Review

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Microbial control of host gene regulation and the evolution of host–microbiome interactions in primates

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Recent comparative studies have found evidence consistent with the action of natural selection on gene regulation across primate species. Other recent work has shown that the microbiome can regulate host gene expression in a wide range of relevant tissues, leading to downstream effects on immunity, metabolism and other biological systems in the host. In primates, even closely related host species can have large differences in microbiome composition. One potential consequence of these differences is that host species-specific microbial traits could lead to differences in gene expression that influence primate physiology and adaptation to local environments. Here, we will discuss and integrate recent findings from primate comparative genomics and microbiome research, and explore the notion that the microbiome can influence host evolutionary dynamics by affecting gene regulation across primate host species.

This article is part of the theme issue ‘The role of the microbiome in host evolution’.

1. Introduction

Primates are an ecologically and behaviourally diverse order, thriving in a wide range of habitats across the globe [1]. It is hypothesized that one driver of primate species diversification are differences in host gene regulation [2–4]. In the gut, one of the factors that can influence host gene expression is the microbiome [5–8]. Microbiome-induced regulatory changes can have immediate effects on host health and physiology, as they alter metabolic and immunological pathways [5]. Thus, variation in the composition of the gut microbiome has widespread effects on host health, and these effects can be mediated by regulation of host gene expression. Therefore, understanding the relationship between microbiome composition and gene regulation is key for quantifying how changes in microbial communities can lead to changes in host physiology and, ultimately, host fitness [9,10]. However, we do not yet understand which traits of the microbiome—i.e. overall microbiome community composition or dynamics of particular taxa or strains—affect host gene expression, how microbial regulation of gene expression differs between host species or across environments, or if such differences could facilitate evolutionary divergence between primate species. Although there is no direct evidence for microbially mediated primate evolution, studies have shown that host-associated microbial communities co-diversify with primate phylogeny [11] and change rapidly with shifts in diet [12] and the physical environment [13], perhaps leading to host species-dependent adaptations to novel conditions (figure 1). In this review, we discuss the role that gene regulation has played in primate evolution (§2), summarize the evidence to date that microbiome variation can influence host gene expression (§3) and describe differences in microbiome composition across primate species, emphasizing the importance of environmental effects (§4). We then discuss how environmentally dependent host–microbe interactions could potentially shape primate evolution (§5). Finally, we suggest future

Figure 1. A schematic of the potential relationship between primate hosts, their environments, their microbes and gene regulation. Host species-specific environmental traits can lead to differences in gut microbiome composition, which in turn can lead to differences in gene expression and resultant effects on host physiology. Figure components from [14]. (Online version in colour.)

approaches for quantifying the relationship between primate hosts, their microbes and their evolutionary trajectories (§6).

2. Evolution of gene regulation in primates

Different primate species are closely genetically related; for example, the divergence between human and chimpanzee genome sequences is estimated to be between 1.1 and 1.4% [15]. Despite this similarity, there are clear physiological, cognitive and behavioural differences between primate species. A central aim of evolutionary genetics is to describe how these vast phenotypic differences can be driven by genetic differences between species. A common hypothesis is that this phenotypic divergence is driven by variation in gene regulation between species [16,17]. In their seminal paper from 1975, King & Wilson argued that gene regulatory changes can drive some of the phenotypic differences between humans and chimpanzees, as these differences cannot be explained by changes in protein sequence alone [18]. This study supported the theory on the importance of gene regulation raised by Britten & Davidson in 1971, namely, that mutations in regulatory elements can lead to differences in gene regulation that drive evolutionary change [19].

Since then, many studies have illustrated the importance of regulatory variation in driving evolutionary adaptations within and between species. For example, in sticklebacks, the regulation of the *Pitx1* gene, which controls pelvic fin morphology, was shown to be under positive selection, driving repeated instances of pelvic reduction [20,21]. In Darwin's finches, variation in the expression of *Bmp4* is thought to control diversity in beak morphology, which is associated with adaptation to different ecological niches [22]. In humans, the loss of an enhancer of the *AR* gene has resulted in the loss of penile spines and sensory vibrissae, both of which are anatomical features common in non-human primates [23]. Among human populations, adaptive changes in the regulation of the *LCT* gene underlie the ability of some individuals to digest lactose, a trait that has been under strong positive selection [24,25].

Recent advances in sequencing technologies have allowed genome-wide investigation of regulatory differences in an evolutionary context [17]. By comparing gene expression patterns in individuals from different species, it is possible to infer the action of natural selection on the regulation of genes in specific lineages. For example, by comparing gene expression levels in six organs from 10 mammalian and bird species, Brawand *et al.* [26] found that gene expression evolution in mammals was strongly shaped by purifying selection—namely, that the regulation of most genes is conserved across species. Focusing on primates, and by comparing gene expression in three organs from humans, chimpanzees and rhesus macaques, a similar study found evidence of species-specific expression patterns that indicated the action of directional selection on gene regulation—i.e. evidence that for many genes, natural selection has modified regulation in one species, while maintaining consistent expression in other species [2]. An additional study has shown that natural selection may act on gene regulation in primates by affecting patterns of alternative splicing [27], and other work has further clarified the regulatory mechanisms controlling these expression patterns [28–30].

Although many studies have identified genes for which regulation may evolve under selection in primates, understanding the potential physiological and organismal traits that may be controlled by these genes is more challenging, as regulatory mechanisms are multilayered and complex. For example, one way that these genes may affect host phenotypes is by altering host metabolism. Indeed, several studies have found that genes whose regulation evolves under positive selection are enriched for metabolic functions [2,27]. In addition, genes whose promoters show evidence of selection are also enriched with genes involved in metabolism and nutrition [31]. Moreover, comparative analysis of metabolomics data show that there are significant differences in liver metabolite concentrations across primate species, and that these differences are associated with interspecies differences in the expression of host enzymes that are involved in the same metabolic pathways as these compounds [32].

3. Influence of the microbiome on host gene regulation

Altering gene expression in neighbouring host cells is one possible mechanism by which the gut microbiome can influence host physiology. In animal models, *in vivo* studies comparing germ-free and conventionally raised animals have shown that the gut microbiome can influence host gene regulation by altering epigenetic signatures in the host [5–8]. These studies generally use transcriptomics, chromatin accessibility profiling, DNA methylation profiling and other functional genomics techniques to assess how the presence or absence of gut microbiota alters gene regulation in epithelial cells along the host's gastrointestinal tract. Results show that the microbiome can influence host gene expression by altering epigenetic programming, such as histone modification, transcription factor binding and methylation [5–8]. For example, Pan *et al.* [7] found that the microbiome influences DNA methylation in gut epithelial cells in mice. Similarly, Camp *et al.* [6] showed that the microbiome influences differential gene expression of certain transcription factors.

The technical complexity, ethical issues and expense of *in vivo* experiments in primates makes them impractical, and so research on this topic is limited when it comes to how the gut microbiome may affect its primate host. As an experimental alternative, *in vitro* strategies have been employed to assess how host cells respond to specific microbial taxa [33]. Cell lines derived from human cancer tissues have been cultured with single, known pathogenic microbial species; these experiments have demonstrated how the interaction of microbes with host cells result in morphological and gene expression changes in cancer cells associated with relevant diseases [34,35]. For example, *Fusobacterium nucleatum*, which has been detected in higher abundances in colorectal cancer patients, has been shown to increase cell proliferation of colon cancer cell lines *in vitro* [34]. Other microbial species may have a protective effect on the host; for example, *Bifidobacterium adolescentis* has been shown to inhibit growth of colon cancer cell lines, probably by producing butanol that alters cell morphology and increases tumour necrosis factors TNF- α [35].

Similar experimental designs have expanded beyond testing single microbes to using entire intact faecal microbiota communities. One strategy employs colonic epithelial cells, which are treated with such communities to assess how microbiome variation regulates host gene expression [36,37]. Richards *et al.* [37] used this strategy with faecal microbiomes derived from healthy humans and showed that host genes show differential response to variation in microbial communities. Furthermore, specific microbial taxa can drive the expression of certain genes, and individual gene-microbe relationships can be further validated by manually adjusting the abundance of microbial taxa of interest in the same experimental system [37]. These *in vitro* and *in vivo* experimental systems can potentially be extended to characterize the impact of between-species variation in the microbiome on host gene regulation and relevant host traits. Host traits that may be of particular interest are those related to immunity and metabolism. It is compelling to hypothesize that gut microbes can regulate the expression of host genes involved in metabolic processes, which may be relevant for host species-specific adaptations to different environments and diets. Similarly, it is likely that the microbiome can impact the regulation of immune system genes, as commensal microbes are known to interact with the immune system [5], and genes that are associated with microbiome composition in humans are enriched for immune-related functions [38].

4. Microbiome variation in primate host species

To understand why differences in gene regulation between primate species may be influenced by microbiome dynamics, it is important to understand how primate host species differ in their microbiome composition. Across primate species, the gut microbiome often has similar microbial features, especially at higher taxonomic levels. Human guts are generally dominated by the microbial phyla Bacteroidetes, Firmicutes, and to a lesser extent Actinobacteria and Proteobacteria [10,39]. These phyla also comprise a substantial portion of the microbial community in non-human primates, although their relative abundances are variable between different primate species [40,41]. Despite these similarities, non-human primates tend to harbour several microbial phyla that are much rarer in humans, including Euryarchaeota, Tenericutes and Verrucomicrobia [42,43]. At lower taxonomic levels, different microbial genera and species are

characteristic of certain host species' microbiomes [42,44,45]. For example, Ochman *et al.* found that humans have a higher relative abundance of Bacteroides compared to chimpanzees, while chimpanzees have a high relative abundance of Coriobacteriaceae, which is rare in humans [42]. It is worth noting that in comparative studies, humans are almost always found to be microbially distinct from other primates; in addition to the previously mentioned differences in phyla abundances, human guts tend to harbour overall lower microbial diversity than their non-human primate counterparts [11,42,45,46].

These differences in microbiome composition between primate species are probably owing to a combination of genetic, morphological, behavioural and environmental factors. Even closely related primate species living in the same area cluster more strongly with geographically disparate members of their same host species than with sympatric members of a related species [40,47]. Microbial divergence falls along expected phylogenetic relationships in some primate taxa, including within the great apes [11,48], and between Old World monkeys, New World monkeys, apes and lemurs [49]. However, host ecology and physiology, especially as it relates to dietary adaptations, may be more important than phylogenetic relatedness in structuring microbiome similarity between many primate species [49–51]. For example, Amato *et al.* found that human microbiomes are more similar to Cercopithecines, a branch of Old World monkeys whose ecology is thought to resemble that of early hominids [52], than to more closely related great ape species [50]. It is important to note that the relative contribution of various factors in shaping microbiome composition may vary based on the scale of microbiome analysis. For example, Groussin *et al.* [53] found that finer grained bacterial resolution correlated with mammalian phylogeny, while dietary distances matched to more ancient (i.e. phylum level) bacterial divergences.

The importance of some aspects of host ecology in shaping the microbiome may also vary between species. Diet is a major predictor of microbiome composition [54–56], and primates that live in seasonal ecosystems undergo changes in the types of food available [13,55,57,58]. Other characteristics of the environment, including soil properties, forest type, geographical location and habitat disturbances, may likewise alter the microbiome, perhaps reflecting exposure to different environmental microbes [43,59–61]. Social structure effects on the microbiome may vary between primate species [62]; social interactions, such as grooming behaviour, can result in higher similarity of microbiota between individuals, and social groups have distinctive microbiota [60,63]. Sex and age effects on microbiome composition may reflect both behavioural and physiological differences [55,60,64,65]. Lastly, in non-human primates, captivity has been shown to influence the gut microbiome, as captive individuals harbour microbiomes that are more similar to human microbiomes than those of wild individuals [45]. Despite our understanding of the myriad of factors that can affect the microbiome, it is unclear how the resulting variation in gut microbiome composition between primate host species interacts with other selective pressures to drive evolutionary divergence in primate physiology.

5. Host-microbiome interactions and primate evolution

In addition to the environmental factors mentioned above, host genotype is also associated with microbiome composition

[38,66–68]. Microbiome phenotypes under host genetic influence include overall microbial composition and diversity, as well as the relative abundance of certain taxa [38,69,70]. The relationship between host genetic variation and microbiome composition has been tested using twin studies to measure microbial heritability [68–70], comparing geographically distinct populations of the same host species [38,65], cross-host species comparisons [50,51,71], sampling over hybrid zones [43,72] and through transplant studies [73,74]. In primates, host genetic effects on the microbiome are much weaker than environmental effects; one recent study estimated average heritability of human gut microbiota at 1.9% [75]. Further, one limitation with disentangling host genetic and environmental effects on the microbiome is that genetic relatives often also share the same environment and, in humans, live in the same household, potentially confounding genetic and environmental estimates [76]. Despite these caveats, there is experimental evidence for health effects in humans from microbe-by-host-genotype associations, including differences in diet, metabolism and immunity [68,70].

Several examples of evolutionary adaptation that may be driven by host–microbiome interactions have been found in humans. Some are diet related; for example, dairy consumption correlates with the abundance of the genus *Bifidobacterium* and genetic variation in and around the *LCT* gene, which encodes the lactase enzyme and has been under strong positive selection in several human populations [24,25,38,70,75,77]. In addition, salivary amylase gene copy number, which is under selection in humans, is associated with both gut and salivary microbiome taxa abundances [78,79]. Other examples are immune- or disease-related; divergence patterns of the pathogen *Helicobacter pylori* matches human migration patterns [80,81], and parasite–gut microbiome interactions are thought to have played an important role in the evolution of the human allergenic immune system [82]. However, humans face unique selection pressures compared to non-human primates, such as antibiotic use, unique diets and living in a built environment. Thus, the path of host–microbe adaptations might function differently in humans compared to non-human primates.

Although host genetic variation in humans has been linked to the microbiome, the extent of the role that host–microbiome interactions play in driving non-human primate evolution is largely unknown. This is owing in part because microbiome variation, host genetic diversity and environmental factors covary along host phylogeny, making it difficult to determine causality [83–86]. As a result, quantifying the direct links between variation in the abundance of particular microbial taxa, changes in host genetic variation or gene expression and resultant changes in host physiology and fitness over evolutionary timescales remains a rich field for future microbiome research [87]. However, to date, there is still some evidence that host–microbiome interactions may have played a role in primate evolution. As described above, the gut microbiome varies by primate host species [41,58,88,89], with some work suggesting that microbial evolution may parallel that of the host [58,71,88]. For example, Moeller *et al.* [71] found evidence for cospeciation of the families Bacteroidaceae and Bifidobacteriaceae across humans and apes. Additionally, Amato *et al.* [49] showed that Old World monkeys, New World monkeys, apes and lemurs have phylogenetically distinct microbiota, which is linked to evolutionary differences in host physiology. Other data suggest that host–microbe interactions have the potential to also influence primate evolution as decoupled

from host phylogeny. Species-specific microbial signatures can be driven by diet-related changes in host physiology [56], as well as by differences in environment use or geography [90]. In support, different species of primates that overlap in range have more similar microbiota than those who don't overlap [40,47], and habitat characteristics are the main predictors of microbiome composition across a baboon hybrid zone [43]. It is also worth noting that although most work on host–microbiome evolution in primates has focused on the gut, there is evidence of host genetic control of the microbiome across various body sites in humans [38,91,92]; and there are primate species-specific microbial communities associated with the reproductive tract [93,94], skin [95,96] and skin-related volatiles (i.e. odours; [97]). Recent work has shown that the skin microbiome affects expression of immunity-related genes [98], suggesting that future work which expands our understanding of host–microbe relationships across body sites is key for developing a comprehensive picture of the potential for microbially mediated host evolution.

There are at least two non-exclusive mechanisms by which microbiome-driven host evolution could occur [86]. First, microbiome variation may lead to an increase in host fitness [99,100]. This effect of the microbiome on host fitness may be caused by microbiome variation leading to greater host phenotype variation than would occur without microbial influences [101,102]. Second, hosts that are capable of acquiring and using local microbes, especially microbes that allow the host to adapt to a stressful environment, may have an advantage over hosts who cannot use local microbes. Both mechanisms are probably applicable in primate hosts, and both could involve changes in host gene expression. There is indirect evidence to support the first mechanism; primates live in a diverse range of environments and demonstrate both considerable microbial flexibility and great variation in microbiome-related host physiology [1,49]. For example, variation in microbial-associated dietary adaptations allows some primates to access difficult-to-process food sources, such as digesting plant fibres in folivores [103]. This in turn could promote fitness in environments with limited alternative food resources. In support of the second mechanism, there is evidence that primates who move into a novel environment acquire the local microbes. For example, the microbiomes of humans undertaking fieldwork in a novel location cluster more closely with their wild Cercopithecine study organisms than they do with non-resident humans [51]; and, length of current group residency predicts microbial similarity to social group members in immigrant baboons [104]. Primate hosts could potentially use local microbes to adapt to their new environment [49]. For example, such microbial plasticity could promote greater diet flexibility, allowing individuals to adjust quickly to environmental changes and resultant changes in food availability that occur not only when individuals immigrate to a new location, but also occur seasonally [13,51,54]. It could be adaptive for environmentally dependent changes in microbiome composition to regulate how primate hosts digest and metabolize different foods, especially given that some genes whose regulation evolves under selection in primates are enriched for metabolic functions [32].

As wild primates' environments undergo increased environmental disruptions via deforestation and major weather events, we may see a change in the relationship between the microbiome and primate hosts [105,106]. Habitat disruption leads to a decrease in microbial diversity in howler monkeys

[59], and dietary and lifestyle changes associated with captivity lead to the ‘humanization’ of the gut microbiome across multiple primate taxa [45,107]. Under increasingly variable environmental conditions, microbial plasticity may become more advantageous. Indeed, microbial flexibility in early humans is proposed to have played a key role in adapting to climate-induced environmental changes [108], and Stumpf *et al.* [109] suggest monitoring microbiomes as part of wild primate conservation.

6. Proposed future work and concluding remarks

To quantify the role of microbially mediated gene regulation in primate evolution, future work will take advantage of multifaceted approaches, integrating results from experimental laboratory work with observational field studies. Long-term studies of wild primate populations with available faecal samples will be important for linking microbial signatures with host fitness and reproductive success [110]. Furthermore, using the kinship information available for several such wild systems will allow researchers to identify heritable microbes; i.e. microbes for which abundance is probably controlled by host genetic variation, and so can be the target of natural selection. To understand the causal effect of microbiome variation on the host, several experimental approaches can be used.

For example, to study how the microbiome can impact host gene regulation, *in vitro* or *in vivo* experimental systems could be used to characterize host regulatory response to gut microbiota from different host species. Linking species-specific regulatory changes with functional pathways may provide insight into how microbial variability between primate species could lead to host species-specific phenotypes. In addition, meta-omic approaches, which integrate metabolomics, metatranscriptomics and metagenomics in cross-host species comparisons could be useful in elucidating the mechanisms of host–microbiome interactions and their evolutionary implications [111]. Combining the outcomes from such studies with longitudinal data and fieldwork will provide insight into host gene–microbiome relationships, and shed light on a potential mechanism by which microbial communities can drive primate evolution.

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