JOURNAL OF BACTERIOLOGY, May 2006, p. 3415–3419 Vol. 188, No. 9
0021-9193/06/$08.00+0 doi:10.1128/JB.188.9.3415–3419.2006
Copyright © 2006, American Society for Microbiology. All Rights Reserved.

Functional Characterization of *Escherichia coli* GlpG and Additional Rhomboid Proteins Using an *aarA* Mutant of *Providencia stuartii*

Katy M. Clemmer,[1] Gwen M. Sturgill,[3] Alexander Veenstra,[3] and Philip N. Rather[1,2]*

Laboratories of Microbial Pathogenesis, Atlanta VA Medical Center, Atlanta, Georgia[1]; Department of Microbiology and Immunology, Emory University School of Medicine, Atlanta, Georgia[2]; and Department of Molecular Biology and Microbiology, Case Western Reserve University School of Medicine, Cleveland, Ohio[3]

Received 23 November 2005/Accepted 16 February 2006

The *Providencia stuartii* AarA protein is a member of the rhomboid family of intramembrane serine proteases and required for the production of an extracellular signaling molecule that regulates cellular functions including peptidoglycan acetylation, methionine transport, and cysteine biosynthesis. Additional *aarA*-dependent phenotypes include (i) loss of an extracellular yellow pigment, (ii) inability to grow on MacConkey agar, and (iii) abnormal cell division. Since these phenotypes are easily assayed, the *P. stuartii aarA* mutant serves as a useful host system to investigate rhomboid function. The *Escherichia coli* GlpG protein was shown to be functionally similar to AarA and rescued the above *aarA*-dependent phenotypes in *P. stuartii*. GlpG proteins containing single alanine substitutions at the highly conserved catalytic triad of asparagine (N154A), serine (S201A), or histidine (H254A) residues were nonfunctional. The *P. stuartii aarA* mutant was also used as a biosensor to demonstrate that proteins from a variety of diverse sources exhibited rhomboid activity. In an effort to further investigate the role of a rhomboid protein in cell physiology, a *glpG* mutant of *E. coli* was constructed. In phenotype microarray experiments, the *glpG* mutant exhibited a slight increase in resistance to the β-lactam antibiotic cefotaxime.

In the gram-negative pathogen *Providencia stuartii*, the AarA protein is a member of the rhomboid family of intramembrane serine proteases that are widely distributed in prokaryotes and eukaryotes (6, 7, 13, 15, 19, 20). AarA is required for the production of an extracellular signaling molecule that activates gene expression in a manner that may involve quorum sensing (4, 5, 12, 13–15). The first rhomboid protein, rhomboid-1, was identified in *Drosophila melanogaster* as a locus required for pattern formation in the ventral ectoderm. The active site serine of rhomboid-1 is within the membrane bilayer and allows rhomboid-1 to carry out regulated intramembrane proteolysis (1, 19). The rhomboid-1-mediated cleavage of the membrane protein Spitz, Gurken, or Keren releases a cleavage product that serves as a ligand for the epidermal growth factor receptor (19, 20). Rhomboid proteins can recognize substrates via helix-breaking residues in the transmembrane domain or by recognition of sequences in the cytoplasmic domain (9, 21).

Studies have demonstrated that the rhomboid-1 protein of *Drosophila* and the AarA protein of *P. stuartii* can functionally substitute for each other (7). Proteins related to AarA/rhomboid are widespread in bacteria, suggesting that important functions may be dependent on these serine proteases (7, 20). Homology comparisons with AarA suggest that the *Escherichia coli* GlpG protein is a member of the rhomboid family. Despite this similarity, the chromosomal context of these genes is quite different. The *glpG* gene is part of the *glpEGR* operon (17, 23). In contrast, *aarA* appears to be monocistronic, with the surrounding genes having no similarity to *glpEGR* (13). The *glpE* gene encodes a sulfur transferase that transfers sulfane from thiosulfate to cyanide and dithiols (16). The *glpR* gene encodes a repressor that regulates various members of the *glp* regulon in the absence of glycerol (17). With respect to GlpG, the native substrates and its role in *E. coli* are unknown (10, 23). Expression of GlpG in eukaryotic CHO cells resulted in cleavage of the rhomboid substrates Spitz, Gurken, and Keren (20), and purified GlpG exhibited proteolytic activity in vitro (8, 10, 22). Recent studies in *E. coli* have shown that GlpG can cleave an artificial substrate composed of a periplasmic β-lactamase fused to a transmembrane region from LacY (10).

The GlpG protein of *E. coli* can functionally replace AarA in *P. stuartii*. To determine whether GlpG or another *E. coli* protein could function as a rhomboid protein and substitute for AarA, the *P. stuartii* strain XD37.A (*cma37*::*lacZ* Δ*aarA*) was electroporated with an *E. coli* (PB103) genomic library of 2- to 5-kb partial Sau3A fragments in pET21a that was obtained from P. deBoer (Case Western Reserve University). Plasmids that complemented the *aarA* mutation were identified as colonies with restored production of an extracellular yellow pigment. One class of inserts contained overlapping fragments of the *glpG* region of the chromosome. One plasmid, pLibG, was used for further studies and contained a 3.8-kb insert with the entire *glpG* gene. A library of random Tn*7Cm* insertions in pLibG was constructed in vitro using a Tn*7Cm* transposon contained in the GPS-LS Linker Scanning System (New England Biolabs). A pool of random insertions was prepared in *E. coli*, and the resulting plasmid DNA was used to transform *P. stuartii* XD37.A (Δ*aarA*) by electroporation. Plasmids containing Tn*7Cm* insertions that inhibited the ability of pLibG to complement the *aarA* allele in *P. stuartii* XD37.A (Δ*aarA*) were identified as transformants that failed to produce pigment. Three noncomplementing plasmids were isolated,

* Corresponding author. Mailing address: Department of Microbiology and Immunology, Emory University School of Medicine, 3001 Rollins Research Center, Atlanta, GA 30322. Phone: (404) 728-5079. Fax: (404) 728-7780. E-mail: prather@emory.edu.

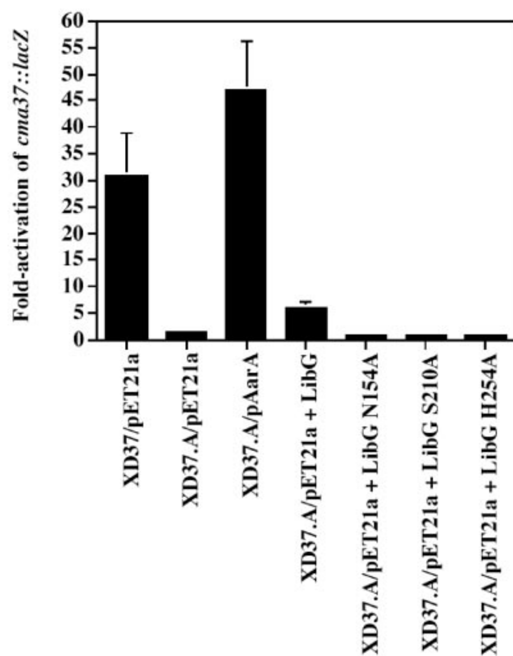

FIG. 1. Ability of GlpG and various mutants to restore signal production to a *P. stuartii aarA* mutant. Conditioned medium was prepared at an optical density of A_{600} = 1.0 from *P. stuartii* strain XD37 wild type and XD37.A (Δ*aarA*) containing pET21a derivatives encoding the wild-type GlpG protein or various GlpG mutant proteins with single alanine substitutions. Each preparation of conditioned medium was tested for activation of the *cma37*::*lacZ* fusion in *P. stuartii* cells at a low density (A_{600} = 0.35) as described previously (15, 18). Values represent the activation (*n*-fold) by conditioned medium from GlpG-containing strains relative to the values obtained with conditioned medium from XD37.A containing the vector control pET21a. Values were calculated from quadruplicate samples obtained from two independent experiments.

and DNA sequence analysis using primers that read outward from the end of Tn*7Cm* indicated that the transposon had inserted into the *glpG* coding sequence in all three plasmids.

The ability of *glpG* to complement several additional *aarA*-dependent phenotypes was investigated. First, the production of extracellular activating signal was tested using an *aarA*-dependent *cma37*::*lacZ* reporter gene fusion (Fig. 1). Conditioned medium was prepared from *P. stuartii* strain XD37.A containing pLibG or from XD37.A containing the vector control pET21a. These plasmids are unstable in the absence of selection, and LB broth was supplemented with ampicillin at 100 μg/ml to maintain pET21a and pLibG. The conditioned medium prepared from *P. stuartii* XD37.A/pLibG exhibited significant production of extracellular signal with a sixfold activation of the *cma37*::*lacZ* reporter gene fusion, compared to the activation observed with *P. stuartii* XD37.A/pET21a containing only the vector. For comparison purposes, conditioned medium prepared from wild-type *P. stuartii* XD37/pET21a under the same conditions activated the *cma37*::*lacZ* reporter gene fusion 31-fold and XD37.A Δ*aarA* containing the *aarA* gene in a high copy number activated the *cma37*::*lacZ* fusion 47-fold (Fig. 1). This difference in conditioned medium activity may result from less efficient rhomboid activity of GlpG in *P. stuartii* relative to the native AarA protein. The ability of the *E. coli glpG* gene to rescue additional phenotypes that resulted from the loss of *aarA* in *P. stuartii* was also examined. The chain-forming phenotype of *P. stuartii* XD37.A was rescued by the *glpG* gene, as was pigment production and the ability of *P. stuartii* XD37.A to grow on MacConkey medium (Table 1).

Mutagenesis of *glpG*. The rhomboid family of proteins contains three highly conserved residues that are proposed to form a catalytic triad for protease activity (19). In GlpG, these residues are Asn154, Ser201, and His254. Single alanine substitutions were made at these residues in the GlpG protein using plasmid pLibG as a template and the QuikChange mutagenesis system (Stratagene, La Jolla, CA). DNA sequence analysis of the entire *glpG* gene confirmed that only the desired change was present in each mutant.

Each of the *glpG* mutant constructs containing N154A, S201A, and H254A was unable to restore signal production to the *aarA* mutant based on activation of the *cma37*::*lacZ* reporter gene fusion (Fig. 1). Each of the mutant *glpG* genes was also unable to rescue the cell division defect, growth on MacConkey plates, and the lack of pigment production in *aarA* mutant *P. stuartii* (Table 1).

Use of a *P. stuartii aarA* mutant to test rhomboid activity from diverse organisms. Previous studies have demonstrated that expression of various prokaryotic rhomboids in eukaryotic CHO cells resulted in cleavage of the *Drosophila* rhomboid substrates Spitz, Gurken, and Keren (20). However, the ability of these rhomboid proteins to function in a prokaryote has not been tested. The use of the *P. stuartii aarA* mutant provides a powerful screening approach to identify proteins from other bacteria that have rhomboid-like activity. Rhomboid genes from *Pseudomonas aeruginosa* (NP251776), *Bacillus subtilis* (NP390367), *Aquifex aeolicus* (NP213910), *Methanococcus jannaschii* (NP247593), *Pyrococcus horikoshii* (NP143361), *Streptococcus pyogenes* (NP268586), and RHBDL2, a human rhomboid (NM017821), were introduced into *P. stuartii* XD37.A (Δ*aarA*) and tested for rhomboid activity by restored production of the AarA-dependent extracellular activating signal. Table 2 shows the ability of conditioned medium from *P. stuartii* XD37.A containing various plasmid-encoded rhomboids to activate the *cma37*::*lacZ* reporter gene fusion. All the proteins examined exhibited various degrees of rhomboid activity based on restoration of *aarA* mutant phenotypes, with the *P. aeruginosa* rhomboid exhibiting the strongest activity based on the 14-fold activation of the *cma37*::*lacZ* reporter gene fusion

TABLE 1. *E. coli glpG* can complement an *aarA* mutation in *P. stuartii*

Strain	Pigment production[a]	Growth on MacConkey agar[b]	Cell morphology[c]
XD37 wild type	+++	+	Rod
XD37.A Δ*aarA*	−	−	Chain
XD37.A/pET21a	−	−	Chain
XD37.A/pLibG (GlpG wild type)	++	+	Rod
XD37.A/pLibG (GlpG-NA)	−	−	Chain
XD37.A/pLibG (GlpG-SA)	−	−	Chain
XD37.A/pLibG (GlpG-HA)	−	−	Chain

[a] Assayed on LB agar plates. +++, wild-type pigment level; ++, moderate pigment level; −, no visible pigment.
[b] +, normal growth; −, no growth.
[c] Determined by phase-contrast microscopy.

TABLE 2. Rhomboid activity from diverse organisms

Rhomboid source[a]	Pigment production[b]	Growth on MacConkey agar[c]	Fold activation[d]
Pseudomonas aeruginosa	+++	+	14
Bacillus subtilis	+	+/−	6
Human	+	−	5
Aquifex aeolicus	+	−	3
Methanococcus jannaschii	+/−	−	3
Pyrococcus horikoshii	+/−	−	3
Streptococcus pyogenes	+	−	3

[a] With the exception of *S. pyogenes*, all rhomboid genes were present in the vector pcDNA3.1 (Invitrogen) and are described in reference 20. The *S. pyogenes* rhomboid was amplified by PCR and cloned under control of the *lac* promoter in pBC.SK (Stratagene).

[b] Assayed on LB agar plates. +++, wild-type pigment level; +, moderate pigment; +/−, weakly visible pigment; −, no pigment.

[c] +, normal growth; +/−, inability to form single colonies when restreaked; −, no growth.

[d] Determined by the expression of *cma37*::*lacZ* with conditioned medium from XD37.A Δ*aarA* with various rhomboids divided by the expression with XD37.A Δ*aarA*-conditioned medium containing the control vector without insert. Values represent the averages of duplicate experiments with four independent data points.

(Table 2). Next, the rhomboid protein YqgP (GluP) from *Bacillus subtilis* in XD37.A restored signal production, as evidenced by the sixfold activation of *cma37*::*lacZ*. Interestingly, the human rhomboid RHBDL2 exhibited significant activity, with a fivefold activation of *cma37*::*lacZ* by conditioned medium from this strain. The ability of these rhomboid proteins to restore the other AarA-dependent phenotypes, such as loss of pigment and growth on MacConkey agar, was also examined. Pigment production was restored to various degrees and correlated well with rhomboid activity based on extracellular signal production (Table 2). However, the rescue of growth on MacConkey agar was observed only with rhomboid proteins from *P. aeruginosa* and *B. subtilis*, both of which appeared to have the strongest activity based on restoration of signal activity and pigment production (Table 2).

Phenotype microarray analysis of a *glpG* mutant. To address the role of *glpG* in *E. coli*, the PCR-mediated allelic replacement procedure of Datsenko and Wanner was used to construct a *glpG*::*cat* allele that disrupted *glpG* at position 67 of the 276-amino-acid protein (3). This insertion point was chosen because it was known to inactivate function based on Tn*7* insertions that disrupted *glpG* function on a plasmid, and it was upstream of the two promoters within *glpG* that transcribe the downstream *glpR* gene and would not be polar (23). The resulting PCR fragment was used to electroporate *E. coli* MG1655/pKD46 carrying genes for an arabinose-inducible, lambda *red* recombination system (3). Transformants were selected on LB medium containing chloramphenicol (25 μg/ml). The *glpG*::*cat* allele was verified by PCR using the *glpG* ORFmers (Sigma-Genosys) and also by Southern blot analysis.

The role of GlpG in *E. coli* was investigated by the use of phenotype MicroArrays (Biolog, Hayward, CA). These experiments were conducted by Michael Zimon at Biolog. A comprehensive set of 20 plates was used to test metabolic differences with respect to growth under a variety of conditions and sensitivities to a variety of compounds. The only parameter that was verified by our lab as consistently different in the *glpG* mutant was an increased resistance to cefotaxime, a β-lactam antibiotic. These results were followed up by testing the levels of resistance by Kirby-Bauer disk diffusion assays according to CLSI (formerly NCCLS) guidelines (2). These experiments were repeated in triplicate with all experiments exhibiting the pattern of resistance. The cefotaxime zone diameter for wild-type *E. coli* MG1655 was 32.5 ± 0.7 mm, and for the *glpG*::*cat* mutant it was 30.5 ± 0.7 mm. The zone diameters for ampicillin and ceftriaxone (additional β-lactams) and for the structurally unrelated antibiotics ciprofloxacin (a fluoroquinolone) and amikacin (an aminoglycoside) were the same for both wild-type *E. coli* MG1655 and the *glpG* mutant.

We could find no additional phenotypes resulting from the *glpG*::*cat* mutation with respect to colony morphology, growth at 30 to 42°C, and growth on minimal medium with either glucose or glycerol.

***glpG* is not required for extracellular signal production in *E. coli*.** In a previous study, the ability of *E. coli* to produce a factor biologically similar to that of *P. stuartii* was demonstrated by the ability of conditioned medium from *E. coli* to activate the *aarA*-dependent *cma37*::*lacZ* fusion in *P. stuartii* (18). Signal production was examined in conditioned medium prepared from six cultures each from an independent *glpG*::*cat* mutant and six colonies of wild-type MG1655. The degree of *cma37*::*lacZ* activation in the *P. stuartii* biosensor varied from 18- to 29-fold with wild-type MG1655, with an average of 24-fold (±4-fold). For the *glpG*::*cat* mutants, the degree of activation ranged from 15- to 39-fold (average, 27-fold ± 10-fold). The basis for the high variability is unknown. However, the *glpG*::*cat* allele does not appear to significantly alter signal production. Moreover, the frequencies of transduction of the *glpG*::*cat* allele into *E. coli* MG1655/pET21a or MG1655/pLibG were similar, and these transductants exhibited the same variability in signal production (data not shown).

To rule out the possibility that the inability to detect a role for *glpG* in signal production was due to its lack of expression under laboratory growth conditions, we performed Northern blot analysis of *glpG* mRNA in cells at mid-log phase in LB-only medium and LB supplemented with 0.4% glycerol (Fig. 2). The *glpG* mRNA was clearly detectable, and the levels were similar in LB with and without glycerol. The accumulation of *glpG* mRNA was also examined in M9 salts containing glucose (0.2%) or glycerol (0.4%). The levels of *glpG* mRNA were similar under each condition, although the absolute levels were lower than in cells grown in LB (Fig. 2). As a control, the levels of mRNA for the housekeeping gene *secD* did not significantly vary under the growth conditions tested (Fig. 2).

The effect of glycerol on signal activity in conditioned medium was examined from cells grown in LB containing either glucose (0.2%) or glycerol (0.4%). For these experiments, conditioned medium was harvested at an optical density at 600 nm (OD_{600}) of 1.0. In addition, cells grown in conditioned medium were harvested at an OD_{600} of 0.4, a point where the optimal response to the extracellular signal has been observed previously (18). The activation of *cma37*::*lacZ* by conditioned medium prepared from LB plus 0.4% glycerol was 1.3-fold (±0.03-fold) above the levels seen in LB-only-grown cells (data not shown). The addition of glucose had no effect. To rule out the possibility that residual glycerol from conditioned medium stimulated expression, 0.4% glycerol was added to LB-only

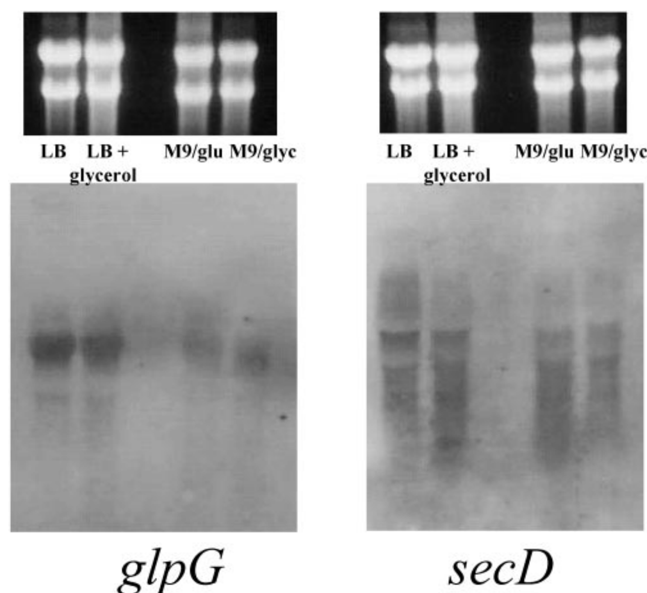

FIG. 2. Accumulation of *glpG* mRNA under various growth conditions. Cells of MG1655 were grown to mid-log phase (OD_{600} = 0.6) under the following growth conditions: LB, LB plus 0.4% glycerol, M9 plus 0.2% glucose, and M9 plus 0.4% glycerol. Total RNA was harvested using the MasterPure RNA purification kit, electrophoresed in 1.2% formaldehyde agarose gels, and transferred to a nylon membrane by capillary action. Probes were generated by PCR using the ORFmer primers from Sigma-Genosys and contained the full-length copy of *glpG* or *secD.* DNAs were labeled with digoxigenin for the Northern blot analysis. The top panel represents the ethidium bromide-stained gel that was used for the respective Northern blots.

medium and β-galactosidase expression from *cma37*::*lacZ* was examined in cells grown to an OD_{600} of 0.4. There was no effect of glycerol on *cma37*::*lacZ* expression (data not shown).

Concluding remarks. The results from this study provide further evidence that GlpG in *E. coli* functions as a rhomboid-like protein. First, GlpG was functionally similar to AarA, a protein previously shown to function as a rhomboid-like serine protease (7, 20). GlpG was capable of complementing the phenotypes in *aarA* mutants of *P. stuartii* including (i) loss of production of an extracellular activating signal, (ii) defective cell division, (iii) loss of an extracellular yellow pigment, and (iv) growth on MacConkey agar. Rhomboid proteins contain conserved asparagine, serine, and histidine residues that comprise a catalytic triad for serine proteases. Single alanine substitutions at these residues in GlpG completely abolished function based on complementation of the above phenotypes (Table 1 and Fig. 1). This result differs from that reported by Meagawa at al., where GlpG containing an N254A substitution still exhibited activity (10). This difference may reflect the use of different substrates and organisms. For example, in our study, GlpG function was addressed in *P. stuartii*, not in *E. coli.* Interestingly, in eukaryotic CHO cells, GlpG N254A was also nonfunctional (20); however, the purified GlpG N254A was active in vitro (8, 10).

In *E. coli*, a chromosomal *glpG*::*cat* null allele resulted in only one detectable phenotype, a slightly increased resistance to cefotaxime. Presently, there are only two examples where a function for the rhomboid family of proteins has been identified in prokaryotes. In addition to the AarA-dependent phenotypes in *P. stuartii* (13, 15), the GluP (formerly YqgP) protein in *B. subtilis* is required for normal cell division and glucose export (11). Additional studies to identify functions for GlpG in *E. coli* under conditions that differ from those of typical laboratory growth are under way. Interestingly, in addition to GlpG, clinical *E. coli* isolates contain a second protein (examples include accession numbers ZP00704902 in *E. coli* HS and NP752671 in *E. coli* CFT073) with features that strongly suggest rhomboid activity. These features include a conserved GASG active site embedded within a membrane bilayer and conserved triad residues asparagine and histidine at positions similar to those of other rhomboids. This protein is missing in K-12 isolates, and its presence in these clinical isolates may indicate a role in virulence.

In summary, this study demonstrates the utility of the *P. sturtii aarA* mutant as a biosensor strain to assess rhomboid activity and conduct structure-function studies. This strain will be useful in identifying or verifying new rhomboids from both prokaryotic and eukaryotic genomes. For example, genomic libraries can be introduced into a *P. stuartii* XD37.A Δ*aarA cma37*::*lacZ* strain and plasmids encoding strong rhomboid activity can be directly selected by growth on MacConkey plates. Plasmids encoding rhomboids with weaker activity can be identified by restored pigment production. In addition, inhibitors of rhomboid activity can be identified using the wild-type *P. stuartii* strain XD37 *cma37*::*lacZ* by sensitivity to growth on MacConkey agar, inhibition of pigment production, or decreased extracellular signal production. Specific inhibitors of rhomboid proteins from other sources can be addressed by placing the respective genes in *P. stuartii* XD37.A Δ*aarA cma37*::*lacZ* and then screening for inhibition based on the above phenotypes.

We thank Matthew Freeman for the gift of rhomboid genes from various organisms. The technical assistance of Julian Wong is acknowledged.

This work was supported by a National Science Foundation award, MCB 0406047, to P.N.R.

REFERENCES

1. **Brown, M. S., J. Ye, R. B. Rawson, and J. L. Goldstein.** 2000. Regulated intramembrane proteolysis: a control mechanism conserved from bacteria to humans. Cell **100:**391–398.
2. **CLSI.** 2005. Performance standards for antimicrobial susceptibility testing. Fifteenth informational supplement 25, p. 34. CLSI, Wayne, Pa.
3. **Datsenko, K. A., and B. L. Wanner.** 2000. One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. Proc. Natl. Acad. Sci. USA **97:**6640–6645.
4. **Dunny, G. M., and B. A. Leonard.** 1997. Cell-cell communication in gram-positive bacteria. Annu. Rev. Microbiol. **51:**527–564.
5. **Fuqua, C., S. C. Winans, and E. P. Greenberg.** 1996. Census and consensus in bacterial ecosystems: the LuxR-LuxI family of quorum sensing transcriptional regulators. Annu. Rev. Microbiol. **50:**727–751.
6. **Gallio, M., and P. Kylsten.** 2001. *Providencia* may help find a function for a novel, widespread protein family. Curr. Biol. **10:**693–694.
7. **Gallio, M., G. Sturgill, P. Rather, and P. Kylsten.** 2002. A conserved mechanism for extracellular signaling in eukaryotes and prokaryotes. Proc. Natl. Acad. Sci. USA **99:**12208–12213.
8. **Lemberg, M. K., J. Menendez, A. Misik, M. Garcia, C. M. Koth, and M. Freeman.** 2005. Mechanism of intramembrane proteolysis investigated with purified rhomboid proteases. EMBO J. **24:**464–472.
9. **Lohi, O., S. Urban, and M. Freeman.** 2004. Diverse substrate recognition mechanisms for rhomboids; thrombomodulin is cleaved by mammalian rhomboids. Curr. Biol. **14:**236–241.
10. **Maegawa, S., K. Ito, and Y. Akiyama.** 2005. Proteolytic action of GlpG, a rhomboid protease in the *Escherichia coli* cytoplasmic membrane. Biochemistry **44:**13543–13552.
11. **Mesak, L. R., F. M. Mesak, and M. K. Dahl.** 2004. Expression of a novel gene, *gluP*, is essential for normal *Bacillus subtilis* cell division and contributes to glucose export. BMC Microbiol. **4:**1–16.

12. **Miller, M. B., and B. L. Bassler.** 2001. Quorum sensing in bacteria. Annu. Rev. Microbiol. **55:**165–199.
13. **Rather, P. N., and E. Orosz.** 1994. Characterization of *aarA*, a pleiotrophic negative regulator of the 2′-*N*-acetyltransferase in *Providencia stuartii*. J. Bacteriol. **176:**5140–5144.
14. **Rather, P. N., M. M. Parojcic, and M. R. Paradise.** 1997. An extracellular factor regulating expression of the chromosomal aminoglycoside 2′-*N*-acetyltransferase of *Providencia stuartii*. Antimicrob. Agents Chemother. **41:**1749–1754.
15. **Rather, P. N., X. Ding, R. R. Baca-DeLancey, and S. Siddiqui.** 1999. *Providencia stuartii* genes activated by cell-cell signaling and identification of a gene required for the production or activity of an extracellular factor. J. Bacteriol. **181:**7185–7191.
16. **Ray, W. K., G. Zeng, M. B. Potters, A. M. Mansuri, and T. J. Larson.** 2000. Characterization of a 12-kilodalton rhodanese encoded by *glpE* of *Escherichia coli* and its interaction with thioredoxin. J. Bacteriol. **182:**2277–2284.
17. **Schweizer, H., W. Boos, and T. J. Larson.** 1985. Repressor for the *sn*-glycerol-3-phosphate regulon of *Escherichi coli* K-12: cloning of the *glpR* gene and identification of its product. J. Bacteriol. **161:**563–566.
18. **Sturgill, G., C. M. Toutain, J. Komperda, G. A. O'Toole, and P. N. Rather.** 2004. Role of CysE in production of an extracellular signaling molecule in *Providencia stuartii* and *Escherichia coli:* loss of *cysE* enhances biofilm formation in *Escherichia coli*. J. Bacteriol. **186:**7610–7617.
19. **Urban, S., J. R. Lee, and M. Freeman.** 2001. *Drosophila* Rhomboid-1 defines a family of putative intramembrane serine proteases. Cell **107:**173–182.
20. **Urban, S., D. Schlieper, and M. Freeman.** 2002. Conservation of intramembrane proteolytic activity and substrate specificity in prokaryotic and eukaryotic rhomboids. Curr. Biol. **12:**1507–1512.
21. **Urban, S., and M. Freeman.** 2003. Substrate specificity of rhomboid intramembrane proteases is governed by helix-breaking residues in the substrate transmembrane domain. Mol. Cell **11:**1425–1434.
22. **Urban, S., and M. S. Wolfe.** 2005. Reconstitution of intramembrane proteolysis in vitro reveals that pure rhomboid is sufficient for catalysis and specificity. Proc. Natl. Acad. Sci. USA **102:**1883–1888.
23. **Yang, B., and T. J. Larson.** 1998. Multiple promoters are responsible for transcription of the *glpEGR* operon of *Escherichia coli* K-12. Biochim. Biophys. Acta **1396:**114–126.