Mechanism uncovered behind Salmonella virulence and drug susceptibility

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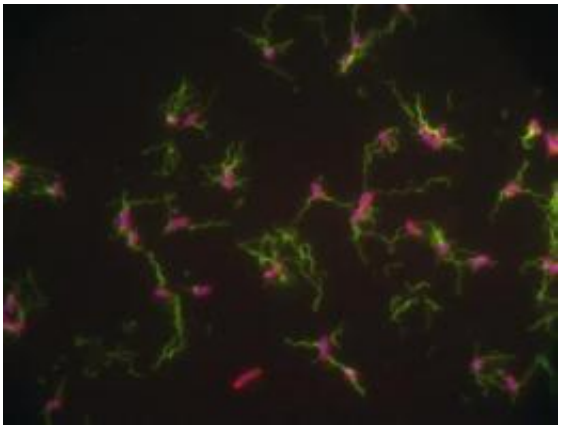

This is an image of *Salmonella* bacteria taken in Dr. Ferric Fang's lab at the University of Washington. The bacteria are stained with reagents to show the flagella (green), DNA (blue) and cell membrane (red). Credit: Joyce Karlinsey

Researchers have discovered a novel mechanism in *Salmonella* that affects its virulence and its susceptibility to antibiotics by changing its production of proteins in a previously unheard of manner. This allows *Salmonella* to selectively change its levels of certain proteins to respond to inhospitable conditions.

Although the mechanism had not been recognized before, the scientists were intrigued to find evidence of a similar mechanism in all five kingdoms of life - animals, plants, fungi, protista, and monera.

The findings were published today, July 29, in *Molecular Cell*. The senior author of the study is Dr. Ferric C. Fang, professor of microbiology, laboratory medicine, and medicine at the University of Washington (UW). Fang also directs the Clinical Microbiology Laboratory at Harborview Medical Center in Seattle. The lead author is William Wiley Navarre, who began the study as a postdoctoral fellow in the Fang lab and is now an assistant professor at the University of Toronto.

Salmonella enters the gut when people eat contaminated food, and can sometimes spread to other parts of the body. Illness outbreaks and grocery recalls related to *Salmonella* are often in the news. Babies, young children, the elderly, and people with cancer or HIV are especially prone to severe illness from *Salmonella*.

Salmonella is adaptable and can withstand many of the body's attempts to fight it. The bacteria live and multiply in a special compartment inside the cells of an infected person or animal. *Salmonella* can alter its physiology as it moves from a free-swimming life to its residence in a host cell. *Salmonella*'s metabolism also changes over time to make use of the nutrients available in the host cell, and to survive damage from the build-up of oxidants and nitric oxide in the infected cell.

While screening mutant *Salmonella* that were resistant to a form of nitric oxide that normally stops the bacteria from dividing, Navarre, Fang and their research collaborators found mutations in two little-known genes. These are the closely linked poxA and yjeK genes. In a number of bacteria, these two genes are associated with a third gene that encodes the Bacterial Elongation Factor P, which is involved in protein production.

The researchers discovered that these three genes operate in a common pathway that is critical for the ability of the *Salmonella* bacteria to cause disease and resist several classes of antibiotics. *Salmonella* with

mutations in either the poxA gene or the yjeK genes, the study noted, appear to be nearly identical and show similar changes in proteins involved in metabolism. Strains with mutations in both genes resemble the single mutant strains, an observation that suggests the two genes work in the same pathway.

The mutant strains exhibited many abnormalities under stressful conditions.

"The wide spectrum of compounds that dramatically inhibited the growth of these mutant strains suggest that the defect lies in a general stress response," the researchers noted. The mutant bacteria measurably differed from the wild-type *Salmonella* under 300 different conditions. In addition, their aberrant production of virulence factors reduces their ability to survive in the host.

The researchers' analysis also suggests that the way poxA and yjeK modify the bacterial protein elongation factor is essential in the production of proteins that allow the bacteria to use alternative energy sources when they are deprived of nutrients, as occurs after they enter host cells.

Unexpectedly the researchers found that the *Salmonella* with mutations in poxA and yjeK continued to respire inappropriately under nutrient-poor conditions in which wild-type *Salmonella* cease respiration.

Perhaps the mutant strains don't know when to quit. Wild-type *Salmonella* might enter a state of suspended animation to weather harsh conditions, whereas the mutants fail to respond properly to environmental stress. The fact that the mutants continue to respire when they are in dire straits might lead to the production of toxic oxygen-containing compounds.

"This might explain," the authors suggested, "why the mutants are broadly sensitive to a large number of unrelated compounds and cellular stresses."

The researchers also noticed a resemblance between the astounding manner in which the poxA gene modifies the bacterial elongation factor to regulate stress resistance, and the way a similarly acting factor is regulated in plant and animal cells.

During the manufacture of a protein, transfer RNA, also called tRNA, normally places an amino acid at the end of a growing chain of protein building blocks. A certain type of enzyme normally hands the tRNA the amino acid for it to place. However, in this study, researchers have shown for the first time that the poxA enzyme steps in and directly attaches an amino acid to the Elongation Factor P protein, rather than to the tRNA.

Fang said, "Sometimes it seems as if the most basic discoveries in biology have already been made. It was fun and unexpected to learn something new about a process as fundamental as protein synthesis."

"This is an interesting illustration of molecular evolution," Fang continued. "This essential, but previously unrecognized mechanism, for regulating the production of proteins appears to have been conserved over evolutionary time and continues to take place in cells belonging to all five kingdoms of life."

Future studies in his lab will address the specific reasons behind the defective stress response in poxA- and yjeK-deficient bacteria and the explanation for its different effects on the amounts of individual proteins. The lab will also look further into the roles of the normal poxA and yjeK proteins, the intriguing way in which the bacterial elongation protein is modified, the apparent universality of this protein-modifying

mechanism in living cells and its conservation throughout the course of evolution.

Provided by University of Washington