Global Regulation by gidA in Pseudomonas syringae

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Analysis of two virulence mutants of Pseudomonas syringae B728a revealed that the Tn5 sites of insertion were within the gidA open reading frame (ORF). These mutations were pleiotropic, affecting diverse phenotypic traits, such as lipodepsipeptide (syringomycin and syringopeptin) antibiotic production, swarming, presence of fluorescent pigment, and virulence. Site-specific recombination of a disrupted gidA gene into the chromosome resulted in the same phenotypic pattern as transposon insertion. Mutant phenotypes were restored by the gidA ORF on a plasmid. The salA gene, a copy number suppressor of the syringomycin-deficient phenotype in gacS and gacA mutants, was also found to suppress the antibiotic-negative phenotypes of gidA mutants, suggesting that gidA might play some role in salA regulation. Reporter studies with chromosomal salA-lacZ translational fusions confirmed that salA reporter expression decreased approximately fivefold in a gidA mutant background, with a concurrent decrease in the expression of the syringomycin biosynthetic reporter fusion syrB-lacZ. Wild-type levels of reporter expression were restored by supplying an intact gidA gene on a plasmid. Often described as being involved in cell division, more recent evidence suggests a role for gidA in moderating translational fidelity, suggesting a mechanism by which global regulation might occur. The gidA gene is essentially universal in the domains Bacteria and Eucarya but has no counterparts in Archaea, probably reflecting specific differences in the translational machinery between the former and latter domains.

Chromosomal replication in Escherichia coli is initiated at a single site, oriC, and proceeds bidirectionally to the replication terminus at terC (25). Early experiments found that initiation at oriC could still be inhibited by rifampin after the point in the replication process that requires protein synthesis (19, 23), indicating that a transcriptional event might be involved. It seemed logical that such a transcriptional event would originate at a promoter proximal to oriC, and so the locale was analyzed for promoter activity (25). The two open reading frames (ORFs) flanking oriC were called mioC and gidA, whose name (glucose-inhibited division) derived from the observation that transposon insertions and deletions within the apparent coding region resulted in a cell elongation phenotype on rich medium supplemented with glucose (36). Data from experiments using a minichromosome model (i.e., oriC on a plasmid [24]) suggested that replication initiation at oriC could be regulated in an adversarial manner by the balance of transcriptional activity at the mioC and gidA promoters. Further evidence reinforcing the proposed role for gidA in replication was found in synchronous cell studies, where it was observed that transcription from the mioC promoter down-regulated before the onset of chromosome initiation, while activity from the gidA promoter fell off after the initiation events were accomplished (29, 35). These and other data were suggestive that transcription beginning from the gidA promoter was involved in the timing and coordination of chromosome initiation and was necessary for efficient chromosome replication.

The basic assumption underlying most of the experiments implicating gidA in the initiation of chromosome replication was that results generated using minichromosome plasmids could be extrapolated to an actual chromosome. While this experimental system produced a wealth of important data on chromosomal synthesis, there have been indications that many replication control requirements are not faithfully reflected in the minichromosome model (2, 4). This seems to be particularly true in the case of the transcriptional events implicated in initiation at oriC (4, 21). Thus, much of the data supporting a direct role for gidA in cell division appear to be either circumstantial or artifacts of the extrachromosomal locale used for the experiments. The gidA gene is widely distributed and highly conserved both in prokaryotes and eukaryotes, implying involvement in some fundamental process within the cell. While probably of a basic nature, the function would not necessarily be essential, as evidenced by the ability to make both insertion and deletion mutants in E. coli (36).

Pseudomonas syringae pv. syringae is the causal agent of bacterial brown spot, an economically significant disease of common snap bean (Phaseolus vulgaris). For some time now, our laboratory has been studying one strain of P. syringae, B728a, in an attempt to gather an overview of the bacterial genes involved in the pathogenic interaction with the host plant. Here we report that mutations in the gidA gene are causal for pleiotropic phenotypes that overlap with those associated with mutations in the two-component regulators gacS and gacA (12, 31, 37). We also demonstrate that gidA affects the level of β-galactosidase activity produced from reporter fusions of salA, a downstream regulator within the gac regulon (17), as well as syrB, an antibiotic synthesis gene regulated by salA. Our data suggest that gidA can act as a global regulator, consistent with the role in translational moderation found for this gene in results from other researchers (see Discussion).
TABLE 1. Bacterial strains and plasmids

| Strain or plasmid | Relevant genotype | Source or reference |
|-------------------|-------------------|--------------------|
| E. coli strain    |                   |                    |
| DH5α              | F’ ΔlacZ ΔM15 Δ(lacZYA-argF)U169 deoR recA1 endA1 hsdR17 (rK− mK+) phoA supE44 λ− thi-1 gyrA96 relA1 | Gibco-BRL |
| P. syringae pv. syringae strains |                   |                    |
| B728a             | RifR, causal agent of brown spot of bean | S. Hirano, University of Wisconsin-Madison |
| B7.41             | RifR, AmpR, salA1::Tn5-HoHo1, B728a derivative containing Tn5-HoHo1-generated SalA::β-galactosidase translational fusion | 17 |
| B7.132            | RifR, AmpR, synB132::Tn5-HoHo1, B728a derivative containing Tn5-HoHo1-generated synB::lacZ transcriptional fusion | 17 |
| BGACX             | RifR, KanR, gacA1::Kan, B728a derivative | 17 |
| KW2163            | RifR, KanR, B728a derivative containing the Tn5-generated gidA2163::Tn5 mutation | 32 |
| KW2803            | RifR KanR B728a derivative containing the Tn5-generated gidA2803::Tn5 mutation | 32 |
| BGIDA             | RifR KanR B728a derivative containing gidA1::Kan | This study |
| BGID.41           | RifR KanR AmpR B7.41 derivative containing gidA1::Kan | This study |
| BGID.132          | RifR KanR AmpR B7.132 derivative containing gidA1::Kan | This study |
| Plasmids          |                   |                    |
| pBluescript KS(+) | AmpR cloning vector | Stratagene |
| pRK2013           | KanR mobilization plasmid | 10 |
| pLAFR3            | TetR cosmid vector | 33 |
| pRK7813           | TetR cosmid vector | 13 |
| pLSG2             | TetR containing the 2-kb StuI-Smal gidA fragment subcloned into pLAFR3 | This study |
| pSSN1             | TetR, the salA gene of B728a in pLAFR3 | 17 |
| pSSN1             | TetR, pLAFR3 containing a β-galactosidase::SalA fusion | 17 |

MATERIALS AND METHODS

Bacterial strains, plasmids, and growth conditions. Strains used in this study are listed in Table 1. P. syringae strains BGIDA, BGID.41, and BGID.132 were constructed by recombinational gene replacement using a modified cycloserine enrichment procedure (see Results and reference 17). Triparental matings using the helper plasmid pRK2013 have been previously described (16). Plasmid pLSG2 contains the 2-kb StuI-Smal gidA fragment subcloned into pLAFR3 as a BamHI-HindIII fragment, using polylinker sites in a pBluescript KS(+) intermediate mediate construct. P. syringae strains were grown at either room temperature (broth) with shaking or at 28°C (plates). King’s medium B (15) was the standard growth medium. E. coli was grown at 37°C on LB broth or agar (26).

Molecular techniques. Standard protocols were followed for general molecular techniques (22). Enzymes were used according to vendor specifications. Chemiluminescent DNA hybridizations were performed using the enhanced chemiluminescence kit (Amersham). The DNA sequence was determined using Molecular techniques.

Phenotype assays. The plate assays for syringomycin and swarming have been described elsewhere (16, 17). Antibacterial (syringopeptin) activity was determined by inoculating P. syringae strains onto 0.3× M9 agar medium (7 mM KH2PO4, 13 mM NaH2PO4, 3 mM NaCl, 6 mM NH4Cl, 2 mM MgSO4, 1% w/ v glucose), incubating at 28°C for 3 days, and then spraying with a 1:10 dilution of an overnight Luria-Bertani culture of Bacillus megaterium grown at 37°C on M9 agar medium. The plate assays for syringomycin and swarming have been previously described (32). Sequence analysis of subclones made from cosmids containing the Tn5 insertions and flanking chromosomal DNA indicated that the two insertion sites mapped to closely placed but separate locations within the gidA gene (Fig. 1) by similarity to numerous other examples in the database. Limited sequence sampling of the area surrounding gidA suggested that the general organization of this region in P. syringae B728a is very similar to that of the analogous region of Pseudomonas putida (28). Polypeptides, including the gidA gene product, predicted from the DNA sequence of the region showed 80 to 90% identity to those from P. putida. The

RESULTS

Genetic manipulation of the gidA locus. The screening and isolation of KW2163 and KW2803 as random Tn5 transposon mutants affected for virulence and syringomycin production have been previously described (32). Sequence analysis of subclones made from cosmids containing the Tn5 insertions and flanking chromosomal DNA indicated that the two insertion sites mapped to closely placed but separate locations within the gidA gene (Fig. 1) by similarity to numerous other examples in the database. Limited sequence sampling of the area surrounding gidA suggested that the general organization of this region in P. syringae B728a is very similar to that of the analogous region of Pseudomonas putida (28). Polypeptides, including the gidA gene product, predicted from the DNA sequence of the region showed 80 to 90% identity to those from P. putida. The
region immediately upstream from gidA in B728a was AT rich and contained a number of potential “DnaA boxes” (25), indicating that it could be an origin of replication.

Wild-type gidA was isolated from a cosmid library prepared in the mobile vehicle pRK7813 from B728a genomic DNA. The library was mated into the gidA mutant KW2163 in triparental matings in grids on agar plates, and individual clones that restored syringomycin production in bioassays were selected. Southern hybridization analysis of the restoring cosmids demonstrated that they fell into two categories, a single cosmid that contained the gidA gene and cosmids that contained the gacS/gacA suppressor gene salA (17). The gidA gene was first subcloned from a cosmid as a 5-kb EcoRI fragment. This subclone restored syringomycin production to the Tn5 mutant compared to the same reporter in a wild-type background (data not shown). This was true using plasmid constructs of salA either with its own promoter (pSSE3) or salA with a presumably constitutive lac promoter (pSSN1) (17). Similar results were obtained for suppression of the antibacterial activity. The presence of salA did not suppress either the swarming or fluorescent pigmentation phenotypes of gidA mutations in B728a, as expected, since mutants disrupted in salA are not affected for these phenotypes (16; this work).

gidA-dependent salA-lacZ and syrB-lacZ reporter expression. The suppressor effect of salA on antibiotic production in gidA backgrounds implied that gidA mutations had some effect on salA expression. The site-specific mutagenic construct that was used to produce BGI DA was also used to mutate the gidA gene in strains containing chromosomal lacZ fusions to the salA gene and the salA-dependent syringomycin biosynthetic gene syrB (17). As shown in Table 2, gidA mutations had dramatic effects on both salA and syrB expression.

(i) Restoration of salA reporter expression by gidA. Expression of the salA-lacZ fusion was reduced over fourfold in a gidA mutant compared to the same reporter in a wild-type background. Reporter expression was returned to near-wild-type levels by the presence of the 2-kb gidA ORF on a plasmid.

(ii) Restoration of syrB reporter expression by gidA. In a similar fashion, expression of the salA-dependent gene syrB is also greatly reduced in a gidA mutant background, with reporter activity being restored by the gidA plasmid construct.

(iii) Effect of salA on salA reporter expression. Reporter activities of salA-lacZ fusions were enhanced in all backgrounds by the presence of plasmid-borne copies of the salA gene, consistent with the autoregulatory amplification of salA expression noted in earlier work (17). As was seen then, amplification of expression was greater when the plasmid-borne salA was under the control of its native promoter (i.e., pSSE3) than with a lac promoter (i.e., pSSN1). This effect was even more pronounced in the gidA mutant background, with pSSN1 suppression being only half of that of pSSE3 and much lower than unsuppressed wild-type expression (Table 2).
(iv) Effect of salA on syrB reporter expression. As expected, the expression of a chromosomal syrB-lacZ fusion was greatly increased by the presence of plasmid-borne salA in both wild-type and gidA mutant backgrounds. Suppression of the effects of gidA mutation on antibiotic production by salA is essentially confirmed by this part of the experiment, with expression of the biosynthetic reporter in a suppressed gidA mutant (Table 2) exceeding that seen with the same reporter in a wild-type background without suppression. This implies sufficient expression of biosynthetic genes to provide for antibiotic production. It might be noted that there is an intact chromosomal copy of the salA gene in the syrB reporter strains, and expression amplification originating at this locus may serve to explain the high reporter activities reported in this part of the table.

These results together appear to confirm that GidA can affect the expression of specific genes. However, the reporter studies do not address whether this regulation occurs directly or via GidA effects on some intervening regulator(s).

Comparative analysis of gidA-like genes in other organisms. It has been known for some time that genes for GidA-like proteins are widely distributed in nature. With the advent of modern genomics, however, it is now possible to examine this distribution more closely. A highly conserved version of GidA exists in nearly all of the searchable completed bacterial genomes as of October 2001, with the only exceptions being in the genus Mycobacterium (National Center for Biotechnology Information). The gene is also found in all sequenced eukaryotic genomes, including those of Homo sapiens, with a level of conservation that overlaps (40 to 45% identity relative to the predicted P. syringae protein) that found among eubacteria (Table 3). While virtually universal in the domains Bacteria and Eucarya, genes encoding a GidA-like protein are completely absent from any of the sequenced members of the domain Archaea (5; this work). Multiple Expectation Maximization for Motif Elicitation (3) motif analysis revealed at least a dozen motifs significantly conserved between members of the GidA protein family from seven different genera.

DISCUSSION

There appears to have existed a colloquial acceptance of gidA and, by extrapolation its gene product, as primary factors in chromosome replication that persisted even after the publication of work that undermined the basis for this functional assignment (see introduction and reference 4). Our initial review of the literature seemed to indicate that there was little doubt that we had disrupted a gene encoding a cell division protein in our mutants. However, at the same time that we were doing our genetic studies in P. syringae, other researchers were producing results that suggested a mechanism by which gidA might exert regulatory control over unlinked genes. In the first study (27), investigators examined temperature-sensitive
mutations that allowed an amber-suppressing strain of *E. coli* to be restored for its ability to grow at elevated temperatures by the wild-type version of the suppressor tRNA<sub>Leu</sub>. Their data suggested some sort of similarity of function for the gene products of *gidA* and *miaA*, a gene involved in tRNA modifications that stabilize codon-anticodon interactions. In a second study (6), data indicated that inactivation of either *gidA* or *mnmE* in *E. coli* greatly increased the occurrence of a 2-base frameshift during the translation of particular sites in mRNA. Since the *mnmE* gene product is known to be involved in the hypomodification of some tRNAs (7), a similar role was proposed for *gidA* by extension. The frameshifts occurred at specific message sites prone to slippage and pausing. Finally, the *gidA* gene product in *Saccharomyces cerevisiae* has been shown to be a mitochondrial protein (9). Mutations in the nuclear gene *MTO1* encoding this protein were pleiotropic on the expression of several mitochondrial genes. The authors suggested that these phenotypes resulted from a defect in translational optimization, possibly at the level of proofreading. Thus, the preponderance of present data suggest that *gidA* plays an important role in translation, suggesting that the global *gidA* regulation observed in this study occurs via a post-transcriptional route.

The profound conservation of GidA in nature indicates that it probably influences gene expression in a wide variety of organisms. Such regulation would exist on two levels, one being direct effects on the efficiency of translation of any particular gene product, as well as broader effects transmitted via the expression of regulators like *salA*. A dependence of situationally required regulons on the *gidA* gene product for efficient expression might explain why *gidA* appears to be an essential gene in some organisms (14). A role for GidA in the modification of some part of the cell’s translational machinery also may explain the striking absence of gene analogs in *Archaec*, since differences in the ribosomes and tRNAs have long been recognized as distinguishing characteristics of that domain. It is possible that the target for GidA modification simply does not exist in the domain *Archaec*.

The frequent localization of the *gidA* gene next to apparent replication origins was one reason why a role in cell division was so appealing for this gene. This gene placement acquires a different significance when viewed from the perspective of GidA involvement in global gene regulation, since it could hypothetically link expression of genes in the *gidA* regulon directly to the cell cycle. Replication activity at the origin by factors such as DnaA might be effectively transduced to other cellular processes by their effects on *gidA* expression. Further coordination of the GidA regulon with the cellular state could be accomplished by the previously demonstrated (34, 35) stringent regulation of the *gidA* gene. Consistent with this, frameshifting of the type associated with *gidA* mutations has been shown to increase during stationary phase (6), when expression of *gidA* would be down-regulated. Ironically, a primary role for GidA in global expression does not preclude functions in chromosome initiation or cell division or indeed in virtually any other cellular process, since genes whose expression are facilitated by GidA may themselves be involved in these processes.

It is important that, while the *gacSigA* two-component system shares some phenotypic overlap with *gidA*, our evidence suggests that they represent largely independent regulatory pathways. For example, extracellular protease production in *B728a* requires intact *gac* genes and has been shown to be separate from antibiotic production within the regulon (17, 18). The production of this protease is unaffected in *gidA* mutants (32). In a similar fashion, loss of pyoverdin production is not a phenotype of *gacS* and *gacA* mutations in *B728a*. It seems unlikely that *gacSigA* and *gidA* sufficiently moderate the other’s expression to fully explain their regulatory role. Thus, the *gac* two-component system and the *gidA* posttranscripational system appear to be separate regulons, and *salA* is a member of both.

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### TABLE 2. Effect of *gidA* on expression of reporter fusions within various genetic backgrounds

| Operation | Strain | Fluorescence (SE) |
|-----------|--------|-------------------|
| Restoration of *salA* reporter expression by *gidA* | B7.41(pLAFR3) | 899 (+13) |
| Restoration of *syB* reporter expression by *gidA* | BGID.41(pLAFR3) | 214 (+30) |
| | BGID.41(pLSG2) | 860 (+10) |
| Effect of *salA* on *salA* reporter expression | B7.41(pSSE3) | 2,177 (+328) |
| | BGID.41(pSSN1) | 1,866 (+490) |
| | BGID.41(pSSE3) | 732 (+113) |
| | BGID.41(pSSN1) | 342 (+52) |
| Effect of *salA* on *syB* reporter expression | B7.132(pSSE3) | 10,388 (+1,265) |
| | B7.132(pSSN1) | 7,741 (+466) |
| | BGID.132(pSSE3) | 1,062 (+158) |
| | BGID.132(pSSN1) | 1,609 (+121) |

* FU per microgram of protein.

### TABLE 3. Similarity of GidA proteins in nature relative to the *P. syringae* B728a GidA protein

| Organism | Identity (%) | Similarity (%) |
|----------|--------------|----------------|
| *E. coli* | 69.7 | 77.0 |
| *Bacillus subtilis* | 50.7 | 60.8 |
| *S. cerevisiae* | 45.8 | 54.9 |
| *Arabidopsis thaliana* | 48.0 | 58.3 |
| *Caenorhabditis elegans* | 41.3 | 51.1 |
| *Drosophila melanogaster* | 48.5 | 59.4 |
| *H. sapiens* | 46.8 | 57.3 |

* Comparisons to the predicted *P. syringae* B728a GidA were performed individually using the Genetics Computer Group BEST-FIT algorithm.
