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Salmonella respiration turns the tables on propionate

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Brief Summary:

Intestinal pathogens must combat host and microbiota-associated resistance to establish an infection. A new study (Shelton, Yoo, *et at.*) highlights how *Salmonella* manipulates the mammalian host to produce anaerobic respiratory electron acceptors, allowing catabolism of propionate and providing a competitive edge to the pathogen in the gut.

Salmonella enterica serovar Typhimurium (S. Typhimurium) is a foodborne bacterial pathogen and a major cause of diarrheal disease worldwide. As with many enteric pathogens, it has long been recognized that an intact microbiota provides a barrier against S. Typhimurium intestinal colonization [1, 2]. The known mechanisms underlying colonization resistance to S. Typhimurium involve both direct inhibition, through microbiota-produced compounds, including bacteriocins [3] and repressive metabolites like short-chain fatty acids (SCFA) [2, 4], as well as indirectly by guiding host metabolism to create a largely anaerobic habitat in the gut adverse to S. Typhimurium expansion [2] (Fig. 1A). To overcome microbiota-associated resistance, S. Typhimurium evolved several virulence factors that allow the pathogen to hijack host immunity and re-engineer the gastrointestinal environment to favor intestinal growth of the pathogen [1, 2].

Writing in *Cell Reports,* Shelton, Yoo, and colleagues [5] highlight an unexpected resource "unlocked" for metabolic use by *S*. Typhimurium after triggering intestinal inflammation: the SCFA propionate. Propionate, a fermentation byproduct produced by commensal microbes, is abundant in the healthy colon and has previously been established as an important inhibitory metabolite towards *S*. Typhimurium growth in mouse models [4]. Yet, the S. Typhimurium genome encodes an operon (*prpBCDE*) that is thought to facilitate the metabolic breakdown of propionate into pyruvate [5, 6]. Parts of the operon have

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Declaration of interests

No interests are declared.

degraded in some *Salmonella* serovars that primarily cause extraintestinal infection, but are conserved in serovars that cause intestinal disease [6], suggesting that the ability to catabolize propionate could be specifically valuable for the pathogen to colonize the inflamed gut. Though *S*. Typhimurium was incapable of utilizing propionate as a sole carbon source for growth *in vitro,* Shelton *et al.* found that addition of the anaerobic respiratory electron acceptor nitrate (NO_3^-) to the media induced expression of *prpBCDE* genes and allowed enhanced bacterial replication using propionate [5].

Prior work has established that the presence of various respiratory electron acceptors such as nitrate, tetrathionate, and oxygen helps *S*. Typhimurium compete for resources with the native microbiota by permitting respiratory metabolism by the pathogen [2]. While not plentiful in the healthy gut, nitrate is generated in the intestine during *S*. Typhimurium-associated inflammation [2, 7]. The pathogen induces inflammation in the gut using two type-III secretion systems (T3SS-1/2) to inject an array of effector proteins into host cells, promoting its own uptake and intracellular survival [8]. T3SS-mediated invasion of host mucosal tissues results in a robust recruitment of monocytes and neutrophils to the gut and activation of antimicrobial programs in the intestine [2], producing antimicrobial reactive oxygen and nitrogen species (ROS and RNS). Intestinal nitrate is produced by the degradation of peroxynitrite, a molecule formed through the reaction of nitric oxide (generated by activated epithelial cells and inflammatory monocytes via inducible nitric oxide synthase) and superoxide (generated by mucosa-recruited phagocytes via NAPDH oxidase) [2]. *S*. Typhimurium in the intestinal lumen can then take advantage of this host-derived nitrate to power anaerobic respiratory growth using a trio of nitrate reductases [7].

To test whether nitrate respiration could fuel propionate use by *S*. Typhimurium during infection, Shelton *et al.* co-inoculated wild-type *S*. Typhimurium and an isogenic *prpC* mutant in mice pretreated with streptomycin, a model commonly used for studying *Salmonella* colitis [1]. After 4 days of infection, wild-type *S*. Typhimurium had outgrown the mutant strain approximately 10-fold. However, neither T3SS virulence mutants deficient in generating colitis nor mutants lacking functional nitrate reductases outcompeted the *prpC* mutant, supporting the hypothesis that propionate provided an advantage to *S*. Typhimurium only when respiratory nitrate was present in the inflamed colon. Blockade of nitrate production also eliminated the *prpC* defect. Furthermore, in germ-free mice the wild-type *S*. Typhimurium advantage over the *prpC* mutant was lost, but mono-colonizing the mice with *Bacteroides thetaiotaomicron* (*B. theta*), a propionate-producing commensal bacterium, promoted wild-type *S*. Typhimurium colonization. Unlike much of the commensal microbiota, *B. theta* can resist inflammatory inhibition via xenosiderophore utilization of S. Typhimurium-produced enterobactin and salmochelin to acquire iron [9], suggesting propionate produced by resilient *Bacteroides* could remain a plentiful carbon source for S. Typhimurium to use in the inflamed gut. Together, these exciting findings demonstrate that *S*. Typhimurium can use microbiota-derived propionate in the gut to help fuel its expansion only once the pathogen has induced a sufficient inflammatory response to generate ample nitrate for anaerobic respiratory growth (Fig. 1B).

Intriguingly, a prior report found that *Bacteroides*-produced propionate is inhibitory towards *S*. Typhimurium growth in the gut [4]. However, the study was focused on evaluating

early stages of chronic *S.* Typhimurium infection in a genetically resistant mouse line, probably resulting in significantly less gut inflammation than the streptomycin-treated mice used by Shelton *et al.,* which instead exhibited high nitrate production and propionate catabolism. Thus, propionate is likely inhibitory during the initial stages of *S.* Typhimurium colonization, until pathogen-triggered inflammation allows *S.* Typhimurium to turn the tables and benefit from the SCFA (Fig. 1B). In this manner, *S.* Typhimurium virulence manipulates host immunity and reshapes the gut ecosystem to benefit the pathogen.

The work from Shelton *et al.* [5] squares well with the growing body of evidence highlighting *Salmonella* virulence in the gut as a process of "ecosystem engineering" to compete with the microbiota [2, 7, 10]. For example, depletion of butyrate-producing microbes –through either antibiotic administration or inflammatory inhibition of the microbiota by ROS and RNS– alters colon epithelial cell metabolism and increases luminal oxygen levels, which *S.* Typhimurium and related facultative anaerobes can use to power aerobic respiratory metabolism with cytochrome oxidases [10] (Fig. 1A and B). Additionally, inflammatory ROS can fuel the generation of tetrathionate ($S_4O_6^{2-}$) from intestinal thiosulfate ($S_2O_3^{2-}$), allowing for anaerobic respiration via tetrathionate reductases encoded by the *S.* Typhimurium *ttr* locus [2]. Overall, respiratory metabolism- whether utilizing nitrate, tetrathionate, or oxygen as electron acceptors-allows *S.* Typhimurium to support its growth using a broader and more diverse pool of carbon sources, including ethanolamine, succinate, and 1,2-propanediol [2]. With the new findings by Shelton *et al.,* propionate can now be added to the list, and future work will undoubtedly uncover additional metabolites and nutrient sources providing *S.* Typhimurium a competitive edge in the inflamed gut.

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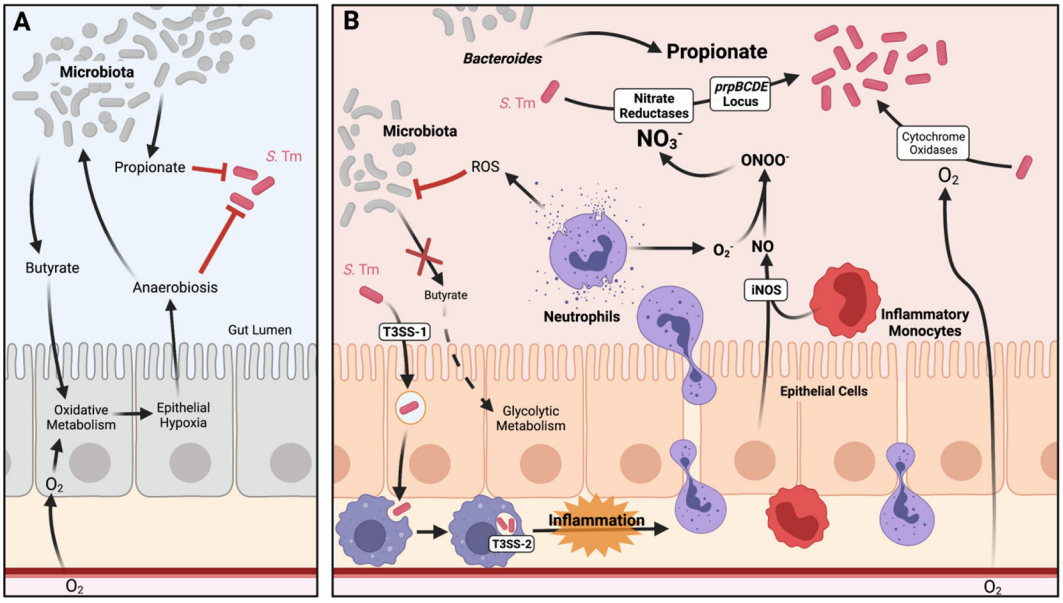

Fig. 1. *S*. Typhimurium overcomes colonization resistance provided by short-chain fatty acids to colonize the inflamed gut.

(A) When initially encountering the microbiota in the colon, *S*. Typhimurium (*S*. Tm) struggles to compete for metabolites in part due to short-chain fatty acid-mediated inhibition. Propionate directly inhibits growth of *S*. Typhimurium in the gut due to acidification of its cytoplasm. Meanwhile, colonocyte metabolism of microbiota-produced butyrate by oxidative phosphorylation drives utilization of local oxygen supplies generating epithelial hypoxia and an anaerobic lumen, favoring growth of the commensal anaerobes over *S*. Typhimurium. (B) Through the use of type-III secretion systems (T3SS-1/2) to invade and manipulate host cells, *S*. Typhimurium triggers a substantial inflammatory response that generates respiratory electron acceptors for the pathogen to better compete with the microbiota. Superoxide from phagocyte NADPH oxidase, and nitric oxide from epithelial cell and inflammatory monocyte iNOS interact in the gut to form peroxynitrite, which quickly degrades into the respiratory electron acceptor nitrate. *S*. Typhimurium can harness nitrate with a set of nitrate reductases (encoded by *narGHI, narZYV,* and *napABC*) to power anaerobic respiratory growth. This nitrate allows *S*. Typhimurium to use the previously inhibitory SCFA propionate (produced by commensal microbes such as *Bacteroides* species) to support the pathogen's expansion via the *prpBCDE* locus. Additionally, antimicrobial ROS inhibits butyrate-producing members of the microbiota, leading to a switch in epithelial metabolism that allows oxygenation of the colon, further impeding obligate anaerobes and providing a competitive edge to the facultative anaerobe *S*. Typhimurium. Created with BioRender.com.