Gut instinct: Salmonella bacteria's molecular tactics to cause illness

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Hundreds of trillions of bacteria make their home in the vertebrate gut. Though many of these microbes perform helpful duties for their host, others—the pathogens—are unwelcome visitors, causing disease.

Salmonella typhimurium is one such pathogenic bacterium. It has evolved sophisticated means of growth, replication, transport and survival within the forbidding environment of the body, where it is responsible for most cases of food-borne illness. Yixin Shi, a researcher at Arizona State University's Biodesign Institute, has taken a keen interest in the regulatory mechanisms that allow *Salmonella* bacteria to overcome their surroundings and continuously modify both their own and their host's responses in order to stay alive.

By cooperating with the Dr. Roy Curtiss' lab in the Biodesign Institute, Shi's research, which appears in the *Proceedings of the National Academy of Sciences*, (*PNAS*) unveils a key survival circuit, which activates a signaling cascade, switching on or off a suite of genes necessary to circumvent the body's multiple defense mechanisms.

A corrosive course

The bacteria are tenacious, surviving acidic pH conditions, digestive enzymes, bile salts, antimicrobial peptides, and other hazards as they pass through the stomach and intestine, and invade the mucosa of the small intestine. Once they make contact with the intestinal lumen, their

goal is to secure a safe haven—within the cells of the intestinal epithelium.

To reach this sanctuary, *Salmonella* first invite themselves in by secreting specific protein factors derived from a region of DNA known as the *Salmonella* Pathogenicity Island 1 or SPI-1. These factors trick the body, inducing the reorganization of the host cell's cytoskeleton. Epithelial cells respond to the *Salmonella* secretions by surrounding the bacterial cell in a membrane-bound balloon—the *Salmonella* Containing Vacuole or SCV. Once the bacterium is taken up in the SCV by the epithelial cell, this secretion system is no longer needed and is switched off. At the same time, another system, SPI-2 is activated and will respond to the altered environment of the internalized *Salmonella*.

Starving the beast

As the *Salmonella* penetrates through the epithelial layer, it encounters a dense population of macrophages that normally act to engulf and digest pathogens and debris. Unlike other gut commensal microbes—*E. coli* for example—*Salmonella* is able to survive and replicate within SCV of these macrophages, which eventually transport it to organs including the liver and spleen. "The host cells isolate nutrients from bacteria," Shi explains. "They may deplete metal ions, nucleotides, and amino acids which are essential for bacterial life and growth. In this way, *Salmonella* are essentially starved to death."

But the *Salmonella* are prepared, and respond— first by sensing the new conditions, then synthesizing proteins allowing them to acquire nutrients from this new environment while switching on genes girding the bacteria against destructive host peptides.

A switch in time

As Shi explains, a regulatory system allowing *Salmonella* to monitor and respond to rapidly changing conditions is made up of two proteins: PhoP and PhoQ. The PhoP/PhoQ regulators act as a master control, switching off invasion proteins when they are no longer required while switching on a new set of protein factors necessary for intracellular survival. In a domino effect, part of this transition activates magnesium transporters, which act to reestablish metal ion levels in the depleted conditions of the vacuole.

But now a problem arises. *Salmonella* must maintain proper Mg2+ concentration in their cytoplasm, though PhoP, once turned on, acts to continually increase these levels. The single master switch PhoP/PhoQ is not sufficient to provide this level of control. *Salmonella* uses an RNA Mg2+ riboswitch to ensure downregulation of Mg2+ transporters without shutting down bacterial resistance to antimicrobial peptides. Likewise, instead of one regulatory switch, two are needed to properly mitigate conditions of resistance to the bacteriocidal peptides, and amino acid starvation. Enter SlyA.

Two to tango

SlyA is a regulatory protein which cleverly integrates itself into the PhoP/PhoQ regulatory system, allowing for multivariable control. Only when both the PhoP/PhoQ and SlyA regulatory systems are activated can the proper activation of genes for intracellular survival be switched on and delicately maintained.

The sequence of events appears as follows: after cellular invasion and formation of the vacuole, PhoP responds to antimicrobial peptides and/or low levels of Mg2+ within the vacuole by switching on, activating Mg2+ transporters and stimulating the production of SlyA. The PhoP/PhoQ system is sufficient to maintain proper Mg2+ levels, but it is SlyA's job to respond to nutrient starvation. Shi believes SlyA does this

by sensing the presence of a particular chemical signal—ppGpp, (guanosine tetraphosphate), indicative of amino acid depletion. If SlyA detects this chemical, it will act in consort with the PhoP/PhoQ system. With both PhoP/PhoQ and SlyA switches thrown, all the necessary regulatory genes are brought into play, as conditions warrant.

Shi emphasizes that the cluster of genes responsible for this sequence of environmental adaptations to adversity in virulent bacteria like *Salmonella* are arranged in particular chromosomal regions, the so-called horizontally acquired loci, which are absent in helpful gut bacteria like E. coli.

Disabling either PhoP or SlyA renders *Salmonella* virtually impotent, its virulence severely attenuated. The bacteria lose their survivability within the macrophage environment, succumbing either to nutrient starvation or direct obliteration by host antimicrobial peptides.

Shi suggests that the specificity of SlyA's activity within *Salmonella*'s regulatory universe may be good news for those hoping to target this system through vaccine development or other therapeutic intervention. Discovering competitive analogs of ppGpp, for instance, could provide an alternate approach, curtailing SlyA's function.

Shi is optimistic that a firmer grasp of such regulatory mechanisms of virulence as PhoP/PhoQ and SlyA will ultimately lead to life-saving applications. "I never think I'm doing basic science," Shi stresses. "I always think I'm working on the first steps of an application. "

Source: Arizona State University