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Review

Functional Redundancy-Induced Stability of Gut Microbiota Subjected to Disturbance

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The microbiota should be considered as just another component of the human epigenetic landscape. Thus, health is also a reflection of the diversity and composition of gut microbiota and its metabolic status. In defining host health, it remains unclear whether diversity is paramount, or whether greater weight is held by gut microbiota composition or mono- or multiple-functional capacity of the different taxa and the mechanisms involved. A network-biology approach may shed light on the key gut players acting to protect against, or promote, disorders or diseases. This could be achieved by integrating data on total and active species, proteins and molecules, and their association with host response. In this review, we discuss the utilization of top-down and bottom-up approaches, following a functional hierarchy perspective.

Microbiota as an Epigenetic Landscape

Microbiota can be regarded as a ubiquitous, symbiotic, and essential organ of the human body, responsible for functions that human cells are unable to carry out [1–3]. A substantial part of this organ is located in the human gut, the natural habitat of a complex microbial community comprising species of archaea, bacteria, viruses, and eukaryotes. Most of these microbes are mutualistic symbionts promoting human health through their contributions to nutrient processing, colonization resistance, immune system development, and stimulation of a wide variety of other host functions [1,2]. Gut microbial community development is an example of ecological succession, starting when the embryonic intestinal organ is developing in the uterus [4]. We can apply Waddington’s notion of an epigenetic landscape [5], and consider the ecological dynamics (see Glossary) of the gut microbiota in a similar way to the development of any other human organ presenting phenotypic changes from ontogeny until death (Figure 1). The taxonomic composition of the microbiota throughout host ontogeny is shaped by the specific species initially colonizing the gut, those gained during development, the niches they occupy (i.e., their intestinal location), developmental time, local and external perturbations, random events, interactions among members within the community and with the host, as well as other factors related to the unique environment that each human being represents [6]. This may explain the time-course compositional and functional variability observed between individuals, which we schematize as microbiotas inhabiting valleys, acting as indicators of stability (Figure 1). From the compositional point of view, the microbiota becomes more and more complex with time, following a sort of dynamic equilibrium where the different trajectories represent the course of the microbiota along the valleys of stability in the ontogenetic landscape.
In this context, the present review discusses the accumulated knowledge evidencing changes in gut-resident microbial populations at all levels of the functional hierarchy during our life. A particular focus is given to understanding and clarifying the importance of functions when a transition of the gut ecosystem to a new state occurs and the consequences of it. The examination of recent top-down and bottom-up functional datasets revealed that the consequences of the different age-, diet-, and disease-induced trajectories (Figure 1) are strongly influenced by metabolic plasticity and functional redundancy.

From Composition to Function and Back

Changes in the microbiota can be also approached from a functional perspective (Figure 1). In fact, the notion of dynamic equilibrium of microbiota at the compositional level can be extrapolated to the upper levels of the functional hierarchy. Thus, a functional microbiota is formed by the repertoire of expressed genes and, maybe even more importantly, by proteins and metabolites. A lot of attention has been paid to the compositional level (i.e., relative abundance of species or genes) but few studies have investigated other levels, such as those corresponding to active genes (metatranscriptomes), proteins (metaproteomes), or metabolites (metabolomes) [7]. However, the latter factors may be as relevant, or even more so, than composition when it...
comes to understanding the role played by microbiota in host function. Table 1 summarizes the type of omics used to characterize microbiota at the compositional level (bacterial composition, metagenomics) and the functional level (metatranscriptomics, metaproteomics and metat metabolomics). The figure also shows key players associated to each study-level as well as associated knowledge and expected limitations. However, we highlight the need to go beyond studying composition and function of microbiota separately by performing integrative modelling of all these levels [8–10].

**Stability, Resistance, Resilience, and Functional Redundancy**

Imagine the gut microbiota continuously responding to food-intake and how variable this can be. The most obvious reaction will be its ability to process a variety of molecules coming from the stomach and the production of new ones from the human host with no appreciable changes in composition and function after such intake. Gut microbiota normally have the ability to react to such disturbance, keeping stable [11]. However, on other occasions, the nature of the disturbance or environmental stress is so strong that microbiota undergoes changes, acquiring a dysbiotic state [12]. As an example, to date, at least 50 pathologies have been associated with gut dysbiosis [13]. This condition depends on the ability of the microbiota to react and return to the predisturbed state, that is, one in which the microbiota is considered stable. This notion of stability, however, must be considered from both the standpoint of composition and function (Figure 1). Within a temporal framework, a given microbiota is considered stable if, after a given disturbance, it does not undergo a change in composition (resistance), restores the initial composition (resilience), or recovers the initial function despite compositional changes (functional redundancy) [14–17]. Functional redundancy is very important because it introduces the idea that, up to a point, species are interchangeable in a given microbiota in terms of function [8]. In fact, two given microorganisms that differ in compositional terms (species and/or genes) may be able to behave similarly at the upper levels of the functional hierarchy, yielding similar protein and metabolite profiles [8]. Resistance, resilience, and functional redundancy are properties exhibited by robust microorganisms: the ability of the microbiota to restore the original function (phenotype). It is a complex issue to determine which factors make certain microbiota robust. Dysbiosis, however, can be interpreted as a breach of robustness and a transition to a new state (Figure 1).

Compositional and functional descriptions of gut microbial communities have been obtained from shotgun metagenomic annotation and from indirect evidence of shifts in taxa annotated by partial 16S ribosomal RNA (rRNA) gene sequences [18], showing that close species may have large genomic differences, distinct responses to environmental factors, and divergent ecological roles [19–23]. Due to these limitations, we lack a functional/mechanistic explanation of the gut microbiota composition that confers stable, or even transferable, metabolic phenotypes when subjected to particular diseases or environmental factors.

One of the questions raised by the observation of greater diet- and disease-induced shifts in the composition and diversity of the total and active community is whether the metabolic performance of gut bacteria may be influenced (and if so, to what extent) by these changes in community structure [24–26].

Studies in microbial ecosystems have shown that the link between community composition and metabolic response is not direct but, rather, is strongly influenced by metabolic plasticity and functional redundancy [14,16]. These two properties influence how bacterial communities respond to the environment and diseases, and modulate the covariation between community composition and metabolic outcome. First, metabolic plasticity reflects the capacity of a community to accommodate environmental changes by adjusting overall performance of existing dominant phylotypes [16]. Second, functional redundancy implies that different
phylotypes can perform similar functional roles in the community. In agreement with this, significant changes in gut microbiota have been shown to cause little consequence to the microbial community’s functional outcome [27].

**Top-down and Bottom-up Functional Approaches**

There is great interest in identifying next-generation information of the gut ecosystem that can be used to treat or prevent diseases and disorders [28]. To this end, vast efforts have been invested in understanding the compositional content, complex dynamics, and molecular mechanisms underlying the links between gut microbiota and several diseases, disorders, and environmental factors [13].

Despite extensive work, no microbial consortia have been identified as specific to any particular disease or intervention because compositional and metagenomic studies are based on the assumption that there is a correlation between ecosystem performance and species abundance or microbiomes. Furthermore, recent studies suggest that metabolic performance of gut microbiota may also govern disease progression independently of its composition, though to what extent remains unknown [27]. Accordingly, in contrast to top-down approaches, bottom-up approaches characterizing how gut bacteria determine function (Figure 2), as a consequence of single-cell properties, such as cell-wall integrity, size, growth, and activity.

**Resilience:** here, resilience refers to the amount of stress or perturbation that can be tolerated before the gut ecosystem changes towards a different state of equilibrium. For the gut environment, stability in the face of a given type of perturbation depends on microbial community resilience.

**Resistance:** a given microbiota is resistant if it does not change in composition after being subjected to disturbance.

**Robustness:** denotes the extent by which the constituents of the microbiota in the human gut can be modified after short- to long-term interventions.

**Stability:** if there is a state of stable equilibrium (no disturbance) of the microbiota, then a stable community is achieved.

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**Figure 2. Model Representing Functional Redundancy in the Gut Ecosystem.** As shown, microbiota species are interchangeable in terms of functions by means of the metabolites produced by the action of gene products contained in the gut bacteria. Metabolites produced by the action of microbiota are the downstream product of gene expression and metabolic activity and, therefore, they can be considered as a final output within the functional hierarchy. Metabolomics can thus provide a reliable snapshot of the actual functional state of the gut ecosystem. According to the model and the functional redundancy concept, the gut ecosystem is formed by a super species with a very large genome, composed of widely divergent microbial lineages whose genomes contain functionally similar sets of genes (represented by triangles) that would give rise to a coordinated single metabolic outcome (represented by circles). The diversity and abundance level of microbes, genes, proteins, and metabolites will influence energy balance, gut motility, inflammatory tone, mucosal integrity, appetite, and signalling, to cite but some. Also, note that the gut key player (i.e., pathogens) may also negatively influence the gut barrier, promoting inflammation (see components in the lower part of the figure).
<table>
<thead>
<tr>
<th>Meta-omics</th>
<th>Microbial Material</th>
<th>Outcome</th>
<th>Advantages</th>
<th>Limitations</th>
<th>Clinical Applications</th>
<th>Key Targets Identified</th>
</tr>
</thead>
</table>
| Phenotyping                | 16S rRNA amplicons generated from DNA or RNA/cDNA | Composition of total microbiota or microbiota with protein synthesis (potentially active) | Fast and cheap sequencing                                                 | 1. Difficulties for phylogenetic assignations at the deepest level of the taxonomic hierarchy  
2. Dominance of major microbial groups mask the identification of low abundant members  
3. Comparisons require amplification of same region                                                                 | 1. Detect imbalances in the taxonomic composition of total and active gut microbiota  
2. Genera/species markers associated to diseases                                                                 | Genera: Prevotella, Ruminococcus, Roseburia, Bacteroides, Blautia, Faecalibacterium, Phascolarctobacterium, Clostridium, Subdoligranulum, Ruminococcus, Coprococcus, Enterococcus, Roseburia, Lactobacillus, Akkermansia, Clostridium, Butyribrio, Phascolarctobacterium.  
Families: Lachnospiraceae, Ruminococcaceae, Enterobacteriaceae  
Orders: Bacteroidales, Enterobacteriales  
Class: Butyrovibrio  
Phylum: Firmicutes, Bacteroidetes                                                                 |
| Metagenomics:              | DNA                       | 1. Gene content profiling and presumptive function analysis  
2. Systematic identification of diverse activities | 1. No amplification bias  
2. Uncovering microbial diversity  
3. Finding new genes  
4. High coverage and deep sequencing of total genes  
5. Identification of genes with assigned functions | 1. Requires high-depth coverage  
2. Assembly complicated due to low coverage and high similarities  
3. No information of active genes  
4. Many unknown genes  
5. Bioinformatics analysis required  
6. Presumptive functions require experimental validations (sequence-based)  
7. Limited activity-based screening protocols available                                                                 | 1. Microbial gene composition dysbiosis  
2. Finding disease-specific genes  
3. Identifying presumptive altered functions  
4. Systematic identification of functions and commensal bacteria impacting host cellular functions | Genes involved in: pathogenic processes, cell wall components biosynthesis, transport, translocation, amino acid metabolism, energy processes, bile acid metabolism, dietary carbohydrate metabolism, oxidative phosphorylation, and the production of mammalian signaling molecules |
| Metatranscriptomics        | mRNA cDNA                 | Gene expression profiling                                                | Reveal different gene expression from active microbiota across multiple conditions | 1. Instability of mRNA  
2. Multiple purification steps  
3. No unique protocol  
4. Lack of reference databases                                                                 | 1. Revealing functional dysbiosis  
2. Enrichment of metagenomics data by focusing on transcriptionally active genes | Genes encoding antibiotic resistance, drug metabolism, stress response pathways and pks genes |

| Table 1. High-Throughput Approaches Used to Study Variations in the Gut Microbiota |
Table 1. (continued)

<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>Metametabolomics</td>
<td>Metabolites</td>
<td>Metabolite profiling</td>
<td>Deeper insights into the metabolic performance of the active fraction of the microbiota at any condition</td>
<td>1. Lack of reference databases 2. No unique protocol 3. Many unknown metabolites in databases 4. Strict identification of compounds is tedious</td>
<td>1. Identifying and confirming new microbiota and host metabolic alterations 2. Finding metabolic biomarkers 3. Ranking the impact of diseases and interventions based on metabolic alterations</td>
<td>Phytochemicals (glucosinolates, polyphenols, glycocones), N-acyl amino acids and polyamides (including arachidoxyl glycine, N-stearoyl proline, N-octoyl (iso)leucine, N-stearoyl tyrosine and N-palmitoyl threonine), short chain fatty acids, long linear and branched saturated and unsaturated fatty acids, lipids (including glyco- and glycerophospho-lipids), bile acids, ceramides and sphingolipids, cholesterol derivatives, amino acids (i.e. tryptophan, histidine, tyrosine, and phenylalanine), metabolites implicated in porphyrin and iron metabolism (ferrooxamine, protoporphyrin IX and mesoporphyrin IX), vitamins, polyols, sugars, trimethylamine-N-oxide (TMAO), carnitine, N-acetyl muramic and N-acetyl neuraminic acids, N-acetylglucosamine, ribose-1,5-biphosphate, thiamine, choline, acetylputrescine, inosine, pseudouridine, hypoxanthine, creatinine, N-acetylhystamine, glyoxylic acid, succinic acid and homoserine lactone</td>
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different environmental factors and diseases, may be interesting to avoid biases due to functional redundancy regardless of taxonomic wealth. Here, functional information about microbial communities is first assessed through 16S rDNA barcoding by comparison with reference sequenced genomes or DNA sequencing. Some examples of the outcomes of top-down approaches are given in Box 1. A network biology approach integrating the study of active species, proteins, and molecules can shed light on the key gut microbial players with a clear and defined metabolic potential [8–10].

The overlap in the functional capacity of different taxa can bestow similar functions on the gut community, as previously observed in other microbial ecosystems [16,29,30]. The thousands of bacterial genome sequences currently available expand the scope of these observations [18]. The fact that all bacterial groups are commonly identified in the gut community, albeit in different abundances, suggests that core functions can be provided by distinct phytypes. Hence, gut microbiota assembly is based on functions encoded in bacterial genomes rather than on species with different growth characteristics dependent on environmental factors, such as diet [31]. Having stated this, as previously observed in other microbial ecosystems, bacteria can respond to shifts in environmental conditions by modifying single-cell properties such as size, physiology, and activity, which ultimately rely on a reversible state between dormancy and viability, depending on the environmental perturbation [32]. For instance, sporulation and biofilm formation are bacterial mechanisms designed to persist and adapt to starvation or escape from the immune system and bactericidal therapy, independently of the metabolic performance [33]. Likewise,

**Box 1. Top-Down Functional Approaches**

Most examples of the rational elucidation of key gut microbial players involve the analysis of species abundances or microbiomes. Here we focus on a selection of examples using top-down approaches.

Many of the microbial clades identified as being differentially abundant as a consequence of disease have commonly been associated with diseases that differ in nature and clinical consequences [59]. For example: (i) at the phylum level, Firmicutes/Bacteroidetes imbalances associated with different diseases, including immune diseases [60], gastrointestinal mucositis [61], obesity [38], and C. difficile-associated diarrhoea [62]; (ii) at the family level, imbalances in Lachnospiraceae and Ruminococcaceae have been found in patients with HIV [63], but also with Crohn’s disease and inflammatory bowel diseases [64,65], obesity [38], and individuals with different diets [43]; imbalances in Enterobacteriaceae associated with inflammatory bowel disease and colorectal cancer [64,65], and also after treatment with metformin and the probiotic oligofructose [66]; and (iii) at the class level, abundance of Butyribrio has been associated with both HIV [63] and also colorectal cancer [67]. A similar scenario has also been found when examining gene content (by DNA sequencing) and gene expression profile of gut bacteria: specific disease gene microbial biomarkers have rarely been found, as many of them are differentially abundant as a consequence of diseases that are different in nature and etiology. For example, genes involved in various pathogenic processes, cell-wall component biosynthesis, various transport systems, bacterial translocation, amino acid metabolism, and energy processes are systemic markers in HIV-infected individuals [63], but also in patients with colorectal cancer [68] and inflammatory bowel disease [64]. As a consequence of C. difficile infection, studies found that PTS transport and the metabolism and regulation of carbohydrates and sugar alcohols were over-represented functions, while aromatic amino acid family biosynthesis was significantly under-represented [62]. Genes encoding fiber-degrading enzymes are altered in patients with colorectal cancer; accordingly, it has been suggested that degradation of host glycans might be related to the etiology of colorectal cancer [68]. However, such functional consequences have also been observed in patients receiving antibiotic therapy [8], patients with inflammatory bowel disease [64,69], and patients with systemic lupus erythematosus [60], which are characterized by a different aetiology: Dysbiosis in patients with systemic lupus erythematosus is also associated with an over-representation of oxidative phosphorylation [60], but this has also been observed in lean individuals as compared to obese subjects [38,70,71], and also in response to intestinal colonization in healthy individuals [72]. Gene expression profiles have also been investigated; however, such studies are limited. For example, the differential expression of the so-called pks pathogenic island (containing about 66 virulence genes) from a bacterium (Escherichia coli) in the microbiota is driven by colorectal cancer and by inflammatory bowel disease, suggesting that the cancer and inflammation micro-environments may impact the functional potential of bacteria to promote cancer or inflammation progression [65]. However, this particular study introduced a pathogenic bacterium into the gastrointestinal environment, and found that adaptation time may influence gene expression as compared to host genotype and disease phenotype. Therefore, metabolic outcomes due to functional redundancy may be underestimated or masked by top-down functional approaches (see Figure 2 in main text) that characterize which bacteria and genes are found in individuals experiencing disease, or after interventions, in order to determine what they do based on inferring function from taxonomic and DNA sequencing data and expression profiles.
Box 2. Bottom-Up Functional Approaches

Functional redundancy can reflect evolutionary convergence of unrelated taxa, further complicating our understanding of variations in microbial community structure. Here we focus on some studies using bottom-up approaches performed in various ways to first identify key molecular agents (metabolites and also proteins/genes) and then gut bacteria involved in their metabolism or production.

Ng and collaborators [73] studied the abundance of mice-derived free sialic acid in the gut during antibiotic intake. By using a bottom-up functional approach, authors linked the presence of such molecules with the presence of enteric pathogens that were identified as Salmonella enterica serovar Typhimurium and Clostridium difficile. Both bacteria are redundant in their ability to catabolise mucosal carbohydrates.

Recent data have shown that one or two strains are sufficient to swiftly regulate the gastrointestinal microbial metabolism [27,74]. Accordingly, the functional status of the host-associated microbial ecosystem may be driven by a limited number of microbial components. This was demonstrated using a bottom-up approach, by first examining the metabolomes of mice [74] and human [27] faecal and/or urine samples, and then by demonstrating that it alters the level of metabolites associated with community compositional changes and the presence of specific microbes.

Host secretions such as bile acids or secretory immunoglobulin A, together with fluctuations in the host’s macronutrient intake (in particular carbohydrate type), can act in synergy as environmental stressors, altering microbial growth along the gut epithelium and modulating microbial metabolism [17]. Besides, these processes may reshape the gut microbiota in a generalized way or target specific bacteria with distinct properties. Indeed, recent data suggest that the gut microbiota possesses a high level of functional response diversity, as rare but functionally similar species fill the niche of abundant phylotypes upon dietary pressure, and thus community diversity and microbial growth lack relevance for functional composition [15,16].

Metatranscriptomic analyses of the gut microbiota during exposure to dietary [34] and xenobiotic [35] interventions have also revealed significant alterations in the gene expression profile of the microbial community, but often without large changes in overall community structure. However, the functional consequences of differential gene expression have yet to be determined on a large scale. First, the gene expresses a protein with an expected function, but this does not imply that it is active after an intervention or a disease that directly, or indirectly, affects the gut environment. Also, multiple gene sequences may encode a single type of protein whose functional consequences are currently unpredictable. For example, until recently it was thought that sequence and structural differences in bacterial bile salt hydrolase enzymes, rather than differences in gene expression, were responsible for influencing bile-acid metabolism efficiency, a single function of relevance in the gut [36]. This suggests that metabolic outcomes may not be directly predictable from the differential abundance of microbes, genes, and proteins, but that function should also be taken into account (Box 2).

The Gut Environment: A Monofunctional Perspective

Functional redundancy in soil microbes has been demonstrated by examining single ecosystem functions, such as respiration and biomass production [37]. This has also been observed in the gut environment by considering single functions, such as dietary carbohydrate degradation and short-chain fatty acid (SCFA) production. Indeed, proteomic analyses binned genes encoding sugar-degrading enzymes from lean individuals to Prevotella of the phylum Bacteroidetes, whereas over 90% of those from obese subjects binned to Firmicutes, especially Ruminococcus [38]. These observations connect different active phylotypes (Prevotella and Ruminococcus) to the same function, namely, the processing of dietary carbohydrates. Therefore, any selective pressure influencing their abundance, independent of total community structure, will likely result in the maintenance of degrading functions. However, obese subjects possess a 10-fold higher anabolic capacity for dietary carbohydrates than do lean subjects, which also suggests that, despite being redundant, both members show different degradation rates, possibly having long-term health consequences [39]. Furthermore, nine predominant
These Clostridium bacterial functions, a consequence of various bacterial metabolic activities, are also functionally redundant in terms of their ability to modify bile acids, even though their abundance differs in patients with distinct diseases [45].

Other examples of functionally redundant bacteria are Sutterella, Akkermansia, Blautia, Clostridium, Faecalibacterium prausnitzii, which all promote interleukin secretion [46,47]. Clostridium clusters IV and XIVA (Firmicutes phylum) and Bacteroides fragilis have redundant roles in promoting differentiation, expansion, and colonic homing of Treg cells [48], whereas Roseburia and Clostridium XIVA promote impaired insulin sensitivity [49]. Two taxa from the family Lachnospiraceae, Roseburia spp. and Ruminococcus gravis, and some species of Lactobacillus such as Lactobacillus reuteri, also have a redundant role associated with increased body fat and insulin levels [50]. However, Akkermansia muciniphila has an inverse role in those metabolic traits, demonstrating that a number of bacterial phylotypes may show inverted functions, even though redundancy is a common event in the gut environment. Redundancy is also evident at the gene level. For example, multiple transporters are involved in, and compete for, vitamin transport (i.e., B12) and vitamin-related processes; at least 260 out of 313 human gut bacterial species contain vitamin (B12) transporters, thus suggesting that apparent redundancies exist for capturing these molecules [51].

Note also that redundancy has been observed not only in the gut bacteria, but also in the host. Thus, recent results reveal unexpected redundant roles among NOD-like receptor (NLRs) in host defence against intracellular pathogens in vivo [52]. Therefore, components of the gastrointestinal tract in direct contact with the microbiota may also be considered as functionally redundant environments.

The Gut Environment: A Multifunctional Perspective

Recent investigations have demonstrated that multifunctional redundancy is generally lower than monofunctional redundancy in environmental communities [29]. Conversely, however, recent works indicate that multifunctional redundancy is an intrinsic property of the gut. In this context, different gut microbial configurations generated similar metabolomics profiles in patients with lupus erythematosus, thus indicating extensive multifunctional redundancy [27]. A similar observation was made in patients with Clostridium difficile-associated diarrhea [53].

These bottom-up investigations suggest that there is a high degree of multifunctional redundancy in the gut ecosystem, and that the gut environment can be regarded as a single super species (Figure 2). This super species has a huge genome composed of widely divergent microbial lineages, whose genomes contain a similar functionally redundant set of genes, as compared to environmental communities where sharing common orthologues among species is a key mechanism responsible for low multifunctional redundancy [29]. This may partially be a consequence of the fact that gene transfer is at least 25 times higher between gut bacteria than between bacteria from other environments, and this may promote the evolution of specialized core functions in the intestinal ecosystem [54] that are acquired through intrinsic mechanisms for replication of horizontally acquired genetic material.

The link between community diversity and multifunctionality may range from being strong, weak, or even absent. Moreover, in this process there is also a strong relationship between community richness, plasticity, and redundancy: communities characterized by high metabolic plasticity (defined as the rate of change in single-cell properties), low microbial richness, and low functional diversity, also tend to express higher functional redundancy [13,29]. This is of special
significance as bacterial density and variety, and thus function, in the gut of healthy individuals develop from a simple structure, usually dominated by bifidobacteria in early life [55,56], to a complex one reaching between 1183–3180 bacterial phylotypes in the adult intestinal microbiome [57]. Accordingly, redundancy in the infant gut may be much higher than that found in the adult gut. To date, we do not know when exactly a stable metabolic state is reached or whether this correlates with the successions and replacements of bacterial phylotypes. Moreover, reduced gut microbiota has also been associated with disease and intervention, such as antibiotic treatment [8]. Therefore, it is also plausible that disease and intervention may be accompanied by a loss of functional redundancy over time, the extent of which has yet to be revealed.

Concluding Remarks
To date, analyses of alterations in the gut microbiota have focused on time-course compositional descriptions. By contrast, examples from recent research cited here demonstrate the importance of taking into account the contribution of stability, resistance, resilience, and redundancy of the functional status of microbiota undergoing disturbance within a temporal framework. As stated above, our understanding of the mechanisms underlying the effects of such properties is limited (see Outstanding Questions), emphasizing the need to develop more functional and integrative studies of microbiota within the field of biomedical research.

In human gut microbial ecology, a major challenge is to identify the active members influencing host metabolic processes and response to environmental factors and diseases. We need to determine the major driving forces capable of reshaping the phylogenetic and metabolic configurations of the active gut microbiota. To do so, bottom-up functional approaches are increasingly important to define the active community of phenotypes, that is, to characterize ‘what they do’ in order to determine ‘who they are’. Whatever advances are made and datasets generated at the different levels of the functional hierarchy (meta-omics) in intestinal microbiota, it is clear that we need an integrated framework to provide insights able to unravel causality in the relationship between the host and the gut microbiota in the context of different diseases and interventions. Not only do we require the generation of knowledge about microbiota, but also information about the host. This is of particular importance as host–microbe interactions are mediated by proteins and molecules that are either secreted or present in microbial cells but also on the surface of the gastrointestinal tract. In this context, by using a functional metagenomics-based pipeline, Cohen and collaborators [58] have identified unique commensal bacteria effector genes that impact host cellular functions and are implicated in disease models of autoimmunity and atherosclerosis.

Taken together, imbalances in the composition and functions of gut bacteria are not only a consequence of the architectural defects in the gut mucosa associated with diseases, but also can contribute to the recovery or the maintenance of persistent defects of the entire human health system. In this context, the present review revealed the importance of integrative top-down and bottom-up research in which both the gut ecosystem and human functions may constitute a key variable to investigate, as they are the final outcome of any biological system.

Acknowledgments
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