

# Symbiosis and Development: The Hologenome Concept

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All animals and plants establish symbiotic relationships with microorganisms; often the combined genetic information of the diverse microbiota exceeds that of the host. How the genetic wealth of the microbiota affects all aspects of the holobiont's (host plus all of its associated microorganisms) fitness (adaptation, survival, development, growth and reproduction) and evolution is reviewed, using selected coral, insect, squid, plant, and human/mouse published experimental results. The data are discussed within the framework of the hologenome theory of evolution, which demonstrates that changes in environmental parameters, for example, diet, can cause rapid changes in the diverse microbiota, which not only can benefit the holobiont in the short term but also can be transmitted to offspring and lead to long lasting cooperations. As acquired characteristics (microbes) are heritable, consideration of the holobiont as a unit of selection in evolution leads to neo-Lamarckian principles within a Darwinian framework. The potential application of these principles can be seen in the growing fields of prebiotics and probiotics. **Birth Defects Research (Part C) 93:56–66, 2011. © 2011 Wiley-Liss, Inc.**

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## INTRODUCTION

Animals and plants arose from microorganisms and have remained in close association with them ever since. These associations, or symbioses, take many forms, mostly different levels of mutualism, where both the host and the symbiont benefit from the interaction, and to a much smaller degree—parasitism, where the symbiont benefits and the host suffers damage. These types of symbioses may change under different local conditions. Until recently, studies on symbiosis have concentrated on a single primary symbiont and its host. However, with the advent of molecular

(culture-independent) techniques in microbiology during the last 15 years, it is now clear that all animals and plants live in close association with hundreds or thousands of different microbial species. In many cases the number of symbiotic microorganisms and their combined genetic information far exceed that of their host. In the last few years, it has been demonstrated that these diverse microbiota with their large microbiomes play a remarkable role in the lives of animals and plants.

Evolutionary developmental biology is based on the principle that evolution arises from heritable changes in development (Gilbert

et al., 2010). In the past, the focus of these changes has been on the host genome (genetic and epigenetic) and occasionally on the genome of a specific primary symbiont (coevolution). In this review, we shall first summarize the hologenome concept and then discuss the contribution of the diverse microbial symbionts (referred to in the past as commensal) to fitness (adaptation, survival, development, growth, and reproduction) and evolution of representative animals and plants, thereby demonstrating the idea that holobionts (host + symbionts) have developed, lived, survived, and evolved together.

## THE HOLOGENOME CONCEPT

The hologenome theory of evolution considers the holobiont with its hologenome, acting in consortium, as a unit of selection in evolution (Rosenberg et al., 2007; Zilber-Rosenberg and Rosenberg, 2008; Sharon et al., 2010). The holobiont has been defined as the host organism and all of its symbiotic microbiota (Rohwer et al., 2002). The hologenome is the sum of the genetic information of the host and its microbiota. The hologenome theory posits that (1) all animals and plants harbor abundant and diverse microorganisms acquiring from their host a

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sheltered and nutrient-rich environment, (2) these microbial symbionts affect the fitness of the holobiont and in turn are affected by it, (3) variation in the hologenome can be brought about by changes in either the host genome or the microbial population genomes (microbiome), and (4) these variations, including those of the microbiome, can be transmitted from one generation to the next with fidelity and thus may also influence evolution of the holobiont.

Fitness in the case of a holobiont must include beneficial interactions between the host and its symbionts, including those that may influence development, reproduction, and adaptation. In addition, it has to include beneficial interactions between the symbionts themselves as well as between the holobiont and other holobionts and the environment.

Variation in a holobiont can arise from changes in either the host or the symbiotic microbiome. Genetic variation in the host (occurring in the gametes or during development) as well as in individual microorganisms can be generated by the well-recognized mechanisms of recombination, chromosome rearrangement, and mutation, in addition to epigenetic variation. Stochastically produced variants followed by selection of the fittest are the essence of neo-Darwinian evolution. Consideration of the hologenome, namely the host genome combined with that of its microbiota, brings forth three additional modes of variation, which are unique to the holobiont. The first is microbial amplification, the increase of one group of symbionts relative to others, which can occur when conditions change. The holobiont is a dynamic entity with certain microorganisms multiplying and others decreasing in number as a function of local conditions within the holobiont. An increase in the number of a particular microbe is actually equivalent to gene amplification. Considering the large amount of genetic information encoded in the diverse microbial population of holobionts, microbial

amplification can be a powerful mechanism for affecting adaptation and development. Examples of environmental factors that can lead to changes in symbiont populations and thereby to variation in hologenomes are nutrient availability, disease, light intensity, pH, and temperature. The second mechanism for introducing variation into holobionts is acquisition of new symbionts from the environment. All animals and plants come into contact with billions of microorganisms during their lifetime. One can reasonably assume that occasionally, as a random event, some of these microbes will find a niche and become established in the host. Under the appropriate conditions, the novel symbionts may become more abundant and affect the phenotype of the holobiont. Unlike microbial amplification, acquiring new symbionts can introduce entirely new genes into the holobiont. The third mechanism is horizontal transfer of genes from transient or nonassociated bacteria to resident microbiota.

The applied fields of prebiotics and probiotics involve attempts to modify microbiota of animals, including man, and plants, by changing the diet or adding specific bacteria, respectively. In effect prebiotics targets variation by amplification and probiotics can lead both to amplification and acquisition of novel bacteria.

Microbial amplification and acquisition of novel microbes into holobionts closely fit the Lamarckian first principle of "use and disuse." The holobiont loses characteristics (microbes) it does not use and gains characteristics (microbes) that are useful. As these amplified or acquired microbes can be transmitted to offspring, it satisfies the second principle of Lamarckism. Thus, the hologenome theory of evolution contains Lamarckian principles within a Darwinian framework (Rosenberg et al., 2009). In addition, it should be pointed out that microbial variation in a holobiont can be considered an epigenetic variation, in that it involves inher-

ited changes in the phenotype caused by mechanisms other than changes in the underlying host DNA sequence (Gilbert et al., 2010).

## INVERTEBRATE: MICROBE SYMBIOSES

### Coral Holobionts

No biological construct compares to coral reefs in terms of size and beauty. The ability to build these magnificent structures depends on the close association of the coral host with several different types of microorganisms (Koren and Rosenberg, 2006; Bourne et al., 2009): endosymbiotic algae of the genus *Symbiodinium* (commonly referred to as zooxanthellae), Bacteria and Archaea. Zooxanthellae provide a large part of the energy requirements of their coral hosts by transferring photosynthetically fixed carbon to the coral (Fallowski et al., 1984). Another less-appreciated function of algal photosynthesis in this system is the production of large amounts of molecular oxygen during daylight that allows for efficient respiration by the coral and associated microorganisms. Moreover, without the transfer of organic compounds and oxygen to the coral host, carbon dioxide concentration would be low, production of the calcium carbonate skeleton would be greatly inhibited and coral reefs would not exist.

The hundreds of different bacterial species that are associated with the coral mucus, tissues, and skeleton are also essential for coral health. A substantial part of the coral nitrogen requirement is provided by nitrogen-fixing coral bacteria (Shashar et al., 1994; Lesser et al., 2004). Some of the bacterial symbionts degrade complex polysaccharides, such as chitin (Ducklow and Mitchell, 1979), thereby providing nutrients to the coral holobiont; others are able to protect the coral against pathogens by producing antibiotics (Ritchie, 2006; Nissimov et al., 2009; Shnit-Orland and Kushmaro, 2009).

Microbial symbionts of corals also play a major role in adaptation to changing environmental conditions. When seawater temperature exceeds the normal maximum by a few degrees, corals lose their symbiotic zooxanthellae, a process referred to as bleaching. If the process is not reversed in a reasonable time, the coral will die. The adaptive hypothesis of coral bleaching (Buddemeier et al., 2004) puts forth the concept that expulsion of the algae allows more temperature-resistant zooxanthellae to infect the coral and establish a more favorable symbiosis. The acquisition of a novel heat-resistant zooxanthellae fits within the hologenome theory of evolution. Another adaptive process of bleached corals is the amplification of cyanobacteria in the coral skeleton. The photosynthetic products of these bacteria are transferred to the host and help them survive the bleaching episode (Fine and Loya, 2002). One can conclude that rapid changes in coral microbiota can assist the holobiont in adaptation and evolution.

### Insect Holobionts

Insects are the most diverse animal group on earth, embracing several million species (Wilson, 1992). Insect-microbe symbioses take many forms: some are endocellular and termed primary (P-symbionts) and many more are extracellular symbionts. In some insects, obligate mutualistic bacteria are harbored in specialized host cells called bacteriocytes. For example, *Buchnera aphidicola* is harbored intracellularly within bacteriocytes in the abdominal body cavity of almost all aphids and provides essential amino acids that are lacking in the phloem sap diet of the insects (Douglas, 1998), and *Wigglesworthia glossinidia* is localized in a midgut-associated bacteriome of tsetse flies and plays pivotal roles in biosynthesis of B vitamins that are deficient in the blood diet of the insects (Akman et al., 2002). These obligate P-symbionts usually share long evolutionary histor-

ies with their hosts and, in most cases, the host cannot survive without the endosymbiont, or the elimination of the endosymbiont has a deleterious effect on the fitness of the host (Baumann et al., 2006).

*Wolbachia*, a Gram-negative bacterium of the alpha-proteobacteria group, is a common obligate intracellular parasite of insects and other invertebrates. It is probably the most ubiquitous endosymbiont on the planet (Dedeine et al., 2001) and is maternally transmitted through the cytoplasm of eggs. First recognized as the cause of some incompatible crosses in insects (Yen and Barr, 1971), *Wolbachia* have since been identified as a cause of parthenogenesis, feminization of male hosts, and male killing in different arthropod taxa (Veneti et al., 2005). *Wolbachia* are of special interest in the study of development and the evolution of symbiosis, because they seem not to fit current theory. It is generally accepted that vertically transmitted microorganisms should tend to evolve to be beneficial to their hosts, or at least evolve toward a benign state. Although *Wolbachia* is generally detrimental to its host, there is at least one known case where it is beneficial: *Wolbachia* is essential for the production of mature oocytes in a parasitic wasp (Dedeine et al., 2001). This appears to be a rare example of the transition of a symbiont from parasitism to obligate mutualism.

The hologenome concept emphasizes the importance not only of the intracellular symbionts but also of the diverse and dynamic extracellular microbial symbionts that are present in all animals. The enormous genetic richness of these symbionts plays a major role in adaptation and evolution of holobionts during times of environmental change. Recent studies using molecular techniques have brought new insights into the mechanisms by which the insects and their microbial symbionts digest cellulose in the small intestine (Watanabe and Tokuda, 2010). If the available

diet of an insect changes from simple sugars to complex polysaccharides, those symbionts, which contain the appropriate polysaccharidases will amplify in number, depolymerize the polysaccharides and allow the insect to grow efficiently. Termites, for example, have a multitude of different microorganisms in their hind gut (Warnecke et al., 2007) that are largely responsible for the breakdown of lignocelluloses (Breznak and Brune, 1994) and nitrogen-fixation (Golichenkov et al., 2002). It has been shown that different bacterial phylogenetic groups are present in the different gut compartments (Schmitt-Wagner et al., 2003).

It has been suggested that microbes have been powerful selective agents in the development of social behavior in insects, such as ants, bees, wasps, and termites (Stow and Beattie, 2008): On one hand, close contact ensures that beneficial microorganisms are transmitted from one generation to the next; on the other hand, it provides ideal conditions for transfer of contagious diseases. To help solve this problem, many social insect holobionts contain symbiotic bacteria, which produce antibiotics active against pathogens (Currie et al., 2006).

Diet-induced mating preference in *Drosophila* was reported many years ago (Dodd, 1989); however, the mechanism was unknown until a recent demonstration that changing the diet caused an amplification of a particular bacterial symbiont, *Lactobacillus plantarum*, and that this bacterium was responsible for the mating preference (Sharon et al., 2010). Analytical data suggested that the symbiotic bacteria influence mating preference by changing the levels of cuticular hydrocarbon sex pheromones. The combination of partial geographic separation and bacterial-induced mating preference could reduce interbreeding of the populations. Slower changes in the host genome would further enhance the mating preference. The stronger the mating preference, the greater the chance that

two populations will become sexually isolated, and biologists (Coyne, 1992; Schluter, 2009) have argued that the emergence of sexual isolation is the central event in the evolution of species.

### **Squid Light Organ: *Vibrio* Symbiosis**

The symbiosis between the Hawaiian bobtail squid *Euprymna scolopes* and the luminous bacterium *Vibrio fischeri* is one of best studied systems that demonstrate how a bacterial symbiont can play a role in the development of an animal organ (Ruby, 1996; Nyholm and McFall-Ngai, 2004). Both partners can be cultured independently in the laboratory, which has allowed experimental manipulation of the partners both as individuals and as dual participants in the association. Following fertilization of the eggs within the female, the embryos develop an immature light organ that is free of bacteria but has three pores leading to separate epithelial-lined crypts. The female host lays clutches of hundreds of eggs, which hatch almost synchronously at dusk. Within hours after hatching, the juvenile squid becomes colonized by *V. fischeri*, which triggers morphogenesis of the light organ (Montgomery and McFall Ngai, 1994). The cells lining the crypts differentiate, becoming more cubical and swelling to four times their original size, and the microvilli become lobate and branching, surrounding, and supporting the symbionts. Over the next 4 days, the crypt spaces enlarge and the ciliated, microvillous epithelial structure regresses as a result of bacteria-induced cell death. This modification of squid tissue by a specific bacterium is a remarkable example of interspecies signaling leading to morphogenesis. The presence of as little as 10 *V. fischeri* in the crypts for 12 h is sufficient to induce the 4-day morphogenetic program (Doino and McFall-Ngai, 1995). In the absence of the specific symbiont, no morphogenesis takes place.

Transmission of *V. fischeri* from parent to offspring has been studied extensively (Nyholm and McFall-Ngai, 2004). The adult squid releases large amounts of *V. fischeri* into the water at dawn every day, assuring that sufficient symbionts are available to colonize the hatchlings (McFall-Ngai, 1999). Furthermore, the squid provides a habitat in which only *V. fischeri* that emits light is able to maintain a stable association (McFall-Ngai, 1999; Visick et al., 2000). Thus, even in transfer via the environment (often referred to as horizontal transmission), the holobiont is reconstituted faithfully.

### **PLANT: MICROBE SYMBIOSES**

Plant abundance, diversity, and activities are essential for life on the planet and microorganisms play a central role in all three phenomena. Microorganisms supply plants with nutrients, play a role in establishment of plants and the development of root systems and in protection against pathogens and other environmental stress conditions. Moreover, it is estimated that about 20,000 species of plants are obligatorily dependant on microbial cooperation for development, growth, and survival (van der Heijden et al., 2008). Studies on the microbiology of plants have been performed with microorganisms found in three main locations: around the roots (rhizosphere), on the leaves, stems, flowers, and fruit (phyllosphere), and inside plant cells (endophytes). The great majority of microorganisms have different degrees of beneficial relationships with plants, whereas only a small minority is parasitic. The close cooperation between plants and microorganisms necessitates overcoming the plant's immune response and often using some of its components together with others of the plant and some of the microbiota for enabling this interaction to occur (Bucher et al., 2009; Bednarek et al., 2010).

### **Mycorrhiza**

Most of the higher plant species enter into a mutualistic root endosymbiosis with arbuscular mycorrhiza, in which plant sugar, primarily glucose, is traded for fungal minerals, mainly phosphorus and water in addition to protection against pathogens and other environmental stresses such as drought (Augé, 2001). In addition, this symbiosis plays an important role in carbon cycling between atmosphere and biosphere (Zhu and Miller, 2003). This is an ancient symbiosis, which has been detected in fossils of early land plants (Remy et al., 1994), and the fungi involved are obligate symbionts all grouped into a single phylum the Glomeromycota (Schüßler, 2001). Development of the mycorrhiza begins with invasion of the plant root by a soil fungus; growth of the fungus toward the root is stimulated by plant excretion into the soil of certain compounds, including flavonoids (Zhang et al., 2009) and strigolactones (Akiyama et al., 2005). The fungal mycelium penetrates the root cells and develops intracellularly, forming structures called arbuscules. However, a large fraction of the mycelium remains in the soil contributing to its assembly. Development of this highly compatible association requires the coordinate molecular and cellular differentiation of both symbiont and host to form specialized interfaces over which bidirectional nutrient transfer occurs (Bucher et al., 2009). Mutations in plant or fungal genes involved in the symbiosis inhibit mycorrhiza development (Harrison, 1999) and lead to stunted plant growth.

### **Rhizosphere and Phyllosphere Bacteria**

In addition to fungi, many bacterial species interact with plant roots. The multitude of bacterial species contribute to carbon transfer to soil, nitrogen-fixation, nitrate reduction, mineralization of organic materials, maintenance of soil structure and water cycling,

and protection against pathogens and other stress conditions, all of which promote plant growth directly or indirectly (Singh et al., 2004; Lugtenberg and Kamilova, 2009). Rhizosphere microbial communities differ between plant species (Innes et al., 2004; Berg and Smalla, 2009), between ecotypes within species (Micallef et al., 2009), between different developmental stages of a given plant (Weisskopf et al., 2006), and from those present in bulk soil (Broz et al., 2007). Microorganisms in the rhizosphere are selected for their functional abilities no less than for their taxonomy (Singh et al., 2004). Moreover, it has been shown that plant's specific exudates are major contributors to the plant specificity of rhizosphere microbiota (Somers et al., 2004; Singh et al., 2004; Berg and Smalla, 2009).

Large populations of microorganisms also live in the phyllosphere. Archaea, filamentous fungi, and yeast are present in the phyllosphere, but bacteria are considered to be the dominant microbial inhabitants present on the plant surface and within the plant tissue (Whipps et al., 2008). Stressful conditions on the leaves, such as extreme temperatures and dryness, irradiation, and oxidative stress in addition to poor nutrient availability determine the kinds of bacteria, their mode of growth, and their activities (Lindow and Brandle, 2003). Most information on the microbial communities in the phyllosphere has been established using culture-dependant methods and much of it is on pathogenic bacteria and fungi. The global surface area of the phyllosphere, estimated to be  $4 \times 10^8$  km<sup>2</sup>, harbors a bacterial population in the region of  $10^{26}$  cells including  $2-3 \times 10^6$  species (Whipps et al., 2008). Interestingly, culture-independent techniques have revealed that similarly to the human gut, these species fall within a relatively small number of dominant phyla, the proteobacteria being the most abundant on leaves (Delmotte et al., 2009; Redford et al., 2010). This phe-

nomenon is in accordance with the special conditions known to occur in the phyllosphere, demanding specific adaptations and activities (Delmotte et al., 2009). Given the high mass of phyllosphere microbiota, it is likely that they play an important role in global transformation of matter, including recycling of carbon and nitrogen. In addition, they contribute to the plant's fitness mainly through spatial protection against pathogens, promotion of growth, and deterrence of herbivores (Lindow and Brandle, 2003; Whipps et al., 2008). Microorganisms are transferred onto the areal part of plants via seeds, the atmosphere, insects, and animals and also via the vascular plant system from the roots. They are unevenly distributed, mainly on the lower part of leaves, as single or aggregated microorganisms (Whipps et al., 2008). Culture-independent methods have shown that by and large community pattern of the phyllosphere bacteria correlate with the tree phylogeny even across continents (Yang et al., 2001; Redford et al., 2010), though not all studies are in agreement (Whipps et al., 2008). In addition, though bacterial leaf communities differ between seasons, similar ones are found on leaves sampled during the same season, and this pattern is predictable from year to year (Ercolani, 1991; Redford and Fierer, 2009).

### Nitrogen-Fixing Legume Holobionts

Several specialized kinds of bacteria, including the most studied, *Rhizobium*, engage in symbiotic relationships with peas, soybeans, and other legumes to convert nitrogen gas into ammonia and further into organic nitrogen-containing compounds. *Rhizobia* are highly specific for their plant host. Their specificity arises, in part, from chemical "cross-talk" between the bacteria and plant (Long, 2001). The interaction begins when legumes secrete flavonoids into the rhizosphere. When a bacterium recognizes this

signal, it responds by synthesizing a specific oligosaccharide (Nod factor), which is responsible for host specificity. The bacteria invade tiny hairs on the roots of the legume, penetrating into the root tissue. There, the bacteria differentiate into larger cells referred to as bacteroids. The appropriate Nod factor triggers the developmental nodulation program in the plant that ultimately leads to the formation of bacteroid-filled root nodules, where nitrogen fixation takes place. The bacterial enzyme responsible for nitrogen fixation, nitrogenase, is extremely sensitive to inactivation by oxygen. Low partial pressures of oxygen are maintained in the nodule by synthesis of leghemoglobin, which becomes concentrated in the root cytoplasm surrounding the vacuoles that enclose the bacteroids. This imparts the pink color to the actively nitrogen-fixing nodules. Interestingly, neither the plant nor the bacterium can synthesize leghemoglobin individually; the apoprotein is encoded by a plant gene (Ott et al., 2009), and the heme moiety is synthesized by bacterial enzymes (Hardison, 1996). Thus, a two-way conversation between the bacterium and its plant host is responsible for the development of the nodule and its nitrogen-fixing capability.

### VERTEBRATE: MICROBE SYMBIOSES

Symbioses between diverse microbiota and vertebrates have been studied in a variety of animals, including ruminants (Dehority, 2003), chickens (Abbas Hilmi, 2007), whales (Olsen et al., 1994), gorillas (Frey, 2006), and rats (Brooks et al., 2003). We would like to present here briefly what is probably the best-studied metabolic system in vertebrates, namely, the human/mouse gut symbiosis. This system has provided a wealth of detailed information on how diverse extracellular symbionts contribute to the health of the human holobiont. We shall

use it for demonstrating the principles and outcomes of the hologenome theory.

### Abundance and Diversity of Microorganisms Associated with Humans

The number of bacteria in and on a typical healthy human is  $10^{14}$  to  $10^{15}$ . Most of these microbes are not merely in transit but rather inhabit defined niches. In the 1970s, it was believed that the number of different bacterial species in the human gastrointestinal tract was a few hundred. This assumption was based on isolation and viable counts. Recently, a new estimate was published using metagenomic techniques (Frank and Pace, 2008) claiming a minimum number of 40,000 species. Using the latter number, the number of unique bacterial genes in the human gut alone is 200 times greater than that of the human host.

In addition to the gastrointestinal tract, there is a high abundance and diversity of microbes on all surfaces of the human body, including skin, oral cavity, nasal cavity, pharynx, esophagus, and urogenital tract. In 2008, the NIH launched a \$157 million, 5-year human microbiome project (HMP) to begin determination of the genomes and proteomes of human microbiota. In 2010, the first report of the HMP was published, an analysis of 178 genomes from microbes that live in or on the human body ([www.nih.gov/news/health/may2010/nhgri-20.htm](http://www.nih.gov/news/health/may2010/nhgri-20.htm)).

The report describes many novel genes and proteins that serve functions in human health and disease.

### Microbial Symbionts Affect the Fitness of the Human Holobiont

Considering the holobiont as a unit of selection in evolution, we argue that the cooperation between the normal microbiota and the host generally leads to improved fitness of the holobiont, by the host outsourcing (Gilbert et al., 2010) different kinds of functions to its microbiota and vice versa. Table 1 summarizes some of the diverse beneficial interactions between humans and their microbiota.

Protection against infectious disease is one of the important attributes of the resident microbiota. Most bacterial pathogens infect their human hosts predominantly via mucosal surfaces of the respiratory, urogenital, or gastrointestinal tracts. In addition to mechanical and immunological barriers, mucosal surfaces are protected against pathogen infection by the high concentration of microbiota colonizing the mucosa. The exact mechanism is unknown, but it has been suggested that resident bacteria occupy binding sites needed by pathogens for adhesion in addition to releasing antibacterials active against pathogens. As an experimental example of bacterial protection against infection, mice were treated with *Bifidobacterium longum*, part of the normal microbiota, and then infected with the

pathogen *Salmonella typhimurium*. The mice that received *B. longum* survived, whereas the control group (*Salmonella* alone) all died within a few days (Silva et al., 2004). Interestingly, it has been demonstrated that immune response to integral microbiota via IgM differs from its reaction to pathogenic microorganisms (Hapfelmeier et al., 2010).

One of the most important known beneficial functions of microbiota is participation in the development and normal function of the innate and adaptive immune systems in the gut (O'Hara and Shanahan, 2006; Ivanov and Littman, 2011) while creating a permissive, noninflammatory environment for their own presence (Hapfelmeier et al., 2010). Bacteria are critical in promoting the early development of the gut's mucosal immune system both in terms of its physical components and its function and continue to play a role later in life in its operation. The microbiota also plays a key role in angiogenesis, the structural buildup of blood vessels (Stappenbeck et al., 2002). Bacteria found in the gut synthesize and excrete vitamins in excess of their own needs, which can be absorbed as nutrients by their host. For example, in humans, enteric bacteria secrete Vitamin K and Vitamin B12, and lactic acid bacteria produce certain B-vitamins (Mai et al., 2010). Moreover, germ-free animals are deficient in Vitamin K to the extent that it is necessary to supplement their diets. The human gut microbiota is a complex ecosystem that plays an essential role in the catabolism of dietary fibers, the part of plant material in our diet that is not metabolized in the upper digestive tract, because the human genome does not encode adequate enzymes (Dethlefsen et al., 2006). Germ-free animals, born and grown under sterile conditions, are a useful tool for studying the relationship between host and its microbiota. Studies on germ-free mice exhibit significant differences in gut development, function, and regulation when compared with

**TABLE 1. Contribution of Microorganisms to the Fitness of Humans and Mice**

Contribution	References
Protection against pathogens	Silva et al., 2004; Stecher and Hardt, 2008
Stimulation of immune system	O'Hara and Shanahan, 2006; Ivanov and Littman, 2011
Angiogenesis	Stappenbeck et al., 2002
Vitamin synthesis	Mai et al., 2010
Fiber breakdown	Dethlefsen et al., 2006
Fat metabolism, obesity, and related disorders	Ley et al., 2005; Turnbaugh et al., 2006; Cani and Delzenne, 2009

mice grown conventionally, that is, possessing normal gut microbiota. The germ-free mice demonstrate enlarged caeca (Wostmann, 1981), a slow digested food transit time (Abrams and Bishop, 1967), altered kinetics of epithelia turn-over in the small intestine (Savage et al., 1981), an increased caloric intake (Wostmann et al., 1983) and a greater susceptibility to infection (Silva et al., 2004). The influence of microbiota on energy metabolism in germ-free conventionalized mice was observed within 2 weeks of the introduction of microbiota (Bäckhed et al., 2007). It included microbial fermentation of polysaccharides not digested by the host, absorption of the microbially produced short-chain fatty acids, more efficient absorption of the monosaccharides from the intestine, conversion of breakdown products in the liver to more complex lipids and microbial regulation of host genes that promote fat deposition in adipocytes. These events were accompanied by lower food intake and higher metabolic rate. In addition, it has been shown in mice (Ley et al., 2005; Turnbaugh et al., 2006) and humans (Ley et al., 2006) that obesity is correlated with different bacterial communities and that a gradual transition occurs in humans from the obese microbiota to the lean microbiota during a course of a restrictive energy intake (Ley et al., 2006). Moreover, obese microbiota have been implicated in obesity-related metabolic disorders such as type 2 diabetes, inflammation, disordered lipid metabolism, and atherosclerosis, in addition to fatty liver, primarily via bacterial Gram-negative LPS (lipopolysaccharide) metabolic effects (Cani and Delzenne, 2009; Caesar et al., 2010; Abu-Shanab et al., 2010).

### Microbial Symbionts Are Transmitted from Parent to Offspring

The hologenome theory of evolution relies on ensuring the continuity of partnerships between hol-

obiont generations. In recent years, it has become clear that microbial symbionts can be transmitted from parent to offspring by a variety of methods, including being in the reproductive cell (cytoplasmic inheritance of endosymbionts), feeding feces of the adult to juveniles (e.g., termites), and direct contact (many animals). In mammals, including humans, the initial population of symbionts is derived during passage through the birth canal and subsequently by close physical contact with parents. Support for vertical transmission of microbiota in humans comes from a study, which showed a greater similarity of microbiota within family members when compared with between families (Zoetendal et al., 2001) in addition to similarity between microbiota of vaginally delivered infants and their mother's vaginal microbiota (Dominguez-Bello et al., 2010). Moreover, it has also been observed that a correlation exists between mother's BMI, weight, and weight gain during pregnancy and infant's microbiota implying a possible effect on fetal and child metabolic development. The conserved transmission of microbiota from parent to offspring for many generations has been used as a window into human migration (Devi et al., 2006).

### Genetic Variation in Holobionts

Variation is the raw material for evolution. According to the hologenome theory of evolution, genetic variation can arise from changes in either the host or the symbiotic microbiota genomes. Variation in host genome occurs during sexual reproduction and development, by recombination, chromosome rearrangements, mutation, and epigenetic variations. Variation in the microbiota occurs not only by these mechanisms but also by three other processes that are unique to the hologenome theory of evolution: microbial amplification, acquisition of novel strains, and horizontal gene transfer. These latter three

processes can occur rapidly under environmental demand and are important elements in the adaptation, development, and evolution of animals and plants

The first, microbial amplification is the most rapid and easy to understand mode of variation in holobionts. It involves changes in the relative numbers of the diverse types of associated microorganisms that can occur as a result of changes in environmental conditions. The holobiont is a dynamic entity with certain microorganisms multiplying and others decreasing in number as a function of local conditions. An increase in the number of a particular microbe is equivalent to gene amplification. Considering the large amount of genetic information encoded in the diverse microbial population of holobionts, microbial amplification is a powerful mechanism for adapting to changing conditions. In fact, changes of symbiont populations as a function of external factors are well documented in many biological systems, including humans. Children on a high fiber diet had a high abundance of bacteria from the genus *Prevotella* and *Xylanibacter*, known to contain a set of bacterial genes for cellulose and xylan hydrolysis, whereas children on a high carbohydrate diet had abundant *Shigella* and *Escherichia* (De Filippo et al., 2010). Further support for amplification of certain bacteria following a change in diet comes from a study of infant gut microbiota (Koenig et al., in press) in which ingestion of solid table foods caused a change in infant gut microbiota with sustained increase in the abundance of bacteroidetes. In a study performed on humanized gnotobiotic mice, it was observed that a one day change in diet from high fiber to high fat brought about an immediate change in microbiota (Turnbaugh et al., 2009).

The second mechanism for introducing variation into holobionts is acquiring new symbionts from the environment. Animals and plants come in contact with billions of microorganisms during their lifetime. It is reasonable to assume

that occasionally, as a random event, one of these microorganisms will find a niche and become established in the host. Under the appropriate conditions, the novel symbiont may become more abundant and affect the phenotype of the holobiont. Unlike microbial amplification, acquiring new symbionts can introduce entirely new genes into the holobiont. Examples of such amplification, or in some circumstances of acquisition of novel strains, are probiotics and prebiotics (Hord, 2008). By introducing specific strains of bacteria known to contribute to the health of the holobiont one can achieve in humans recovery from *Clostridium difficile*-associated diarrhoea (MacConnachie et al., 2009) and changes in metabolic characteristics (Laitinen et al., 2009). The third mechanism is the microbe-microbe interaction of horizontal gene transfer by which new traits can be transferred from microorganisms not generally associated with the holobiont to resident microbes. An example of the latter is the transfer of genes coding for porphyranases, agarases, and associated proteins from a marine bacteria member of the bacteroidetes to the human gut bacterium *Bacteroides plebeius* in Japanese population (Hehemann et al., 2010).

We suggest that once a beneficial genetic variation in a holobiont has occurred as a result of changes in the microbiota (in a single specific symbiont or in multiple symbionts), two general pathways may be possible for ensuring that any useful genetic information is conserved in future holobiont generations: (1) The microbial genes can be inserted into the host genome, as in the transfer of carotenoid biosynthetic genes from a fungus to aphids (Moran and Jarvik, 2010), and/or (2) the host and microbe can undergo secondary changes that stabilize and benefit the interactional symbiosis. This latter kind of adaptation can occur in primary symbiosis as in corals and their algae (Fallowski et al., 1984) or in secondary symbiosis

as in the bovine rumen which fosters the growth of anaerobic cellulose-degrading microorganisms, which benefit the host not only by conversion of the cellulose to utilizable fatty acids but also by satisfying its protein and vitamin requirements (Dehority, 2003). In this regard, two interesting unresolved questions come to mind: What determines, during evolution, which functions in the holobiont will be taken on by the host and which by the symbiont? Which microbial genes will be inserted into the host genome and which will be kept external within the microbiota?

## CONCLUSIONS AND IMPLICATIONS

The hologenome theory considers all of the diverse microbiota associated with the animal or the plant as part of the developing, growing, reproducing, surviving, adapting, and evolving holobiont and that changing the microbial community by amplification and acquisition of novel microbes or genes from the environment provide additional mechanisms for adaptation and evolution. To support this concept, we have discussed published results that exemplify the large diversity of symbionts of higher organisms, their ability to be transmitted from one generation to the next, their contributions to the fitness of the holobiont and their potential to change rapidly under environmental shifts. Interestingly, the hologenome theory incorporates principles of both Darwinism and Lamarckism. Individual organisms evolve by selection of random variants, whereas the holobiont can also evolve by adaptive processes.

Approaching the holobiont with its hologenome as a single entity can lead to better understanding of mechanisms of fitness and health, adaptation to new environments, developmental processes, and also the evolution of species. The practical aspect of this approach is the potential of manipulating the holobiont microbiota and its microbiome with pro-

biotics and prebiotics to change relevant characteristics. On the other hand, it is clear that attempting to treat animals or plants with nutrients, such as iron supplementation in humans (Zimmermann et al., 2010) or with medications, such as antibiotics (Jernberg et al., 2010) may have detrimental effects. As in addition to achieving a positive goal, these attempts can also select for unwanted microbiota or reduce the potential microbiota gene pool available for future use by the holobiont and as a result cause more harm than benefit.

Another interesting aspect of the hologenome concept is its relationship to the developmental origin hypothesis, which based on epidemiological and animal data (Barker, 1995; Roseboom et al., 2006; Waterland and Michels, 2007), posits that during critical embryonic and postnatal development, nutrition, and other environmental factors affect developmental processes in humans and other mammals by inducing persistent changes in metabolism that may lead to chronic disease susceptibility (Harding, 2001; Gluckman and Hanson, 2004). Epigenetic mechanisms have been suggested to be responsible for these phenomena. It might be valuable to consider the maternal and the postnatal progeny microbiota as an additional factor programming health and disease in later life. The vast size and diversity of the microbial community within the mammalian host, the interrelation of the microbiota with host metabolism, and its transmission from parents to offspring make it an interesting candidate for influencing developmental programming.

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