Much of the increase in NPP would, however, be in short-lived tissues that do not accumulate from year to year; that part of increased NPP going into woody tissues would be subject to death and decay, and the full amount of that increased woody NPP would not accumulate in storage as biomass. We know of no evidence of increased wood storage in natural forests because of CO_2 increases, but have no way to calculate with assurance the possible effect.
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45. Net ecosystem production is the net storage (or loss) of carbon by a unit of landscape. It drops to zero when total respiration equals gross production in old-growth forests and is normally positive during midsuccession in forest seres [G. M. Woodwell and R. H. Whittaker, *Am. Zool.* **8**, 19 (1968)]. Sample calculation: The plant biomass of tropical forests was estimated as 461×10^{15} g of carbon (Table 1). If 1 percent of old-growth forest were cut per year, 4.6×10^{15} g of carbon per year could be released as CO_2. However, some 5 percent of this, or 0.2×10^{15} g, might go into long-term storage in structures and thus only 4.4×10^{15} g per year would be released. If 10 percent of tropical forests have been cut, and half of this area now supports forest regrowth, the carbon going into storage in that regrowth would equal tropical forest productivity, estimated to total 22×10^{15} g of carbon per year (Table 1), times ½ of 10 percent, times 0.6 of that productivity that goes into woody tissues, or 0.66. If the regrowing forests have a net production 1.4 times greater than the old-growth forests, the amount into storage (increased NEP) would be about 0.9×10^{15} g of carbon, and the net release of carbon as CO_2 would be $4.6 - 0.2 - 0.9 = 3.5 \times 10^{15}$ g of carbon per year.
46. In preparing this article we have had the advantage of extensive discussions with various colleagues at the series of meetings held over recent months, including the Dahlem Conference in West Berlin, the Conference on the World Carbon Budget in Ratzeburg, West Germany, and the Miami Beach Conference arranged under ERDA auspices. Individuals who have contributed to these discussions include B. Bolin, E. Ericsson, E. Degens, R. Rotty, E. Lemon, L. Machta, W. Broecker, A. Weinburg, H. Brooks, and others. Research supported by the Ecosystems Center, Marine Biological Laboratory, Woods Hole, Mass.

Phosphorylated Proteins as Physiological Effectors

Protein phosphorylation may be a final common pathway for many biological regulatory agents.

Paul Greengard

Within the past few decades, the individual steps in many essential metabolic pathways have been elucidated. In contrast, we are only now beginning to understand the homeostatic mechanisms by which multicellular organisms regulate and coordinate their metabolic and physiological processes in the face of a constantly changing internal and external environment. An understanding of such systems would clearly be of great importance for our comprehension of basic biological processes. In addition, such information would be of profound medical significance. It seems probable that derangements of homeostatic processes are responsible for many disease states. Conversely, it seems likely that the effects of many therapeutic and toxic agents are exerted on such homeostatic systems. In this article I outline a conceptual framework within which many features of biological regulation may be understood. This framework attributes a role of central importance to phosphorylated proteins in the control of diverse biological processes.

About 10 years ago, a cyclic AMP (adenosine 3′,5′-monophosphate)-dependent protein kinase was discovered in skeletal muscle, and evidence was presented that this protein kinase mediates the effects of cyclic AMP in causing the breakdown of glycogen (*1*). Sub-

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sequently, it was proposed (*2*) that regulation of protein kinases, leading to an altered state of phosphorylation of specific substrate proteins, may be a general mechanism through which the diverse biological effects of cyclic AMP are mediated. Much evidence has now accumulated in many laboratories which supports the concept (*2*) (Fig. 1) that regulation of protein kinase activity is involved in many, if not all, of the actions of cyclic AMP in eukaryotic cells.

Recent studies with my co-workers (*3–9*) were designed to test the possibility that phosphorylated proteins may play a much broader role, and may act as general physiological effectors for many classes of regulatory agents in addition to those whose effects are mediated through cyclic AMP. The results indicate that several classes of regulatory agents affect the phosphorylation of specific proteins in their target cells. In addition to cyclic AMP and substances that work through it, these regulatory agents include cyclic GMP (guanosine 3′,5′-monophosphate) (*3–5*), steroid hormones (*6, 7*), insulin (*8*), and calcium (*9*). Moreover, in studies in other laboratories, effects on phosphorylation of specific proteins have been observed in response to some of the above substances and to a variety of other agents, including interferon (*10*), thyroid hormone (*11*), vaccinia virus (*12*), hemin (*13, 14*), and light (*15*). Thus, various studies indicate that a diverse group of regulatory agents, including but by no means limited to those agents acting through cyclic AMP, may achieve certain of their biological actions through effects on the phosphorylation of specific proteins.

The diversity of stimuli that have already been shown to affect the phosphorylation of specific proteins suggests a general framework within which to investigate possible molecular mechanisms of biological regulation: namely, a study of the phosphorylation of endogenous proteins may provide an effective approach to the elucidation of a common mechanism by which numerous regulatory agents elicit some of their specific biological responses (Fig. 2). This is not meant to imply that all effects of all regulatory agents, or even all effects of the regulatory agents discussed in this article, are mediated through protein phosphorylation. Rather, it seems likely that certain of the biological effects of diverse regulatory agents are mediated through protein phosphorylation. In such cases, we can expect that the biological response will correlate more closely with the level of phosphorylation of the specific protein involved than with the level of second messengers such as cyclic AMP, cyclic GMP, or Ca^{2+}. Further, I would suggest that two types of roles exist for these protein phosphorylation systems in biological regulation, one mediatory and one modulatory (Fig. 3).

Phosphorylation of Specific Proteins

Many investigations have provided evidence that protein phosphorylation is involved in diverse types of enzymological and physiological responses to cyclic AMP (see *16–19*). Only a few recent examples will be given here. First, the inhibition by cyclic AMP of protein synthesis at the translational level appears to be mediated through a cyclic AMP-dependent protein kinase; a catalytic subunit prepared from cyclic AMP-dependent protein kinase strongly inhibits polypeptide chain initiation in reticulocyte, wheat germ, and *Artemis salina* systems (*14*). Second, a somatic genetic analysis of the mechanism of cyclic AMP action in S49 mouse lymphoma cells has provided strong evidence that protein kinase, and consequently phosphorylated proteins, are obligatory mediators of the known biological responses of these cells to cyclic AMP; the responses include phosphodiesterase induction, inhibition of cell proliferation, and inhibition of the G1 phase of growth (*20*). Third, injection of regulatory and catalytic subunits of cyclic AMP-dependent protein kinase into *Xenopus* oocytes has provided evidence that this enzyme controls meiosis in these cells (*21*). Fourth, 8-azido-cyclic AMP, a photoaffinity analog of cyclic AMP (*22*), has been used to identify the biological receptors for cyclic AMP; it was found that a large proportion of the cyclic AMP-binding material of all tissues examined cochromatographs with, and is associated with, cyclic AMP-dependent protein kinase activity (*23*). Finally, in the case of the nervous system alone, there is now evidence that cyclic AMP-dependent protein kinases may be involved in several distinct functional processes, including regulation of neurotransmitter biosynthesis in presynaptic terminals (*24*), mediation of the postsynaptic actions of certain neurotransmitters (*18, 25*), and regulation of microtubule function (*26, 27*).

Far less is known about the biological roles of cyclic GMP than of cyclic AMP. A number of hormones and neurotransmitters, including acetylcholine (*28*), histamine (*29*), norepinephrine (*30, 31*), epinephrine (*31*), and glutamic acid (*32*) raise cyclic GMP in their target tissues, suggesting that this cyclic nucleotide may mediate or modulate certain of the effects of these regulatory substances. This possibility is supported by the demonstration that exogenous cyclic GMP or its derivatives (or both) can mimic certain of the effects of acetylcholine on some target tissues, including the superior cervical sympathetic ganglion (*33*),

Summary. A variety of neurotransmitters, hormones, and other regulatory agents affect the phosphorylation of specific proteins in their target tissues. The types of stimuli that share this common effect on protein phosphorylation include numerous substances that do not act through cyclic AMP. These and other observations suggest that many different classes of regulatory substances achieve certain of their biological effects by altering the phosphorylation of specific proteins.

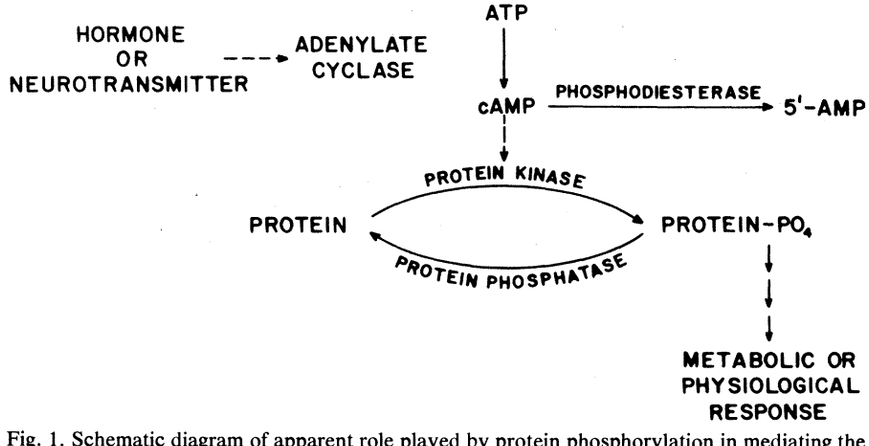

Fig. 1. Schematic diagram of apparent role played by protein phosphorylation in mediating the biological effects of those hormones and neurotransmitters acting through cyclic AMP.

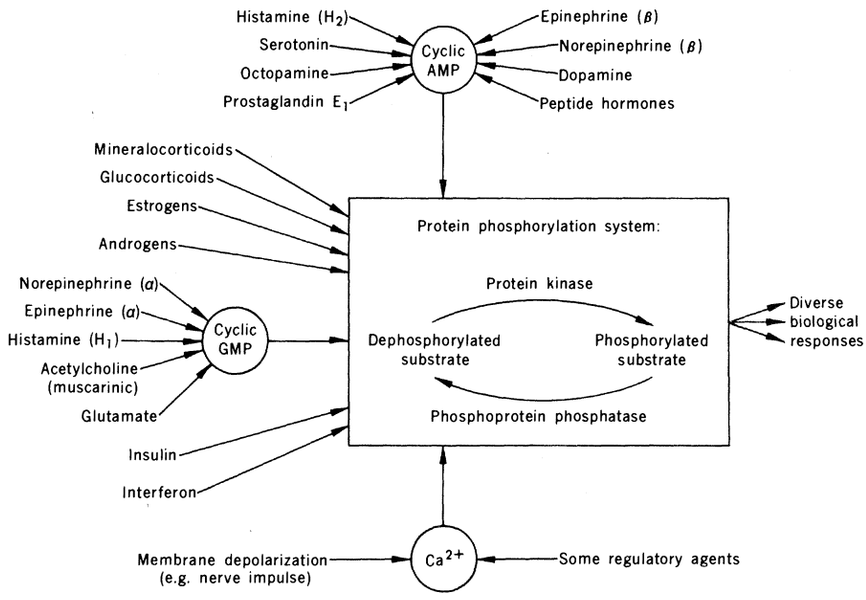

Fig. 2. Schematic diagram of postulated role played by protein phosphorylation in mediating some of the biological effects of a variety of regulatory agents. The diagram gives examples of regulatory agents, some of whose effects may be mediated through regulation of the phosphorylation of specific proteins, and is not intended to be complete. In addition to cyclic AMP and a variety of neurotransmitters and hormones whose effects are mediated through cyclic AMP, these regulatory agents include cyclic GMP, a variety of neurotransmitters and hormones whose effects are mediated through cyclic GMP, Ca^{2+}, and agents whose effects are mediated through Ca^{2+}, as well as several classes of steroid hormones, insulin, and interferon. For brevity, the numerous peptide hormones whose effects are known to be mediated through cyclic AMP, and the various regulatory agents believed to act through translocation of Ca^{2+}, are not listed individually. As indicated in the text, it seems likely that some, but not necessarily all, of the biological responses elicited by any given regulatory agent are mediated through the protein phosphorylation system; for simplicity, pathways from regulatory agent to biological response that do not involve protein phosphorylation are not shown.

neutrophils (*34*), cerebral cortex (*35*), exocrine pancreas (*36*), and heart (*37*). Cyclic GMP–dependent protein kinases (a family of enzymes that are activated by cyclic GMP rather than by cyclic AMP), have been demonstrated in several tissues (*3–5, 38*). In addition, endogenous substrate proteins for cyclic GMP–dependent protein kinases have been found in mammalian smooth muscle (*5*), intestinal epithelium (*39*), and cerebellum (*40*). Acetylcholine-induced increases in cyclic GMP, membrane-bound cyclic GMP–dependent protein kinase, and membrane-bound endogenous substrates for this cyclic GMP–dependent protein kinase have all been demonstrated in primary cultures of isolated smooth muscle cells (*41*). The results suggest that some of the actions of cyclic GMP, as well as of those regulatory substances that elevate intracellular cyclic GMP, are achieved through regulating the activity of cyclic GMP–dependent protein kinases.

Calcium is a regulatory agent of widespread importance. As one example, membrane depolarization in presynaptic nerve terminals (and in other secretory structures) causes an influx of extracellular calcium through specific voltage-sensitive calcium channels (*42*). The biosynthesis of neurotransmitters (*43*) and their release (*44*) from the terminal are among the physiological responses regulated by the entry of Ca^{2+}. It is therefore of significance that calcium ions also regulate the level of phosphorylation of a number of endogenous proteins in intact nerve terminals apparently through activation of a Ca^{+}-sensitive protein kinase (*9, 44a*).

It is clearly possible that the phosphorylation of these proteins may be involved in some of the physiological effects of calcium in nerve terminals. Moreover, much experimental evidence indicates that, in various tissues, appropriate physiological stimuli, including hormones, neurotransmitters, membrane depolarization, and light alter the influx of Ca^{2+} from extracellular fluid or the translocation of Ca^{2+} from one intracellular compartment to another. It seems quite possible that many of the physiological effects of such stimuli are mediated through Ca^{2+}-dependent protein kinases. Finally, Ca^{2+} activates phosphorylase kinase in cell-free systems, and it has been postulated, on the basis of the low protein substrate specificity of this enzyme, that it may function as a calcium-dependent protein kinase of general significance (*45*).

Several of the regulatory agents that increase intracellular levels of cyclic GMP also increase Ca^{2+} entry into cells. As has been discussed above, both Ca^{2+} and cyclic GMP can stimulate phosphorylation of intracellular proteins. The Ca^{2+}-dependent protein phosphorylation and the cyclic GMP–mediated protein phosphorylation appear to be regulated by separate enzyme systems: cyclic GMP, derivatives of cyclic GMP, and phosphodiesterase inhibitors do not mimic Ca^{2+} in stimulating endogenous membrane phosphorylation in intact synaptosomes (*9*); conversely, Ca^{2+} does not mimic cyclic GMP in stimulating endogenous phosphorylation in membranes of smooth muscle (*46*). The relative importance of the two pathways in regulating protein phosphorylation in response to appropriate hormonal or neurotransmitter stimulation, and the precise relationship of these two types of phosphorylation to the physiological effects produced by the hormones or neurotransmitters, is an important area for future investigation.

Steroid hormones have multiple actions on their target tissues. Many of these actions are either synergistic with, or antagonistic to, the effects of those hormones whose actions are known to be mediated through cyclic AMP. Although steroid hormones have been found to affect cyclic AMP levels and the enzymes (adenylate cyclase and phosphodiesterase) involved in cyclic AMP metabolism in a variety of tissues (*47*), only a few aspects of steroid-hormone action can be accounted for by the effects of these hormones on cyclic AMP levels (*48*). It is of significance, therefore, that representatives of all of the major classes of steroid hormones, including the mineralocorticoid, glucocorticoid, estrogen, and androgen classes, affect protein phosphorylation systems in their target tissues (*6, 7*). In every instance, administration in vivo of the appropriate steroid hormone affects the autophosphorylation of the regulatory subunit of a cyclic AMP–dependent protein kinase by the catalytic subunit of this enzyme; the effect is specific for the respective target tissue, occurs with low doses of the steroid hormones, can be demonstrated within 1 hour of steroid hormone administration, and is associated with alterations in the level of cyclic AMP–dependent protein kinase activity (*7, 49*). The detailed mechanism by which the steroid hormones affect the autophosphorylation of the regulatory subunit of cyclic AMP–dependent pro-

tein kinase is not yet understood. However, the effect of the steroids is indirect and requires de novo protein synthesis, in contrast to the direct effect of cyclic AMP on protein kinases. The effect of steroid hormones and of cyclic AMP on the same cyclic AMP-dependent protein kinase (Fig. 4) may provide the molecular basis for the ability of the steroid hormones to act synergistically (permissive action of the steroid hormones) or antagonistically with those hormones whose effects are mediated through cyclic AMP (*7*).

Administration of androgen (*7, 50*), estrogen (*7, 51*), or glucocorticoid (*7, 52*) hormones leads to alterations in the phosphorylation of other proteins in the appropriate target tissues. Further studies will be required to determine whether the effects of the steroid hormones on the phosphorylation of these other proteins are direct consequences of the effects on the phosphorylation of the regulatory subunit of the cyclic AMP-dependent protein kinase. This possibility is supported by the demonstration (*53*) that phosphorylation of the regulatory subunit alters the state of dissociation and, therefore, the activity of cyclic AMP-dependent protein kinase.

As with the steroid hormones, insulin also affects several biological processes that are regulated by hormones known to work through cyclic AMP. The effects of insulin and of those hormones that act by increasing intracellular cyclic AMP levels are, in general, antagonistic. However, on the basis of a review of the literature, it was concluded (*54*) that the lowering of cyclic AMP levels does not seem to be an obligatory step in the mechanism by which insulin achieves its effects in target tissues. It is, therefore, noteworthy that insulin affects the phosphorylation of specific proteins in intact fat cells (*8, 55*). The lipolytic hormones norepinephrine and adrenocorticotropic hormone (ACTH) stimulate the phosphorylation of specific proteins in isolated intact fat cells, and these effects on protein phosphorylation parallel the effects of the hormones on lipolysis in these cells (*8*). Concentrations of insulin that prevent, or reverse, the actions of norepinephrine or ACTH on lipolysis also prevent or reverse the effects of these two hormones on protein phosphorylation. Under the various experimental conditions in which lipolytic and antilipolytic hormones were studied, the rate of lipolysis correlated better with the level of phosphorylation of these specific proteins in intact cells than with the level of cyclic AMP (*8*). Such results can be explained by a scheme in which the protein phosphorylation system is closer to the biological response than is cyclic AMP (Fig. 2).

Preliminary treatment of intact cell preparations with insulin leads, under various conditions, to alterations of protein phosphorylation as measured in extracts of such cells (*19, 54, 56, 57*). Among these alterations, prior treatment of cells with insulin promotes the dephosphorylation and activation of both glycogen synthetase and pyruvate dehydrogenase, actions that can account for certain of the metabolic effects of this hormone. Finally, the phosphorylation of a specific (S6) rat liver ribosomal protein is increased by experimental diabetes, and this effect is reduced toward normal by administration of insulin (*58*).

Independent studies in three laboratories (*10*) have provided another illustration of the great diversity of biological effects in which protein phosphorylation may be involved. Thus, a protein kinase has been implicated in the mechanism of action of interferon. Interferon is a protein that is induced in host cells in response to viral infection and endows cells with antiviral activity. Incubation of many cell types with interferon induces the formation of an inactive form of a cyclic AMP-independent protein kinase, which can then be activated by very low concentrations (300 nanograms

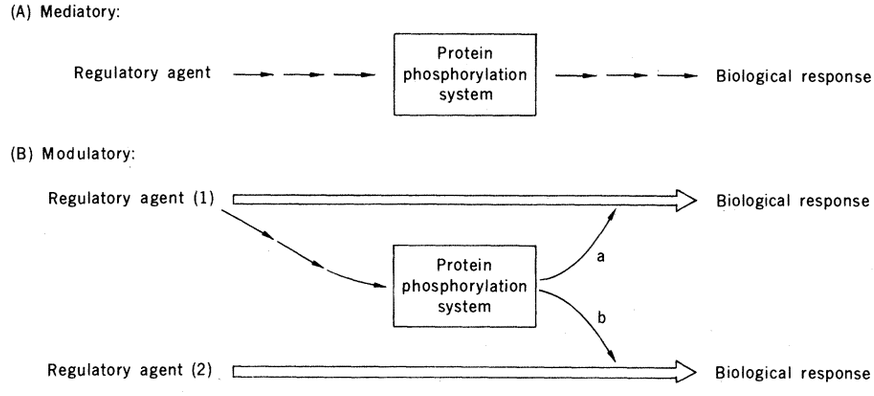

Fig. 3. Two possible types of roles for protein phosphorylation in the action of regulatory agents: (A) Obligatory: protein phosphorylation is an obligatory mediatory step in the primary chain of molecular events by which the regulatory agent elicits its biological response; (B) Modulatory: protein phosphorylation either (a) acts as a fine-control feedback mechanism which modulates activity in the primary chain of molecular events by which the regulatory agent elicits its biological response or (b) modulates the response to a different regulatory agent. Either or both roles may apply to any given regulatory agent.

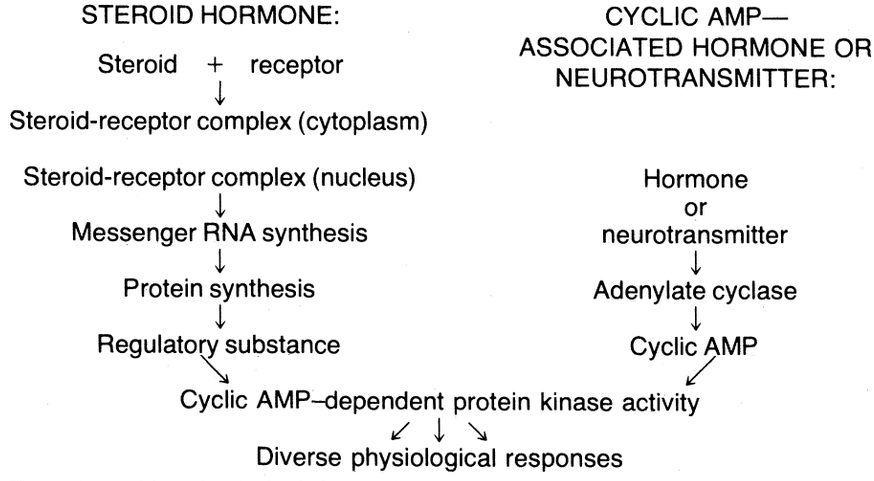

Fig. 4. A possible molecular basis for the biological interactions of steroid hormones with those hormones and neurotransmitters whose effects are mediated through cyclic AMP. The steroid hormones, through a mechanism involving de novo protein synthesis but not involving cyclic AMP, regulate cyclic AMP-dependent protein kinases in their target tissues. The cyclic AMP-associated hormones and neurotransmitters, through a mechanism not involving de novo protein synthesis, cause an increase in cyclic AMP which directly affects protein kinase activity. For simplicity, mechanisms of steroid hormone action independent of the cyclic AMP-dependent protein kinase system are not shown.

per milliliter) of double-stranded RNA. This protein kinase may be a mediator of the antiviral action of interferon.

Other types of stimuli, in addition to those discussed above, have been reported to affect specific phosphorylation systems in intact or in broken cell preparations. For example, thyroid hormone regulates nuclear protein kinase activity of rat liver (*11*). Vaccinia virus affects the phosphorylation of a ribosomal protein in HeLa cells (*12*). Hemin, through a mechanism that involves a cyclic AMP-dependent protein kinase (*14*), regulates the activity of a cyclic AMP-independent protein kinase, which phosphorylates factors involved in the initiation of hemoglobin synthesis (*13*). Light, through an indirect mechanism, stimulates the phosphorylation of rhodopsin by an endogenous protein kinase (*15*); the phosphorylation reaction may play a role in the regulation of the rod's light sensitivity.

General Considerations

The precise mechanism by which various regulatory agents affect protein phosphorylation may vary considerably. For instance, cyclic AMP activates protein kinases directly by dissociating inhibitory regulatory subunits from the catalytic subunits of the enzyme (*59*). In contrast, the mechanism through which steroid hormones alter the autophosphorylation of cyclic AMP-dependent protein kinases involves de novo protein synthesis (*7*) (Fig. 4). In principle, regulatory agents might affect protein phosphorylation systems in any of several ways, including altering the amount or properties of (i) the protein kinase that phosphorylates the substrate protein, (ii) the substrate protein itself, or (iii) the phosphoprotein phosphatase that dephosphorylates the phosphorylated form of the substrate protein. In addition, the regulatory agent might affect the amount or activity of an activator or inhibitor of the kinase or phosphatase or affect the accessibility of any two components of the phosphorylation system to one another.

The evidence demonstrating effects of various regulatory agents on phosphorylation of specific proteins is stronger than the evidence linking the phosphorylation of specific proteins to alterations in cell function. Nevertheless, correlations between protein phosphorylation and one or another enzymological or physiological response have been observed for biological processes as diverse as metabolism, transport across membranes, actomyosin function, and expression of genetic information. In addition to the examples mentioned above, a few other examples follow.

The evidence for a role of protein phosphorylation in the regulation of biological processes is probably strongest in the case of carbohydrate and lipid metabolism (*16, 19, 54, 56*). Thus, cyclic AMP-dependent protein kinase regulates the activity of phosphorylase kinase, glycogen synthase and lipase, and, largely through these actions, appears to bring about the breakdown of glycogen and triglyceride.

There is increasing evidence that protein phosphorylation may be involved not only in the regulation of metabolic processes such as carbohydrate and lipid metabolism, but also in the regulation of processes that have traditionally been considered the domain of physiologists. One group of physiological studies has been concerned with the possible involvement of protein phosphorylation in the regulation by hormones and neurotransmitters of ion transport across membranes (*18*). In these investigations, the state of phosphorylation of specific membrane proteins has been found to correlate well, under a variety of experimental conditions, with ion transport across the plasma membrane of intact turkey erythrocytes (*60*), across toad bladder epithelium (*61*), and across the sarcoplasmic reticulum of myocardial cells (*62*).

Protein phosphorylation also appears to play an important regulatory role in actin-myosin interaction in both smooth muscle and blood platelets. Phosphorylation of myosin has been found to occur on specific light-chain myosins isolated from skeletal muscle, cardiac muscle, smooth muscle, and platelets (*63, 64*). The phosphorylation is catalyzed by a specific enzyme, myosin light-chain kinase (*63, 65*). Myosin light-chain kinase from muscle is dependent on Ca^{2+} for activity, whereas that from platelets is not. Actin-activated mysoin adenosine triphosphatase activity is increased on phosphorylation of the myosin light chain in preparations from platelets (*66*) or smooth muscle (*67*).

Two other types of evidence for the ubiquity of protein phosphorylation reactions that are independent of cyclic AMP are worth mentioning.

1) When the protein phosphorylation pattern of many tissues—for example, nucleated erythrocytes (*60*), adipocytes (*8, 55*), toad bladder epithelium (*61*), or synaptic membranes (*68*)—is examined by high-resolution gel electrophoresis and autoradiography, it is evident that relatively few of the phosphorylated proteins are subject to regulation by cyclic AMP. This is true both in studies of intact cells exposed to hormones whose effects are mediated through cyclic AMP and in studies of subcellular organelles exposed to cyclic AMP itself. Such observations suggest that a number of additional agents also regulate the state of phosphorylation of phosphoproteins.

2) Cyclic AMP-independent as well as cyclic AMP-dependent protein kinase activities occur throughout the animal kingdom (*2*). In addition, cyclic AMP-independent protein kinases that catalyze the phosphorylation of specific viral polypeptides have even been found in a variety of enveloped animal viruses [for review, see reference (*69*)]. In the one case studied, the viral protein kinase is a viral gene product (*70*). Moreover, two recent studies (*71, 72*) present evidence that, in RNA viruses, phosphorylation by viral protein kinase of a viral substrate protein has important biological consequences. In one of these studies Rauscher murine leukemia virus and simian sarcoma-associated virus were used, and each contains a core protein (p12) that binds specifically to the viral RNA; both the specificity and stoichiometry of the p12-RNA interactions suggest that these RNA tumor virus proteins have a regulatory role in the functioning of the virus (*73*). It is of considerable interest, therefore, that phosphorylation of viral p12 proteins regulates their extent of binding to viral DNA (*71*). The other study implicating protein kinase in the functioning of viruses concerns the avian myeloblastosis virus, which has been reported to contain a protein kinase that can phosphorylate a protein associated with DNA polymerase of the virus (*72*). Phosphorylation of this protein is associated with a severalfold increase in DNA polymerase activity. The widespread occurrence of cyclic AMP-independent protein kinases and the evidence suggesting a functional role for these enzymes provide further support for the postulate that protein phosphorylation plays a ubiquitous role in biological regulation.

Conclusion

It should be clear, even from the foregoing brief survey, that many neurotransmitters, hormones, and other substances that can be classified broadly as regulatory agents affect the phosphorylation of specific proteins in their target tissues. The specificity of the effects of these agents on protein phosphorylation

suggests that, in many cases, the observed phosphorylation will prove to be of physiological significance. Nevertheless, in most of the instances cited, many important questions remain unanswered. In certain instances—for example, phosphorylation of a high-molecular-weight protein intrinsic to microtubules (*27*)—the regulatory agent responsible in vivo for the alteration in protein phosphorylation and biological response has not yet been identified. In other instances (for example, that of insulin), the nature, and even the existence, of the intermediate messenger between the regulatory agent and the protein phosphorylation step is unknown. In most instances, the details of the relation between the phosphorylation of specific proteins and the ultimate biological response are still not well understood; in fact, in most cases, it is not yet possible to distinguish between a mediatory role and a modulatory role (Fig. 3) for the protein phosphorylation step in the biological response.

Nevertheless, the pattern of experimental results that has begun to emerge suggests the concept that protein phosphorylation represents a basic and ubiquitous regulatory mechanism. Within the framework of this concept, it is worth considering the following ancillary postulates: (i) In addition to cyclic AMP, a number of other intracellular regulatory agents use this phosphorylation system to achieve certain of their effects; among these agents are cyclic GMP and calcium and possibly other still unknown substances. (ii) Many other compounds, which act as extracellular regulatory agents, including many hormones and neurotransmitters (and possibly stimuli such as light, or change in membrane potential) utilize the intracellular regulatory agent-protein phosphorylation system to achieve certain effects. (iii) Still other regulatory agents, through mechanisms that do not involve cyclic AMP, cyclic GMP, or Ca^{2+} as intracellular regulatory agents, also use this phosphorylation system to achieve some of their effects. (iv) Various pairs of regulatory agents (for example, vasopressin and aldosterone in kidney and norepinephrine and hydrocortisone in liver) can act on the same phosphorylation system to produce synergistic or antagonistic effects. (v) The known ability of pairs of intracellular regulatory substances (Ca^{2+} and cyclic AMP; Ca^{2+} and cyclic GMP; cyclic AMP and cyclic GMP) to mutually affect each other's intracellular concentrations may in some cases be achieved through molecular mechanisms involving protein phosphorylation. (vi) In those instances in which protein phosphorylation is involved in mediating the biological response to a regulatory agent, the response should correlate more closely with the level of phosphorylation of the specific protein involved than with the level of second messengers such as cyclic AMP, cyclic GMP, or Ca^{2+}. (vii) Protein phosphorylation may play a mediatory or a modulatory role in the actions of regulatory agents. The domain of the validity and applicability of the various components of this thesis can be subjected to experimental testing.

The validity of the scheme shown in Fig. 2 can be tested experimentally by certain general approaches. One such approach is to determine whether correlations exist, under various experimental conditions, between the state of phosphorylation of a specific protein and the biological response in a given target tissue, upon application of the appropriate regulatory agent; one can exploit various pharmacological, developmental, and genetic techniques for this purpose. A second such approach is to extract from the target tissue the various components of the protein phosphorylation system—namely the protein kinase, the substrate protein, and the phosphoprotein phosphatase—to purify these components to homogeneity, and to prepare antibodies to them; one should then be able to determine whether introducing these components themselves or the antibodies to these components into the target cells will modify the biological response to the regulatory agent in a manner predicted by the scheme shown in Fig. 2. To the extent that this conceptual framework is valid, it will be important to determine the mechanisms by which these regulatory agents control protein phosphorylation and the mechanisms by which these alterations in protein phosphorylation mediate or modulate the appropriate biological response.

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NEWS AND COMMENT

Nuclear Weapons History: Japan's Wartime Bomb Projects Revealed

A little-publicized chapter in the history of atomic weapons is the Japanese effort to develop an atomic bomb during World War II. The effort centered around Japan's university physics laboratories, and its chief figure was Yoshio Nishina, who was Japan's leading scientist and a physicist of international stature.

Although the effort was unsuccessful—and was probably doomed from the start because of lack of manpower, funds, uranium, and the disorganization of its military sponsors—the project is highly significant to the history of nuclear weapons, to Japan's subsequent self-denial of nuclear weapons, and to the relationship that developed between Japan and the United States after the U.S. atomic bombing of the cities of Hiroshima and Nagasaki in August 1945 (see inserts).

In addition, the Japanese "Manhattan Project"—such as it was—may have been the reason for the destruction of Japan's five cyclotrons and the dumping of them in Tokyo Bay by U.S. occupation forces in November 1945.

Because of its alleged mindlessness, the destruction caused an international protest and was denounced by U.S. scientists. It played a role in the battle then being waged in Congress for civilian control of atomic energy.

Much has been written about how the United States and Britain during the war were concerned that the Germans, who had discovered atomic fission in the 1930's, would develop the world's first superbomb based on this principle. The story has often been told of the heroic attempts by the Allies to destroy heavy water production at the Norwegian plant at Vemork, from which the Germans were demanding increased production, clearly destined for their atomic research. One of the most extraordinary scientific intelligence missions in history was the Alsos mission, in which an internationally prominent physicist, Samuel A. Goudsmit, was put in charge of a team of specialists that accompanied the Allied armies into Germany, seized uranium stocks and equipment, and interviewed the German scientists about how far they had gotten. Indeed, the German wartime atomic research effort—which was known through such clues as the Vemork plant's activity—was a major rationale for the Manhattan Project in the United States.

But in the case of Japan, the United States appears to have known very little—and knows very little to this day—about the fact that the Japanese scientists were also ordered to do whatever they could to develop an atomic bomb. As authoritative a source as General Leslie R. Groves, the chief of the Manhattan Project, devotes only a single paragraph of his memoirs to the issue. Groves writes that he never took the possibility of a Japanese atomic bomb seriously because of Japan's want of enough scientists, uranium, and industrial backup capacity. But he admitted that, "It would have been extremely difficult for us to secure and get out of Japan any information of the type we needed." In other words, he had far less intelligence about Japan than he had about Germany.

Indeed, with the single exception of the period before the November 1945 cyclotron incident, it seems that no one in the U.S. government took the possibility

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