Review

The Other Side of the Coin: What Beneficial Microbes Can Teach Us about Pathogenic Potential

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Abstract

Koch's postulates and molecular Koch's postulates have made an indelible mark on how we study and classify microbes, particularly pathogens. However, rigid adherence to these historic postulates constrains our view of not only microbial pathogenesis but also host–microbe relationships in general. Collectively, the postulates imply that a "microbial pathogen" is a clearly identifiable organism with the exclusive capacity to elicit disease through an arsenal of pathogen-specific "virulence factors." This narrow definition has been repeatedly contradicted. Advances in DNA sequencing technologies and new experimental systems have revealed that the outcomes of host–microbe interactions are highly contextual and dynamic, especially those involving resident microbiota and variable aspects of host biology. Clarifying what differentiates pathogenic from non-pathogenic microbes, including their paradoxical ability to masquerade as one another, is critical to developing targeted diagnostics and treatments for infectious disease. Such endeavors will also inform the design of therapeutic strategies based on microbiome engineering by providing insights into how manipulating entire host–microbe systems may directly or indirectly alter the pathogenic potential of microbial communities. With these goals in mind, we discuss the need to develop experimental models that better capture the contexts that determine the nature of host–microbe relationships. To demonstrate the potential of one such model—the zebrafish and its resident microbiota—we describe recent work that has revealed the thin line between pathogenic and mutualistic relationships, how the intestine physically shapes bacterial populations and inflammation, and the ability of microbial transmission to override the host's innate immune system.

Our Evolving View of Host–Microbe Relationships: A Brief History

> "We perceive only that part of nature that our technologies permit and, so too, our theories about nature are highly constrained by what our technologies enable us to observe."—S.F. Gilbert *et al.* [1]

In the late 19th century, the belief that afflictions such as plague and cholera were caused by "bad air" was replaced with the germ theory of disease, which asserted that many diseases are in fact caused by tiny life forms invisible to the naked eye. Stoking this nascent view of disease were improvements in microscopy techniques at the time that revealed that the human body is teeming with microscopic wildlife [2]. However, the ubiquity of these microorganisms presented a conundrum: Which ones cause disease? In response to this problem, in 1890 Robert Koch introduced a set of rigorous criteria—later known as Koch's postulates—that provided microbiologists with a framework for moving beyond making mere associations to establishing causal connections between infectious microbes and specific diseases [2]. Koch proposed that satisfying these criteria (which are summarized below) served as evidence of causality.

1. The parasite occurs in every case of the disease in question and under circumstances which can account for the pathological changes and clinical course of the disease.

2. The parasite occurs in no other disease as a fortuitous and non-pathogenic parasite.
3. After being fully isolated from the body and repeatedly grown in pure culture, the parasite can induce the disease anew.

Nearly 100 years later, techniques for isolating and manipulating the instruction manual of life, DNA, were developed, which facilitated the molecular dissection of the virulence traits encoded within the genomes of pathogens. With this technological advance, the field of infectious disease research found itself in familiar territory, which was captured by Stanley Falkow when he wrote, "In some ways, our capacity to isolate genes has outstripped the experimental means to document the essentiality of a given genetic property to pathogenicity." [3] In essence, just as the multitude of microbes that thrive in and on the human body obscured the identification of those that are truly pathogenic, so too did the vast number of genes carried by microbes make it difficult to ascertain which of them encode the capacity for true pathogenic behaviors. This challenge spurred Falkow to formulate "molecular" Koch's postulates (summarized below), which were meant to encourage the methodical examination of microbial genes and their causal relationship with infection and disease [3].

1. The phenotype or property under investigation should be associated with pathogenic members of a genus or pathogenic strains of a species.
2. Specific inactivation of the gene(s) associated with the suspected virulence trait should lead to a measurable loss in pathogenicity or virulence.
3. Reversion or allelic replacement of the mutated gene should lead to restoration of pathogenicity.

Koch's postulates and molecular Koch's postulates together have had an undeniable influence on infectious disease research. Empirically, their application led to the identification and genetic interrogation of the bacteria that cause anthrax (*Bacillus anthracis*), tuberculosis (*Mycobacterium tuberculosis*), and plague (*Yersinia pestis*) [4–7]. Perhaps the most enduring impact of the postulates has been on how we conceptually approach and investigate microbial pathogens and, by extension, other host-associated microbes. The postulates were introduced as a framework for obtaining sufficient evidence to prove causality but embedded within them is a working hypothesis of what defines a "pathogen." For example, an interpretation of the second and third criteria of Koch's postulates is that pathogens will only be found in the context of a diseased host and that when the pathogen is introduced to a new host, disease should manifest. Furthermore, an interpretation of the first and second criteria put forth in molecular Koch's postulates is that genes encoding pathogenic behaviors—known as "virulence factors"—will be conserved among closely related pathogens of the same lineage and that bona fide virulence factors are necessary in all cases of disease. However, these hypothetical, pathogen-specific properties have been contradicted numerous times since the introduction of the postulates.

Koch and Falkow were both aware of the limited ability of the postulates to capture all aspects of what distinguishes pathogens from other microbes [3,5,8]. For example, Koch knew that *Vibrio cholerae*, which he had discovered and suspected was the causative agent of cholera, could be isolated from healthy individuals without signs of disease and thus violated Koch's second postulate [5]. Furthermore, *V. cholerae* did not conform to Koch's third postulate when Max Joseph von Pettenkofer, a chemist and hygienist, "… swallowed 1.0 cm^3 of a freshly grown broth culture from a case of cholera and did not develop the disease." [5] Echoing the quandary that pathogens were capable of colonizing a host without causing disease, over a hundred years later Falkow wrote, "In some ways, the main conundrum to the study of pathogenicity has been the uncomfortable fact that many (most?) of the pathogens to which humans are susceptible show two faces." [8]

In a similar way, the limitations of molecular Koch's postulates have also been exposed. For example, extraintestinal pathogenic *Escherichia coli* (ExPEC)—which causes urinary tract infections, bacteremia, and neonatal meningitis—is recognized for expressing the well-known virulence factor α-hemolysin (a pore-forming toxin) [9–11]. However, only half of all ExPEC isolates encode this toxin. In addition, for some hemolysin-positive ExPEC strains, hemolysin is dispensable for causing disease, presumably due to functional redundancy with other virulence factors [12]. The ExPEC lineage also displays a remarkable degree of strain-level individuality, with some isolates relying on phyletically rare genes for pathogenicity [13]. These violations of molecular Koch's postulates underscore the limitations of defining pathogens purely based on gene content.

Koch's postulates and molecular Koch's postulates were not intended to be a rigid rule book for proving causality or identifying a pathogen. Rather, as Falkow stated, "…it must be possible to modify the postulates as new technology permits us to examine new aspects of the pathogen and the host." [3] Indeed, the postulates and our concept of host–microbe relationships have needed to adapt numerous times as new technologies and experimental models were developed [2,5,14,15]. For example, in 1957, Robert Huebner described the "virologist's dilemma," which arose as it became easier to isolate viruses, revealing that many viruses are in fact commonly associated with otherwise healthy individuals [14]. Huebner saw that this dilemma was, at its core, the same as the one Koch faced 60 years prior, writing, "Thus, our increasing proficiency in demonstrating viruses has produced a disconcerting but not entirely unwelcome paradox—the spectacle of new information leading to confusion." [14] This confusion almost led Huebner to declare an "epidemic of good health" when he found that 43 out of 43 infants surveyed in a Washington, DC, orphanage harbored an ECHO-like enterovirus without signs of disease, making it appear as if the virus in some way *promoted* health [14]. Ultimately, the ability of viruses to sometimes be carried asymptomatically while at other times cause acute seasonal illnesses or cancer later in life required a unique set of Koch-like postulates to be devised [5]. Presently, the explosion in high-throughput DNA sequencing, multi-omics technologies, and "big data" over the last two decades has unveiled an even more complex picture of host–microbe relationships. The apparent entanglement between the diverse microbial communities that inhabit our bodies and a rapidly growing number of diseases with no previous microbial links has demanded, yet again, reformulation of how we investigate and classify host–microbe relationships [2,15].

The picture that has emerged is one that is highly contextual and dynamic. It is clear that the ability of a particular microbe to cause disease is heavily dependent on host biology, resident microbiota, and environmental factors. Calling attention to this fact, Casadevall and Pirofski [16] recently admonished infectious disease researchers to "ditch the term pathogen," which they asserted imposes a limited, microbe-focused view of host–microbe interactions. Indeed, the view of host–microbe relationships has evolved over the last two decades from one that is dichotomous and declares microbes as either pathogens or non-pathogens, to one that recognizes microbes exist along a spectrum of pathogenic potential. A major challenge associated with this new perspective is that we know little about the factors that move microbes along this spectrum, pushing those that are normally pathogenic to act more commensal or those that are commensal to act more pathogenic. Moreover, the health-promoting and disease-causing potentials of our microbiota are poorly understood and there are many open questions about the origins, assembly, and transmission of our resident microbial communities.

Research into these questions has exposed the shortcomings of conventional experimental approaches [17,18]. For example, microbe-centric and host-centric approaches focus almost exclusively on the role of one partner while oversimplifying or neglecting the other. Both of these approaches are considered reductionist and benefit from maximal tractability but risk losing sight of the complexity of biological systems. On the other extreme are holistic approaches, which compromise precision in an attempt to capture the breadth of biological systems. Furthermore, most approaches are concentrated on interactions at the scale of a single host, which omits the broader ecology of microbial life cycles; namely, transmission between hosts [19]. Critically, a more fluid view of host–microbe relationships is at odds with our need to clearly delineate microbes based on their pathogenic potential so that they can be effectively monitored and managed. In this review, we do not intend to propose any new postulates or identify what makes a pathogen a pathogen. Rather, our goal is to highlight experimental approaches and emerging areas of research that we expect will broaden our mechanistic understanding of what governs the nature of host–microbe relationships.

Context Is Everything: A Need for New Experimental Systems

> "The shared properties of commensals and of pathogens of the same host species can be extraordinarily close and a source of consternation when trying to define a pathogen…"—S. Falkow [8].

Technological leaps over the last two decades have exposed a grander view of the microbial life that inhabits the bodies of animals [1,20,21]. At the same time, we are now confronted with the overwhelming complexity and dynamic nature of the relationships that form between animals and their resident microbiota. Just as pathogens often defy their assigned identity by not causing disease in every host they encounter, members of the healthy microbiota that are typically recognized as "commensal" or "beneficial" can incite disease under certain conditions [22,23]. Exemplifying the reaches of this continuum is *Helicobacter pylori*. *H. pylori* is widely recognized for its ability to cause gastric disorders, including gastric cancer. However, the majority of people colonized with *H. pylori* are asymptomatic, and some studies suggest that *H. pylori* is associated with protection against esophageal adenocarcinoma and childhood asthma [23,24]. Further hindering our ability to classify host–microbe relationships, polymicrobial communities—rather than individual microbes acting alone—exhibit emergent behaviors that can either promote disease (e.g., in the case of periodontitis) or promote health (e.g., by resisting colonization of pathogenic species) [22,25]. From these and countless other examples, however, at least one clear lesson about host–microbe relationships has emerged: context matters.

The context of a host–microbe relationship can be delineated along three axes (Fig. 1) [26]. The first axis is the catalog of system components; for example, the types of molecules, genes, cells, tissues, organisms, and so on, that are involved. The second axis is the spatial topography of all components within the system; for example, the degree to which components are mixed, stratified, and compartmentalized. The third axis is how each

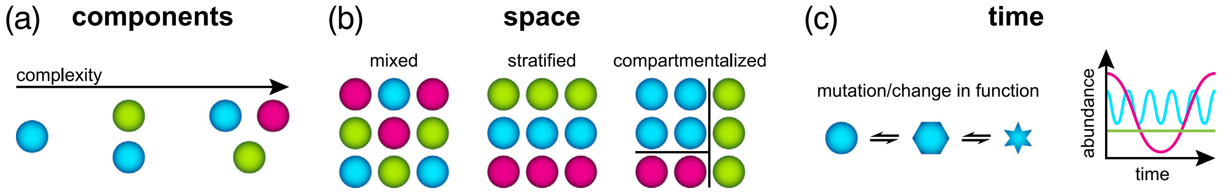

Fig. 1. The three axes of context that determine the nature of host–microbe relationships. In all panels, colored symbols and lines represent distinct components of host–microbe systems across scales. For example, bacterial and host organisms; cells, tissues, and organs; and genes, proteins, and metabolites. (a) The complexity of host–microbe systems increases with the number of components. (b) The spatial topography of host–microbe systems determines the landscape of potential interactions. Mixed systems allow for a high number of interactions, stratified systems limit interactions to neighboring components, and compartmentalized systems largely restrict interactions. In both stratified and compartmentalized systems, the potential for highly specialized interactions or those requiring particular environmental conditions is increased. (c) Left: The function of components within host–microbe systems can change over time through mutation or different gene expression patterns. Right: System components can change relative to each other at different rates; for example, fluctuations in the abundance of each component can give rise to multiple discrete system states.

component and its interactions change over time; for example, through mutation, shifts in expression or physiology, and growth or death. Most experimental approaches and model systems are inadequate for capturing all three axes of context [16–18,27]. Another limitation is that many studies of host–microbe interactions only focus on one or the other partner, which constrains the view of the relationship. Microbe-centric approaches tend to consider the role of the host as constant or negligible (e.g., in terms of immune status, development, or sex), often treating in vitro conditions as a surrogate host. Likewise, host-centric approaches tend to consider the role of the microbe as constant or negligible (e.g., in terms of antigenic profile, physiology, or physical location), often substituting living microbes with a heat-killed concoction or single antigen. And until the turn of the 21st century, both microbe- and host-centric approaches were almost completely blind to the role of the microbiome.

In our laboratory, we study interactions between zebrafish and their resident intestinal microbiota, a model system that provides unrivaled access to all three axes that make up the context of host–microbe relationships [28]. Larval zebrafish can be reared germ-free, which means that we can control the complexity of the host–microbe system parts list from simple to complex microbial consortia [29]. We can further control host–microbe system components because both zebrafish and many of its native bacterial symbionts can be genetically manipulated [30]. The small size and optical transparency of larval zebrafish make them amenable to a variety of live imaging techniques, allowing comprehensive studies of spatial and temporal dynamics. For example, using light sheet fluorescence microscopy, the entire three-dimensional architecture of the larval zebrafish intestine can be captured with single bacterial cell resolution [31,32]. Moreover, the speed and low phototoxicity of light sheet fluorescence microscopy allow dynamics of host tissues and bacterial populations to be monitored with high temporal resolution. The tractability of the zebrafish–microbiota system is further enabled by the high fecundity of zebrafish. Hundreds of larvae can be obtained from a single clutch, which means large population sizes can be used to test many different conditions in parallel and to resolve subtle phenotypes and high inter-host variation. In addition, microbial transmission between zebrafish is mediated through the aqueous environment they inhabit, which can be easily sampled and controlled through various housing designs [33–35]. The attributes of the zebrafish make a powerful experimental instrument for linking interactions across multiple scales. Below we describe recent examples of discoveries we have made about the contextual nature of host–microbe relationships using this model system.

The Pathogenic Side of Mutualism: The Potential for Beneficial Bacteria to Employ Virulent Tactics

The host immune system is a major barrier to all colonizing microbes, from those that are harmful to those that are beneficial. However, our knowledge of the microbial strategies for overcoming this obstacle—especially those used by members of the indigenous microbiota—remains limited. Pathogens are well recognized for deploying virulence factors that enable them to subvert and sabotage antimicrobial countermeasures launched by the host [36]. Some of these mechanisms involve direct modulation and suppression of immune signaling cascades within host cells. For example, lethal factor produced by *B. anthracis* and YopJ produced by *Y. pestis* inhibit inflammatory MAPK signaling, and pathogenic *E. coli* lineages can interfere with MyD88 signaling through effectors such as Tir and TcpC [36,37]. Targeted suppression of the immune system is normally viewed as a virulence strategy that allows pathogens to survive and persist within their host, ultimately leading to disease. However, could similar virulence factor-mediated immune modulation play a role in establishing mutualistic relationships?

Non-pathogenic and beneficial members of the resident microbiota can offer insights into this possibility because they too must weather attacks from the host's immune system [38]. One of the best-studied examples that generally illustrates the need for resident microbes to actively contend with host defenses is *Vibrio fischeri*, which forms a mutualistic relationship with the bobtail squid *Euprymna scolopes* [39]. Initiation into this union is anything but peaceful. During colonization of the squid's light organ—which is critical for squid development and predator evasion—*V. fischeri* is welcomed by numerous host-derived biochemical stresses including antimicrobial peptides and nitric oxide [40–42]. This onslaught is similar to those faced by pathogens during host colonization. *V. fischeri* counters this barrage by inducing biofilm formation and upregulating genes encoding efflux pumps and proteins involved in repairing oxidative damage [43,44]. Mirroring this contentious relationship, numerous bacterial members of the mammalian intestinal microbiota were found to be highly resistant to antimicrobial peptides associated with host inflammation [45]. In the case of *Bacteroides thetaiotaomicron*, this trait was demonstrated to be due to modification of the cell wall component lipopolysaccharide [45], which is a familiar tactic used by pathogens to resist antimicrobial agents and evade the immune system.

With regard to immune modulation by indigenous microbes as a means to engender mutualism, one of the first examples was shown to involve the capsular polysaccharide PSA produced by *Bacteroides fragilis* [46]. PSA acts to reinforce mucosal tolerance and guard against intestinal inflammation by modulating T-cell development and activity [47]. More recently, our laboratory came across a particularly intriguing scenario involving immune modulation by a resident microbe that reveals the fine line that can exist between mutualistic and pathogenic relationships (Fig. 2).

Using zebrafish as a host model, we discovered a novel secreted bacterial protein with anti-inflammatory properties that is produced by species of *Aeromonas* [48]. *Aeromonas* is a prevalent member of the zebrafish intestinal microbiota and helps promote normal development [49]. The protein we identified, which we named *Aeromonas* immune modulator A (AimA), functions to reduce the presence of intestinal neutrophils in response to chemical and bacteria-derived (e.g., lipopolysaccharide) proinflammatory stimuli (Fig. 2, left). Remarkably, when we deleted *aimA* along with its paralog *aimB* from the *Aeromonas* genome, the relationship between host and symbiont transformed dramatically. We found that the total abundance of *aim* mutants in the intestine was significantly reduced, and nearly half of all zebrafish hosts died within days of colonization (Fig. 2, right). Both bacterial and host survival could be restored to normal levels by addition of purified AimA protein to the water. The model that emerges from this study is that *Aeromonas* is able to mask its intrinsically hyperinflammatory nature by tempering potentially lethal host responses through the Aim proteins. Stated another way, the Aim proteins represent the dividing line between *Aeromonas* acting as a mutualist and *Aeromonas* acting as a pathogen.

In light of the AimA example, we can reimagine immunomodulatory virulence factors of pathogens as factors that enable the establishment of asymptomatic infections similar to commensalism. Viewed from the opposite perspective, we can see that beneficial microbes often display behaviors, such as immune modulation and suppression, that are typically considered hallmarks of pathogens. It is notable that the genomes of a diverse range of beneficial microbes contain predicted virulence factors, toxin-related genes, and virulence regulators. As an extreme case, beneficial symbionts of *Bathymodiolus* mussels as well as other free-living bacteria that dwell near deep-sea hydrothermal vents (up to 2600 m in depth) have been found to possess unexpectedly large repertoires of genes resembling virulence factors [50–52]. From these studies, genes associated with type 3 and 6 secretion systems, a number of “hemolysins,” and RTX- MARTX-, and YD repeat-related genes were detected. The widespread distribution of so-called virulence factors harbored by non-pathogenic and beneficial bacteria further suggests that these gene products may moonlight—or possibly have originated—as mutualism factors.

From Bacterial Behavior and Dynamics to Community Assembly and Host Inflammation

In many ways, pathogens portray a “fugitive” lifestyle in which they rapidly infiltrate, grow, and disseminate throughout host tissues [53]. By comparison, relatively little is known about the colonization dynamics of indigenous microbes. Using live imaging to monitor bacterial populations during mono-association of the zebrafish intestine, we found that commensal bacteria also experience rapid growth kinetics *in vivo*, similar to that of logistic growth observed in a test tube [54,55]. By examining the physical architecture of bacterial populations—which is mostly an unexplored feature of any microbe,

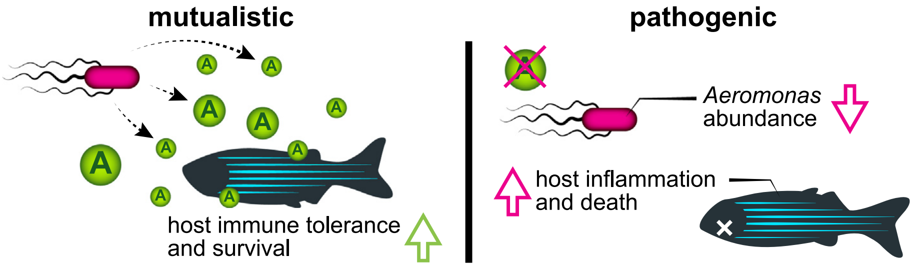

Fig. 2. Immune modulation as a bacterial strategy for promoting mutualistic host–microbe relationships. Left: Species of *Aeromonas* (magenta bacterium) can promote mutualistic relationships by secreting Aim proteins (green orbs) that temper host (blue zebrafish) inflammation and increase tolerance to bacterial colonization. Right: The absence of Aim proteins produces a pathogenic relationship characterized by reduced *Aeromonas* abundance within the intestine and increased host inflammation and death. Note that the original work that is summarized in Fig. 2 was carried out using larval zebrafish, but for clarity, the adult form of zebrafish is depicted.

pathogen or not—we found a striking diversity of colonization patterns across different species [32]. These patterns vary with regard to their spatial distribution along the intestine and the fraction of cells that exist as planktonic individuals or within large multicellular aggregates. Live imaging revealed that aggregated bacterial populations surrender to the propulsive movements of the intestine, which drives cycles of expulsion and regrowth (Fig. 3, left) [55,56]. By contrast, populations predominantly containing planktonic and motile individuals are highly stable and readily adapt to the dynamic contours of the intestine like water filling a cup. Exploring how two species displaying contrasting population architectures (i.e., planktonic *versus* aggregated) come together within a community, we observed a strong competitive interaction with the planktonic population dominating [55]. Remarkably, this competitive interaction is largely controlled through the physical environment of the intestine. In hosts with reduced intestinal transport due to genetic disruption of the enteric nervous system (ENS), competition is neutralized, and the two species coexist (Fig. 3, right).

In a parallel study, we found evidence that the interplay between the intestinal environment and bacterial populations impacts the assembly of complex communities and host inflammation [57]. Zebrafish hosts with reduced intestinal transport (via deletion of the ENS), assemble a pathogenic community that sparks spontaneous TNFα-dependent intestinal pathology (Fig. 3). Profiling of these dysbiotic communities revealed that they contain an overabundance of pro-inflammatory *Vibrio* lineages with a concomitant decline in anti-inflammatory lineages of *Escherichia*. Therapeutic administration of an *Escherichia* strain or restoring the ENS ameliorated inflammation. Together, these studies demonstrate how bacterial behaviors playing out at the micron scale interact with host processes at the organ-level scale to give rise to emergent properties such as pathogenic inflammation.

Thinking Outside the Host: The Role of Inter-host Transmission in Host–Microbe Relationships

Like a contradiction in terms, microbes classified as pathogens do not always elicit disease. Equally paradoxical is that some pathogens can establish long-term, asymptomatic residence within their host. In some cases, asymptomatic colonization may facilitate transmission to other susceptible hosts where disease subsequently manifests. An example of this phenomenon is the “extraordinary predicament of Mary Mallon,” who became known as Typhoid Mary and in 1907 was dubbed a “typhoid factory” by a local New York newspaper [58,59]. Mallon was a healthy, asymptomatic carrier of *Salmonella* Typhi and infected dozens of people through her occupation as a cook. Another example involves *V. cholerae*. Epidemiological and mathematical modeling studies indicate that the rapid and global spread of the seventh pandemic El Tor lineage is attributable to the relatively high rate at which it causes mild and asymptomatic infections [60–63]. These examples not only highlight the importance of knowing how pathogens cause life-threatening disease in one host while behaving as benign residents in another, but they also emphasize the need to understand more generally how microbes move among hosts.

The majority of experimental models are set up to only extract information about host–microbe relationships at the scale of a single host [19,33]. This narrow focus is imposed by the constraints of traditional animal infection models dating back to Robert Koch [6]. In these models, the microbe is directly administered by the experimentalist, rather than acquired through a more natural route. Using the zebrafish, in which microbes can be administered via the aqueous environment to populations of hosts, we have come to challenge the assumptions derived from single host investigations (Fig. 4).

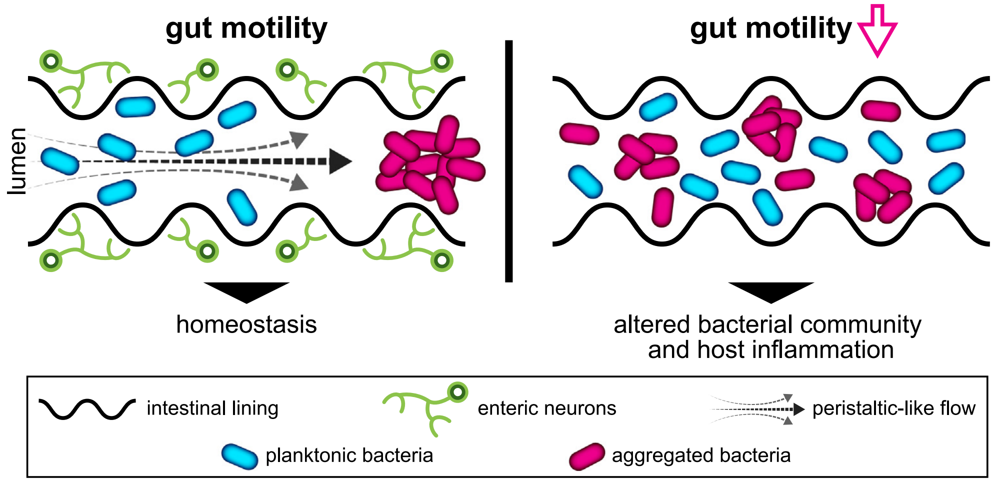

Fig. 3. The physical and dynamic activity of the intestine shapes the outcome of host–microbe relationships. Left: Through the activity of enteric neurons (green), waves of propulsive contractions generate anterior to posterior flow (hashed arrows) within the intestinal lumen (outlined by wavy black line). Bacteria are differentially affected by intestinal transport, with planktonic bacteria (cyan) being more resistant to expulsion and aggregated bacteria (magenta) being more sensitive to expulsion. Note that many additional and yet to be characterized host and bacterial factors likely contribute to the physical landscape within the intestine. In total, the physical interplay between the host intestine and resident bacteria leads to homeostasis in conventionally raised zebrafish (i.e., reared in the presence of a complex microbiota). Right: In mutant hosts lacking enteric neurons and displaying reduced gut motility, bacterial community assembly is altered. In gnotobiotic mutant hosts, it was found that both planktonic and aggregated bacteria co-exist, whereas in conventionally raised mutant hosts, there was an imbalance of pro-inflammatory and anti-inflammatory bacterial lineages that ultimately resulted in host inflammation.

In one example, we used experimental evolution to identify traits that increase the host association of a native zebrafish bacterial symbiont through serial transfer (Fig. 4a) [33]. As opposed to acquiring enhanced competitive fitness within a single host, we found that the first adaptive leap was an increased capacity to migrate from the aqueous environment into the host. The competitive advantage between evolved clones and the ancestor could be erased by delivering bacteria directly into the intestine via gavage and thus, bypassing the natural environmental route of colonization. In a separate but related study, our group assessed the relative contributions of host innate immunity *versus* inter-host transmission

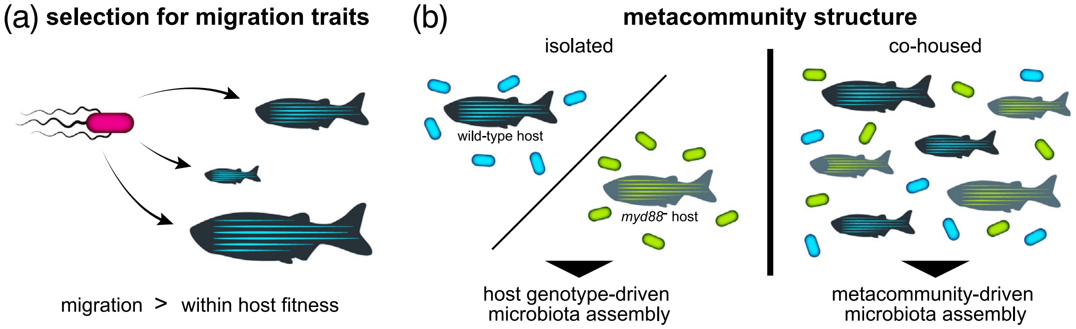

Fig. 4. Migration and metacommunity structure are important factors in establishing host–microbe relationships. (a) Many experimental models omit natural routes of bacterial colonization. By contrast, zebrafish are colonized by inoculation of bacteria into their aqueous environment. Experimental evolution revealed that traits facilitating migration from the environment are potentially the first major adaptations that enhance host colonization rather than those that increase within host fitness. Note that the original work that is summarized in panel a was carried out using larval zebrafish and the adult form of zebrafish is depicted for clarity. (b) Left: Individual zebrafish hosts raised in isolation assemble resident bacterial communities that are genotype-specific; in this case, comparing wild-type animals (cyan) to mutant animals lacking the gene *myd88* (green), which encodes a signaling protein critical for host immune responses to microbes. Right: When animals are co-housed—regardless of genotype—they assemble similar and overlapping bacterial communities that are distinct from those assembled in isolation, highlighting how metacommunity structure can drive microbiota assembly.

on the assembly of a complex microbiome (Fig. 4b) [34]. Specifically, we surveyed the microbiomes of individual wild-type and immunocompromised hosts (mutants lacking the common adaptor protein MyD88 important for immune signaling) raised in different housing conditions. When raised in isolation, animals harbored distinct, genotype-specific microbial assemblages. However, when animals were co-housed with individuals of the same genotype or with individuals of both genotypes, the intestinal communities of all hosts—independent of genotype—became similar.

Together, these studies indicate that the structure of the metacommunity, which comprises all the microbes circulating within a population of hosts, can override the ability of individual hosts to selectively filter their microbiome. In other words, the factors governing microbiome composition are not necessarily inherent to an individual host, but rather influenced by the greater host–microbe system. From our intrinsically human host-centric perspective, the inability to control our microbial associations through our extensive repertoire of evolutionarily honed innate immune receptors may seem surprising. From a microbial perspective, however, a single host is just a temporary stop along their ecological life cycle.

Perspective

> “It really doesn't matter how we define a pathogen! To underestimate the evolutionary potential of microorganisms and their ability to survive, even in the face of enormous pressure to eradicate them, would be a mistake.”—S. Falkow [64].

What we know about host–microbe interactions has historically been informed by our efforts to identify and eliminate diseasing-causing pathogens. But as new technologies continue to expose the microbial world we inhabit, what distinguishes a pathogen from an innocuous commensal has become a deceptively difficult question to answer. When we look closer, we find that many “pathogens” can reside as members of a normal, healthy microbiome for years to decades without consequence. Also defying expectations, “commensals” can be agents of disease and often harbor genes that are typically recognized to encode virulence behaviors. These realizations have eroded the longstanding dichotomy between pathogens and commensals, and in its place has emerged a highly contextual and fluid spectrum of microbial behaviors. To resolve this complex picture, we propose shifting from trying to understand what divides microbes to what unites them: their shared need to fulfill an ecological life cycle that involves the colonization of a host, growth and replication within that host, and transmission to a new host. By studying these processes specifically in commensal and beneficial microbes, which have largely been unexplored compared to pathogens, we will obtain a more complete view of the diversity of microbial life cycles and their relationship to health and disease. Critical to achieving this goal are experimental models like the zebrafish that allow the dissection of host–interactions along all three axes of context (components, space, and time) and across multiple scales of complexity—from the molecular to the metacommunity. Understanding the broader ecological facets of our resident microbial wildlife will uncover new strategies to prevent disease and engineer health.

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Abbreviations used:
ExPEC, extraintestinal pathogenic *E. coli*; AimA, *Aeromonas* immune modulator A; ENS, enteric nervous system.

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