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Proteobacteria: microbial signature of dysbiosis in gut microbiota

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Recent advances in sequencing techniques, applied to the study of microbial communities, have provided compelling evidence that the mammalian intestinal tract harbors a complex microbial community whose composition is a critical determinant of host health in the context of metabolism and inflammation. Given that an imbalanced gut microbiota often arises from a sustained increase in abundance of the phylum *Proteobacteria*, the natural human gut flora normally contains only a minor proportion of this phylum. Here, we review studies that explored the association between an abnormal expansion of *Proteobacteria* and a compromised ability to maintain a balanced gut microbial community. We also propose that an increased prevalence of *Proteobacteria* is a potential diagnostic signature of dysbiosis and risk of disease.

Proteobacteria in dysbiosis

Since the introduction of the small subunit ribosomal RNA gene as a molecular evolutionary clock [1], continuous advances have been made in the field of bacterial phylogeny. More recently, the development of next-generation sequencing techniques and biological computational tools has allowed cost-effective, large-scale multiplexing analyses, which have transformed our understanding of the interactions between microbial communities and their niche [2–6]. This approach has opened a window onto the enormous taxonomic and functional diversity of the microbial community of the human body, called the microbiota [7].

Of the 52 currently recognized bacterial phyla on Earth, approximately five to seven phyla are known to be resident in the mammalian gastrointestinal tract (GIT). Generally, the phyla *Firmicutes* and *Bacteroidetes* dominate the gut microbial community, while members of *Proteobacteria*, *Actinobacteria*, *Verrucomicrobia*, and the candidate phylum *TM7* are less abundant (Box 1) [8]. Despite the relatively few dominant phyla, the number of bacterial species is estimated to reach 1000 or more, and the number of their genes is more than 150-fold greater than those of the genome of their host [9]. This vast repertoire of the microbiome provides the host with complementary genetic resources, such as pathways for energy harvesting, production of essential vitamins, intestinal maturation, and development of the immune system. The healthy adult human gut microbiota is known to be stable over time [10,11]; however, diseases associated with metabolism and immune responses drive the microbial community to an imbalanced unstable state.

Here, we review studies that have explored the associations between an abundance of *Proteobacteria* in the microbiota and the difficulty for the host of maintaining a balanced gut microbial community. Based on this analysis, we propose that an increased prevalence of the bacterial phylum *Proteobacteria* is a marker for an unstable microbial community (dysbiosis) and a potential diagnostic criterion for disease.

Host nutrition and metabolic disorders

Diet is considered one of the most critical environmental factors shaping gut microbial structures [12,13]. Cumulative evidence has demonstrated differences in the taxonomic and functional composition of the gut microbiota between healthy and obese individuals, in both humans and rodents [14–17]. In addition, the transmissibility of the obese phenotype through fecal transplantation [16,17] suggests that an altered gut microbial community, as a primary trigger, is causative rather than consequential.

An imbalance in the taxonomic composition of gut microbiota, called dysbiosis, is well documented in metabolic disorders and is seen as an increment in relative abundance of *Firmicutes* with respect to *Bacteroidetes* (F:B ratio) [14,15]. Although consistent findings have commonly supported this concept, dysbiosis during metabolic disorders often includes an increased prevalence of *Proteobacteria* (Table 1). For example, a study of gut microbiota in children found more *Proteobacteria* in European children who consumed a calorie-dense, high-fat, low-fiber diet compared with children from Burkina Faso who were low-fat, high-fiber consumers [13]. This difference revealed an adaptation of the gut microbial community to the diet of African children, which could improve their ability to harvest energy from indigestible polysaccharides. In addition, several factors causing deleterious metabolic effects, such as the consumption of noncaloric artificial sweeteners and emulsifiers (which are commonly used as additives in processed foods), also impaired glucose control and induced a *Proteobacteria* bloom [18,19]. Particularly, the artificial sweetener-mediated elevations in the relative abundances of the family *Enterobacteriaceae* and class *Deltaproteobacteria* were in line with results from patients with type 2 diabetes mellitus

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Box 1. *Proteobacteria* in the ecosystem and in human microbiota

The phylum *Proteobacteria*, named after the Greek god Proteus, has the largest phylogenetic composition, comprising 116 validated bacterial families. On average, there are 10.1 families per validated phylum (median, 3.0) according to the *List of Prokaryotic Names with Standing in Nomenclature* (http://www.bacterio.net/). As the name suggests, members of the phylum *Proteobacteria* have greatly variable morphology and versatile physiology, which gives them a competitive edge in surviving in various ecological niches. *Proteobacteria* has been observed to be ubiquitous in habitats such as soil [2], plants [4], seawater [5], freshwater [3], the atmosphere [6], and mammalian body sites [7,48] (Figure I). The phylum comprises six bacterial classes: *Alpha-*, *Beta-*, *Gamma-*, *Delta-*, *Epsilon-*, and *Zetaproteobacteria*. With the exception of the *Zetaproteobacteria*, all classes of *Proteobacteria* have been observed in various human body sites, including the oral cavity, skin, vaginal tract, and GIT [7,11,48]. Despite interstudy variation, the oral cavity microbiota of healthy humans has the highest relative abundance of *Proteobacteria* (17.2–36.8%), followed by skin (6.8–30.0%), GIT (2.5–4.6%), and vaginal tract (2.3%). In the GIT, *Proteobacteria* was more abundant in intestinal biopsy samples (mucosa-associated fraction) than in fecal samples (luminal fraction) from both healthy subjects and patients with IBD [43,47]. While most microbes in the GIT are obligate anaerobes, members of *Proteobacteria* are facultative anaerobes. This unique oxygen requirement of *Proteobacteria* may influence the relation between the abundance of *Proteobacteria* and oxygen homeostasis or concentration in the GIT.

Host genetic factors and extrinsic environmental factors, such as diet and antibiotics, continuously influence the taxonomic and functional composition of gut microbiota. Given that a balanced gut microbiota with high stability has symbiotic interactions with the immune system of the host, which is capable of suppressing uncontrolled expansion of *Proteobacteria*, a bloom of *Proteobacteria* in the gut can reflect an unstable structure of the gut microbial community; this unstable structure can be observed in nondisease states (e.g., neonatal period [55] and after gastric bypass surgery [56]) and disease states (e.g., metabolic disorders [22] and intestinal inflammation [43]) (Figure II). During the initial colonization of the neonatal GIT, facultative anaerobic *Proteobacteria* make the intestinal niche favor colonization by obligate anaerobes; the latter are soon replaced by obligately anaerobic *Firmicutes* and *Bacteroidetes*, which dominate the gut microbiota of healthy adults. The gastrointestinal rearrangement by gastric bypass surgery can alter pH, bile flow, and intestinal hormones, all factors that influence the abundance of *Proteobacteria*.

Relative abundance (%)

	Soil	Plant leaf	Air	Ocean	Freshwater	Human gut
Proteobacteria	36.5	62.0	77.9	57.9	61.3	4.5
Bacteroidetes	11.2	7.0	3.9	32.8	10.8	65.4
Firmicutes	2.9	7.0	4.8	0.0	0.0	24.4
Actinobacteria	13.0	0.0	4.6	2.6	15.8	2.2
Acidobacteria	30.9	20.0	0.0	0.0	0.0	0.0
Cyanobacteria	0.0	0.0	0.0	4.5	1.3	0.0
Verrucomicrobia	0.0	0.0	0.0	1.4	1.5	0.7
Others	5.5	4.0	8.9	0.7	9.5	2.8

TRENDS in Biotechnology

Figure I. Abundance of *Proteobacteria* in various ecosystems, including soil, plant leaf surface, atmosphere, seawater, freshwater, and human gut. Figures in the table refer to the 'relative abundance (%) of the phylum'.

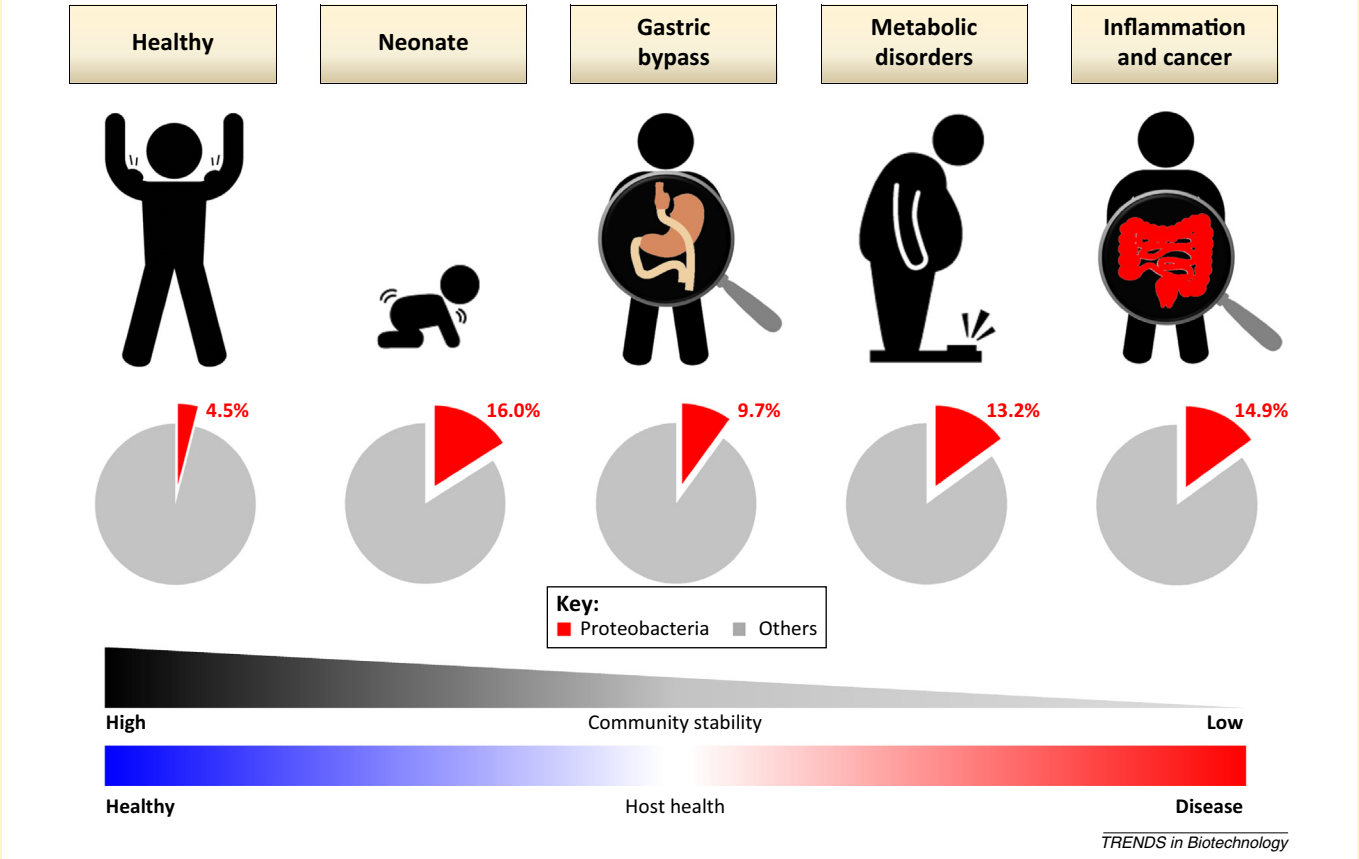

Figure II. Expansion of *Proteobacteria* in gut microbiota under different host conditions.

Table 1. Abundance comparison of enteric *Proteobacteria* in metabolic disorders[a]

Disease type	Taxonomic lineage[b]	Comparison (relative abundance (%))[c]			Model organism	Technique (region)	Refs
Diet-induced obesity	*Proteobacteria*	Standard chow diet (2.6 ± 0.82)	vs	High-fat diet (5.85 ± 0.26)	C57BL/6J mice	Pyrosequencing (16S rRNA V3)	[57]
	Proteobacteria; *Deltaproteobacteria*; *Desulfovibrionales*; *Desulfovibrionaceae*	Standard chow diet (3.49)	vs	High-fat diet (6.30)	C57BL/6J mice	Pyrosequencing (16S rRNA V3)	[58]
	Proteobacteria	Standard chow diet (1.39 ± 0.17)	vs	High-fat diet (3.09 ± 0.39)	C57BL/6J mice	Illumina Hiseq (metagenome)	[59]
Fecal microbiota transplantation	*Proteobacteria*; *Deltaproteobacteria*; *Desulfovibrionales*; *Desulfovibrionaceae*	Lean twin's microbiota recipient (0.09)	vs	Obese twin's microbiota recipient (0.22)	Humanized germ-free C57BL/6J mice	Pyrosequencing (16S rRNA V2)	[27]
Genetically induced diabetes	*Proteobacteria*	Lean littermate (0.01 ± 0.01)	vs	*db/db* (2.04 ± 0.57)	Leptin receptor-deficient *db/db* mice	Pyrosequencing (16S rRNA V3)	[60]
Genetically induced obesity	*Proteobacteria*; *Betaproteobacteria*; *Burkholderiales*; *Alcaligenaceae*; *Parasutterella*	*ob/ob*-prebiotics (0.17 ± 0.08)	vs	*ob/ob* (0.60 ± 0.10)	Leptin-deficient *ob/ob* mice	Pyrosequencing (16S rRNA V3)	[61]
Obesity	*Proteobacteria*	Non-obese control (0.87 ± 0.28)	vs	Obese patient (3.13 ± 0.87)	Human	Pyrosequencing (16S rRNA V4)	[62]
T2DM	*Proteobacteria*; *Betaproteobacteria*	Nondiabetic control (0.81)	vs	Patient with T2DM (2.09)	Human	Pyrosequencing (16S rRNA V4)	[63]
	Proteobacteria; *Betaproteobacteria*	Nondiabetic control (0.15)	vs	Patient with T2DM (0.32)	Human	Pyrosequencing (16S rRNA V4)	[64]

[a]The significantly different proportions of the *Proteobacteria*-assigned 'operational taxonomic unit' or 'metagenomic linkage group' between individuals with metabolic disorders and healthy controls were obtained from recent metagenomics studies, and represented as relative abundance (%).

[b]Taxonomic lineage: phylum; class; order; family; genus.

[c]Relative abundances (%) are expressed as the mean or mean ± standard error mean.

Box 2. *Proteobacteria* in the neonatal gut

The microbiota in the neonatal gut is of particular interest, because it reflects not only the fragile structure of the bacterial communities, but also the true origin of mammalian gut microbiota. Bacterial communities in the neonatal gut are unstable due to its rapid temporal variation. However, this fragility is linked to colonization by more important gut flora, such as strict anaerobes. Specifically, due to the abundance of oxygen in the neonatal gut, microbiota in the first week of life are frequently dominated by facultative anaerobes, mainly *Proteobacteria* species (e.g., *Escherichia*, *Klebsiella*, and *Enterobacter* species) [65]. These facultative anaerobes make the habitat suitable for colonization by strict anaerobes, by consuming oxygen, altering the pH, lowering the redox potential, and producing carbon dioxide and nutrients [66,67]. Thus, one can speculate that *Proteobacteria* has a role in preparing the neonatal gut for successive colonization by strict anaerobes, which are abundant in the gut of healthy adults. A recent study of the maternal placental microbiome described the presence of commensal bacterial communities with the greatest abundance of *Escherichia coli* [68]. Despite arguments regarding the viability and origin of the placental microbiota, these intriguing bacterial communities found in maternal placenta overlap with those from both the maternal amniotic fluid and neonatal meconium [69,70]. Therefore, *Proteobacteria* in the neonatal gut may be transmitted from the maternal placenta through fetal swallowing of amniotic fluid *in utero*. Interestingly, the proportion of *Proteobacteria* in the gut of pregnant women increased during the later period of pregnancy [71], implying the transfer of this specific bacterial group in the mother's microbiota to the newborn infant.

It is likely that the duration of *Proteobacteria* blooming observed in the neonatal GIT is under maternal control. Indeed, the neonatal microbiota can be affected by various maternal factors, such as mode of delivery, diet, and exposure to antibiotics during pregnancy [55,72–74]. Above all, the abundance of *Proteobacteria* in the neonatal gut is affected by the type of feeding, with a higher frequency of these bacteria in formula-fed infants, but a scarcity in breast-fed infants (reviewed in [75]). Human milk oligosaccharides [76] and secretory IgA production [77] are involved in the selective suppression of *Proteobacteria* during the initial intestinal colonization process. Therefore, a reduction in the abundance of *Proteobacteria* in a timely manner after its blooming is increasingly thought to be a normal part of the initial microbial colonization, and disturbance of this colonization pattern is linked to an increased risk of neonatal diseases [78,79].

Box 3. *Proteobacteria* in patients with gastric bypass surgery

Studies that monitored the daily variations of human gut microbiota in both a healthy man (followed for 15 months) and a woman (followed for 6 months) reported a relatively low abundance of the phylum *Proteobacteria* (average of 2.5% for the man and 4.1% for the woman) and a stable composition, despite large, abrupt, and long-lasting changes at the class level within the phylum [11,80]. Although these observations are based on only two individuals, the results are intriguing because they open the possibility that the adult human gut regulates the proportions of enteric *Proteobacteria*. We currently do not know much about the mechanisms underlying the interactions between host factors and the abundance of this phylum. Nonetheless, we can infer potential host factors from specific circumstances, such as the alteration of the gut microbiome by the surgical Roux-en-Y gastric bypass (RYGB) procedure, a highly effective treatment for morbid obesity, and T2DM. Interestingly, several studies of patients and animals treated with RYGB demonstrated that changes in gut microbiota after surgery are mostly characterized by a dominance of the phylum *Proteobacteria* regardless of the host species, and by the type of diet and metabolic phenotype before surgery [56,81–84]. Similar to the neonatal gut, where facultative anaerobes grow due to abundant oxygen, the GIT in patients who have ventrotomy for RYGB may be subject to a transient aerobic condition. However, this situation is unlikely because the abundance of the phylum *Proteobacteria* in sham-operated subjects did not change significantly [56,82]. Instead, because RYGB involves surgical reconfiguration of the GIT, it induces several changes in the physical condition of the intestine that affect the gut microbiota. In particular, an altered pH due to reduced input of gastric acid, changes in bile flow, hormonal fluctuations (especially of glucagon-like peptide 1 and peptide YY), and modification of the total length of small bowel, are all factors that may influence the spatiotemporal abundance of enteric *Proteobacteria*. One major difference in the blooming pattern of *Proteobacteria* between the neonatal gut and patients with RYGB is the duration of that pattern. A high proportion of *Proteobacteria* is soon replaced by other bacterial taxa during the initial intestinal colonization in healthy neonates, whereas longitudinal analyses of gut microbiota from RYGB recipients demonstrated sustained increases in the abundance of *Proteobacteria* for 8 weeks in a rat model [82], 12 weeks in operated mice [56], and 8–15 months in humans [83]. So far, it remains unclear whether the increased abundance of *Proteobacteria* is a causative factor in the RYGB-induced metabolic improvement.

(T2DM) [20], suggesting a link between glucose homeostasis and intestinal *Proteobacteria*. By contrast, Karlsson *et al.* demonstrated a negative correlation between the abundance of *Proteobacteria* and diabetic phenotype [21], challenging the notion of a high abundance of *Proteobacteria* in patients with metabolic diseases.

In support of the relation between a metabolic disorder and the expansion of *Proteobacteria*, the obesogenic potential of *Proteobacteria* has been identified in a monoassociation study in germ-free mice [22]. Given that the relative abundance of the *Enterobacteriaceae* gradually diminished during a weight-loss trial for one morbidly obese volunteer, Fei and Zhao [22] hypothesized that *Enterobacter* has a causative role in metabolic deteriorations. Monocolonization of germ-free mice with *Enterobacter cloacae* B29, isolated from the obese human gut, was sufficient to induce obesity and insulin resistance. This finding supports the hypothesis that an unstable gut microbial community, characterized by an abundance of *Proteobacteria*, may represent an active feature, rather than a passive consequence, of metabolic disturbances.

Undernutrition causes other health problem, such as marasmus and kwashiorkor. Malnutrition is a life-threatening condition for children younger than 5 years of age in developing countries [23]. The primary etiology of malnutrition is a chronic negative energy balance resulting from macronutrient deprivation and micronutrient deficiency during maternal pregnancy or the first 3 years of postnatal life. However, recent studies demonstrated that the structure and gene contents of the gut microbial community in malnourished children from Bangladesh and Malawi were distinct from those of well-nourished children [24,25]. In these studies, a dominance of *Proteobacteria* and a low diversity of gut microbiota were commonly observed in undernourished children and regarded as hindrances to postnatal maturation of the gut microbiota. Furthermore, a recent study revealed a mechanistic interrelation between the *Enterobacteriaceae* and the gut mucosal immunoglobulin A (IgA) response under malnutrition, which elicited enteropathy and interrupted the development of mucosal immunity and assembly of a healthy microbiota [26].

Given that the dysbiosis-driven selective pressure seems to interfere with the stability of the microbial

community, *Proteobacteria* subsequently take the opportunity to increase their fitness. Instability of the microbial community in abnormal metabolic conditions has been explained by impaired resistance to colonization. When gnotobiotic mice inoculated with cultured bacteria from an obese human donor ('obese-recipient mice') were cohoused with mice harboring bacterial species from a lean donor (on a low-fat, high-fiber diet), they were effectively colonized by lean donor-derived bacterial strains and their obese phenotype was ameliorated. By contrast, the lean-recipient mice were not colonized by the exogenous or allochthonous bacterial strains from the obese-recipient mice [27]. This finding indicates that dysbiosis is characterized by an attenuated transmissibility and resistance to colonization. Given that the gut is microbially immature and enriched with enteropathogens in children with kwashiorkor [24,26], malnutrition is thought to be associated with a defective resistance to colonization. Collectively, this circumstantial evidence leads to the notion that the expansion of gut *Proteobacteria* reflects an energy disequilibrium of the host and an unstable microbial community. Intriguingly, an unstable structure of the gut microbial community with a high abundance of *Proteobacteria* is also observed in nondisease states, such as the neonatal period (Box 2) and after gastric bypass surgery (Box 3).

Immune disorders: inflammation and cancer

Owing to the massive collection of exogenous antigens in the intestinal luminal, the immune system must strictly regulate its responses to maintain the symbiotic relation with commensal bacteria. Commensals transmit a signal that induces a tolerogenic response of host immunity [28,29]. Hence, the host can discriminate between beneficial autochthonous microbes and harmful pathogens, and establish a healthy microbiota [30]. To prevent an inflammatory response to commensal bacteria, gut-residing immune cells, such as mononuclear phagocytes (macrophages and dendritic cells) and $CD4^+$ T cells, are hyporesponsive or display a mutualistic response to microbial stimulation [31,32]. At the same time, the mucosal immune system is responsible for clearing pathogens, a process that requires an active proinflammatory signaling cascade. Accordingly, an inappropriate immune response destroys the intestinal homeostasis, triggers dysbiosis, and contributes to local and systemic inflammation and metabolic dysfunction. This state of chronic, progressive intestinal inflammation is clinically diagnosed as inflammatory bowel disease (IBD), which encompasses ulcerative colitis (UC) and Crohn's disease (CD). A precise etiology for IBD is still unavailable, but emerging evidence points to the gut microbiota as the prime suspect in this disease.

Mice lacking Toll-like receptor (TLR)-5 developed transmissible spontaneous colitis and dysbiosis, which was associated with an abnormal expansion of *Proteobacteria* (family *Enterobacteriaceae*) [33]. Concurrently with the *Proteobacteria* bloom, colitic *Tlr5*$^{-/-}$ mice exhibited a disorganized colonic mucous layer and had delayed clearance of infectious pathogens compared with their noncolitic *Tlr5*$^{-/-}$ siblings. These results suggest that a transiently unstable gut microbiota, especially a *Proteobacteria*-dominated community, predisposes genetically susceptible mice to chronic colitis. The hypothesis that dysregulated innate immune responses drive the outgrowth of *Proteobacteria* that, in turn, promotes intestinal inflammation, is supported by studies in other mice models with mutations affecting the adaptive immunity. For example, interleukin (IL)-10 is the main immunoregulatory cytokine required for immune tolerance to indigenous microbiota. IL-10-deficient mice exhibited spontaneous colitis due to their intolerance of intestinal microbiota [34]. Along with the onset and progression of colonic inflammation, in IL-10$^{-/-}$ mice colonized with conventional microbiota or microbiota lacking particular pathogens, there were more *Proteobacteria* and *Escherichia coli* than in wild-type mice [34]. In another study of IL-10-deficient mice, a diet rich with saturated milk fat perturbed the gut microbial assemblage and resulted in a bloom of the sulfite-reducing deltaproteobacterium *Bilophila wadsworthia*. This pathobiont induces a proinflammatory mucosal immune response and promotes the incidence and severity of spontaneous colitis in IL-10$^{-/-}$ mice; it also promotes dextran sodium sulfate (DSS)-induced colitis in wild-type mice fed a high milk-fat diet [35].

In addition to the positive correlation between the susceptibility to colitis and the relative abundance of gut *Proteobacteria*, evidence to support a causative role of *Proteobacteria* in intestinal inflammation has been provided by studies of mice deficient in both the innate and adaptive immune systems. Mice lacking the transcription factor T-bet and the recombinase activating gene *Rag* (T-bet$^{-/-}$ × Rag2$^{-/-}$ or 'TRUC' mice) developed spontaneous UC-like inflammation, and this colitic phenotype was transmissible to T-bet-sufficient Rag2$^{-/-}$ mice and wild-type mice through coprophagy by cross-fostering or cohousing [36]. The significant expansion of *Proteobacteria* in colitis was reproduced in a more recent study that compared the gut microbiome of TRUC mice with active colitis to those in remission due to treatment with gentamicin, metronidazole, or antitumor necrosis factor (TNF)-α [37]. Notably, transfer of two *Enterobacteriaceae* species (*Klebsiella pneumoniae* and *Proteus mirabilis*) isolated from feces of TRUC mice was sufficient to provoke colitis even in recipient mice without any genetic immune defects [38]. However, the colitogenic potential of these two microbes was not reproduced in germ-free TRUC mice, indicating that other commensal members are required for the pathogenesis of colitis. Oral administration of *Helicobacter typhlonius*, another *Proteobacteria* species enriched in TRUC mice, also triggered colitis in noncolitic TRUC mice that had a robust production of proinflammatory cytokines (e.g., TNF-α) [39].

The dysbiosis in mice genetically prone to colitis is of particular relevance to human IBD, because risk alleles or polymorphisms linked to IBD are associated with innate and adaptive immune components [40–42]. Similar to studies in mice, two studies in humans have shown that the gut microbial community of patients with IBD is characterized by a low microbial diversity, an outgrowth of *Proteobacteria* (particularly *Enterobacteriaceae*), and a concomitant depletion of *Firmicutes* compared with healthy subjects [43,44]. A human cohort study found that the nucleotide-binding oligomerization domain (NOD)-2

risk allele dosage correlated positively with the relative abundance of *Enterobacteriaceae* in intestinal specimens from patients with IBD [45]. In patients with UC, a significantly higher level of *Proteobacteria* was observed in the severe stage compared with the moderate and mild stages of inflammation [46]. A recent study by Gevers and colleagues demonstrated clear differences in the mucosa-associated microbiome of ileal and rectal biopsies (but not in stool samples) between treatment-naïve pediatric patients with new-onset CD, and nonIBD control subjects [47]. An increase in the relative abundance of *Proteobacteria*, including the families *Enterobacteriaceae*, *Pasteurellaceae*, and *Neisseriaceae*, discriminated the CD-related bacterial community from healthy control subjects. Consistent with chronic inflammation, an altered gut microbial community accompanying the preponderance of *Proteobacteria* was seen not only in acute inflammation due to infectious pathogenic bacteria or a protozoan parasite, but also in experimental and human colitis-associated colorectal cancer (see Table S1 in the supplementary material online).

Concluding remarks and future perspectives

Taken as a whole, numerous studies to date endorse the concept that a bloom of *Proteobacteria* in the gut reflects dysbiosis or an unstable gut microbial community structure. In addition to the exogenous enteropathogenic *Proteobacteria*, the healthy mammalian gut contains several members of commensal bacterial species belonging to this phylum as its natural gut flora [11,48]. As pointed out above, these bacteria seem benign when they are in minor proportion, whereas, under certain gut environments, they become colitogenic microbes that can trigger inflammatory responses. One thing to keep in mind is that the hologenome theory, namely, that the host and all of its associated microbiota form a unit of selection in evolutionary change, contains Lamarckian aspects: (i) hologenome evolution is regulated by the use and disuse of microbes; and (ii) changes in the hologenome are transmitted to offspring [49,50]. Thus, one can speculate that enteric *Proteobacteria* have as yet unidentified functions, and so a better understanding of the ecological roles of these bacteria is key to identifying the symbiotic and/or pathological relations between host and microbes in the mammalian gut.

The phylum *Proteobacteria* is the most unstable over time among the four main phyla (*Firmicutes*, *Bacteroidetes*, *Proteobacteria*, and *Actinobacteria*) in the gut microbiota [10]. Under a healthy steady state, the relative abundance of *Proteobacteria* in the human gut transiently increases to 45% (average: 2.5% for 15 months) without clinical signs [11]. It could be presumed that *Proteobacteria*, as a front-line responder, responds sensitively to environmental factors, such as diet. However, a chronic enrichment of *Proteobacteria* in the gut can represent an imbalanced unstable microbial community structure or a state of disease of the host. Thus, time-series monitoring, rather than cross-sectional studies, could be a better way to determine disease risk according to the proportion of *Proteobacteria* in the gut.

There are also possible mechanistic insights into the ecological niche that allows an outgrowth of *Proteobacteria*. In healthy intestine, the commensal microbiota has a protective role in immune responses against infection or inflammation by inducing anti-inflammatory IL-10 and suppressing proinflammatory IL-17 or transforming growth factor (TGF)-β production [28,29]. The importance of this mutualistic relation is implicated in metabolic disorders in terms of the metabolic inflammatory state [51,52]. Furthermore, commensal *Enterobacteriaceae*, which are benign in a healthy state, are able to occupy the inflamed niche by using nitrate generated from the inflammatory response of the host [53]. Thus, anaerobic respiration using host-derived nitrate as an alternative electron acceptor enables *Enterobacteriaceae* to outcompete the obligately anaerobic *Firmicutes* and *Bacteroidetes* that rely on fermentation for growth. Indeed, the genetic variability and high frequency of conjugation-mediated horizontal gene transfer in bacterial strains belonging to *Enterobacteriaceae* might contribute to their fitness advantage over other members of the gut microbial community [54]. This possibility suggests the existence of a positive feedback loop. Disruption of homeostasis, by environmental or host factors, such as a low-fiber diet and acute or chronic inflammation, has a selective force and causes dysbiosis with a bloom of *Proteobacteria* in the gut. The uncontrolled expansion of *Proteobacteria*, resulting from the inability of the host to keep commensal *Proteobacteria* in a minor fraction and from reduced resistance to colonization by the microbial community, can further facilitate inflammation or invasion by exogenous pathogens. Therefore, a strategy to sever the feedback loop could comprise optimization of the partnership between the gut microbiota and host. Given that most studies have described the microbial community state in a context of correlation with host physiology, identifying the causes of the bloom of *Proteobacteria* is required for development of effective treatments.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tibtech.2015.06.011.

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