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Article

Lactobacillus plantarum Promotes *Drosophila* Systemic Growth by Modulating Hormonal Signals through TOR-Dependent Nutrient Sensing

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SUMMARY

There is growing evidence that intestinal bacteria are important beneficial partners of their metazoan hosts. Recent observations suggest a strong link between commensal bacteria, host energy metabolism, and metabolic diseases such as diabetes and obesity. As a consequence, the gut microbiota is now considered a "host" factor that influences energy uptake. However, the impact of intestinal bacteria on other systemic physiological parameters still remains unclear. Here, we demonstrate that *Drosophila* microbiota promotes larval growth upon nutrient scarcity. We reveal that *Lactobacillus plantarum,* a commensal bacterium of the *Drosophila* intestine, is sufficient on its own to recapitulate the natural microbiota growth-promoting effect. *L. plantarum* exerts its benefit by acting genetically upstream of the TOR-dependent host nutrient sensing system controlling hormonal growth signaling. Our results indicate that the intestinal microbiota should also be envisaged as a factor that influences the systemic growth of its host.

INTRODUCTION

For historical reasons and biomedical concerns, bacteria have been mainly studied for their harmful effects on human health. However, growing evidence suggests that bacteria are also important beneficial partners of metazoans (Fraune and Bosch, 2010). Interactions between bacteria and their animal hosts can be viewed in terms of a continuum ranging from symbiosis or commensalism to pathogenicity (Hooper and Gordon, 2001). The term commensalism comes from the Medieval Latin "commensalis," meaning "eating at the same table," and refers to a host-microbial interaction that does not result in perceptible host damage (Casadevall and Pirofski, 2000). As opposed to saprophytes that live independently of an animal host, commensal bacteria colonize their host generally at birth, through vertical transfer, and are acquired constantly during the host life from the environment through ingestion. Therefore, numerous commensal bacteria reside in the host intestine, a nutrient-rich environment, where they form a vast, complex, and dynamic consortium of indigenous microbial species collectively referred as the microbiota (Hooper and Gordon, 2001).

Although it has been known for decades that humans carry ten times more bacterial cells than their own cells (Savage, 1977), the human microbiota characterization has previously been hampered by the difficulty of cultivating most gut bacterial species in laboratory conditions. Thanks to the revolution of deep-sequencing technologies, the commensal metagenome now starts to be unraveled (Furrie, 2006). Recent studies suggest that it contains about 150 times more genes than the human gene complement and shows a significant enrichment in genes encoding metabolic activities (Gill et al., 2006; Nelson et al., 2010; Qin et al., 2010). Hence, the idea that the intestinal microbiota constitutes an additional organ has recently re-emerged (Bocci, 1992; O'Hara and Shanahan, 2006). Intestinal bacteria communities shape the nutrient environment of the host by contributing enzymatic activities that break down otherwise non-digestible carbohydrates (Hooper et al., 2002). They also salvage energy through carbohydrate fermentation, leading to the production of short-chain fatty acids (Venema, 2010). In this light, the gut microbiota is now deemed a "host" factor that influences energy uptake (Bäckhed et al., 2005). The link between commensal bacterial communities and energy metabolism is further supported by recent evidence suggesting a strong association between the composition of the intestinal microbiota and metabolic diseases such as diabetes and obesity (Burcelin et al., 2009; Cani and Delzenne, 2009). However, the molecular mechanisms through which microbiota exerts its beneficial or detrimental influences remain largely undefined (Sekirov et al., 2010). Important unsolved basic questions are still standing in the field. For instance, do specific bacterial strains account for the benefit or the damage caused by the microbiota, and if so, which ones? In addition, besides optimizing energy harvest, do commensal bacterial species influence other systemic physiological parameters? Bacterial complement referred as "probiotics" have now been used for decades in the farming industry to promote growth of poultry, calves, and pigs; however, the precise mechanisms underlying these enhancements are still highly debated (Delzenne and Reid, 2009; Ehrlich, 2009; Raoult, 2009; Simon, 2005). These debates highlight the need of using experimental models to evaluate the role of intestinal bacteria as animal growth promoters.

To tackle these biological questions, we used *Drosophila melanogaster* as a host model. Indeed, over the last 4 years *Drosophila* has emerged as a powerful animal model to study host-commensal biology. Wild or lab-raised *Drosophila* carry simple bacterial communities composed of a maximum of 20 species with usually 3–4 dominant Lactobacillale and Acetobacteraceae species (Corby-Harris et al., 2007; Cox and Gilmore, 2007; Ren et al., 2007; Ryu et al., 2008). Recent reports, including our work, have begun to illustrate the molecular dialog between the microbiota and the intestinal epithelium. The *Drosophila* microbiota promotes immunomodulation by triggering the expression of negative regulators of innate immune signaling in intestinal epithelial cells (Lhocine et al., 2008; Ryu et al., 2008) and influences epithelial homeostasis through the promotion of intestinal stem cell activity (Buchon et al., 2009). A previous report suggested that the indigenous bacteria promote *Drosophila* lifespan (Brummel et al., 2004), supporting the idea that the *Drosophila* microbiota contributes somehow to its host biology; however, this observation is now seriously questioned (Ren et al., 2007). Although it has recently been shown that *Drosophila* commensal bacteria influence their host mating preference and are likely to severely impact *Drosophila* ecology in its natural environment (Sharon et al., 2010), the contribution of the microbiota to its host physiology is currently unknown. In this study, we demonstrate that the *Drosophila* gut microbiota promotes larval growth upon nutrient scarcity. We further identify the bacterial species present in the gut of our laboratory fly strain and show that one of them, *Lactobacillus plantarum*, recapitulates the microbiota growth-promoting effect. Finally, we show that *L. plantarum* exerts its beneficial effect on larval growth through the host nutrient sensing system, which relies on tissue-specific TOR activity controlling systemic hormonal growth signaling.

RESULTS

Drosophila Microbiota Sustains Optimal Larval Development upon Nutrient Scarcity

The growth phase of insects is restricted to the larval stages, where size gain can be spectacular in certain species. In *Drosophila melanogaster*, individuals increase their size by about 200-fold during the three larval instars (Robertson, 1963). This massive larval growth is fully dependent on food richness, since culture on poor-nutrient medium severely impacts *Drosophila* systemic growth and results in a marked delay of adult emergence (Layalle et al., 2008; Robertson, 1963). In order to test the putative contribution of *Drosophila* microbiota to its host systemic growth, we compared the timing of adult emergence of germ-free (GF) and conventionally reared (CR) siblings. Although no significant difference was observed between GF and CR larvae raised on rich medium (Figures 1A and 1B), spectacular growth delays were noticed when larvae were reared on poor-nutrient conditions. Consistent with previous reports, reduction of the amount of yeast extract in the medium results in about 2.5 days delay of adult emergence for CR individuals (Figures 1A and 1B) (Layalle et al., 2008). Strikingly, this delay was more than doubled for individuals raised in GF conditions, since GF adults emerged 2.9 days later than their CR siblings (Figures 1A and 1B; Table S1). These data demonstrate that although the *Drosophila* microbiota is dispensable for larval growth, it is necessary for optimal larval development upon nutrient scarcity.

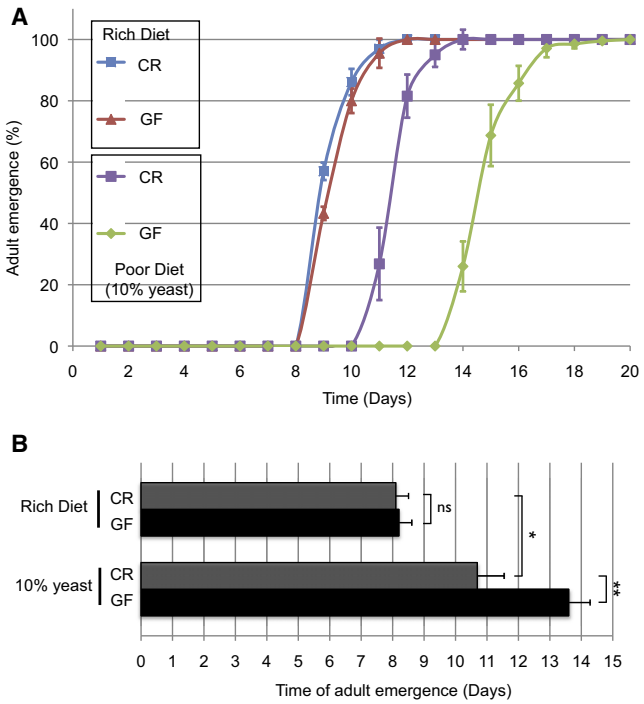

Figure 1. *Drosophila* Microbiota Sustains Optimal Larval Development upon Nutrient Scarcity

(A) Developmental timing of germ-free (GF) or conventionally reared (CR) individuals grown on either rich or poor diet (10% yeast). The cumulative percentage of the adult population emergence is shown over time. Data represent the mean of n biological replicates containing at least 30 individuals each ± SEM (n = 8 for CR*yw*/poor diet, purple square; n = 7 for GF*yw*/poor diet, green diamond; n = 6 for CR*yw*/rich diet, blue square; n = 6 for GF*yw*/rich diet, red triangle).

(B) Mean time of the emergence of 10% of the whole germ-free (black) or conventional (gray) adult population grown on either rich or poor diet (10% yeast). Statistical significance of the results is included (Student's t test, ns $p > 0.05$; *$p < 0.05$; **$p < 0.01$).

Lactobacillus plantarum Colonizes *Drosophila* Midgut

In order to identify which commensal bacterial species mediate this effect, we characterized the bacterial communities associated with our CR fly strain. To this end, we generated bacterial 16S rRNA gene libraries from whole flies and dissected midguts. Analyses of clone sequences indicate that each library contains 16S clones of three bacterial phylotypes, one unique to each library (an *Aerococcus* spp. strain identified in whole flies and a *Corynebacterium variabile* strain identified in midguts) and two common dominant species (*Enterococcus faecalis* and *Lactobacillus plantarum*) (Table 1). These latter species were previously found to be associated with adult *Drosophila* intestines and are likely to be commensal with *Drosophila* (Cox and Gilmore, 2007; Ryu et al., 2008). We then tested whether *L. plantarum* and *E. faecalis* have the ability to colonize *Drosophila* gut. To this end, GF embryos were cultured on rich or poor medium supplemented with 10^8 cfu of either bacterial

Table 1. Bacterial Species Associated with Our Conventionally Reared Wild-Type Fly Strain

CR*yw* Whole Body Library		
Phylotype	Closest strain	% identity
Enterococcus faecalis	*Enterococcus faecalis* V583	99%
Lactobacillus plantarum	*Lactobacillus plantarum* WCFS1	99%
Aerococcus spp.	*Aerococcus viridans* ATCC11563	97%
CR*yw* Adult Midgut Library		
Phylotype	Closest strain	% identity
Enterococcus faecalis	*Enterococcus faecalis* V583	99%
Lactobacillus plantarum	*Lactobacillus plantarum* WCFS1	99%
Corynebacterium variabile	*Corynebacterium variabile* DSM20132	98%

species, and internal bacterial loads were quantified at different developmental stages following this inoculation (Figures 2A and S1A). One day after the inoculation, both *L. plantarum* and *E. faecalis* were detected in larvae, suggesting that both species can colonize young larvae. However, kinetics between *L. plantarum* and *E. faecalis* began to diverge the following day. *L. plantarum* load kept on increasing during larval development, whereas *E. faecalis* titers constantly dropped down, ultimately reaching an undetectable level at late larval stage (Figures 2A and S1A). These data suggest that *L. plantarum*, unlike *E. faecalis*, has the ability to remain associated with *Drosophila* long after an initial colonization. The fact that similar *L. plantarum* quantities were found in whole individuals and in dissected midguts demonstrates that *L. plantarum* resides in the midgut after colonization (Figures 2B, 2C, and S1B). Finally, we tested whether the presence of *L. plantarum* in the gut required constant reassociation by feeding on contaminated medium. To this end, young larvae colonized by *L. plantarum* were surface sterilized and transferred to GF culture medium. The bacterial loads of larvae as well as the bacterial load on the medium were quantified over time. In this experimental setting, *L. plantarum* titers were similar to those observed in nontransferred larvae. In addition, *L. plantarum* was able to efficiently recolonize the medium (Figures 2D, 2E, S1C, and S1D). These results demonstrate that *L. plantarum* remains associated with its host upon transfer and that larval gut-derived *L. plantarum* has the ability to recolonize the entire larval niche upon transfer. Although these results highlight the commensal behavior of *L. plantarum* and *Drosophila* larvae, we cannot exclude that *L. plantarum* is constantly recolonizing its host by repeated ingestion. Taken collectively, these results reveal the potent ability of *L. plantarum* to efficiently colonize the whole larval niche, including its host midgut and the external media, and to resist the passage through the digestive tract of its host.

L. plantarum Recapitulates Conventional Microbiota Association

We next asked whether the parameters of the *Drosophila* monoassociation with *L. plantarum* (kinetics of persistence and internal loads) mirror those of indigenous bacteria in CR individuals. Indeed, *L. plantarum* loads fluctuated in between different developmental stages but in a very stereotyped and reproducible manner. *L. plantarum* loads constantly increase during the larval stages, reaching a maximum at midpupal stage (Figure 2A). This was followed by a dramatic fall during late metamorphosis and by a reassociation upon adult emergence, illustrated by an increasing amount of bacteria during the adult life (Figure 2A). Similar kinetics of the whole bacterial population persistence and loads were observed during the larval, pupal, and early adult stages of CR individuals (Figure 2G). In contrast, internal bacterial loads following adult emergence were slightly different between *L. plantarum*-associated and CR adults (Figures 2A and 2H). Since vertical transfer is a hallmark of the natural process of microbiota acquisition, we tested whether *L. plantarum* could be efficiently transmitted from the parents to their progenies. As shown in Figure 2F, *L. plantarum* loads and kinetics of persistence in progenies of *L. plantarum*-associated parents followed the same pattern as the one observed in artificially *L. plantarum*-associated flies or as the whole bacterial population in CR flies (Figures 2A, 2F, and 2G). Taken together, these experiments demonstrate that the protocol used to associate GF individuals with *L. plantarum* faithfully recapitulates a natural pattern of bacterial colonization of CR individuals, at least during larval, pupal, and early adult stages.

L. plantarum Association Sustains Larval Development upon Nutrient Scarcity

Having demonstrated that *L. plantarum* colonizes the larval niche as a natural microbiota, we tested whether *L. plantarum* on its own sustains the development of larvae raised on poor-nutrient media. *L. plantarum* association in poor-condition medium was sufficient to accelerate larval growth and resulted in earlier emergence of adults (Figures 3A and 3B; Table S1). This effect was not observed in rich-medium condition (Figures 3A and 3B). This growth-promoting effect, which was observed in different poor-medium conditions, results in a reduction of all three larval instars (Figures 3C and 3D). Strikingly, the presence of *L. plantarum* was sufficient to allow development of larvae in the complete absence of yeast extract, a condition that normally led to lethality of GF late first instar larvae (Figure 3C). Importantly, this beneficial effect was neither observed upon colonization of GF larvae with another bacterial species, *E. faecalis*, which does not persist in its host, nor with another strain of *L. plantarum* isolated in our lab and fully capable of colonizing the larvae and the medium (Figures 3A, 3B, and 3E–3H). Importantly, several other strains of *L. plantarum* isolated independently from flies cultivated in our or other labs are beneficial, as well as the reference *L. plantarum* strain, whose genome is sequenced (data not shown; Figures S2 and S4). This suggests that many *L. plantarum* strains exert a specific effect on systemic larval growth that is not a mere trophic effect of adding organic matter to the fly medium, but rather relies on a specific biological activity of these strains. Finally, we show that the beneficial effect of *L. plantarum* on the developmental timing is also vertically transmitted from *L. plantarum*-associated parents to their progenies (Figure 3I). Altogether, these observations demonstrate that association with several strains of *L. plantarum* accelerates larval development upon nutrient scarcity and results in an earlier emergence of adults compared to GF animals. These data reveal

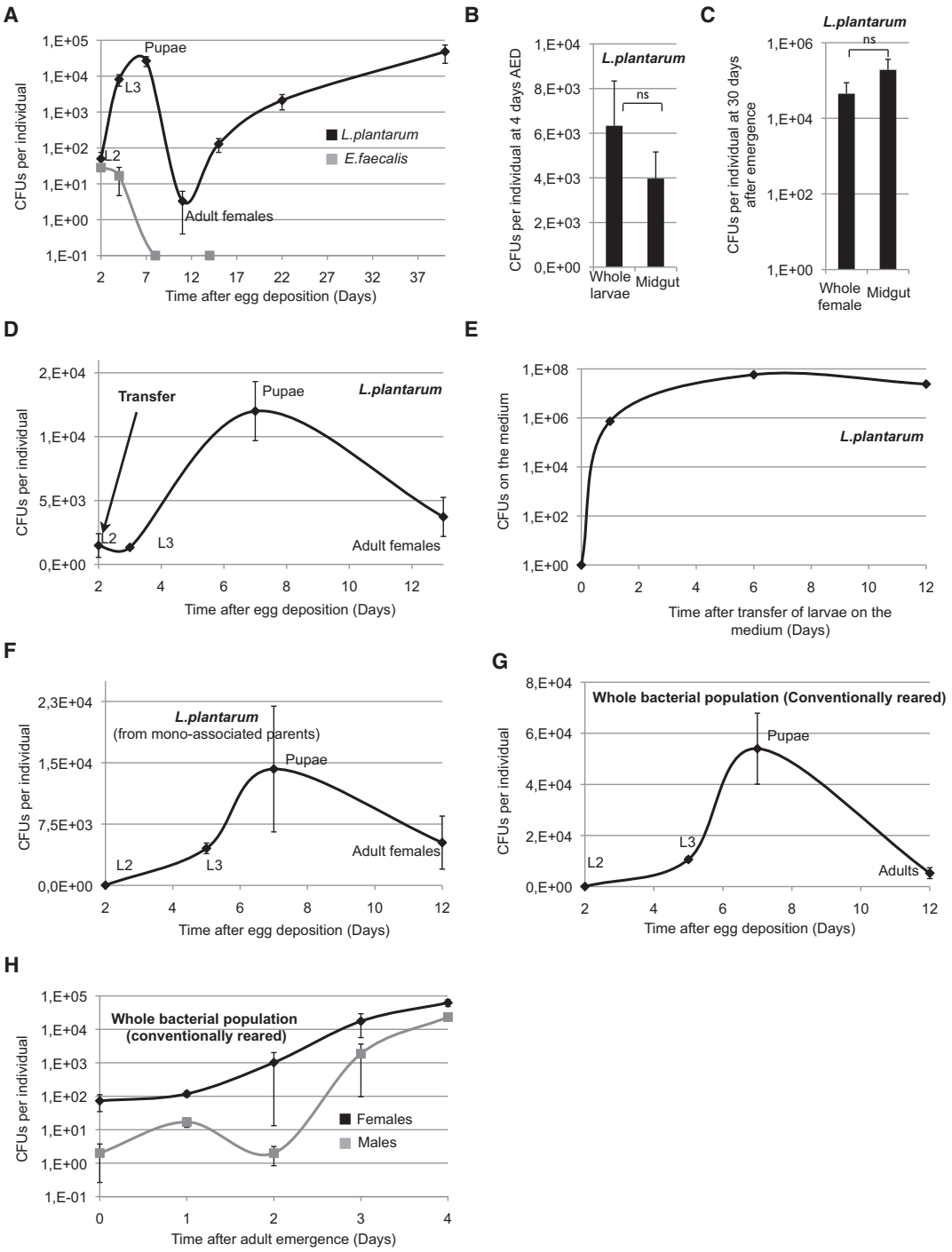

Figure 2. *Lactobacillus plantarum* Association Recapitulates Conventional Microbiota Association

(A) Internal bacterial load of germ-free *Drosophila* individuals after contamination of the fly medium with *E. faecalis* (gray) or *L. plantarum* (black).

(B and C) Internal and midgut load of *L. plantarum* in larvae collected 4 days after egg deposition (AED) (B) or adults collected 30 days after emergence (C).

(D) Internal bacterial load of *Drosophila* individuals colonized with *L. plantarum* at day 1 AED and transferred to germ-free medium after surface sterilization at day 2 AED.

(E) Fly medium bacterial load after transfer of surface-sterilized *L. plantarum*-associated larvae.

(F) Internal bacterial load of the progenies of *Drosophila* adults monoassociated with *L. plantarum*.

(G and H) Internal load of the whole bacterial population of conventionally reared *Drosophila* individuals after egg deposition (G) and female (black) or male (gray) emergence (H). All experiments were performed on rich diet. Each graph represents the mean of three biological replicates ± SEM. Bacterial load is illustrated as the colony forming units (cfu). Developmental stages of individuals are indicated on the graphs. For (B) and (C), statistical significance of the results is included (Student's t test, ns $p > 0.05$).

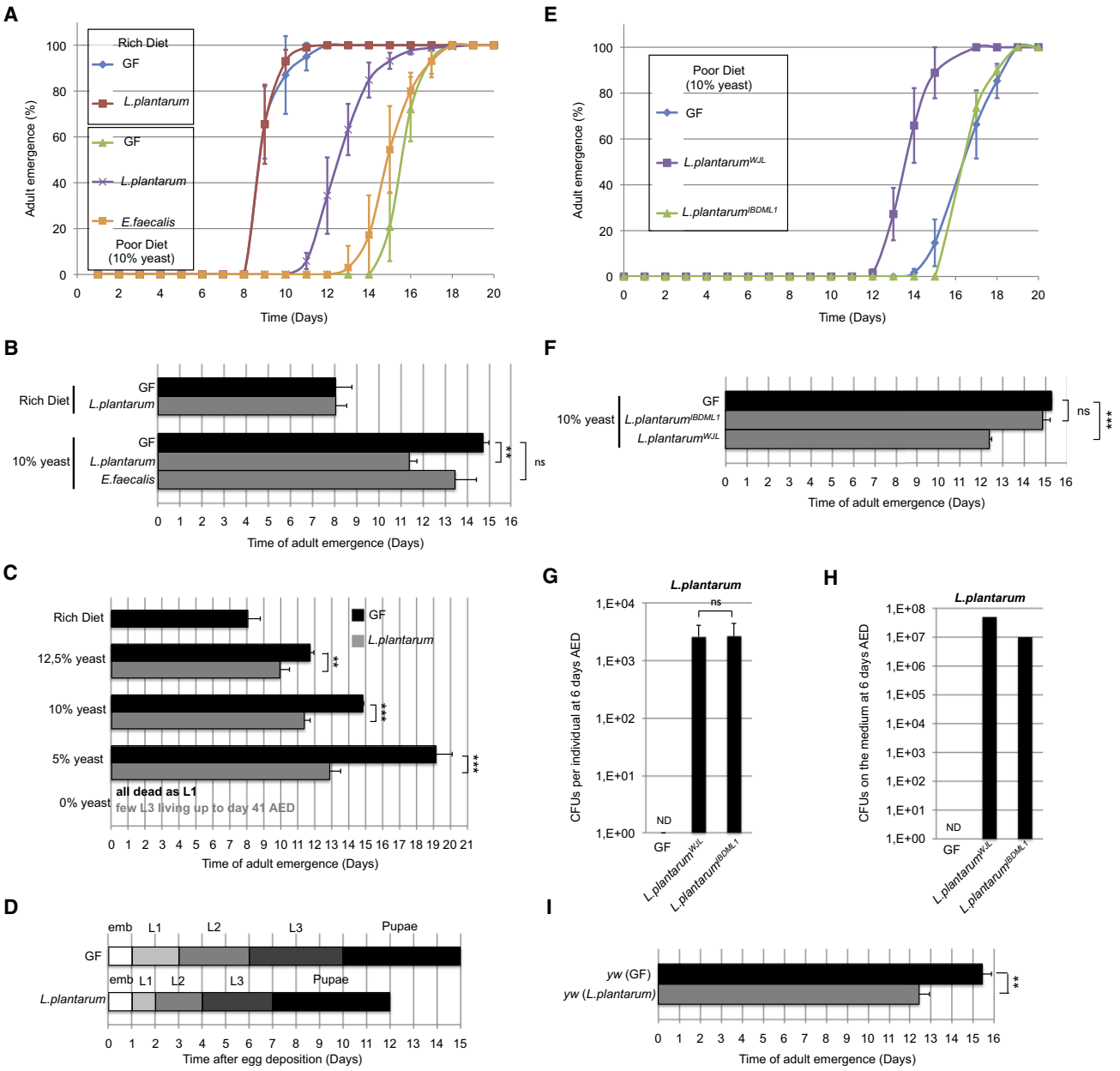

Figure 3. *L. plantarum* Association Sustains Larval Development upon Nutrient Scarcity

(A) Developmental timing of germ-free (GF), *L. plantarum*-, or *E. faecalis*-associated individuals grown on either rich or poor diet. The cumulative percentage of the adult population emergence is shown over time. Data represent the mean of three biological replicates containing at least 30 individuals each ± SEM.

(B) Mean time of the emergence of 10% of the whole germ-free, *L. plantarum*-, or *E. faecalis*-associated adult population grown on either rich or poor diet (10% yeast).

(C) Mean time of the emergence of 10% of the whole germ-free or *L. plantarum*-associated adult population grown on rich or poor diets containing, respectively, 12.5%, 10%, 5%, or 0% of the yeast extract content of a rich diet.

(D) Time of appearance of the first germ-free (GF) or *L. plantarum*-associated individuals at each different developmental stage when grown on poor diet (10% yeast).

(E) Developmental timing of germ-free (GF), *L. plantarum*^{WJL}- or *L. plantarum*^{IBDML1}-associated individuals grown on poor diet. The cumulative percentage of the adult population emergence is shown over time. Data represent the mean of three biological replicates containing at least 30 individuals each ± SEM.

(F) Mean time of the emergence of 10% of the whole germ-free, *L. plantarum*^{WJL}- or *L. plantarum*^{IBDML1}-associated adult population grown on poor diet (10% yeast).

(G and H) Internal larval (G) and fly medium (H) load of *L. plantarum* 6 days AED upon association with *L. plantarum*^{WJL} or *L. plantarum*^{IBDML1}.

(I) Mean time of the emergence of 10% of the F1 adult population of GF or *L. plantarum*-associated parents grown on poor diet (10% yeast). For (B), (C), (F), and (I), gray is *L. plantarum*-associated and black is GF condition. Statistical significance of the results is included (Student's t test, ns $p > 0.05$; **$p < 0.01$; ***$p < 0.001$). For (G) and (H), ND: not detected.

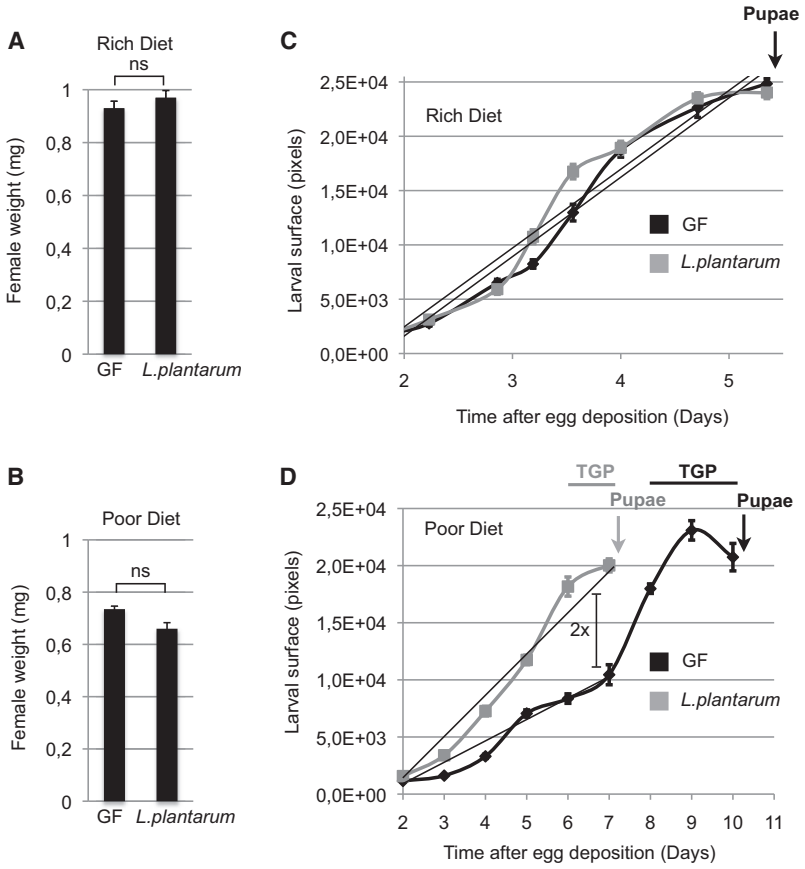

Figure 4. *L. plantarum* Association Promotes Larval Growth Rate

(A and B) Weight of 3-day-old GF or *L. plantarum*-associated females emerging from individuals grown either on rich (A) or poor diet (10% yeast) (B). Statistical significance of the results is included (Student's t test, ns $p > 0.05$).

(C and D) Larval surface of GF (black) or *L. plantarum*-associated (gray) larvae over time when grown on rich (C) or poor diet (10% yeast) (D). Linear regression curves are included (GF/rich diet, $y = 7254\times - 12{,}051$; *L. plantarum*/rich diet, $y = 7308.5\times - 13{,}006$; GF/poor diet, $y = 2013\times - 3736$; *L. plantarum*/poor diet $y = 4029\times - 7771$). A 2-fold increase in the growth rate of *L. plantarum*-associated larvae is illustrated, as well as the terminal growth periods (TGPs) and time of pupae emergence in each condition.

that some strains of a single *Drosophila* commensal bacterial species, *L. plantarum*, are sufficient to recapitulate the beneficial effect of a naturally acquired microbiota.

L. plantarum Promotes Larval Growth Rate

To further characterize how *L. plantarum* impacts larval growth, we analyzed the final adult size, a parameter that is directly dependent on the larval growth phase. To this end, we compared the weight of young adults appearing from larvae raised on GF or *L. plantarum*-contaminated media. As for the length of the larval stages, we did not observe any significant differences in the weight of adults developing from GF and *L. plantarum*-associated larvae grown on rich diet (Figure 4A). Similarly, when larvae were grown on poor medium, no significant difference was observed between GF and *L. plantarum*-associated individuals (Figure 4B). However, adults developing from either GF or *L. plantarum*-associated larvae grown on poor diet were lighter than individuals grown in rich conditions (Figures 4A and 4B). Given that *L. plantarum* reduces the length of the growth phase without affecting the final size of the individual, we hypothesized that *L. plantarum* increases the larval growth rate. To test this, we compared the size of *L. plantarum*-associated versus GF larvae from L1 larvae to pupae. Data presented in Figures 4C and 4D clearly show a 2-fold increase in the growth rate of *L. plantarum*-associated larvae raised on a poor diet, whereas no impact on the growth rate is observed when larvae are raised on a rich diet. These results demonstrate that *L. plantarum* association enhances systemic growth upon nutrient scarcity by promoting larval growth rate and reducing the duration of the growth period.

L. plantarum Association Correlates with Enhanced Hormonal Growth Signaling

In *Drosophila*, the duration of the larval period and the larval growth rate are controlled by two circulating hormones: the steroid hormone Ecdysone (Ecd) and the *Drosophila* insulin-like peptides (dILPs), respectively (Hietakangas and Cohen, 2009). To test if the presence of *L. plantarum* directly impacts these growth signals, we compared the levels of molecular readouts of these signals in GF and *L. plantarum*-associated larvae. The expression of the transcription factor *E74B*, one of the "early" genes that responds to increasing Ecd titers, is classically used as a molecular marker of Ecd activity (Karim and Thummel, 1991). Figure 5A shows that *L. plantarum* association did not increase the *E74B* mRNA levels until day 7 AED; however, from then *E74B* mRNA levels sharply peaked in *L. plantarum*-associated larvae, while the peak was less acute and delayed in GF larvae. Of note, in GF larvae *E74B* mRNA levels were already increased (albeit with low statistical significance) at day 9 AED, but no larvae pupariated at this time point (pupariation started at day 11 AED, Figure 3D). These results indicate that *L. plantarum* association correlates with an earlier and stronger Ecd peak in third instar larvae. We then used the *InR* gene expression as a readout for systemic dILP activity. Indeed, the *InR* gene transcription is under the direct negative regulation of the InR signaling pathway via the activity of the FoxO transcription factor. *InR* expression is therefore used as a negative molecular marker of systemic dILP activity: low *InR* expression correlating with high dILP activity (Puig and Tjian, 2005). As shown in Figure 5B, *InR* expression was always lower in *L. plantarum*-associated larvae than in GF larvae. These results show that *L. plantarum* association correlates with increased systemic InR signaling during larval growth. Taken together, our observations support the notion that *L. plantarum* association, albeit with distinct kinetics, enhances the systemic production of two hormonal growth signals.

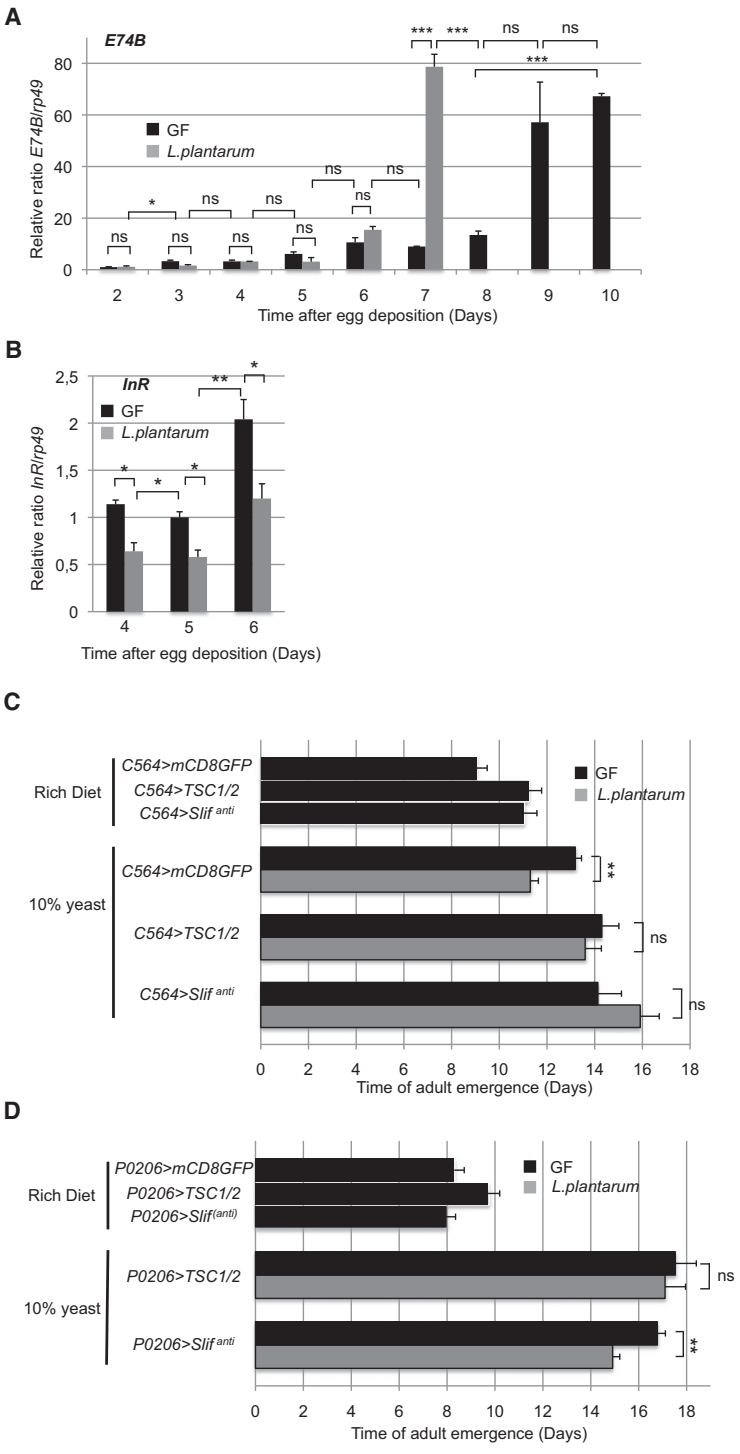

Figure 5. *L. plantarum* Enhances TOR Activity via Increased Nutrient Sensing

(A) *E74B* mRNA levels from day 2 AED to day 10 AED in GF (black) or day 2 AED to day 7 AED for *L. plantarum*-associated larvae (gray) grown in poor condition (10% yeast). The RT-qPCR value of the relative $\Delta Ct^{E74B}/\Delta Ct^{rp49}$ ratios is represented for each day AED, and the $\Delta Ct^{E74B}/\Delta Ct^{rp49}$ ratio calculated for GF larvae at day 2 AED was anchored to 1 to indicate fold induction.

(B) *InR* mRNA levels from day 4 AED to day 6 AED in GF (black) and *L. plantarum*-associated larvae (gray) grown in poor condition (10% yeast). Relative $\Delta Ct^{InR}/\Delta Ct^{rp49}$ ratios are represented, and the $\Delta Ct^{InR}/\Delta Ct^{rp49}$ ratio calculated for GF larvae at 5 days AED was anchored to 1 to indicate fold induction.

(C) Mean time of the emergence of 10% of the whole germ-free (black) or *L. plantarum*-associated (gray) adult population grown on either rich or poor diet (10% yeast). Genotype tested were: (1) w;C564-GAL4/UASmCD8::GFP (C564 > mCD8GFP), (2) w;C564-GAL4/UAS-TSC1,UAS-TSC2 (C564 > TSC1/2), and (3) w;C564-GAL4/UAS-*Slif*^{anti} (C564 > *Slif*^{anti}).

(D) Mean time of the emergence of 10% of the whole germ-free (black) or *L. plantarum*-associated (gray) adult population grown on either rich or poor diet. Genotype tested were: (1) w;P0206-GAL4,UASmCD8::GFP /UASmCD8::GFP (P0206 > mCD8GFP), (2) w;P0206-GAL4,UASmCD8::GFP/UAS-TSC1,UAS-TSC2 (P0206 > TSC1/2), and (3) w;P0206-GAL4,UASmCD8::GFP/UAS-*Slif*^{anti} (P0206 > *Slif*^{anti}). Statistical significance of the results is included (Student's t test, ns $p > 0.05$; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$).

L. plantarum Effect on Growth Requires Optimal TOR Activity

In *Drosophila*, TOR pathway modulates hormonal signals regulating larval growth in a tissue-specific manner (Hietakangas and Cohen, 2009). While TOR directly controls Ecd production by the prothoracic gland during the mid-third larval instar (Layalle et al., 2008), the regulation of InR signaling is more complex and implicates cross-talks between different tissues. It has been shown that systemic InR signaling is regulated by a remote control of dILP secretion by neurons through TOR activity in the fat body (Colombani et al., 2003; Géminard et al., 2009). Since our results suggest that *L. plantarum* association impacts both InR and Ecd signaling in larvae, we wondered whether the TOR pathway mediates these effects. To test this hypothesis, we analyzed the impact of reduced TOR kinase activity on *L. plantarum*-mediated benefit using a mild and tissue-specific expression of TSC1 and TSC2, two negative regulators of TOR (Tapon et al., 2001). The developmental timing of larvae in which TSC1 and TSC2 were selectively expressed in the fat body (by using *ppl*-GAL4 or C564-GAL4 drivers) was no longer influenced by the presence of *L. plantarum*. Indeed, adult emergence timing of *L. plantarum*-associated and GF individuals with reduced TOR activity were identical (Figure 5C; Supplemental Information and Figure S3). Using the P0206-GAL4 driver, we reduced TOR kinase activity specifically in the prothoracic gland (Layalle et al., 2008). As observed for the fat body, TSC1 and TSC2 expression in this organ also abolished the beneficial effect of *L. plantarum* on the timing of adult emergence (Figure 5D; Supplemental Information and Figure S3). Taken together, these experiments demonstrate that optimal TOR kinase activity is required in both fat body and prothoracic gland to promote enhanced systemic growth upon *L. plantarum* association.

L. plantarum Effect on Growth Relies on the Host Nutrient Sensing System

Diet-derived branched-chain amino acids are the main activators of TOR kinase activity (Avruch et al., 2009). However, the precise molecular mechanisms responsible for amino acid sensing remain elusive. In *Drosophila*, genetic studies have implicated the product of the *slimfast* gene in the regulation of TOR kinase activity in the fat body (Colombani et al., 2003). Silencing *slimfast* expression selectively in this tissue causes a systemic growth defect similar to what is seen in *Drosophila* raised under poor nutritional conditions or TOR inhibition (Colombani et al., 2003) (Figure 5C; Supplemental Information and Figure S3). *Slimfast* encodes a transporter involved in the intracellular uptake of diet-derived circulating amino acids, suggesting that TOR activity in the fat body is regulated by the availability of these micronutrients (Colombani et al., 2003). Using UAS-*slif* antisense transgene and fat body or prothoracic gland GAL4 drivers, we tested the consequence of *slif* inactivation on the beneficial effect mediated by *L. plantarum* association on its host developmental timing. Figures 5C and 5D show that the selective extinction of *slif* in the fat body severely impacts this process, demonstrating that *L. plantarum* association requires a fully functional host nutrient sensing system to promote systemic growth.

DISCUSSION

Our results establish the importance of the *Drosophila* microbiota to sustain larval development upon nutrient scarcity. They emphasize the role of the microbiota in the adaptation of its host to different nutritional conditions that can be encountered in the wild and support the hologenome theory of evolution (Fraune and Bosch, 2010; Zilber-Rosenberg and Rosenberg, 2008). This theory posits that the holobiont (the host plus its associated micro-organisms) acts as a unit of selection in evolutionary change and that commensal microbes, thanks to their genetic wealth, may play an important role in both adaptation and evolution of metazoans to their environment (Zilber-Rosenberg and Rosenberg, 2008). Indeed, we demonstrate that the fly microbiota confers optimal adaptation to its environment and that this beneficial effect is transferred from one generation to the other. Both characteristics are essential for the ecological and evolutionary success of a given species. Together with the recent indication that commensal bacteria influence *Drosophila* mating preference (Sharon et al., 2010), our results highlight the key role of the *Drosophila* microbiota to its host biology.

The two major bacterial species identified in our *Drosophila* strain (*Lactobacillus plantarum* and *Enterococcus faecalis*) were previously identified in commensal communities of lab-reared or wild-captured *Drosophila* (Corby-Harris et al., 2007; Cox and Gilmore, 2007; Ren et al., 2007; Ryu et al., 2008; Sharon et al., 2010). We did not find Acetobacteraceae species, although they were found in previous studies. This could be due to fly food composition favoring selection of specific bacterial strains. Our fly food, which does not contain simple sugars but starch as a carbohydrate source, could also explain the limited number of bacterial species found associated with flies. Indeed, Sharon et al. have recently shown that flies reared on starch medium hold low microbiota diversity with the dominance of *Lactobacillus plantarum* and the absence of Acetobacteraceae, while flies reared on molasses medium show higher diversity and contain Acetobacteraceae (Sharon et al., 2010).

We show that unlike *E. faecalis*, *L. plantarum* has the potent ability to reside in the *Drosophila* intestine and to be vertically transmitted to its progenies. This ability most likely stems from the extreme flexibility and versatility of this bacterial species. Indeed, *L. plantarum* is encountered in a variety of environmental niches, including dairy, meat, and vegetable or plant fermentations and is a natural inhabitant of the human mouth, intestine, and vagina. Of note, *L. plantarum* is the most common bacterium used in silage inoculants, and a selected strain, *L. plantarum*^{299v}, is marketed as a probiotic supposed to confer various health benefits to the consumer. The ecological flexibility of *L. plantarum* is reflected by the observation that this species has one of the largest genomes known among lactic acid bacteria and is equipped with a large number of genes encoding regulatory, transport, and extracellular proteins (Kleerebezem et al., 2003).

Strikingly, we reveal that monoassociation of young GF larvae with *L. plantarum* mirrors bacterial colonization patterns seen upon vertical transfer or upon colonization by a natural microbiota (i.e., in CR conditions). The colonization pattern revealed in this study strictly correlates with the ones described in past reports (Bakula, 1969; Ren et al., 2007). This robust ability of *L. plantarum* to colonize and reside in its host actually suggests that this bacterial strain is adapted to occupy the intestinal niche of *Drosophila* individuals. Taken collectively, our results reveal that *L. plantarum* monoassociation is a faithful gnotobiotic model to address the functional impact of a unique commensal strain to its host physiology. Hence, we demonstrate that *L. plantarum* sustains larval development upon nutrient scarcity and recapitulates, on its own, the effect of a natural microbiota. This reveals that, at least for *Drosophila* systemic growth, a single bacterial species can recapitulate the beneficial effect of a more complex natural microbiota.

We next wondered how *L. plantarum* exerts its growth-promoting effect. Since the hallmark of *L. plantarum* metabolic activity is the massive production of lactic acid from homofermentation of sugars (Ferain et al., 1996), we tested whether *L. plantarum* strain producing lower amounts of lactate was still beneficial for the host. Indeed, lactic acid or lactate in its reduced form is a particularly mobile fuel for aerobic metabolism, and recent evidence reveals that eukaryote lactate dehydrogenase produces pyruvate that in turn fuels the mitochondrial Krebs cycle to produce energy (Gladden, 2004). The fact that a strain of *L. plantarum* that was genetically engineered to produce minute amounts of lactate was still fully beneficial suggests that lactate production is not a limiting activity of *L. plantarum* to sustain larval growth (Figure S4). Several lines of evidence support the notion that *L. plantarum* is not acting via a diet-derived sugar metabolism, but rather by promoting protein assimilation by the host. First, numerous publications have previously established that *Drosophila* systemic growth is influenced by nutrient availability and more specifically by the protein content of the diet (Layalle et al., 2008). Second, the benefit of *L. plantarum* association for its host growth is only revealed upon nutrient scarcity, but not in rich-diet

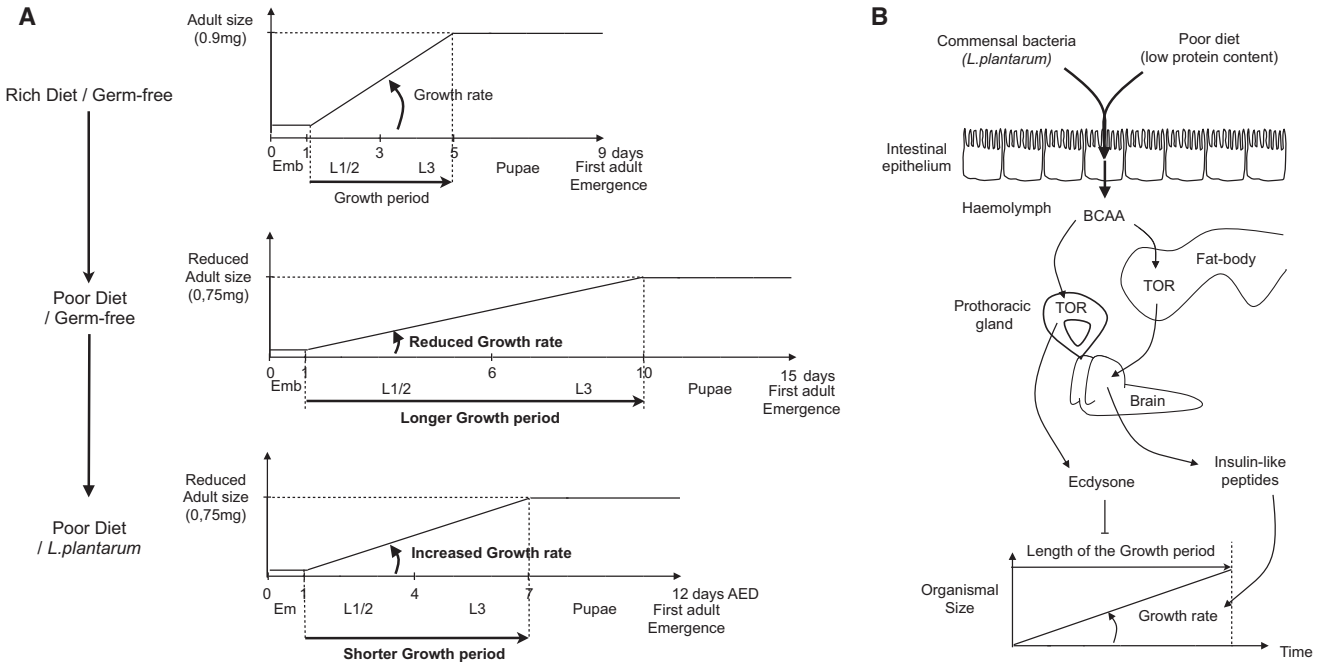

Figure 6. Model of the Impact of *L. plantarum* Association on Host Systemic Growth Parameters upon Nutrient Scarcity

(A) Both nutrient contents of the diet and *L. plantarum* association influence the larval growth rate and the length of the growth period, two parameters controlling the adult final size. The reduction of the yeast content in the diet reduces the growth rate and increases the length of the growth period. On a poor diet, the association of larvae with *L. plantarum* increases the growth rate and reduces the length of the growth period. As a consequence, those individuals more quickly reach the optimal size to develop into viable adults.

(B) *L. plantarum* association promotes protein assimilation from the diet, optimizing diet-derived branched-chain amino acid (BCAA) levels in the hemolymph. This in turn stimulates TOR kinase activity in the fat body, leading to increased *Drosophila* insulin-like peptides production by the brain. The dILPs, released in the hemolymph, increase systemic InR signaling and promote growth rate. In parallel, increased levels of BCAA activate TOR kinase activity in the prothoracic gland, which potentiates Ecdysone production during late larval stage and impacts on the length of the growth phase. This integrated action of hormonal signals via increased TOR activity leads to optimal systemic larval growth upon nutrient scarcity.

condition, which in our case is very concentrated in proteins compared to other fly food recipes (http://flystocks.bio.indiana.edu/Fly_Work/media-recipes/media-recipes.htm). Moreover, the *L. plantarum*-mediated benefit is only observed upon reduction of the yeast extract content, but not of the cornmeal in the culture medium (data not shown). Of note, in our fly food, the yeast extract is the main protein source. In addition, *L. plantarum* association is not able to sustain growth of *Drosophila* larvae on an agar/banana/grape media, which contains high titers of simple and complex sugars but less than 1% of protein (data not shown), while it can sustain larval growth on an agar/cornmeal medium in absence of yeast extract, which still contains proteins from cornmeal but low titers of simple sugars (Figure 3C). Finally, we show that on the same poor diet (10% yeast), *L. plantarum* association impacts its host developmental timing, similar to the mere addition of 2.5% yeast extract to the poor diet of GF larvae (Figure 3C, compare timing of emergence of GF on 10% yeast to GF on 12.5% yeast and *L. plantarum* on 10% yeast). Taken collectively, these data imply that *L. plantarum* exerts its beneficial effect through enhanced protein assimilation.

This notion is further supported by our functional data, which demonstrate that the host TOR kinase activity and the amino acid transporter Slimfast are essential for *L. plantarum* beneficial effect on growth. These molecules participate to the host nutrient sensing system, which governs *Drosophila* systemic growth (Hietakangas and Cohen, 2009). Our functional data demonstrate that *L. plantarum* exerts its beneficial effect on systemic growth genetically upstream of this host amino acid sensing system. Interestingly, optimal TOR activity is required both in the fat body and the prothoracic gland, two key endocrine tissues, to allow *L. plantarum* association to promote growth. Actually, TOR activity in the fat body controls larval growth rate by influencing systemic InR signaling (Colombani et al., 2003), while TOR activity in prothoracic gland influences the duration of the growth phase by controlling the length of the terminal growth period (TGP) through the regulation of Ecd production at late larval stage (Layalle et al., 2008). Accordingly, we show that both parameters are influenced by *L. plantarum* association upon nutrient scarcity: the host growth rate is enhanced and the TGP is reduced (see Figure 4), and thus both systemic InR signaling and Ecd production are modified upon *L. plantarum* association. The observed *E74B*/*InR* expression patterns and the TOR dependence again support a simple model where *L. plantarum* association enhances protein assimilation from the food by the larvae. Taken collectively, our results reveal that *L. plantarum* influences both growth parameters, leading to an optimal systemic larval growth on a poor diet and allowing individuals to reach sooner the critical size needed to pupariate and form viable adults (Figure 6). Based on these results, we propose the following model where *L. plantarum* association

with its host promotes protein assimilation from the diet, optimizing diet-derived branched-chain amino acid levels in the hemolymph. This in turn stimulates TOR kinase activity both in the fat body and the prothoracic gland. In the fat body, TOR activity optimizes systemic InR signaling and promotes growth rate, while in the prothoracic gland, TOR potentiates Ecd production during late larval stage to reduce the length of the growth phase. This integrated action on hormonal signals via increased TOR activity leads to optimal systemic growth (Figure 6). Importantly, our results suggest that in addition to influencing host energy uptake, the microbiota, at least in *Drosophila*, can promote host systemic growth by influencing nutrient sensing system, controlling hormonal signals through enhanced nutrient assimilation. We therefore propose that the microbiota should not be considered only as a "host" factor influencing energy uptake, but should also be deemed as a "host" factor influencing growth. Finally, since microbiota-mediated growth-promoting effect in flies can be recapitulated by a single lactic acid bacterial species, it would be of great interest to test whether in mammals, in which lactic acid bacteria have been used for decades as alimentary complements in the farming and agroalimentary industry, these bacteria favor the systemic production of growth-promoting hormones.

EXPERIMENTAL PROCEDURES

Drosophila Stocks and Breeding

Drosophila stocks were cultured at 25°C on a yeast/cornmeal medium (rich diet). For 1 l of food, 8.2 g agar (VWR, cat. #20768.361), 80 g cornmeal flour (Westhove, Farigel maize H1), and 80 g yeast extract (VWR, cat. #24979.413) were cooked for 10 min in boiling water; 5.2 g Methylparaben sodium salt (MERCK, cat. #106756) and 4 ml of 99% propionic acid (CARLO ERBA, cat. #409553) were added when the food had cooled down. Poor-nutrient food was obtained by reducing the amount of yeast extract to 12.5% (10 g/l), 10% (8 g/l), 5% (4 g/l), or no yeast extract. Fresh food was prepared every week to avoid desiccation, and no yeast paste was added to the medium. CR stocks carry a conventional microbiota, which was removed in GF individuals by bleaching and cultivating embryos on autoclaved conventional medium. GF stocks were maintained on a rich diet supplemented with a cocktail of four antibiotics (Ampicillin/Kanamycin/Tetracyclin/Erythromycin at 50 μg/ml final each). *Drosophila yw* flies were used as the reference strain in this work. The following GAL4 drivers were used: C564-GAL4 (Harrison et al., 1995) and *ppl*-GAL4 (Colombani et al., 2003) for mild larval fat body expression and P0206-GAL4 for mild prothoracic gland expression (Janning, 1997). The following UAS transgenes were used: UAS-*mCD8::GFP* (Bloomington stocks #5137), UAS-*TSC1*, UAS-*TSC2* (Tapon et al., 2001), and UAS-*Slif*^{anti} (Colombani et al., 2003). All crosses were performed using GF stocks at 25°C on our conventional medium supplemented with antibiotics. Mated GF females were transferred on appropriate medium for egg laying, followed by bacterial association at day 1 AED.

Bacterial Strains

Apart from the *Lactobacillus plantarum*^{WCFS1}, which is a sequenced strain isolated from human saliva (Kleerebezem et al., 2003), all the other *L. plantarum* strains used in this study have been isolated from lab-raised flies: *L. plantarum*^{WJL} in Won-jae Lee's lab (Seoul), *L. plantarum*^{cnw10} in Angela Douglas' lab (Ithaca, NY), and *L. plantarum*^{NAB} in Bruno Lemaitre's lab (Lausanne). *L. plantarum*^{IBDML1} has been isolated from a 20-day-old CR*yw* female upon plating of serial dilutions of the fly homogenate on nutrient agar plates. Single colonies were recovered and species identification was performed by sequencing 16S rRNA gene amplicons. We recovered 30 distinct isolates of *L. plantarum*, all sharing more than 98% identities on their full-length 16S DNA sequences, from which we identified *L. plantarum*^{IBDML1} as being capable of colonizing the whole larval niche but unable to promote larval growth upon nutrient scarcity. This strain has a growth rate in MRS media similar to beneficial *L. plantarum* strains (data not shown). All the experiments were performed using the *L. plantarum*^{WJL} strain unless otherwise stated. For *E. faecalis* association, we used the *E. faecalis* JH2-2 *Rifampicin*^{r} strain (Hols et al., 1992). All *L. plantarum* strains were grown overnight at 37°C standing in Man, Rogosa, and Sharpe (MRS) broth (BD Bioscience), and *E. faecalis* was grown overnight at 37°C under agitation in Brain Heart Infusion (BHI) broth (BD Bioscience) supplemented with 50 μg/ml rifampicin. Heat inactivation of bacterial culture was achieved by incubating bacterial culture (OD_{600} = 1) for 10 min at 100°C. The *L. plantarum ldhD*$^{-}$,*ldhL*$^{-}$ strain produces minute amounts of lactate and has been described previously (Ferain et al., 1996).

Monoassociation of GF Individuals

GF females laid GF embryos on appropriate culture medium (rich or poor GF diets). Bacterial culture (150 μl, OD_{600} = 1) was then added directly on the embryos and the fly food after the egg-laying period. Emerging larvae were allowed to develop on the contaminated media. For transfer experiments (Figure 2D), larvae were collected 1 day after inoculation of the medium, surface sterilized for 1 min under agitation in 70% EtOH, rinsed in sterile water, and transferred to a fresh GF medium.

Bacterial Load Analysis

Bacterial load of surface-sterilized individuals was quantified by plating serial dilutions of lysates obtained from five individuals (larvae or adults) or five dissected midguts (from larvae or adults) on nutrient agar plates (BHI-rif for *E. faecalis* or MRS for *L. plantarum*). Midguts were isolated from whole dissected guts where the foregut, the hingut, and the Malphighian tubules were removed. Biological triplicates were collected for each experimental condition. Homogenization of individuals or tissues was performed using the Precellys 24 tissue homogenizer (Bertin Technologies, France) and 0.75/1 mm glass beads in 500 μl of the appropriate bacterial culture medium. The *L. plantarum* load on the fly medium after transfer of larvae (Figures 2E and S1D) was quantified by plating several dilutions of 1 ml MRS, previously deposited on the surface of the contaminated fly medium and vortexed thoroughly.

Developmental Timing and Larval and Adult Size Measurements

Developmental timing of individuals raised in different conditions was quantified by counting the number of adults emerging over time. These data were represented either as the cumulative percent of the whole adult population emerging per day or as the day at which 10% of the whole adult population has emerged. Each graph represents the mean of at least three biological replicates, including at least 30 individuals each. Larval stages were identified based on the morphology of mouth hooks and anterior spiracles (Demerec, 1950). Larval size was estimated by collecting and freezing larvae ($n > 20$) every day when grown on poor diet or twice a day (morning and evening) when grown on rich diet after an initial 3 hr period of egg deposition and appropriate bacterial inoculation 24 hr later. Larvae were frozen and mounted in 80% glycerol in PBS. Pictures were taken on a black background using a ProgResC5 CCD camera (JenOptik) mounted on a stereomicroscope. The body surface of each larva was calculated using ImageJ. Masks covering the surface of the larvae were generated using the threshold tool. Surface values were displayed in pixels. Adult size was estimated based on the weight of 3-day-old females. For each condition, the weight of multiple replicates (minimum of three) of a pool of five females was weighed using a precision balance (Mettler Toledo, AG245).

Additional methods are in Supplemental Information available online.

SUPPLEMENTAL INFORMATION

Supplemental Information includes one table, four figures, Supplemental Text, Supplemental Experimental Procedures, and Supplemental References and can be found with this article online at doi:10.1016/j.cmet.2011.07.012.

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REFERENCES

Avruch, J., Long, X., Ortiz-Vega, S., Rapley, J., Papageorgiou, A., and Dai, N. (2009). Amino acid regulation of TOR complex 1. Am. J. Physiol. Endocrinol. Metab. *296*, E592–E602.

Bäckhed, F., Ley, R.E., Sonnenburg, J.L., Peterson, D.A., and Gordon, J.I. (2005). Host-bacterial mutualism in the human intestine. Science *307*, 1915–1920.

Bakula, M. (1969). The persistence of a microbial flora during postembryogenesis of Drosophila melanogaster. J. Invertebr. Pathol. *14*, 365–374.

Bocci, V. (1992). The neglected organ: bacterial flora has a crucial immunostimulatory role. Perspect. Biol. Med. *35*, 251–260.

Brummel, T., Ching, A., Seroude, L., Simon, A.F., and Benzer, S. (2004). Drosophila lifespan enhancement by exogenous bacteria. Proc. Natl. Acad. Sci. USA *101*, 12974–12979.

Buchon, N., Broderick, N.A., Chakrabarti, S., and Lemaitre, B. (2009). Invasive and indigenous microbiota impact intestinal stem cell activity through multiple pathways in Drosophila. Genes Dev. *23*, 2333–2344.

Burcelin, R., Luche, E., Serino, M., and Amar, J. (2009). The gut microbiota ecology: a new opportunity for the treatment of metabolic diseases? Front. Biosci. *14*, 5107–5117.

Cani, P.D., and Delzenne, N.M. (2009). The role of the gut microbiota in energy metabolism and metabolic disease. Curr. Pharm. Des. *15*, 1546–1558.

Casadevall, A., and Pirofski, L.A. (2000). Host-pathogen interactions: basic concepts of microbial commensalism, colonization, infection, and disease. Infect. Immun. *68*, 6511–6518.

Colombani, J., Raisin, S., Pantalacci, S., Radimerski, T., Montagne, J., and Léopold, P. (2003). A nutrient sensor mechanism controls Drosophila growth. Cell *114*, 739–749.

Corby-Harris, V., Pontaroli, A.C., Shimkets, L.J., Bennetzen, J.L., Habel, K.E., and Promislow, D.E. (2007). Geographical distribution and diversity of bacteria associated with natural populations of Drosophila melanogaster. Appl. Environ. Microbiol. *73*, 3470–3479.

Cox, C.R., and Gilmore, M.S. (2007). Native microbial colonization of Drosophila melanogaster and its use as a model of Enterococcus faecalis pathogenesis. Infect. Immun. *75*, 1565–1576.

Delzenne, N., and Reid, G. (2009). No causal link between obesity and probiotics. Nat. Rev. Microbiol. *7*, 901, author reply 901.

Demerec, M., ed. (1950). Biology of Drosophila (New York: John Wiley & Sons, Inc).

Ehrlich, S.D. (2009). Probiotics - little evidence for a link to obesity. Nat. Rev. Microbiol. *7*, 901, author reply 901.

Ferain, T., Hobbs, J.N., Jr., Richardson, J., Bernard, N., Garmyn, D., Hols, P., Allen, N.E., and Delcour, J. (1996). Knockout of the two ldh genes has a major impact on peptidoglycan precursor synthesis in Lactobacillus plantarum. J. Bacteriol. *178*, 5431–5437.

Fraune, S., and Bosch, T.C. (2010). Why bacteria matter in animal development and evolution. Bioessays *32*, 571–580.

Furrie, E. (2006). A molecular revolution in the study of intestinal microflora. Gut *55*, 141–143.

Géminard, C., Rulifson, E.J., and Léopold, P. (2009). Remote control of insulin secretion by fat cells in Drosophila. Cell Metab. *10*, 199–207.

Gill, S.R., Pop, M., Deboy, R.T., Eckburg, P.B., Turnbaugh, P.J., Samuel, B.S., Gordon, J.I., Relman, D.A., Fraser-Liggett, C.M., and Nelson, K.E. (2006). Metagenomic analysis of the human distal gut microbiome. Science *312*, 1355–1359.

Gladden, L.B. (2004). Lactate metabolism: a new paradigm for the third millennium. J. Physiol. *558*, 5–30.

Harrison, D.A., Binari, R., Nahreini, T.S., Gilman, M., and Perrimon, N. (1995). Activation of a Drosophila Janus kinase (JAK) causes hematopoietic neoplasia and developmental defects. EMBO J. *14*, 2857–2865.

Hietakangas, V., and Cohen, S.M. (2009). Regulation of tissue growth through nutrient sensing. Annu. Rev. Genet. *43*, 389–410.

Hols, P., Baulard, A., Garmyn, D., Delplace, B., Hogan, S., and Delcour, J. (1992). Isolation and characterization of genetic expression and secretion signals from Enterococcus faecalis through the use of broad-host-range alpha-amylase probe vectors. Gene *118*, 21–30.

Hooper, L.V., and Gordon, J.I. (2001). Commensal host-bacterial relationships in the gut. Science *292*, 1115–1118.

Hooper, L.V., Midtvedt, T., and Gordon, J.I. (2002). How host-microbial interactions shape the nutrient environment of the mammalian intestine. Annu. Rev. Nutr. *22*, 283–307.

Janning, W. (1997). FlyView, a Drosophila image database, and other Drosophila databases. Semin. Cell Dev. Biol. *8*, 469–475.

Karim, F.D., and Thummel, C.S. (1991). Ecdysone coordinates the timing and amounts of E74A and E74B transcription in Drosophila. Genes Dev. *5*, 1067–1079.

Kleerebezem, M., Boekhorst, J., van Kranenburg, R., Molenaar, D., Kuipers, O.P., Leer, R., Tarchini, R., Peters, S.A., Sandbrink, H.M., Fiers, M.W., et al. (2003). Complete genome sequence of Lactobacillus plantarum WCFS1. Proc. Natl. Acad. Sci. USA *100*, 1990–1995.

Layalle, S., Arquier, N., and Léopold, P. (2008). The TOR pathway couples nutrition and developmental timing in Drosophila. Dev. Cell *15*, 568–577.

Lhocine, N., Ribeiro, P.S., Buchon, N., Wepf, A., Wilson, R., Tenev, T., Lemaitre, B., Gstaiger, M., Meier, P., and Leulier, F. (2008). PIMS modulates immune tolerance by negatively regulating Drosophila innate immune signaling. Cell Host Microbe *4*, 147–158.

Nelson, K.E., Weinstock, G.M., Highlander, S.K., Worley, K.C., Creasy, H.H., Wortman, J.R., Rusch, D.B., Mitreva, M., Sodergren, E., Chinwalla, A.T., et al; Human Microbiome Jumpstart Reference Strains Consortium. (2010). A catalog of reference genomes from the human microbiome. Science *328*, 994–999.

O'Hara, A.M., and Shanahan, F. (2006). The gut flora as a forgotten organ. EMBO Rep. *7*, 688–693.

Puig, O., and Tjian, R. (2005). Transcriptional feedback control of insulin receptor by dFOXO/FOXO1. Genes Dev. *19*, 2435–2446.

Qin, J., Li, R., Raes, J., Arumugam, M., Burgdorf, K.S., Manichanh, C., Nielsen, T., Pons, N., Levenez, F., Yamada, T., et al; MetaHIT Consortium. (2010). A human gut microbial gene catalogue established by metagenomic sequencing. Nature *464*, 59–65.

Raoult, D. (2009). Probiotics and obesity: a link? Nat. Rev. Microbiol. *7*, 616.

Ren, C., Webster, P., Finkel, S.E., and Tower, J. (2007). Increased internal and external bacterial load during Drosophila aging without life-span trade-off. Cell Metab. *6*, 144–152.

Robertson, F.W. (1963). The ecological genetics of growth in Drosophila 6. The genetic correlation between the duration of the larval period and body size in relation to larval diet. Genet. Res. *4*, 74–92.

Ryu, J.H., Kim, S.H., Lee, H.Y., Bai, J.Y., Nam, Y.D., Bae, J.W., Lee, D.G., Shin, S.C., Ha, E.M., and Lee, W.J. (2008). Innate immune homeostasis by the homeobox gene caudal and commensal-gut mutualism in Drosophila. Science *319*, 777–782.

Savage, D.C. (1977). Microbial ecology of the gastrointestinal tract. Annu. Rev. Microbiol. *31*, 107–133.

Sekirov, I., Russell, S.L., Antunes, L.C., and Finlay, B.B. (2010). Gut microbiota in health and disease. Physiol. Rev. *90*, 859–904.

Sharon, G., Segal, D., Ringo, J.M., Hefetz, A., Zilber-Rosenberg, I., and Rosenberg, E. (2010). Commensal bacteria play a role in mating preference of Drosophila melanogaster. Proc. Natl. Acad. Sci. USA *107*, 20051–20056.

Simon, O. (2005). Micro-organisms as feed additives - probiotics. Advances in Pork Production, 161–167.

Tapon, N., Ito, N., Dickson, B.J., Treisman, J.E., and Hariharan, I.K. (2001). The Drosophila tuberous sclerosis complex gene homologs restrict cell growth and cell proliferation. Cell *105*, 345–355.

Venema, K. (2010). Role of gut microbiota in the control of energy and carbohydrate metabolism. Curr. Opin. Clin. Nutr. Metab. Care *13*, 432–438.

Zilber-Rosenberg, I., and Rosenberg, E. (2008). Role of microorganisms in the evolution of animals and plants: the hologenome theory of evolution. FEMS Microbiol. Rev. *32*, 723–735.