Involvement of Phosphotransacetylase, Acetate Kinase, and Acetyl Phosphate Synthesis in Control of the Phosphate Regulon in *Escherichia coli*

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Two controls of the phosphate (PHO) regulon require sensor proteins that are protein kinases that phosphorylate the regulator, PhoB, which in turn activates transcription only when phosphorylated. P control requires the P sensor PhoR; the other control is P independent and requires the sensor CreC (formerly called PhoM). Here we describe an additional control of the PHO regulon which is P independent and requires neither PhoR nor CreC. This control is regulated by a two-step pathway in carbon metabolism in which acetyl coenzyme A, P, and ADP are converted into acetate, coenzyme A, and ATP via the enzymes phosphotransacetylase (Pta) and acetate kinase (AckA). It responds to the synthesis of acetyl phosphate, an intermediate in the Pta-AckA pathway. Since the synthesis of acetyl phosphate via this pathway leads to the incorporation of P into ATP, the primary phosphor donor in metabolism, we propose that a regulatory coupling(s) may exist between the PHO regulon, which encodes genes for P uptake, and genes for enzymes in central metabolism for incorporation of P into ATP. Regulatory interactions of this sort may be important in global control. Further, it provides a functional basis for the concept of cross-regulation in the PHO regulon. This is also the first evidence that acetyl phosphate may have a role as an effector of gene regulation.

The PHO regulon includes several genes and operons for the uptake and degradation of extracellular phosphorus sources. These genes are repressed when P, the preferred P source, is present in excess; they are induced many hundredfold when P, is growth limiting. This induction requires the partner proteins PhoB and PhoR, which make up a two-component regulatory system for P control (22, 23). PhoR autophosphorylates itself and acts as a histidine protein kinase that is thought to phosphorylate the response regulator PhoB when the level of P is growth limiting. PhoB is a transcriptional activator that functions only when it is phosphorylated (11).

The PHO regulon is highly regulated in *phoR* mutants even though P control is abolished. Instead, PHO regulon genes are regulated by the carbon source via a control that is not apparent in *phoR* cells (21, 26, 27). Investigators studying this control have used *phaA*, the bacterial alkaline phosphatase (Bap) structural gene, as a reporter of PHO regulon gene expression. The presence of glucose or mutations in the adeny1 cyclase (crp) or cyclic AMP (cAMP) receptor protein (crp) gene leads to induction of the *phaA* gene in *phoR* mutants. This induction requires the sensor CreC and the regulator PhoB (27). However, CreC probably does not detect glucose per se. CreC more probably detects an intermediate in central metabolism because many carbon sources (such as acetate, malate, and succinate) can mimic the effect of glucose (28). Therefore a *crp* or *crp* mutation may indirectly affect CreC-dependent control by altering the expression of genes that are important in carbon metabolism. The *creC* gene is in the *creABCD* operon, which also encodes CreB. CreC and CreB are partner proteins of a two-component regulatory system whose function is unknown (15, 19, 23). CreC, like PhoR, autophosphorylates itself and acts as a histidine protein kinase which can phosphorylate both CreB and PhoB in vitro. From its sequence CreB is probably a regulator of some unknown gene(s) (1).

Cells undergo numerous adjustments of central metabolic pathways in response to the carbon source. In particular, the carbon flow differs in cells grown on glucose, pyruvate, or acetate (Fig. 1). Glycolysis is the principal pathway for glucose metabolism. During growth on glucose, phosphoenolpyruvate (PEP) is formed via glycolysis and pyruvate is formed (mainly) during glucose uptake via the PEP-dependent phosphotransferase system. The pyruvate is metabolized to acetyl coenzyme A (acyt1-CoA), which mostly enters the tricarboxylic acid cycle for biosynthesis. Therefore very little pyruvate is metabolized via the phosphotransacetylase (Pta) and acetate kinase (AckA) pathway (hereafter called the Pta-AckA pathway) during growth on glucose. However, when pyruvate is the sole carbon source, acetyl-CoA is made in excess and much more carbon is metabolized via the Pta-AckA pathway. When acetate is the carbon source, acetate is metabolized (primarily) via an inducible acetyl-CoA synthetase (3, 7, 14, 16). Some acetate is also metabolized via the Pta-AckA pathway, which is reversible in vivo, even though this pathway is dispensable for growth on acetate (4).

Here we report an additional P-independent control of the PHO regulon, which responds to the synthesis of acetyl phosphate, an intermediate of the Pta-AckA pathway. Since the Pta-AckA pathway leads to the incorporation of P into ATP, the primary phosphor donor in metabolism, the Pta-AckA pathway is formally a pathway in both carbon and P metabolism. We describe an hypothesis in which P control and P-independent controls of the PHO regulon are linked for the purpose of global control(s) of P metabolism. Such linkage may lead to cross-regulation by phosphorylation of regulators of different general control systems (24).
We also discuss the role of acetyl phosphate in gene regulation.

MATERIALS AND METHODS

Bacteria and phage. All bacteria are Escherichia coli K-12 strains. CAG18484 (zej-223::Tn10) was from C. Gross via M. Levinthal (18); ECL546 was from E. C. C. Lin (6); TA3514 (pta-200), TA3515 (ackA200), and TA3516 (pta-ackA) were from G. F.-L. Ames (9). Other bacteria are listed in Table 1. pMK800 is a pBR322 derivative with a 9.5-kbp chromosomal fragment that includes the ackA gene, and pMK814 is a derivative of pMK800 with a 1.7-kbp fragment that includes the ackA gene; both were from A. Nakata (8). pMW2 was described previously (29). Transductions with P1ke and DNA transformations were done as described elsewhere (25). Tn5 and Tn5-112 encode kanamycin resistance, and Tn5-132 encodes tetracycline resistance; Tn5, Tn5-112, and Tn5-132 elements were switched (swapped) as described previously (13).

The Δcreate ABCD) mutant, BW13075, is a tetracycline-sensitive (Tc') recombinant of BW12812 which has two Tn5 insertions in the creABC operon (Table 1). In strains with nearby Tn5 insertions, deletions can occur by homologous recombination between directly repeated IS50 elements. Such events result in the loss of the DNA between the sites of the Tn5 insertions; the deletions can be verified in genetic tests (13). Tn5 insertions in the creABC operon pMW2 were isolated after mutagenesis with λ::Tn5. The creAI14::Tn5 and creD104::Tn5 insertions were mapped with restriction enzymes to near the 5' end of the creA gene and in the creD gene, respectively. Both were crossed onto the chromosome by linear transformation as described elsewhere (29). Mutations were switched to a Tn5-132 and/or a Tn5-112 insertion and shown to be linked to the creABC operon. Their effect on the Bap phenotype were tested after crossing each into a phoR mutant with P1 (28).

In the course of making strains with mutations in the creABC operon, we found that some strains had a leaky mutation, denoted arcA*, in the arcA gene, which is adjacent to the creABC operon. During anaerobic growth ArcA negatively regulates genes for enzymes in central pathways such as ones for the succinate dehydrogenase (sdh) complex (6). To rule out effects of the arcA* allele, we separated insertions in the creABC operon from the arcA* allele in P1 crosses. We tested the arcA phenotype by crossing mutations into an sdh-lacZ fusion strain, in which arcA* strains were white, an arcA::Tn5 mutant was red, and arcA* mutants gave an intermediate color during anaerobic growth on lactose MacConkey agar. The arcA* allele was from strain MG1655Mc (21); we had fortuitously crossed it into the parent of BW11450, BW11174, when we made JC7623 Thr+ with P1 on MG1655Mc (Table 1) (27).

Media. In general, media were described previously (20). M63 agar with 0.4% potassium acetate was used to score pta and ackA mutants, which grow poorly on acetate. 5-Bromo-4-chloro-3-indolylphosphate-p-toluidine (X-Pi; Bachem, Torrance, Calif.) was used as an indicator of Bap activity.

Cell growth and enzyme assays. Cells were pregrown on glucose-morpholinepropanesulfonic acid (MOPS) agar with 2 mM P, and inoculated into 0.04% glucose-MOPS with 2 mM P, 0.4% glucose-MOPS with 0.1 mM P, 0.1% potassium pyruvate-MOPS with 2 mM P, or 0.2% potassium acetate-MOPS with 2 mM P, liquid media and grown for 16 h. Transformants were grown similarly, except that all media contained 100 μg of ampicillin per ml. Because ackA phoR mutants grow poorly and can give rise to faster-growing mutants which are Bap+, cultures of ackA phoR mutants were tested by streaking samples onto Bap indicator agar. The cell culture optical density was measured at 420 nm. Bap activities were measured as described previously (20); units are nanomoles of p-nitrophenol made per minute at 37°C.

RESULTS

Evidence for an additional PHO regulon control. The amount of Bap made in phoR mutants depends on both the carbon source and the strain background. Typically, phoR mutants make 10 to 50% as much Bap when grown on glucose with excess P, as phoR+ cells make during Pi limitation. This synthesis requires CreC and is abolished in creC phoR mutants. Such mutants are Bap− on glucose minimal, acetate minimal, and TYE media, on which they make less than 0.5% as much Bap as phoR+ cells make during Pi limitation. Although certain other media can induce Bap synthesis in creC phoR mutants, such mutants are also Bap− on asparagine, glycerol, malate, and succinate minimal media (22, 26; data not shown).

Several lines of evidence indicated that an additional control of the PHO regulon existed. In particular, we found that creC phoR mutants readily mutated to Bap+ when
selected for growth on glucose agar with X-P, as the sole P source. This was true both of creC point mutants and of creC deletion mutants. However, such Bap⁺ pseudorevertants were difficult to study because they generally grew very poorly and frequently gave rise to faster-growing mutants that were Bap⁻.

In addition, we discovered that creC phoR mutants were Bap⁺ on energy-rich media such as TYE agar with high glucose (unpublished data cited in reference 27). This was also true of both point mutations and deletion mutations of the creC gene. However, this was true only when mutants were grown on agar (it was not true for mutants grown in liquid culture). Furthermore, this induction occurred only on TYE agar with high (1%) glucose and not on TYE agar with low (0.2%) glucose.

To study this additional control, we isolated transposon-induced Bap⁺ mutants of a creC phoR strain. Four such mutants that grew poorly had insertions in the ackA gene. We determined this by cloning the transposon in one mutant, sequencing its insertion site, and identifying the ackA gene in a DNA database search. Its junction sequence was identical to a segment of the ackA structural gene. We then verified its map location and ackA phenotype. Both ackA phoR and ackA (creABC) phoR mutants were Bap⁺ on glucose minimal or TYE agar. Bap synthesis in an ackA creC phoR mutant was dependent on both the phoA and phoB genes and was repressed by P, when made phoR⁺ (28).

**Effects of the Pta-AckA pathway on Bap synthesis in phoR mutants.** The effect of glucose on Bap synthesis in cells grown on TYE agar showed that an additional PHO regulon control involved carbon metabolism. This was substantiated by isolating Bap⁺ pseudorevertants of a creC phoR mutant.
that proved to be mutated in the ackA gene. Further, this pointed to an involvement of the Pta-AckA pathway in this control.

We assessed the role of the Pta-AckA pathway in PHO regulon control in two ways. We examined Bap synthesis in cells grown with glucose, pyruvate, or acetate as the sole carbon source, and we tested the effects of pta and ackA mutations. Our results for PhoR- and CreC-dependent regulation were similar to ones reported previously (22) in that PhoR was needed during P$_i$ limitation and CreC was needed in phoR mutants when grown on glucose or acetate. Our results on the additional P$_i$-independent control showed that it led to induction in phoR mutants when grown on pyruvate and that it required neither PhoR nor CreC.

The wild-type strain, BW13711, made 2,000-fold more Bap during P$_i$ limitation than when P$_i$ was in excess (Table 2). The phoR mutant, BW13713, made 15, 130, and 30% as much as the wild-type on glucose, pyruvate, and acetate, respectively, when P$_i$ was in excess. Synthesis on glucose or acetate required CreC, because it was abolished in the Δ(creABCD)phoR mutant, BW13736, which made less than 0.2% as much on these carbon sources. This mutant also consistently made about threefold more on acetate than on glucose, although such levels were very low. In contrast, the Δ(creABCD)phoR mutant made 35% as much on pyruvate as the wild-type made during P$_i$ limitation. Therefore pyruvate leads to a substantial induction in the absence of both PhoR and CreC.

Induction in a Δ(creABCD)phoR mutant is due to the Pta-AckA pathway because pta and ackA mutations can result in induced or decreased Bap synthesis in a manner that depends on the carbon source and the mutation. Further, the control involving the Pta-AckA pathway appears to be associated with the synthesis of acetyl phosphate, an intermediate in the pathway. This is because conditions expected to result in an accumulation of acetyl phosphate led to induction, whereas conditions expected to result in decreased synthesis of acetyl phosphate led to decreased Bap synthesis.

The Pta-AckA pathway is reversible (4, 7). In cells grown on glucose or pyruvate, acetyl phosphate is made from acetyl-CoA via Pta and broken down via AckA (Fig. 1). In cells grown on acetate, acetyl phosphate is made via AckA and broken down via Pta. Therefore an ackA mutation is expected to result in an accumulation of acetyl phosphate on glucose or pyruvate and in decreased synthesis on acetate.

Conversely, a pta mutation is expected to result in decreased synthesis of acetyl phosphate on glucose or pyruvate and an accumulation on acetate. A Δ(pta-ackA) deletion mutant is expected to block acetyl phosphate synthesis altogether.

The ackA Δ(creABCD)phoR mutant, BW16469, made 150% as much Bap on glucose or pyruvate but only 0.5% as much on acetate as BW13711 made during P$_i$ limitation (Table 2). The pta Δ(creABCD)phoR mutant, BW16546, made less than 0.1% as much on glucose or pyruvate but 16% as much on acetate. Furthermore, the Δ(pta-ackA) Δ(creABCD)phoR mutant, BW16470, made less than 0.1% as much on all media. The same Δ(ackA-pta) mutation also abolished Bap synthesis in Δ(creABCD)phoR mutants under other conditions that were known to lead to induction, e.g., on TYE agar containing 1% glucose (28). Therefore the Pta-AckA pathway may be solely responsible for induction of the PHO regulon in the absence of PhoR and CreC.

**Effect of an ackA mutation on growth.** We noticed that ackA phoR mutants grew very poorly. In Luria broth (LB) and glucose minimal medium liquid cultures, ackA mutants grew more slowly than otherwise isogenic wild-type cells did. An ackA phoR double mutant always grew even more poorly, although the effect was more pronounced in LB than in glucose minimal medium liquid cultures (Table 3). A phoR mutation alone had no effect on the growth rate. However, in combination with an ackA mutation, a phoR mutation had a severe negative effect on growth, an effect that was especially apparent on agar. As expected, the effect was also more dramatic on TYE agar than on glucose minimal agar. No effect due to a phoR mutation was noticeable on acetate agar, on which all ackA mutants grew very poorly.

Mutations other than phoR which lead to derepression of the PHO regulon also resulted in a severe growth defect in an ackA mutant on agar. Mutants that grew poorly included ackA pstSCAB, ackA phoU, ackA phoR, and ackA creC phoR mutants. Mutants that appeared to grow well on agar included ackA mutants that were otherwise wild type and ackA phoB mutants. No effect due to a phoA mutation was apparent. Furthermore, faster-growing mutants accumulated in cultures of slow-growing ackA mutants. Faster-growing mutants were mostly Bap$^+$; most had apparent compensatory mutations in the phoB or pta gene (28). In agreement, both the pta Δ(creABCD)phoR and Δ(pta-ackA) Δ(creABCD)phoR mutants studied above grew well.

**Effect of AckA overproduction on Bap synthesis.** While this study was in progress, Lee et al. (8) reported that overproduction of AckA complemented (partially) a creC phoR mutant. The effect required PhoB, was overcame by PhoR, and was correlated with elevated intracellular ATP concentrations. They concluded that the effect was due to an increased ATP level or a direct effect of AckA. Because both interpretations were in apparent contradiction with our results, we repeated the supplementation experiment with

**TABLE 2.** P$_i$ control and P$_i$-independent controls of Bap synthesis

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype*</th>
<th>P$_i$ control</th>
<th>P$_i$-independent controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW13711</td>
<td>Wild type</td>
<td>0.1, 280d</td>
<td>42.7, 361, 82.4</td>
</tr>
<tr>
<td>BW13713</td>
<td>phoR</td>
<td>0.2, 104, 0.6</td>
<td>422, 409, 1.4</td>
</tr>
<tr>
<td>BW16469</td>
<td>phoR creC</td>
<td>0.2, 0.2</td>
<td>0.2, 0.2</td>
</tr>
</tbody>
</table>

*See Table 1.

**TABLE 3.** Effect of an ackA mutation on growth rate

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype*</th>
<th>Doubling time (min) in:</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW13711</td>
<td>Wild type</td>
<td>22, 58</td>
</tr>
<tr>
<td>BW15891</td>
<td>ackA</td>
<td>45, 85</td>
</tr>
<tr>
<td>BW13713</td>
<td>phoR</td>
<td>22, 56</td>
</tr>
<tr>
<td>BW15892</td>
<td>phoR ackA</td>
<td>73, 100</td>
</tr>
</tbody>
</table>

*See Table 1.

a $P_i$ was in excess.
TABLE 4. Effect of AckA overproduction on Bap synthesis

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Plasmid</th>
<th>Bap sp act*</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW8882</td>
<td>phoR</td>
<td>None</td>
<td>56.2</td>
</tr>
<tr>
<td>BW521</td>
<td>phoR creC</td>
<td>None</td>
<td>0.3</td>
</tr>
<tr>
<td>BW521</td>
<td>phoR creC</td>
<td>pMK800</td>
<td>2.0</td>
</tr>
<tr>
<td>BW521</td>
<td>phoR creC</td>
<td>pMK814</td>
<td>2.3</td>
</tr>
</tbody>
</table>

*See Table 1.

b Cells were grown for 16 h in glucose-MOPS medium with 2 mM Pi, plus ampicillin, as appropriate, and assayed as described in Materials and Methods. Units of specific activity are given in Table 2, footnote b.

their plasmids (8). Overall, our results were in good agreement with theirs. However, the effect due to AckA overproduction was far smaller than the effect due to an ackA mutation. The effect due to AckA overproduction is therefore probably due to an effect on acetyl phosphate synthesis.

Two AckA-overproducing plasmids, pMK800 and pMK814 (see Materials and Methods), gave Bap+ transformants of the creC phoR mutant, BW521, in that they were blue on Bap indicator agar. However, measurements showed that Bap synthesis was increased only about sixfold in our transformants (Table 4). This amount is only 3% of what is made in the nearly isogenic creC+ phoR mutant, BW8882. Lee et al. (8) showed an approximate 10-fold increase in the synthesis of Bap in similar transformants, which corresponded to 25% of what was made in creC+ (phoM+) plasmid transformants of the same strain. Such quantitative differences may be due to the use of different media (8).

DISCUSSION

We found an additional control of the PHO regulon which involves the Pta-AckA pathway. Previously, only two controls were known to exist: Pi, control which requires the sensor PhoR, and one P1-independent control which requires the sensor CreC. Like PhoR and CreC-dependent controls, the Pta-AckA pathway leads to gene activation because induction is abolished in mutants lacking this pathway. Induction via the Pta-AckA pathway does not require PhoR or CreC, however. Like the CreC-dependent control, the control involving the Pta-AckA pathway is P1 independent and is apparent only in phoR mutants. However, both P1-independent controls are quite substantial. Upon induction, either can lead to a level of synthesis that is 50% or more of the maximal level of induction during Pi limitation. All three controls of the PHO regulon require the regulator PhoB as a transcriptional activator.

Our results show that induction via the Pta-AckA pathway is associated with acetyl phosphate synthesis. Accordingly, acetyl phosphate may be an effector for gene activation. Although we have not ruled out other metabolites, our data indicate that the synthesis of a different hypothetical effector would also have to be similarly affected by pta and ackA mutations. Acetyl-CoA is a possible candidate for an alternative effector. During growth on glucose or pyruvate, conditions that lead to an accumulation of acetyl phosphate may simultaneously drain acetyl-CoA levels. Therefore, under these conditions, induction may occur in response to a decreased level of acetyl-CoA or a product of a reaction requiring acetyl-CoA for synthesis. However, it is harder to explain the effects during growth on acetate. It is particularly difficult to rationalize how a pta mutation specifically leads to induction by acetate. Therefore, a control in which acetyl phosphate is an effector is much more likely, although

unprecedented. Of course, the synthesis of an effector in a side reaction involving Pta and AckA is also feasible on the basis of our data.

PhoR and CreC activate the PHO regulon by phosphorylation of PhoB. Acetyl phosphate may therefore act indirectly on PhoB via a sensor which in turn phosphorylates PhoB in an ATP-dependent reaction. However, no sensor for acetyl phosphate is known. Also, attempts to find mutants lacking such a putative sensor have so far proven unsuccessful (28). Alternatively, acetyl phosphate may act directly on PhoB by binding or covalently modifying PhoB and thereby converting PhoB into an activator. If acetyl phosphate acts directly on PhoB, its role may relate to the chemical properties of acetyl phosphate as a phosphoryl or an acetyl donor. In the Pta-AckA pathway, acetyl phosphate is a phosphoryl donor in the synthesis of ATP and an acetyl donor in the synthesis of acetyl-CoA. Therefore acetyl phosphate may have a direct role as a substrate in a phosphorylation, or an acetylation, reaction involving PhoB. A variety of other mechanisms are also possible. If acetyl phosphate has a direct role, no new protein factor is necessarily required for it to activate PhoB. If, instead, acetyl phosphate has an indirect role, an additional (unknown) factor is also likely to be involved.

Our Bap+ ackA creC phoR mutants grew very poorly. Although we do not understand the basis for the slow-growth phenotype, it may be a physiological effect. An ackA mutation alone has a modest effect on the growth rate. This effect may result from a drain on acetyl-CoA levels owing to an accumulation of acetyl phosphate. Derepression of the PHO regulon is also expected to place heavy demands on biosynthesis. Therefore derepression of the PHO regulon in a cell that accumulates acetyl phosphate may overwhelm the biosynthetic capacity of the cell. Alternatively, it is possible that a regulatory interaction(s) exists between the PHO regulon and the Pta-AckA pathway and that a disruption of this interaction(s) is responsible for the severe growth defect.

The effect of AckA overproduction on Bap synthesis in a creC phoR mutant (8) may also reflect an effect on acetyl phosphate synthesis. This is because AckA catalyzes the reversible formation of acetate and ATP from acetyl phosphate and ADP. Clearly, the effect due to AckA overproduction is small compared with the effect of an ackA mutation. Furthermore, the same ackA+ plasmids complemented an ackA creC phoR mutant both by restoring normal growth on acetate and by reducing Bap synthesis greatly, in comparison with the level in the same strain without a plasmid (data not shown). However, we cannot rule out the possibility that acetate kinase has two separate effects on PHO regulon control.

It is especially interesting that acetyl phosphate is an intermediate in a pathway in which P1 is incorporated into ATP (Fig. 2), which is the primary phosphoryl donor in biosynthesis. Our results imply that increased acetyl phosphate synthesis leads to induction of the PHO regulon. Since the control of a pathway often responds to its end product, regulation involving acetyl phosphate synthesis may respond to the ATP-to-acetyl phosphate ratio, with a lowered ratio leading to induction.

Our finding that the PHO regulon is subject to regulation by the Pta-AckA pathway, which has a role in both P1 and carbon metabolism, implies that the overall global control of the PHO regulon may involve regulation by multiple steps in P1 metabolism, in addition to its regulation by P1 in the media. Since the regulation of the PHO regulon involving
CreC is also subject to carbon control, CreC-dependent regulation of the PHO regulon may also be related to a pathway in central metabolism, which may also lead to the incorporation of \( P_i \) into ATP. However, CreC-dependent control would have to involve a different pathway because the \( P_i \)-independent controls involving the Pta-AckA pathway and CreC are functionally independent.

The ways in which \( P_i \) control and \( P_i \)-independent controls of the PHO regulon may be related to different steps in \( P_i \) metabolism are illustrated in Fig. 3. Further, our results imply that a regulatory coupling may exist between \( P_i \) and carbon metabolism, which may lead to cross-regulation. Such a regulatory coupling may be an example in which cross talk has a fundamental biological role in the control of genes in central pathways (24). Regulatory interactions of this sort may be especially important in a network of global controls for the overall control of cell growth.

Multiple controls of the PHO regulon may be important in \( P_i \) metabolism as a means of coordinating \( P_i \) uptake with the incorporation of \( P_i \) into ATP. This implies that \( P_i \)-independent controls have a role in normal cells, although effects due to the Pta-AckA pathway or CreC are apparent only in \( phoR \) mutants. The reason why effects may not have been seen in wild-type cells may simply be that the proper gene or growth condition has not been tested. Most studies on \( P_i \)-independent control of the PHO regulon used the \( phoA \) gene as a reporter and were directed toward understanding gene regulation during aerobic growth on glucose. \( P_i \)-independent controls may have regulatory effects on a different PHO regulon gene under other growth conditions in wild-type cells. For instance, these controls may be important during \( P_i \) limitation on other carbon sources or during shifts from one carbon source to another. Whether an individual PHO regulon gene is affected by a particular \( P_i \)-independent control in a wild-type cell may depend on its promoter structure. Indeed, regulatory differences among \( P_i \)-regulated genes have been seen previously (22).

Since the enzymatic synthesis of acetyl phosphate was first demonstrated nearly 50 years ago (10), there has been much speculation on its role. Some evidence has suggested that acetyl phosphate acts as an energy source in binding protein-dependent transporters (5). Other data suggest that acetate kinase has a role in catabolite repression or the PEP-dependent phosphotransferase system (2, 17), which might involve the synthesis of acetyl phosphate. However, this is the first evidence that acetyl phosphate may regulate gene expression.

Our results show that acetyl phosphate (or a closely related molecule) is likely to have an important role as an effector of gene regulation. Such a role is independent of whether it has also a bona fide role in the control of the PHO regulon in wild-type cells. Acetyl phosphate may be especially important in the control of another system(s), in particular one(s) linked to a central pathway in carbon and energy metabolism. If so, the PHO regulon in E. coli may still provide an attractive system for studying the role of acetyl phosphate in biology.

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