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Molecular call-and-response: how *Salmonella* learns the gospel from its host

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Host–microbe interactions are often portrayed as a game of molecular hide-and-seek or tug-of-war where one partner seeks to establish an upper-hand over the other. Perhaps a more useful analogy is the traditional call-and-response preaching method used so effectively in churches of the southern USA to encourage participation by the assembled parishioners. The preacher calls out a line of a gospel or hymn and the congregation responds as one to the cue. A recent paper identifies Nramp as a potential molecular preacher, and *Salmonella*, and probably other pathogenic bacteria, are singing back full-throated.

Salmonella enterica serovar Typhimurium normally causes self-limiting gastroenteritis. This pathogen uses type III pili to deliver effector proteins to its preferred host cell, the macrophage. The bacterial colony proliferates in an intracellular vacuole. As macrophages wander the body, the bacteria are disseminated by escape into various cellular compartments. Death can occur, but only in host mice that lack the product of the *Nramp* gene. Here, bacterial loads in the liver and spleen reach lethal levels. This *nramp* loss-of-function mutation was first defined as a natural variation between strains of inbred mice. The strain lacking *Nramp* was killed by a variety of bacterial pathogens, including several species of *Mycobacterium* and *Leishmania*, and *Candida albicans* and *Toxoplasma gondii* (reviewed in Ref. [1]). In fact, *Nramp,* like many an itinerant preacher, went by several names in the inbred mouse strain literature (*Ity*/*Bcg*/*Lsh*). *Nramp* was cloned based on its genomic position in one of the earliest chromosome walks in mouse [2], and was subsequently shown to encode a protein that resides in a late endocytic vacuole of macrophages, the same subcellular address inhabited by *S. enterica* Typhimurium. The function of Nramp is still somewhat unclear, but it is thought to pump divalent ions pleiotropically. It might also function to scavenge iron from dead red blood cells in the infected host.

Nramp gathers the congregation

A quirk of the cell biology edifice of *Salmonella* that has emerged over the past ten years is the heavy reliance on

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Nramp-defective mice (genotype *nramp*) (or cell lines derived from them) as experimental hosts. Among other things, this system has revealed that genes in the *S. enterica* Typhimurium SPI2 pathogenicity island are required for systemic proliferation. These genes include the type III structural genes and effectors delivered by it into the host cell. Although instructive for understanding how systemic disease results, and thus potentially a reasonable model for typhoid fever in humans, the ancestral wild-type allele must have been *Nramp*. Therefore, the interaction most reflective of the co-evolved natural history of *S. enterica* Typhimurium and mice would be with *Nramp*-positive host cells, and would result in self-limiting gastroenteritis. How then does Nramp preach to the congregation of bacteria growing in the privileged confines of the late endocytic vacuole? And which Psalm do they sing?

Enter Zaharik *et al.* [3] who address the effects of Nramp on the growth and survival of *S. enterica* Typhimurium, and the effect of Nramp on a variety of pathogen genes involved in virulence. We know that intimate host–microbe associations result in signals being passed back-and-forth, and that these signals usually result in transcriptional re-programming in one or both partners. Therefore, if Nramp is a key molecule preaching a direct message to the pathogen, as opposed to simply a molecule whose presence conditions resistance independent of the bacterial genotype, its presence should generate a chorus of transcriptional 'amens' from the pathogen.

Choir soloists are virulence factors

First, Zaharik and colleagues demonstrated that *S. enterica* Typhimurium does indeed survive in Nramp-positive mice. Splenic bacterial numbers increase over the same four-day time-course that kills most of the Nramp-negative mice. This result indicates that Nramp is required to keep the slowly proliferating bacteria from growing uncontrollably. The growth observed in both Nramp-positive and Nramp-negative mice is eliminated by mutation in SPI2, proving that the type III system is key in this interaction. As the interaction in Nramp-negative mice is self-limiting, it would have been interesting to know the splenic bacterial titer at a time-point when infection is limited; presumably it drops to baseline levels.

Because the SPI2 system was required for bacterial survival in Nramp-positive mice, Zaharik *et al.* postulated that genes in, or controlled by, SPI2 might respond to the presence of *Nramp*. They assayed promoter–reporter fusions for induction or repression in a macrophage-like cell line derived from an *nramp* mouse but complemented by stable transfection with *Nramp*. Three SPI1-associated genes were in fact upregulated in an *Nramp*-dependent manner, whereas two others associated with SPI1 functions were not. This result was reproduced for at least two of the three SPI2-associated genes following intraperitoneal injection of the relevant *S. enterica* Typhimurium strains into *Nramp* mice. Importantly, these mice are congenic with *nramp* knockout mice used as controls [4]. The authors conclude that Nramp is required for transcriptional upregulation of critical pathogen virulence genes. This result raises the question of what influences the host *Nramp* genotype has on the overall transcriptional status of SPI1- and SPI2-associated genes, or indeed on the whole *S. enterica* Typhimurium genome. Given that the full sequence of *S. enterica* Typhimurium is available, these questions could certainly be answered using microarrays.

If SPI2-associated genes are singing to an *Nramp*-dependent tune, what mediates the message? What gospel is being preached by the host? The authors returned to the issue of what might be transported by the Nramp proteins. They found that chelation of iron resulted in SPI2-selective gene activation. More importantly, they demonstrated that, following iron chelation, addition of only iron (among those ions tested) could restore normal expression from the SPI2-associated test genes. They conclude that Nramp removes iron from the vacuole, and that *Salmonella* responds by upregulating genes required for iron acquisition. The call, therefore, is for iron, and the response is movement out of the particular host to get more.

Preaching to many

A broader implication of the Nramp sermon is that the course of infection of several pathogenic bacteria is affected. One presumption is that each of these species of pathogen will respond similarly, namely by upregulating iron acquisition strategies. However, like most slick preachers, *Nramp* might have a multitude of messages that it sends to different vacuolar inhabitants. Its message might also be tailored to those inhabitants. For example, Nramp has also been implicated in the production of reactive oxygen and nitric oxide, and, in turn, SPI2-associated functions are known to diminish the oxidative burst in *nramp* macrophages. Alternatively, Nramp could pump different divalent cations out of the pathogen haven, driving differential responses from different pathogens. Thus, if the overall set of pleiotropic Nramp functions results in regulation of a similar set of pathogen responses, this particular call-and-response could take many Sundays to explore.

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