and non-repetitive sequences and of course for the RNA transcribed for them.

HORMONES AND GENE EXPRESSION

Cell differentiation involves drastic changes in cell metabolism. The synthesis of many proteins is stopped and that of new ones is begun. This

type of regulation could be called "Macroregulation" in contrast to "Microregulation", where the synthesis of a particular protein, or set of genes in an operon, is specifically regulated by induction (or derepression) and repression, according to Jacob and Monod (1961). There is no doubt that the principle of microregulation operates in cellular differentiation. A hormone is an effector molecule produced in low concentration by one cell which evokes a physiological response in another. Thus, hormones are extracellular compounds and they act at a different site other than the site of their production. In multicellular organisms a "second messenger" or adenosine 3'—5'— cyclic phosphate (cyclic AMP) is responsible for action of those hormones that stimulate its synthesis.

Cyclic AMP

Cyclic AMP acts as a positive allesteric effector for the synthesis of various proteins involved in phosphorylation (Tomkins and Martin. 1970). The hormones which influences cyclic AMP is called first messenger as it is transcribed by the genes. In vertebrates, numerous classes of chemically unrelated compounds such as polypeptides, amino acids, amines, fatty acid derivatives, and steroides have hormonal activity. Certain of these substances probablya re themselves the primary intracellular effectors wherea sothers clearly function at the cell surface where they activate the membrane-bound enzyme, adenyl cyclase, and thereby stimulate the production of cyclic AMP from ATP. (Sutherland *et al.* 1968 ; Robinson *et al.* 1968). A cyclic AMP-dependent kinase has been found in muscle, brain, and a number of vertebrate and invertebrate tissues (Miyamoto *et al.* 1969; Kuo and Greengard, 1969). The macromolecular substrates for the kinase reactions are either enzymes or structural proteins such as histones.Histone phosphorylation permits increased transcription specific genes,which accounts for the cyclic AMP-and glucagon-mediated induction of liver enzymes (Wicks *et al.*,1969). In addition to affecting gene transcription,cyclic AMP can also promote messenger RNA translation since its timulates the fromation of tryptophanase in *E. coli* (Pastan and perlman; 1969). Several lines of evidence using inhibitors of RNA synthesis or mutants in the promotor region suggest that cyclic AMP specifically stimulates the initiation of transcription of the operon. Cyclic AMP may also regulate the translation of messenger RNA's perhaps by stimulating the phosphorylation of certain components for example, ribosomes initiation factors etc , involved in protein synthesis. The regulatory role of cyclic AMP in prokaryotes, however, suggests

that the cyclic AMP could have been the evolutionary precursor of hormonal control in higher organisms.

TRANSCRIPTIONAL CONTROL

It is generally agreed that the fundamental cellular action of the hormones is the stimulation of certain proteins, and it appears that a specific hormone-receptor protein complex is involved in this effect. Receptor proteins are found in each target tissue, associated both with the cytoplasm and the nucleus. They appear to be heat labile proteins with sedimentation coefficients of 8 to 10 s at low ionic strength and 3 to 5 s at ionic strength greater than that of 0.3 M Kcl, (Fang et al, 1969). Little is known about the mechanism of interaction of hormone with receptors. "Two step" mechanisms for hormone interaction with a target tissue has been proposed (Tomkins and Martin, 1970). The first step involves the interaction of hormone with its specific cytoplasmic receptor. The second step is the transfer of the hormone receptor complex to the nucleus. The hormones are supposed to act as inducers by antagonizing specific gene repressors (frequently identified as histones or other chromosomal proteins) thereby increasing the rates of synthesis of specific messenger RNA's. The arguments in favour of this view, aside from the analogy with prokaryotes are : (a) Steroid hormones administered *in vivo* cause increased rate of labelled precursor incorporation into RNA (Hamilton, 1968) (b) chromatin isolated from hormone treated tissues shows increased template activity when used to direct RNA synthesis in cell-free preparation. (c) Hormonal induction is inhibited by inhibitors of RNA synthesis. (d) New species, or increased concentrations of pre-existing species, of RNA appear in hormone-treated tissue (Church and McCarthy, 1970). (e) Nuclear localisation of some steroid hormones and their binding to chromatin (Haussler and Norman, 1969) are frequently observed (f) Chromosomal "puffing" of specific loci occurs in polytene chromosomes from ecdysone-treated insect larvae. Taken together these results constitute a strong argument in favour of a primary action of the hormones in facilitating gene transcription. At present, most of the evidence suggests that the accumulation of specific RNA's accounts for enzyme induction.

POST-TRANSCRIPTIONAL CONTROL

In addition to the arguments that steroid hormones control DNA transcription directly, the possibility has also been considered that they may

function by regulating post-transcriptional events in gene expression. Hormonal control of post-transcriptional processes was first suspected because of the "Paradoxical" stimulation of induced enzyme synthesis produced when inhibitors of RNA synthesis were adminstered to rats previously injected with cortisol (Garren et al., 1964). A model proposing that gene expression is controlled both at transcriptional and post-transcriptional levels has recently been advanced (Tomkins and Martin, 1970). This model involves two genes, a structural gene (G^s) and a regulatory gene (G^r) which in dividing cells have two possible states of activity (Fig. 1). During the inducible periods of the cell cycle both genes are transcribed actively whether or not the inducer is present. In the non-inducible periods, both genes are inactive and connot be activated by the hormone inducers. During the inducible periods (when both genes are active) transcription of the structural gene messenger RNA is inhibited by a labile post-transcriptional repressor (R), a product of the regulatory gene. The hormones are known to induce enzyme synthesis by antagonizing the post-trans criptional repressor, therby stabilizing active mssenger RNA and promoting its accumulation. The evidence for a harmone-insensitive control over gene expression comes from the fact that the steroids connot stimulate enzyme synthesis during the noninducible periods. The exact mechanism of this inducer-insensitive regulation is unknown, although it is presumed to be at the level of gene transcription and to be specific rather than general.

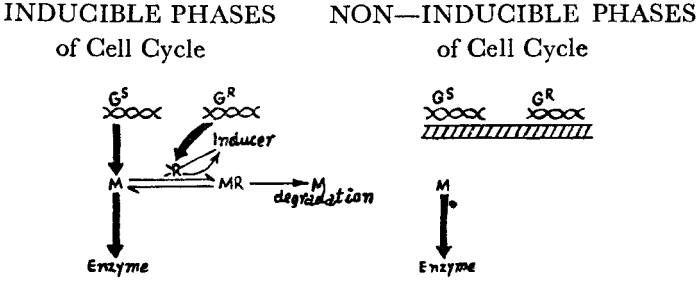

Fig. 1. Theory of homonal enzyme induction in higher organisms. The configuration shown on the left is assumed to exist during the inducible phases of the cell cycle (late G_1 and S), while that on the right, during the non-inducible phases. The G^s refers to the structural gene for the inducible enzyme, while G^r refers to the regulatory gene. During the inducible periods, G^s is transcribed and the resulting messenger RNA, M, can be translated to

form the enzyme. The G^r is likewise transcribed and its product is the labile post-transcriptional repressor, *R*. *R* combines with *M* to produce the inactive complex *MR* which leads to *M* degradation. *R* itself is labile, as shown by the arrow leading way from it. The figure shows that inducer some-how inactivates *R*. During the noninducible phases of the cycle, neither G^s nor G^r is transcribed, but pre-existing *M* can be translated. (after Tomkins et al., 1969).

VII. CONCLUSION :

In this article several questions on the nature of the mechanisms responsible for gene regulation in higher organisms have been discussed. The operon, defined in prokaryotes, is a group of contiguous genes which, on derepression, are transcribed into a single strand of messenger RNA. Both translation and transcription proceed from the operator end of the operon. Recent results indicate that the operator is not the site for the initation of transcription, but a separate locus called the "promotor" must bear the responsibility for this. This locus could be the site where RNA polymerase is recognized and commences transcription. Furthermore, a protein factor more recently termed as "sigma" factor acts at the very first step in initiation of transcription. It helps in the binding of RNA polymerase to the promotor site to form a "preinitiation Complex" for transcription. Mutations to the triplets for chain termination in any of the genes of the operon cause polarity, a reduction in the activity of genes distal to the operator, as a consequence of the translation of the polygenic messenger RNA as a unit. Operon function is controlled hy two sorts of genes, operator genes and regulator genes. In higher orgnisms however, the units of transcription and of translation are not always of the same length as in microorganisms. Since thc chromosomal DNA is confined to the nucleus and protein synthesis occurs largely in the cytoplasm, the process of transcription and translation are physically separated. Recent experimental information claims that the genetic material in higher organisms contain repetitive and nonrepetitive DNA sequences. The significance of both repectitive and nonrepetitlve sequence with regard to their specific function has been considered. A new model of gene regulation consisting of five elements; producer gene, receptor gene, activator RNA, integrator gene, sensor gene, and battery of genes, in eukaryotic organisms has been described. The Major events in evolution may have required changes in patterns of gene regulation.These changes most likely consist of additions of novel patterns of regulation of the reorganisation of pre-existing patterns in prokaryotes. The appearance of new struc-

tural (producer) genes may represent a minor part of the changes involved. The model supports the motion of the theory of gene regulation, that gene activities may be co-ordinated in higher cell genomes, and provide a framework for considerations of the nature of change in the regulatory programmes The elements of the model taken together appear to have the potentiality of establishing a pattern of gene regulation which determine a particular cell state, and probably are sufficient to establish an orderly process of development leading to the full set of cell types and states of an orgnisms.

It is recognised that differential control of gene action accompanies the orderly sequence of events in the development of eukaryotic organisms. This control is accomplished throuth various cellular mechanisms. Basic to all of them, however, are those genetic systems that serve to initiate or programme the gene sequences. The manner in which hormones help in gene expression during the course of the development of an organism is discussed. The facts that hormones localize in specific organs and are concentrated and retained by these organs suggest the existence of specific "receptor" substances in target cells capable of recognizing a particular hormone. Certain of the hormones probably are themselves the primary intracellular offectors, whereas others function at the cell surface. The major question about the mechanism of action of these hormones has now become that of the intracellular action of cyclic AMP itself. In multicellular organisms cyclic AMP is a positive allosteric effector for the phosphorylation of various proteins by ATP.

VIII SUMMARY

Pertinent problems concerning the nature of the mechanisms responsible for gene regulation in higher oranisms have been discussed. The operon concept as applied to both prokaryotic and eukaryotic organisms for functioning of the gene has been elaborated. The significance of both repetitive and non-repetitive DNA sequences is enumerated. A new model of gene regulation consisting of five elements; producer gene, receptor gene, activator RNA, integrator gene, sensor gene, and battery of genes has been described. The element of this model possesses the potentiality of establishing a pattern of gene ragulation which determine a particular cell state and types. Some aspects of the mechanism of action of hormones through transcription or post-transcriptional regulation of gene expression have also been discussed.

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