

Why bacteria matter in animal development and evolution

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While largely studied because of their harmful effects on human health, there is growing appreciation that bacteria are important partners for invertebrates and vertebrates, including man. Epithelia in metazoans do not only select their microbiota; a coevolved consortium of microbes enables both invertebrates and vertebrates to expand the range of diet supply, to shape the complex immune system and to control pathogenic bacteria. Microbes in zebrafish and mice regulate gut epithelial homeostasis. In a squid, microbes control the development of the symbiotic light organ. These discoveries point to a key role for bacteria in any metazoan existence, and imply that beneficial bacteria-host interactions should be considered an integral part of development and evolution.

Keywords:

■ evolution; host-microbe; holobiont interaction; *Hydra*; microbiota

Introduction

'Understanding evolution's inner workings requires understanding the full range of life's possibilities' [1]

The human body represents a scaffold upon which multitudes of mutualistic and symbiotic species build residence, creating a diverse ecosystem composed of members of five (bacteria, protozoa, fungi, archaea and viruses) of the six kingdoms of life. These are our normal microbiota and they include 'good' microbes that can help us and microbes that can hurt us. By colonising germ-free animals with single

microbial species, researchers have begun to evaluate each one's contribution to the hosts development and health. The good microbes contribute to health in ways that range from efficient nutrient digestion and absorption, to controlling pathogens by effective and balanced barrier function, to triggering developmental programs. This article reviews the latest evidence that the animal host and its microbiota have evolved into a homeostatic, symbiotic relationship.

From *Hydra* to man: Epithelia select their microbiota

Hydra have been model systems in developmental biology since the 1980s. Clonally growing mass cultures can be kept in plastic or glass dishes for decades. Different species are cultivated under standard conditions at constant temperature, getting the same food (freshly hatched *Artemia*). When examining the microbiota in two *Hydra* species kept in the lab for more than 20 years under identical conditions, we found that the epithelium was colonised by a complex and dynamic community of microbes (Fig. 1) [2]. More surprisingly, we observed that – although cultured under identical conditions – individuals from different species differed greatly in their microbiota [2]. Even more astonishing was the finding that individuals living in the wild were colonised by a group of microbes similar to that in polyps grown in the lab, pointing to the maintenance of specific microbial communities over long periods of time [2]. This finding strongly indicates that distinct selective pressures are imposed on and within the *Hydra* epithelium, and suggest that in each species of host the active selection that shapes the specific bacterial community is genetically encoded in the epithelium.

Hydra has one of the simplest epithelia in the animal kingdom, with only two cell layers (a mesoderm is missing), and few cell types derived from three distinct stem cell lineages: the ectodermal and endodermal epithelial stem cells, and the interstitial stem cells. How intimate is the relationship between microbes and this epithelium? Do microbes react to changes in the *Hydra* epithelium? To decipher putative links between epithelial homeostasis and species-specific bacterial phylotypes, we removed the cells of one of the three stem cell

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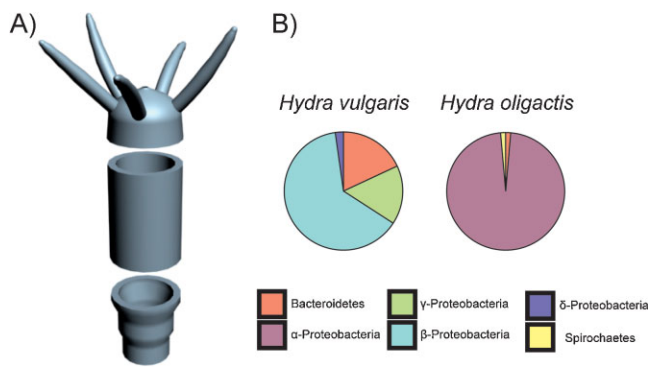


Figure 1. The basal metazoan *Hydra* is colonised by species-specific microbiota. (A): Schematic view of *Hydra* indicating the simple body plan consisting of a tube-like directive axis with an oral ('head') and an aboral ('foot') end. (B): Distinct compositions of the bacterial communities identified from *Hydra vulgaris* and *Hydra oligactis*.

lineages, the interstitial cell lineage, from the tissue while leaving both epithelial stem cell lineages untouched. These changes in the epithelia cell composition led to significant modulation of the microbial community [3]. In particular, two bacterial phylotypes changed drastically when one host cell lineage was removed. The dominant bacterial phylotype in control polyps belonging to the β -Proteobacteria was decreased in polyps lacking the interstitial cell lineage. In contrast, a bacterial phylotype belonging to the *Bacteroidetes* abundantly increased in polyps lacking interstitial cell lineage compared to control polyps [3]. These changes in the bacterial composition depending on the loss of the interstitial cell lineage imply a direct interaction between epithelia and microbiota.

What is the function of the bacteria in *Hydra*? Long before the profound effects of beneficial microbiota were explored in mammals, Rahat and Dimentman showed [4] that bacteria have profound effects on tissue proliferation in *Hydra*. *Hydra* polyps were sterilised by treatment with a mixture of antibiotics, and cultured under axenic conditions. Unexpectedly, although well fed with sterile shrimps, these polyps were unable to proliferate asexually by budding. Interestingly, this effect could be rescued by inoculating the culturing medium with bacteria from a stock culture of *Hydra* [4]. While this probiotic effect in *Hydra* remains mostly unexplained, in several invertebrates and vertebrates there is accumulating evidence that beneficial microbes have a crucial role in metabolism, immunity and development.

Probably the best-documented case of the ancient and crucial role of host-microbe coevolution comes from the analysis of gut microbiota. Investigating the intestinal microbiome in human gut biopsies and faecal samples from more than 60 species of mammals by large-scale 16S rRNA gene sequencing revealed that bacterial communities codiversified with their vertebrate hosts, and that the gut microbiota are, at least to a certain degree, selected by the gut epithelium [5]. Although more than 80% of the identified phylotypes belong to only two phyla, the *Firmicutes* and *Bacteroidetes*, the genera and species diversity are large. Interestingly, the bacterial community structure is more similar within mammalian species than between different species [5]. Host diet appears

to affect the microbiota composition since herbivore, omnivore and carnivore groups of mammals cluster discretely with regard to their microbiota [5] (for review see ref. [6]). The general conclusion was that in vertebrates, similar to *Hydra*, there is a coevolved relationship between hosts and their gut microbiota.

Microbes assist in digestion of otherwise inadequate diets

Some animals have escaped from the metabolic requirement for a balanced dietary supply of essential amino acids by forming symbioses with microorganisms that both synthesise and provide them with essential amino acids [7]. The gastrointestinal tract of ruminant animals, for example harbours dense and complex microbial communities. Amongst the beneficial effects, gastrointestinal tract microbial communities are involved in digestion and fermentation of plant polymers, an activity that is of particular importance in herbivorous animals [8]. The indigenous gut microbiota are also responsible for the synthesis of vitamins, bioconversion of toxic compounds to non-toxic residues, stimulation of the immune system and maintenance of intestinal mucosal integrity [8].

Like ruminants, termites also have a series of stomachs, each harbouring a distinct community of microbes under precisely controlled conditions. Inside termites' hindguts reside several hundred, largely unique, microbe species that vary among termite species. Termites digest wood with the help of their large and complex community of intestinal microorganisms. The metabolism of the microbes converts the polysaccharides in wood to acetate, which the termites absorb as their major source of nutrition. A metagenomic analysis of this bacterial community in the hindgut of a Costa Rican *Nasutitermes* termite species [9] uncovered the presence of a diverse set of bacterial genes for cellulose and xylan (the main component of hemicellulose) hydrolysis, indicating that the bacteria with their hydrolytic enzymes indeed are engaged in the decomposition of these polysaccharides. Obviously, these microbes are tasked with particular steps along the conversion pathway of woody polymers to sugars, which can then be fermented into fuels such as ethanol. Therefore, this first system-wide gene analysis of microbes specialised towards the breakdown of plant polysaccharides not only provides insights into important symbiotic functions, but also could eventually lead to the production of novel enzymes for better biofuel production [9].

Aphids are insects that feed only on plant phloem sap, a sugary mix rich in carbohydrates but low in protein and deficient in nitrogenous compounds [10–12]. This nutritional specialisation has been attributed to their symbiotic microorganism, a γ -Proteobacterium termed *Buchnera aphidicola* [13], which belongs to the *Enterobacteriaceae* family. Although genetic and physiological studies have indicated that *Buchnera* can synthesise the essential amino acids methionine, cysteine and tryptophan, and supply them to the aphid host [14], it was only recently that Gündüz and Douglas [15], in an elegant study, succeeded in demonstrating that the capacity of aphids to exist solely on plant phloem sap can be directly attributed to their symbiotic bacteria.

The genome sequences of both the pea aphid *Acyrtosiphon pisum* (<http://www.hgsc.bcm.tmc.edu/projects/aphid/>) and the endosymbiont *Buchnera sp* [16], provides a unique window into a long history of parallel evolution and interdependence of *Buchnera* and aphids. The aphid, with an estimated 34,604 protein-coding genes, has about twice the number of genes found in *Drosophila*. Among them are several novel, taxonomically restricted genes, not known in other species (for review see ref. [17]), that code for saliva proteins that likely help to keep plant juices flowing once the aphid has inserted its mouthparts into the plant phloem. Intriguingly, the aphid has apparently given up some of the genes that normally help to fight infections by gram-negative bacteria such as *Buchnera*. That loss might account for the evolutionary success of aphids to obtain and maintain beneficial symbionts. In return, the genome of *Buchnera* is tiny, consisting of only one 640,681 base pair chromosome and two small plasmids, and encodes only about 640 genes. This reduced gene set reflects the tight mutualism between *Buchnera* and the aphid hosts at the genomic level, and includes key genes for providing the aphid with about nine amino acids that are missing from the sap on which these insects feed. Notably, *Buchnera* lacks the enzyme needed for the last step in making leucine, but it is present in the aphid genome, so the synthesis is finished in the aphid. This division of anabolic labour dramatically underscores the dependent nature of the *Buchnera*-aphid relationship at the molecular level.

Life in the mammalian intestine

The human gastrointestinal tract is an important and challenging system for exploring (i) how microbial communities become established within their hosts, (ii) how their members maintain stable ecological niches and (iii) how these dynamics relate to host health and disease (Fig. 2A). The human gut is home to trillions of bacteria comprising thousands of species-level phylotypes. In a human individual this huge bacterial load amounts to 2 kg of bacteria in the gastrointestinal tract. Because the number of bacterial cells is ten times the number of somatic cells in the human body [18], man seems more microbial than human. Some microbes are firmly rooted in place, forming densely populated communities. Others are only transiently present in the gastrointestinal tract often associating with food material as it passes through. Life in the mammalian gut is certainly not easy. However, despite the continuous flow of colonic material and the opportunity for microbes to get constantly washed out, the bacterial density and diversity of the colon is high and remains remarkably stable [19]. How is the constancy of this complex community structure maintained?

While there are as yet no definite answers, it is interesting to note that research at the Duke University School of Medicine, drawing upon a cladistic approach, has provided evidence [20, 21] that the mammalian caecal appendix may be a place where the beneficial microbiota can be stored safe and undisturbed until they are needed to repopulate the gut after, for example intestinal stress. Parker and coworkers found recently [21] that rather than being a mere vestigial or useless structure appealing only to a small group of specialists, the

appendix may play a key role as a refugia maintaining the complex gut microbiota.

As stated above, two bacterial phyla, the *Firmicutes* and the *Bacteroidetes*, commonly dominate this gut ecosystem [22], as they do in the guts of many other mammalian species [5, 6]. Comparative analysis of the *Bacteroides thetaiotaomicron* genome shows that it contains a large number of genes dedicated to breaking down complex carbohydrates that cannot be processed by host-encoded enzymes in the human gut [23, 24]. By simultaneously profiling the relative abundance of tens of thousands of *B. thetaiotaomicron* mutants across multiple conditions [23], hundreds of genes have been identified that encode glycoside hydrolases and polysaccharide lyases. Additionally, numerous bacterial outer membrane proteins involved in recognition and import of specific carbohydrates, as well as a large number of environmental sensors and regulators, have been detected [25]. Thus, *B. thetaiotaomicron* clearly has the capacity to sense the presence of polysaccharides within the human digestive system and to activate the proper set of enzymes to harvest these nutrients. This capacity is beneficial for the human host because *B. thetaiotaomicron* breaks down the polysaccharides into products that can only then be absorbed by the human body.

Microbial component of obesity

Obesity has become a major public health issue worldwide. Important observations from the Gordon laboratory at Washington University in St. Louis (Wash. U.) [26–28] of the microbiota of mice and man indicate that obesity has a microbial component. For instance, the mouse caecum is dominated by *Firmicutes* (60–80% of phylotypes) and *Bacteroidetes* (20–40%) species (Fig. 2B). By comparing mice that were identical except for mutations in the ‘obese gene’ leptin, guts of the lean mice were found [27] to have a high proportion of *Bacteroidetes* bacteria, while the guts of the genetically obese *ob/ob* mice were even more dominated by the *Firmicutes*, with a proportional 50% decrease in the abundance of *Bacteroidetes*, relative to lean mice (Fig. 2C). Are the changes in the gut microbiota a cause or consequence in obesity? Were specific types of bacteria contributing to the weight loss or a result of it? Answers came after implanting specific combinations of microbes into the guts of germ-free mice and monitoring the resulting impact on weight gain. When microbes were transplanted from obese and lean mice into mice raised in a sterile environment, those that got microbes from the obese mice gained twice as much fat. Transplanting the gut microbiota from the caecum of conventionally raised mice into germ-free recipients increased their body fat content by 60% after 14 days without any increase in food consumption [26]. The lean body mass decreased 7%, leaving total body weight unchanged [26]. While the germ-free mice were able to feast on rodent chow and remain thin, their genetically identical counterparts with bacteria in their guts ate 29% less but increased body fat. Thus both the presence and the composition of the microbial community in the gut clearly affect the amount of energy extracted from the diet.

What is true for mice is apparently also true for man. In a follow-up-study in humans, the relative proportion of

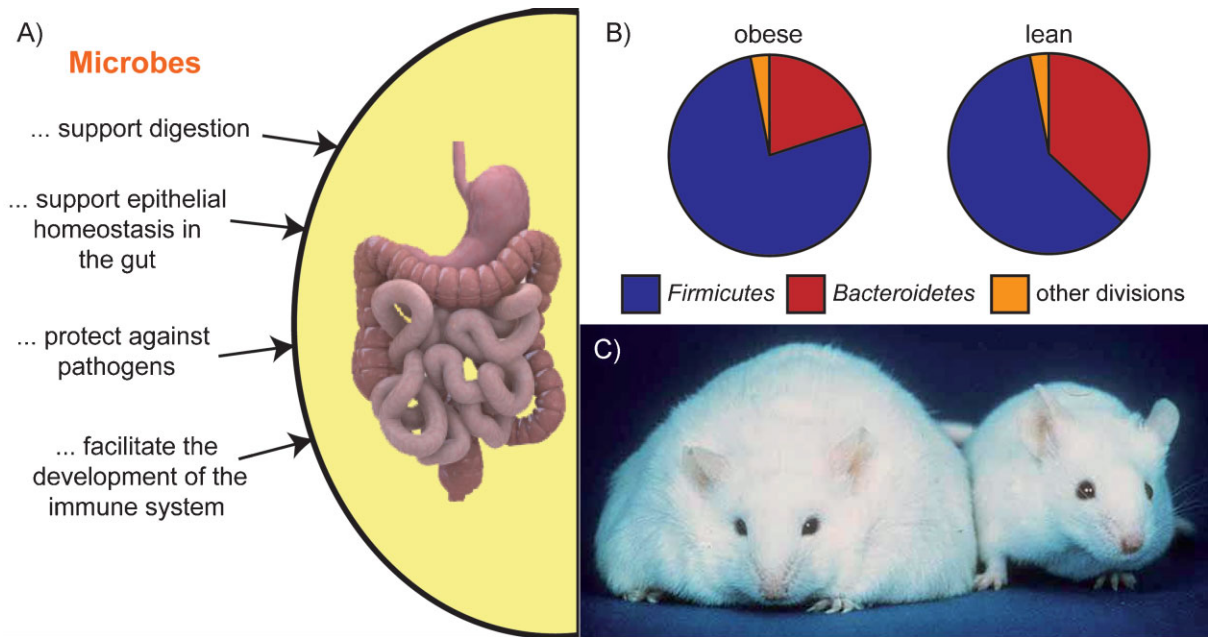


Figure 2. Microbes in the mammalian intestine. **(A):** Summary of some of the influences that the intestinal microbiota have on the biology of host organisms. **(B):** Proportion of *Bacteroidetes* and *Firmicutes* in the caecal microbiota of lean versus obese mice (data taken from Ref. [27]). **(C):** Mice lacking the hormone leptin cannot monitor the amount of fat in their body and quickly become obese. Analysis of the gut microbiota in these mice revealed 50% fewer *Bacteroidetes* in their caeca than in the control mice (picture taken from <http://notexactlyrocketscience.files.wordpress.com/2007/01/fatmouse.jpg>).

Bacteroidetes was decreased in obese people in comparison with lean people, but this proportion recovered with weight loss on two types of low-calorie diet [28]. This implies that people with certain communities of gut microbes may get more calories from their food than people with a different set of microbes. While the specific microbial components that can change the proportion of the *Bacteroidetes* and *Firmicutes* community remain to be uncovered, these studies convincingly demonstrate that weight regulation is influenced by the gut microbiota – and that manipulation of this community may provide additional avenues for the treatment of obesity.

Bacteria shape the complex immune system

Several years ago the Russian immunologist Vladimir Klimovich speculated that ‘the immune system appeared and developed not only under conditions of contact with potential pathogens, but also on the background of the coexistence and interaction with genetically foreign organisms, *i.e.* under conditions of symbiosis’ [29]. Supporting this view (see also ref. [30] for a discussion on the relationship between beneficial symbionts and the evolution of the host’s immune system), the central importance of bacteria in shaping the immune system was impressively documented in studies

comparing germ-free and conventionally raised animals. Germ-free animals have serious problems with their immune systems, including higher susceptibility to infections as well as fewer lymphocytes, less active intestinal macrophages and reduced vascularity, digestive enzyme activity, muscle wall thickness, cytokine production, serum immunoglobulin levels and CD4 T cells compared to conventionally colonised mice [31, 32]. Reconstitution of the germ-free mice with a normal intestinal microbiota is sufficient to restore the mucosal immune system [33]. These bacteria profoundly influence the development of humoral components of the gut mucosal immune system [34] and also modulate the fine-tuning of T cell repertoires and T helper (Th) cell type 1 or type 2 cytokine profiles [35, 36].

Colonisation of germ-free mice with a single species, *B. thetaiotaomicron*, can restore mucosal barrier function and the proper development of the enteric nervous system [37]. Moreover, a specific surface molecule from a single strain of symbiotic bacteria, *i.e.* capsular polysaccharide A (PSA) from *Bacteroides fragilis*, influences the normal development and function of the mucosal immune system [38]. When the Mazmanian laboratory colonised one group of germ-free mice with whole *B. fragilis* and another group with a strain of *B. fragilis* that lacked PSA but displayed the seven other capsular polysaccharides [38], wild-type *B. fragilis* restored CD4⁺ T cell levels to those of animals with the normal community of hundreds of bacteria. In mice that were colonised with the mutant bacteria lacking the zwitterionic PSA, CD4⁺ T cell levels were no better than in germ-free mice [38]. A histological examination of spleens and lymph nodes indicated that germ-free mice exposed to *B. fragilis* without PSA lacked the well-defined follicular structures that are a hallmark of healthy immune-cell development. Mice colonised with wild-type *B. fragilis* contained abundant follicles [38]. Recently it has been reported [39] that PSA not only initiates the activation of T regulatory cells and dampens inflammation by producing the cytokine IL-10, but also suppresses proinflammatory Th17

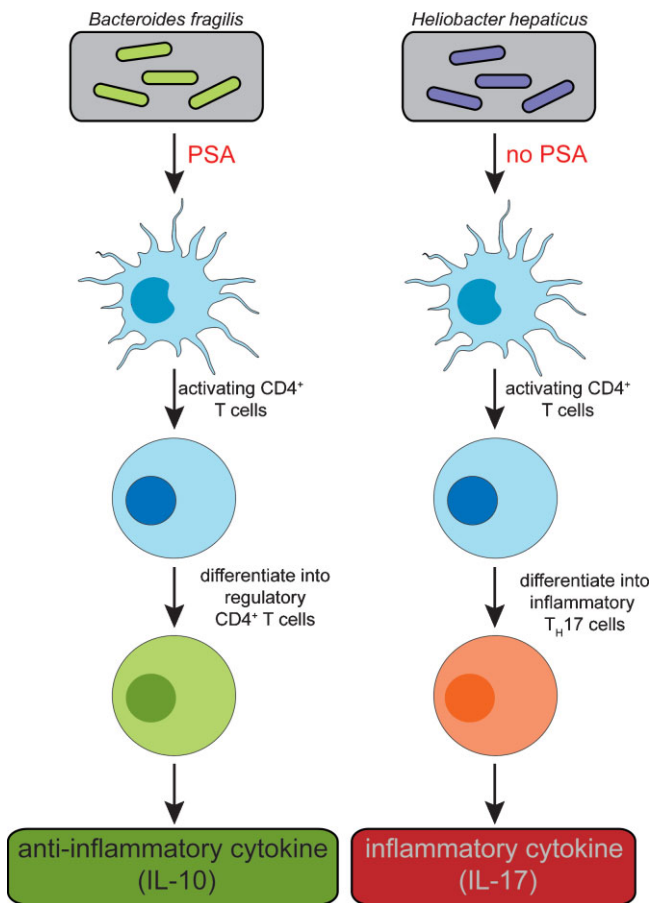


Figure 3. Bacteria regulate the immune system in vertebrates. Dendritic cells stimulated with PSA from *B. fragilis* presenting the antigen to CD4⁺ T cells responding with the production of the anti-inflammatory cytokine IL-10. In contrast, dendritic cells activated by *Helicobacter hepaticus* present the antigen also to CD4⁺ T cells that then differentiate into inflammatory Th₁₇ effector T cells and produce the inflammatory cytokines IL-17.

cells (Fig. 3). When germ-free mice are given either *B. fragilis* or purified *B. fragilis* PSA at the same time as inflammation-inducing agents, intestinal inflammation is prevented [39]. In the mouse intestine, PSA is presented by dendritic cells that traffic locally to mesenteric lymph nodes (reviewed in ref. [40]). Thus, a single molecule produced by *B. fragilis* appears to induce a coordinated and complex immune profile that is associated with intestinal health. The molecular mechanisms by which PSA prevents *H. hepaticus*-induced colitis remain to be uncovered.

B. fragilis is not the only important symbiont in the human gut [41]. Do all bacteria have a similar ability to induce intestinal Th cell development? Or, instead, have defined members of the microbiota evolved the unique ability to direct specific aspects of immune system maturation? Gaboriau-Routhiau *et al.* [42] recently revealed that colonisation of the mucosal epithelium with segmented filamentous bacteria (SFBs) induces the expression of a variety of innate and adaptive immune genes in the gut. Furthermore, germ-free animals mono-associated with SFBs develop Th cell populations that were similar to those in animals colonised with complex microbiota.

It appears, therefore, that SFBs have the ability to uniquely coordinate the intestinal T cell profile. It remains to be shown which bacterial molecule(s) are mediating this effect, or what type of host cells sense and respond to the SFB-derived microbial signals, and how this signalling affects the health of the host.

Imbalanced gut microbiota cause inflammatory bowel disease

Crohn's disease and ulcerative colitis are chronic inflammatory bowel diseases (IBDs) characterised by chronic and relapsing intestinal inflammation of unknown aetiology. Both genetic and environmental components are involved in these diseases, which affect about 2 million people in the United States [43]. Several recent studies show that immune activation by an imbalanced intestinal bacterial microbiota plays a major role in initiation and/or maintenance of the disease. First hints came from the observation that none of the genetic-defect models described to date develop IBD if they are kept in a pathogen-free environment [43]. Subsequently, culture-independent, DNA-based methodologies showed that the species diversity of the microbiota in Crohn's disease patients is reduced to 50% that of normal controls [44]. This reduction in diversity is primarily due to loss of normal anaerobic bacteria such as *Bacteroidetes* species [44]. Finally, intestinal microbes that affect Toll-like receptor (TLR) expression, or modulate TLR signal transduction pathways, influence host responses by the induction of distinct transcriptional responses. Non-invasive members of the normal microbiota, for example, activate TLRs on the apical surface of intestinal epithelial cells [45, 46], inducing a partial and protective activation of NF- κ B, a key regulator of the innate immune response. Invasive pathogens, on the other hand, appear to activate TLRs on basolateral mucosal surfaces, leading to a robust inflammatory response [47]. As pointed out by Leser and Molbak [19], this activation may enable pathogenic bacteria to invade spaces that are usually devoid of bacteria.

How beneficial microbiota are discerned from pathogens and are tolerated by the host is one of the fundamental questions in symbiosis research – and the answer is far from understood [19]. It is also not clear whether the presence of certain members of the normal microbiota triggers IBD, or whether this is triggered by the absence of protective symbiotic strains. Can good microbes help control bad ones? The answer is very likely 'yes'. Recent evidence suggests that products of 'friendly' intestinal microbes can counter IBD and Type 1 diabetes. Bacteria in the colon are known to ferment dietary fibres to produce short-chain fatty acids. Maslowski *et al.* [48], using a mouse model of chronic colitis, have demonstrated that these fatty acids down-regulate innate and inflammatory responses by stimulating the chemoattractant receptor GPR43 on neutrophils.

Microbes are required for gut development

Both mammalian and fish intestines initially develop in an initially sterile environment, but complete their maturation in

the presence of microbes. Do microbes play an active role in shaping the mature intestine architecture?

To study the mutualism between vertebrates and their associated microbial communities researchers at

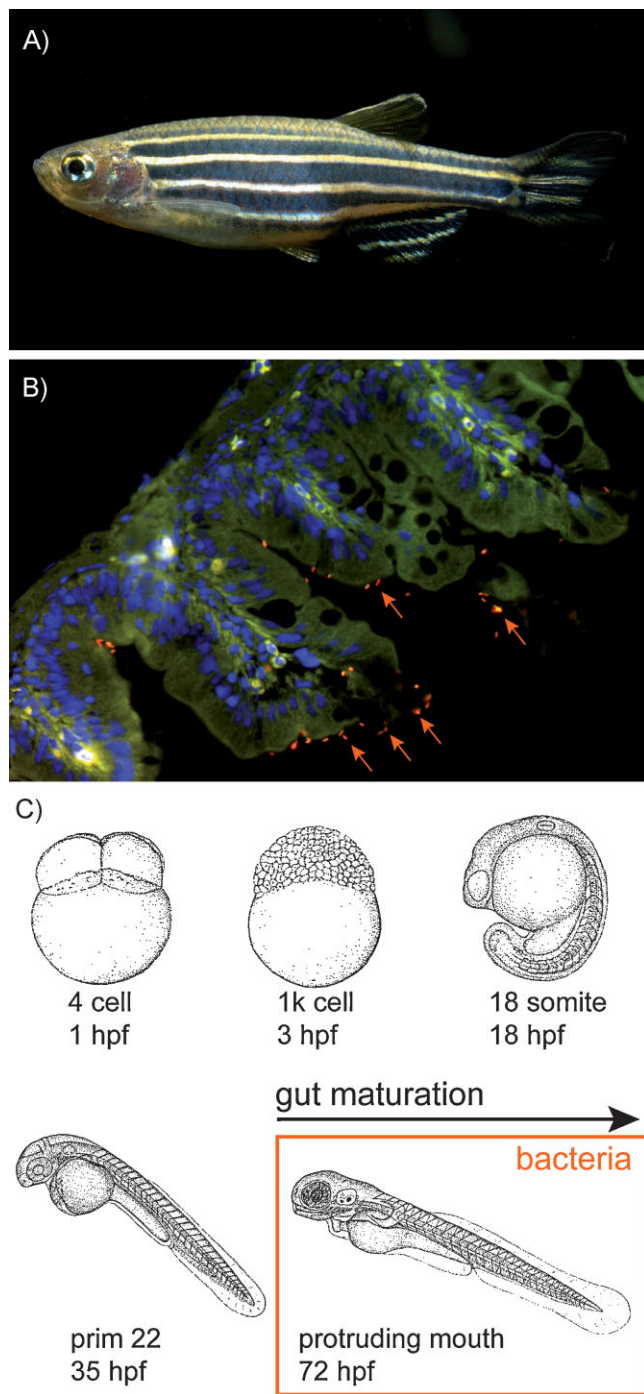


Figure 4. Zebrafish are a useful model to monitor gut development in the absence or presence of bacteria. **A:** Adult zebrafish (*Danio rerio*). **B:** Micrograph of an adult zebrafish intestine with bacteria (marked with orange arrows) in orange, host cell nuclei in blue, and tissue autofluorescence in green (reprinted with permission from ref. [74]). **C:** Stages of embryonic development of the zebrafish. Note: full gut maturation at 72 hours post fertilisation is dependent on the presence of bacteria [54].

Washington University have developed a germ-free zebrafish model [49] as a complementary vertebrate gnotobiotic model (Fig. 4A). Zebrafish digestive-tract development and physiology are very similar to that of mammals [50] but zebrafish are transparent until they reach adulthood. Thus, unlike mice, these fish provide researchers with unique opportunities to watch the gut develop with and without the beneficial effects of symbiotic bacteria (Fig. 4B). Similar to those in germ-free mice, several biological processes including the ability to process nutrients and the proper development of the immune system were compromised in germ-free zebrafish. In addition, the cells that line the intestine were not renewed as rapidly. Because the lining of the gut is continuously exposed to potentially toxic substances, this renewal of the gut lining is critical for maintaining health. To decipher how the bacteria communicate with the host, the genetic profile of fish raised under conventional conditions was compared to the profile of fish raised in a germ-free environment and to that of an initially germ-free group that was later colonised with normal gut bacteria. The comparison revealed that the microbiota induce specific gut gene responses. While some host responses are quite specific for the presence of a given type of bacteria, others are more general. In comparing the zebrafish gut-microbe interactions to those of mice, some responses were apparently conserved across millions of years of evolution [51]. Finally, to examine how host environments influence the composition of gut microbial communities, conventional gut microbiota from zebrafish and mice were swapped between host species. A survey of the two microbial communities revealed that the zebrafish and mice guts significantly shape the relative abundance of the different types of bacteria composing their bacterial communities [51]. Recently, some insights have been obtained on the molecular mechanisms by which animal hosts influence the composition of the colonising microbiota. In mice [52] and drosophila [53] a new role of antimicrobial peptides was revealed in controlling the homeostasis of the commensal microbiota.

By tagging bacteria with a fluorescent molecule to observe how and when they take up residence in the developing fish, University of Oregon scientists have discovered that bacteria colonise the immature gut soon after the larvae hatches, and before the animal is fully mature [54]. In the absence of microbiota, the zebrafish gut does not develop fully, but instead is arrested in specific aspects of differentiation, and the animals have trouble absorbing nutrients (Fig. 4C). All these defects can be reversed by the introduction of bacteria later in development [54]. Thus, during embryogenesis the gut microbiota in zebrafish have important roles in intestinal epithelial maturation, cell homeostasis and cell-type specification [54]. When microbial communication with the developmental programme of the fish is interrupted, growth and development are arrested. Conserved components of bacterial cell walls such as lipopolysaccharide (LPS) are involved in this microbe-host cross-talk [54].

Taken together, in both zebrafish and mice [55], microbial colonisation induces changes in gut epithelial homeostasis such as increases in cell proliferation and the relative number of secretory cells [49, 54, 55]. Gene expression profiling of germ-free and conventionalised mice and zebrafish reveals striking parallels in their transcriptional responses to the

microbiota, with marked changes in expression of genes involved in cell proliferation, nutrient utilisation and immune function [49, 56]. Vertebrates, therefore, appear to possess a conserved programme of interactions with the microbes with which they have coevolved. In sum, because signals from the bacterial symbionts are required for the completion of gut differentiation, microbes must be considered as an integral part of this process.

Bacteria induce organ formation in a squid

Pioneering work led by Margaret McFall-Ngai and Edward Ruby of the University of Wisconsin, Madison, has shown that morphogenesis of the light organ of the Hawaiian bobtail squid *Euprymna scolopes* is actively induced by one member of the complex seawater microbial community to which the developing squid is exposed in its natural environment. *E. scolopes* is a small, nocturnal, marine invertebrate (Fig. 5A) that spends its nights hunting for prey in shallow waters. The squid has a specialised light-emitting organ within its mantle cavity, which is colonised by luminescent *Vibrio fischeri* bacteria. The bacteria emit light downward, and the squid can manipulate the intensity of this bioluminescence to match the intensity of down-welling moon and starlight, thereby erasing its shadow on the sea floor and evading bottom-dwelling predators [57]. The light organ of *E. scolopes* is capable of both generating and modulating luminescence. It can also

sense the light that it produces and, therefore, seems to function as an extraocular photoreceptor [58].

The squid recruits *V. fischeri* bacteria from the surrounding environment during gill ventilation by passing seawater over the entrances to the light organs [59]. The juvenile light organ is covered by ciliated epithelia that create currents to entrain bacterial cells from the seawater (Fig. 5B). In response to a common bacterial cell-wall component, peptidoglycan, the epithelial cells secrete mucus [60] in which captured gram-negative bacteria form aggregates. After several hours, symbiotic *V. fischeri* migrate from these aggregates through pores on the surface of the light organ into the crypt spaces deep within the organ. Once colonised, the squid undergoes a dramatic morphogenesis in which the ciliated appendages, and subsequently over a period of 4–5 days the whole 'symbiont-harvesting apparatus', are reduced in size through a coordinated program of cell death (Fig. 5C) [60–62]. In the absence of symbionts, the ciliated appendages persist. By exposing germ-free squid to *V. fischeri*-derived envelope components LPS and peptidoglycan [62], much of the host morphogenetic program associated with symbiosis including onset of programmed cell death and regression of the ciliated epithelium can be induced. Through the rest of the life time, the squid nurtures its symbionts by supplying them with nutrients like peptides and proteins [63].

In the natural environment, the juvenile squid hatches into seawater teeming with microorganisms. Intriguingly, although *V. fischeri* cells make up less than 0.1% of the natural bacterial community, the light organ becomes colonised within hours after the squid hatches, and contains only strains of *V. fischeri* [60, 64]. Moreover, the squid can select from different *V. fischeri* strains to find the most suitable symbionts [65]. How can certain bacteria get exclusive access to the light organ crypts? Which mechanisms control this strict host symbiont specificity and exclude other bacteria from stable colonisation? At the initiation of colonisation, the juvenile squid expresses a glycan-rich mucus around the pores that connect the light organs to the outside environment [60]. This mucus binds gram-negative bacteria and selects for *V. fischeri* from the complex mixture of seawater microbes. Once the squid is colonised, it down-regulates mucus production; however, if it loses its symbionts, then it will reinitiate mucus production. Thus, McFall-Ngai and colleagues hypothesise that the secret lies in mucus production as a regulated process that allows the colonised host to control when it solicits microbes from its environment. Although this appears to open the door to studies of an entirely new mechanism for regulating symbiont specificity, it remains to be shown how exactly *V. fischeri* changes the secretory activity of the squid's epithelia of the symbiotic organ [66]. In a recent paper, Mandel *et al.* showed that during establishment of the symbiosis *V. fischeri* contribute also to the adhesion through their own symbiosis-induced exopolysaccharide. A specific regulator encoded by the symbiont is sufficient for conferring efficient colonisation of *E. scolopes* [67]. Interestingly, the factors regulating the exclusive entry of *V. fischeri* are not installed at very early stages of embryogenesis [66]. During the first hour after hatching, the crypts are transiently open to non-specific bacteria and particles. Within 2 hours after inoculation, however, further entry by non-specific bacteria or particles appeared to be

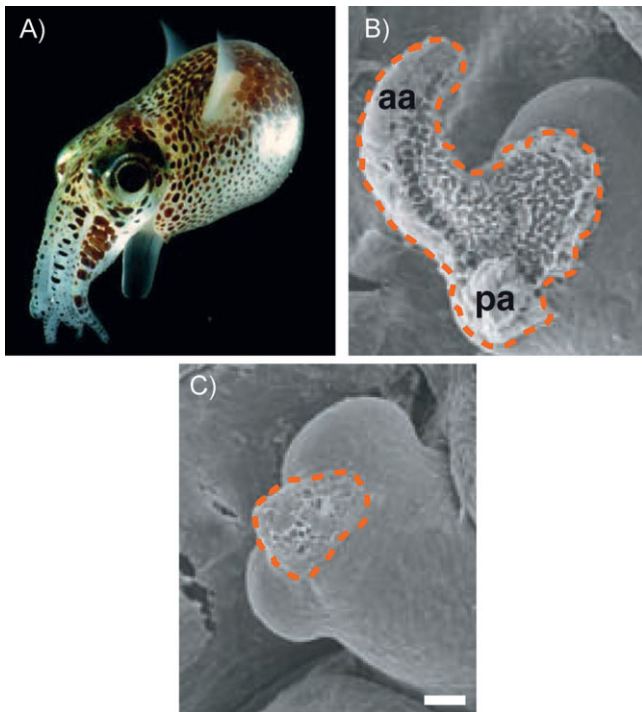


Figure 5. Bacterial symbionts induce light-organ morphogenesis in squid. **A** Adult squid (*E. scolopes*). SEM images of epithelial fields before **B** and after **C** regression of ciliated appendage. Scale bar, 50 μm (reprinted with permission from ref. [62]). Ciliated appendages are marked by an orange dashed line.

blocked [66]. Taken together, the squid-*Vibrio* partnership is a fascinating example of how symbionts affect animal development. It not only provides researchers with unique opportunities for studying the beneficial effects of bacteria, but it also reveals that these microbes are indispensable: they actually help orchestrate the squid's body plan early in life.

Understanding microbe-host interactions: The importance of being simple

For a long time, the main purpose of host-associated microbiology was to study pathogenic bacteria and infectious disease; the potential benefit of good bacteria remained unrecognised. The above-cited examples have demonstrated that individuals from *Hydra* to man are not solitary, homogenous entities but consist of complex communities of many species that likely evolved during a billion years of coexistence. Bacterial contributions not only assist in digestion of otherwise inadequate food but also are indispensable in shaping the immune system and development of organs such as the vertebrate intestine or the squid light organ. Additionally, the solution to pathological processes such as IBD apparently relies on understanding fundamental aspects of microbe-host interactions. Thus, contrary to previous assumptions, most bacteria may be beneficial and crucial to the fitness of many eukaryotes. From an evolutionary and ecological perspective, the complexity of these issues at the interface with the microbial environment is invigorating (Fig. 6). But within that complexity lie answers to the fascinating question of how cross-kingdom interactions have so profoundly shaped the evolution of life. Defining the individual microbe-host conversations in these consortia, therefore, is a challenging but necessary step on the path to understanding the function of the associations as a whole. How do we confront this complexity? In our view, untangling the complex interactions between hosts and their beneficial bacteria requires simple animal models with only a few specific bacterial species. Such models can function as living test tubes, and may be key to identifying

symbiosis-specific genes and dissecting the fundamental principles that underlie all host-microbe interactions.

Microbe-host interactions on an evolutionary scale

A strictly microbe-dependent life style has profound evolutionary consequences. The hologenome theory of evolution [68] considers the holobiont with its hologenome as a unit of selection in evolution. While the holobiont is defined as the host organism and all of its symbiotic microbiota [69–71], the hologenome is the sum of the genetic information of the host and its microbiota. The hologenome theory suggests that all animals and plants harbour abundant and diverse microorganisms and that these microbial symbionts affect the fitness of the holobiont [72].

Selection fundamentally acts on genes or individuals of distinct species. At the individual level, the success of a collection of interacting genes is mediated through the fitness of an individual phenotype. But what is the phenotype? What is a species? It may be worth remembering what Alfred Russel Wallace, natural selection's co-discoverer, published as species definition: 'A species . . . is a group of living organisms, separated from all other such groups by a set of distinctive character(istic)s, having relations to the environment not identical with those of any other group of organisms, and having the power of continuously reproducing its like' [73]. Thus, it is the relation to the environment which is one of the features defining a species. The crucial role of many microbes in development demonstrates that environmental and genetic information interact.

Nineteenth-century zoologists were well aware that organisms evolve in the context of their physical environments and of the other organisms with which they interact. Karl August Möbius, for example proposed his novel concept of 'biozönose' or *Lebensgemeinschaft*, the biotic community characterised by the dependence of their members on one another as well as their physical conditions of existence, as early as 1877. However, since then, efforts to add an ecological concept to the consideration of animal development and to recognise the entire system with its inputs, outputs and the interconnections met with an uphill struggle. Increasing awareness of microbial-derived molecules that affect animals' development should now help Moebius's successors to close the loop between ecology and development. That's why bacteria matter.

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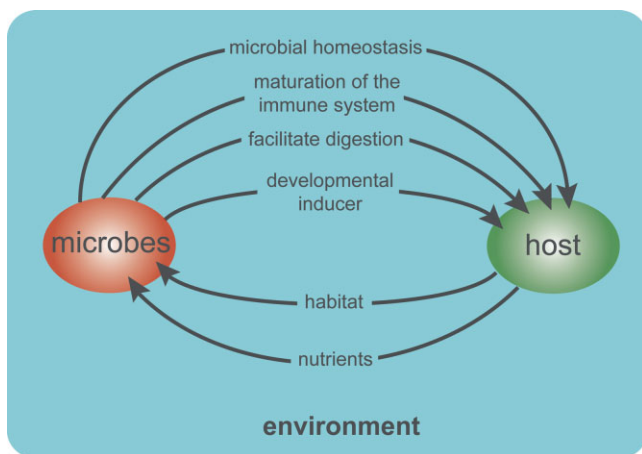


Figure 6. Schematic view of the interactions between host organisms and their microbiota.

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