Phosphoenolpyruvate Carboxykinase Is an Acid-Induced, Chromosomally Encoded Virulence Factor in Agrobacterium tumefaciens

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The pckA gene, encoding phosphoenolpyruvate carboxykinase, catalyzes the reversible decarboxylation and phosphorylation of oxaloacetate to form phosphoenolpyruvate. Located on the circular chromosome of Agrobacterium, this locus is adjacent to the loci chvG and chvI, encoding a two-component regulatory system that has been shown to be important in virulence. Using a reporter gene fusion, studies showed that the pckA gene is induced by acidic pH but not by acetosyringone. This acid induction is regulated by the chvG-chvI regulatory system, which controls acid-inducible genes. A pckA mutant had no demonstrable PckA enzyme activity and grew on AB minimal medium with glucose but did not grow on the same medium with succinate as the sole carbon source and was more inhibited in its growth than the wild-type strain by an acidic environment. A pckA mutant was highly attenuated in tumor-inducing ability on tobacco leaf disks and was severely attenuated in vir gene expression. Although vir gene induction was completely restored when a constitutive virG gene was introduced into the mutant strain, virulence was only partially restored. These results suggest that avirulence may be due to a combination of the inhibition of this mutant in the acidic plant wound environment and the poor induction of the vir genes.

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it is important to identify additional target genes of this regulatory system. It is well known that genes under the control of a two-component system often map in the vicinity of the regulatory genes (7). This potential candidate gene downstream of chvGI encodes phosphoenolpyruvate carboxykinase (PckA), the first enzyme of gluconeogenesis.

Mutations in the pckA locus have profound biological effects in a variety of other bacteria. For example, a pckA-deficient Mycobacterium bovis BCG mutant was attenuated in infection of both macrophages and mice (22). The pckA mutation in Rhizobium sp. strain NGR234 resulted in different nodulation phenotypes depending on the host plant (31). In Sinorhizobium meliloti, a pckA mutant fixed nitrogen at 70% of the level of the wild type, whereas the pckA mutant of Rhizobium leguminosarum fixed nitrogen at the same efficiency as wild-type cells.

In this paper, we demonstrate that pckA is indeed under the control of chvGI and that, like other genes under this control system, the expression of pckA is induced by acid and is important for maximum virulence. Other properties of the gene and its protein product are described.

MATERIALS AND METHODS

Bacterial strains, plasmids, and media. The bacterial strains and plasmids used in this study are listed in Table 1. Agrobacterium tumefaciens strains were grown in Mg/L or AB minimal medium at 28°C with shaking (4). Escherichia coli strain DH5α was grown in LB medium at 37°C with shaking (34). The following antibiotics were used at the indicated concentrations (in μg/ml): for A. tumefaciens, carbenicillin (100), kanamycin (100), and gentamicin (50); for E. coli, carbenicillin (100), kanamycin (100), and gentamicin (10). vir gene induction was measured in cells grown in induction broth as previously described (28). The detergents sodium dodecyl sulfate (SDS), sodium decyloxycholate (DOC), and Sarkosyl were used at concentrations (in mg/ml) of 0.2, 2, and 2, respectively (7).

Construction of pckA-lacZ fusion. To determine whether pckA is acid inducible, the pckA gene was fused with the lacZ reporter gene. To make this construct, two primers, pckA1 (CGTTCGATCTCGAGTTGCGTTTCCAAAAG CTC) and pckA2 (GCTAGTTAGAATTCTTATACGCCGCGAGCAG), were used to amplify the pckA gene from A. tumefaciens genomic DNA, using Taq-polymerase. Xhol and EcoRI restriction sites were then introduced into primers pckA1 and pckA2, respectively. The 1.5-kb pckA PCR product was digested with Xhol and EcoRI and ligated to a 4.95-kb Xhol-EcoRI fragment of pBR1MCS-4 to create pLP200. The 3.5-kb EcoRI fragment of pAB2002 containing the lacZ and gentamicin resistance genes was cloned into pLP200, which was then digested with EcoRI, creating pLP201.

Construction of the pckA in-frame deletion mutant. The unmarked A. tumefaciens pckA deletion mutant was generated as described previously (16). In brief, 1.5-kb regions were amplified from the upstream and downstream regions flanking the region targeted for replacement, using primers that included specific restriction enzyme sites. After restriction enzyme digestion, the upstream and downstream fragments were ligated into the vector pEX18Gm using a directional three-way ligation. The plasmid was introduced into strain C58 by electroporation, and after incubation for 3 h to allow homologous recombination, the cells were plated on LB medium with 5% sucrose for the first selection. Colonies growing on the sucrose plates were streaked onto plates of Mg/L medium and M gimm/L medium plus 25 μg/ml gentamicin for the second selection. The deletion mutant cannot grow on Mg/L medium containing 25 μg/ml gentamicin. Putative mutations were verified by sequencing the junction fragment generated by the deletion using PCR fragments that spanned the open reading frame selected for deletion. All experiments that involved a mutation of the pckA locus used an in-frame deletion mutation of the entire gene unless otherwise indicated.

Gene expression measurements. Expression of the pckA gene was measured as β-galactosidase activity using the pckA-lacZ fusion. The expression of the virG, virB, and virE genes was assayed by using the virG::lacZ, virB::lacZ, and virE::lacZ fusions, respectively (Table 1). For the assay of β-galactosidase, A. tumefaciens cells containing the appropriate fusions were grown in AB minimal liquid medium with shaking at 28°C overnight and then transferred to induction broth (4) at pH 5.5 or fresh AB minimal medium (pH 7.0) and incubated for 24 h. The β-galactosidase activity reported was assayed as described previously (28). All β-galactosidase activities represent an average of three independent determinations.

Virulence assay. Virulence assays were performed on Nicotiana tabacum leaf disks according to the method of Banta et al. (2). Briefly, A. tumefaciens cells were grown in liquid Mg/L medium to mid-log phase and harvested by centrifugation, and the cell concentration was adjusted to an optical density at 600 nm (OD600) of 0.4 to 0.5 with MS medium (29). The cells were cocultivated with 40 leaf squares of Nicotiana tabacum leaf disks for 24 h at 28°C with shaking (4). Escherichia coli DH5α cells were grown in LB medium at 37°C with shaking (34). The following antibiotics were used at the indicated concentrations (in μg/ml): for A. tumefaciens, carbenicillin (100), kanamycin (100), and gentamicin (50); for E. coli, carbenicillin (100), kanamycin (100), and gentamicin (10) (vir gene induction was measured in cells grown in induction broth as previously described (28)). The detergents sodium dodecyl sulfate (SDS), sodium decyloxycholate (DOC), and Sarkosyl were used at concentrations (in mg/ml) of 0.2, 2, and 2, respectively (7).

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Complementation. The plasmid pSY204 (24), containing the constitutive virG gene, which induces the vir regulon in the absence of AS and sugar and at a neutral pH, was electroporated into C58 containing the virB-lacZ or virE-lacZ construct.

Assay of PckA enzyme activity. Cell extracts were prepared from cells grown in 50 ml AB medium grown to the stationary phase. Cells were harvested by centrifugation in a Sorvall RC-58 centrifuge (10 min; 4°C; 10,000 × g) and then washed twice with cold 20 mM Tris-HCl buffer containing 1 mM MgCl2 (pH 7.4) and resuspended in the same buffer. The cells were sonicated on ice six times for...
RESULTS

Identification and characterization of the \textit{pckA} gene. The \textit{pckA} gene encodes the enzyme phosphoenolpyruvate (PEP) carboxykinase, which catalyzes the decarboxylation and phosphorylation of oxaloacetic acid to produce the glycolytic intermediate PEP. This enzyme is the first enzyme in gluconeogenesis. The gene, coding for a predicted protein of 537 amino acids, was initially identified in \textit{Agrobacterium} from the similarity of its nucleotide sequence to those of previously identified genes. The predicted protein encoded by the gene has 52.4\% identity to the \textit{E. coli} (26) and 78.3\% identity to the \textit{S. meliloti} \textit{PckA} proteins (30). The \textit{PckA} enzyme has the specific domain (IGGTYSYAGE-KKS; 190 to 202), which is required for its activity (30), and a phosphate-binding site (G--EG) could also be identified in residues 226 to 229. A BLAST search of the \textit{C58} genome did not reveal any other copies of this gene (39). Nutritional and biochemical data support the contention that this protein, identified as \textit{PckA} by a bioinformatics analysis, is the only protein in \textit{Agrobacterium} strain \textit{C58} with \textit{PckA} enzyme activity. This activity is not activated by calcium or cell growth to stationary phase (Table 2), which differs from what has been observed in \textit{E. coli} (26). Furthermore, crude extracts from cells with a deletion of the gene lack \textit{PckA} enzyme activity (Table 2). Cells with a mutation in the gene cannot grow on succinate as a sole carbon and energy source, presumably because they lack this key enzyme in gluconeogenesis and therefore cannot synthesize the sugars required for macromolecule synthesis. This suggests that an alternative pathway from oxaloacetate to PEP does not exist in \textit{A. tumefaciens} strain \textit{C58}. The genome sequence indicates that two putative malic enzymes that convert malate to pyruvate are encoded (atu1652 and atu3356), but no evidence for phosphoenolpyruvate synthase, the enzyme that synthesizes PEP from pyruvate, could be seen from a BLAST search of the genome.

The \textit{pckA} gene maps to the circular chromosome and is downstream of genes encoding a two-component system, \textit{chvG} and \textit{chvl} (7, 25, 39), but is transcribed in a direction opposite to that of the regulatory genes (Fig. 1). Downstream of the \textit{pckA} gene is a locus that by BLAST analysis codes for a conserved hypothetical protein and is transcribed in the same direction as the \textit{pckA} gene.

\textbf{Regulation of \textit{pckA} expression.} Since two-component regulatory systems often regulate genes that map nearby, we determined whether \textit{chvGI} regulates the expression of \textit{pckA}. Since a previous study (21) showed that this two-component system regulates many acid-inducible genes in \textit{Agrobacterium}, we first determined whether \textit{pckA} is acid inducible. To test this possibility, \textit{pckA} was fused with the reporter gene \textit{lacZ}; the \textit{pckA-lacZ} fusion was then introduced into strain \textit{C58}, and the \(\beta\)-galactosidase activity was assayed. As shown in Table 3, \textit{pckA-lacZ} expression increased 4.2-fold when the cells were grown in minimal medium at pH 5.5 compared with growth in the same medium at pH 7.0. Moreover, AS in the induction medium did not affect the expression of \textit{pckA} (data not shown). This observation was expected, since no \textit{vir} box, which is found in the promoter regions of \textit{AS}-induced genes, could be identified in the upstream region of the gene. These results indicate that \textit{pckA} is an acid-inducible gene.

To determine whether \textit{ChvGI} plays a role in the regulation of \textit{pckA}, the \textit{pckA-lacZ} fusion was introduced into a \textit{chvG} \textit{Tn-phoA} insertion mutant (7). We found that the expression of \textit{pckA} in this \textit{chvG} mutant was reduced about 90-fold compared to its expression in wild-type cells grown at pH 5.5 (2,468 versus 27) (Table 3). Moreover, the expression of \textit{pckA} in the \textit{chvG} mutant is the same in cells growing at pH 5.5 and pH 7.0 but is 10-fold lower than the expression in the wild-type strain growing at pH 7.0. It appears that \textit{chvG} is very important for the expression of \textit{pckA}.

In \textit{Sinorhizobium meliloti}, the expression of \textit{pckA} is modulated by the carbon source, and arabino is a stronger inducer of \textit{pckA} expression than glucose (30). To determine if the expression of \textit{pckA} in \textit{Agrobacterium} is also controlled by the carbon source, cells containing the \textit{pckA-lacZ} fusion were cultured in AB minimal medium at pH 7.0 with either glucose,

<table>
<thead>
<tr>
<th>Conditions</th>
<th>AB medium</th>
<th>+ 6 mM MgCl₂</th>
<th>+ 6 mM MgCl₂ and 6 mM CaCl₂</th>
<th>+ 1 mM MnCl₂</th>
<th>+ 1 mM MnCl₂ and 6 mM CaCl₂</th>
<th>Log phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sp act (nmol of ADP/mg of protein/min)</td>
<td>7.0 ± 0.3</td>
<td>7.6 ± 0.4</td>
<td>8.5 ± 0.4</td>
<td>7.2 ± 0.3</td>
<td>6.8 ± 0.3</td>
<td>6.7 ± 0.2</td>
</tr>
</tbody>
</table>

\(\textit{C58}\) and \(\textit{pckA} \textit{mutant}\)

\(^{a}\) \textit{A. tumefaciens} strains were cultured to stationary phase in AB minimal medium (pH 7.0) plus different compounds. The values in the table are means ± standard deviations from at least three independent experiments.

\[\begin{array}{|c|c|c|c|}
\hline
\text{Strain} & \text{\(\beta\)-Galactosidase activity} & \text{Change (fold)} \\
& \text{pH 5.5} & \text{pH 7.0} & \\
\hline
\text{C58 (wild type)} & 2,468 ± 145 & 583 ± 45 & 4.2 \\
\text{chvG mutant} & 27 ± 8 & 31 ± 9 & 0 \\
\hline
\end{array}\]

\(^{a}\) All strains were grown for 20 to 24 h in AB minimal medium at the pH indicated. The cultures were then assayed for \(\beta\)-galactosidase activity as described in Materials and Methods. The data represent the average of three independent experiments. Data are expressed in Miller units (28).
arabinose, glycerol, or sucrose as the sole carbon source. No difference in the expression of pckA was seen in cells grown on these different carbon sources (data not shown).

The pckA mutant is inhibited by an acidic environment. Since pckA is an acid-inducible gene, we next determined if a pckA deletion mutant was acid sensitive by monitoring the growth behavior of both the pckA mutant and its parent strain, C58 (Fig. 2). The pckA mutant grew more slowly than its parent at pH 7.0, and this growth defect was even more pronounced at pH 5.5. Furthermore, we grew the pckA mutant and wild-type C58 in the acidic medium and adjusted to the same OD and then plated serial dilutions of the bacteria on AB solid medium at pH 7.0; the number of pckA mutants growing on AB solid medium was threefold less than that of the wild-type C58 strain. These data suggest that the pckA mutant is indeed inhibited by acid. The fact that gene expression is acid inducible suggests that the gene plays a role in overcoming growth inhibition under acidic conditions. Therefore, it is not surprising that a strain lacking the gene would be acid sensitive.

Virulence of pckA mutant. To determine if the pckA locus is important in tumor formation, we inoculated tobacco leaf disks with the pckA deletion mutant, as well as the C58 strain and the C58 strain cured of its Ti plasmid (A136), as described in Materials and Methods. The results are shown in Fig. 3 (compare B and C). The pckA deletion mutant was significantly less virulent than the wild-type C58 strain.

Vir gene induction of mutant. The attenuated virulence of the pckA mutant may be due to the poor growth of the mutant in the acidic plant wound environment, which likely alters the overall physiology of the cell, and/or some other requirement for tumor formation. A readily testable possibility is that the mutation reduces the level of vir gene induction in some way. This seemed like a real possibility, since so many other mutations (ivr211, miaA, and chvD), and another gene in carbohydrate metabolism (38), also reduced vir gene induction. The plasmid pSM243cd containing the virB-lacZ translational fusion was introduced into the pckA deletion mutant and the wild-type C58 strain. As shown in Table 4, expression of the virB-lacZ fusion was reduced 84% in the pckA deletion mutant compared to the wild-type strain. We also introduced a virE-lacZ fusion on a plasmid (pSM358cd) into the same strain. Under optimal inducing conditions, the expression of virE-lacZ was reduced approximately the same amount, 80%. These results show that the expression of both virB and virE decreased in the pckA mutant to an extent that could likely explain its attenuated phenotype. To further explore this possibility, a virG-lacZ translational fusion, pWT160, was introduced into the pckA deletion mutant. In the presence of AS, the expression level of virG-lacZ decreased 88% compared with the wild type under inducing conditions (Table 4). This greatly reduced induction of the response regulator virG could certainly ac-

FIG. 2. Growth curve of ΔpckA in AB minimal medium. Log-phase cultures of C58, C58 with constitutive virG, ΔpckA, and ΔpckA with constitutive virG were diluted in AB minimal medium (top, pH 7.0; bottom, pH 5.5) with a starting OD_600 calculated to be 0.001. The cells were grown at 28°C with shaking. The OD_600 was measured at 2-h intervals over a 36-h period.

FIG. 3. Virulence assay of pckA mutant on tobacco leaf disks. A. tumefaciens cells were grown and inoculated onto tobacco leaf disks as described in Materials and Methods. Tumor formation on leaf disks (n = 40) is shown.

FIG. 4. A136 and C58 on AB solid medium. A136 and C58 were grown at 28°C (top, pH 7.0; bottom, pH 5.5) with a starting OD_600 calculated to be 0.001. The cells were grown at 28°C with shaking. The OD_600 was measured at 2-h intervals over a 36-h period.
TABLE 4. vir gene induction by AS and acidic pH in a pckA deletion mutant

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>CS8 pH 7.0</th>
<th>pH 5.5 + AS</th>
<th>ΔpckA pH 7.0</th>
<th>pH 5.5 + AS</th>
<th>ΔpckA with constitutive virG pH 7.0</th>
<th>pH 5.5 + AS</th>
</tr>
</thead>
<tbody>
<tr>
<td>virB::lacZ</td>
<td>3 ± 2</td>
<td>579 ± 15</td>
<td>5 ± 4</td>
<td>95 ± 11 (83.5%)</td>
<td>523 ± 56</td>
<td>592 ± 76</td>
</tr>
<tr>
<td>virE::lacZ</td>
<td>11 ± 2</td>
<td>629 ± 23</td>
<td>5 ± 3</td>
<td>126 ± 10 (80.0%)</td>
<td>579 ± 67</td>
<td>606 ± 45</td>
</tr>
<tr>
<td>virG::lacZ</td>
<td>7 ± 5</td>
<td>1,043 ± 25</td>
<td>21 ± 5</td>
<td>125 ± 15 (88%)</td>
<td>907 ± 134</td>
<td>1,278 ± 63</td>
</tr>
</tbody>
</table>

* A. tumefaciens strains were cultured in AB minimal medium (pH 7.0) or induction medium at pH 5.5 and assayed for β-galactosidase activity between 20 and 24 hours. The data are means ± standard errors of the mean calculated from three independent experiments. The percentages in parentheses represent the reduction in expression in the ΔpckA mutant relative to the C58 wild-type strain grown at the same pH. Data are expressed in Miller units (28).

Some role in the virulence of Agrobacterium. Furthermore, it appears to function upstream of the critically important two-component system VirAG, which responds to environmental signals and activates all vir genes on the Ti plasmid. Since acidic conditions represent one of the key environmental signals for a plant wound site, the identification and characterization of genes that respond to acid conditions is important to an overall understanding of the physiology of Agrobacterium, both as a ubiquitous inhabitant of soils, which often are acidic, and as a plant pathogen, which requires an acidic environment to activate the entire pathogenesis program. The importance of the chvGI regulatory system is further underscored by its importance in the interaction of other α-proteobacteria with their hosts. In S. meliloti, the synthesis of succinoglycan, which is required for the nodulation of alfalfa, is under the control of ExoS/ChvI, the homologs of ChvGI (8). In Brucella, BvrS/BvrR, the homolog of ChvGI, controls the synthesis of two outer membrane proteins, at least one of which is required for virulence (13).

The pckA locus falls into the class of acid-inducible genes under the control of ChvGI, which are important in virulence. Although only acid-inducible genes have been found thus far to be under the control of chvGI, not all such genes are regulated by chvGI. For example, the chromosomally encoded acvB locus, which is induced about threefold at pH 5.5 compared to pH 7.0 and which plays a role in virulence, is expressed to the same extent in a wild-type cell and a chvI mutant (P. Liu, unpublished observation). A purine biosynthesis gene, purB, is also acid inducible but is not under the control of chvGI (Y. Liang and P. Liu, unpublished observation).

The relationship between acid inducibility and growth inhibition of mutations at pH 5.5 is variable. A gene that is induced at pH 5.5 is likely to be especially important in a physiological process that occurs at that pH. This process might be related to the growth or survival of the bacteria in acidic soils or in the interaction with wounded plants in an acidic environment. In either case, a mutation in the gene would be magnified by a greater inhibitory effect on growth at the lower pH and perhaps a loss of virulence. This is the situation that has been observed. Thus, mutants of pckA and another chv gene, acvB, are inhibited in their growth at pH 5.5 much more than are their wild-type parents (M. Brodhagen, unpublished observation). It might be expected that a chv gene that plays a role in the physiology of Agrobacterium in the absence of a wounded plant would have several functions, only one of which is related to virulence. It would not be surprising for a mutation in a gene whose function is important for bacterial physiology under...
acidic conditions to result in a greatly reduced rate of growth. On the other hand, mutants of virG or virB grow as well as the wild-type strain at pH 5.5 and 7.0. A mutation in a vir gene dedicated solely to virulence would not be expected to exhibit general physiological changes manifested by a lower growth rate at pH 5.5.

At least two factors apparently play roles in the attenuation of virulence in pckA mutants. One may be related to the general physiological consequences of the mutation and the other to effects on a specific interaction with a host plant. The first relates to the inhibition of growth of the pckA mutant at pH 5.5, which approximates the acidic conditions at the wound site on a plant. Although wild-type Agrobacterium does not grow as well at pH 5.5 as it does at pH 7.0, the mutation in pckA amplifies this growth inhibition. This increased generation time certainly must alter the overall physiology of the cell significantly, which in turn might lead to reduced DNA transfer. However, a reduced growth rate does not always reduce virulence. Liu et al. (23) reported that a mutation in chvD resulted in an avirulent strain that grew slowly in a complex medium but could be complemented with a plasmid that restored virulence without affecting the generation time of the cells. In the present study, the constitutive virG locus significantly enhanced the virulence of the pckA mutant without significantly affecting its growth rate (Fig. 2 and 3).

The second, and probably more interesting, factor that plays a role in reduced virulence in the pckA mutant is the major reduction in the expression of vir under acidic conditions with AS. This reduction in vir expression, in turn, results in the reduced expression of all vir genes. Thus, the induction of virB and virE is reduced over 80%. The basis for this reduction is not at all clear, but it apparently relates to an early step in the signaling process. Since a constitutive virG gene can overcome the reduction in the expression of all three vir genes, the pckA gene in some way must be involved in the signaling cascade upstream of virG gene expression. Furthermore, since the restoration of vir gene expression is not accompanied by a complete restoration of virulence, an additional factor(s) must be involved in reducing virulence. One possibility, as already discussed, is the sensitivity of the pckA mutant to growth under acid conditions.

The reason(s) why a mutation in pckA reduces signal transduction so significantly is not at all clear. This enzyme is the first step in gluconeogenesis, and therefore, the synthesis of sugars would be reduced. Although sugars are critical for gene induction, especially in strain CS8, glucose was supplied, which should have provided the sugar needed for induction. Thus, the pckA mutant can be added to the list of chromosomal-gene mutants in Agrobacterium that are significantly reduced in vir gene induction. These include the chvD (23), ivr211 (27), and miaA mutants (12). In none of these cases is the basis for this reduction understood. We have observed recently that a mutation in citrate synthase also results in reduced vir gene induction (38). The question of whether the reductions in vir gene induction in the two mutations in carbohydrate metabolism share a common basis awaits further study.

One of the interesting features that these studies have revealed is the difference in regulation of pckA shown by Agrobacterium compared to all of the other prokaryotes studied thus far. There is no evidence for catabolite repression by glucose, sucrose, or glycerol in Agrobacterium, all of which are found in Rhizobium. Furthermore, in contrast to Rhizobium, there is no evidence from the lacZ fusion expression data in pckA that the enzyme is induced in the stationary phase of growth (data not shown). This induction in Rhizobium requires cyclic AMP (32), but there is no evidence for a cyclic AMP binding site in the promoter region of the pckA gene of Agrobacterium.

The biological significance of the divergent cation binding site is also uncertain. In E. coli, Ca\(^2+\) binds to this site and activates the enzyme allosterically (26). We could not demonstrate that Ca\(^2+\) activates the pckA enzyme in Agrobacterium. We conclude that the control of pckA differs in Agrobacterium and in E. coli. This may reflect the possibility that this enzyme plays somewhat different roles in Agrobacterium, Rhizobium, and E. coli. In all cases, PCKa is the first enzyme in gluconeogenesis, and all three organisms can synthesize sugars from succinate. However, in Agrobacterium, it likely plays an additional as-yet-undefined role, which may involve the interaction of Agrobacterium with its hosts.

Although we can add the pckA gene to the list of genes that are under the control of the ChvGI regulatory system, it is clear that additional genes remain to be identified. This conclusion is based on the phenotypic characterization of mutants of chvGI and the mutants known to be under its control. chvGI mutants grow poorly under acidic conditions, do not grow on a complex medium, and are inhibited in their growth by detergents; the last phenotype suggests a defect in the cell envelope. Some of the mutants under the control of ChvGI have these properties. The pckA mutant grows poorly under acidic conditions, and the aopB mutation involves a protein on the surface of the cell. However, whether it confers sensitivity to detergents has not been reported. Since the ChvGI regulatory system plays a crucial role in the acidic signaling process between plants and Agrobacterium, it is of considerable interest to identify all of the genes under its control. Probably the most direct approach is to identify genes that are up or down regulated in a chvI mutant, using microarray technology. Such experiments are under way.

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