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MINIREVIEW

Is Acetyl Phosphate a Global Signal in *Escherichia coli*?

WILLIAM R. McCLEARY,[1] JEFFRY B. STOCK,[1] AND ALEXANDER J. NINFA[2]*

Department of Molecular Biology, Princeton University, Princeton, New Jersey 08544,[1] and Department of Biochemistry, Wayne State University School of Medicine, Detroit, Michigan 48201[2]

INTRODUCTION

In this article we discuss recent evidence indicating that acetyl phosphate may be a global signal in *Escherichia coli* and related bacteria. We shall start by reviewing the evidence that acetyl phosphate is involved in signal transduction by a family of related phosphorylation-dependent switches known as the two-component systems. We shall also review the data indicating the mechanism by which acetyl phosphate affects the function of these switches. Finally, we shall review the factors affecting the intracellular concentration of acetyl phosphate in an attempt to deduce the significance of the signal provided by acetyl phosphate. We conclude by offering two suggestions for the role of acetyl phosphate as a potential global signal.

THE PHOSPHORYLATION OF RESPONSE REGULATOR PROTEINS IS USED TO CONTROL MANY CELLULAR PROCESSES IN BACTERIA

Many regulatory phenomena in bacteria, ranging from control of cell cycle progression to adaptations to environmental stimuli, are achieved by phosphorylation-dependent signal transduction switches consisting of homologous proteins. These systems, known in the aggregate as the two-component systems, have been the subject of several recent reviews (3, 20, 25, 30, 33, 34, 37). The switches have the following important properties. One component, known as the response regulator (also referred to as the receiver or effector), contains a domain (response regulator domain [RRD]) that is transiently phosphorylated on a conserved aspartyl residue (13, 31, 32, 40). Response regulator proteins usually contain additional domains whose activities are controlled by the phosphorylation of the RRD. For example, many response regulators are transcriptional factors that are able to activate transcription upon phosphorylation of the RRD (11, 22). In another case, the protein domain controlled by the RRD is an enzyme (17). Indeed, the target controlled by phosphorylation of an RRD may even be located on another protein or protein complex (e.g., CheY [26]). In all cases, however, the key to the signalling seems to be the regulation of the intracellular concentration of the phosphorylated form of the response regulator protein.

The second component of the switches formed by the two-component systems is a histidine kinase protein (HK), also referred to as the sensor or transmitter (25, 33). This protein binds ATP and is autophosphorylated at a conserved histidine residue (9, 21, 40, 43). Phosphoryl groups are then transferred from this histidine residue to the conserved aspartate residue in the RRD, activating the response regulator protein (9, 40, 43).

Many different mechanisms are used in different signal transduction systems to regulate the intracellular concentration of the phosphorylated form of the response regulator protein. For example, control can be exerted at the level of regulation of the autophosphorylation of the histidine kinase (2, 23). In other cases, the intracellular concentration of the phosphorylated response regulator is controlled by regulated phosphatase activities (11, 13, 22). In some cases, this regulated phosphatase activity is found in the same protein that has the histidine kinase activity, in other cases, a separate protein has the phosphatase activity. The sensory input for these regulatory events may be via sensory domains located on the HK proteins themselves, via dissociable regulatory subunits that communicate information from a distinct sensory apparatus to the HK protein, or via the incorporation of the HK protein into an assembled protein complex containing sensory components (reviewed in reference 33).

It has been estimated that there may be as many as 50 different two-component regulatory systems in *E. coli* (25, 33). To prevent cross-signalling, it is essential that the phosphorylation of each response regulator can be separately controlled (37). The required specificity is obtained by control of the phosphorylation of response regulators and, in some cases, by the highly specific control of the dephosphorylation of response regulators by the regulated phosphatase activities.

RESPONSE REGULATOR PROTEINS CATALYZE THEIR OWN PHOSPHORYLATION AND DEPHOSPHORYLATION

It has been known for some time that when an autophosphorylated HK protein is incubated with the appropriate unphosphorylated response regulator, transfer of the phosphoryl group to the response regulator occurs (9, 40, 43). An important clue regarding the mechanism of this phosphotransfer reaction comes from the observation that certain low-molecular-weight phosphorylated compounds can transfer phosphoryl groups to the RRD in the absence of an HK protein. For example, phosphoramidate (NH_2PO_3), acetyl phosphate, and carbamyl phosphate are able to serve as phosphoryl group donors for the chemotaxis response regulator CheY (16). In addition to CheY, six other purified response regulator proteins (CheB, NR_I, PhoB, OmpR, ArcA, and AlgR [7, 8, 16, 19]) have been phosphorylated by using one of these three small phosphorylated compounds. Thus, it is apparent that the response regulator proteins catalyze their own phosphorylation, i.e., the phosphotransfer reaction from the HK phosphohistidine to the RRD

* Corresponding author.

aspartate target is an autophosphorylation reaction catalyzed by the RRD. The different response regulator proteins show widely different reactivities toward the three small phosphorylated compounds that serve as potential phosphoryl group donors. Acetyl phosphate is the preferred substrate for CheY, while phosphoramidate is a better substrate for NR_I and CheB autophosphorylation (19). The rates of autophosphorylation also differ dramatically between different response regulators. For example, in the presence of acetyl phosphate, CheY is phosphorylated faster than NR_I, which is phosphorylated faster than PhoB (19). Interestingly, it has been known for some time that acetyl phosphate can directly transfer phosphoryl groups to certain ATPases that typically become transiently phosphorylated as part of the catalytic cycle; in these cases as well, the site of phosphorylation is an aspartyl residue (29).

Response regulators phosphorylated by acetyl phosphate behave identically to those phosphorylated in the presence of their cognate HK proteins. CheB catalyzes the demethylation of glutamyl methyl esters located on the methylated chemotaxis receptor proteins (MCPs). Phosphorylation of CheB by phosphotransfer from the HK protein CheA increases the methylesterase activity at least 10-fold (17). A similar increase in CheB methylesterase activity results from incubation of CheB with phosphoramidate (16). The response regulator of the Ntr (nitrogen-regulated) regulon, NR_I, is an enhancer-binding transcription factor that, upon phosphorylation, becomes an ATPase and activates transcription by RNA polymerase containing σ^{54} (22, 39). Both the ATPase activity of NR_I and the ability to activate transcription by σ^{54}-RNA polymerase are greatly increased when NR_I is incubated with either phosphoramidate, carbamyl phosphate, or acetyl phosphate (8). These experiments demonstrate that the phosphorylated response regulators formed by phosphotransfer from small molecules have the same properties as those resulting from phosphotransfer from their cognate HK proteins.

It has been known for some time that certain phosphorylated response regulators hydrolyze very rapidly, in some cases with half-lives of seconds, at neutral pH. In other cases, the phosphorylated response regulators are relatively stable, with half-lives of several hours. Thus, it is clear that the stability of the phosphorylated RRD is an intrinsic property that has been optimized for different regulatory functions (reviewed in reference 20). Since the most unstable phosphorylated response regulators are stabilized upon denaturation of the protein or upon removal of the coordinated metal ion, this instability has been designated an "autophosphatase activity" (13). Response regulator proteins can therefore be considered phosphatases whose transient covalent intermediate has a signalling function (31). The substrates for these phosphatases are the phosphorylated HK proteins and low-molecular-weight compounds such as acetyl phosphate.

ACETYL PHOSPHATE METABOLISM

Acetyl phosphate is a high-energy phosphate compound with a ΔG^0 of hydrolysis of -43.1 kJ/mol, compared to -30.5 kJ/mol for ATP (35). Acetyl phosphate was first identified as a precursor of acetic acid during fermentation and has since been shown to be involved in the activation of acetate for its metabolic utilization (5). The metabolic pathway responsible for the synthesis and degradation of acetyl phosphate is shown in Fig. 1. Synthesis of acetyl phosphate from acetyl-CoA and P_i is catalyzed by phosphotransacetylase, the product of the *pta* gene. Synthesis of acetyl phosphate from ATP and acetate is catalyzed by acetate kinase, the product of *ackA*. Both reactions are readily reversible. In *E. coli*, *Salmonella typhimurium*, and related bacteria, the *pta* and *ackA* genes are contiguous on the chromosome, mapping at 49 min (14, 38). The expression of these genes is nearly constitutive, with only a twofold induction upon anaerobiosis (14, 24). It has been reported that these genes do not form an operon in *E. coli* (38). However, insertions in *ackA* have a polar effect on *pta* expression in *S. typhimurium*, suggesting that they constitute an operon in that organism (14).

Levels of acetyl phosphate vary dramatically depending on the carbon source in the growth medium (1, 10, 19). For example, in defined medium under limiting phosphate conditions, very low levels of acetyl phosphate are observed when cells are grown on glycerol (<40 μM), moderate levels of acetyl phosphate are observed when cells are grown on glucose (300 μM), and high levels of acetyl phosphate are observed when cells are grown on pyruvate (1.5 mM). The level of acetyl phosphate seems to correlate with the amount of acetate that is produced under these growth conditions. The production of acetyl phosphate is completely abolished in an *E. coli ackA pta* double mutant (19). Acetyl phosphate synthesis is also blocked in a *pta* mutant unless acetate is provided (19). In an *ackA* mutant, the levels of acetyl phosphate are elevated because conversion to acetate is blocked (19).

ROLE OF ACETYL PHOSPHATE IN THE REGULATION OF THE BACTERIAL PHO, NTR, AND MOTILITY REGULONS

It has been known for some time that expression of genes and regulons controlled by two-component systems is often not eliminated by null mutations in the HK proteins (reviewed in reference 33). This phenomenon has been referred to as crosstalk or cross regulation (20, 37) and was attributed to the phosphorylation of response regulator proteins by noncognate HK proteins. In several cases such crosstalk has been observed in vitro by using purified components (reviewed in references 20 and 33). The first indications that acetyl phosphate may be responsible for the activation of response regulator proteins (in the absence of the cognate HK proteins) came from studies of bacterial chemotaxis and the Pho regulon (15, 41). It was observed that a *cheA* mutant lacking the HK protein of the chemotaxis signal transduction system, which is normally unable to tumble (or rapidly change swimming direction), could be made to tumble by exposure of the cells to acetate (41). Furthermore, one of the regulated genes of the Pho regulon, *phoA*, could be expressed in the absence of the two cognate HK proteins, PhoR and CreC, by increasing the copy number of *ackA* (15). These results stimulated further investigations which revealed that, in both of these cases and in the case of the Ntr regulon, activation of the response regulators in the absence of the cognate HK proteins is due to the intracellular accumulation of acetyl phosphate, as discussed below.

E. coli regulates the synthesis of many genes in response to the rate of transport of phosphate across the cell membrane by the phosphate-specific transport system (Pst system); these genes constitute the Pho regulon (reviewed in reference 36). Among these regulated genes and operons is the *phoA* gene, encoding alkaline phosphatase. The transcription of the Pho regulon is controlled by a two-component system in which PhoB is the response regulator and

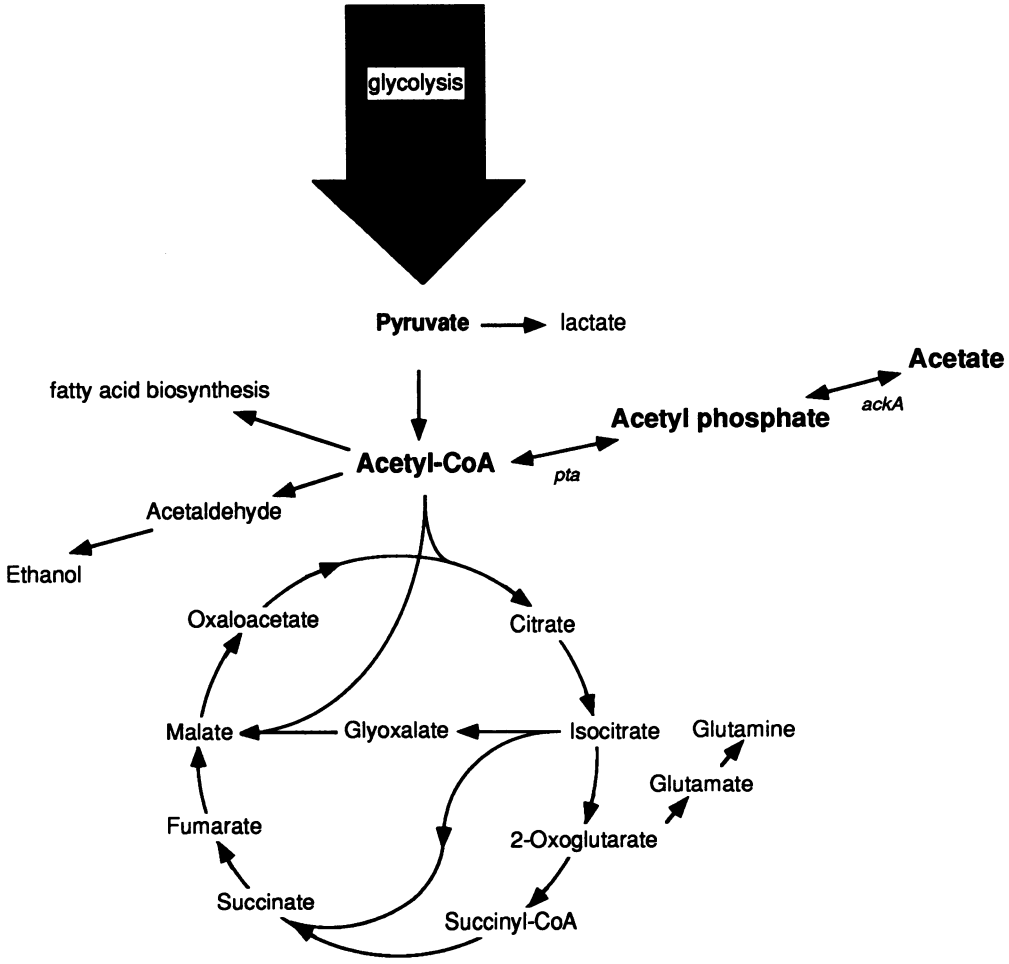

FIG. 1. Central metabolic pathways in *E. coli* that affect levels of acetyl phosphate.

PhoR is the histidine kinase and PhoB~P phosphatase. Another histidine kinase, CreC (PhoM), can also donate phosphoryl groups to PhoB but cannot dephosphorylate PhoB~P. PhoB~P activates transcription of *phoA* and the Pho regulon. In a *phoR creC* double mutant, the induction of alkaline phosphatase is completely dependent upon the ability to produce acetyl phosphate (38). In this strain, alkaline phosphatase is produced when cells are grown on pyruvate under conditions of excess phosphate. This effect is particularly pronounced in an *ackA* background, as would be expected, since *ackA* strains accumulate acetyl phosphate. In contrast, there is no production of alkaline phosphatase in a *creC phoR ackA pta* mutant (38). These results clearly indicate a role for acetyl phosphate in activating Pho regulon expression in intact cells lacking PhoR and CreC.

In *E. coli*, the transcription of the *glnA* gene (encoding glutamine synthetase) and the Ntr [nitrogen-regulated] regulon) is activated in response to nitrogen limitation (reviewed in reference 18). Regulation is achieved by a two-component system in which NR_I (NtrC) is the response regulator and NR_{II} (NtrB) is the histidine kinase and NR_I~P phosphatase. The partitioning between the kinase and phosphatase activities of NR_{II} depends upon the availability of a regulatory subunit, designated P_{II}, that elicits the phosphatase activity of NR_{II}. Under conditions of nitrogen limitation, when this phosphatase activity is inappropriate, P_{II} is rendered innocuous by reversible covalent modification catalyzed by a sensory apparatus. Conversely, P_{II} is made available for interaction with NR_{II} under conditions of nitrogen excess, and the resulting NR_I~P phosphatase activity prevents the activation of *glnA* and Ntr transcription. Several lines of evidence indicate that the activation of *glnA* transcription requires only a low intracellular concentration of NR_I~P, but activation of transcription of the other members of the Ntr regulon requires about a 10-fold-higher intracellular concentration of NR_I~P (18).

Not only are cells lacking NR_{II} able to activate *glnA* expression, but under certain conditions this expression is nitrogen regulated (8, 28). For example, glucose minimal medium is often used to quantitate nitrogen regulation by ammonia in intact cells; under such conditions glutamine synthetase is regulated about 6- to 10-fold by ammonia in the presence of NR_{II} and about 4-fold in the absence of NR_{II} (8). It has been known for some time that the change in the rate of *glnA* transcription upon shift from excess nitrogen medium to nitrogen deprivation medium and vice versa is very rapid in cells containing NR_{II} but is much slower in cells lacking NR_{II} (28). The slow nitrogen regulation in the absence of NR_{II} is apparently due to changes in the intracellular concentration of acetyl phosphate. Mutation of *pta* and *ackA* prevent the activation of *glnA* transcription in the absence of NR_{II}, and conditions that result in elevated acetyl

phosphate, such as growth on pyruvate or growth of a *pta* mutant on acetate, result in the inappropriate activation of *glnA* (8). Thus, acetyl phosphate is responsible for nitrogen regulation of *glnA* in glucose-grown cells lacking NR_{II}.

As mentioned above, the effect of exogenously added acetate on the tumbling frequency of bacteria has been known for some time (41). This effect was observed in mutants that were deleted for both the CheA HK protein and the CheY~P phosphatase, CheZ (41). Apparently this effect is due to intracellular acetyl phosphate, which also affects, either directly or indirectly, the expression of flagellar and chemotaxis proteins (42).

CONTROL OF RESPONSE REGULATOR PHOSPHORYLATION AND DEPHOSPHORYLATION

The discovery that small molecules can donate phosphoryl groups to activate response regulators emphasizes the importance of the regulated phosphatase activities. Different response regulators differ in their sensitivity to phosphorylation by small phospho-donor molecules, and different levels of these compounds could thus trigger various responses. The regulated phosphatase activities ensure that inappropriate responses do not occur. This was demonstrated in the experiments with the Pho and Ntr regulons (discussed above), in which inappropriate effects of acetyl phosphate were observed only in cells lacking the regulated phosphatase activity. Thus, the intracellular concentration of the phosphorylated RRD is controlled at the level of the rates of formation and hydrolysis of the phosphorylated RRD (Fig. 1).

ACETYL PHOSPHATE AS A GENERAL SIGNAL

In nature, *E. coli* experiences a wide variety of growth conditions from the intestinal tract to the soil. Within these diverse environments, levels of oxygen, carbon, nitrogen, phosphorus, sulfur, iron, etc., vary greatly, as do toxins, temperature, pH, and osmolarity. Bacterial regulatory systems insure survival and optimal growth rates in these diverse environmental conditions. In general, enzymes for the catabolism of various carbon and nitrogen sources are not produced unless the substrates of these enzymes are present and, even then, only if a more suitable carbon or nitrogen source is not present (reviewed in reference 18). In many cases, a particular small molecule, often a product of metabolism, is the signal which, through interaction with a regulatory protein, achieves control. For example, allolactose regulates transcription of the *lac* operon through its interaction with *lac* repressor, and cyclic AMP (cAMP) regulates transcription of *lac* through its interaction with cAMP receptor protein. These two examples represent in one sense opposite poles in regulatory strategy; in the case of the *lac* repressor, the regulator is highly specific, while in the case of cAMP-cAMP receptor protein, the regulator is global. Effective regulation is often achieved by the combination of a global signal, such as cAMP, with a highly specific one, such as allolactose. Acetyl phosphate is likely to affect the regulation of many targets, such as cAMP, and thus might constitute a global signal. The specific signal for control of these targets would be through the histidine kinase/phosphatase protein(s). What role could the global signal play in regulating the targets of individual two-component systems?

For some two-component systems, the regulators are present in the cell at very low concentrations and are subject to autoregulation. For example, in the Ntr system NR_I and NR_{II} are present at levels of just a few molecules per cell under conditions of nitrogen excess, but the concentration of NR_I is elevated at least 10-fold upon severe nitrogen limitation (27). One possible role for the global signal in such cases might be to prime the system by initiating the response.

Another possible role for a global signal provided by acetyl phosphate could be to provide a baseline level of phosphorylated response regulator protein, facilitating a more rapid response upon a shift to the inducing conditions or providing a higher absolute level of the phosphorylated response regulator under inducing conditions.

Finally, small phospho-donor molecules might in some cases eliminate the necessity for a histidine kinase altogether. There exist a number of response regulator proteins for which the cognate kinase has not been identified. Since acetyl phosphate or some other low-molecular-weight phospho-donor provides a mechanism for the phosphorylation of these "orphan" response regulators, these proteins could represent "one-component" systems.

WHAT DOES ACETYL PHOSPHATE SIGNAL?

It seems possible that acetyl phosphate is part of a signal that indicates that *E. coli* is in the intestinal tract. *E. coli* is a minor component of the intestinal flora and is vastly outnumbered by anaerobes such as *Bacteroides* spp. (4). These other organisms ferment carbohydrates and produce large amounts of acetate (up to 75 mM following digestion of complex carbohydrates [6]). In an anaerobic environment, high levels of acetyl phosphate would be expected to accumulate because the bacteria cannot metabolize acetate in the absence of respiration. Thus, acetyl phosphate may be a signature molecule for enteric residence. It is interesting that the regulation of the enzymes of the tricarboxylic acid (TCA) cycle and of the glyoxylate shunt are mediated by the Arc two-component system, in which the phosphorylated form of the response regulator, ArcA, is the repressor of the enzyme genes (12). We can speculate that this repression would be facilitated by the transfer of phosphoryl groups from acetyl phosphate to ArcA. Furthermore, it is known that expression of the OmpC porin, associated with enteric residence, is stimulated by an elevated intracellular concentration of the phosphorylated OmpR response regulator (reviewed in reference 33). Since OmpR is phosphorylated by acetyl phosphate, we can speculate that the expression of the OmpC porin during enteric residence might be facilitated by the accumulation of acetyl phosphate. It must be stressed, however, that while this proposed role of acetyl phosphate is logical, there is as yet no evidence to support or refute the hypothesis.

Another role for the global signal provided by acetyl phosphate may be to indicate an imbalance between carbon flowing through glycolysis and into the TCA cycle. Specifically, acetyl phosophate is elevated when entry of carbon into the TCA cycle is slower than entry into glycolysis. There are many possible causes for such an imbalance. For example, under conditions of ammonia deprivation, 2-ketoglutarate is not withdrawn from the TCA cycle by the reactions catalyzed by glutamate synthase and glutamate dehydrogenase (Fig. 2). A decreased flow through the TCA cycle would be expected to cause an increase in the acetyl coenzyme A pool. If such were the case, acetyl phosphate levels might increase upon ammonia starvation. This may account for the slow nitrogen regulation observed in cells lacking NR_{II} (28).

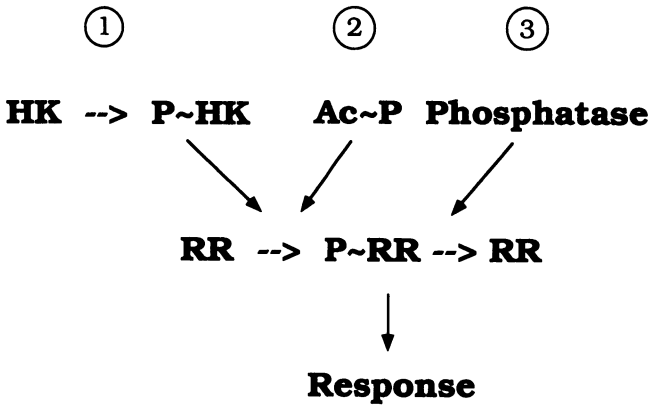

FIG. 2. Control of intracellular concentrations of activated response regulators depend upon their rates of phosphorylation and dephosphorylation. These rates are modulated by: (1) histidine kinases, (2) small molecule phospho-donors, and (3) phosphatases.

In conclusion, low-molecular-weight phosphorylated compounds such as phosphoramidate, acetyl phosphate, and carbamyl phosphate donate phosphoryl groups to response regulator proteins. This activity has confirmed that response regulators are themselves enzymes that catalyze their own phosphorylation. Indeed, the response regulators can be viewed as phosphatases whose transient covalent intermediates have a signalling function (32). The demonstration that small phosphorylated metabolic intermediates can serve as substrates for response regulator autophosphorylation has revealed some subtle aspects of regulation of the two-component signal transduction pathways. These pathways can be regulated by controlling the levels of different phospho-donors (phospho-histidine kinases or small molecules) or by controlling the rates of dephosphorylation of the activated response regulators.

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