HHMI
HOWARD HUGHES MEDICAL INSTITUTE

RESEARCH NEWS

SEPTEMBER 29, 2000

How Salmonella Bacteria Protect Against Death by Iron

Researchers have discovered a survival mechanism that *Salmonella* bacteria employ to detect and protect themselves from high levels of iron in the environment. The newly discovered iron sensor provides double-barreled protection since it allows *Salmonella* to fend off the antibiotic polymyxin, which is produced by a soil microbe. The research offers a hint of the tools that *Salmonella* calls upon to survive and thrive in hostile environments.

The discovery by Howard Hughes Medical Institute investigator Eduardo Groisman and colleagues at Washington University School of Medicine in St. Louis was reported in the September 29, 2000, issue of the journal *Cell.*

> "These findings also help us understand the nature of environments where *Salmonella* prospers when not infecting an animal or human host."
> **— Eduardo A. Groisman**

The scientists investigated the function of PmrA/PmrB, a two-component regulatory system found in *Salmonella* and other gram-negative bacteria. Bacteria use two-component regulatory systems to sense environmental stimuli and to respond to those stimuli by activating or repressing the expression of different genes. In the PmrA/PmrB partnership, the scientists already knew that PmrB was the sensing element of the pair, but they did not know what signal activated PmrB. "We knew that PmrA/PmrB was a regulatory system that controlled resistance to the antibiotic polymyxin B," said Groisman. "Earlier, we had established that PmrA/PmrB was activated by another two-component system called PhoP/PhoQ."

A clue to the nature of the PmrB activation signal came when the scientists compared the sensing segment of PmrB to domains of yeast and mammalian proteins that bind iron. Their studies showed that PmrB also contained protein sequences, or motifs, that could respond to iron.

"Because the PmrA/PmrB system was activated by iron, we reasoned that it must be required for growth in the presence of toxic levels of iron," said

Groisman. "Similarly, the PhoP/PhoQ system is activated in low magnesium and is necessary for growth in low magnesium."

In their experiments, the scientists showed that iron specifically turns on the genes activated by PmrA. They also showed that iron binds to the periplasmic segment of the PmrB protein that extends through the cell membrane.

In studies with mutant strains of *Salmonella* , they found that those with malfunctioning PmrB sensor proteins that lack the periplasmic segment did not respond to extracellular iron. And, they discovered a *Salmonella* strain with a malfunctioning PmrA is overly sensitive to killing by iron. Groisman and his colleagues also found that iron inhibits the growth of *Salmonella* strains lacking functioning versions of either PmrA or PmrB, or certain genes activated by PmrA.

"It was quite a surprise that the PmrA/PmrB system responded to high iron concentrations because all the textbooks tell you that it is low iron that promotes expression of bacterial genes," said Groisman. In such responses, the bacteria are attempting to maintain sufficient internal iron concentrations for essential bacterial processes, he said.

Groisman and his colleagues found that the PmrA/PmrB system is highly sensitive to ferric iron, or Fe^{3+} , as opposed to ferrous iron, Fe^{2+} , which is the form found within cells. For example, they showed that treating *Salmonella* with chemicals that selectively scavenged Fe^{3+} from the cells abolished transcription of one of PmrA-regulated genes. The researchers also showed that these genes were not activated by a wide array of other positively charged atoms, called cations.

The iron concentrations that activated the protective response, said Groisman, are typical of those found in the soil and water that the bacteria encounter once they leave the gut environment of animals or humans. High iron concentrations may also be found in the stomachs of animals and humans, he said.

Iron promotes resistance to polymyxin by working through PmrA, said Groisman. The speculation is that such resistance may prove beneficial for *Salmonella* by protecting it against chemical assaults by the soil polymyxin-producing bacterium *Paeniacillus polymyxa* . This beneficial effect would have been preserved in evolution because it offers an advantage, said Groisman.

Understanding how ferric iron kills bacteria by damaging their macromolecules and how the protective genes function are going to be major challenges, said Groisman. "Our working hypothesis is that these genes modify the lipopolysaccharide that covers the surface of bacteria so that iron does not bind to them," he said.

"In addition to helping us understand the protective mechanism of *Salmonella* and other gram-negative bacteriaall of which possess the PmrA/PmrB systemthese findings also help us understand the nature of environments where *Salmonella* prospers when not infecting an animal or human host," he said.