absent or rare in other archaeal and bacterial organisms, supporting the possibility that some of them were horizontally transferred from eukaryotic organisms (e.g., Figure 5 of [2]). Thus, data and alternative explanations weaken the missing link interpretation of the eukaryote-like features of Lokiarchaeota. In comparison, giant viruses increased the number of universal ABEV FSFs from 370 to 395 and resulted in a ~6% increase in total viral FSFs (from 619 to 654). Thus, they were 6 times more impactful in changing the Venn distribution than *Lokiarchaeum*.

To conclude, the *Lokiarchaeum* genome shows some crucial 'eukaryotic' genes are shared with Archaea that could have served as early 'starter-kit' in eukaryotic evolution [2]. However, FSF distribution patterns in Venn diagrams (Figure 1B) make it unlikely that eukaryotes originated from an archaeal ancestor that was more complex than any of the contemporary archaeal species. Furthermore, in order to consider Lokiarchaeota a missing link in eukaryotic evolution, possible gains of putative starter-kit genes independently from viruses or even eukaryotes must be ruled out. Thus, checking the virosphere (group of all known and unknown viruses in the biosphere) near Loki's castle would be revealing. Interestingly, while Archaea and Eukarya show some striking resemblances at the molecular level, the archaeal mobilome (viruses, plasmids, insertion sequences, and transposons associated with a particular domain of life) resembles more that of Bacteria than Eukarya [9]. In fact, the differential selection of virosphere by cellular domains is often ignored in cellular evolution. In this case, it does not explain the origin of Eukarya from Archaea, as many groups of eukaryotic RNA viruses are yet to be detected in archaeal species [7]. Because RNA viruses are believed to be very ancient, their *de novo* origin in eukaryotes after the split from an Archaea-like ancestor is deemed to be a less likely scenario (discussed in [9]). Therefore, carefully exploring viral mobilome differences between Lokiarchaeota, other archaeal species, and Eukarya could support or challenge the missing link hypothesis. Of course, this requires undertaking the technically more challenging isolation of archaeal viruses [10].

Regardless of interpretations, the discovery of Lokiarchaeota inches us closer in the quest to reduce the gap between the sequenced minority and the unknown majority. It also encourages us to unearth unexplored biodiversity, showcasing the many hues of microbial physiology and the complexity of phylogenetic reconstruction. Moreover, missing links in microbial dark matter probably hold clues about the timing of mitochondrial acquisition and the origin of the nucleus, important hallmarks in the origin and evolution of the eukaryotic domain.

References

1 Claverie, J.M. *et al.* (2006) Mimivirus and the emerging concept of 'giant' virus. *Virus Res.* 117, 133–144
2 Spang, A. *et al.* (2015) Complex archaea that bridge the gap between prokaryotes and eukaryotes. *Nature* 521, 173–179
3 Kurland, C. *et al.* (2006) Genomics and the irreducible nature of eukaryote cells. *Science* 312, 1011–1014
4 Woese, C.R. *et al.* (1990) Towards a natural system of organisms: proposal for the domains Archaea, Bacteria, and Eucarya. *Proc. Natl. Acad. Sci. U.S.A.* 87, 4576–4579
5 Wang, M. *et al.* (2007) Reductive evolution of architectural repertoires in proteomes and the birth of the tripartite world. *Genome Res.* 17, 1572–1585
6 Martijn, J. and Ettema, T.J. (2013) From archaeon to eukaryote: the evolutionary dark ages of the eukaryotic cell. *Biochem. Soc. Trans.* 41, 451–457
7 Nasir, A. *et al.* (2015) Untangling the origin of viruses and their impact on cellular evolution. *Ann. N. Y. Acad. Sci.* 1341, 61–74
8 Andreeva, A. *et al.* (2008) Data growth and its impact on the SCOP database: new developments. *Nucleic Acids Res.* 36, D419–D425
9 Forterre, P. (2013) The common ancestor of Archaea and Eukarya was not an archaeon. *Archaea* 2013, 372296
10 Nasir, A. *et al.* (2014) The distribution and impact of viral lineages in domains of life. *Front. Microbiol.* 5, 194

The giant panda gut microbiome

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Giant pandas (*Ailuropoda melanoleuca*) are bamboo specialists that evolved from carnivores. Their gut microbiota probably aids in the digestion of cellulose and this is considered an example of gut microbiota adaptation to a bamboo diet. However, this issue remains unresolved and further functional and compositional studies are needed.

Remarkable progress in microbiological research over the past decade has revealed the critical role of gut microbial symbionts to host immunity, nutrient use, and disease, and genome sequencing has contributed much to this progress. When the working draft of the human genome was completed in 2001, microbiologists quickly suggested using genome sequencing techniques to identify molecular foundations of the commensal relationships between humans and their gastrointestinal bacterial communities [1]. Human gut microbiome diversity was initially described using 16S ribosome RNA gene sequencing [2], and later shotgun sequencing showed that the gut
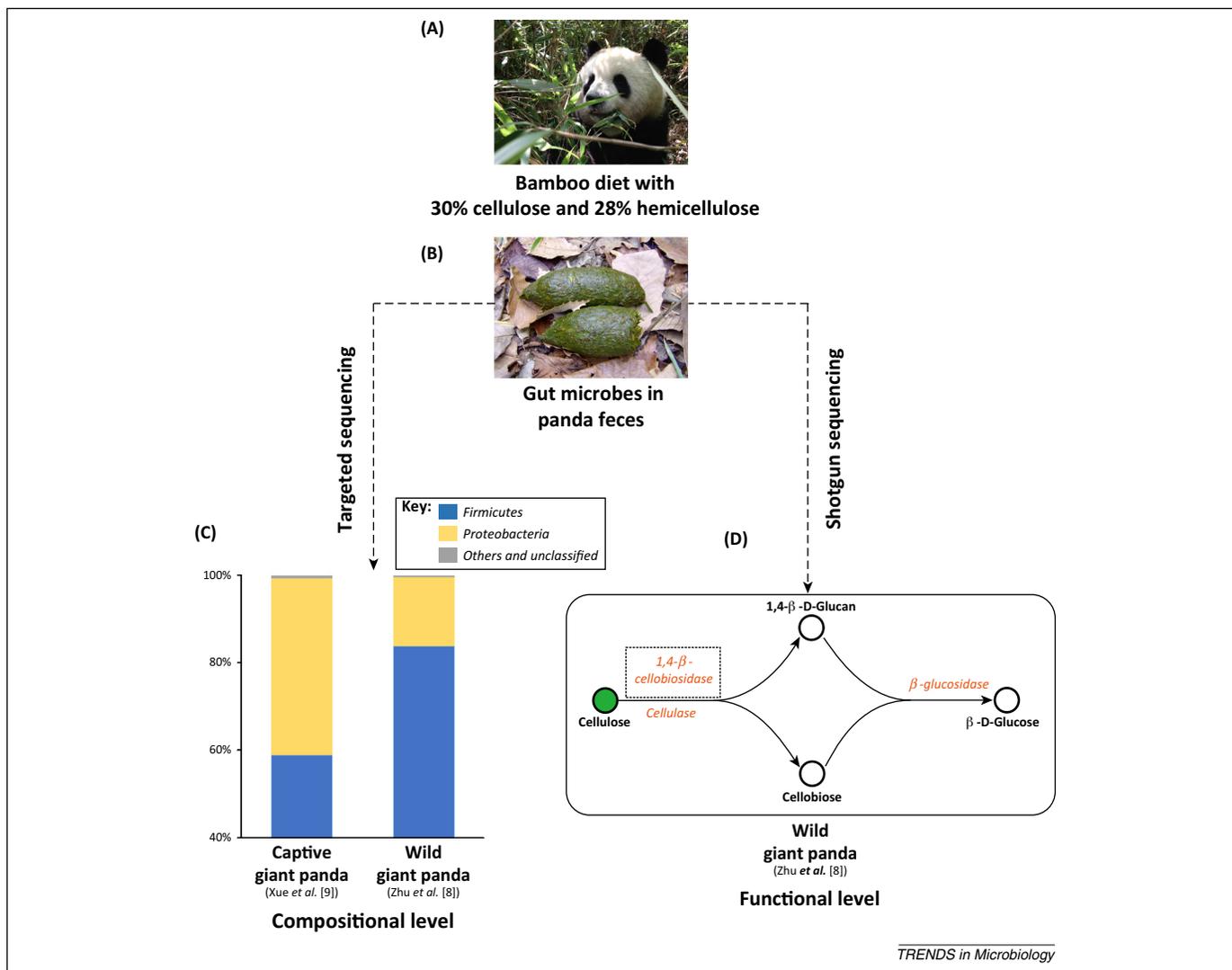
Figure 1. The composition and function of the giant panda gut microbiome. (**A**) The giant panda forages a large amount of bamboo diet daily, which contains high percentage of cellulose and hemicellulose. (**B**) Gut microbe samples collected from fresh feces of giant pandas. (**C**) Targeted sequencing of the 16S ribosome RNA gene reveals the gut microbe community structure of both captive and wild giant pandas [8,9], which indicates markedly different of composition of gut microbiomes of giant pandas living in different environments. (**D**) Pathway of cellulose digestion by the giant panda microbiomes, which indicates that the cellulose can be digested to glucose through cellulase, 1,4-β-cellobiosidase, and β-glucosidase identified in the gut microbiomes of giant pandas with the shotgun sequencing method [8].

microbiome is closely associated with host physiology, health, and disease [3].

These approaches have been now widely used to explore the adaptation and evolution of gut microbiomes of wild animals. For example, 16S ribosome RNA analysis of fecal microbiota of humans and 59 other mammals (including giant pandas) showed that host diet and phylogeny influence bacterial diversity and that this increases as mammals go from being carnivores to omnivores to herbivores [4]. Later, 16S ribosome RNA and shotgun metagenomics from the feces of 33 mammals and 18 people confirmed that the adaptation of microbiota to diet follows similar patterns across different mammalian lineages [5]. Thus, the general rule that microbiota tend to adapt to the diet of hosts at compositional and functional levels was borne, and evidence of this in other mammals is now widespread. However, trying to understand this relationship in giant pandas has been tricky.

The giant panda is interesting because it is a bamboo specialist that evolved from carnivores. Giant pandas have a digestive system typical of carnivores, but have

developed morphological, behavioral, ecological, genetic, and genomic adaptations to their cellulose-rich bamboo diet [6]. Work on its intestinal microbiota can be traced back to the 1980s [7], but these early studies failed to detect cellulose digestion functionality due to the limitation of pure culture techniques. That changed when Zhu *et al.* [8] used 16S ribosome RNA gene and next-generation sequencing metagenomics on feces and uncovered the dominant presence of *Firmicutes*, a phylum of bacteria with putative genes coding for cellulose and hemicellulose-digesting enzymes such as cellulase, β-glucosidase, and xylan 1,4-β-xylosidase (Figure 1). This data also showed that the abundance of oligosaccharide-degrading enzymes in giant panda microbiomes was lower than that in the cow, and the abundance of cellulases and endohemicellulases was the lowest of all known herbivores. Not surprisingly, the gut microbial composition of wild and captive animals was markedly different, and captive animals had a higher proportion of *Proteobacteria* (compared to *Firmicutes*) than wild animals [8].

A recent and large study by Xue *et al*. [9] used 16S ribosome RNA methods to uncover gut symbionts across three different seasons in 45 giant pandas born in captivity. Much variation was found in the diversity and structure of the gut microbiota across seasons and between individuals, and low gut microbial diversity dominated by *Proteobacteria* and *Firmicutes* confirmed the findings of Zhu *et al*. [8] (Figure 1). However, this latest study suggests that the gut microbiota structure in giant pandas deviates from the general pattern in mammals explaining microbiota adaptations to diet [5] and the giant panda gut microbiota may not be well adapted to their highly fibrous bamboo diet. For example, most members of the class Clostridia identified in the giant panda gut microbiota were absent in other herbivores and phylogenetically unrelated with known cellulolytic lineages. This is in contrast to the earlier work by Zhu *et al*. [8] that did identify potential and unique cellulolytic bacteria in the class Clostridia in the gut of wild giant pandas. It is clear that gut microbial composition data alone cannot resolve whether gut microbes of giant pandas aid in the digestion of cellulose and hemicellulose and are an adaptation to a bamboo diet, and metagenomics and meta-transcriptome analysis may be the way forward.

The giant panda is a member of a family of carnivores and its microbial composition more closely resembles that of other carnivores [4] even though it may possess special gut bacteria as a consequence of its bamboo diet [8]. Host phylogeny is known to influence gut microbes [4] and so it is not surprising that giant pandas have different gut microbes compared to other herbivores. Giant pandas digest 27% of the hemicelluloses and 8% of the celluloses in their bamboo diet [10]; the gut microbes of wild and captive giant pandas may play the same role in cellulose and hemicellulose digestion and reflect evolutionary adaptation to host diet, although captive and wild environments are very different.

In summary, recent research has provided a preliminary understanding of the composition and function of the giant panda gut microbiome and differences between wild and captive animals. However, to better reflect natural characteristics of the microbiome of giant pandas, future work will need to be based on wild animals and metagenomics and meta-transcriptome approaches, especially when attempting to address unresolved aspects of this system. The next challenge is uncovering direct evidence of the digestion of cellulous and hemicellulose by giant panda gut microbes. The second is to know how microbes develop over time, which is made difficult by the need for sufficient fecal samples from wild cubs and mothers. The third is to document microbiota seasonal fluctuations and functions, and then to see how this changes in providing nutrients to wild giant pandas. The last challenge, but by no means least, is to map interactions between gut microbes and disease in this endangered species.

References

1 Hooper, L.V. *et al*. (2001) Commensal host–bacterial relationships in the gut. *Science* 292, 1115–1118
2 Eckburg, P.B. *et al*. (2005) Diversity of the human intestinal microbial flora. *Science* 308, 1635–1638
3 Turnbaugh, P.J. *et al*. (2006) An obesity associated gut microbiome with increased capacity for energy harvest. *Nature* 444, 1027–1031
4 Ley, R.E. *et al*. (2008) Evolution of mammals and their gut microbes. *Science* 320, 1647–1651
5 Muegge, B.D. *et al*. (2011) Diet drives convergence in gut microbiome functions across mammalian phylogeny and within humans. *Science* 332, 970–974
6 Wei, F.W. *et al*. (2015) Giant pandas are not an evolutionary cul-de-sac: evidence from multidisciplinary research. *Mol. Biol. Evol.* 32, 4–12
7 Hirayama, K. *et al*. (1989) The faecal flora of the giant panda (*Ailuropoda melanoleuca*). *J. Appl. Bacteriol.* 67, 411–415
8 Zhu, L.F. *et al*. (2011) Evidence of cellulose metabolism by the giant panda gut microbiome. *Proc. Natl. Acad. Sci. U.S.A.* 108, 43 17714–17719
9 Xue, Z.S. *et al*. (2015) The bamboo-eating giant panda harbors a carnivore-like gut microbiota, with excessive seasonal variations. *Mbio* 6, e00022-15
10 Dierenfeld, E.S. *et al*. (1982) Utilization of bamboo by the giant panda. *J. Nutr.* 112, 636–641

Entry and exit of bacterial outer membrane proteins

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The sites of new outer membrane protein (OMP) deposition and the fate of pre-existing OMPs are still enigmatic despite numerous concerted efforts. Rassam *et al*. identified mid-cell regions as the primary entry points for new OMP insertion in clusters, driving the pre-existing OMP clusters towards cell poles for long-term storage.

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Scientists have long sought to determine how the outer membranes of Gram-negative bacteria develop from new materials and what happens to the old membrane. To analyze outer membrane proteins (OMPs), the experimental setup has frequently involved transient production of OMPs by means of employing a temperature sensitive allele [1], adding an operon-specific transcriptional inducer for a short period of time [2], or growing cells in media that either repressed or promoted the production of a major OMP [3]. The detection of newly-arrived OMPs on the cell surface was aided by OMP-specific bacteriophages [1,2] or ferritin-conjugated OMP-specific antibodies [3], and