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Role of gut commensal bacteria in juvenile developmental growth of the host: insights from *Drosophila* studies

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ABSTRACT

The gut microbiome plays a crucial role in maintaining health in a variety of organisms, from insects to humans. Further, beneficial symbiotic microbes are believed to contribute to improving the quality of life of the host. *Drosophila* is an optimal model for studying host–commensal microbe interactions because it allows for convenient manipulation of intestinal microbial composition. Fly microbiota has a simple taxonomic composition and can be cultivated and genetically tracked. This permits functional studies and analyses of the molecular mechanisms underlying their effects on host physiological processes. In this context, we briefly introduce the principle of juvenile developmental growth in *Drosophila*. Then, we discuss the current understanding of the molecular mechanisms underlying the effects of gut commensal bacteria, such as *Lactiplantibacillus plantarum* and *Acetobacter pomorum*, in the fly gut microbiome on *Drosophila* juvenile growth, including specific actions of gut hormones and metabolites in conserved cellular signaling systems, such as the insulin/insulin-like (IIS) and the target of rapamycin (TOR) pathways. Given the similarities in tissue function/structure, as well as the high conservation of physiological systems between *Drosophila* and mammals, findings from the *Drosophila* model system will have significant implications for understanding the mechanisms underlying the interaction between the host and the gut microbiome in metazoans.

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Introduction

Ellie Metchnikoff first recognized that certain bacteria are host-friendly and could improve health and delay aging. Since then, numerous researchers have attempted to classify the bacterial species that constitute the gut microbiome and determine how specific species or combinations of species affect host physiology in vertebrates. Despite the use of advanced techniques, such as next-generation sequencing and metatranscriptomic analysis, reports on the effects of particular bacterial species or gut microbiota complexes on host physiology are inconsistent. This can be attributed to the extreme variability in the gut microbiome, which depends on environmental factors as well as the difficulty in artificially changing intestinal microbial communities. The advantages of the *Drosophila* model system have been emphasized for studying host–gut microbe interactions, specifically to overcome the abovementioned limitations. In contrast to mammals, the gut microbiome of *Drosophila* is relatively simple as it is composed of approximately 30 species (Cox and Gilmore 2007; Chandler et al. 2011; Fink et al. 2013; Han et al. 2017). This provides a more accurate method for assessing the effects of different combinations of microbial communities on the host. Additionally, it also provides the perfect opportunity for artificial colonization of single bacterial taxa, co-cultures of multiple taxa, and undefined microbiota in the feces or dissected guts of conventionally reared (CR) flies.

Furthermore, *Drosophila* shares many similarities with mammals in various aspects such as functional organs and physiology (Hyun 2013; Buchon et al. 2014; Lee SH and Kim 2021). For example, insulin/insulin-like (IIS) and target of rapamycin (TOR) signaling pathway are a highly conserved signaling pathway that plays a major role in metazoan growth and development in response to the nutritional status of the host. Thus, *Drosophila* research has been helpful in the current understanding of the role of microbial communities and their functions in host physiology. In this review, we discuss the regulation of body growth and juvenile development and its association with the IIS/

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TOR pathway and summarize the important findings on how gut commensal bacteria regulate appropriate body growth and developmental processes through the IIS/TOR pathway in *Drosophila*.

IIS/TOR *Drosophila* nutrient-dependent pathway and juvenile growth

Organisms must modify their metabolism to adapt to the changing conditions for developmental growth and survival. To achieve this adaptation, they must be able to sense changes in the external environmental conditions and adjust their internal states accordingly. Metabolic adaptability in response to changes in nutritional status is necessary to maintain energy balance and developmental progress (Leopold and Perrimon 2007; Owusu-Ansah and Perrimon 2014). The IIS/TOR signaling pathway is an evolutionarily conserved signaling cascade across metazoans that coordinates organismal growth and development by sensing the internal nutritional status (Wullschleger et al. 2006; Hyun 2013).

The *Drosophila* nervous system plays a vital role in sensing internal nutrient conditions, coordinating metabolic processes, and regulating energy metabolism. When glucose levels rise, insulin-producing cells (IPCs) in the brain release *Drosophila* insulin-like peptides (Dilps) into the bloodstream, mainly Dilp2, Dilp3, and Dilp5 (Hyun 2013; Owusu-Ansah and Perrimon 2014). These Dilps bind to insulin receptors (InR) and trigger downstream signaling. This signaling cascade involves the phosphorylation of Chico, which activates phosphoinositide-3-kinase (PI3 K). PI3 K converts phosphatidylinositol 4,5-diphosphate (PIP2) to phosphatidylinositol 3,4,5-triphosphate (PIP3) (Auger et al. 1989; Lee JO et al. 1999), leading to the recruitment and activation of phosphoinositide-dependent kinase 1 (PDK1) and Akt (Oldham and Hafen 2003; Mora et al. 2004; Lee JE et al. 2021). Akt suppresses the activity of *Drosophila* forkhead box O (dFOXO), a transcription factor that regulates stress responses and cell growth (Arden 2008). Imaginal morphogenesis protein-late 2 (Imp-L2) acts similarly to human IGF-binding protein 7 (IGFBP-7) by binding directly to Dilps and inhibiting their activity, reducing systemic IIS levels (Honegger et al. 2008; Alic et al. 2011). When amino acid levels, especially branched-chain amino acids, increase in the hemolymph, they enter cells through Slimfast amino acid transporters and activate the TOR pathway, which mediates cellular nutrient responses (Colombani et al. 2003). TOR exists in two complexes: TOR complex 1 (TORC1) and TOR complex 2 (TORC2). TORC1 is sensitive to amino acid changes and diet, promoting cell growth by enhancing protein translation and ribosome biogenesis through phosphorylation of the translation initiation factor 4E-binding protein (4EBP) and ribosomal protein S6 kinase (S6 K), respectively (Hara et al. 2002; Yang et al. 2013). On the other hand, TORC2, which does not respond to amino acids or rapamycin, is more involved in insulin signaling and cell regulation (Jacinto et al. 2004; Hietakangas and Cohen 2007; Yang et al. 2013; Jevtov et al. 2015).

During the juvenile growth period, the nutritional status significantly influences the growth and final adult size. In *Drosophila*, the fat body acts as an endocrine organ, much like the mammalian liver and adipose tissues, and plays a pivotal role in regulating body size through the insulin/TOR signaling pathway. Depletion of Slimfast in the fat body leads to systemic growth defects by reducing S6 K phosphorylation, indicating that the fat body can slow down growth in response to decreased amino acids, closely tied to the TOR pathway (Colombani et al. 2003). Circulating Dilps in *Drosophila* promote body growth by binding to InR in the larval fat body, activating the PI3 K signaling pathway and repressing dFOXO. Undernourishment reduces InR-dependent PI3 K activity, causing dFOXO to inhibit cell proliferation and reduce the final body size (Britton et al. 2002; Kramer et al. 2003; Puig et al. 2003).

In humans, the cessation of growth coincides with increased circulating steroid hormones. High-fat and high-protein imbalances in nutrition can lead to early and excessive sex hormone production, causing precocious puberty and a shortened growth period (Tanner and Davies 1985; Calcaterra et al. 2021; Tang et al. 2022), highlighting the close link between nutritional status-driven insulin/TOR signaling and steroid hormone production. In *Drosophila*, ecdysone, a major steroid hormone, is produced in the prothoracic glands through the IIS/TOR pathway (Colombani et al. 2005; Layalle et al. 2008). Ecdysone affects body size by regulating peripheral IIS directly or indirectly. miR-8 in *Drosophila* plays a role in ecdysone-dependent growth regulation by targeting *U-shaped* (*Ush*), the repressor of PI3 K, and the upregulation of miR-8 increases peripheral IIS activity and final body size (Hyun et al. 2009; Jin et al. 2012). Additionally, ecdysone-induced Imp-L2 production in the larval fat body suppresses peripheral IIS activity and body growth (Lee GJ et al. 2018).

In summary, these studies suggest that the IIS/TOR pathway, body growth rate, and sex hormone secretion are interconnected, with the final size determined by the length of the juvenile growth period and the rate of body growth (Hyun 2013, 2018) (Figure 1).

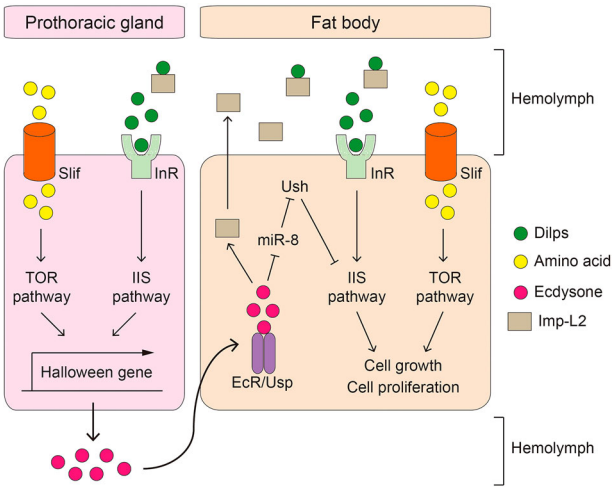

Figure 1. The molecular pathways involved in *Drosophila* juvenile development and growth. Amino acids in the hemolymph enter cells through Slimfast (Slif) and thereafter activate TOR signaling. *Drosophila* insulin like peptides (Dilps) bind to insulin receptor (InR), thereby activating the insulin/insulin-like growth factor signaling (IIS) pathway. Both signaling pathways stimulate transcription of Halloween genes in the prothoracic gland, which facilitates the synthesis of ecdysone. The release of ecdysone into the hemolymph promotes fly development. Additionally, Dilps and amino acids stimulate IIS and TOR signaling in the larval fat body, respectively, to promote cell growth and proliferation. Activation of IIS/TOR signaling in the larval fat body is thought to contribute to body growth rate through regulation of non-autonomous tissue growth; however, the underlying mechanisms are still elusive. Ecdysone in the hemolymph penetrates the larval fat body and immediately binds to the ecdysone receptor (EcR). When EcR is activated, it secretes imaginal morphogenesis protein-Late 2, which inhibits the activity of Dilps and prevents the activation of the systemic IIS pathway.

Anatomy and functions of the *Drosophila* gut

The gut is one of the largest organs in the body and plays a key role in the digestion and absorption of nutrients. Additionally, it forms the largest immune epithelial barrier and serves as the first line of defense against ingested pathogens. The structure and functions of the *Drosophila* gut are analogous to those of mammals, which is why it is a widely used alternative model for research on the human gastrointestinal system. The *Drosophila* gut can be divided into the midgut and hindgut, which correspond to the small and large intestines, respectively. Similar to mammal, the distribution of predominant bacteria may vary depending on the intestine tract (Donaldson et al. 2016; Chiang et al. 2022). After ingestion, food passes through the esophagus to the midgut, where it is broken down into small units by digestive enzymes, including proteases, carbohydrates, and lipases. Subsequently, the hindgut selectively absorbs nutrients, water, and electrolytes. The *Drosophila* gut comprised four cell types: intestinal stem cells (ISCs), enteroblasts (EBs), differentiated enterocytes (ECs), and enteroendocrine cells (EEs). ISCs and EBs are undifferentiated and differentiated ISC daughter cells, respectively, and the latter can differentiate into two types of functional cells: ECs and EEs. Similar to mammalian goblet cells, ECs secrete digestive enzymes, absorb and transport nutrients, and maintain epithelial barriers by secreting mucus. EEs are scattered throughout both *Drosophila* and mammalian guts, and sense various environmental stimuli and secrete hormonal peptides to regulate the host's nutritional status. For detailed information on the structure and function of the *Drosophila* gut, readers can refer to other excellent reviews (Wang and Hou 2010; Apidianakis and Rahme 2011; Miguel-Aliaga et al. 2018).

The microbiome composition and effective bacteria species involved in *Drosophila* juvenile growth

For many years, numerous studies have investigated the *Drosophila* intestinal microbiome using 16S rRNA pyrosequencing. These studies have found that flies generally support relatively simple microbial communities, composed of approximately 30 species, represented by two phyla, Firmicutes and Proteobacteria, and further dominated by two major families, *Acetobacteraceae* and *Lactobacillaceae,* and two minor families, *Enterococceae* and *Enterobacteriaceae* (Cox and Gilmore 2007; Chandler et al. 2011; Fink et al. 2013; Han et al. 2017). Interestingly, *Drosophila* reared on complex polysaccharide diets, such as cornmeal or soy flour, have a high abundance of *Lactiplantibacillus* species, whereas *Drosophila* reared on sugar-rich diets have microbiomes dominated by *Acetobacteraceae*, particularly *Acetobacter* and *Gluconobacter* species (Chaston et al. 2014; Huang and Douglas 2015). According to the nutrient niche theory, ecological niches in the gut can be defined by available nutrients, and only a few bacterial species that efficiently use partially limiting nutrients can be colonized (Pereira and Berry 2017). Several analyses of fly gut microbiota collected from various geographic locations and laboratory sources have shown that bacterial community abundance is dependent on food preferences, implying that gut microbial composition is primarily determined by the host's diet and nutritional environment (Staubach et al. 2013; Wong AC et al. 2013). Despite considerable variation at the species level, *Lactiplantibacillus* and *Acetobacter* mostly inhabit the gut and both genera likely play important roles in the physiology of their hosts. Researchers have attempted to understand the specific commensal bacteria-dependent effects on host traits,

and focused on how specific commensal bacteria affect body growth and development during the juvenile period in *Drosophila*. Germ-free (GF) animals can be easily created using the *Drosophila* model system and numerous tools are available for temporal expression, down-regulation, and tissue-specific gene expression. Moreover, *Drosophila* dynamically exhibits changes in body size and developmental metamorphosis during its short life cycle, thereby facilitating quick and easy observation of bacteria-dependent effects.

Lactiplantibacillus species

Lactiplantibacillus species (formerly known as *Lactobacillus*) are found in a wide range of ecological niches including the mucosal surfaces of several animals. Although the probiotic effects of diverse *Lactiplantibacillus* species have been explored in detail, there is a limited understanding of the processes through which *Lactiplantibacillus* species alter animal physiology (Oelschlaeger 2010). Members of the genus *Lactiplantibacillus*, including *Lactiplantibacillus plantarum*, *Lactiplantibacillus fructivorans*, and *Lactiplantibacillus brevis* are dominant in the *Drosophila* gut and are gram-positive, rod-shaped, lactic acid-producing microaerophilic bacteria from the Firmicutes phylum. Among these, *L. plantarum*^{WJL} (hereinafter referred to as Lp) was reported to promote *Drosophila* larval development (Shin et al. 2011; Storelli et al. 2011; Lee J et al. 2020). Under nutritional scarcity, GF larvae showed developmental delays and lower body growth rates than CR larvae, which was reversed when GF larvae were mono-associated with Lp (Storelli et al. 2011). The body growth and development-promoting effects of Lp were lost when the larvae were fed a nutrient-rich diet but were noticeable when the host had nutritional deficits. Further, inactivation of the amino acid transporter Slimfast in the larval fat body and PG was sufficient to disrupt Lp-induced body growth and juvenile development, respectively (Storelli et al. 2011). Therefore, Lp is hypothesized to promote both body growth and juvenile development by providing the host with essential amino acids for proper TOR activity (Figure 2).

Upon microbial infection, *Drosophila* rapidly induces the expression of a series of antimicrobial peptides (AMPs) including diptericins, drosomycin, and attacins. Induction of AMPs is primarily controlled by the *Drosophila* homolog of the Nuclear factor kappa B transcription factor. In flies, two different pathways respond to microbial infections: the Toll and IMD pathways. In general, the Toll pathway recognizes Gram-positive bacteria with lysine-type peptidoglycan in the cell wall, while the IMD pathway recognizes Gram-negative and

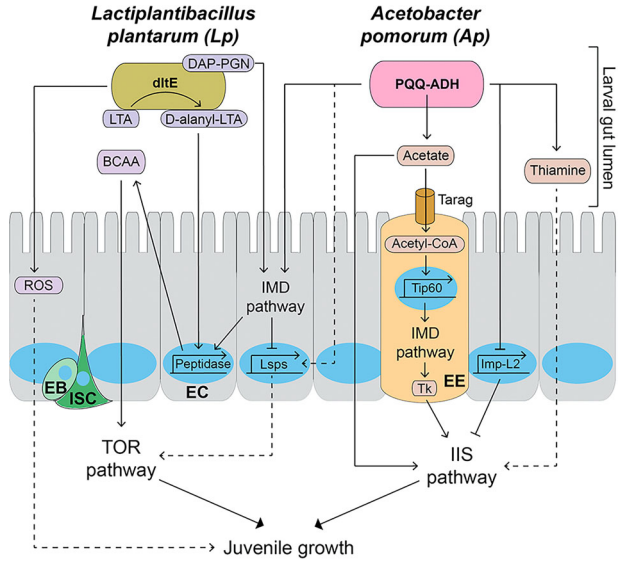

Figure 2. The molecular pathways mediating the effects of *Lactiplantibacillus plantarum* (Lp) and *Acetobacter pomorum* (Ap) on *Drosophila* juvenile development and growth. The IMD pathway recognizes the diaminopimelic acid-type peptidoglycan in cell walls of Lp and Ap, and promotes peptidase gene transcription in enterocytes (ECs). D-alanylated-lipoteichoic acid in Lp is modified from lipoteichoic acid by dltE and induces peptidase transcription in ECs, independently of the IMD pathway. The pyrroquinoline quinone-dependent alcohol dehydrogenase enzyme in Ap produces acetate, which stimulates the IIS pathway, promoting body growth and development. Acetate in the gut lumen enters enteroendocrine cells (EEs) via a monocarboxylic acid transporter called Tarag and is converted into acetyl-CoA. Acetyl-CoA pools stimulate the Tip60 histone acetylase complex, leading to an increase in the IMD pathway at the transcriptional level. The IMD pathway in EE activates the systemic IIS pathway by increasing the expression of Tk. Ap promotes the expression of larval serum proteins (Lsps) the IMD pathway in ECs, which is suppressed by IMD pathway. Lsps in the hemolymph are likely up taken in nutrition-sensing tissues such as fat bodies by endocytosis and stimulate the TOR pathway and larval development. Ap suppresses the expression of Imp-L2 via ecdysone signaling in ECs, which in turn stimulates the IIS pathway. Thiamine secreted from Ap can regulate larval development; however, it's the underlying mechanism is not clear. DAP-PGN: diaminopimelic acid-type peptidoglycan. Nucleus is described as a blue circle or an oval. Dotted lines indicate putative pathways. LTA: Lipoteichoic acid. D-alanyl-LTA: D-alanylated-lipoteichoic acid. ROS: Reactive oxygen species. BCAA: Branched-chain amino acids. Lsps: Larval serum proteins. PQQ-ADH: Pyrroquinoline quinone-dependent alcohol dehydrogenase. Tk: Tachykinin. EC: Enterocyte. EE: Enteroendocrine cell. EB: Enteroblast. ISC: Intestinal stem cell.

Gram-positive bacteria with diaminopimelic acid (DAP)-type peptidoglycan (Leulier et al. 2003; Buchon et al. 2014). Over-activation of the IMD pathway during the juvenile growth period results in smaller final size, developmental delay, and disruption of metabolic homeostasis via decreased IIS/TOR signaling, but the mechanism underlying the influence of gut commensal bacteria on

IMD pathway-mediated modulation of larval development remains unknown (Davoodi et al. 2019). By comparative transcriptomic analysis of GF flies and CR flies, it was reported that multiple peptidase genes are significantly elevated in the presence of gut commensal bacteria, and their expression was partly dependent on the IMD pathway (Erkosar et al. 2014). Based on this finding, the authors hypothesized that Lp, which has DAP-type peptidoglycan, regulates peptidase expression through the IMD pathway. Mono-association of Lp was sufficient to induce an overall increase in midgut-enriched peptidases, and ectopic overexpression of *Jon66Cii* peptidases in the ECs of GF larvae was sufficient to promote larval body growth (Erkosar et al. 2015). Consistent with previous observations, the loss of the IMD pathway partially abolished Lp-induced peptidase expression and growth promotion. Interestingly, D-alanylated teichoic acids (D-Ala-TAs) in the cell wall of Lp promote body growth and juvenile development via gut peptidase expression during chronic nutrition deficiency, and their effect is independent of the IMD pathway (Matos et al. 2017). Teichoic acids (TAs) are anionic polymers found within the cell walls of Gram-positive bacteria, contributing up to 50% of the cell envelope weight, and are present in two forms: wall TAs and lipoteichoic acids (LTAs). Encoded-*Dlt* genes from *pbpX2-Dlt* operon in other *Lactiplantibacillus* species are involved in bacterial cell wall biogenesis, and the inactivation of *Dlt* genes causes a significant reduction in the esterification of TAs by D-alanine. Deletion of the entire *Dlt* operon in the Lp^{NC8} strain (*Lactiplantibacillus plantarum*^{NC8}) leads to a reduction in the D-alanine esterification of TAs, which slightly increases gut peptidase expression and larval growth. Recently, only D-Ala-LTAs produced by *Dlt* genes have been shown to be direct triggers that promote intestinal peptidase expression and body growth (Nikolopoulos et al. 2023). This suggests that the detection of specific cell envelope structures of commensal bacteria is critical for *Drosophila* to benefit from their interactions with symbiotic microorganisms.

Reactive oxygen species (ROS) were initially thought to be harmful to cell integrity, being the underlying cause of numerous diseases and aging. However, ROS plays an important roles in maintaining proper physiology and homeostasis in insects and humans (Lopez-Otin et al. 2013; Liu et al. 2018; Sinenko et al. 2021). The gut microbiome affects many aspects of host physiology, such as intestinal cell proliferation and pathogen resistance, by producing ROS (Ballard and Towarnicki 2020). Specifically, Lp stimulates *Drosophila* NADPH oxidase-dependent ROS production in ECs and subsequently activates Nrf2-responsive cytoprotective genes (Jones et al. 2013, 2015). In addition to contributing to host nutrition, Lp-induced ROS effectively inhibits phenotypic variation in host developmental traits under low-nutrient stress (Ma et al. 2019). Consistently, Lp-associated larvae had fewer developmental trait abnormalities than GF larvae, including smaller larval body length and delayed developmental time. The mechanism underlying this buffering effect is still unclear; however, the antioxidant N-acetylcysteine compromises the buffering capacity of Lp (Ma et al. 2019).

Acetobacter species

Drosophila is often referred to as 'vinegar flies,' most likely because they are constantly present near vinegar manufacturers. *Drosophila* has been shown to play a key role in acetic acid synthesis through its function as a vector for microorganisms required for acetic acid production. Members of the genus *Acetobacte*r, including *Acetobacter pomorum*, *Acetobacter pasteurianus*, *Acetobacter tropicalis*, and *Acetobacter aceti* are dominant in the gut microbiota of *Drosophila* and are Gram-negative acetic acid-producing bacteria (Ren et al. 2007; Ryu et al. 2008; Wong CNA et al. 2011). Shin et al. found *A. pomorum* (hereinafter referred to as Ap) modulates host homeostatic programs that control the developmental rate and final body size by regulating the IIS pathway. Genetic investigations have revealed that the pyrroquinoline quinone-dependent alcohol dehydrogenase (PQQ-ADH) enzyme in Ap, which catalyzes the oxidation of alcohols into acetic acid, plays a crucial role in the modulatory effect of Ap on the host IIS pathway (Yakushi and Matsushita 2010; Shin et al. 2011). However, extensive research has not yet been conducted on the underlying mechanisms through which AP-derived acetic acid regulates insulin signaling.

Acetate, a primary short-chain fatty acid, is secreted by gut bacteria, including *Acetobacter* species. Acetate deficiency in the gut interferes with the maintenance of several physiological functions, including body weight control (Hernandez et al. 2019; Fan and Pedersen 2021). Watnick et al. proposed a mechanism by which acetate produced by bacteria, including Ap, regulates host insulin signaling. Initially, they found that *Vibrio cholerae*, a pathogen that causes the diarrheal illness cholera in humans, affects host insulin signaling and lipid metabolism via acetate depletion through the acetate-switch process in the *Drosophila* gut (Hang et al. 2014). Subsequent studies have shown that microbial-derived acetate activates host insulin signaling by modulating the secretion of tachykinin through the IMD pathway in EEs in the gut (Kamareddine et al. 2018). Tachykinin is a gut endocrine peptide hormone

produced by EEs that regulates lipogenesis in ECs via the PKA/SREBP signaling pathway (Song et al. 2014). Mono-association of Ap and supplementation with dietary acetate activates the IMD pathway and restores body growth and puparium morphogenesis in GF larvae by elevating IIS signaling, but these effects were abolished in the EE-specific IMD pathway knockdown mutant. Acetate generated by gut bacteria enters EEs through the monocarboxylic acid transporter Tarag, and is converted into acetyl-CoA by acetyl-CoA synthase. Tip60 histone acetyltransferase activates EcR signaling by acetylating H2Av using intracellular Acetyl-CoA as a substrate, thereby activating the IMD pathway (Jugder et al. 2021).

In line with this, our group recently reported that Ap can modulate larval development independently of acetate derived from microbes. Mono-association of Ap increases systemic IIS activity by suppressing Imp-L2 production in ECs in the larval gut (Lee J et al. 2022). Increased production of Imp-L2 in ECs was followed by a decrease in the activity of the PI3K–Akt axis in both the larval gut and fat bodies of GF larvae; however, these effects were restored by mono-association with Ap in GF larvae. Neither the mono-association of the PQQ-ADH mutant nor acetate treatment altered Imp-L2 production. Ap modulates ecdysone signaling-dependent Imp-L2 production from ECs, as indicated by the observation that inactivation of EcR prevents the decrease in Imp-L2 production noted in mono-association of Ap. Additionally, our group reported that Ap can regulate the rate of fly larval development by modulating the expression of Larval serum proteins (Lsps) (Lee J et al. 2023). During the larval stage, Lsps provide a source of amino acids, allowing insects to efficiently store excess nutrients in the hemolymph (Roberts et al. 1977; Powell et al. 1984; Telfer and Kunkel 1991; Burmester 1999; Short et al. 2020). We found that mono-association of Ap increased the expression of *Lsps* in *Drosophila* larval gut, which is suppressed by IMD immune pathway. Knockdown of *Lsps* in gut enterocytes delayed larval developmental rate, which became severe when Ap was mono-associated. Thus, multiple pathways, including IMD, appear to regulate Ap's impact on larval developmental rate in opposing directions (Lee J et al. 2023).

Vitamins are essential micronutrients that function as precursors to various coenzymes required for important biochemical reactions in all living cells. It has been hypothesized that the gut microbiota provides the host with various types of vitamin B, such as thiamine, riboflavin, and folate (Hill 1997; LeBlanc et al. 2013). Thiamine is an essential cofactor for several metabolic pathways. Specifically, thiamine pyrophosphate is a coenzyme involved in various enzymatic activities associated with cell regulatory processes and biosynthesis (Rindi and Laforenza 2000). A recent study reported that the gut microbiota of *Drosophila*, particularly Ap, produces thiamine, which is involved in juvenile development of the host (Sannino et al. 2018). The authors found that although GF larvae rarely develop on a thiamine-free diet, dietary thiamine promotes the larval development of *Drosophila* in a dose-dependent manner. Further, they found that Ap could restore the developmental failure of GF larvae by producing thiamine. Although it is unknown how thiamine affects developmental processes in *Drosophila* during the juvenile stage, a few studies have suggested that thiamine regulates the IIS pathway. Thiamine deficiency significantly inhibits insulin production and secretion in vertebrates. High levels of thiamine are stored in human pancreatic beta cells and islets, and its absorption is carrier-mediated and adaptively controlled by the prevailing vitamin levels via transcriptional processes (Rathanaswami et al. 1991; Mee et al. 2009). Consistently, rats fed a thiamine-deficient diet had lower blood insulin levels and transmembrane glucose transport (Debski et al. 2011). High-dose thiamine supplementation may relieve clinical signs of the condition, and may reduce or ablate the requirement for exogenous insulin (Olsen et al. 2007). These findings indicate that metabolites, such as acetate and thiamine, secreted by Ap regulate body growth and developmental processes of the host by activating various signaling pathways.

Enterococcus species

Enterococci are lactic acid-producing Gram-positive bacteria (Hanchi et al. 2018) that reside in the gastrointestinal tract of a wide range of mammals (Mundt 1963) and insects (Steinhaus 1941; Martin and Mundt 1972). A small proportion of *Enterococcus* species inhabit the *Drosophila* gut (Han et al. 2017). The most prevalent of these are *Enterococcus faecalis*, followed by *Enterococcus durans*, *Enterococcus faecium*, and *Enterococcus gallinarum,* which together account for less than 10% of the overall enterococcal population (Cox and Gilmore 2007). Although *Enterococcus* are known as pathogenic bacteria that cause hospital-acquired infections, and infection with *E. faecalis* reduces adult *Drosophila* survival (Huycke et al. 1998; Cabrera et al. 2023), a few species have also been utilized as probiotics (Huycke et al. 1998; Edwards 2000; Hanchi et al. 2018). Little is known about the effects of colonized *Enterococci* in the gut on juvenile development of other insects. Inoculation of *E. mundtii* and *E. gallinarum* in fall armyworm promoted body growth compared with GF larvae.

Colonization of *E. faecium* in the gut of honey bees (*Apis mellifera*) increases midgut and hindgut weight and transcriptome analysis and microRNA profiling showed *E.faecium* in the gut influences host developmental genes including TOR pathway (Du et al. 2021). However, *E. faecalis* did not directly affect the body growth and pupation time of *Bombyx mori* (Chen et al. 2022; Zhang et al. 2022). These findings suggest that most enterococcus species have distinct functions, and the effect changes depending on the type of host. Thus, it is necessary to further investigate the characteristics of each *Enterococcus* species as well as their effects on host physiology, including body growth.

Effects of microbial metabolites produced from gut microbiome on host growth and gut microbial community

The gut microbiome connects the hosts to themselves through a network of nutrition and metabolism, allowing these interactions to directly affect host nutrition and metabolism. It is believed that an imbalance in gut microbiome composition is involved in the pathogenesis of a variety of diseases, such as cancers, metabolic disorders, and developmental disabilities, and that the gut microbiome composition that is favorable to the host or functions as a pathogen will exist separately (Gagniere et al. 2016; Gomaa 2020; Fan and Pedersen 2021). Microbial metabolites are considered major environmental factors that determine the genetically encoded growth potential of organisms and enable them to sustain distinct gut microbiota communities (Sommer and Backhed 2013; Moya and Ferrer 2016; Krautkramer et al. 2021). Malnourished children have an immature gut microbiome, and the gut microbiome compositions of healthy and growth-stunted twins are clearly different (Smith et al. 2013). When the fecal microbiome of the healthy or stunted twin is transplanted into genetically identical GF mice fed a poor diet, recipients of the microbiome from stunted twins exhibit lower growth and body weight recovery. Even the immature gut microbiome in severely malnourished children interferes with the restoration of healthy growth through classical re-nurturing treatments (Subramanian et al. 2014). Previous studies have proposed the following: (1) there may be an optimal gut microbiome composition that supports body growth; (2) microbial metabolites produced in the gut microbiome are used as nutrients for body growth and may be difficult to replace; (3) microbial metabolites play key roles in maintaining or modifying the composition of the gut microbiome. Therefore, understanding the types and mechanisms of microbial metabolites produced in the gut microbiome will broaden insights into host–gut microbiota interactions.

The *Drosophila* model system can be used to analyze the contribution of gut commensal bacteria to organismal body development and their composition in the gut under strictly controlled nutritional conditions using a holidic diet (HD) with a chemically specified fly medium. It was reported that GF larvae are auxotrophic for ten essential amino acids, one non-essential amino acid (Asn), six types of vitamin B (1, 2, 5, 6, 7, and 9), vitamin E, cholesterol, choline, Mg ions, and Zn ions during the juvenile phase, and mono-association with Lp or Ap differentially allows GF larvae to develop normally (Consuegra, Grenier, Baa-Puyoulet, et al. 2020). Except for in liquid HD without a few traces, Ap grew well in other conditions; however, Lp^{NC8} did not grow in an environment lacking the most necessary amino acids (Arg, Ile, Leu, Met, Phe, Thr, and Val), several non-essential amino acids (Ala, Asp, and Cys), biotin, and pantothenate. Given that Ap and Lp are prevalent in the fly gut microbiome independent of changes in the majority of the internal/external environment, it is speculated that Ap and Lp aid each other's growth by supplying essential nutrients, and that their metabolic cooperation enhances the development of the host. A subsequent study found that the bi-association of Ap and Lp^{NC8} not only increased body growth during larval development but also enhanced bacterial growth in the gut through exchange of microbial metabolites (Consuegra, Grenier, Akherraz, et al. 2020). During nutrient scarcity, Ap helps the growth of Lp^{NC8} by supplying essential amino acids and vitamins. Conversely, Lp^{NC8} secretes lactate, which promotes the production of amino acids by Ap. This metabolic cooperation enhances the promotive effects of body growth; however, the amino acids generated by Ap using lactate had no direct effect on larval body growth. Together, these findings suggest that microbial metabolites from gut commensal bacteria act as essential nutrients for the developmental processes of the host and for bacterial growth in the gut.

Concluding remarks

The juvenile stage is a key phase in most metazoans that determines the final adult size, as well as defines the quality of life and ability to survive in the wild. Many environmental factors, including nutritional conditions, affect body development during the juvenile stage. The IIS/TOR pathway is a core effector of body growth and developmental maturation, and is regulated by nutritional availability. Over the past decade, it has been highlighted that the relationship between host nutrition and gut microbiome is crucial for maintaining

host physiology. The physiological systems of animals, including tissue structure/function and developmental processes, are well conserved in the *Drosophila* model system. Furthermore, *Drosophila* is recognized as an excellent model for studying the interactions between gut commensal bacteria and the host because it has a relatively simple gut microbiota complex and can be readily sterilized and colonized by specific bacterial species. *Lactiplantibacillus* and *Acetobacter* species are major constituents of the fly gut microbiome, and both Lp and Ap contribute to body growth and developmental maturation of the host by primarily regulating the IIS/TOR pathway under nutrient-deficient conditions. In short, gut commensal bacteria affect host physiology in three main ways: (1) through recognition of the bacterial cell wall structure via immune pathways; (2) production of bacteria-derived metabolites that confer beneficial effects to the host and/or other gut commensal bacteria; and (3) modulation of the nutritional availability and other physiological pathways in the gut. In particular, recent studies have extensively explored the types and formation principles of gut microbiome-derived or gut microbiome-modified metabolites, and have challenged us to expand our understanding of the functions and mechanisms underlying metabolic activities in host–gut microbiome interactions (Agus et al. 2021; Krautkramer et al. 2021). Future elucidation of the mechanisms underlying the regulation of body growth and microbial metabolites during the juvenile phase in *Drosophila* could provide new insights into the pathogenesis of various growth disorders, hormonal imbalances, and metabolic dysfunctions.

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