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Love Thy Neighbor: Sharing and Cooperativity in the Gut Microbiota

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To persist in the competitive gastrointestinal ecosystem, microbes often enforce selfish strategies that limit resource loss to neighboring bacteria. In contrast, a recent study in *Nature* by Rakoff-Nahoum et al. (2016) reveals that one commensal bacterium releases nutrients to benefit another species, which reciprocally provides growth-promoting factors to the producer.

Hundreds of different bacterial species coexist at high density in the distal intestine of a typical human (Qin et al., 2010). Although the nutrients that sustain this commensal community include host products such as mucus, the majority of nutrients come from dietary fiber, a chemically diverse group of complex carbohydrates (Koropatkin et al., 2012). However, the quantities and identities of fiber polysaccharides change from meal to meal, raising the question: how can a diverse microbial community remain resilient in the face of a constantly changing nutrient landscape?

Many microorganisms in the gut work in "supply chains," with lower organisms in the chain feeding off the metabolic products, sometimes the waste, of others. Well-studied examples are hydrogen consumers (such as methanogens, acetogens, and sulfate reducers), which work at the end of the fermentative chain to consume inhibitory gases like carbon dioxide and hydrogen to generate methane, acetate, and hydrogen sulfide, respectively. Another example is *Ruminococcus bromii*, a more recently recognized player in the gut microbiota that uses a cellulosome-like extracellular apparatus to digest resistant starch particles that are inaccessible to many other gut bacteria—even those that are adept at utilizing soluble starches (Ze et al., 2015). *R. bromii* is proposed to act as a keystone member of the microbiota due to its attributes as a "messy eater"; by digesting resistant starch outside the cell, *R. bromii* unlocks a significant portion of soluble starch fragments for other, less-specialized microbes (Ze et al., 2012).

As important as these examples are, they exemplify relationships in which one microorganism benefits from another and not necessarily a reciprocal transfer of beneficial services (although, removal of hydrogen by the organisms noted above increases overall fermentation efficiency in the gut). In this light, Rakoff-Nahoum et al. (2016) investigated whether or not gut bacteria involved in dietary fiber degradation share some of their nutrient products with organisms around them, possibly with the advantage of getting other factors in return.

Although cataloging the species and gene inventories present in the human gut microbiome has been the focus of intense effort in the past decade, functional understanding of the hundreds of individual species—let alone their myriad ecological interactions—is still just emerging. Previously, Rakoff-Nahoum et al. (2014) surveyed a group of intestinal-dwelling Bacteroidales for the ability to create nutrient-driven ecological networks by sharing some of their dietary fiber digestion products with other species. Consistent with findings from other groups (Cuskin et al., 2015), some of the species surveyed were "selfish" in their behavior, releasing sparingly little of some polysaccharides into the extracellular environment. However, other species generated significant amounts of freely available fiber digestion products (as oligosaccharides), which the investigators attributed to secretion of membrane-associated degradative enzymes that were packaged in outer-membrane vesicles (OMVs). For example, thin-layer chromatography analysis revealed that strains capable of growth on the common prebiotic fiber inulin produced very different amounts of extracellular breakdown products. Inulin-degrading species, like *Bacteroides caccae* and *Bacteroides ovatus*, digested the majority of full-length inulin to smaller mono- and oligosaccharides, which remain freely available in the extracellular environment during exponential growth. On the other hand, *Bacteroides fragilis* and *Bacteroides uniformis* grew at similar rates but generated far fewer inulin oligosaccharide products, suggesting that these species captured more of this nutrient for their own use by either importing the products more efficiently or not digesting them as freely soluble products to begin with. Interestingly, spent media from inulin-grown *B. ovatus* (or inulin media treated with outer membrane vesicles of *B. ovatus*) could support the growth of a non-inulin degrader, *Bacteroides vulgatus*. Moreover, expression of two putative surface-expressed enzymes from *B. ovatus* in *B. vulgatus* enabled the latter to degrade inulin on its own, indicating that *B. vulgatus* lacks only the initial step in the degradation of inulin. Spent media from *B. fragilis*, which displays a more "selfish" phenotype, did not promote *B. vulgatus* growth.

Rakoff-Nahoum's recent work (Rakoff-Nahoum et al., 2016) extends this line of investigation by addressing the question of whether or not freely available oligosaccharides reinforce relationships that are mutually beneficial to both the recipient (*B. vulgatus*) and the producer (*B. ovatus*). The authors unexpectedly found that deletion of two *B. ovatus* surface-associated glycoside hydrolases, which they hypothesized to be critical for inulin degradation, had no impact on the direct ability of this species to use inulin. Instead, deleting these enzymes, alone or together, eliminated only the extracellular accumulation of oligosaccharide products. Separately

deleting an energy-dependent import apparatus, encoded in the same gene cluster as the two surface enzymes, eliminated growth on inulin, suggesting that *B. ovatus* is capable of directly importing the longer inulin chains and degrading them selfishly with periplasmic enzymes.

Based on their observations, Rakoff-Nahoum et al. (2016) hypothesized that the *B. ovatus*-secreted enzymes could serve an altruistic role either by helping to unlock oligosaccharides for other *B. ovatus* cells in the same colony but far away from the primary nutrient source, or for other species like *B. vulgatus*, which they previously demonstrated to grow on *B. ovatus* inulin breakdown products. Data suggested that other *B. ovatus* cells in a colony did not benefit from this behavior. However, in vitro co-culture data demonstrated that wild-type *B. ovatus*, which releases free oligosaccharides, promotes the abundance of *B. vulgatus*. Co-culture with the *B. ovatus* enzyme mutant did not completely abolish the benefit to *B. vulgatus*, indicating that *B. vulgatus* receives more than one benefit from the presence of *B. ovatus*, and at least one of these involves sharing inulin oligosaccharides.

Likewise, *B. ovatus* also benefits from *B. vulgatus*. During co-culture together on solid medium, *B. ovatus* counts increased by ~10-fold when co-cultured with *B. vulgatus*. This occurred irrespective of the availability of surface enzymes, indicating that *B. ovatus* does not need to share products directly with *B. vulgatus* to receive a benefit in return. However, in triculture experiments in which *B. vulgatus* was present with both wild-type and mutant *B. ovatus* strains, there was an increased proportion of the wild-type (both in vitro and in vivo in gnotobiotic mice). Thus, within the spatially restricted environment of a colony or intestinal contents, there may be a distinct benefit for wild-type *B. ovatus* to release oligosaccharides in its immediate vicinity, which will in turn recruit *B. vulgatus* and reinforce the mutualistic interaction (Figure 1). These interactions could enable both parties to survive or thrive in frequently changing nutrient conditions that might otherwise be unfavorable for either species alone.

Mechanistic studies of the gut microbiome have been accelerated by the Human Microbiome Project, MetaHit, and other efforts but are still likely to be a rate-limiting step to understanding in detail how individual bacteria function (Martens et al., 2014). An even more exciting scientific horizon will be using mechanistic studies to connect individual bacteria together into functional models that incorporate ecological concepts like cooperation, competition, food-chains, and succession to explain the gut microbiome and predict its responses to diet and other interventions in precise ways. Clever and well-executed experiments like those reported by Rakoff-Nahoum et al. (2016) are a deliberate step toward this horizon.

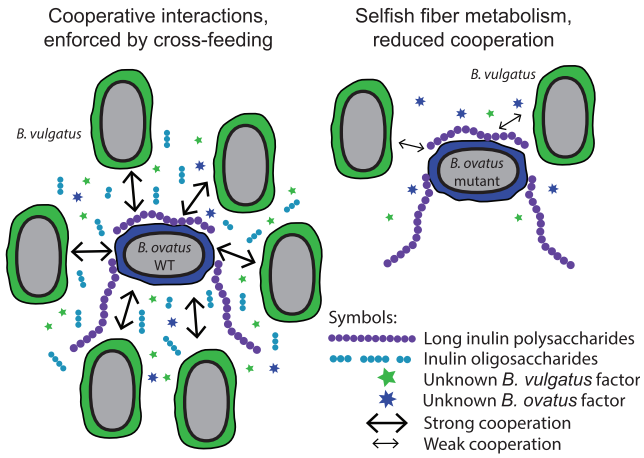

Figure 1. Cooperative Behavior among Microbiota Bacteria
Left: in the presence of the dietary fiber inulin, wild-type *Bacteroides ovatus* uses surface-associated enzymes to generate oligosaccharides that are used by another species, *B. vulgatus*, which is alone unable to directly utilize long-chain inulin. Both species produce unknown factors (green and blue stars) that stimulate the growth of the other. Right: a mutant *B. ovatus* that lacks inulin-cleaving surface enzymes (or another non-cooperative strain) does not generate oligosaccharide products to support *B. vulgatus*. As a consequence, *B. vulgatus* numbers are lower, leading to weaker exchange of beneficial growth products.

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