

FOREWORD

HORIZONTAL GENE TRANSFER: PERSPECTIVES AT A CROSSROADS OF SCIENTIFIC DISCIPLINES

Barth F. Smets* and Tamar Barkay[‡]

Horizontal gene transfer (HGT) has a crucial role in microbial evolution, in shaping the structure and function of microbial communities and in controlling a myriad of environmental and public-health problems. Here, Barth F. Smets and Tamar Barkay assess the importance of HGT and place the selection of articles in this Focus issue in context.

Recent years have witnessed an increased appreciation for the role of microorganisms and their metabolism in shaping and sustaining life on Earth. Most of life's fundamental processes were 'invented' during the first 2 billion years of life on Earth, prior to the appearance of the first eukaryote. This achievement is especially striking considering that it was accomplished by organisms lacking sexual reproduction, the long-presumed major mechanism of genetic innovation. Horizontal gene transfer (HGT) is a process that can compensate for the otherwise clonal mode of prokaryotic life, affecting microbial adaptation, speciation and evolution. Microorganisms occupy — and adapt to occupy — a plethora of ecological niches on earth, and their activities in large part control global homeostasis. Through its attendant effects on microbial adaptation, HGT poses both challenges and opportunities in the control of global human and environmental health.

For any gene to be horizontally transferred from one genome to another, at least four (sometimes five) distinct steps need to occur (FIG. 1). First, a nucleic-acid molecule (DNA or RNA) in the donor organism is prepared for transfer. This might entail the active packaging of nucleic acids into phage particles, plasmid replication from an origin that leads to conjugal transfer, integron assembly or passive release of DNA into the environment upon cell death. Second, the transfer step, which might or might not require physical contact between the donor and recipient organism, takes place. Third, the nucleic acid enters the recipient organisms through

specific or non-specific means. Fourth, the nucleic-acid molecule is established in the recipient either as a self-replicating element or through recombination with, or transposition into, the recipient's chromosome. This existence can be transitory, as is the case with many plasmids of which maintenance by the recipient genome depends on selective pressure. Last, in step 5, stable inheritance in the recipient genome might ensue.

Several scientific disciplines are addressing HGT, each providing their unique perspective and each using different approaches and methodologies. In general, evolutionary biology considers HGT events that have gone to completion (that is, through step 5). Molecular ecology, on the other hand, tends to focus on HGT events at the level of step 4. Finally, molecular biology is most interested in the mechanisms controlling steps 1 through 4. With such distinct perspectives, conflicts are bound to arise, but opportunities for synthesis are certain to emerge. The goal of this Focus issue of *Nature Reviews Microbiology* is to present HGT from the perspective of these different disciplines and to provide a path towards the construction of a holistic picture of HGT and its effects on extant microbial communities. We argue that efforts towards such synthesis will accelerate our understanding of the mechanisms and factors that control HGT, the impacts of HGT on the evolutionary history of prokaryotes, the effect of HGT on microbial interactions with each other and their environment, and the means by which HGT can be controlled to affect human and environmental health.

*Institute of Environment & Resources, Technical University of Denmark, Kongens Lyngby, DK 2800, Denmark.

[‡]Department of Biochemistry and Microbiology, Rutgers University, New Brunswick, New Jersey 08901, USA. Correspondence to B.F.S. e-mail: bfs@er.dtu.dk

Molecular evolution

Molecular evolution employs a retrospective approach to infer HGT by examining the signatures left by HGT in microbial genomes. This approach has benefited enormously from the availability of complete microbial genome sequences. As is described in this Focus issue by **Peter Gogarten and Jeffrey Townsend**, HGT can be inferred from phylogenetic dependent or independent inspections of genes. In the first approach, the atypical distribution of genes, inferred from incongruence between various gene phylogenies, is taken as evidence of HGT. In the latter approach, genes that seem unusual in their genomic context are considered to have arrived in their current genome relatively recently through horizontal transfer. In addition, experimental approaches that specifically aim at the isolation and identification of heterologous 'gene islands' in closely related strains¹ are also employed.

Molecular evolution examines HGT from a post-step-5 position (FIG. 1), and whereas evidence of past HGT in current genomes is pervasive, the ramification of these observations to our understanding of life's history is hotly contested. Certainly, HGT has challenged our view of the evolutionary history of organisms and genes from a tree-like paradigm² to a network-like paradigm³, and therefore its influence on microbial speciation and diversification. This area of ongoing controversy — the concept of the prokaryotic species — is discussed in detail by **Dirk Gevers and colleagues**, who also propose approaches to find a taxonomic framework that can accommodate the vast differences in biology presented by prokaryotes.

Understanding the way HGT has contributed to microbial evolution can help identify intra- and extracellular processes that affect the stable inheritance of transferred genes in a new genome (step 5 in FIG. 1). Crucial among them, according to Gogarten and Townsend, is the question of selective pressure for the inheritance of transferred genes in their

new host in light of their observation of selective neutrality of transferred genes. There remain, therefore, fundamental questions on the actual (if any) ecological role of such transferred genes and the mechanism that controls their maintenance in a host genome. If harmful genes (for example, antibiotic-resistance or virulence genes) are to be prevented from spreading horizontally, or beneficial genes (for example, biodegradative genes) are to be stimulated to do so, information on the processes that facilitate inheritance after HGT is essential, and bioinformatic analyses should provide useful clues.

The traditional view that obligate intracellular parasitic microorganisms have 'fixed' minimal genomes with little influence of intra- and inter-genomic genetic (ex)change is challenged by **Seth Bordenstein and William Reznikoff**. These authors argue that the extensive presence of mobile genetic elements (MGEs) such as prophages, plasmids and transposons in recently sequenced genomes of obligate parasites suggests a more complex picture. As the association of eukaryotic organisms with obligate intracellular parasites often leads to pathologies, the issues raised by this review could have far-reaching practical implications.

Molecular biology

Molecular biology has long been examining the mechanisms that govern the first steps in the gene-transfer process (steps 1–4 in FIG. 1), in part because MGEs are at the core of much molecular biological experimentation. Diverse elements and elegant mechanisms have been discovered and elucidated. The molecular processes that govern, as well as those that serve as barriers for, gene transfer have been thoroughly, yet incompletely, characterized, as reviewed by **Christopher Thomas and Kaare Nielsen**. The authors observe that any identified explicit barrier to HGT (for example, surface exclusion, restriction and so on) is subject to genetic and/or physiological modulation, and is therefore not impermeable. Remarkably little, however, is known about environmental and molecular signals that control expression (or overexpression, if such exists) of the HGT processes. Yet this question is central to a correct assessment of HGT in microbial communities. Is HGT an adaptive phenomenon that is stimulated in challenging environments or is it a random process of which the outcome is controlled by natural selection?

Molecular biology has supplied microbial ecologists with the tools to interrogate the mobile gene pool in microbial communities from diverse habitats. The surprise and lesson from such studies is that the diversity of mobile elements is much broader — and probably underestimated — than what has been gleaned from the original molecular biological work that focused primarily on pathogenic microorganisms. The diversity of MGEs, and especially the challenges and opportunities in annotation and cataloguing that arise as their rate of discovery has accelerated with

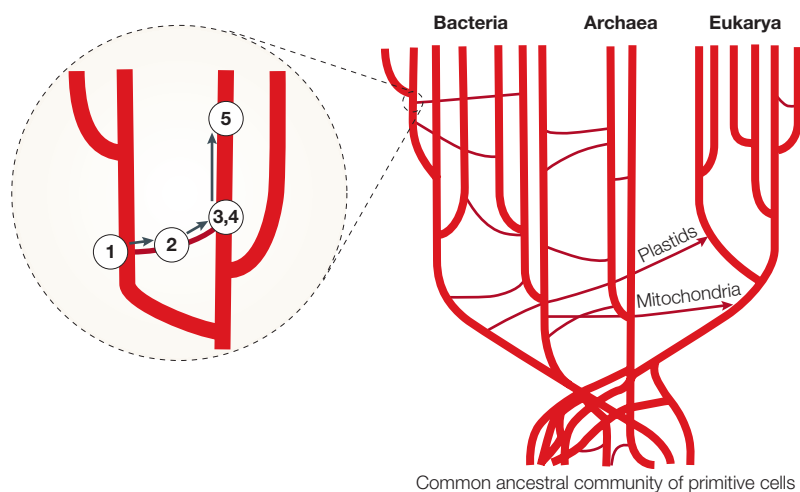


Figure 1 | **The 5 steps of horizontal gene flow.** Horizontal gene transfer and how it has impacted the evolution of life is presented through a web connecting bifurcating branches that complicate, yet do not erase, the tree of life. The inset illustrates the continuum of 5 steps that leads to the stable inheritance of a transferred gene in a new host.

the sequencing of microbial genomes, is addressed by [Anne Summers and colleagues](#). Together, these elements are in the process of being transferred (step 2) and could be considered to be 'genes in transit'. Comparison of this gene pool — with the exclusion of genes involved in the transfer processes themselves, such as viral genes or plasmid maintenance genes — with those that are identified in complete genome sequences by bioinformatic approaches as laterally transferred genes should generate new insights and testable hypotheses on the processes that favour stable inheritance of transferred genes in a new genomic context (the transition from step 4 to 5 in FIG. 1).

Our expanded view on the diversity and distribution of MGEs has, to a large extent, been made possible by the progression in microbial ecology from the study of pure cultures (and the genetic elements residing within them) to the direct isolation of nucleic acids and mobile elements from microbial communities (for example, exogenous plasmid isolation, direct sequencing of viral DNA). This newly found diversity should be matched by an attempt at characterizing these mobile elements beyond their sequence composition and understanding the manner in which they might enhance microbial genome evolution. An essential tool for progress towards this goal is the establishment of curated and carefully annotated databases and repositories of molecular information specifically for the mobile gene pool, or 'mobilome', which spans all kingdoms of life. Challenges associated with this effort are further discussed by Summers and colleagues.

Microbial ecology

The role of HGT in adaptation of microbial communities to changing environmental conditions has intrigued microbial ecologists for at least three decades. Although the advantage of spreading 'ready made' genes that enhance fitness under altered conditions relative to their *de novo* evolution by the slow process of mutations acted upon by natural selection is obvious, obtaining solid evidence of this occurrence in extant microbial communities has been elusive. Most evidence to date consists of observations that imply HGT's role in response to changing environments. Chief among them is the frequent association of environmentally beneficial genes, such as antibiotic- and metal-resistance genes and xenobiotic-compound-degradation genes, with MGEs, as described by Summer and colleagues in this Focus issue. Observations of such MGEs in man-impacted environments, and in related but pristine environments, has led to the fascinating hypothesis that the horizontal transfer events that led to the dissemination of such genes are induced by the introduction of substances such as antibiotics, metals or organic contaminants into the environment. However, the observation of HGT under apparently selective conditions does not necessarily imply that the environmental forces caused HGT; it could simply mean that these elements were enriched to detectable concentrations.

The documented incidence of HGT, as revealed from comparative genome-sequence analysis, and the discovery of an increased diversity of MGEs have nevertheless given credence to the notion that HGT could be an important determinant in shaping the microbial community metagenome. Analytical and experimental tools developed by molecular evolution and molecular biology are now routinely used to examine strains and nucleic-acids pools from different environments. For example, incongruence between gene trees has been invoked to suggest horizontal transfer of metal-homeostasis⁴ and 2,4-dichlorophenoxyacetic-acid-degradation genes⁵ among microorganisms from aquatic and terrestrial environments.

Perhaps most exciting are new experimental approaches that facilitate the real-time demonstration of HGT in undisturbed microbial communities. [Søren Sørensen and colleagues](#) describe the issues, challenges and achievements in the study of HGT in extant microbial communities. These methods are largely driven by technological advances in optical detection and biomarker construction to permit observations of single-cell and single-MGE dynamics in undisturbed microbial communities. Whereas achievements to date have mostly focused on methods development, future research employing these methods should place HGT within the context of contemporary microbial communities and their activities. The consequences of such studies to enhance our ability to modulate interactions with and among the microorganisms around us might result in a better control of disease processes and improved environmental management (see below).

HGT: why care?

While the concept of HGT frequently engenders joyful intellectual contemplation and lively philosophical exchanges, it carries more than just ivory tower relevance. The evidence indicates that HGT is a central process in microbial activities that control our health and the environment, and that it holds promise as a tool for their improvement.

The increased global documentation of human pathogenic bacteria (for example, *Streptococcus pneumoniae*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*) that are resistant to multiple classes of antibiotics — identified as one of the key challenges to contemporary infectious-disease control — is one example in which proficient HGT has resulted in undesirable consequences⁶. The improper and excessive administration of antibiotics (conferring selective advantage), combined with the ready bacterial ability to transfer antibiotic-resistance genes through plasmids and transposons and the presence of large transfer communities (for example, the gastrointestinal tract) in places such as hospitals or animal husbandry facilities, promotes the widespread dissemination of these genes. An urgent need for a more prudent use of antibiotics, combined with a better grasp of the ecology of HGT, is essential to avoid a return to a pre-antibiotic area of infectious-disease control⁷.

Transgenic organisms hold great promise for improved food production. Concerns about HGT from these organisms have, however, shrouded and limited their application. The appropriateness of current risk-assessment models⁸ and monitoring protocols⁹ to depict the potential for recombinant gene transfer through HGT to unintended target organisms are issues that are subject to fierce debate. A fuller understanding of the mechanisms and constraints for HGT could ensure development of effective gene-containment strategies within target species and ultimately allow the full realization of the promise of biotechnology.

On the other hand, the spread of genes by HGT to microorganisms in contaminated environments is a desired outcome of gene-augmentation strategies¹⁰. In these strategies, donor cells carrying an MGE that encodes essential genes for the biodegradation of a target contaminant are introduced into the relevant environment, and dissemination of the genes to indigenous bacteria, followed by expression of the degradative genes in their new hosts, leads to accelerated contaminant degradation. Although some promising results have been obtained to date, more studies are required to evaluate whether the concept of HGT-based environmental management can be sustained.

The ability to control harmful effects and to enhance desired attributes of HGT depends on the integrated understanding of HGT as a continuum spanning steps 1–5 of the gene-transfer paradigm (FIG. 1) and its integration within an ecological framework.

Conclusions

Clearly, HGT has contributed to prokaryotic evolution and is an ongoing process in extant microbial communities. The 'mobilome' is therefore receiving

unprecedented attention from a range of scientific disciplines. The purpose of this themed issue is to synthesize the state of our knowledge from these different perspectives. We believe that such a synthesis will be mandatory to obtain a more precise appraisal of HGT as a force in shaping prokaryotic evolution, diversity and activity and, therefore, in modulating the history of life on Earth.

1. Nesbo, C. L. & Doolittle, W. F. Targeting clusters of transferred genes in *Thermotoga maritima*. *Environ. Microbiol.* **5**, 1144–1154 (2003).
2. Woese, C. R. Interpreting the universal phylogenetic tree. *Proc. Natl Acad. Sci. USA* **97**, 8392–8396 (2000).
3. Baptiste, E. *et al.* Do orthologous gene phylogenies really support tree-thinking? *BMC Evol. Biol.* **5**, 33 (2005).
4. Coombs, J. M. & Barkay, T. Molecular evidence for the evolution of metal homeostasis genes by lateral gene transfer in bacteria from the deep terrestrial subsurface. *Appl. Environ. Microbiol.* **70**, 1698–1707 (2004).
5. McGowan, C., Fulthorpe, R., Wright, A. & Tiedje, J. M. Evidence for interspecies gene transfer in the evolution of 2, 4-dichlorophenoxyacetic acid degraders. *Appl. Environ. Microbiol.* **64**, 4089–4092 (1998).
6. Monroe, S. & Polk, R. Antimicrobial use and bacterial resistance. *Curr. Opin. Microbiol.* **3**, 496–501 (2000).
7. Levy, S. B. & Marshall, B. Antibacterial resistance worldwide: causes, challenges and responses. *Nature Med.* **10**, S122–S129 (2004).
8. Heinemann, J. A. & Traavik, T. Problems in monitoring horizontal gene transfer in field trials of transgenic plants. *Nature Biotechnol.* **22**, 1105–1109 (2004).
9. Nielsen, K. M. & Townsend, J. P. Monitoring and modeling horizontal gene transfer. *Nature Biotechnol.* **22**, 1110–1114 (2004).
10. Springael, D. & Top, E. M. Horizontal gene transfer and microbial adaptation to xenobiotics: new types of mobile genetic elements and lessons from ecological studies. *Trends Microbiol.* **12**, 53–58 (2004).

Acknowledgements

The authors would like to thank the US National Science Foundation (BES programme) and the US Department of Energy (NABIR programme) for support of research on HGT in their laboratories. This article and special issue were inspired by a workshop on 'Horizontal Gene Flow in Microbial Communities' that was co-chaired by the authors in Warrenton, Virginia, USA, in June 2004, and sponsored by the National Science Foundation (MO/MIP programme) and the Department of Energy (NABIR programme). These agencies, as well as the US National Aeronautics and Space Agency (Astrobiology Programme) provided gracious support to the production of this issue.