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Lateral gene transfer mechanisms and pan-genomes in eukaryotes

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Abstract

Lateral gene transfer (LGT) is well known as an important driver of genome evolution in bacteria and archaea, but its importance in eukaryote evolution has yet to be fully elucidated. There is now abundant evidence indicating that LGT has played a role in the adaptation of eukaryotes to new environments and conditions, including host-parasite interactions. However, the mechanisms and frequency of LGT across the tree of eukaryotes remain poorly understood. Here we review evidence for known and potential mechanisms of LGT into diverse eukaryote lineages with a particular focus on protists, and discuss trends emerging from recently reported examples. We also explore the potential role of LGT in generating ‘pan-genomes’ in diverse eukaryotic species.

The role and prevalence of lateral gene transfer in genome evolution

Lateral (or horizontal) gene transfer (LGT or HGT) is an important mechanism by which genetic material is inherited in a non-vertical fashion. In bacteria, DNA is transmitted horizontally by **transduction, transformation** and **conjugation** (see Glossary) (e.g., [1]). Although the frequency of LGT varies greatly amongst taxa, these processes are largely responsible for gene content variation between and within prokaryotic species. Indeed, the total gene content of a given species – the **pan-genome** – can exceed the number of ‘core’ genes shared between all strains of that species by an order of magnitude or more; those accessory genes are commonly acquired by LGT (e.g., [2] and references therein). It remains unclear what proportion of the genes found in some but not all strains of a bacterial species are adaptive versus neutral or deleterious [3,4], but there is no doubt that LGT has a major role in prokaryote genome evolution.

By contrast, the prevalence, mechanisms and roles of LGT in eukaryote evolution are poorly understood. Putative LGTs are increasingly reported as the number of comparative genomic analyses of diverse unicellular and multicellular eukaryotes rapidly grows (Box 1). It is generally accepted that LGT likely occurs less frequently in eukaryotes relative to most prokaryotes (e.g., [5]), but this does not mean that it is an unimportant mechanism in eukaryotic genome evolution [6]. Laterally acquired genes can provide eukaryotic organisms with new functions that impact their biology and evolutionary trajectory (e.g., [7]); LGT has been suggested to play a role in the adaptation of eukaryotes to ‘extreme’ conditions such as high temperature (e.g., [8]), high salt (e.g., [9]), and anaerobiosis (e.g., [10, 11]). LGT also appears to have contributed to the evolution of parasitism (e.g., [12] and references therein, [13,14]) and in the reversal from a parasitic to a free-living lifestyle (e.g., [15]).

How do eukaryotes acquire genes? Numerous potential sources of foreign DNA have been suggested (Figure 1) but the evidence supporting their involvement in LGT varies. Some phenomena, like **endosymbiotic gene transfer** (EGT), are well supported by evidence. Others, such as inter-domain conjugation or natural transformation, have been documented in the lab [16] but their frequencies and relevance in nature is unknown. Regardless of the source, a ‘successful’ LGT must involve several steps including: (i) the introduction of foreign DNA (or RNA) into the recipient eukaryotic cell; (ii) translocation of the DNA to the nucleus; and (iii) incorporation of the DNA into one or more chromosomes, either accidentally by host DNA repair and recombination pathways, or by an active integration mechanism. For transferred DNA to be faithfully inherited, it must be stably integrated in all sister chromosomes and, for multicellular eukaryotes, must exist in the germline. To persist over longer time periods, newly acquired DNA must be fixed in the population by neutral drift and/or positive natural selection. If the transferred genes are expressed and provide a function that is useful (e.g., a novel adaptive trait, or compensation for the loss of a pre-existing gene), they can be maintained by purifying selection. Otherwise, further mutations may erode these genes into pseudogenes (e.g., [17]) and they will eventually be lost or become unrecognizable intergenic DNA. Clearly the likelihood of any given LGT event being successful is multifactorial, and a number of factors potentially influence the frequency of LGT in any given species (Box 2).

Here we review evidence for several means by which foreign DNA is introduced into eukaryotic cells and potential mechanisms that integrate such DNA into eukaryotic genomes. We also discuss aspects of genome biology that may influence the rate of LGT and discuss its importance to adaptive evolution in eukaryotes by acting as a ‘catalyst’ for the acquisition of niche-specific traits.

Viruses and their hosts exchange genetic material

Virus-mediated transduction has long been recognized as an integral part of the biology of prokaryotic cells. A growing body of literature supports the notion that viruses can act as vectors of LGT from prokaryotes to eukaryotes and between eukaryotes (reviewed in [18]). The nature of viral transmission (horizontal), replication (inside the host cell) and survival strategies (e.g., integration into the host genome) together lead to intimate associations with their host's genetic material, providing ample opportunity for gene exchange. At least some viruses can infect a wide range of hosts and/or change hosts (e.g., [19]) and some eukaryotes harbour multiple divergent viruses, each of which may enable gene exchange beyond species boundaries. The virus-mediated export of nuclear DNA in plants was recently documented in real time by Catoni et al. [20], where *Beta vulgaris* chromosomal DNA integrated into the viral genome of a single-stranded DNA virus during infection was encapsidated into viral particles and spread throughout the plant population via subsequent viral infections. Notably, the *B. vulgaris* DNA present in the hybrid minicircles was replicated and transcribed in the newly infected host.

It is well established that host genes can be integrated into a viral genome and persist, particularly in double stranded DNA (dsDNA) viruses that infect a variety of eukaryotes. In the case of nucleocytoplasmic large DNA viruses, host DNA can make up a substantial portion of the virus' genome (e.g., [21]). A recent study of the giant DNA virus of the green alga *Tetraselmis*, TetV, identified 110 protein coding genes (17% of its protein coding genes) that appeared to be acquired from eukaryotes, including many specifically of Viridiplantae origin [21]. At least four of the eukaryotic homologs identified in TetV were novel viral genes that suggested recent incorporation of genes from both its host (*Tetraselmis*) and other unicellular eukar-

yotes that likely allow manipulation of the host fermentation pathways during anaerobic conditions. Giant virus genomes are highly mosaic – not only do they acquire genes from their eukaryotic host(s), they also pick up genes from bacteria and other viruses present within the host cell (e.g., [22]).

The fact that genes of host origin exist in viral genomes implies that host DNA is regularly introduced into viral genomes with varying consequences on fitness; while some genes are fixed, others presumably persist temporarily over a limited number of replication cycles. Short-lived insertions, however, could still allow the foreign genes to be transferred to a new host. This was shown experimentally by Gilbert et al. [23], who investigated the frequency of gene transfer in 21 baculovirus (AcMNPV) populations infecting two moth species. Around 5% of AcMNPVs were found to harbor at least one moth-derived DNA sequence at a given time, all of which persisted only temporarily. There is nevertheless evidence for a continuous flow of genetic material from the host to AcMNPV and for repeated transfers of the short-lived insertions from the viral genome into the one of the various insects they infect. Host DNA was predominately inserted into viral genomes via transposition. However, there is also evidence for a variety of microhomology-mediated and homology-independent recombination events at double stranded breaks. Whether these recombination events were mediated by host or viral factors, or some combination of the two is currently unknown, but Gilbert et al. [23] speculate that as long as there is a double stranded break, viral and host DNA have the potential to be joined.

A large number of viral genomes and genome fragments from a wide range of both retroviruses and other viruses have been found in eukaryote genomes, including those of multicellular organisms (e.g., [24-26]). In order to replicate, retroviruses become endogenized, i.e., integrated in their hosts genome via an autonomous mechanism using enzymatic machinery they encode.

Beyond retroviruses, it has been suggested that viral DNA is inserted via accidental chromosomal integration at double stranded DNA (dsDNA) breaks through homology mediated recombination, non-homologous end joining repair, or a hybrid mechanism that utilizes the enzymatic machinery of retrotransposons [24]. For example, inactive remnants of endogenous florendoviruses (non-retroviral viruses that lack their own integrases) found across a broad diversity of plant genomes are frequently inserted in transposable element (TE)-rich regions, specifically at TA dinucleotide simple repeats [27]. These repeats are thought to give rise to fragile chromosome regions, making them more prone to dsDNA breaks – breaks that create potential integration sites for foreign DNA.

Further clear evidence for the flux of genetic material from virus to host and *vice versa* has been reported in various eukaryotes. Substantial gene exchange is particularly evident between the amoebozoan *Acanthamoeba castellanii* and its large DNA viruses [28]. 267 genes of probable viral origin were identified in the genome of *A. castellanii*, many of which were transcriptionally active and clearly integrated in the nuclear genome. Phylogenetic analyses also detected a kinase gene that appears to have been transferred on multiple occasions – at least once from eukaryotes to viruses and at least twice subsequently from viruses to amoebae [28]. Recently, experimental evidence suggested that two densovirus genes transferred to the nuclear genome of pea aphids from a densovirus that infects rosy apple aphids are responsible for their ability to produce winged offspring during population crowding, a phenotype with clear adaptive benefit [29]. Similarly, endogenous chromosomally-transmitted bracoviruses in parasitic wasps produce dsDNA circles encoding virulence genes that are injected into host caterpillars, integrate into their genomes and are expressed suppressing the immune response allowing the wasp larvae to survive in the lepidopteran body. These endogenous viruses appear to have been vectors for

transfer of C-type lectin genes from the wasps to lepidopterans that have been domesticated by the latter and allow them to resist baculovirus infection [30].

Transposable elements—agents of intra- and inter-genomic mobility

Transposable elements (TEs) have an intrinsic ability to move around within a genome, which may make it easier for them to be transferred to other genomes. Numerous examples of laterally transferred TEs have been reported in animals, plants, insects and fungi (e.g., [31–34]). A large-scale survey of TEs in 195 diverse insect genomes identified 2,248 lateral transfers of TEs, constituting 2–24% of a given insect genome [33]. Reiss et al. [34] recently extended this survey to screen 460 insect genomes for patterns of TE transfer. They concluded that DNA transposons were the most frequently transferred TE class in arthropods, followed closely by Long Tandem Repeats (LTRs), and that there was a clear excess of TE transfer in species hosting baculoviruses.

With the exception of the studies mentioned above, large-scale surveys of TEs in eukaryotes are generally lacking, especially in protists, making it difficult to make general conclusions about the extent of inter-genomic TE mobility and its global impact on eukaryotic genome evolution. Regardless, studies looking at the rate of TE transfer have found that geographic proximity as well as genetic distance between the host and recipient lineage impact the rate of LGT (e.g., [33]). Some of the most abundant retrotransposon families in eukaryotes were suggested to have been transferred multiple times between aquatic organisms (L1) and between animals (BovB) via various parasite vectors [35]. Some viral genomes contain TEs acquired from eukaryotes (e.g. [23,36]), including the widespread invasion of miniature inverted-repeat TEs found in high copy numbers in viruses that use host machinery to transpose themselves [37]. Several TEs have been

shown to produce extrachromosomal circular forms during transposition [38], which if encapsidated in a viral particle may be more likely to undergo virus-mediated LGT than linear DNA [18].

Under stress conditions, LTR retrotransposons are activated and generate double-stranded breaks in DNA (e.g., [39]); this could potentially facilitate integration of foreign DNA at these breakpoints via normal DNA repair pathways. Furthermore, imperfect excision of a TE from a chromosome, as has been observed in bacteria [40], can result in large adjacent portions of the genome accidentally being carried along with a TE during transposition or horizontal transfer. Due to the ability of TEs to capture and transduce sequences at a high frequency, it has been suggested that horizontal TE transfer could be responsible for the direct movement of genes among eukaryotes as well [41]. Consistent with this hypothesis, transferred genes are often associated with TE-rich regions in metazoan genomes [14]. The transfer of a wheat virulence protein (*ToxA*) between three fungal wheat pathogens in two independent events was likely facilitated by a type II DNA transposon [42]. Long-read sequencing based genome assemblies for several of these fungi showed that the genomic context of the two *ToxA* LGTs included 14 kbp of both coding and non-coding DNA, including conserved terminal inverted repeats bearing strong resemblance to features of type II DNA transposons, suggesting LGT and lateral TE transfer can occur simultaneously [43]. Altogether, this strongly suggests that TEs are not only transferred laterally, but that they also can facilitate the movement of other genes between species.

Endosymbiosis and gene transfer

A large variety of eukaryotes harbour prokaryotic and/or eukaryotic **endosymbionts** exhibiting varying degrees of cellular and genomic integration. In a number of cases (e.g., mito-

chondria and plastids), the endosymbionts have co-evolved with the host to the point where they have become permanent, semi-autonomous organelles. In any case, the close proximity of the host nucleus and endosymbionts or endosymbiont-derived organelles provides an opportunity for the movement of genetic material [43,44]. Endosymbiont-to-host nucleus gene transfers played an important role in the evolution of mitochondria and plastids, a process specifically referred to as endosymbiotic gene transfer (EGT) [45,46]. In these instances, if the transfer of genes from the endosymbiont-derived organelle to host is followed by the retargeting of the gene product to the organelle, the copy of the gene in the organellar genome can be lost. In the fullness of time, the result of EGT is the existence of complementary gene inventories and coordinated metabolisms in host and endosymbiont-derived compartments [10,44]. The substrate for EGT is thought to be organelle lysis; the frequency of EGT over short evolutionary timescales appears to be positively correlated with the number of organelles per cell (see [16,47] and references therein).

The extent to which bacteria other than the endosymbiotic progenitors of mitochondria and plastids contributed to organelle establishment remains controversial (e.g., [10, 48–50]). In the case of plastids, phylogenetic evidence suggests that LGTs from diverse bacteria – not just cyanobacteria – may have facilitate organelle evolution (e.g., [51]). Beyond canonical plastids, the contribution of LGT to the recently evolved ‘chromatophores’ in the rhizarian amoeba *Paulinella chromatophora* is clear. Sequencing of the *P. chromatophora* genome revealed 228 nuclear genes of various bacterial affinities, only ~25% of which appear to come from the cyanobacterium-derived chromatophore [52]. The suite of EGTs and LGTs, along with native host nuclear genes, contribute to many essential biosynthetic pathways partially encoded by the substantially reduced chromatophore genome, and encode transporters facilitating host-chromatophore metabolite exchange [52,53]. The phagotrophic nature of the ancestors of *P.*

chromatophora likely played an important role in sourcing these various bacterial genes, driving LGT and, ultimately, chromatophore integration.

Various other examples of endosymbiosis-associated gene transfers have also been documented. Some of these transfers are directly from endosymbionts to host, while others seem to be LGTs from ‘third party’ organisms that allow endosymbionts to lose the corresponding genes. The latter is exemplified by the mealybug *Planococcus citri*, in which the loss of enzymes for synthesizing eight amino acids in its endosymbiont *Tremblaya* were made possible by >20 LGTs from various bacteria to the *P. citri* nuclear genome [54]; a related mealybug harbors a less genomically streamlined *Tremblaya* endosymbiont that still encodes all the enzymes necessary for the synthesis of these amino acids. This endosymbiosis actually involves a tripartite relationship between *P. citri* and two bacteria – a betaproteobacterium (*Tremblaya*) and its gammaproteobacterial endosymbiont *Moranella* – which exhibit complementary gene losses and are themselves metabolically intertwined [54]. Peptidoglycan biosynthesis is just one pathway that appears to be a mosaic of *P. citri* encoded LGTs and endosymbiont encoded genes. Using a variety of techniques including Nanoscale Secondary Ion Mass Spectrometry (NanoSIMS), Bublitz et al. [55] recently showed that the laterally acquired genes for peptidoglycan synthesis are translated in the cytoplasm of *P. citri* and the resulting proteins are transported across multiple membranes to the *Moranella* endosymbiont where peptidoglycan is assembled. These results clearly show that LGT can give rise to stable, complex, mosaic biochemical pathways in endosymbioses outside the bounds of mitochondria and plastids.

Several species of single-celled Foraminifera harbor intracellular bacteria capable of denitrification – a process widespread in prokaryotes but not in eukaryotes, which allows for the storage and use of nitrate in energy metabolism [56]. Recently, a novel denitrification pathway

was identified in the genome of the foraminiferan *Globulimina* where two of the key enzymes are clearly of prokaryotic origin and likely originated from the denitrifying endosymbiotic bacteria [57]. In the ctenophore *Mnemopsis leidyi*, various LGTs have been suggested to be a result of the association between these enigmatic invertebrates with various bacteria and protists such as amoebae [58]. Numerous studies have shown a variety of LGTs from *Wolbachia* endosymbionts into various insect and nematode hosts ([59] and references therein), ranging from smaller insertions to genome-sized fragments. These are just a few of many examples illustrating that endosymbionts contribute DNA to their hosts and that gene transfers from various sources during and/or prior to the establishment of a permanent endosymbiont can influence host-endosymbiont integration. The fact that EGTs have occurred in diverse lineages across the eukaryotic tree shows that there are few – if any – barriers to the uptake and integration of foreign DNA into eukaryote nuclear genomes.

“You are what you eat”

Many protists feed on other organisms via phagocytosis. Doolittle’s ‘you are what you eat’ hypothesis [60] posits that both prokaryotic and eukaryotic prey could act as sources of new genes simply as a by-product of digestive vacuole rupture and release of nucleic acids. In this respect, the overall mechanism of gene transfer from phagocytosed prey is similar to gene acquisition from endosymbionts. Even if food vacuole or symbiont rupture is a very rare occurrence, both generate free DNA that could enter the nucleus and be incorporated into the nuclear genome at a low frequency. Such sequences will accumulate mutations and may be deleted. But if the gene within the inserted DNA happens to be expressed, it could be fixed in the population by drift or, if some advantage is conferred, positive selection. Because of their overall similarity in

mechanism and outcome, it is difficult if not impossible to determine if gene transfer events originated from transient ancestral endosymbionts or phagocytosed food. A number of potential examples of the latter have nevertheless been suggested.

Adaptive gene transfers of bacterial origin in the bacterivorous protist *Halocafeteria seosinensis* allow it to produce an osmoprotectant that relieves significant osmotic stress found in its hypersaline habitat. This, in combination with other LGTs from bacteria, likely facilitated the adaptation of *H. seosinensis* to a high salt environment [9]. In the anaerobic oxymonad *Monocercomonoides*, a laterally acquired SUF-system from a bacterial donor (with genes verified to be in the nucleus by fluorescent in situ hybridization) is likely linked to the ability of *Monocercomonoides* to ingest and digest bacterial prey [61]. This laterally acquired pathway allows for iron-sulfur cluster assembly to take place in the cytosol rather than *via* the typical ISC pathway in mitochondrion, ultimately making the last essential function of *Monocercomonoides*' mitochondria-related-organelle redundant and dispensable [61]. Eukaryovores are relatively rare compared to bacterivorous protists, which may contribute to apparent differences in the frequency of gene transfer between eukaryotes and between eukaryotes and prokaryotes [62]. However, these differences may also reflect the difficulties associated with confidently identifying eukaryote-to-eukaryote gene transfer events (Box 3).

Parasites as LGT recipients and vectors

LGT has played an important role in the adaptation of many successful parasite lineages to their host and interactions with other host-associated microbes. Cases of adaptive LGT from prokaryotes to a variety of unicellular and multicellular parasitic eukaryotes have been well documented (e.g., [7,12]). For example, LGTs have been reported in diplomonad protozoa (e.g.,

Spiroplasma and *Giardia intestinalis* (e.g., [63]), *Plasmodium*, *Toxoplasma*, *Leishmania*,
Trichomonas and *Entamoeba* [12,64] as well as parasitic nematodes (e.g., [14, 65]).

Several trends in the patterns of laterally transferred genes into parasitic lineages have
been noted. For example, there is an excess of genes received from organisms that live in the
same ‘host’ environment as the parasites (e.g., [12, 64]). Furthermore, the functions of the ac-
quired genes tend to reflect the lifestyles of the different parasite types. For example, the muco-
sal parasite *Trichomonas vaginalis* has acquired genes related to harvesting glycans from host
epithelial tissues and secretions (Box 4). A recent analysis of the anaerobic gut mucosal parasite
Blastocystis sp. is consistent with these observations. 2.5% of the protein-coding genes in this
organism were acquired laterally from a wide range of both prokaryotes and eukaryotes, allow-
ing for successful host infection, evasion of host immune systems, modulation of other gut mi-
crobes (e.g., via production of indole and polyketides) and metabolic adaptation to the gut envi-
ronment [13]. Several of the LGTs in *Blastocystis* appear to have come directly from animal
hosts. Others appear to have been transferred multiple times between this organism and other
mucosal protists (e.g., *Trichomonas*, *Giardia* and *Entamoeba*), suggesting the potential existence
of a pool of adaptive genes shared between anaerobic mucosal parasites. Numerous other strong
examples of LGT facilitating parasite adaptation have been reported across the eukaryotic tree.
For example, Dean et al. [66] used a combination of ancestral gene reconstruction and functional
assays to show that the acquisition of a single nucleotide transporter from bacteria to the com-
mon ancestor of *Microsporidia* species allowed them to become energy parasites that steal host
ATP, GTP and NAD⁺ and subsequently lose the capacity to synthesize nucleotides on their own.

In phytoparasitic nematodes, functionally important genes encoding enzymes involved in
plant cell-wall degradation and related metabolism were identified as LGTs from bacteria that

are plant pathogens of the same hosts, as well as from a variety of eukaryotes (including the plant pathogen *Phytophthora infestans*) [14]. Interestingly, LGTs in these nematodes are often found in TE regions of the genome, and the bacterial homologs are found on plasmids, suggesting a mechanistic role for mobile genetic elements in LGT. Another example of host-parasite mediated LGT is the acquisition of mammalian TE families by the blood-sucking insect *Rhodnius prolixus* and the pond snail *Lymnaea stagnalis*. These TEs had extremely high sequence identities to TEs in the genomes of host mammals of the insect and trematode parasites for which the snail acts as an intermediate host [67], implicating host-parasite interactions in horizontal transmission of the TEs. Based on the foregoing examples, it seems likely that parasites that infect a wide range of hosts or species that exist in general symbiotic associations could be particularly potent vectors for LGT.

Conjugation and natural transformation in eukaryotes

A wide variety of eukaryotes have been transformed in the lab. While artificial in nature, such experiments indicate that there is no fundamental obstruction to LGT when barriers to transfer are intentionally removed. Established eukaryotic transformation systems are frequently exploited for commercial applications such as the production of genetically modified crops. For example, *Agrobacterium tumefaciens*, which uses a type IV secretion system to inject a tumor inducing (Ti) plasmid carrying specific genes into plant cells, has been used to modify plant species to confer pest resistance [68]. Once in the host cell, the Ti plasmid utilizes the plants machinery to enter the nucleus and integrate into the genome, using eukaryotic promoters to express its genes. Importantly, there is evidence that this transformation occurs naturally in plants as well. Genes from *Agrobacterium* have been found to reside and be expressed in the germ line of

various plant species that have not been artificially transformed (e.g., [69,70]), suggesting that these gene transfers are maintained and functional.

Recently a 100% efficient transformation system was developed in the red alga *Porphyridium purpureum* where the transformation vectors are maintained episomally as extrachromosomal high-copy number plasmids [71]. This shows that different bacterial origins of replication can function in a eukaryote system and result in stable, autonomous replication within the nucleus. Naturally occurring extrachromosomal plasmids of unknown origin have been found in some red algae; these plasmids contain both bacterial and viral ORFs (e.g., [72]). The apparent genetic compatibility between plasmid replication systems of bacteria and components of DNA replication machinery in the red algal nucleus [73] raises intriguing questions about algal genome evolution. The extended presence and replication of a nuclear episomal element could provide opportunities for gene transfer to the nuclear genome and may have facilitated the contribution of bacterial genes to red algae (estimated to be between 1% and 9.3% of the total gene content, depending on the lineage [73,74]).

Interestingly, a conjugation-based transformation system capable of delivering episomal vectors from *E. coli* has been developed for the diatoms *Phaeodactylum tricornutum* and *Thalassiosira pseudonana* [75]. Although direct conjugation has not been observed in nature, this system suggests that bacterial conjugation may be one mechanism that could explain the high percentage of putative bacterial-derived genes (5-10% of genes) in diatom genomes [76]. The transfer of plasmids via bacterial conjugation has been observed in yeast [77], as has the natural transformation of exogenous DNA under non-artificial conditions [78].

Difficulties in detecting LGT in eukaryotes

Eukaryotes typically have relatively large and complex genomes compared to prokaryotes, making genome sequencing, assembly and analysis difficult. The paucity of high-quality genome data from diverse eukaryotes, especially from closely related species and multiple strains of the same species, is a present barrier to the study of LGT [79]. In regions of the eukaryotic tree in which there is denser genomic sampling of lineages, the extent of adaptive LGT is becoming clearer. For example, Ropars et al. [80] analyzed 10 high-quality genomes from *Penicillium* in the context of genome data from 416 strains of 65 fungal species. The authors confidently identified 104 LGTs between *Penicillium* species, including some large recently transferred genomic regions spanning more than 10 kb, some of which show signatures of TE mediated insertion [80]. Gene expression analysis and fitness experiments confirmed that at least some of the LGTs encode functional genes that provide metabolic adaptations to a cheese environment (e.g. lactose metabolism) or antifungal proteins used to out-compete other fungal species [80]. Until a similar density of high-quality genomic sampling is available for other branches of the eukaryote tree— particularly for microbial eukaryote lineages – it is impossible to know whether the dynamic nature of fungal genomes is the exception or the rule.

Distinguishing true LGTs from contamination (see 81) can be difficult and mitigated by careful inspection of candidate genes (Box 3). Many (but by no means all) published studies have used appropriately stringent phylogenetic criteria for reporting LGT. Initial bioinformatic steps generally lean towards the exclusion of bacterial/symbiont sequences from assemblies, which means that the impact of LGT to a given genome can be underestimated. Additional difficulties arise when trying to determine the scale of how far back in evolutionary time one has to go to consider a gene as having been vertically or laterally transferred. More ancient LGTs are harder to detect as the sequence similarity of a transferred gene to the gene of the donor lineage

decays over time. At the same time, confidently detecting eukaryote-eukaryote LGT is generally more difficult than prokaryote-eukaryote LGT due to the more recent common ancestry shared between donor and recipient, resulting in less obvious phylogenetic incongruence. A ‘patchy’ gene distribution across the eukaryote tree is often interpreted as evidence of LGT between eukaryotic lineages. However, secondary loss of ancestral genes is well known to have occurred in parallel in many eukaryotic lineages (e.g. genes in mitochondrial genomes, reviewed in [10]) and is sometimes an equally plausible alternative explanation for the gene distribution pattern. Therefore, strongly supported single gene trees that contradict the expected species tree relationships are needed to support suspected LGT cases. This standard is sometimes challenging to achieve due to the limited phylogenetic signal within many single gene alignments. Identifying gene transfers in eukaryotes also requires a sufficient level of taxonomic sampling of the given gene of interest, which is often lacking. Strategic sequencing of diverse eukaryote lineages will make LGT detection easier in the future.

LGT and pan-genomes in eukaryotes

In prokaryotes, within-species variation in gene content is such that the core genome (i.e., gene families found across all or most strains) represents 3-84% of the total gene families found in all sequenced strains of a given species (reviewed by [3]). The accessory genome includes genes found in a single or subset of strains (Figure 2). This pan-genome pattern is due to the combined effects of LGT and differential gene loss.

The extent to which a pan-genome-like pattern is observed in various eukaryote lineages – and, in particular, protists – is less clear, as inferences are limited by the paucity of genomes that cover the breadth of diversity for a given species outside of animals, plants and fungi [79].

Another problem is the difficulty in obtaining accurate and comprehensive gene predictions in eukaryotes, as gene prediction is much more difficult than in prokaryotes because of the presence of spliceosomal introns and alternative splicing. As a result, potential pan-genome patterns must be cross-checked to be certain they are not simply artefacts of failure to predict existing genes. Nevertheless, across various model fungal species where a dozen or more genomes have been sequenced, pan-genomes have been observed with core genomes ranging from 80-90% of the species coding capacity [e.g., 82 and references therein]. Some of the accessory genes are inferred to be a result of LGT; others appear to be a consequence of gene duplication, pseudogenization and outright gene loss. In plants, the accessory genome component of the species pan-genome seems to be more extensive (up to ~47% of the pan-genome). For example, across >3,000 genomes of *Oryza sativa* L., 46.5% of the pan-genome was found to be accessory genes, most of which are related to immune and defense responses [83].

In the world of protists and algae, fascinating insight into the eukaryotic pan-genome has come from investigation of the coccolithophorid *Emiliania huxleyi* [84]. In analyzing the genomes of 14 highly-similar strains of *E. huxleyi* (>98% 18S ribosomal DNA (rDNA) identity), Read et al. [84] found a core genome (experimentally confirmed via microarrays) comprised of only 69.5% of the total gene set. While Read et al. were unable to confidently assign LGTs from bacteria or viruses as the main source of the accessory genes, they did find that *E. huxleyi* genomes contain a lot of repetitive elements, which may foster accessory gene formation and serve as preferential target sites for LGT.

As with prokaryotes, there are serious problems in defining the ‘pan-genome’ because it is confounded with how species are defined and delimited. For example, Worden et al. [85] found significant gene content differences between two isolates of *Micromonas* that were origi-

nally considered the same species on the basis of morphology and 18S rDNA similarity. The fact that these two isolates were found to share only 90% of their genes was interpreted to mean that they were different species. Yet, an alternative interpretation is that these two isolates are members of the same pan-genome-containing species. In the absence of evidence for sexual recombination between individuals of a putative species – information that is lacking for the majority of protists – defining a species is difficult. Without a clear species definition for most microbial eukaryotes, it is difficult to distinguish between these two scenarios.

Concluding Remarks

The past few years have seen a rapid increase in the sequencing of complete genomes from diverse eukaryotes and, concordantly, abundant evidence for LGT into eukaryotic genomes. Many questions nevertheless remain unanswered (see Outstanding Questions). Indeed, for most documented cases that are not obviously EGTs, the means by which the transferred DNA was introduced into cell and incorporated into the chromosomes remain unclear. Many of the mechanisms that could have been involved, and their relative importance, are ‘known unknowns’. For example, endosymbiont- or ‘you-are-what-you-eat’ mediated transfers are probably much more common in eukaryotes that commonly harbor endosymbionts and phagocytose prey, respectively. On the other hand, virus-mediated transfer will depend on the number and types of viruses that infect particular lineages and the degree to which they can integrate into host genomes and mobilize host DNA. To address these questions the following data are needed. First, a greater density of sampling of eukaryotic genomes from each of the major eukaryotic groups should allow us to ‘catch’ more very recent transfer events that may still bear the hallmarks of their origins (e.g., flanking TEs, viral genes, breakpoints of recombination events etc.). This should also

allow us to assess the extent to which pan-genomes are a common feature of diverse eukaryote species, assuming we can get reliable information about how such species are delimited (i.e., evidence for recombination within species). Our understanding of eukaryote LGT will also be greatly enhanced by the development of new model systems and long-term laboratory evolution experiments that allow the process of LGT to be studied in real time. Ideally, organisms from different branches of the eukaryotic tree should be developed as model systems and the various insights they yield considered in isolation and together. Long-term progress in our understanding of the frequency and significance of LGT in eukaryotes will rely on the synthesis of information gleaned from both experimental and bioinformatic approaches.

References

- 1 Soucy, S. et al. (2015) Horizontal gene transfer: building the web of life. *Nat. Rev. Genet.* 16, 472–482
- 2 Land, M. et al. (2015) Insights from 20 years of bacterial genome sequencing. *Funct. Integr. Genomics* 15, 141–161
- 3 McInerney, J.O. et al. (2017) Why prokaryotes have pangenomes. *Nat. Microbiol.* 2, 17040
- 4 Vos, M. and Eyre-Walker, A. (2017) Are pangenomes adaptive or not? *Nat. Microbiol.* 2, 1576–1576
- 5 Danchin, E.G. (2016) Lateral gene transfer in eukaryotes: tip of the iceberg or of the ice cube? *BMC Biol.* 14, 101
- 6 Leger, M.M. et al. (2018) Demystifying eukaryote lateral gene transfer. *BioEssays* 40, 1700242
- 7 Lukeš, J. and Husník, F. (2018) Microsporidia: a single horizontal gene transfer drives a great leap forward. *Curr. Biol.* 28, R712–R715
- 8 Schönknecht, G. et al. (2013) Gene transfer from bacteria and archaea facilitated evolution of

- an extremophilic eukaryote. *Science* 339, 1207–1210
- 9 Harding, T. et al. (2017) Adaptations to high salt in a halophilic protist: differential expression and gene acquisitions through duplications and gene transfers. *Front. Microbiol.* 8, 944
- 10 Roger, A.J. et al. (2017) The origin and diversification of mitochondria. *Curr. Biol.* 27, R1177–R1192
- 11 Stairs, C.W. et al. (2018) Microbial eukaryotes have adapted to hypoxia by horizontal acquisitions of a gene involved in rhodoquinone biosynthesis. *eLife* 7, e34292
- 12 Hirt, R.P. et al. (2015) Lateral gene transfers and the origins of the eukaryote proteome: a view from microbial parasites. *Curr. Opin. Microbiol.* 23, 155–162
- 13 Eme, L. et al. (2017) Lateral gene transfer in the adaptation of the anaerobic parasite *Blastocystis* to the gut. *Curr. Biol.* 27, 807–820
- 14 Paganini, J. et al. (2012) Contribution of lateral gene transfers to the genome composition and parasitic ability of root-knot nematodes. *PLoS One* 7, e50875
- 15 Xu, F. et al. (2016) On the reversibility of parasitism: adaptation to a free-living lifestyle via gene acquisitions in the diplomonad *Trepomonas* sp. PC1. *BMC Biol.* 14, 62
- 16 Bock, R. (2017) Witnessing genome evolution: experimental reconstruction of endosymbiotic and horizontal gene transfer. *Annu. Rev. Genet.* 51, 1–22
- 17 Strese, Å. et al. (2014) A recently transferred cluster of bacterial genes in *Trichomonas vaginalis* – lateral gene transfer and the fate of acquired genes. *BMC. Evol. Biol.* 14, 119
- 18 Gilbert, C. and Cordaux, R. (2017) Viruses as vectors of horizontal transfer of genetic material in eukaryotes. *Curr. Opin. Virol.* 25, 16–22
- 19 Longdon, B. et al. (2014) The evolution and genetics of virus host shifts. *PLoS Pathog.* 10, e1004395
- 20 Catoni, M. et al. (2018) Virus-mediated export of chromosomal DNA in plants. *Nat. Commun.* 9, 5308
- 21 Schvarcz, C.R. and Steward, G.F. (2018) A giant virus infecting green algae encodes key fermentation genes. *Virology* 518, 423–433

- 22 Moreira, D. and Brochier-Armanet, C. (2008) Giant viruses, giant chimeras: the multiple evolutionary histories of Mimivirus genes. *BMC Evol. Biol.* 8, 12
- 23 Gilbert, C. *et al.* (2016) Continuous influx of genetic material from host to virus populations. *PLoS Gene.* 12, e1005838
- 24 Feschotte, C. and Gilbert, C. (2012) Endogenous viruses: insights into viral evolution and impact on host biology. *Nat. Rev. Genet.* 13, 283–296
- 25 Marchi, E. *et al.* (2014) Unfixed endogenous retroviral insertions in the human population. *J. Virol.* 88, 9529–9537
- 26 Filée, J. (2014) Multiple occurrences of giant virus core genes acquired by eukaryotic genomes: The visible part of the iceberg? *Virology* 466, 53–59
- 27 Geering, A.D. *et al.* (2014) Endogenous florendoviruses are major components of plant genomes and hallmarks of virus evolution. *Nat. Commun.* 5, 5269
- 28 Maumus, F. *et al.* (2016) Study of gene trafficking between *Acanthamoeba* and giant viruses suggests an undiscovered family of amoeba-infecting viruses. *Genome Biol. Evol.* 8, 3351–3363
- 29 Parker, B.J. and Brisson, J.A. (2019) A laterally transferred viral gene modifies aphid wing plasticity. *Curr. Biol.* 29, 1–6
- 30 Gasmi, L. *et al.* (2015) Recurrent domestication by Lepidoptera of genes from their parasites mediated by bracoviruses. *PLoS Genet.* 11:e1005470
- 31 Hill, T. and Betancourt, A.J. (2018) Extensive exchange of transposable elements in the *Drosophila pseudoobscura* group. *Mobile DNA* 9, 20
- 32 Baidouri, M. *et al.* (2014) Widespread and frequent horizontal transfers of transposable elements in plants. *Genome Res.* 24, 831–838
- 33 Peccoud, J. *et al.* (2017) Massive horizontal transfer of transposable elements in insects. *Proc. Natl. Acad. Sci. U.S.A.* 114, 4721–4726
- 34 Reiss, D. *et al.* (2019) Global survey of mobile DNA horizontal transfer in arthropods reveals Lepidoptera as a prime hotspot. *PLoS Genet.* 15, e1007965

- 35 Ivancevic, A.M. et al. (2018) Horizontal transfer of BovB and L1 retrotransposons in eukaryotes. *Genome Biol.* 19, 85
- 36 Gilbert, C. et al. (2014) Population genomics supports baculoviruses as vectors of horizontal transfer of insect transposons. *Nat. Commun.* 5, 3348
- 37 Zhang, H. et al. (2018) Unexpected invasion of miniature inverted-repeat transposable elements in viral genomes. *Mobile DNA* 9, 19
- 38 O’Brochta, D.A. et al. (2009) Transpositionally active episomal hAT elements. *BMC Mol. Biol.* 10, 1–15
- 39 Hedges, D.J. and Deininger, P.L. (2007) Inviting instability: Transposable elements, double-strand breaks, and the maintenance of genome integrity. *Mutat. Res.* 616, 46–59
- 40 Frost, L.S. et al. (2005) Mobile genetic elements: the agents of open source evolution. *Nat. Rev. Microbiol.* 3, 722–732
- 41 Schaack, S. et al. (2010) Promiscuous DNA: horizontal transfer of transposable elements and why it matters for eukaryotic evolution. *Trends Ecol. Evol.* 25, 537–546
- 42 McDonald, M.C. et al. (2019) Transposon-mediated horizontal transfer of the host-specific virulence protein ToxA between three fungal wheat pathogens. *mBio* 10, e01515-19
- 43 Sieber, K.B. et al. (2017) Lateral gene transfer between prokaryotes and eukaryotes. *Exp. Cell Res.* 358, 421–426
- 44 Husnik, F. and McCutcheon, J.P. (2017) Functional horizontal gene transfer from bacteria to eukaryotes. *Nat. Rev. Microbiol.* 16, 67–79
- 45 Martin, W. et al. (1993) Evidence for a chimeric nature of nuclear genomes: eubacterial origin of eukaryotic glyceraldehyde-3-phosphate dehydrogenase genes. *Proc. Natl. Acad. Sci. U.S.A.* 90, 8692–8696
- 46 Timmis, J.N. et al. (2004) Endosymbiotic gene transfer: organelle genomes forge eukaryotic chromosomes. *Nat. Rev. Genet.* 5, 123–135
- 47 Smith, D.R. et al. (2011) Correlation between nuclear plastid DNA abundance and plastid number supports the limited transfer window hypothesis. *Genome Biol. Evol.* 3, 365–371

- 48 Ku, C. et al. (2015) Endosymbiotic gene transfer from prokaryotic pangenomes: Inherited chimerism in eukaryotes. *Proc. Natl. Acad. Sci. U.S.A.* 112, 10139–10146
- 49 Archibald, J.M. (2015) Genomic perspectives on the birth and spread of plastids. *Proc. Natl. Acad. Sci. U.S.A.* 112, 10147–10153
- 50 Pittis, A.A. and Gabaldón, T. (2016) Late acquisition of mitochondria by a host with chimaeric prokaryotic ancestry. *Nature* 531, 101–104
- 51 Qiu, H. et al. (2013) Assessing the bacterial contribution to the plastid proteome. *Trends Plant Sci.* 18, 680–687
- 52 Nowack, E.C. et al. (2016) Gene transfers from diverse bacteria compensate for reductive genome evolution in the chromatophore of *Paulinella chromatophora*. *Proc. Natl. Acad. Sci. U.S.A.* 113, 12214–12219
- 53 Singer, A. et al. (2017) Massive protein import into the early-evolutionary-stage photosynthetic organelle of the amoeba *Paulinella chromatophora*. *Curr. Biol.* 27, 2763–2773
- 54 Husnik, F. et al. (2013) Horizontal gene transfer from diverse bacteria to an insect genome enables a tripartite nested mealybug symbiosis. *Cell* 153, 1567–1578
- 55 Bublitz, D.C. et al. (2019) Peptidoglycan production by an insect-bacterial mosaic. *Cell* 179, 703–712
- 56 Kamp, A. et al. (2015) Nitrate storage and dissimilatory nitrate reduction by eukaryotic microbes. *Front. Microbiol.* 6, 1492
- 57 Woehle, C. et al. (2018) A novel eukaryotic denitrification pathway in foraminifera. *Curr. Biol.* 28, 2536–2543
- 58 Hernandez, A.M. and Ryan, J.F. (2018) Horizontally transferred genes in the ctenophore *Mnemiopsis leidyi*. *PeerJ* 6, e5067
- 59 Doudoumis, V. et al. (2012) Tsetse-*Wolbachia* symbiosis: comes of age and has great potential for pest and disease control. *J. Invertebr. Pathol.* 112, S94–S103
- 60 Doolittle, F.W. (1998) You are what you eat: a gene transfer ratchet could account for bacterial genes in eukaryotic nuclear genomes. *Trends Genet.* 14, 307–311

- 61 Karnkowska, A. et al. (2016) A eukaryote without a mitochondrial organelle. *Curr. Biol.* 26, 1274–1284
- 62 Keeling, P.J. and Palmer, J.D. (2008) Horizontal gene transfer in eukaryotic evolution. *Nat. Rev. Genet.* 9, 605–618
- 63 Andersson, J.O. et al. (2007) A genomic survey of the fish parasite *Spironucleus salmonicida* indicates genomic plasticity among diplomonads and significant lateral gene transfer in eukaryote genome evolution. *BMC Genomics* 8, 51
- 64 Alsmark, C. et al. (2013) Patterns of prokaryotic lateral gene transfers affecting parasitic microbial eukaryotes. *Genome Biol.* 14, R19
- 65 Mayer, W.E. et al. (2011) Horizontal gene transfer of microbial cellulases into nematode genomes is associated with functional assimilation and gene turnover. *BMC Evol. Biol.* 11, 13
- 66 Dean, P. et al. (2018) Transporter gene acquisition and innovation in the evolution of *Microsporidia* intracellular parasites. *Nat. Commun.* 9, 1709
- 67 Gilbert, C. et al. (2010) A role for host–parasite interactions in the horizontal transfer of transposons across phyla. *Nature* 464, 1347–1350
- 68 Quispe-Huamanquispe, D.G. et al. (2017) Horizontal gene transfer contributes to plant evolution: the case of *Agrobacterium* T-DNAs. *Front. Plant Sci.* 8, 2015
- 69 Matveeva, T.V. et al. (2012) Horizontal gene transfer from genus *Agrobacterium* to the plant *Linaria* in nature. *Mol. Plant-Microbe Interact.* 25, 1542–1551
- 70 Kyndt, T. et al. (2015) The genome of cultivated sweet potato contains *Agrobacterium* T-DNAs with expressed genes: An example of a naturally transgenic food crop. *Proc. Natl. Acad. Sci. U.S.A.* 112, 5844–5849
- 71 Li, Z. and Bock, R. (2018) Replication of bacterial plasmids in the nucleus of the red alga *Porphyridium purpureum*. *Nat. Commun.* 9, 3451
- 72 Lee, J. et al. (2016) Reconstructing the complex evolutionary history of mobile plasmids in red algal genomes. *Sci. Rep.* 6, 23744
- 73 Bhattacharya, D. et al. (2013) Genome of the red alga *Porphyridium purpureum*. *Nat. Commun.* 4, 1941

- 74 Rossoni, A.W. et al. (2019) The genomes of polyextremophilic cyanidiales contain 1% horizontally transferred genes with diverse adaptive functions. *eLife* 8, e45017
- 75 Karas, B.J. et al. (2015) Designer diatom episomes delivered by bacterial conjugation. *Nat. Commun.* 6, 6925
- 76 Bowler, C. et al. (2008) The *Phaeodactylum* genome reveals the evolutionary history of diatom genomes. *Nature* 456, 239–244
- 77 Heinemann, J.A. and Sprague, G.F. (1989) Bacterial conjugative plasmids mobilize DNA transfer between bacteria and yeast. *Nature* 340, 205–209
- 78 Nevoigt, E. et al. (2000) Cells of the yeast *Saccharomyces cerevisiae* are transformable by DNA under non-artificial conditions. *Yeast* 16, 1107–1110
- 79 Sibbald, S.J. and Archibald, J.M. (2017) More protist genomes needed. *Nat. Ecol. Evol.* 1, 145
- 80 Ropars, J. et al. (2015) Adaptive horizontal gene transfers between multiple cheese-associated fungi. *Curr. Biol.* 25, 2562–2569
- 81 Richards, T.A. and Monier, A. (2016) A tale of two tardigrades. *Proc. Natl. Acad. Sci. U.S.A.* 113, 4892–4894
- 82 McCarthy, C.G. and Fitzpatrick, D.A. (2019) Pan-genome analyses of model fungal species. *Microb. Genomics* DOI: 10.1099/mgen.0.000243
- 83 Wang, W. et al. (2018) Genomic variation in 3,010 diverse accessions of Asian cultivated rice. *Nature* 557, 43–49
- 84 Read, B.A. et al. (2013) Pan genome of the phytoplankton *Emiliana* underpins its global distribution. *Nature* 499, 209–213
- 85 Worden, A.Z. et al. (2009) Green evolution and dynamic adaptations revealed by genomes of the marine picoeukaryotes *Micromonas*. *Science* 324, 268–272
- 86 McDonald, S.M. et al. (2010) The mixed lineage nature of nitrogen transport and assimilation in marine eukaryotic phytoplankton: a case study of *Micromonas*. *Mol. Biol. Evol.* 27, 2268–2283

- 87 Savory, F. et al. (2018) Ancestral function and diversification of a horizontally acquired oomycete carboxylic acid transporter. *Mol. Biol. Evol.* 35, 1887–1900
- 88 Milner, D.S. et al. (2019) Environment-dependent fitness gains can be driven by horizontal gene transfer of transporter-encoding genes. *Proc. Natl. Acad. Sci. U.S.A.* 116, 5613–5622
- 89 Kominek, J. et al. (2019) Eukaryotic acquisition of a bacterial operon. *Cell* 176, 1356–1366
- 90 Dunning, L.T. et al. (2019) Lateral transfers of large DNA fragments spread functional genes among grasses. *Proc. Natl. Acad. Sci. U.S.A.* 116, 4416–4425
- 91 McVey, M. and Lee, S. (2008) MMEJ repair of double-strand breaks (director's cut): deleted sequences and alternative endings. *Trends Genet.* 24, 529–538
- 92 Huang, J. (2013) Horizontal gene transfer in eukaryotes: The weak-link model. *BioEssays* 35, 868–875
- 93 Matsuo, M. et al. (2018) Characterization of spliced leader trans-splicing in a photosynthetic rhizarian amoeba, *Paulinella micropora*, and its possible role in functional gene transfer. *PLoS One* 13, e0200961
- 94 Doolittle, W. et al. (2003) How big is the iceberg of which organellar genes in nuclear genomes are but the tip? *Philos. Trans. R. Soc. London, Ser. B* 358, 39–58
- 95 Vembar, S. et al. (2016) Complete telomere-to-telomere de novo assembly of the *Plasmodium falciparum* genome through long-read (>11 kb), single molecule, real-time sequencing. *DNA Res.* 23, 339–351
- 96 Funkhouser-Jones, L.J. et al. (2015) Wolbachia co-infection in a hybrid zone: discovery of horizontal gene transfers from two *Wolbachia* supergroups into an animal genome. *PeerJ* 3, e1479
- 97 Brelsfoard, C. et al. (2014) Presence of extensive *Wolbachia* symbiont insertions discovered in the genome of its host *Glossina morsitans morsitans*. *PLoS Negl. Trop. Dis.* 8, e2728
- 98 Pinheiro, J. et al. (2018) The protozoan *Trichomonas vaginalis* targets bacteria with laterally acquired NlpC/P60 peptidoglycan hydrolases. *mBio* 9, e01784-18
- 99 Pereira-Neves, A. and Benchimol, M. (2007) Phagocytosis by *Trichomonas vaginalis*: new insights. *Biol. Cell* 99, 87–101
- 100 Liu, L. et al. (2012) The human microbiome: A hot spot of microbial horizontal gene trans-

fer. Genomics 100, 265–270

101 Parfrey, L. et al. (2011) Microbial eukaryotes in the human microbiome: ecology, evolution, and future directions. Front. Microbiol. 2, 153

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Glossary

Transduction

Virus-mediated transfer of genetic material. In bacteria, generalized transduction involves the integration and transfer of random pieces of bacterial DNA into the viral genome, while in specialized transduction only defined regions of the donor genome can be packaged into the virus and transferred.

778 **Transformation**

779 Uptake of exogenous DNA by a cell. This can occur naturally or in an experimental context.

780 Both prokaryotic and eukaryotic cells are capable of being transformed.

781

782 **Conjugation**

783 Transfer of genetic material mediated by cell-cell contact; single-stranded DNA moves through a
784 thin connection between the donor and recipient cells. Although best known to occur between
785 bacteria, artificial conjugation between bacteria and eukaryotes such as diatoms has been carried
786 out.

787

788 **Pan-genome**

789 The term refers to the entire set of genes in the genomes of members of a given species. The
790 word was invented to underscore the fact that individuals or strains of a given species can vary in
791 their gene content. In bacterial and archaeal species genome content variability can be immense
792 such that the set of genes found in all members of a given species (the ‘core’) can represent a
793 small fraction of the total pan-genome. This variability in gene content has a number of causes
794 including gene duplications, gene losses, *de novo* gene formation and the acquisition of new
795 genes by lateral gene transfer (LGT).

796

797 **Protists**

798 Protists are any eukaryotic organisms that are not animals, plants or fungi. Protists are an ances-
799 tral ‘grade’ of eukaryotic organization and encompass the bulk of eukaryotic diversity. Although

most protist species are unicellular microbes, charismatic multicellular forms are well known (e.g., macroalgae like giant kelp).

Endosymbiont

An organism that thrives within another organism, typically without killing its host. Endosymbiosis of bacteria, archaea and microbial eukaryotes (e.g., algae) is relatively common in free-living protists, but more rare in parasites. Organelles such as mitochondria and chloroplasts evolved from ancient bacterial endosymbionts. Readers should keep in mind that (endo)symbiosis encompasses a continuum from mutualism to parasitism, and determining where a given symbiotic system lies along this continuum can be very difficult.

Endosymbiotic gene transfer (EGT)

The transfer of genetic material from endosymbionts and their organellar derivatives to the host cell nuclear genome. This process involves endosymbiont / organelle lysis, migration of released DNA into the nucleus, and integration of random pieces of foreign DNA into nuclear chromosomes by DNA repair enzymes.

Box 1: Robust examples of LGT in eukaryotes

This review focuses on a select few of the numerous reports of LGT in eukaryotes. A recent example of adaptive LGT concerns the acquisition of the gene *rquA*, which is involved in rhodoquinone (RQ) biosynthesis, in various anaerobic protistan lineages [11]. The RQ produced by RquA allows for fumarate to be used as an alternative terminal electron acceptor during mitochondrial ATP production under anaerobic conditions, allowing these protists to adapt to low oxygen conditions. Phylogenetic analyses suggest that *rquA* was transferred on at least two separate occasions from bacteria to eukaryotes, followed by multiple eukaryote-to-eukaryote transfers.

Single gene-encoded transporters may be especially amenable to LGT as they could provide a direct selective advantage to the recipient by allowing for the uptake and utilization of a particular metabolite. Numerous examples of LGTs involving transporter genes have been reported (e.g., [68,86-88 and references therein]). Milner et al. [88] identified seven cases of transporter-gene LGT between fungal phyla and demonstrated that they conferred a competitive advantage in certain environments. They proposed a “transporter gene acquisition ratchet” where the range of transporters encoded in a given genome are continually being altered by various mechanisms, including LGT.

Another notable report of LGT is the acquisition of an entire operon involved in siderophore biosynthesis from Enterobacteriaceae into a group of closely related yeasts [89]. The co-linearly arranged genes were found to be expressed and functional (via transcriptomics, metabolic assays, and mass spectrometry), allowing for the sequestering and uptake of iron from the environment – a likely beneficial feature as the yeast reside in the iron-limited insect gut environment co-inhabited with numerous Enterobacteriaceae. After the initial LGT, the operon struc-

ture appears to have undergone a ‘eukaryotification’ process that included the addition of polyadenylation sites, various structural rearrangements, addition of some eukaryotic genes, and loss of some genes encoded by the operon in closely related yeast lineages. In principle, acquiring an entire operon in a single LGT would allow for complex multi-gene pathways to spread rapidly and effectively from donor to recipient.

Analyses of chromosome-level genomes for the grass *Alloteropsis semialata* also revealed recent acquisitions of chunks of foreign DNA encoding multiple genes, including genes related to disease resistance, abiotic stress tolerance and C4 photosynthesis [90]. Phylogenetic analyses including genomes from >140 other grass species led to the discovery of 59 unambiguous, recent LGTs into *A. semialata* from at least nine distantly related donor grasses. These genes were found to be mostly expressed and clustered in 23 transferred fragments up to 170 kbp long, indicating that large stretches of DNA containing multiple functional genes as well as non-coding regions were transferred in a single event. In some cases, TEs were also encoded on the transferred region.

Box 2: Factors influencing the frequency of lateral gene transfer

The frequency of gene transfer into a given species may hinge on a number of genomic and environmental factors. For example, non-homologous end joining repair could be a major mechanism of incorporation of foreign DNA [44] and thus impact the frequency of LGT in a given genome. It is generally thought that successful LGTs can occur more frequently between closely related organisms due to similarities between their genomes (codon usage, cis-acting regulatory elements, etc.); high levels of sequence identity also foster homologous recombination, which can lead to the transfer of alleles from one species to another [1]. Genetic exchange,

however, is not completely limited to very similar sequences. As long as there are conserved flanking regions, divergent alleles can recombine by homologous recombination [1] and the presence of small short repeats may allow for micro-homology mediated recombination as well [91].

Dynamic and repetitive regions of the genome that are more prone to duplication are likely to have increased rates of gene integration. LGTs have been observed in regions enriched in DNA transposons and retrotransposons and in gene-poor regions such as telomeres or in/near TEs (discussed in [43]). Additionally, selfish genetic elements (e.g., self-splicing introns, inteins etc.) are common in multicellular eukaryotes and could promote LGT and genome rearrangements [1]. ‘Fragile’ genomic regions that are prone to double stranded breaks or environmentally induced DNA damage and repair could also create opportunities for foreign gene integration. LGTs may occur when viruses or parasites manipulate DNA damage/repair pathways. Certain life/developmental stages may be more likely entry points for foreign genes into the nuclei of both unicellular eukaryotes (particularly those who are not phagotrophic), and in particular, the germline of multicellular eukaryotes (see the ‘weak-link’ model [92]).

In the testate amoeba *Paulinella micropora*, Matsuo et al. [93] identified a common ~30 bp sequence added post-transcriptionally by spliced leader (SL) trans-splicing to a diverse set of mRNAs, including those derived by LGT and EGT. Based on these observations, the authors suggested that SL trans-splicing could mitigate the potential detrimental effects of LGT in eukaryotes by ensuring translation of native nuclear genes disrupted by the insertion of foreign genes in their 5’UTRs. This could in principle apply not only to *Paulinella* species but to other trans-splicing organisms including euglenids, dinoflagellates and some metazoans.

Box 3: Detecting LGT with confidence

Genomic features that differ between eukaryotic and prokaryotic genes can be used to differentiate between bona fide LGT and prokaryotic contaminants. One useful distinction is the presence of introns in eukaryotic genes and lack thereof in prokaryotes; if a candidate LGT of bacterial origin contains an intron it most likely resides within a eukaryote genome. Other characteristics such as nucleotide composition, amino acid usage and the presence of a poly-A tail on transcripts can help identify genuine LGTs. Further support can be found by examining the genomes of closely related organisms; unless the transfer is very recent, the suspected LGT will likely be found in multiple genomes. For example, most of the LGTs identified in the gut parasite *Blastocystis* sp. subtype 1 were also found in genomes of seven of the other subtypes [13].

Phylogenetic analysis is essential for differentiating candidate LGTs from gene duplications and differential loss. A patchy gene distribution, combined with a phylogenetic relationship that contradicts the species relationship, is suggestive of an LGT. If it is assumed that all patchily distributed eukaryotic genes are due to differential gene loss rather than lateral acquisitions, then there becomes a genome of Eden problem [94] – the unlikely scenario that the last eukaryotic common ancestor (LECA) had almost every gene seen in modern eukaryotes and an enormous range of metabolic capabilities. Phylogenetic reconstruction of LGT is not without its limitations, with certain transfers being more difficult to detect and interpret (such as sequential transfers, which results in more complex gene distributions [62]). The lack of resolution associated with single gene trees can make it tough to infer the validity and origin of candidate LGTs. Phylogenetic artifacts, leading to falsely resolved relationships that may mislead interpretations of a gene's evolutionary history, are also of concern.

Some of the other most conclusive bioinformatic evidence for LGT can come from investigations of genomic context. A candidate LGT contained within a larger genomic scaffold clearly of host origin is likely to be real (e.g., viral LGTs in *Acanthamoeba* that are surrounded by amoebal host genes [28]). This can be difficult to assess if a reference genome is poorly assembled and highly fragmented. Fortunately, long read sequencing technologies make it possible to produce more highly contiguous genome assemblies, allowing for greater insight into genomic context, particularly in repetitive regions and near telomeric repeats that typically do not assemble well (e.g., [95]). Classical molecular biology techniques can also be used to verify genomic integration of candidate LGTs into host chromosomes (e.g., fluorescence in situ hybridization (FISH) has been used to confirm insertions of *Wolbachia* DNA on multiple chromosomes in the grasshopper *Chorthippus parallelus* and Tsetse flies (*Glossina* spp.) [96, 97].

Box 4: *Trichomonas* and gene transfer in the microbiome

Trichomonas vaginalis is a sexually transmitted parasite that colonizes an environment occupied by other parasites and bacteria. 149 LGTs of both prokaryotic and eukaryotic origin were identified in the *T. vaginalis* genome, including genes likely facilitating pathogenicity and to take advantage of the rich metabolic opportunities in its environment (e.g., glycan degradation) [64]. Further study on LGTs in *T. vaginalis* identified and confirmed the functionality of NIPC/P60 peptidoglycan hydrolases that were acquired laterally from bacterial co-members of the microbiota [98]. Pinheiro et al. [98] showed that the bacterial NIPC/P60 hydrolases in the genome of *T. vaginalis* were capable of degrading bacterial cell envelopes and that their expression was significantly upregulated in the presence of bacteria. The transfer of NIPC/P60 hydrolases in *T. vaginalis* appears to be critical in controlling bacterial abundance in its environment

and the establishment of a successful infection. This example shows that co-inhabitants of a given microbiome can act as a source of genes for one another, particularly in cases of phagocytic species like *T. vaginalis* [99].

The human microbiome has been referred to as “a hotspot of microbial horizontal gene transfer” [100]. While studies on LGT in the human microbiome has almost entirely focused on the extensive prokaryote-prokaryote transfers (e.g., 13,514 high confidence LGTs between 308 human microbiome bacteria [100]), diverse eukaryotes with lifestyles ranging from parasitic to beneficial also reside in and are important components of the human microbiome [101]. The abundance of a variety of species and phages in close proximity presumably creates a favorable environment for LGT for microbial eukaryotes; by increasing the frequency of donor DNA in the environment you increase the exposure of a species to a given DNA source [13,44,64].

Figure 1. Possible sources of foreign DNA contributing to LGT in eukaryotes. Eukaryotic cells are exposed to many potential sources of exogenous DNA including from: i) lysed endosymbiont-derived organelles (mitochondria and chloroplasts), prokaryotic or eukaryotic endosymbionts, ii) viral infection, iii) bacterial conjugation (e.g., *Agrobacterium tumefaciens*) and iv) lysed phagosomes containing food organisms.

Figure 2. The pan-genome concept. The pan-genome consists of all genes found in all strains of a given species and is depicted at the top of the figure as the union of all ellipses (each ellipse is the set of genes present in that strain) in the Venn diagram. The pan-genome can be further broken down into the ‘core’ genome (genes found in all strains shown in dark teal) and the ‘accessory’ genome (shown colours ranging from green to blue) which are genes found in a single strain (i.e. strain specific) or a subset of strains (i.e. strain select).