INFECTION AND IMMUNITY, Nov. 1990, p. 3706–3710
0019-9567/90/113706-05$02.00/0

Vol. 58, No. 11

Characterization of Defensin Resistance Phenotypes Associated with Mutations in the *phoP* Virulence Regulon of *Salmonella typhimurium*

SAMUEL I. MILLER,[1,2]* WENDY S. PULKKINEN,[1] MICHAEL E. SELSTED,[3] AND JOHN J. MEKALANOS[2]

Infectious Disease Unit, Massachusetts General Hospital, Boston, Massachusetts 02114[1]; Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Massachusetts 02115[2]; and Department of Pathology, University of California at Irvine, Irvine, California 92717[3]

Received 29 March 1990/Accepted 4 August 1990

The defensin sensitivities of *Salmonella typhimurium* strains with mutations in the *phoP/phoQ* two-component virulence regulon were tested by using purified defensins NP-1 and NP-2. Strains with mutations in either gene of the regulatory pair (*phoP* [transcriptional activator] or *phoQ* [membrane sensor kinase]) had increased sensitivities to defensin. The predicted periplasmic domain of the PhoQ protein contained a markedly anionic domain that could interact with cationic proteins and that could be responsible for resistance to defensin. Because insertion mutations in *phoP* are polar on *phoQ*, we constructed strains that expressed the PhoQ protein in the absence of PhoP to test whether resistance to defensin requires only the *phoQ* gene product. We found that resistance to defensin requires the function of both components of this regulatory system, because strains expressing PhoQ without PhoP were still markedly sensitive to defensins. This implied that a *pag* (*phoP*-activated gene) product is responsible for defensin resistance. We also tested for the ability of defensins NP-1, NP-5, and HNP-1 to activate *pag* expression and found that these peptides have no effect. Defensin resistance is not the only virulence characteristic controlled by the PhoP-PhoQ regulon because mutations in *pagC*, as well as ones in the *phoP* locus that resulted in constitutive *pag* activation (phenotype PhoP^c), had no effect on defensin resistance, even though they rendered the organism avirulent and deficient in survival within macrophages. The virulence defect conferred by mutations in the *phoP-phoQ* two-component regulatory system is not completely explained by alterations in resistance to cationic proteins and involves the control of other proteins necessary for *S. typhimurium* survival within macrophages.

Salmonella species are facultative intracellular pathogens that are capable of survival and persistence within mammalian host macrophages (2). The pathogenesis of typhoid fever, and possibly the diarrheal disease caused by these organisms, is related to this pathogenic characteristic (14, 16). *Salmonella typhimurium* is an intracellular pathogen for mice that has been used extensively as a model of typhoid fever (3, 15). Little is known about the molecular basis of *Salmonella* survival within macrophages, but genetic studies have documented a correlation between in vitro survival within macrophages and virulence defects in vivo (7). Recently, we, as well as other investigators, have documented that mutations in the *phoP* genetic locus render *S. typhimurium* avirulent and deficient in survival within macrophages (6, 9, 22). The *phoP* locus is a two-component transcriptional regulatory system that controls a number of unlinked genes (12, 22). One of these genes, termed *pagC* (*phoP*-activated gene C), is necessary for full in vivo virulence as well as survival within macrophages (22). Other *pag* genes, including *pagA*, *pagB*, *psiD*, and *phoN*, which encodes a periplasmic acid phosphatase, do not, as single mutations, have an effect on virulence (12, 22).

Fields et al. (6) observed that mutations in the *phoP* locus were 100- to 1,000-fold more sensitive to cationic proteins, including the rabbit defensin NP-1. Present in mammalian neutrophils and macrophages, defensins are small cationic peptides of 29 to 34 amino acids which possess wide antibacterial, antifungal, and antiviral activities (10, 18, 28–30, 33). The molecular basis of their action is unknown, but these peptides are widely present in many mammalian species and make up 30 to 50% of the phagocytic granule protein (33). The predicted amino acid sequence of the PhoQ protein contains a markedly basic amino acid region in the predicted periplasmic domain of the protein that might interact with defensins (22). Therefore, we tested the defensin sensitivities of our *phoP* locus mutant *Salmonella* strains in order to determine whether mutations in either *phoP* or *phoQ* conferred the same phenotype of defensin sensitivity. Since *phoP* and *phoQ* form an operon (22; S. Miller unpublished observations), we also tested whether the defensin sensitivity of *phoP* mutants was simply due to a polar transcriptional effect on *phoQ* or the absence of a gene(s) regulated by PhoP and PhoQ.

We also hypothesized that the PhoP-PhoQ system is a coordinate virulence regulator of *S. typhimurium* that is similar to those described for the ToxR protein of *Vibrio cholerae* (5, 20, 24, 26, 32) and the *bvg* locus of *Bordetella pertussis* (5, 13, 20, 34). These regulators control several genetically unlinked virulence factors, including toxins and colonization factors, by transcriptional *trans* activation in response to environmental stimuli. Previous work by Kier et al. (17), as well as work in our laboratory, has documented that starvation conditions as well as low pH activate genes that require PhoP for transcriptional activation. Because of the marked anionic periplasmic region of the PhoQ protein, we tested whether defensins can also serve as a signal to activate *phoP*-regulated genes. We also tested other strains with virulence defects related to the *phoP-phoQ* regulon for defensin resistance to determine whether other virulence

* Corresponding author.

TABLE 1. Properties of the bacterial strains and plasmids used in this study

Strain or plasmid	Description	Acid phosphatase[b]	Defensin resistance	Source or reference
Strain				
10428	Wild type	180	+	ATCC[a]; 22
CS009	*phoQ101*::MudJ	25	−	22
CS015	*phoP102*::Tn*10*d-Cam	<10	−	22
CS003	Δ*phoP* Δ*purB*	<10	−	22
CS053	*phoP103*::MudJ	<10	−	22
CS008	*phoN104*::Tn*10*d-Kan	<10	+	22
CS019	*phoN2zxx*::6251-Tn*10*d-Cam	<10	+	22
CS012	*pagA1*::MudJ		+	22
CS013	*pagB1*::MudJ		+	22
CS119	*pagC1*::Tn*phoA*		+	22
	phoN2zxx::6251-Tn*10*d-Cam		+	22
CS022	*pho-24*	1,720	+	23
CS024	CS003(pSM003)	205	+	22
CS025	CS003(pSM004)	190	+	This study
CS026	CS003(pSM005)	<10	−	This study
CS027	CS015(pSM004)	200	+	This study
CS028	CS009(pSM004)	225	+	This study
CS029	CS015(pSM005)	<10	−	This study
CS030	CS009(pSM005)	185	+	This study
Plasmid				
pSM003	*purB*$^+$ *phoP*$^+$ *phoQ*$^+$			
pSM004	*phoP*$^+$ *phoQ*$^+$			
pSM005	*phoQ*$^+$			

[a] ATCC, American Type Culture Collection, Rockville, Md.
[b] Units as defined by Miller (21).

factors besides those related to cationic protein resistance are controlled by this regulon.

MATERIALS AND METHODS

Bacterial strains and genetic methods. The bacterial strains used in this study are listed in Table 1 and were cultured in Luria broth for 16 h at 37°C. Subcultures were grown to mid-logarithmic-growth-phase density in Luria broth at 37°C. The cultures were washed and diluted in 0.9% saline and adjusted to contain the appropriate CFU per milliliter by measuring the optical density at 620 nm by spectrophotometry.

Molecular biology techniques. All enzymes were purchased from New England BioLabs, Beverly, Mass., and Bethesda Research Laboratories, Inc., Gaithersburg, Md. Transformation of *Salmonella* strains with plasmid DNA was done as described by MacLachlan and Sanderson (19). Conditions of enzymatic reactions were as described by the manufacturer.

Enzymatic assays. Acid phosphatase assays were done as described previously (17, 22) and were performed in 2-(*N*-morpholino)ethanesulfonic acid (MES) buffer at pH 5.5. β-Galactosidase assays were performed by the method of Miller (21). Assays for regulation of *pag* gene activity were all performed on strain CS013 containing the *pagB*::MudJ gene fusion. This strain was grown to the mid-logarithmic growth phase (optical density, 0.5), and then cells were washed and diluted into 1.0% tryptone. The cells were then incubated at 37°C under conditions which included 0.5% tryptone; defensins NP-1, NP-5, and HNP-1 at concentrations of 0.01 to 5 mg/ml with 0.5% tryptone; 0.1 M triethanolamine (TEA) buffer (pH 5.5) with 0.5% tryptone; and defensins, 0.5% tryptone, and TEA. At 15-, 30-, 45-, 90-, and 180-min intervals, cells were removed from the 37°C incubation and assayed for fusion protein activity. Controls were also performed to test the effects of TEA and defensins on fusion protein activity assays.

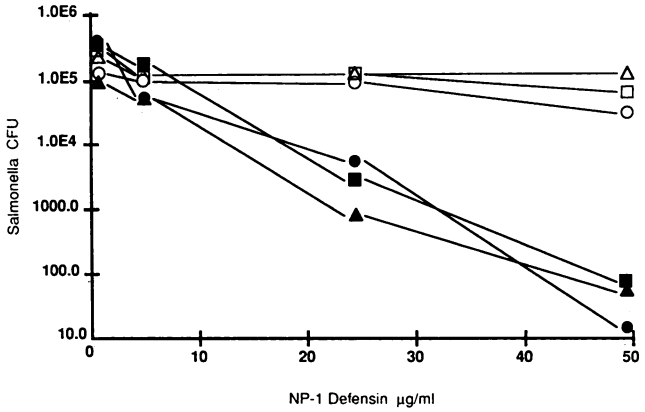

FIG. 1. Sensitivities of *Salmonella* strains with mutations in the *phoP* locus to defensins. The *y* axis is *Salmonella* CFU, and the *x* axis is defensin NP-1 concentration (in micrograms per milliliter). Symbols: △, wild-type strain 10428; □, CS022 the PhoP^c (constitutive mutant) strain; ○, strain CS030, a *phoQ* mutant complemented with a plasmid containing *phoQ*; ▲, strain CS009, a PhoQ mutant strain; ●, strain CS015, a PhoP mutant strain; ■, strain CS029, a PhoQ$^+$ PhoP$^-$ strain.

Defensins. NP-1, NP-2, and NP-5 were purified from rabbit peritoneal neutrophils as described previously (28, 30) and were homogeneous, as determined by polyacrylamide gel-acid urea electrophoresis and high-performance liquid chromatography. HNP-1 was purified from human granulocytes as described previously (10).

Bactericidal assay. Bacteria were assayed in 0.5% tryptone in a 100-μl volume (6, 30). Typically, 10^5 CFU was exposed to 1 to 50 μg of defensin per ml. The assays were performed at 37°C for 2 h, and then appropriate dilutions were plated in duplicate to determine the numbers of surviving bacteria. The reactions were stopped by diluting the reaction in 0.9% NaCl (30).

RESULTS

Both *phoP* and *phoQ* mutants are equally sensitive to defensins. We previously found (22) that the *phoP* locus is composed of two genes located in an operon (*phoP* and *phoQ*) and that transposon insertions in either gene result in reduced virulence. Therefore, we wished to test mutants with transposon insertions in these two genes to see whether sensitivity to defensins was characteristic of both *phoP* and *phoQ* insertions and to confirm the results of Fields et al. (6). The results in Fig. 1 document the fact that strains with either *phoP* or *phoQ* mutations are equally sensitive to the defensin NP-1. There was a marked difference in the sensitivities of these mutants compared with those of wild-type organisms. The defensin sensitivities of mutations in each gene product had a similar quantitative effect, with 25 to 50 μg/ml always showing a marked reduction in bacterial survival of at least 100-fold. Identical results (data not shown) were also obtained with the rabbit defensin NP-2 as well as with several other strains with distinct *phoP* and *phoQ* mutations (Table 1). This documented the fact that it is not the lack of synthesis of the *phoP* gene product alone that is

responsible for a defensin-sensitive phenotype and that the PhoQ protein is also essential for resistance to defensin.

Construction of a PhoQ$^+$ PhoP$^-$ strain of *S. typhimurium*. Since the *phoP* and *phoQ* genes form an operon, a polar effect of a transposon insertion in *phoP* would include loss of the *phoP* and *phoQ* gene products. Therefore, we wished to construct strains that expressed *phoQ* but not *phoP* to test whether sensitivity to defensins was simply due to the lack of the PhoQ protein. Therefore, we deleted the 406-base-pair *Eco*RV restriction endonuclease-generated DNA fragment of pSM004 to create plasmid pSM005. pSM004 is a derivative of pSM003 (Table 1) (22) and contains the entire *phoP* and *phoQ* genes and promoter. This plasmid (pSM004), when inserted in several *phoP* and *phoQ* mutant strains, complements acid phosphatase activity as well as the defensin resistance phenotype (Table 1 and Fig. 1). The deleted DNA fragment of pSM005 is within the *phoP* gene and encodes 135 amino acids of the 224-amino-acid PhoP protein. This deletion preserved 22 nucleotides upstream of the methionine start codon predicted for PhoQ translation and likely contained the necessary sequences for ribosomal binding and effective translation of PhoQ, but it was predicted to not make a functional PhoP protein. Plasmid DNA was digested with the restriction enzyme *Eco*RV, and after incubation at 65°C, to heat inactivate the enzyme, the plasmid was religated with T4 DNA ligase. This religated plasmid DNA was then transformed into strains CS015 and CS009, and transformants were screened for blue colony phenotype when plated on the chromogenic phosphatase substrate 5-bromo-4-chloro-3-indolyl phosphate (i.e., complementation of the phenotype of PhoP and PhoQ mutants). Plasmid DNA was then purified from populations of blue and white colonies, and this DNA was digested with the restriction enzyme *Eco*RV to determine whether a deletion was present. One of these plasmids, designated pSM005, contained the deletion in *phoP* described above, and this plasmid DNA, in fact, complemented *phoQ* mutant strains for acid phosphatase production, indicating that it allows the production of the PhoQ protein. This plasmid, as expected, did not complement the acid phosphatase defect of *phoP* mutant strains (Table 1). PhoP mutant strains containing pSM005 had a phenotype of PhoP$^-$ PhoQ$^+$.

Sensitivity of *phoP* mutant *S. typhimurium* to defensins is not simply due to the absence of the PhoQ protein. We then wished to test the sensitivity of the PhoQ$^+$ PhoP$^-$ *Salmonella* strain to defensins to determine whether sensitivity to defensins was related to the absence of the PhoQ protein per se or the loss of the regulatory function of the PhoP-PhoQ system. As shown in Fig. 1, this plasmid did not complement the sensitivity to defensins of strains with a transposon insertion or deletion in *phoP-phoQ*, even though identical strains containing a plasmid (pSM003, pSM004) with the entire intact *phoP-phoQ* operon were resistant to defensins.

Defensin sensitivity phenotype of *S. typhimurium* with mutations in *phoP*-activated genes. The data presented above demonstrated that the defensin resistance of *S. typhimurium* requires both PhoP and PhoQ regulatory proteins and suggested that a gene product regulated by the *phoP-phoQ* system is necessary for defensin resistance. We therefore tested strains with mutations in a number of genes that require *phoP-phoQ* for transcriptional activation. The data in Fig. 2 demonstrate that all of these strains were as resistant to defensins as wild-type organisms were, even though one strain contained a mutation in a *phoP*-regulated gene (*pagC*) that is necessary for full mouse virulence and survival within macrophages.

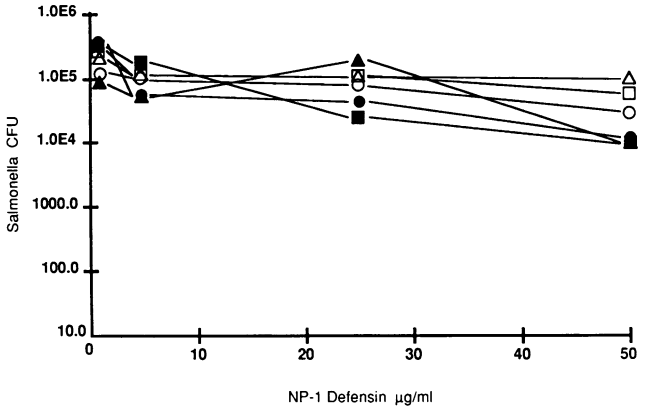

FIG. 2. Sensitivities of *Salmonella* strains with mutations in the *phoP*-regulated genes to defensins. The *y* axis is *Salmonella* CFU, and the *x* axis is defensin NP-1 concentration (in micrograms per milliliter). The strains are CS019 (●), 10428 (▲), CS008 (■), CS012 (○), CS013 (△), and CS119 (□).

Effect of defensins on *pag* gene activation. The deduced amino acid sequence of the PhoQ protein as well as alkaline phosphatase fusion protein data derived from Tn*phoA* mutagenesis strongly suggested that PhoQ has a periplasmic domain that may serve as a sensory transducer (22). This domain contains a markedly anionic region that could bind defensins and signal the activation of *pag* gene transcription. Therefore, we used an *S. typhimurium* strain containing a *phoP*-activated β-galactosidase transcriptional gene fusion (*pagB*) to measure transcriptional activation under different conditions with defensins. We observed no difference in fusion protein activity when defensins were added to cells growing at pH 7.4 or 5.5 (data not shown). We tested concentrations of NP-5 up to 50 μg/ml as well as concentrations up to 5 mg/ml for NP-1 and HNP-1 defensins. With 250 μg of NP-1 per ml and 500 μg of HNP-1 per ml, we began to see toxicity against these strains, despite the lower pH and higher numbers of organisms used in these assays. Results of control experiments indicated that the presence of TEA and the three defensins tested had no effect on fusion protein assays when they were added to cells immediately before the assay. Therefore, we concluded, within the limits of our ability to assay this phenomenon, that defensins probably do not act as a signal to activate *phoP*-regulated genes.

An attenuated *phoP* constitutive *Salmonella* mutant is also resistant to defensins. Recently, we observed that a mutation in the *phoP* locus yielding the phenotype termed PhoP^c can constitutively activate *pag* gene expression while repressing the synthesis of other protein species (23). The *phoP* constitutive strain CS022 was also found to be attenuated for virulence and survival within macrophages (23). We therefore tested this strain for its sensitivity to defensins to evaluate whether the protein species that are repressed in this strain are essential to defensin resistance. The strain with the PhoP^c phenotype also has normal sensitivity to defensins, even though it is deficient in virulence and survival within macrophages (Fig. 2). These data were also consistent with the supposition that a *phoP*-activated gene product is essential to defensin resistance.

DISCUSSION

Intracellular survival and growth within phagocytes is an essential feature of *Salmonella* pathogenesis. The ability to

withstand the hostile environment of the phagolysosome, where the organism persists despite the oxygen-independent and -dependent antimicrobial armamentarium of the phagocyte, is a pathogenic strategy of a number of human pathogens including *Yersinia* (31), *Mycobacterium* (1), and *Leishmania* (4) species, in addition to *Salmonella* species. The wide sensitivities of many organisms to phagocytic cationic granule proteins, defensins, and the abundance of these protein species in vacuoles suggest that these proteins are important to the host defense against microorganisms (33). It is logical that resistance to defensins should be required for organisms with the ability to survive intracellularly in phagocytes.

The coordinate regulation of virulence in a number of gram-negative pathogens has been shown to be transcriptionally controlled by proteins with domains that are similar to those of the PhoP (phosphorylated transcriptional activator) -PhoQ (sensor kinase) two-component regulatory family (6, 20, 27). The fact that not all strains with *phoP* regulon mutations, including those in *pagC*, with reduced survival within macrophages have reduced sensitivity to defensins is consistent with the complex array of known phagocyte antimicrobial factors (33) and suggests that the *phoP-phoQ* regulatory system positively activates factors involved in resistance to multiple host cell killing mechanisms.

The *phoP* and *phoQ* gene products are both required for defensin resistance, and this suggests that the sensor function of the PhoQ periplasmic domain and the transfer of this information to the activator protein (PhoP) by phosphorylation is essential to resistance to defensins. Given these results, it is logical, as was postulated by Fields et al. (7), that defensin resistance must involve gene products regulated by the transcriptional activator PhoP. We tested strains with mutations in several different *pag* genes, including one that is essential for full virulence (*pagC*), but no strain had defensin resistance different than that of wild-type organisms. However, a recent two-dimensional protein gel analysis indicated that more than 20 different protein species are likely *phoP*-activated gene products (23) and supported the supposition that one or several of these proteins, which remain to be characterized, are essential to defensin resistance.

We have previously postulated that the PhoQ protein may be involved in sensing or binding cationic proteins because of its marked anionic region (22). Results of our studies on *pag* activation by defensins, while limited by the toxicity of defensins and our ability to replicate the correct microenvironment that may occur intracellularly, suggest that this is not the case. However, it is still possible that a cationic protein that remains to be characterized, such as those encoded by mouse Paneth cell cryptdin genes (25), is the major signal for *pag* activation.

We have recently observed that the *phoP-phoQ* system, when fully activated by a constitutive *phoP* locus mutation that results in unregulated expression of *pag* genes, can repress the synthesis of some protein species (postulated to be encoded by genes termed *prg* [*phoP*-repressed genes]) (23). Strains with a *phoP* constitutive phenotype ($PhoP^c$) have marked virulence defects in vivo and are attenuated for survival within macrophages in vitro (23). The fact that strains with this mutation have normal resistance to defensins seems to rule out the possibility that *prg* gene products are involved in resistance to defensins.

When tested in vitro, defensins are most bactericidal at neutral pH. The phagosome is at neutral pH only for the period immediately after phagocytosis (11). Resistance to defensins seems likely to be most important to *S. typhimurium* before acidification of the phagosome occurs (18, 33). Recently, it has been observed that *phoP* locus mutants are more sensitive to killing by acid (pH 3.3) than wild-type *S. typhimurium* is (8). It seems likely that defensin resistance and acid sensitivity are related to the lack of synthesis of different *phoP*-related gene products since defensins are most active at neutral pH. It also appears that activation of *phoP*-regulated genes would be desirable both before phagolysosomal fusion (possibly for defensin resistance) as well as after acidification. However, our study did not address the possible bacteriostatic effects of defensins that could occur after phagolysosomal fusion at low pH but, rather, addressed the dramatic bactericidal effect noted in PhoP and PhoQ mutant strains of *Salmonella*. Therefore, given the complex environments and different signals for expression of *pag* genes, such as starvation and low pH, as well as the different phenotypes of *phoP* regulon mutants, it appears that the *phoP* regulon can modulate the gene products necessary to survive defensins or other factors active in multiple environments with different pHs.

It is also possible that resistance to defensins may be less important to *Salmonella* survival within macrophages than to survival from attacks by polymorphonuclear leukocytes and gut Paneth cells. The pathogenesis of the human diarrheal disease caused by *S. typhimurium* may be related to the ability to avoid killing by polymorphonuclear leukocytes because the pathology of this disease involves invasion of the bowel mucosa, polymorphonuclear leukocyte infiltration, and microabscess formation (14). The NP-1 and NP-2 defensins are derived from leukocyte granules, and these are the most cationic defensins that have been described (28) and the ones with the greatest activity against *phoP* mutant *Salmonella* strains. The predicted sequence and charge of Paneth cell cryptdin are most similar to NP-1 and NP-2 (25). The importance of defensin resistance to the virulence defect of *phoP-phoQ* mutants in mouse typhoid remains to be determined and will require the identification of *pag* gene products which, when deleted, confer increased defensin (cryptdin) sensitivity on *S. typhimurium*.

ACKNOWLEDGMENTS

This work was supported by Public Health Service grants AI-18045 and AI-26289 from the National Institute of Allergy and Infectious Diseases. S.I.M. was a fellow of the Medical Foundation when most of this work was performed and is a recipient of a Milton Fund Award from Harvard University.

LITERATURE CITED

1. **Armstrong, J. A., and P. D'Arcy Hart.** 1971. Response of cultured macrophages to *Mycobacterium tuberculosis* with observations on fusion of lysosomes with phagosomes. J. Exp. Med. **134**:713–740.
2. **Buchmeier, N. A., and F. Heffron.** 1989. Intracellular survival of wild-type *Salmonella typhimurium* and macrophage-sensitive mutants in diverse populations of macrophages. Infect. Immun. **57**:1–7.
3. **Carter, P. B., and F. M. Collins.** 1974. The route of enteric infection in normal mice. J. Exp. Med. **139**:1189–1203.
4. **Chang, K. P., and D. M. Dwyer.** 1976. Multiplication of a human parasite (*Leishmania donovani*) in phagolysosomes of hamster macrophages in vitro. Science **193**:678–680.
5. **Dirita, V. J., and J. J. Mekalanos.** 1989. Genetic regulation of bacterial virulence. Annu. Rev. Genet. **23**:455–482.
6. **Fields, P. I., E. A. Groisman, and F. Heffron.** 1989. A *Salmonella* locus that controls resistance to microbicidal proteins from phagocytic cells. Science **243**:1059–1062.
7. **Fields, P. I., R. V. Swanson, C. G. Haidairis, and F. Heffron.**

1986. Mutants of *Salmonella typhimurium* that cannot survive within the macrophage are avirulent. Proc. Natl. Acad. Sci. USA **83**:5189–5193.
8. **Foster, J. W., and H. K. Hall.** 1990. Adaptive acidification tolerance response of *Salmonella typhimurium*. J. Bacteriol. **172**:771–778.
9. **Galen, J. E., and R. Curtiss III.** 1989. Virulence and vaccine potential of *phoP* mutants of *Salmonella typhimurium*. Microb. Pathog. **6**:433–443.
10. **Ganz, T., M. E. Selsted, D. Szklarek, S. S. L. Harwig, K. Daher, D. F. Bainton, and R. I. Lehrer.** 1985. Defensins: natural peptide antibiotics of human neutrophils. J. Clin. Invest. **76**:1427–1435.
11. **Geisow, M. J., P. D'Arcy-Hart, and M. R. Young.** 1981. Temporal changes of lysosome and phagosome pH during phagolysosome formation in macrophages: studies by fluorescence spectroscopy. J. Cell Biol. **89**:645–652.
12. **Groisman, E. A., E. Chiao, C. J. Lipps, and F. Heffron.** 1989. *Salmonella typhimurium* virulence gene *phoP* is a transcriptional activator. Proc. Natl. Acad. Sci. USA **86**:7077–7081.
13. **Gross, R., B. Arico, and R. Rappuoli.** 1989. Genetics of pertussis toxin. Mol. Microbiol. **3**:119–124.
14. **Hook, E.** 1989. Salmonella species (including typhoid fever), p. 1700–1715. *In* G. Mandell (ed.), Principles and practice of infectious disease. Churchill Livingstone, Ltd., Edinburgh.
15. **Hormaeche, C. E.** 1979. Natural resistance to *Salmonella typhimurium* in different inbred mouse strains. Immunology **37**:311–318.
16. **Hornick, R. B., S. E. Greisman, T. E. Woodward, H. L. Dupont, A. T. Dawkins, and M. J. Snyder.** 1970. Typhoid fever: pathogenesis and immunological control. N. Engl. J. Med. **283**:686–691, 739–745.
17. **Kier, L. K., R. M. Weppleman, and B. N. Ames.** 1979. Regulation of nonspecific acid phosphatase in *Salmonella*: *phoN* and *phoP* genes. J. Bacteriol. **138**:155–161.
18. **Lehrer, R. I., M. E. Selsted, D. Szklarek, and J. Fleischmann.** 1983. Antibacterial activity of microbicidal cationic proteins 1 and 2, natural peptide antibiotics of rabbit lung macrophages. Infect. Immun. **42**:10–14.
19. **MacLachlan, P. R., and K. E. Sanderson.** 1985. Transformation of *Salmonella typhimurium* with plasmid DNA: differences between rough and smooth strains. J. Bacteriol. **169**:3945–3951.
20. **Miller, J. F., J. J. Mekalanos, and S. Falkow.** 1989. Coordinate regulation and sensory transduction in the control of bacterial virulence. Science **243**:916–922.
21. **Miller, J. H.** 1972. Experiments in molecular genetics, p. 352–355. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
22. **Miller, S. I., A. M. Kukral, and J. J. Mekalanos.** 1989. A two component regulatory system (*phoP* and *phoQ*) controls *Salmonella typhimurium* virulence. Proc. Natl. Acad. Sci. USA **86**:5054–5058.
23. **Miller, S. I., and J. J. Mekalanos.** 1990. Constitutive expression of a virulence regulon results in attenuation of *Salmonella typhimurium*. J. Bacteriol. **172**:2485–2490.
24. **Miller, V. L., and J. J. Mekalanos.** 1984. Synthesis of cholera toxin is positively regulated at the transcriptional level by *toxR*. Proc. Natl. Acad. Sci. USA **81**:3471–3475.
25. **Ouellette, A. J., R. M. Greco, M. James, D. Frederick, J. Naftilan, and J. T. Fallon.** 1989. Developmental regulation of cryptdin, a corticostatin/defensin precursor mRNA in mouse small intestinal crypt epithelium. J. Cell Biol. **108**:1687–1695.
26. **Peterson, K. M., and J. J. Mekalanos.** 1988. Characterization of the *Vibrio cholerae* ToxR regulon: identification of novel genes involved in intestinal colonization. Infect. Immun. **56**:2822–2829.
27. **Ronson, C. W., B. T. Nixon, and F. M. Ausubel.** 1987. Conserved domains in bacterial regulatory proteins that respond to environmental stimuli. Cell **49**:579–581.
28. **Selsted, M. E., D. M. Brown, R. J. Delange, S. S. L. Harwig, and R. I. Lehrer.** 1985. Primary structure of six antimicrobial peptides of rabbit peritoneal neutrophils. J. Biol. Chem. **260**:4579–4584.
29. **Selsted, M. E., S. S. Harwig, T. Ganz, J. W. Schilling, and R. I. Lehrer.** 1985. Primary structures of three human neutrophil defensins. J. Clin. Invest. **76**:1436–1439.
30. **Selsted, M. E., D. Szklarek, and R. I. Lehrer.** 1984. Purification and antibacterial activity of antimicrobial peptides of rabbit granulocytes. Infect. Immun. **45**:655–659.
31. **Straley, S. C., and P. A. Harmon.** 1984. *Yersinia pestis* grows within phagolysosomes in mouse peritoneal macrophages. Infect. Immun. **45**:655–659.
32. **Taylor, R. K., V. L. Miller, D. B. Furlong, and J. J. Mekalanos.** 1987. Use of *phoA* gene fusions to identify a pilus colonization factor coordinately regulated with cholera toxin. Proc. Natl. Acad. Sci. USA **84**:2833–2837.
33. **Thomas, E. L., R. I. Lehrer, and R. F. Rest.** 1988. Human neutrophil antimicrobial activity. Rev. Infect. Dis. **10**:S450–S456.
34. **Weiss, A. A., and S. Falkow.** 1984. Genetic analysis of phase change in *Bordetella pertussis*. Infect. Immun. **43**:263–269.