BIOCHEMICAL ACTIONS OF HORMONES

Edited by Gerald Litwack

Volume II

Biochemical Actions of Hormones

Edited by GERALD LITWACK

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Preface

This collection of papers by researchers in the field of hormone action surveys the significant developments in our progress toward understanding the primary effects of hormones in cellular receptors at the molecular level. During the last six years, there have been enormous developments in this field. The extent of progress is reflected in the size of this two-volume work. An advantage in having two volumes is the prompt publication in Volume I of those manuscripts completed at an early date, an important consideration in a rapidly expanding area of research.

Some informational overlap between contributions was unavoidable, but, hopefully, has been held to a minimum. It seemed more sensible to tolerate a small degree of redundancy than to tamper with cohesiveness. There are certain areas in which relatively little progress has been made. Accordingly, a few gaps in coverage will be evident, such as the absence of a contribution on intestinal hormones.

The coverage is broad enough to make this work useful as a modern reference text for the endocrinologist. In many cases, new data from the contributors' laboratories are presented. Thus, the purpose of these two volumes is to provide in one source an up-to-date survey of molecular and biochemical approaches bearing on the problem of hormone mechanism.

GERALD LITWACK

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CHAPTER 1

The Present Status of Genetic Regulation by Hormones

Gordon M. Tomkins and Thomas D. Gelehrter

I. SIMPLE AND COMPLEX REGULATION IN MICROORGANISMS

Even small bacterial viruses containing three genes control their development and function by very sophisticated means (Stavis and August, 1970). Therefore, before considering the detailed mechanisms of hormone action in multicellular eukaryotic organisms, it is of interest to consider some aspects of the basic biology of regulation in much simpler systems. Regulatory processes can be regarded as either "simple" or "complex": simple meaning that a biological effector (substrate, feedback inhibitor, etc.) influences only one or a very limited number of processes; and complex indicating that regulation of a variety of cellular processes are controlled by a common mechanism.

The first simple control mechanism to be understood was gene repression (Jacob and Monod, 1961), in which a specific negative regulatory protein (repressor) combines with a particular operator site on the DNA of the chromosome to block the transcription into RNA of the gene lying

distal to (and therefore under control of) the operator. Specific small molecules (inducers) can combine with the operator–repressor complex and cause its dissociation (Gilbert and Müller-Hill, 1966, 1967; Riggs and Bourgeois, 1968; Riggs *et al.*, 1968; Ohshima *et al.*, 1970; Ptashne, 1967), thereby permitting transcription of the previously repressed genes, provided, however, that an RNA polymerase molecule is correctly attached to the promotor site on the DNA so that transcription can be initiated.

Since the latter condition is fulfilled only under physiologically appropriate circumstances, the initiation of transcription by the polymerase is also under positive control (Hinkle and Chamberlin, 1970; Zillig *et al.*, 1970; H. Travers, 1971). In some bacterial operons, a single regulatory protein may both promote and repress gene transcription. Whichever activity of the regulatory protein is favored appears to be determined by the small effector molecule (e.g., arabinose) which induces the operon (Englesberg *et al.*, 1969). Gene expression therefore requires the organism to make two independent "decisions": to remove the repressor and to initiate transcription.

The termination of transcription at a specific site is also controlled. The factor ρ is needed to arrest the polymerase at a particular point on the chromosome of bacteriophage λ (Roberts, 1970; Maitra *et al.*, 1970), and it may be that whether transcription is actually terminated at that point or continues on to a further "stop signal" is determined by the product of another regulatory gene (Roberts, 1970). These simple mechanisms are summarized in Fig. 1.

Prokaryotes also regulate the translation of genetic information into amino acid sequence, although less is known about this process. For

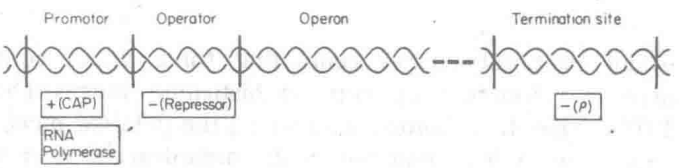

FIG. 1. "Simple" mechanisms controlling operator transcription. The figure illustrates a hypothetical operator with a promotor, operator, and termination site. A regulatory protein designated "+" is shown ready to attach to the promotor region which will then facilitate the interaction of the RNA polymerase with the DNA. "CAP" refers to the cAMP-binding protein of *E. coli* which is required, together with the cyclic nucleotide, for the expression of certain operons (see p. 4). The second protein designated "—" (for example, a repressor) is shown associating with the operator site. The attachment of repressor at this site will block the transcription of the operon by the RNA polymerase. A negative element designated "ρ," shown attaching to the termination site, prevents transcription from progressing beyond this point.

example, the coat proteins of the small RNA bacteriophages act as specific repressors of the translation of the polymerase cistron (Lodish and Robertson, 1969; Sugiyama, 1969). This mechanism, like repression of DNA transcription, depends on a specific protein blocking a unique site on a polynucleotide, except that in the case of the virus, the site is on a messenger RNA molecule rather than on DNA. Evidence for a different sort of regulatory mechanism comes from studies showing that ribosomes from virus-infected bacteria are unable to translate host–cell messenger RNA's but function normally with viral messengers (Hsu and Weiss, 1970). Alterations in the tertiary structure of the messenger itself may also control its own translation (Lodish and Robertson, 1969; Fukami and Imahori, 1971), and it has been proposed that the rate of messenger degradation is also regulated (Mosteller *et al.*, 1970). These mechanisms of control are summarized in Fig. 2.

Specific allosteric control of protein conformation is a widespread mechanism of biological regulation (Tomkins and Yielding, 1961; Monod *et al.*, 1965; Koshland *et al.*, 1966). Besides simply regulating enzyme activity, effectors may also influence the three-dimensional structure of certain proteins, modulate their intracellular degradation, and therefore their concentration (Schimke *et al.*, 1967).

In complex regulation, rather than a single process, an entire set or program of events is controlled by a single mediator. An example is the

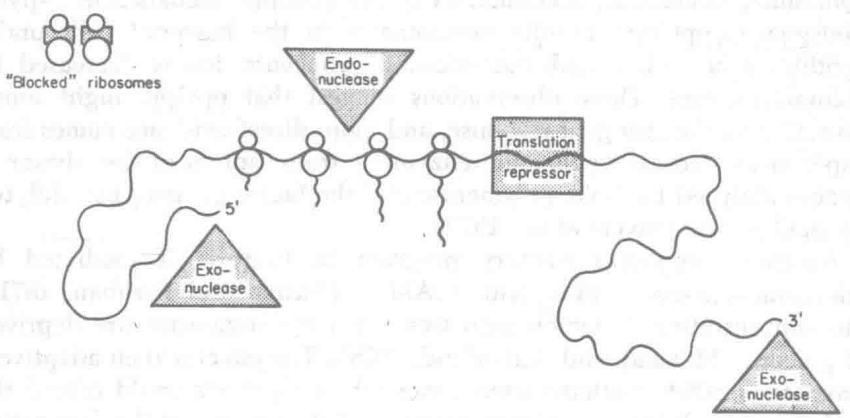

FIG. 2. Control of messenger RNA translation. The figure illustrates a hypothetical messenger RNA in the process of translation. Several untranslated areas of secondary structure are shown as loops. A specific translational repressor molecule is shown blocking the progress of ribosomes, and three types of nuclease are depicted attacking the messenger; an endonuclease and two exonucleases beginning messenger destruction at the 5′ and 3′ ends, respectively. In addition, several ribosomes are shown which are unable to attach to the messenger because they carry regulatory molecules which inhibit this process.

stringent response in bacteria controlled by the RC, or *rel* gene which governs the behavior of organisms starved for an essential amino acid (Stent and Brenner, 1961). Under conditions of amino acid deprivation, there follows:

1. Slowing of the synthesis of ribosomal and transfer RNA's with a relatively smaller effect on the formation of messenger RNA's (Lazzarini and Winslow, 1970; Primakoff and Berg, 1970).
2. Inhibition of the uptake of certain nucleic acid precursors, and their phosphorylation (Edlin and Neuhard, 1967; Nierlich, 1968).
3. Stimulation of the degradation of intracellular proteins (Goldberg, 1971) and of normally stable RNA (Lazzarini and Winslow, 1970).
4. Depression of glucose transport (Sokawa and Kaziro, 1969).

Several lines of evidence indicate that a common mechanism is responsible for all of these alterations. For example, all are restored to normal when the missing amino acid is supplied and none occur in organisms bearing mutation in the RC gene. Furthermore, chloramphenicol and certain other inhibitors of protein synthesis reverse several parameters of the stringent response (Pardee and Prestidge, 1956; Aronson and Spiegelman, 1961). Recent work (Cashel and Gallant, 1968, 1969; Cashel, 1969; Cashel and Kalbacher, 1970) has shown that a particular guanine-containing nucleotide, identified as 3′-pyrophosphorylguanosine 5′-pyrophosphate (ppGpp) rapidly accumulates in the bacterial cells under conditions of amino acid starvation. Its concentration is decreased by chloramphenicol. These observations suggest that ppGpp might somehow control the stringent response, and more direct evidence comes from experiments indicating that the enzymatic transcription of the ribosomal genes catalyzed by RNA polymerase and the factor ψR may be inhibited by ppGpp (A. Travers *et al.*, 1970).

Another complex regulatory program in bacteria is mediated by adenosine-3′,5′-cyclic phosphate (cAMP) (Pastan and Perlman, 1971), the concentration of which increases when the organisms are deprived of glucose (Makman and Sutherland, 1965). The effector then adaptively promotes mRNA synthesis from genes whose products could relieve the carbohydrate deficiency. The mechanism of its action is still under active study, but the results to date show that cAMP promotes the attachment of a protein factor, CR or CAP, directly to the DNA (Riggs *et al.*, 1971). This observation, together with those showing that the cyclic nucleotide promotes mRNA synthesis from the operons under its control (Varmus *et al.*, 1970; Zubay *et al.*, 1970), suggests that cAMP and CAP direct the polymerase to the appropriate promotors. It should be recalled from our

earlier discussion that transcription also requires an operon-specific inducer to remove the repressor.

The full implications of cAMP-mediated control in bacteria have not yet been explored, since the cyclic nucleotide may also control the translation of certain mRNA's (Pastan and Perlman, 1969). Furthermore, there are perhaps indications of even more complex interactions, since some processes may be controlled both by ppGpp and cAMP (Varmus *et al.*, 1970), and since another cyclic nucleotide cGMP, antagonizes the cAMP stimulation of the CAP–DNA association (Riggs *et al.*, 1971).

II. THE METABOLIC CODE

From this survey, we infer that specific metabolites (sugars, amino acids, etc.) influence only one or a few cellular processes, such as the function of a certain operon. On the other hand, mediators such as ppGpp and cAMP arise in response to more general environmental changes such as amino acid or carbon starvation and control an entire set of adaptive responses. We also note that complex mediators are not major metabolites themselves, but rather act as "symbols" for these substances, which act at much lower concentrations than the nutrients themselves. If this point of view is valid, we can regard the correspondence between a class of energy-yielding or structural molecules and a specific mediator as a metabolic code, similar to the genetic code in which a triplet of nucleic acid bases stands for an amino acid.

Using this logic, we might regard the hormones as having evolved by a further process of metabolic coding, in which an intracellular mediator such as cAMP become represented by intercellular effectors such as ACTH or TSH.

III. EVOLUTIONARY ORIGINS OF CELL–CELL INTERACTION

Hormones are chemical mediators of communication between different cells of the same organism. As such, they are part of a much larger universe of molecules which pass from cell to cell or from organism to organism by means of which complicated ecological systems are established and maintained (Whittaker and Feeny, 1971). In pursuing the

question of the origins of hormone action on the molecular level, we can again find simpler models of intercellular interaction. Rather surprisingly, extensive communication exists between different types of prokaryotic cells, although the effect of one cell type on another tends to be deleterious rather than helpful. For example, the colicins secreted by one bacterium act specifically to poison, sometimes in an extremely sophisticated way, an "enemy" bacterium which presumably threatens the survival of the coliciogenic organism (Nomura, 1967). The colicins themselves are rather complicated molecules which interact with specific sites on the membrane of the receptor cells, a mechanism quite familiar to endocrinologists, although in a somewhat different context.

Chemical cooperation between cells also occurs in simple organisms. Hormones, secreted by one mating type of certain fungi, attract the opposite mating type, ultimately leading to genetic recombination. In at least one case, these primitive sex hormones are steroids (Barksdale, 1969).

A more familiar example of metabolic cooperation between single cells leading temporarily to metazoan morphology is the role of cyclic AMP in the slime mold (*Dictyostelium discoideum*). As long as the individual cells (myxamoeba) are well nourished, they exist as independent, free-living organisms. However, under starvation conditions, they secrete cyclic AMP which causes the aggregation of a large number of individual cells (Konijn *et al.*, 1967). Then, under the further influence of cyclic AMP, the aggregates ("slugs") move about presumably in "search" of nutriment. If their quest is successful, the multicellular slug disaggregates to form individual cells. However, if no food source is found, the organism sporulates, an activity which requires complex differentiation of the newly formed slug. The negative limb of a feedback loop is also found in the slime mold, since under certain circumstances these organisms also secrete the enzyme cyclic AMP phosphodiesterase, which catalyzes in the inactivation of the effector (Bonner, 1970). This organism illustrates the important biological principle that an effector (cyclic AMP) used in bacteria only for metabolic regulation can, in more complex cases, physically propel an organism to search for food.

Since adenyl cyclase in bacteria is an intracellular enzyme (Hirata and Hayaishi, 1967), sometime during evolution the cyclase must have become associated with the cell membrane, allowing it to be regulated by diffusible extracellular substances, giving rise to the present day hormone-modulated cyclase systems (Sutherland *et al.*, 1968). We might speculate that electrically excitable cells arose from hormone-producing progenitors, and that electrical "symbolism" ultimately became the dominant means of information transfer in sophisticated metazoan organisms,