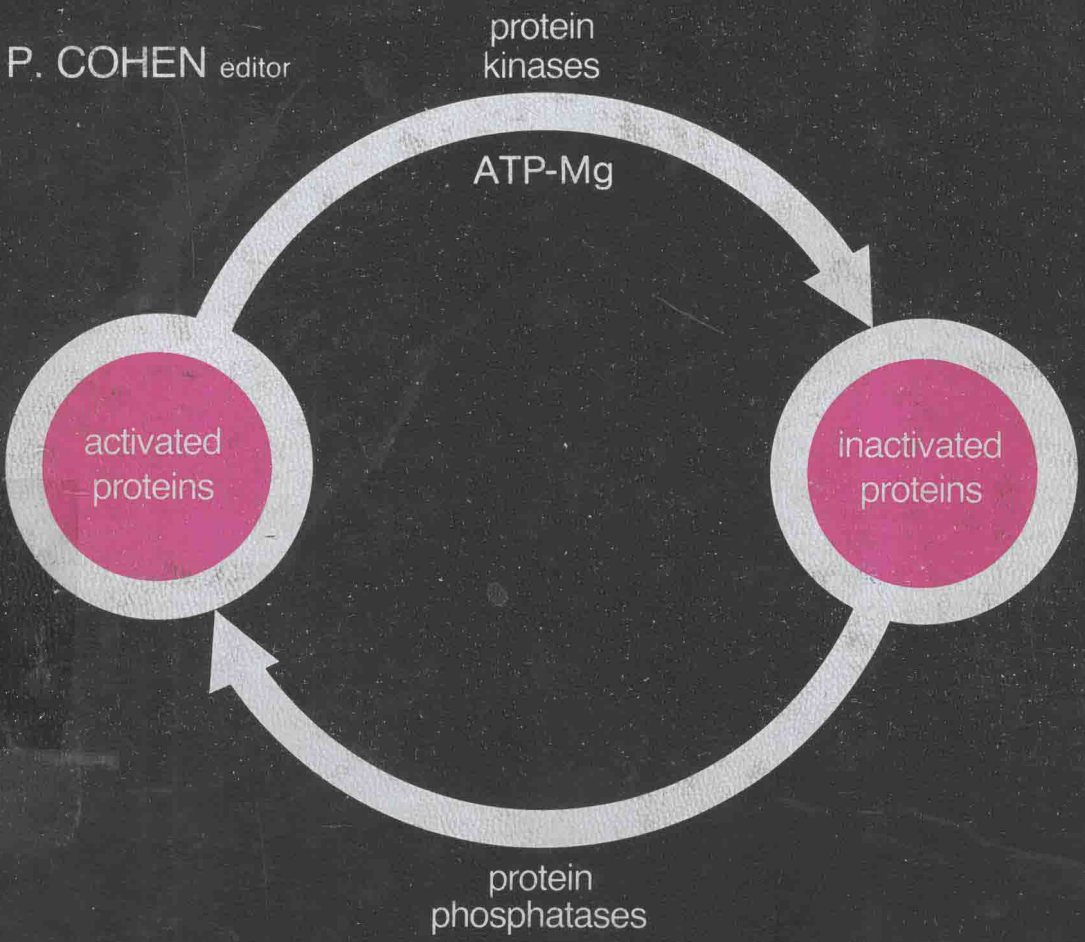
MOLECULAR ASPECTS OF CELLULAR REGULATION
VOLUME 1

# recently discovered systems of enzyme regulation by reversible phosphorylation

P. COHEN editor

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# RECENTLY DISCOVERED SYSTEMS OF ENZYME REGULATION BY REVERSIBLE PHOSPHORYLATION

*Edited by*

P. COHEN

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# Editor's foreword

As the structures of metabolites and macromolecules, and the pathways by which they are synthesized and degraded have been elucidated, the main emphasis of biochemical research has progressively shifted towards problems of cellular organisation and control, a trend which has been particularly marked over the past five years.

While a number of series dealing with cellular control mechanisms started to appear in 1970s, most of these books were merely collections of reviews dealing with unrelated topics. This was perhaps inevitable since knowledge of control mechanisms at the molecular level had not advanced sufficiently for common underlying themes to have emerged. However the past few years have seen considerable advances and cellular regulation has now reached an exciting stage where unifying concepts are now linking areas of research which were previously thought of as being quite separate.

The purpose of *Molecular Aspects of Cellular Regulation* is not to produce a regular annual volume, but to publish occasional books on multidisciplinary topics which illustrate general principles of cellular regulation. The first volume concerns the role of protein phosphorylation in co-ordinating the control of intermediary metabolism. Volume 2 will deal with the molecular actions of bacterial toxins, viruses and interferon.

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# Contents

CHAPTER 1

# Well established systems of enzyme regulation by reversible phosphorylation

PHILIP COHEN

## 1. *Discovery of enzyme regulation by phosphorylation–dephosphorylation*

Although it has been known for almost a hundred years that proteins contain covalently bound phosphorous, it is only since the discovery of enzyme regulation by reversible phosphorylation that interest in protein phosphorylation has gathered momentum (Figure 1). In 1956, Krebs and Fischer [1] discovered that glycogen phosphorylase the rate limiting enzyme in glycogenolysis could be converted from a dephosphorylated '*b*'-form whose activity was dependent on the allosteric activator 5′-AMP to a phosphorylated '*a*'-form which was largely active in the absence of 5′-AMP. In 1959 the same workers demonstrated that phosphorylase kinase, the enzyme which converted phosphorylase *b* to *a* was itself an 'interconvertible' enzyme which could exist in a low activity dephosphorylated state or a high activity phosphorylated state [2]. The third enzyme shown to be regulated by this mechanism was also in the field of glycogen metabolism. In 1964 Joseph Larner and his associates showed that glycogen synthase the rate limiting enzyme in glycogen synthesis could be converted from a dephosphorylated form of high activity to a phosphorylated form which required that allosteric activator glucose-6P for activity [3].

The idea that this mechanism of regulation might exist in other systems was slow to take root. It was only after the discovery of cyclic AMP-dependent protein kinase in 1968 by Walsh, et al. [4], also as a result of studies of the control of glycogen metabolism by hormones, that the field started to move rapidly and there are now some 25 enzymes whose activities have been demonstrated to be regulated by phosphorylation–dephosphorylation in vitro (Figure 1). However, the number of phosphoproteins (as opposed to phospho-

*Cohen (ed.) Recently discovered systems of enzyme regulation by reversible phosphorylation*

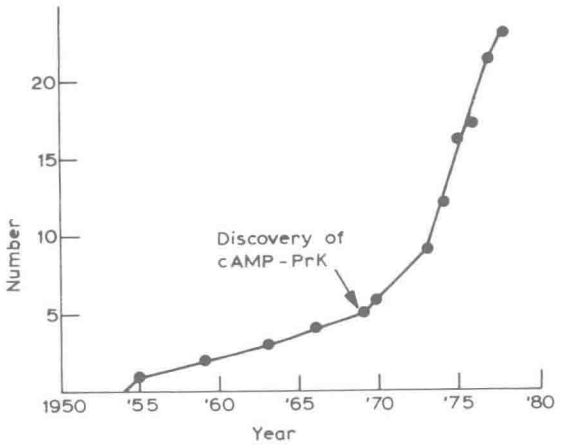


Figure 1. Enzymes reported as undergoing regulation by phosphorylation–dephosphorylation. (Reproduced by permission of Edwin G. Krebs.)

enzymes) that have been identified is now greater than one hundred.

The past five years have established protein phosphorylation as the major general mechanism by which intracellular events in mammalian tissues are controlled by nervous and hormonal stimuli. The purpose of this book is to describe some of these newly discovered systems of enzyme regulation by phosphorylation–dephosphorylation which have been instrumental in developing our current understanding of how the major pathways of intermediary metabolism are controlled in a co-ordinated manner in response to physiological stimuli. It will become apparent from the book that the regulation of carbohydrate metabolism, lipid, steroid and protein synthesis, and chromosome condensation show striking similarities and the general themes that will emerge are discussed in Chapter 11.

Although this book is concerned primarily with newly discovered systems, it is apparent from the foregoing discussion that the first three enzymes shown to be regulated by phosphorylation–dephosphorylation were those concerned with the regulation of glycogen metabolism in mammalian skeletal muscle, and this system continues to acts as the model to which all others are compared. It is therefore important the summarize briefly our current understanding of this system and its implications for other cellular processes which respond to neural and hormonal stimuli.

## 2. *Regulation of glycogen metabolism in mammalian skeletal muscle*

Glycogen metabolism in muscle is regulated by the hormones adrenaline and insulin as well as by the contractile state of the tissue. Electrical excitation of muscle or the release of adrenaline into the circulation stimulate glycogenolysis, while insulin promotes glycogen synthesis. These stimuli act by changing the activities of glycogen phosphorylase and glycogen synthase the rate limiting enzymes in glycogenolysis and glycogen synthesis respectively. The interrelationships between the protein kinases and phosphorylated proteins involved in this system are summarized in Figure 2. Progress up to the end of 1977 has been described in two reviews [5,6].

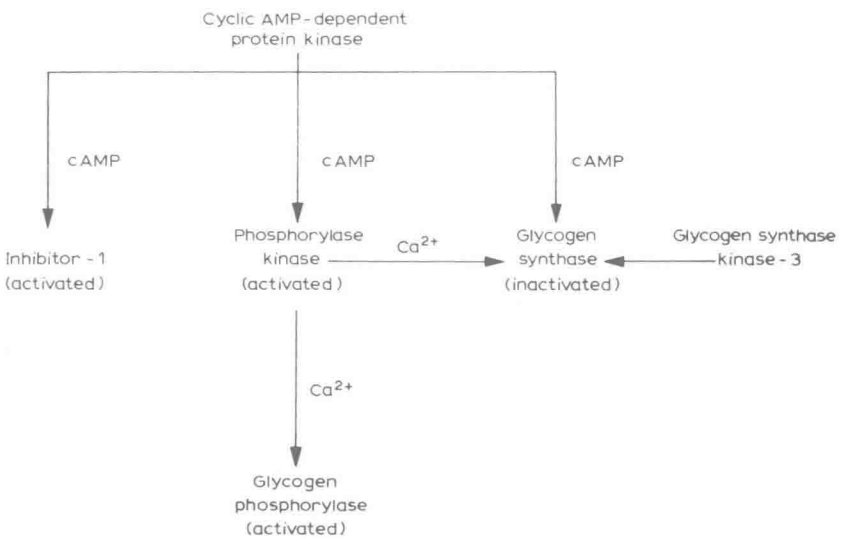


Figure 2. Interrelationship between the protein kinases and phosphorylated proteins involved in the regulation of glycogen metabolism in mammalian skeletal muscle.

The interaction of adrenaline with its $\beta$-receptors on the outer surface of the plasma membrane leads to the activation of adenylate cyclase located on the inner surface on the membrane. This elevates the intracellular level of cyclic AMP leading to the activation of cyclic AMP-dependent protein kinase. Cyclic AMP-dependent protein kinase possesses the structure $R_2C_2$, where R, the regulatory subunit, binds cyclic AMP and C is the catalytic subunit. The

binding of cyclic AMP to the inactive $R_2C_2$ complex causes it to dissociate thereby liberating the active catalytic subunit.

$$\underset{\text{(inactive)}}{R_2C_2} + \text{cAMP} \rightleftharpoons R_2 - \text{cAMP} + \underset{\text{(active)}}{2C}$$

The catalytic subunit controls glycogen metabolism by phosphorylating the three proteins inhibitor-1 (see below), phosphorylase kinase and glycogen synthase. The phosphorylation of phosphorylase kinase and glycogen synthase increases and decreases the activities of these two enzymes respectively. Thus the two opposing pathways of glycogenolysis and glycogen synthesis can be regulated in a synchronous manner in response to adrenaline.

Electrical excitation of muscle causes calcium ions to be released from the sarcoplasmic reticulum into the cytoplasm. The calcium ions not only activate actomyosin ATPase and initiate muscle contraction, but also activate phosphorylase kinase which is almost completely dependent on this divalent cation. The activation of phosphorylase kinase promotes the conversion of phosphorylase *b* to *a*, thereby stimulating glycogenolysis to provide the energy which sustains muscle contraction.

Recently, phosphorylase kinase has been shown to phosphorylate glycogen synthase and to decrease its activity [7–11]. Thus the two opposing pathways of glycogenolysis and glycogen synthesis, as well as the muscle contractile apparatus itself, can be controlled in a co-ordinated manner in response to neural stimulation of the tissue.

Phosphorylase kinase possesses the structure $(\alpha\beta\gamma\delta)_4$. The $\alpha$- and $\beta$-subunits are the components phosphorylated by cyclic AMP-dependent protein kinase and the $\gamma$-subunit appears to be the catalytic subunit [12]. Recently, the $\delta$-subunit was identified as the calcium binding protein termed calmodulin [13–15]. This protein is also a subunit of a number of other calcium dependent enzymes and will be discussed further in Chapter 7 and in Chapter 11. It is becoming apparent that calmodulin is the major cytoplasmic calcium receptor in eukaryotic cells and that it plays a role analogous to that of the regulatory subunit of cyclic AMP-dependent protein kinase.

The amino acid sequence of calmodulin has demonstrated a 50% identity with troponin-C [16], the protein which confers calcium sensitivity to the actomyosin ATPase reaction in the muscle contractile apparatus. Troponin-C and calmodulin both bind four calcium ions per mole with affinities in the micromolar range [17]. These findings allow one to start to visualize at a molecular level how the processes of muscle contraction and glycogen metabolism are so closely synchronized.

Glycogen synthase is also phosphorylated by at least one further enzyme