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PROSPECTS SYMPOSIUM

Introduction: Protein Phosphorylation and Signal Transduction in Bacteria

Milton H. Saier, Jr.

Department of Biology, University of California at San Diego, La Jolla, California 92093-0116

Abstract A single type of reversible protein-phosphorylating system, the ATP-dependent protein kinase/phosphatase system, is employed in signal transduction in eukaryotes. By contrast, recent work has revealed that three types of protein-phosphorylating systems mediate signal transduction in bacteria. These systems are (1) classical protein kinase/phosphatase systems, (2) sensor-kinase/response-regulator systems, and (3) the multifaceted phosphoenolpyruvate-dependent phosphotransferase system. Physiological, structural, and mechanistic aspects of these three evolutionarily distinct systems are discussed in the papers of this written symposium. © 1993 Wiley-Liss, Inc.

Key words: prokaryotic protein phosphorylation systems, sugar attractant, bacteria, eukaryotes, protein kinases, sensor kinases, response regulators, phosphotransferase system

The existence of bacterial protein kinases remained in doubt until 1978 [1]. Even as late as 1986, a leading expert in the protein phosphorylation field concluded that "the extent to which protein phosphorylation is employed in bacteria is much less than that in higher organisms" [2]. Just six years later, we find that this statement must be re-evaluated in view of our rapidly expanding knowledge of protein phosphorylation in prokaryotes.

Signal Transduction and Protein Phosphorylation

Post-translational modification of proteins by phosphorylation has long been known to serve as one of the principal mechanisms for the regulation of cellular functions in response to external stimuli in eukaryotes. The protein kinases and protein phosphate phosphatases that catalyze the reversible phosphorylation of target systems are frequently responsive to extracellular signals such as hormones, growth factors, neurotransmitters and light, the actions of which may be mediated by cytoplasmic metabolites and second messengers such as cAMP, cGMP, Ca^{2+}, and diacylglycerol [3]. These agents allosterically control the catalytic activities of protein kinases and protein-phosphate phosphatases in eukaryotic cells. Processes regulated include transmembrane nutrient and salt transport, cellular metabolism, protein synthesis, differentiation, and cellular motility [3,4].

In recent years, our thinking about signal transduction in bacteria has become revolutionized by the realization that protein kinase-like systems function as key regulatory elements in prokaryotes as they do in eukaryotes [5,6]. We are now faced with the probability that protein phosphorylating enzymes mediate sensory transduction throughout the living world. Furthermore, the importance of protein kinases to growth regulation and cancerous transformation in animal cells, which has become apparent as the catalytic characteristics of the protein products of certain oncogenes have been elucidated, has emphasized the need for easily manipulatable model systems for elucidation of the transduction mechanisms involved. It is now clear that bacteria provide these requisite model systems.

Examination of the roles played by protein kinases in bacteria reveals parallels with eukaryotic systems but shows unique characteristics as well. Thus, we now know that bacterial protein phosphorylating enzymes, some of which resemble animal protein kinases, control intermediary metabolism, carbohydrate transport, gene transcription, and cellular differentiation, as is the case for animal cell protein kinases. They also appear to regulate bacterial-specific processes, such as chemotaxis, bacteriophage infection, virulence, and bacterial photosynthetic CO_2 fixa-

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tion [5–9]. Normally we think of bacteria as being simple and of eukaryotes as being more complex. In the case of protein phosphorylation mechanisms, however, the reverse now appears to be true. Thus, while a single general mechanism involving the ATP-dependent protein kinases is currently believed to be the mediator of phosphorylation-triggered regulatory responses in animal cells, three distinct mechanisms, involving three evolutionarily unrelated protein phosphorylating systems, clearly operate in *E. coli* and other bacteria. These three systems are illustrated in Figures 1–3. They form the basis for dividing this written symposium into three major sections, the first dealing with classical ATP-dependent protein kinase/phosphatase systems, the second dealing with sensor-kinase/response-regulators, and the third dealing with the bacterial phosphotransferase system.

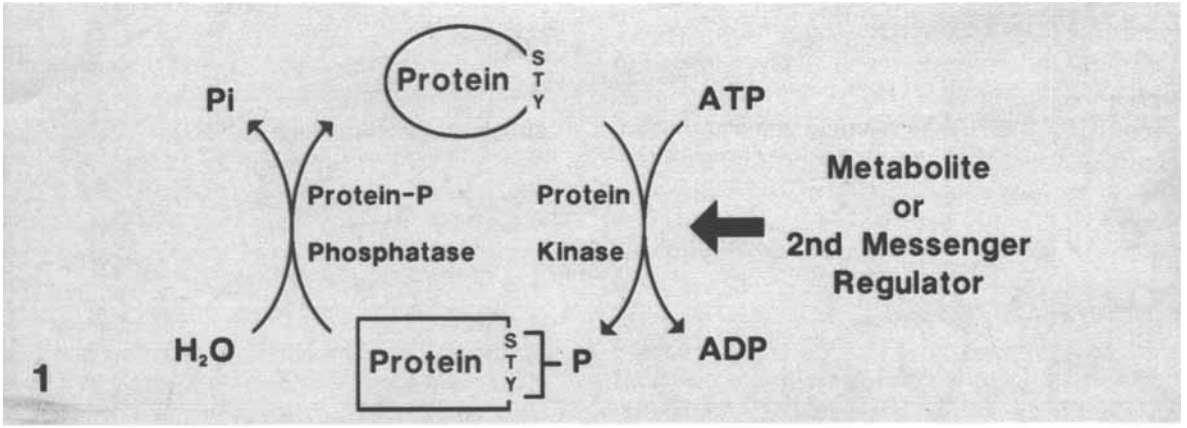
Classical ATP-Dependent Protein Kinase/Phosphatases

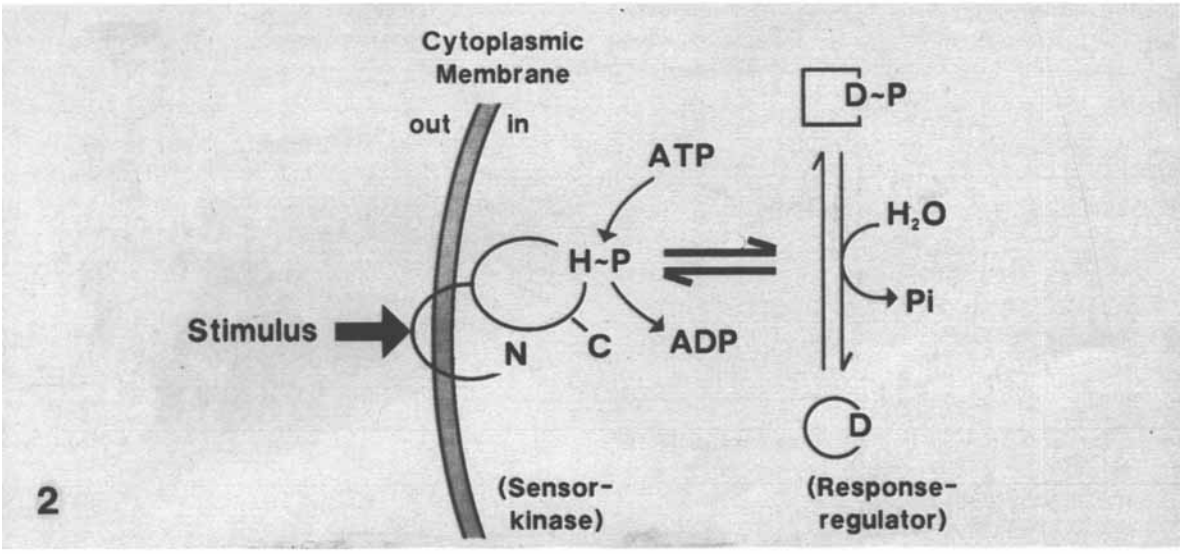
The first of the three classes of bacterial protein-phosphorylating systems discussed in this symposium includes the classical ATP-dependent protein kinases which resemble those that have been extensively characterized from animal cells (Fig. 1). These enzymes phosphorylate seryl, threonyl, and tyrosyl residues in their substrate proteins (see article by Alain Cozzone, this issue). Interestingly, most of the currently known bacterial kinases of this class are allosterically activated by cellular metabolites rather than second messengers, although regulation by cytoplasmic Ca^{2+} has also been reported. The best characterized of the metabolite-regulated bacterial protein kinases are the isocitrate dehydrogenase kinases of enteric bacteria, which are allosterically inhibited by a variety of metabolic intermediates (e.g., isocitrate, NADPH, adenosine phosphates, phosphoenolpyruvate). These enzymes are discussed in the contribution of David LaPorte (this issue). The kinases in Gram-positive bacteria, which phosphorylate HPr, a phosphocarrier protein of the phosphoenolpyruvate:sugar phosphotransferase system (PTS) is also well characterized from a biochemical standpoint (see article by Reizer et al., this issue). The recent results reported in the paper by Reizer, Romano, and Deutscher have led to the possibil-

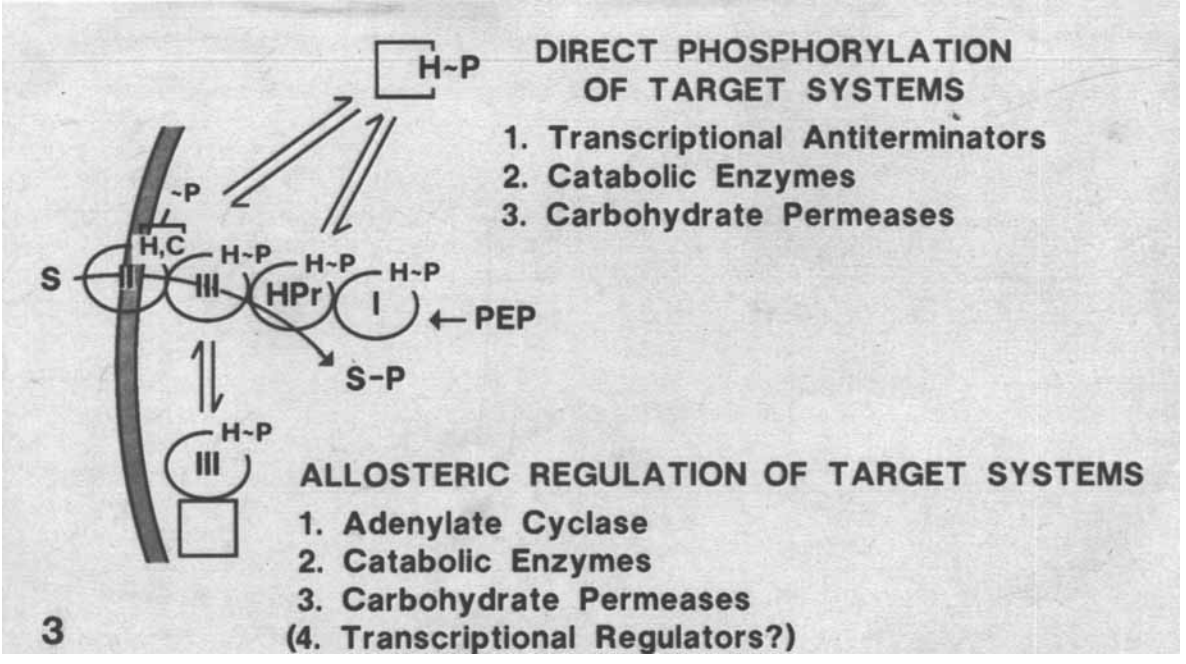
Fig. 1. Schematic illustration of the classical metabolite- or second messenger-activated protein kinases These enzymes phosphorylate seryl (S), threonyl (T), or tyrosyl (Y) residues in target proteins All bacterial protein kinases characterized to date use ATP preferentially as the phosphoryl donor, but other phosphoryl donors may prove to be utilized For any particular system, the protein kinase and protein-phosphate phosphatase may be present either as two separate proteins, or both reactions may be catalyzed by a single bifunctional protein

Fig. 2. Schematic representation of sensor-kinase/response-regulator systems These systems utilize ATP first to phosphorylate a histidyl (H) residue in the sensor kinase, which is usually but not always membrane bound The phosphoryl group is then transferred to an aspartyl (D) residue to generate an unstable mixed anhydride bond in the response regulator Phosphorylation of the response regulator controls its activity The activity of the histidyl kinase (which may also function as a protein [asp ~ P] phosphatase) is allosterically regulated by the stimulus which may be present in the extracytoplasmic medium as shown It should be noted that this generalized scheme is an oversimplification, as some of the homologous systems consist of a single polypeptide chain containing both the sensor-kinase and the response-regulator functions, while others consist of three or more proteins (see text) Some of the histidyl kinases (i e , NtrB and CheA of the nitrogen regulatory system and the chemotaxis system, respectively) are cytoplasmic proteins rather than transmembrane proteins Only the transmembrane sensors are presumed to respond to extracytoplasmic stimuli N and C represent the N-terminus and the C-terminus, respectively, of the sensor-kinase

Fig. 3. Schematic depiction of protein phosphorylation and regulation of target systems by the phosphoenolpyruvate (PEP)-dependent phosphotransferase system (PTS) In most proteins of the PTS, histidyl (H) residues are phosphorylated, but the Enzymes II (IIB proteins or protein domains) may be phosphorylated either on histidyl (H) or cysteyl (C) residues Two related PTS-mediated regulatory mechanisms are depicted Direct phosphorylation of the target systems The transcriptional antiterminators of the β-glucoside (*bgl*) operon in *E coli* and the sucrose (*sac*) regulon in *B subtilis* are believed to be directly phosphorylated and dephosphorylated in an equilibrium reaction controlled by the availability of an extracellular substrate-inducer and catalyzed by an Enzyme II or Enzyme II-like protein in the membrane By contrast, phosphorylation of catabolic enzymes and carbohydrate permeases is believed to occur as a result of phosphoryl transfer from HPr(his ~ P) In the latter case, the availability of an extracellular sugar substrate of the PTS controls the phosphorylation state of HPr, and, consequently, that of the target protein Phosphorylation of an allosteric regulatory protein, an Enzyme IIA (Enzyme III) of the PTS Adenylate cyclase is believed to be allosterically activated by the phosphorylated derivative of the glucose-specific Enzyme IIA or Enzyme III (III^{glc} ~ P) while the catabolic enzymes and non-PTS carbohydrate permeases are inhibited by the free (dephosphorylated) form of the protein The mechanisms by which Enzymes IIA potentially function in transcriptional regulation have not yet been elucidated







Figures 1, 2, and 3.

ity that protein kinases function in the phenomenon of catabolite repression in Gram-positive bacteria. The details of the many mechanisms of catabolite repression [10] are poorly understood, particularly in the Gram-positive bacteria, and thus this area of study represents a new frontier in prokaryotic research (see the article by George Stewart, this issue). Finally, protein kinases which are homologous to the eukaryotic enzymes have recently been identified in the myxobacterium, *Myxococcus xanthus* and shown to be important in the process of fruiting body formation. These exciting findings are presented and discussed in the paper by Muñoz-Dorado, et al., this issue.

Sensor-Kinase/Response-Regulators

The second type of bacterial protein phosphorylation system is represented by the recently discovered sensor-kinase/response-regulator elements which respond to environmental stimuli and control gene transcription or cellular behavior (see Fig. 2). In response to a stimulus, a sensor kinase uses ATP to phosphorylate itself on a histidyl residue within a conserved C-terminal domain. This phosphoryl group is then transferred to an aspartyl residue within a conserved N-terminal domain of a second protein, the response regulator. The sensor kinase may also act as a phosphatase and remove the phosphoryl group from the response regulator, although spontaneous dephosphorylation is also known to occur. The first of the sensor-kinase/response-regulator phosphorylation cascades to be characterized, and the one that may still be the best understood from a biochemical standpoint, is the nitrogen regulatory system discussed in the article by Boris Magasanik, this issue.

All sensor kinases share a homologous C-terminal domain, in addition to similar sequences that surround the conserved histidyl residue. The response regulators all possess a conserved N-terminal domain. In the case of the chemotaxis system, described in the paper by Lukat and Stock (this issue), the CheY response regulator, the three-dimensional structure of which has been elucidated, consists only of this domain and must therefore exert its effect by interacting with components of its target system, proteins in the basal region of the bacterial flagellum. The conserved domains from these types of phosphorylation regulated systems together with additional domains which differ according to the system under study allow a variety of signaling capabilities.

The term *two-component system,* sometimes used to describe these regulatory proteins, is now recognized to be inaccurate. Some of these systems consist of a single polypeptide chain bearing both the histidyl protein kinase and the response-regulator functions. FrzE, which controls motility and development in myxobacteria, is an example of such a one-component system. In the chemotaxis system of *Escherichia coli,* the sensor function is separate from the histidyl protein kinase. Thus, the CheA kinase functions with a set of transmembrane sensors, each of which binds on its external surface and responds to a number of chemical stimuli. The phosphate-responsive regulatory system discussed in the speculative synopsis authored by Barry Wanner (this issue) describes one in which the phosphate-specific transport system (Pst) serves as the sensor that controls the activities of the response-regulator kinase and phosphatase. In the case of sporulation (see the paper by Jim Hoch, this issue), two separate kinases feed into a phosphorelay that controls cellular differentiation in *Bacillus subtilis.* Many of the details of this transduction process are understood, but the signals detected by these kinases have not yet been identified.

At least several dozen such homologous sensor-regulator systems are believed to be encoded within the bacterial genome, each controlling a specific cellular activity in response to one or a few agents or conditions. The structural similarities of these systems, as determined by their sequence homologies, probably reflect a unified mechanism. Interestingly, the sensor kinases are homologous to plant phytochromes, but the mechanism of action of the latter proteins may be quite different [11] [also unpublished observations].

The Bacterial Phosphotransferase System

The third bacterial protein kinase-like system is the complex, but well-characterized, phosphoenolpyruvate:sugar phosphotransferase system (PTS). In this system, a phosphoryl group donated by phosphoenolpyruvate is passed down a chain of phosphorylatable proteins which are known collectively as the phosphoryl transfer chain of the PTS. The best known function of the PTS is group translocation of sugars across bacterial cytoplasmic membranes. However, the

phosphoryl transfer chain also mediates chemoreception, somehow controlling the direction of rotation of the bacterial flagellum (see article by Titgemeyer, this issue). In addition, the system is known to regulate a variety of other bacterial physiological processes (Fig. 3). The PTS phosphoryl transfer chain normally consists of five proteins or protein domains: Enzyme I and HPr are general (non-sugar-specific) energy coupling proteins, while the Enzymes IIA,B, and C, which must be present in the membrane as a complex for normal function, are specific for different sugars. Most of the soluble proteins of the PTS known to function in energy coupling and/or regulation are phosphorylated on histidyl residues. Phosphorylation of the imidazole ring of histidine on either the N-1 or the N-3 position gives rise to a high-energy phosphoramidate bond.

In *E. coli* and other enteric bacteria, the catalytic activities of the PTS serve to regulate adenylate cyclase as well as various carbohydrate permeases and catabolic enzymes by a well-characterized mechanism in which the allosteric effector is the phosphorylatable PTS protein, Enzyme IIA^{glc}, also referred to as the Enzyme III^{glc} (Fig. 3). This phosphorylated glucose-specific protein is believed to somehow activate adenylate cyclase, while the free (dephospho) form is known to inhibit the permeases and catabolic enzymes as considered in the symposium article by the present author. The three-dimensional structures of the PTS regulatory proteins, IIA^{glc} and HPr, have recently been determined both by multidimensional NMR and by X-ray crystallography. These dynamic structures are presented in the contribution by Chen, et al., (this issue). In Gram-positive bacteria, HPr may regulate catabolic enzyme synthesis by at least two mechanisms which involve phosphorylation of two distinct residues in this protein as discussed by Reizer et al.

A regulatory mechanism distinct from any of those described above involves PTS-mediated phosphorylation of transcriptional antiterminator proteins specific to particular carbohydrate-catabolic operons (see the article by Amster-Choder and Wright, this issue). The β-glucoside (*bgl*) operon of *E. coli* and the sucrose (*sac*) regulon of *Bacillus subtilis* are both regulated by such a mechanism. In these mechanisms, antitermination is dependent on an inducer (β-glucoside and sucrose, respectively), whereas the promoters are inducer independent. Thus, induced synthesis of the encoded catabolic enzymes is apparently due to suppression of a transcriptional termination event that occurs at a site preceding the first structural gene.

Functional Overlap of the Three Prokaryotic Protein Phosphorylation Systems

The *sacB* operon of *B. subtilis* is controlled both by a two-component sensor-kinase/response-regulator and by PTS-mediated antiterminator phosphorylation. Two distinct phosphorylative regulatory mechanisms may thus function in the control of a single process, allowing detection of an increasing number of environmental stimuli. Moreover, phosphoenolpyruvate- and ATP-dependent protein phosphorylating systems undoubtedly interact functionally in the chemotaxis response of *E. coli* toward PTS sugar substrates. In this case, the sugar attractant is presumably detected by the PTS components via a PEP-dependent phosphorylation mechanism, but the eventual behavioral response is probably produced through interactions with the cytoplasmic CheA and CheY proteins that exhibit the standard features of ATP-dependent sensor-kinase/response-regulator systems. These examples suggest that the three distinct types of protein phosphorylating systems in bacteria interact functionally to increase the regulatory sensitivities of the target system as a whole.

Although the three classes of prokaryotic regulatory protein phosphorylation systems evidently evolved independently of each other, it seems likely that elucidation of the mechanisms of their action will require the application of fundamentally similar experimental approaches and will reveal universal biochemical principles applicable to eukaryotes as well as prokaryotes. Molecular genetic approaches have made it possible to produce gram quantities of soluble proteins such as isocitrate dehydrogenase, the CheY chemotaxis protein and the phosphoryl transfer proteins of the PTS. This technical advance has already allowed use of the most refined physicochemical tools to the elucidation of structural features of these enzymes and regulatory proteins. It is clear that the application of multipronged approaches to the structures and modes of action of proteins that mediate signal transduction via protein phosphorylation mecha-

nisms will be required for a full understanding of these processes.

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