Transcriptional Regulation of the 4-Amino-4-deoxy-l-arabinose Biosynthetic Genes in Yersinia pestis

Received for publication, December 9, 2004, and in revised form, January 28, 2005 Published, JBC Papers in Press, February 14, 2005, DOI 10.1074/jbc.M413900200

Mollie D. Winfield, Tammy Latifi, and Eduardo A. Groisman‡

From the Department of Molecular Microbiology, Howard Hughes Medical Institute, Washington University School of Medicine, St. Louis, Missouri 63110

Inducible membrane remodeling is an adaptive mechanism that enables Gram-negative bacteria to resist killing by cationic antimicrobial peptides and to avoid eliciting an immune response. Addition of 4-amino-4-deoxy-l-arabinose (4-aminoarabinose) moieties to the phosphate residues of the lipid A portion of the lipopolysaccharide decreases the net negative charge of the bacterial membrane resulting in protection from the cationic antimicrobial peptide polymyxin B. In Salmonella enterica serovar Typhimurium, the PmrA/PmrB two-component regulatory system governs resistance to polymyxin B by controlling transcription of the 4-aminoarabinose biosynthetic genes. Transcription of PmrA-activated genes is induced by Fe³⁺, which is sensed by PmrA cognate sensor PmrB, and by low Mg²⁺, in a mechanism that requires not only the PmrA and PmrB proteins but also the Mg²⁺-responsive PhoP/PhoQ system and the PhoP-activated PmrD protein, a post-translational activator of the PmrA protein. Surprisingly, Yersinia pestis can promote PhoP-dependent modification of its lipid A with 4-aminoarabinose despite lacking a PmrD protein. Here we report that Yersinia uses different promoters to transcribe the 4-aminoarabinose biosynthetic genes pbgP and ugd depending on the inducing signal. This is accomplished by the presence of distinct binding sites for the PmrA and PhoP proteins in the promoters of the pbgP and ugd genes. Our results demonstrate that closely related bacterial species may use disparate regulatory pathways to control genes encoding conserved proteins.

The lipopolysaccharide (LPS) is a major component of the outer membrane of Gram-negative bacteria (1, 2). It consists of a poly- or oligosaccharide region that is linked to a glycolipid molecule termed lipid A (3). The lipid A is a major determinant of the interaction between Gram-negative bacteria and the host innate immune system because: 1) it is the molecule responsible for activation of host cell receptors such as TLR4 (4, 5); and 2) the phosphate residues in lipid A provide the LPS with a negative charge that constitutes the target of cationic antimicrobial proteins and peptides (6, 7). Certain Gram-negative species have the ability to modify the lipid A, which enables them to resist killing by cationic antimicrobial compounds and to avoid eliciting an immune response (8–14).

The covalent modification of the phosphate residues in lipid A with 4-amino-4-deoxy-l-arabinose (4-aminoarabinose) confers resistance to the cationic peptide antibiotic polymyxin B (14–21). The enzymes mediating the biosynthesis of 4-aminoarabinose are encoded by the pbgP operon (also referred to as pmrHPIJKLM, Ref. 19 and arn, Ref. 22) and the ugd gene (19, 23, 24). In Salmonella enterica serovar Typhimurium, expression of these genes is controlled by the PmrA/PmrB two-component system (25). Transcription of PmrA-activated genes is induced by Fe³⁺, which is the signal sensed by the PmrA cognate sensor PmrB (26), and by low Mg²⁺, in a mechanism that requires not only the PmrA and PmrB proteins but also the Mg²⁺-responsive PhoP/PhoQ system and the PhoP-activated PmrD protein, a post-translational activator of the PmrA protein (27, 28) (Fig. 1A).

The PmrA protein binds to the pbgP and ugd promoters at regions that include the hexanucleotide repeat (C/T)TTAAT separated by 5 bp, which has been termed the PmrA box (29–32). The PmrA protein promotes transcription of the pbgP and ugd genes using the same start sites whether the inducing condition is Fe³⁺, which is a PhoP/PhoQ- and PmrD-independent process, or low Mg²⁺, which is dependent on both PhoP/PhoQ and PmrD (28). Thus, wild-type Salmonella expresses PmrA-regulated genes and is resistant to polymyxin B following growth in the presence of Fe³⁺ (26) and/or low Mg²⁺ (27), whereas pmrD and phoP mutants are resistant to polymyxin B if grown in the presence of Fe³⁺ but sensitive if the inducing condition is low Mg²⁺ (26, 27).

Salmonella modifies its lipid A with 4-aminoarabinose during infection of murine macrophages (33). This modification appears to be PhoP/PhoQ-dependent because it was detected only under PhoP-inducing conditions when bacteria were grown in defined media (33), and because expression of the ugd gene inside macrophages required a functional PhoP/PhoQ system even though ugd transcription can be promoted by other two-component systems independently of PhoP/PhoQ (34). Escherichia coli cannot modify its lipid A with 4-aminoarabinose in response to the low Mg²⁺ signal that induces the PhoP/PhoQ system (33, 35) because its highly divergent PmrD protein fails to activate the PmrA protein, which prevents E. coli from expressing PmrA-dependent genes in low Mg²⁺ (36).

The plague agent Yersinia pestis can promote PhoP-dependent modification of its LPS with 4-aminoarabinose (14). This is surprising because Yersinia lacks a PmrD protein. Moreover, it indicates that Salmonella and Yersinia must use different strategies to promote expression of lipid A modifying genes under PhoP-inducing conditions. Here we report the mechanism by which Y. pestis regulates expression of the 4-amino-
**Transcription of Lipid A-modifying Genes in Yersinia**

**Table I**

| Strain   | Description | Ref. |
|----------|-------------|------|
| KIM6    | Pgm~ *Pst* Lcr~ Fra~ (pmrT1, pPC1) | 38   |
| EG14325 | KIM6 ΔpbgP::CmR  | This work |
| EG14737 | KIM6 ΔpbgP::CmR  | This work |
| EG14738 | KIM6 ΔpbgP::CmR ΔpmrA::KnR | This work |
| EG14796 | KIM6 ΔpbgP::KnR  | This work |

**Plasmids**

| Plasmid | Description |
|---------|-------------|
| PKD3    | rep*F17 Kmp* FRT CmR FRT | 41   |
| pKD4    | rep*F17 Kmp* FRT KnR FRT | 41   |
| pKD46   | rep*F17 Kmp* Amp* pRpsAD y β expo | 41   |

Aribinose biosynthetic genes mediating resistance to polymyxin B. Our results demonstrate that closely related bacterial species adopt distinct regulatory strategies for the expression of conserved genes encoding structural proteins, and highlight the difficulty of deducing bacterial behavior solely on the basis of gene content.

**MATERIALS AND METHODS**

**Bacterial Strains and Growth Conditions**—Bacterial strains used in this study are listed in Table I. *Y. pestis* strains were derived from KIM6 (37, 38), and grown at the optimal growth temperature of 28 °C (39) in a modified defined medium (40), pH 7.0, supplemented with 0.1% casamino acids, 10 mM (g)-glucosamine, and 10 μM MgSO₄, 10 mM MgSO₄, 10 mM MgSO₄, 10 mM MgSO₄, +100 μM FeSO₄ as indicated. Ampicillin was used at 50 μg/ml, chloramphenicol at 25 μg/ml, and kanamycin at 50 μg/ml.

**Construction of Strains with Defined Deletions**—All mutant strains were constructed according to the one-step disruption protocol (41) using the following primer pairs: 3387 (5'-GCCCGCTCTTATAAGAAGATTTATCCAGAACTACGAGCTCATAATGACTCCTC-3') and 3388 (5'-ATCGCCGGCGATTTAAGAGGATCGTTCGACAGAGGATCGTTCCGACATCAGGCTCATAATGACTCCTCCTCAGT-3') and 3386 (5'-CGAAGGGGAAGGTTATATTATTATTTTTGAGCAGTATGTTAGTTAGTCGAGGTCTGTCCTC-3') for *phoP*. The *Y. pestis* *phoP* pmrA double mutant was constructed using the one-step method to delete the *pmrA* gene from the *phoP* mutant strain EG14738. The chromosomal structure of the mutated loci was verified both by PCR as described (41) and by Southern hybridization using probes specific to: (i) the antibiotic resistance genes and conserved genes encoding structural proteins, and (ii) the chromosomal deletions, and (ii) footprinting with the PhoP and PmrA protein and primers 5297, 5298 for footprinting with the PmrA protein. Primers 3383 and 5297 were labeled for the coding strand and primers 3384 and 5298 were labeled for the non-coding strand. The Salmonella PhoP and PmrA proteins were purified as described (29, 43). Binding reactions with the PhoP and PmrA proteins were carried out as follows. Proteins were incubated with 25 fmol of DNA probe in 100 μl of 2× Hepes (pH 7.9), 10 mM KCl, 20 μM EDTA, 500 μg/ml of bovine serum albumin, 20 μg/ml poly(dI-dC), and 2% glycerol for 20 min at room temperature. *DNAase I* (Invitrogen) (0.01 units), 100 μM CaCl₂, and 100 μM MgCl₂ were added and incubated for 3 min at room temperature. The reactions were stopped by the addition of phenol-chloroform, and the aqueous phase was precipitated. Samples were analyzed by electrophoresis on a 6% polyacrylamide, 7.5 M urea gel and compared with a Maxam-Gilbert A+G DNA ladder generated from the same DNA probe.

**PmrB Susceptibility Assay**—Strains were grown to logarhythmic phase in the defined medium described above containing either 10 μM MgSO₄, 10 μM MgSO₄, or 10 μM MgSO₄ + 100 μM FeSO₄, washed and incubated in the presence of 5.0 μg/ml polymyxin B at 28 °C for 1 h. Samples were serially diluted in phosphate-buffered saline, plated on BHI and incubated for 36–48 h at 28 °C for viability counts. Survival values were calculated by dividing the number of bacteria following treatment with polymyxin B relative to those incubated in the presence of PBS and then multiplied by 100. Assays were performed in triplicate.

**Identification of Putative Transcription Factor Binding Sites**—The promoter regions of the *pbgP* and *ugd* genes were examined manually, as well as using the Gps program (soar-tools.wustl.edu) to identify putative binding sites for the PhoP (44–46) and PmrA (29–32) proteins.

**RESULTS**

**Transcription of the Yersinia *pbgP* Gene Is Mediated By Distinct PhoP- and PmrA-dependent Promoters**—We searched the genomes of the three sequenced *Y. pestis* strains (37, 47) ([www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov)) but found no open reading frame with sequence similarity to the *Salmonella* PmrD protein. Because *Y. pestis* harbors a Mg²⁺-responsive PhoP/PhoQ system (48), can modify its lipid A with 4-aminoarabinose in a PhoP-dependent manner (14), and encodes a conserved PmrA/PmrB system (37, 47) ([www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov)), we reasoned that the low Mg²⁺ induction of the 4-aminoarabinose biosynthetic genes taking place in *Yersinia* must involve a mechanism different from the PmrD-dependent pathway described in *Salmonella* (27, 28) (Fig. 1A).

To determine the transcription start site of the *pbgP* operon in *Y. pestis*, we conducted S1 mapping experiments using RNA harvested from organisms grown under conditions known to modulate *pbgP* transcription in *Salmonella* (25–27). Because the *Yersinia* strains were unable to grow in the N-minimal media (49) used to analyze *pbgP* transcription in *Salmonella*, we measured transcription of *pbgP* in *Yersinia* grown in a *Yersinia*-specific defined media (40), which differs from the N-minimal media in its salt composition and carbon source. We identified two transcription start sites: an ORF-proximal site that respond to high Mg²⁺, and a condition that represses transcription in *Yersinia* (27, 50).

Deletion of the *pmrA* gene eliminated transcription from the distal but not from the proximal promoter (Fig. 2A). In contrast, inactivation of the *ugd* gene abolished transcription from the proximal but not from the distal promoter (Fig. 2A).
There was no transcription from either promoter in a phoP pmrA double mutant, regardless of the growth condition (Fig. 2A). These results demonstrate that Y. pestis harbors a Fe\(^{3+}\)/H\(^{11001}\)-responding PmrA/PmrB system, which is consistent with the presence of the Fe\(^{3+}\)/H\(^{11001}\)-binding motif in the putative periplasmic region of the PmrB protein (26). Moreover, they indicate that, unlike what happens in Salmonella, the PmrA and PhoP proteins use different promoters to transcribe the pbgP gene (Fig. 2B). Interestingly, pbgP transcription from the pmrA-dependent promoter was still present in a phoP mutant experiencing low Mg\(^{2+}\) (Fig. 2A), indicative that the PmrA protein is activated in this media, albeit at lower levels.

**FIG. 2.** Transcription of pbgP is PhoP- and PmrA-dependent in Y. pestis. A, S1 nuclease protection assay of RNAs extracted from bacteria grown at 28 °C in defined medium (40), pH 7.0, with 10 \(\mu\)M Mg\(SO_4\) \((L, -)\), 10 mM Mg\(SO_4\) \((H, -)\) or 10 \(\mu\)M Mg\(SO_4\) and 100 \(\mu\)M Fe\(SO_4\) \((L, +)\). Lane AG corresponds to the Maxam-Gilbert DNA ladder of the target sequence. The sequences spanning the two transcription start sites are shown, and the start sites are indicated with arrows. B, DNA sequence of the promoter region of the Yersinia pestis pbgP gene. The two transcription start sites are indicated in bold and with arrows, the sequence in blue indicates the PmrA box (29–32), the sequence in red indicates the PhoP box (44–46) and the putative −10 regions are underlined in black. Regions footprinted by the PmrA and PhoP proteins are underlined in blue and red, respectively. The first four amino acids of the pbgP ORF are indicated below the nucleotide sequence. C, DNase I footprinting analysis of the pbgP promoter performed with probes for the coding and non-coding strands (see “Materials and Methods”) and increasing amounts of PhoP protein (0, 25, 50, 100 pmol). D, DNase I footprinting analysis of the pbgP promoter performed with probes for the coding and non-coding strands (see “Materials and Methods”) and increasing amounts of PmrA protein (0, 5, 10, 20 pmol). Solid vertical lines correspond to regions of the pbgP promoter protected by the PhoP and PmrA proteins.
Correspond to regions of the "Methods") and increasing amounts of PmrA protein for the coding (0, 5, 10, 20 pmol) and non-coding (0, 5, 30, 60 pmol) strands.

The first four amino acids of the putative product mediates an earlier step in the biosynthesis of 4-aminodeoxyarabinose than those catalyzed by the enzymes encoded in the pbgP operon (51). Thus, we reasoned that, similar to Salmonella (25–27), Yersinia was likely to coordinate transcription of the ugd gene with that of the pbgP operon. Therefore, we conducted S1 mapping experiments to identify the ugd transcription start site(s) in Yersinia grown under different conditions. We identified two distinct transcription start sites: an ORF-distal site that was induced to maximal levels during growth in low Mg2+ and required a functional phoP gene (Fig. 3A), and an ORF proximal site that was maximally induced in response to low Mg2+ + Fe3+ and required a functional pmrA gene (Fig. 3A).

No ugd transcription was detected in the phoP pmrA double mutant (Fig. 3A). Weak expression was detected from both start sites in wild-type cells grown in high Mg2+ (Fig. 3A). These data indicate that the PhoP and PmrA proteins use distinct promoters to transcribe the Yersinia ugd gene. Moreover, they mimic the results described above for the pbgP promoter, indicative that Yersinia coordinates the expression of the pbgP operon and ugd gene.

The PhoP and PmrA Proteins Bind to the ugd Promoter—Consistent with the presence of distinct PhoP- and PmrA-dependent transcription start sites for the ugd gene (Fig. 3A), we identified PhoP and PmrA boxes in the Yersinia ugd promoter (Fig. 3B). DNase I footprinting carried out with the Salmonella PhoP and PmrA proteins demonstrated that the PhoP protein protected nucleotides –4 to –41 and –16 to –46 relative to the PhoP-dependent start site on the coding and non-coding strands, respectively (Fig. 3C), and that the PmrA protein protected nucleotides –16 to –34 and –14 to –54 relative to
the PhoP and PmrA proteins needed to control expression of the 4-aminoarabinose biosynthetic genes.

**DISCUSSION**

We have determined the mechanism responsible for transcription of the 4-aminoarabinose biosynthetic genes pbgP and ugd in *Y. pestis*, establishing a direct activation by both the PmrA and PhoP proteins (Fig. 1B). This allows *Yersinia* to express these lipid A-modifying determinants in response to two signals: Fe³⁺, which is likely sensed by the PmrB protein as in *Salmonella* (26), and low Mg²⁺, possibly sensed by the PhoQ protein. This model is supported by the following data: (i) transcription of the pbgP and ugd genes is initiated from two different promoters depending on the inducing condition: a PhoP-dependent promoter that is maximally induced during growth in low Mg²⁺, and a PmrA-dependent promoter that is maximally induced in the presence of Fe³⁺ (Figs. 2A and 3A); (ii) the pbgP and ugd promoter regions of the sequenced strains of *Y. pestis* and *Y. pseudotuberculosis* harbor sequences resembling PhoP and PmrA boxes (Fig. 5); and (iii) the *Salmonella* PhoP and PmrA proteins footprinted the pbgP and ugd promoter regions, protecting regions that include the PhoP and PmrA boxes, respectively (Figs. 2 and 3).

**Yersinia and Salmonella Utilize Different Regulatory Strategies to Control the Genes for 4-Aminoarabinose Biosynthesis—**

*Salmonella* relies on the shunt protein PmrD to convey the low Mg²⁺ signal that activates the PhoP/PhoQ system to the PmrA/PmrB system (26, 27) (Fig. 1A), whereas *Yersinia* PhoP protein activates the pbgP operon and ugd gene directly, using a different promoter from that utilized by the PmrA protein in response to Fe³⁺ (Fig. 1B). The *Salmonella* PmrD protein promotes the phosphorylated state of PmrA (28), which binds to its target promoters with higher affinity than unphosphorylated PmrA (29, 53) and results in global activation of the whole PmrA regulon (27). On the other hand, the *Yersinia* strategy demands that binding sites for the PhoP protein be present in the regulatory region of PmrA-regulated genes for expression to occur in low Mg²⁺. This raises the possibility that other genes regulated by the PmrA protein in *Salmonella* might harbor binding sites for both the PmrA and PhoP proteins in *Yersinia*, which would allow for expression in response to both low Mg²⁺ and Fe³⁺ signals. These results demonstrate that closely related bacterial species may respond to the same signals by using different regulatory mechanisms to control expression of conserved structural proteins.

**Role of the PmrA/PmrB and PhoP/PhoQ Two Component Systems in the Yersinia Life Style—**

Our results indicate that *Yersinia* harbors an Fe³⁺-responding PmrA/PmrB system because the PmrB protein has a Fe³⁺-binding motif in its predicted periplasmic region (26) and the PmrA protein directly promotes expression of the pbgP operon and ugd gene when the organism experiences Fe³⁺ (Fig. 2 and 3). The conservation of both the PmrB protein amino acid sequence and the binding sites for the PmrA protein in the promoter regions of the pbgP and ugd loci across the genus *Yersinia* (Fig. 5)
suggests all members of this genus experience environments denoted by Fe³⁺.

The Mg²⁺-responding PhoP protein was previously implicated in the regulation of modifications to the core polysaccharide (54) and lipid A (14) portions of the LPS of Y. pestis, which are important for reduction of the endotoxic properties of the LPS and for resistance to antimicrobial peptides. The conserved ability of Salmonella and Yersinia to promote expression of the 4-aminoarabinose biosynthetic genes in low Mg²⁺ suggests that these two pathogens may encounter similar low Mg²⁺ environments. One such environment could be the mammalian macrophage, where the Salmonella PhoP-activated genes are highly induced (12) and where 4-aminoarabinose modification of its lipid A takes place (33). Furthermore, the Mg²⁺-responding PhoP protein is required for replication of Y. pestis (48) and Y. pseudotuberculosis (55) inside macrophages and for virulence in mice, as reported for Salmonella (56, 57), suggesting that PhoP-dependent lipid A modification may be a common strategy used by these pathogens in their interactions with mammalian hosts. Alternatively or in addition, these modifications may be carried out by Y. pestis when in non-mammalian host environments such as the flea (14), or in soil.

Members of the genus Yersinia demonstrate resistance to antimicrobial peptides (14, 54, 58). We have shown that deletion of both the phoP and pmrA genes is necessary to mimic

**Fig. 5. Conservation of PhoP and PmrA boxes in the pbgP and ugd promoters across the genus Yersinia.** A, DNA sequence of the promoter region of the pbgP gene of Y. pestis CO92, Y. pestis Medievalis, Y. pseudotuberculosis and Y. enterocolitica. The sequence in blue indicates the putative PmrA boxes (29–32), the sequence in red indicates the putative PhoP boxes (44–46), and the putative −10 regions are underlined in black. The first four amino acids of the pbgP or ugd ORF are indicated below the nucleotide sequence.
the hypersensitivity of a strain lacking the \textit{phgB} gene when \textit{Yersinia} experiences low Mg\textsuperscript{2+} + Fe\textsuperscript{3+} (Fig. 4A). This likely reflects the fact that sufficient expression of the 4-aminoarabinose biosynthetic genes is attained by \textit{pmaR} and \textit{phgB} single promoters, respectively, for wild-type levels of resistance to polymyxin B. However, we suspect that there are natural environments where only \textit{PhoP} or \textit{PmrA} is active, resulting in LPS modification and resistance to antimicrobial peptides mediated by the either \textit{PhoP} or \textit{PmrA}-dependent control of the 4-aminoarabinose biosynthetic genes. Similar to what has been reported previously (14, 54), we observed that the \textit{phoP} mutant was more sensitive to polymyxin B than the wild-type strain when the inducing condition was low Mg\textsuperscript{2+} (Fig. 4B). Yet, the susceptibility levels were not the same, possibly reflecting differences in strain backgrounds, \textit{phoP} alleles, growth media, and assay conditions.

The modification of lipid A with 4-aminoarabinose taking place in low Mg\textsuperscript{2+} may also help \textit{Yersinia} and \textit{Salmonella} cope with low Mg\textsuperscript{2+} stress, possibly by making the Mg\textsuperscript{2+} present in the LPS available to other compartments in the bacterial cell (12, 18). Indeed, even organisms that lack the \textit{PmaAPmb} system, such as the insect pathogen \textit{Photorhabdus luminescens}, use the low Mg\textsuperscript{2+}-inducible \textit{PhoP} protein to control transcription of the \textit{phgB} gene directly (20).

The Need for Biochemical Verification of Bioinformatic Predictions—The acquisition and loss of genes is thought to be the major force driving the evolution of bacterial species (59). Therefore, attempts to determine the genetic basis for the diverse ecology of closely related organisms have focused on the identification of species-specific genes. This pursuit, which is greatly facilitated by the completion of increasing numbers of bacterial genome sequencing projects, has resulted in the identification of genes that are critical for the lifestyles of several organisms (60–63). This premise would have led to the prediction that \textit{Yersinia} should not be able to promote transcription of the \textit{phgB} and \textit{ugd} genes in low Mg\textsuperscript{2+} environments because it lacks the \textit{pmaR} gene, which is essential for this property in \textit{Salmonella} (27, 28). Likewise, techniques that rely on the conservation of \textit{cis} regulatory motifs across species, such as phylogenetic footprinting (64), would have predicted that the \textit{PhoP} protein controlled \textit{phgB} transcription given the absence of \textit{PhoP} boxes from the \textit{phgB} promoter of several species, such as \textit{E. coli} and \textit{Salmonella}. Ultimately, our work emphasizes the importance of biochemical and molecular verification of regulatory pathways as changes in \textit{cis} and \textit{trans} regulatory sequences may have profound effects on the behavior of a bacterial species.

Acknowledgments—We thank F. Solomon and S. Winkeler for technical assistance.

REFERENCES

1. Raetz, C. R. (1993) \textit{J. Bacteriol.} 175, 5745–5753
2. Brade, H., Opal, S. M., Vogel, S. N., and Morrison, D. C. (eds) (1999) \textit{Endotoxin in Health and Disease}, pp. 31–38, Marcel Dekker, Inc, New York.
3. Raetz, C. R., and Whitfield, C. (2002) \textit{Annu. Rev. Biochem.} 71, 635–700
4. Takeda, K., Kaisho, T., and Akira, S. (2003) \textit{Annu. Rev. Immunol.} 21, 335–376
5. Polorok, A., He, X., Smirnova, I., Liu, M., van Huffel, C., Du, X., Birdwell, D., Alarcon, F., Kolbe, R., Krajewska-Pietrasik, D., and Krajewski, J. A. (2004) J. Bacteriol. 186, 6796–6801
6. Ochman, H., Lawrence, J. G., and Groisman, E. A. (2000) \textit{Cell} 103, 113–125
7. Kox, L. F., Wosten, M. M., and Groisman, E. A. (2000) \textit{EMBO J.} 19, 1861–1872
8. Kato, A., and Grossman, E. A. (2001) \textit{Science} 293, 2502–2513
9. Wosten, M. M., and Groisman, E. A. (1999) \textit{J. Bacteriol.} 181, 2715–27190
10. Rebeil, R., Ernst, R. K., Gowen, B. B., Miller, S. I., and Hinnebusch, B. J. (2004) \textit{Microbiol. Mol. Biol. Rev.} 68, 1367–1373
11. Ernst, R. K., Guina, T., and Miller, S. I. (1999) \textit{FEMS Immunol. Molec. Biol.} 89, 329–341
12. Nummila, K., Kipelainen, I., Zahringer, W., Vaara, M., and Helander, I. M. (1999) \textit{Microbiol. Mol. Biol. Rev.} 63, 272–278
13. Groisman, E. A., Kayser, J., and Soncini, F. C. (1997) \textit{J. Bacteriol.} 179, 7040–7045
14. Gunz, J. S., Lim, K. B., Krueger, J., Kim, K., Guo, L., Hackett, M., and Miller, S. I. (1998) \textit{Microbiol. Mol. Biol. Rev.} 62, 1171–1182
15. Derzelle, S., Turlin, E., Duchaud, E., Pages, S., Kunst, F., Givaudan, A., and Danchin, A. (2004) \textit{J. Bacteriol.} 186, 1270–1279
16. Moskowitz, S. M., Ernst, R. K., and Miller, S. I. (2004) \textit{J. Bacteriol.} 186, 475–479
17. Trent, M. S., Ribeiro, A. A., Lin, S., Cotter, R. J., and Raetz, C. R. (2003) \textit{J. Biol. Chem.} 278, 42371–42379
18. Zhou, Z., Ribeiro, A. A., Lin, S., Cotter, R. J., Miller, S. I., and Raetz, C. R. (2003) \textit{J. Biol. Chem.} 278, 42455–42460
19. Mouslim, C., and Groisman, E. A. (2003) \textit{Microbiol. Mol. Biol. Rev.} 67, 335–344
20. Zhou, Z., Lin, S., Cotter, R. J., and Raetz, C. R. (1999) \textit{J. Bacteriol.} 181, 15033–15041
21. Winfield, M. D., and Groisman, E. A. (2004) \textit{Proc. Natl. Acad. Sci. U. S. A.} 101, 4405–4409
22. Mouslim, C., and Groisman, E. A. (2003) \textit{Microbiol. Mol. Biol. Rev.} 67, 423–438
23. Hoeijmakers, S. H., Nilles, M. L., Matson, J. S., halls, S.,..
Transcription of Lipid A-modifying Genes in Yersinia

61. Porwollik, S., Wong, R. M., and McClelland, M. (2002) Proc. Natl. Acad. Sci. U. S. A. 99, 8956–8961

62. Wei, J., Goldberg, M. B., Burland, V., Venkatesan, M. M., Deng, W., Fournier, G., Mayhew, G. P., Plunkett, G. I., Rose, D. J., Darling, A., Mau, B., Perna, N. T., Payne, S. M., Runyen-Janecky, L. J., Zhou, S., Schwartz, D. C., and Blattner, F. R. (2003) Infect. Immun. 71, 2775–2786

63. Zhou, D., Han, Y., Song, Y., Tong, Z., Wang, J., Guo, Z., Pei, D., Pang, X., Zhai, J., Li, M., Cui, B., Qi, Z., Jin, L., Dai, R., Du, Z., Bao, J., Zhang, X., Yu, J., Wang, J., Huang, P., and Yang, R. (2004) J. Bacteriol. 186, 5136–5146

64. McCue, L. A., Thompson, W., Carmack, C. S., and Lawrence, C. E. (2002) Genome Res. 12, 1523–1532