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Is the Regulation of Insulin Signaling Multi-Organismal?

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Abstract

The sustained health of an individual animal depends on the composition and activities of its resident microbiota. A major challenge is to identify the processes by which the microbiota and animal interact, recognizing that this research should lead ultimately to novel strategies to promote human health. *Drosophila* is emerging as a tractable model system to investigate these interactions. New evidence reveals that the gut microbiota promotes insulin signaling in *Drosophila*, leading to increased growth and development rates. Different gut bacteria and bacterial effectors were implicated: acetic acid produced by *Acetobacter pomorum* and branched-chain amino acids produced by *Lactobacillus plantarum*, respectively. These findings raise the possibility that multiple bacterial effectors may interact with signaling networks to shape animal health.

Have we made a serious error of omission in our current understanding of the signaling networks that define the form and function of animals? Are we systematically underestimating the complexity of signaling by assuming that all components are of animal origin? These questions arise directly from two publications on the fruit fly *Drosophila melanogaster* (1, 2) that independently implicate the gut microbiota in the integration of cell growth and metabolism through the insulin-TOR (target of rapamycin) signaling cascades.

Drosophila is not only a superb genetic model for developmental and cell biology, it is also a tractable model for multi-organismal interactions between animals and their resident microbiota. The gut lumen of *Drosophila* is colonized by ~20 bacterial taxa—an order of magnitude lower than the 500 to 1000 taxa in the gastrointestinal tract of humans and other mammals (3, 4)—and is dominated by two bacterial groups that are readily culturable: the Gram-negative *Acetobacter* and related “acetic acid” bacteria (Proteobacteria) and the Gram-positive *Lactobacillus* (Firmicutes) (5, 6). Germ-free insects can be obtained by surface-sterilizing the eggs and rearing them under aseptic conditions, and associations with single bacterial taxa are generated by feeding the flies on the cultured bacteria. Germ-free *Drosophila* larvae tend to grow and develop more slowly than individuals bearing gut bacteria (conventional *Drosophila*), although the magnitude of the effect varies with diet (1, 2).

These experimental tools, combined with a simple but technically demanding genetic screen, enabled Shin *et al.* (1) to demonstrate that the gut microbiota modulates insulin signaling. The screen comprised a comparison between the growth of *Drosophila* larvae infected with a wild-type strain of the gut bacterium *A. pomorum* and each of ~3000 transposon mutants. It revealed that the beneficial effect of *A. pomorum* on *Drosophila* requires one metabolic complex: the pyrroloquinoline quinone-dependent alcohol dehydrogenase (PQQ-ADH)-dependent oxidative respiratory chain, which couples energy production with the fermentation of ethanol to acetic acid (7). The gut of *Drosophila* bearing the wild-type *A.*

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pomorum contains 25 to 30 pg of acetic acid, but acetic acid is undetectable in *Drosophila* infected with mutant bacteria lacking functional PQQ-ADH (1). The *Drosophila* with the mutant bacteria also displayed a decreased abundance of DILPs (*Drosophila* insulin-like peptides) that are homologous to the mammalian insulin, as well as multiple traits indicative of reduced insulin signaling, such as elevated sugar concentrations, depressed proliferation of intestinal stem cells, and reduced wing size and cell number. Two sets of analyses defined the bacterial effect on *Drosophila* insulin signaling more precisely. First, the phenotype of *Drosophila* infected with the mutant bacteria was recapitulated when *Drosophila* with genetically reduced insulin signaling were infected with wild-type bacteria. Second, the phenotype of larvae bearing wild-type bacteria could be rescued in larvae bearing the mutant bacteria, either by supplementing the medium with acetic acid or by using *Drosophila* genetically manipulated to overexpress DILPs. These experiments provide persuasive evidence that acetic acid produced by *A. pomorum* promotes insulin signaling in *Drosophila*.

Storelli *et al.* (2) also obtained evidence for reduced insulin signaling in germ-free *Drosophila*, providing assurance of the repeatability of the core conclusion of Shin *et al.* (1) in a different *Drosophila* strain and laboratory context. Nevertheless, the results of the two studies differ in detail, and the several discrepancies are informative (Fig. 1A). Each study identified a single bacterium that could recapitulate the effect of the full microbiota on insulin signaling and larval growth and development rates, but the bacterium was different. Storelli *et al.* obtained unambiguous effects with *L. plantarum*, which was one of four bacterial species derived from *Drosophila* guts that Shin *et al.* reported as supporting larval growth rates no better than germ-free larvae. This difference is important because it means that taxonomically divergent bacteria can affect insulin signaling in *Drosophila*. *Lactobacillus* is not known to release acetic acid but produces copious amounts of lactic acid (8). But we cannot infer acid production as a commonality between the two studies, because a strain of *L. plantarum* with minimal lactic acid production supports high development rates in *Drosophila*, indicative of “normal” insulin signaling (2). Instead, the key products of *L. plantarum* may be protein-derived branched-chain amino acids, which activate TOR signaling in the fat body and have downstream effects on DILP production (9).

The key implication of these two studies is that at least two, and potentially multiple, bacterial products interact with *Drosophila* insulin signaling and the linked signaling networks that define nutrient sensing in the animal. This raises questions of mechanism. Which gut factor interacts with the bacterial products? How is the information conveyed to the signaling networks that integrate insulin, TOR, and other key components in nutrient sensing across multiple organs of the animal? There is also the issue of interactions. Multiple bacterial products may interact competitively, additively, or synergistically with *Drosophila* signaling networks, such that the impacts of *A. pomorum*, *L. plantarum*, and other bacteria differ between *Drosophila* bearing single species [as studied in (1) and (2)] and the full community of 20 to 30 bacterial taxa.

Not only is the mechanism unresolved, so is the ultimate explanation, “why?” Bacterial-mediated enhancement of larval growth and development rates is likely beneficial to *Drosophila* under natural conditions. The female flies deposit eggs onto the transient resource of rotting fruit, and larvae that develop fast are at a competitive advantage and are more likely to pupate before the fruit resource is exhausted (10). Why is the “desired” setpoint of the signaling cascades that control development rate dependent on input of bacterial products? There are two possibilities. Perhaps these bacterial products are cues that inform *Drosophila* of the likely abundance and persistence of resources in the rotting fruit, with the prediction that some bacterial products (indicative of a persistent resource or stressful conditions) may favor long developmental rates by suppressing insulin signaling

(Fig. 1B). Alternatively, the difference in insulin signaling between conventional and germ-free larvae may be the evolutionary consequence of conflict between *Drosophila* and its microbiota. The optimal developmental rate may be lower for the animals, which are selected for lifetime reproductive success, than for the gut microbiota, selected to maximize dispersal through rapid larval development to adults that fly to (and defecate onto) multiple fruits. In this conflict scenario, the observed setpoint of insulin signaling is a titration between the high and low preferred setpoints of the bacteria and animal, respectively (Fig. 1C). If the intrinsic set point of the animal is calibrated constitutively to account for bacterial manipulation [as is likely because the bacteria are always present in naturally occurring *Drosophila* (6)], then the signaling would be depressed in the germ-free flies, which lack the manipulative up-regulation by the bacteria (Fig. 1C).

Associations with bacteria are common to animals and their protistan ancestors (11), and the signaling networks in animals presumably evolved in the context of cues and manipulative effectors produced by their associated bacteria. This raises the possibility that the modulation of *Drosophila* insulin signaling by bacterial products may be explicable not only in the context of fruit fly ecology but also in terms of the long evolutionary history of animal-microbial interactions. In other words, the fundamentals of the interactions between *Drosophila* and its gut microbiota could be general across the animals: set in deep evolutionary time and retained because their elimination would be disruptive to the complex, coevolved, multi-organismal networks. There is much to be done to understand the interactions between bacterial products and animal signaling networks, but one core point is becoming clear: bacterial intervention in animal signaling networks is part of how the resident microbiota keeps flies—and possibly us—healthy.

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References and Notes

1. Shin SC, Kim SH, You H, Kim B, Kim AC, Lee KA, Yoon JH, Ryu JH, Lee WJ. *Drosophila* microbiome modulates host developmental and metabolic homeostasis via insulin signaling. *Science*. 2011; 334:670–674. [PubMed: 22053049]
2. Storelli G, Defaye A, Erkosar B, Hols P, Royet J, Leulier F. *Lactobacillus plantarum* promotes *Drosophila* systemic growth by modulating hormonal signals through TOR-dependent nutrient sensing. *Cell Metab*. 2011; 14:403–414. [PubMed: 21907145]
3. Dethlefsen L, McFall-Ngai M, Relman DA. An ecological and evolutionary perspective on human-microbe mutualism and disease. *Nature*. 2007; 449:811–818. [PubMed: 17943117]
4. Nemergut DR, Costello EK, Hamady M, Lozupone C, Jiang L, Schmidt SK, Fierer N, Townsend AR, Cleveland CC, Stanish L, Knight R. Global patterns in the biogeography of bacterial taxa. *Environ Microbiol*. 2011; 13:135–144. [PubMed: 21199253]
5. Wong CN, Ng P, Douglas AE. Low-diversity bacterial community in the gut of the fruitfly *Drosophila melanogaster*. *Environ Microbiol*. 2011; 13:1889–1900. [PubMed: 21631690]
6. Chandler JA, Lang JM, Bhatnagar S, Eisen JA, Kopp A. Bacterial communities of diverse *Drosophila* species: Ecological context of a host-microbe model system. *PLoS Genet*. 2011; 7:e1002272. [PubMed: 21966276]
7. Yakushi T, Matsushita K. Alcohol dehydrogenase of acetic acid bacteria: Structure, mode of action, and applications in biotechnology. *Appl Microbiol Biotechnol*. 2010; 86:1257–1265. [PubMed: 20306188]
8. Ljungh, Å.; Wadström, T. *Lactobacillus Molecular Biology: From Genomics to Probiotics*. Caister Academic; Norfolk, UK: 2009.

9. Gminard C, Rulifson EJ, Lopold P. Remote control of insulin secretion by fat cells in *Drosophila*. *Cell Metab*. 2009; 10:199–207. [PubMed: 19723496]
10. Nunney L. *Drosophila* on oranges: Colonization, competition, and coexistence. *Ecology*. 1990; 71:1904–1915.
11. Douglas, AE. *The Symbiotic Habit*. Princeton Univ. Press; Princeton, NJ: 2010.

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Fig. 1.

The impact of resident bacteria on insulin signaling in *Drosophila*. **(A)** Insulin signaling is promoted, with consequent enhanced larval developmental rates, by acetic acid produced by *A. pomorum* (1) and by *L. plantarum* (with proposed role of branched-chain amino acids, mediated by TOR signaling) (2). Dashed lines represent putative interactions. **(B)** Hypothesized role of bacterial products (A to E) as cues of resource availability, used by *Drosophila* to regulate developmental rates through insulin signaling, either by promoting (1, 2) or reducing *Drosophila* insulin signaling relative to germ-free animals. **(C)** Hypothesized conflict between the bacteria and animal over the setpoint of insulin signaling and correlated larval development rates, such that the realized setpoint for conventional *Drosophila* (bearing microbiota) is intermediate between the bacterial and animal optimum. The realized setpoint for germ-free *Drosophila* is lower than the optimal setpoint for the animal, which is calibrated constitutively to account for bacterial manipulation.