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# Harnessing the power of microbial genomics for exploring exceptions and shifting perceptions

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The launch of "Frontiers in Evolutionary and Genomic Microbiology" marks 15 years since generation of the first genome sequence from a free-living organism (Fleischmann et al., 1995). In this relatively short time, microbial genome sequencing has allowed enormous advances in our understanding of the genomic basis of microbial life, and it is difficult to think of a sub-field of microbiology that has not been profoundly affected. The volume and diversity of data available from projects that are completed or ongoing (5,465 bacterial genomes and 209 archaeal genomes, according to www.genomesonline.org, accessed December 21, 2010) presents both opportunities and challenges. One of the most exciting challenges is how to best exploit genomic data to understand the exceptions of bacterial and archaeal biology. These exceptions conflict with accepted hypotheses and theories, and challenge the "rules" that we presently teach from microbiology textbooks. They also provide opportunities to uncover new commonalities and interactions between and within the three domains of life.

Such exceptions have sometimes been recognized for many years, but the availability of a new genome sequence can serve as a springboard for new experimental work. The results of this work can demonstrate a functional role for the exception and, in some cases, bring about a shift in perceptions. Many examples could be cited, but rather than provide an exhaustive list, the authors beg the indulgence of readers to focus on a personal area of interest – the cell biology and evolutionary history of members of the Planctomycetes–Verrucomicrobia–Chlamydiae (PVC) superphylum (Wagner and Horn, 2006). Several members of this group have a common cell plan that features a ribosome-free paryphoplasm separated from a ribosome-containing riboplasm by an intracellular membrane (Fuerst, 2005; Lee et al., 2009). Planctomycete bacteria exhibit additional intracellular complexity, such as the double-layered membrane system that surrounds the condensed genomic DNA of *Gemmata obscuriglobus* (Fuerst and Webb, 1991) or the anammoxosome (anaerobic ammonia-oxidizing compartment) in the Brocadiaceae family (Jetten et al., 2001). Both of these findings challenge the validity of restricting the terms "nucleus" and "organelle," respectively, to the domain Eukarya. The availability of genome sequences from members of the PVC superphylum has allowed creative computational and experimental work to demonstrate – uniquely within the domain Bacteria – the presence of protein structures that resemble eukaryotic membrane coat proteins (Santarella-Mellwig et al., 2010). A representative of these coat-like proteins was localized to paryphoplasmic vesicles in *G. obscuriglobus* (Santarella-Mellwig et al., 2010), and, most remarkably, these same vesicles were found to receive proteins endocytosed by the *Gemmata* cell (Lonhienne et al., 2010). Thus the availability of PVC superphylum genomes and subsequent experiments allowed demonstration of commonalities between eukaryotic and bacterial cellular trafficking, and of an exception to the rule of endocytosis as a stereotypically eukaryotic trait. The *G. obscuriglobus* genome sequence also provided a springboard for the discovery of sterols in this organism (Pearson et al., 2003), one of only a handful of examples in the Bacteria for another characteristic eukaryotic property. It remains to be seen whether the sterols of *Gemmata* contribute to the structure of its complex endomembrane system. If so, this would provide an interesting parallel to the presence of other unusual planctomycete molecules (the ladderanes) in the anammoxosome of the anammox planctomycetes (Sinninghe Damsté et al., 2002).

These genome-enabled findings in the PVC superphylum have naturally spurred other efforts to use high-throughput computational analyses to better understand the evolutionary history and uncover the genomic basis of unusual aspects of PVC member biology. Because of the relatively large phylogenetic distances separating members of the PVC phyla, there is an urgent need for closing the gaps with more genome sequences. However, while we wait for these sequences to be generated, it is worthwhile to consider novel analytical approaches that can accommodate the phylogenetic distances of the currently available PVC genomes. Whole-genome scans for positive Darwinian selection are widely used to detect evolution of genome novelty, but commonly used methods (e.g., evaluation of non-synonymous to synonymous substitution rate ratio across evolutionary lineages) are sensitive to saturation of synonymous sites and thus cannot be used to study evolution of distantly related organisms. Such challenges stimulate the development of alternative methodologies such as the analysis of indel (insertion/deletion) events, which occur less frequently than amino acid replacements, accumulate more slowly, and generate functional changes through positive selection. They thus can be employed to characterize evolution of diverged organisms such as members of the PVC superphylum (Kamneva et al., 2010). While these new methodologies have been successfully developed for characterization of this particular group of organisms, they could be applied to any of the relatively newly described phyla where cultured representatives and genomes are sparse, and phylogenetic distances are large.

Arguably the most exciting genome-based insights into microbial ecology have been obtained from metagenomics, the analysis of entire microbial communities (Rondon

et al., 2000). We can turn to another favorite microbial group, the nitrifiers (aerobic oxidizers of ammonia to nitrate) for an example of how metagenomic analysis has shifted our perception of the "key players" in an ecologically important process. Cultured members of the genera *Nitrosomonas*, *Nitrosospira*, and *Nitrobacter* were thought to be the major contributors to nitrification in the natural environment. However, metagenomic analysis has revealed the importance of other bacterial nitrite oxidizers not yet available in axenic culture (Lücker et al., 2010), and novel ammonia-oxidizing archaea (Treusch et al., 2004, 2005; Francis et al., 2005; Leininger et al., 2006; Reigstad et al., 2011). The combination of metagenomics and classical isolation techniques have most recently led to a significant expansion of the former (Lebedeva et al., 2011; Spieck and Lipski, 2011). The latter have now been shown to be a distinct phylum of organisms that perform ammonia oxidation with a novel gene inventory, higher substrate affinity, and different pathways than known for the ammonia-oxidizing bacteria, and also to be the dominant ammonia-oxidizers in the majority of environments where nitrification occurs (Könneke et al., 2005; Prosser and Nicol, 2008; Martens-Habbena et al., 2009; Schleper and Nicol, 2010; Spang et al., 2010). Ecological insight into the function of microbes "closer to home" has also been gained through recent forays into metagenomics of the human microbiome. Among these, metagenomic analysis of the gastrointestinal microbiome has revealed a tremendous diversity of carbohydrate-active enzymes presumed to be responsible for the catabolism of dietary fiber (Li et al., 2009; Turnbaugh et al., 2010). A recent study (Tasse et al., 2010) demonstrated that a targeted functional metagenomics approach could detect carbohydrate-active enzymes from organisms representing a minority of the dominant gut bacteria. Such enrichment strategies provide helpful models for addressing one of the major challenges of metagenomic studies – elucidating the structure–function relationships of microbial communities and (where hosts exist) how those relationships affect host biology.

The few examples discussed here illustrate the interesting paths genome-based research can follow when computational and experimental approaches are creatively combined, and when the realities of the structure of the bacterial tree drive the development of new bioinformatic tools. These combinations can (or could) feature some of the many new genome-based methodologies – ranging from synthetic genomics (Gibson et al., 2010) and single-cell environmental genomics (Ishoey et al., 2008), to genome-based microbial systematics and taxonomy (Konstantinidis and Tiedje, 2005) and powerful functional genomics screens that explore host–pathogen relationships in a high-throughput fashion (Waterfield et al., 2008). We can therefore expect that as we continue to gain unprecedented insight into the gene inventory underlying the biology of bacteria and archaea (both cultured and currently uncultured) we will also discover creative new ways to harness the power of microbial genomics to understand exceptions and challenge current perceptions. "Frontiers in Evolutionary and Genomic Microbiology" provides an ideal publishing platform to describe both novel findings and novel approaches, and we look forward to receiving both types of contributions.

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