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The animal gut as a melting pot for horizontal gene transfer

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Keyword:	Horizontal gene transfer, gut microbiome, Conjugation, Plasmids, Phages

1 **The animal gut as a melting pot for horizontal gene transfer**

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13 **Abstract**

14 In this minireview we examine horizontal gene transfer (HGT) events in the
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Horizontal gene transfer (HGT) is a widespread phenomenon in the prokaryotic world. It is commonly thought of as a key process in the evolution of bacteria and their adaptation to new environments [1]. One of the most interesting microbial communities resides in the mammalian gastrointestinal (GI) tract. These communities have intricate relationships within them, as well as with their mammalian host [2, 3]. The gut bacterial communities are extremely diverse and complex, as well as exceptionally dense with over 10^{10} microorganisms per milliliter of content. The immediate proximity and wide range of neighboring cells create a favorable environment for HGT [4]. Here we review the importance of HGT in the mammalian gut and the mechanisms by which it occurs, as well as the methods available for its study.

Metagenomic approaches for studying HGT

In most studies, HGT events are identified in bacterial genomes by looking at phylogenetic trees, network analysis and sequence properties such as G+C content [5]. By examining bacterial genomes, one can estimate the extent of HGT and the phylogenetic distances crossed by the genes. Nevertheless, a more fundamental understanding of the genes that are being mobilized at a given time can be gained by exploring the mobile genetic elements (MGE) themselves. These include plasmids, viruses and other elements, such as transposons and integrons [6, 7]. Recent developments in sequencing technologies have allowed investigators to look at overall bacterial communities and describe them. These techniques can also be employed to characterize communities of MGE and study the genes that they are carrying.

Such methods have been developed for the analysis of plasmid metagenomes [8-11]. Brown Kav et al. (2012) [9] analyzed the rumen plasmidome (overall plasmid population) and showed the effect of the environment on its plasmids and the genes they carry, as contigs sequenced in that study exhibited similarity to plasmids previously isolated from rumen bacteria. In addition, the plasmid contigs showed great phylogenetic mosaicism, demonstrating the large phylogenetic distances crossed in such an environment. Finally, the contigs were enriched in rumen-relevant functions such as respiration, and carbohydrate, amino acid and protein metabolism [9].

Another category of MGE that has been studied using metagenomic approaches is the virome—the overall virus population in the environment. Several studies have attempted to characterize the mammalian GI tract virome using similar techniques [12-17]. In this case, one of the main challenges encountered by investigators was the extremely low similarity of the obtained sequences to known genes in the different databases. Of the small number of sequences that could be identified, most were associated with DNA metabolism, replication and repair [16, 17]. Other overrepresented functions could not be detected, most likely due to the lack of proper database representation. These studies also identified sequences of prophages, indicating that transduction is one of the mechanisms by which HGT occurs in these systems [12-17].

HGT in individual genomes

Numerous studies have described specific HGT events in microbial genomes. Meehan and Beiko (2012) [18] detected multiple events of HGT of a peptides/nickel transport complex in strains of the gut microbe *Faecalibacterium prausnitzii*, each operon

originating from a different taxonomic source, but all from other gut-inhabiting Clostridiales [18]. Coyne et al. (2014) [19] found HGT events between different Bacteroidales species in the gut of individual humans. These events were likely due to integrative conjugative elements, and encoded fitness-amplifying functions involving orphan DNA methylases, fimbriae synthesis proteins, putative secreted antimicrobial molecules, and a predicted type VI secretion system [19]. An interesting case involved the transfer of porphyranases and agarases from marine Bacteroidetes into the genome of *Bacteroides plebeius*. These genes were further found in the microbiome of Japanese subjects, and were missing from North American ones. As these donating bacteria are associated with seaweed, this finding serves as an indication of the manner in which this bacterium obtained the genes for porphyran and agar degradation: high seaweed consumption in the Japanese population enabled this transfer, and most likely maintained it, as the substrates of these genes are common in the Japanese gut [20].

HGT over phylogenetic distances

A wider view of the effect of HGT on gut microbial genomes was reported by Zaneveld et al. (2010) [21]. These authors characterized gene content conservation with regard to phylogenetic distances and environment by analyzing the gene content conservation in bacterial genomes isolated from different environments and the plasmids sequenced with them. Their analysis showed that gene content in gut bacterial chromosomes is more conserved over long phylogenetic distances than that in non-gut bacterial chromosomes. Reciprocally, gene content over short phylogenetic distances was less conserved in gut vs. non-gut bacterial chromosomes. The same phenomenon was

observed for the analyzed plasmids. The authors suggested that over long phylogenetic distances, the distinctive functional requirements of the gut environment dictate the genetic repertoire, whereas closely related bacteria in the gut are driven to specialization [21]. This suggests that HGT sculpts the microbial community and the similarity among its genomes in the gut environment, and somehow outweighs the constraints imposed by phylogenetic distances, such as G+C content and restriction systems [5, 22, 23]. This is further supported by the analysis of HGT events identified in sequenced genomes. The authors concluded that the main driver of HGT events is the ecological niche, signifying adaptation to that niche [24].

HGT events in the mammalian GI tract have been found to bridge a range of phylogenetic distances, including different domains. Evidence of inter-domain HGT was found in the gut archaeon *Methanobrevibacter smithii*, where 15% of its genes were suspected of having been transferred from co-inhabiting gut bacteria. These genes were in large part adjacent to mobile genetic elements in *M. smithii*'s genome, such as phage-related proteins, integrases, transposases and insertion sequence elements. The newly acquired genes mostly encoded surface-related proteins that contributed to the archaeon's adaptation to the gut environment [25]. Furthermore, Garcia-Vallvé et al. (2000) [26] suggested that many of the glycosyl hydrolases of rumen fungi had been acquired by HGT from bacteria, also exemplifying cross-domain gene transfer [26]. Similarly, the transfer of environment-relevant genes was discovered in rumen ciliates. Ricard et al. (2006) [27] proposed that HGT enabled ciliates to inhabit the rumen by introducing advantageous functions involved in the degradation of plant cell wall—a substrate that is plentiful in the rumen environment [27].

Mechanisms for HGT in the mammalian GI tract

One of the main forces enabling this large extent of HGT in the gut, as well as cross-domain gene transfer, could be physical proximity. This was shown in the aquatic environment, where intra-amoebal microorganisms obtain many genes from nearby bacteria, as well as their amoeba hosts. It was suggested that the amoebae serve as a “genetic melting pot”, creating optimal conditions for HGT between the bacteria residing within them [28]. Rumen ciliates have also been shown to play a role in promoting HGT between bacteria in the rumen. McCuddin et al. (2006) [29] demonstrated that gene transfer occurs, most likely via conjugation, within the ciliate itself. They hypothesized that the conditions inside the ciliate—close proximity of the donor and receptor coupled with stress induced by engulfment by the ciliate—are manifested in increased occurrence of HGT [29].

Such conditions of extreme density also characterize the mammalian gut itself. One of the mechanisms for HGT that can be markedly affected by physical proximity is conjugation [30, 31]. This mechanism is also advantageous in the gut system since it protects the DNA from nucleases and heavy metals that are present in the environment [32]. Viruses also provide such protection during DNA transfer, but unlike marine environments, where viruses are extremely abundant and outnumber bacteria 10:1, this ratio in the mammalian GI tract has been reported to be about 1:1 [33]. This suggests that conjugation has a cardinal role in HGT within gut microbiomes. Therefore, a comparison of these two mobile entities and their contribution to HGT in these environments could be of great importance to understanding the forces driving HGT.

Conclusions

HGT is a central mechanism for genome diversification and the acquisition of novel functions. It is therefore a driver of microbial evolution and ecology. Evidence suggests that in the mammalian GI tract, HGT events have allowed microbes to adapt to this selective and demanding environment by conferring gut-relevant functions as well as antimicrobial drug resistance and virulence genes [34] and enabling the utilization of new niches. Plasmid conjugation in gut environments could be a favored process for HGT due to the dense and diverse nature of the microbial populations that occupy them and the protection it provides to the DNA during its transfer. This environment is essentially a “melting pot” of genetic exchange, resulting in frequent HGT events crossing varying phylogenetic distances. It is thus of major interest for the study of gut microbiomes and HGT-facilitated bacterial evolution.

Although there are many indications of HGT in the mammalian GI tract, a broader view of this evolutionarily important phenomenon is required. Recent technological advances have allowed investigators to access total populations of MGE and investigate HGT processes from a community perspective. While these studies provide important initial information, much work is needed to understand the ecological and evolutionary roles played by HGT in a complex and restrictive environment such as the mammalian GI tract.

Understanding HGT within the mammalian GI tract will provide a better understanding of the microbial population residing there, as well as of the universal evolutionary processes steered by such events.

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123 Mechanisms for HGT in the mammalian GI tract

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