Functional horizontal gene transfer from bacteria to eukaryotes

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Abstract | Bacteria influence eukaryotic biology as parasitic, commensal or beneficial symbionts. Aside from these organismal interactions, bacteria have also been important sources of new genetic sequences through horizontal gene transfer (HGT) for eukaryotes. In this Review, we focus on gene transfers from bacteria to eukaryotes, discuss how horizontally transferred genes become functional and explore what functions are endowed upon a broad diversity of eukaryotes by genes derived from bacteria. We classify HGT events into two broad types: those that maintain pre-existing functions and those that provide the recipient with new functionality, including altered host nutrition, protection and adaptation to extreme environments.

Horizontal gene transfer (HGT). Movement of genetic material between organisms (also called lateral gene transfer) via non-vertical (not parent-to-offspring) transmission.

Germ line
The specialized cellular lineage in multicellular sexual organisms that is used to pass on genetic material to the progeny.

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The genome of an organism is usually passed vertically from the parent to its offspring. As such, in the simplest case, the evolutionary history of a genome should reflect the evolutionary history of the organism. However, genomes are dynamic in content, size and rates of evolution. Genes can be lost, non-coding or selfish genomic regions can expand or contract over short timescales, different loci can evolve at different rates because of unequal selective pressures, and genes can be gained through both duplication within genomes and acquisition from foreign sources through horizontal gene transfer (HGT). HGT can in principle occur between any two organisms that contain DNA genomes, leading to different genes in the same genome exhibiting different evolutionary histories. However, HGT is not likely to occur equally among all branches of life.

HGT is now understood to be a major driver of genome evolution in bacteria and archaea[1–7]. In fact, it is so common that our ability to infer organismal relationships through phylogenetic tree analysis has been questioned for bacteria and archaea[8,9]. By contrast, the frequency and importance of HGT between bacteria and eukaryotes remain controversial and less clear[7,10–14], in particular for animals[15–17]. This lack of clarity — at least partly due to the large size and complexity of animal genomes and the almost inevitable problem of microbial contamination in genome projects — has resulted in misattributions of HGT events in animal genomes (such as human genomes[18–22]) in the past, and more recently, similar misattributions have occurred in the genome of a tardigrade[23,24] (BOX 1). However, this history of sometimes over-ambitious claims of microbial–animal HGT events does not mean that HGT does not happen or that it is not important in eukaryotes.

Many cases of *bona fide* functional inter-kingdom HGT events have been documented, including several recent examples of bacteria-to-animal transfers[25–34] (FIG. 1; TABLE 1). In this Review, we discuss these recent findings, aiming to incorporate examples from a wide diversity of eukaryotes. We focus on gene transfers from bacteria to eukaryotes because they seem to be the most common — or at least the most commonly reported — but we note that HGTs among nearly all branches of life have been described (TABLE 1; Supplementary information S1 (table)). Using this broad taxonomic sampling, we outline mechanistic similarities in HGT events that result in functional genes and organize these transfers into common functional categories. We classify these HGT events into 'maintenance' transfers and 'innovation' transfers and explain how HGT from bacteria has shaped, and continues to shape, eukaryotic genomes.

Mechanisms of DNA transfer

Most transferred DNA does not become functional. Factors that affect the frequency of gene transfer include the ability of an organism to take up foreign DNA, the accessibility of its germ line (specifically, whether it is sequestered away from somatic tissue), its recombinogenic tendencies and the frequency of the donor DNA in the environment. The presence of stably associated germline endosymbionts can dramatically increase the chances of HGT, as the DNA of any lysed endosymbiont cell has direct access to the germline nucleus. Upon acquisition, the foreign DNA must become subject to selection[35], either by being transcribed into RNA or by providing a function at the genome level. For example, a transferred protein-coding gene must be transcribed and translated by the host for it to gain new functionality (FIG. 2). If the transfer does not become subject to

selection, the newly acquired DNA will likely be deleted or otherwise erode in a manner dependent on the mutagenic tendencies of the host. Because of the differences in gene structure between bacteria and eukaryotes (for example, the presence or absence of introns, differences in GC content and differences in transcriptional promoters), gene inactivation and erosion are likely to be the most common outcomes of HGT between these organisms[36–39]. However, despite these structural hurdles, transferred DNA has been shown to become functional in many instances (see below).

Inferring the source of transferred DNA. Two issues hinder the precise identification of the donor organism for many HGT events. The first is that bacterial genomes can contain DNA fragments from many different organisms owing to the high frequency of HGT events among them, so the donor DNA may or may not have the same evolutionary history as most of the other genes in the donor bacterial genome[40,41]. The second is that phylogenetic problems such as long-branch attraction, differential gene loss and inadequate taxon sampling can often make it difficult to infer the origin of the donor DNA[9,42,43].

If we assume that the more common the source organism is in the environment of the recipient organism, the more likely transfer will occur between these organisms, it is not surprising that bacterivorous or parasitic unicellular eukaryotes — in which every cell is a germline cell, and any gene transfer thus has the potential to be transmitted to the next generation — are among the most frequent recipients of genes from bacteria[12,44,45].

Box 1 | **Methodologies and caveats in the identification of horizontal gene transfer events**

Sample preparation. The detection of horizontal gene transfer (HGT) events tends to start with a genome project. As such, the first step in trying to detect HGT events in any organism should be careful sample and sequencing-library preparation, with a particular focus on reducing microbial contamination. For example, tissues known to contain dense communities of bacteria can sometimes be removed, and the outer surface of sequenced individuals can be bleached to reduce contaminating environmental bacteria. Guidelines for reducing reagent and laboratory contamination are well defined for microbiome studies[136], and we recommend using these approaches when generating data that might include the detection of HGT events.

Sequence database searches. Numerous BLAST-based approaches have been developed to identify candidate genes that might have been transferred horizontally into eukaryotic genomes[20,34,72,137,138]. However, the performance of any sequence alignment-based method is only as good as the databases used in the searches. BLAST-based approaches are generally reliable for taxa with numerous sequenced and well-annotated genomes (for example, some bacteria and metazoa), but candidate gene transfers found with these approaches should be interpreted with extreme caution for non-model organisms, for detection of ancient and highly diverged HGT events or if any related reference genomes are of low quality (or possibly contaminated with bacterial sequences).

Phylogenetic evidence. Sequence database searches should be used primarily to narrow down the total number of candidate genes to a computationally feasible number of candidates for phylogenetic analysis. Phylogenetic conflict (that is, incongruence of a single-gene tree with a known species phylogeny) is the method of choice for the detection of HGT events. However, phylogenetic trees are always imperfect inferences of the evolution of the underlying sequences. HGT events are likely to evolve under different selection pressures than native genes, especially at first, and many have long branches. Methods that reduce long-branch attraction[139] can be used with single-gene data sets of candidate genes, and the quality of important parts of phylogenetic trees can be tested with statistical approaches. For example, the approximately unbiased test[140] can be used to compare statistical support for a tree structure with an HGT candidate branching within bacteria to a tree structure where the HGT candidate is constrained to branching within eukaryotes. Finally, differential gene loss in different lineages can lead to situations that appear to be HGT events but are not[18,40], especially when taxon sampling is limited.

Genomic evidence. The co-assembly of a candidate gene with one or more eukaryotic genes on a single genomic scaffold, along with gain of active eukaryotic introns, is probably the best genomic evidence of gene transfer. This does not mean that a promising candidate gene is simply present in the same assembly, which can easily be because of contamination, but rather that it shares the same high-quality contig or scaffold as a high-confidence native gene. Typically, this means that the average depth of sequencing coverage of the scaffold with the candidate gene should be similar to that of other eukaryotic scaffolds. The use of PCR-free library methods can help to reduce unevenness in sequencing depth of coverage. Software such as BlobTools, which bins and displays assemblies by characteristics such as depth of coverage, GC content, codon usage and *k*-mer frequencies, can also be helpful in discriminating contamination from possibly horizontally transferred candidate genes[24]. However, very recent HGT events can be difficult to distinguish from contamination or symbionts[58,59,63].

Population, microscopy and functional evidence. The presence of a candidate gene in individuals from geographically distinct populations can help to distinguish it from most contaminants[51]. Localizing the candidate gene on the host chromosome by use of fluorescence *in situ* hybridization with specific fluorescently labelled probes provides very strong evidence of HGT. This approach was essential in corroborating the whole-genome inserts from *Wolbachia* spp. into arthropod genomes because these HGT events look so similar to normal bacterial chromosomes[58,59,63]. RNA sequencing data can be useful for the detection of expressed and possibly functional horizontally transferred genes. Tissue-specific expression combined with experimental validation of the previously unknown enzymatic function from the target taxon was used as supporting evidence for several HGT events, for example, in arthropods (reviewed in REF. 17).

Endosymbionts
Organisms living within the body or cells of another organism.

Long-branch attraction
A phylogenetic artefact that causes distantly related lineages (often on long branches) to be incorrectly inferred as closely related in phylogenetic trees.

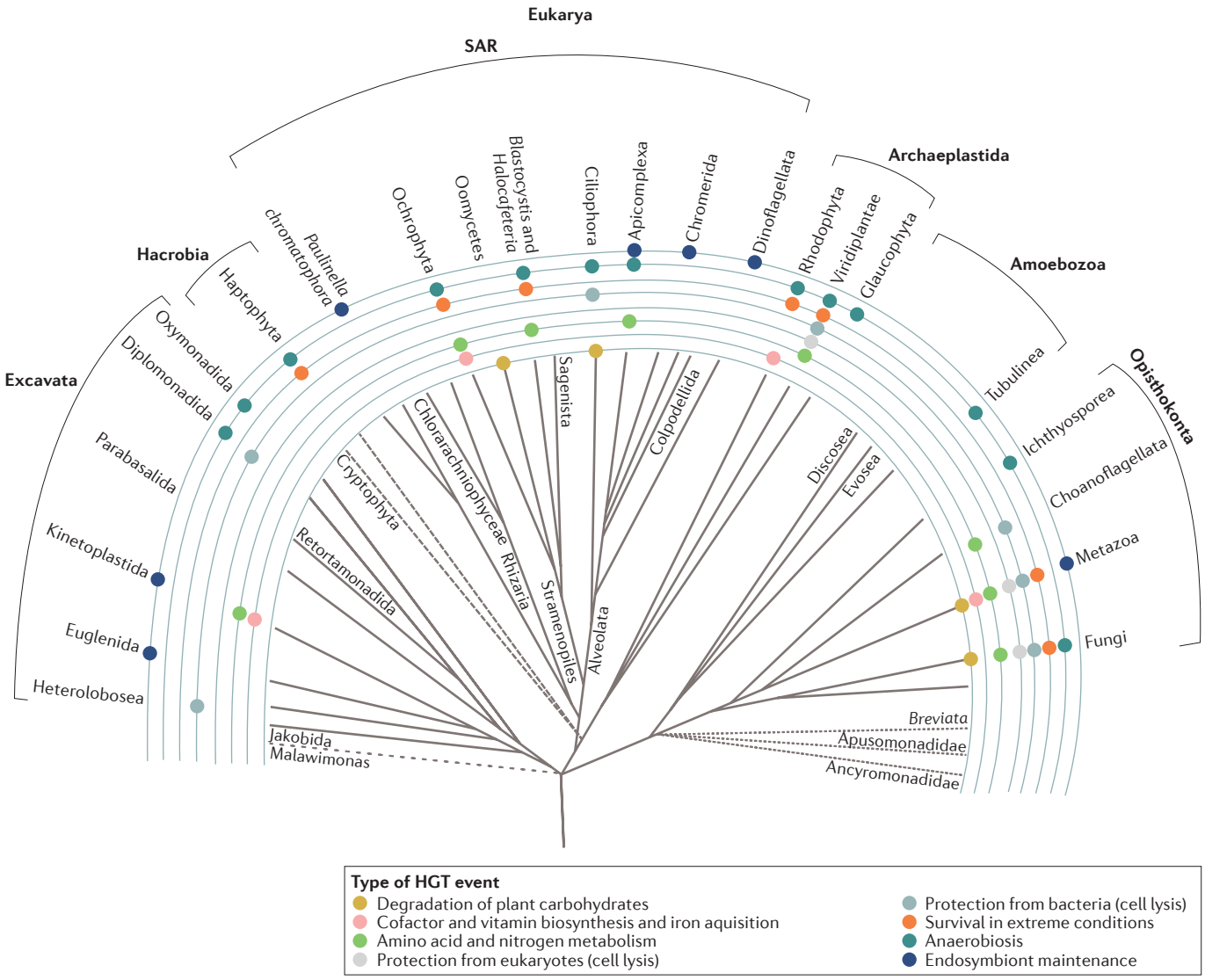

Figure 1 | **Functional horizontal gene transfer events in eukaryotes.** A phylogenetic tree showing functional horizontal gene transfer (HGT) events in eukaryotes (indicated by differently coloured circles). HGT events can be classified into two broad types: those that maintain pre-existing functions (maintenance transfers) and those that add new functionality to the recipient (innovation transfers), including altered host nutrition, protection and adaptation to extreme environments. Of note, many lineages that lack reports of HGT events are microbial eukaryotes such as amoebae, ciliates, dinoflagellates and non-parasitic excavates. These gaps are likely due to sampling bias. Note that gene transfers related to mitochondria and plastids were omitted from the figure for simplicity. Bolded terms indicate supergroups of Eukaryota. SAR, eukaryotic supergroup containing Stramenopiles, Alveolata and Rhizaria.

Many other examples also suggest that proximity matters: soil-dwelling nematodes tend to acquire genes from putative soil bacteria[46]; thermoacidophilic algae acquire genes from putative thermoacidophilic bacteria[47,48]; plant pathogenic oomycetes acquire genes from plant-associated bacteria[49]; stramenopile pathogens of the human digestive tract acquire genes from relatives of common members of the gut microbiome[50]; and many invertebrates acquire genes from common intracellular bacteria such as *Wolbachia* spp.[25,32,34,51]. By contrast, for some organisms that seem to be frequent recipients of foreign DNA via HGT, such as rotifers[27,52] or fungi[53], there is no clear environmental source of transferred DNA, and these organisms seem to acquire genes from multiple donor organisms from diverse environments.

Various models have been put forward to explain the patterns of frequency and taxonomy observed in bacteria–eukaryote HGT[11,54,55]. For example, the weak-link model[56] expands previous proximity-based hypotheses[11,54] by suggesting that genes primarily enter cells of their recipient organisms at particular stages of their life cycle in natural environments. As mentioned above, every cell of a unicellular eukaryote is both a germ cell and somatic cell and therefore has the potential to pass transferred DNA to its offspring[56]. In multicellular eukaryotes, early developmental stages that are exposed to their environment (for example, spores, zygotes or

Table 1 | **Functional and putatively functional horizontal gene transfers reported in eukaryotes**

Function	Supergroup	Eukaryotic lineage	Refs
Maintenance			
Compensation for gene loss in obligate endosymbionts	Opisthokonta	Mealybugs, aphids, psyllids and whiteflies	25,32,34, 44,51,96, 97
	SAR	*Paulinella chromatophora*	
	Excavata	*Angomonas* spp. and *Strigomonas* spp.	
Innovative			
Protection from bacteria (lysis of bacterial cells)	Opisthokonta	Mites and ticks, *Daphnia* spp., *Capitella teleta* and molluscs, lancelets, acorn worms, sea anemones, *Monosiga brevicollis*, hemipteran insects, *Trepomonas* sp. PC1, bdelloid rotifers and fungi	25,30,31, 45,51,72
	Archaeplastida	*Selaginella moellendorffii*	
	SAR	*Oxytricha trifallax*	
	Excavata	*Naegleria gruberi*	
Protection from eukaryotes and their metabolites	Opisthokonta	*Hydra* spp., ticks, fungi, *Nematostella* spp.*, *Epichloe* spp. and herbivorous arthropods	17,26,104
	Archaeplastida	Various plants	
	Excavata	*Leishmania* spp. and *Trypanosoma* spp.	
Amino acid and nitrogen metabolism	Opisthokonta	Herbivorous arthropods, *Monosiga brevicollis* and fungi	17,50,76, 96,97,107, 111,116, 117
	Amoebozoa	*Dictyostelium discoideum* and *Entamoeba* spp.	
	Archaeplastida	*Micromonas* spp.	
	SAR	Diatoms, Apicomplexa spp. and *Blastocystis* spp.	
	Excavata	Kinetoplastida, *Trichomonas vaginalis* and *Giardia intestinalis*	
Cofactor and vitamin biosynthesis and iron acquisition	Opisthokonta	Hemipteran insects, *Brugia malayi*, *Heterodera glycines* and fungi	17,29,47, 96,97,107, 112–115, 119
	Amoebozoa	*Dictyostelium discoideum* and *Entamoeba* spp.	
	Archaeplastida	*Galdieria sulphuraria*	
	SAR	Diatoms	
	Excavata	Kinetoplastida and *Trichomonas vaginalis*	
Degradation of plant carbohydrates	Opisthokonta	Herbivorous arthropods, rumen chytrid fungi, plant-parasitic nematodes, necronemic nematodes (*Pristionchus* spp.) and rotifers	17,46,52, 77,106,108, 121,141
	SAR	Rumen ciliates, oomycetes	
Carbohydrate metabolism	Opisthokonta	Fungi	12,50,107
	Amoebozoa	*Entamoeba* spp. and *Dictyostelium discoideum*	
	SAR	*Cryptosporidium* spp. and *Blastocystis* spp.	
	Excavata	*Trichomonas vaginalis* and Kinetoplastida spp.	
Survival under extreme and toxic conditions and environmental stress	Opisthokonta	Rotifers, *Antonospora locustae* microsporidians and fungi	39,50,53, 114, 121–125
	Amoebozoa	*Dictyostelium discoideum*	
	Archaeplastida	*Coccomyxa subellipsoidea*, *Galdieria sulphuraria* and various land plants	
	SAR	Diatoms, *Halocafeteria seosinensis* and *Blastocystis* spp.	
	Hacrobia	*Phaeocystis antarctica*	
Facultative or obligate anaerobiosis	Opisthokonta	Chytrid fungi and yeasts and *Amoebidium parasiticum*	50,106,111, 142–148
	Amoebozoa	*Entamoeba* spp. and *Mastigamoeba balamuthi*	
	Archaeplastida	*Cyanophora paradoxa*, *Pyropia haitanensis*, Prasinophyte algae and Chlorophyte algae	
	SAR	*Thalassiosira pseudonana*, *Cryptosporidium parvum*, rumen ciliates, *Nyctotherus ovalis* and *Blastocystis* spp.	
	Hacrobia	*Prymnesium parvum*	
	Excavata	*Giardia intestinalis*, *Trichomonas vaginalis* and *Paratrimastix pyriformis*	

Table 1 (cont.) | **Functional and putatively functional horizontal gene transfers reported in eukaryotes**

Function	Supergroup	Eukaryotic lineage	Refs
Innovative (cont.)			
Nucleotide metabolism	Opisthokonta	Microsporidia	12
	Amoebozoa	*Entamoeba* spp.	
	SAR	*Cryptosporidium* spp. and *Plasmodium* spp.	
	Excavata	*Trichomonas vaginalis*, Kinetoplastida, *Giardia intestinalis* and *Spironucleus salmonicida*	
RNA and DNA modifications	Opisthokonta	Mealybugs and psyllids	12,28,32
	Amoebozoa	*Dictyostelium discoideum*	
	SAR	*Plasmodium* spp. and *Toxoplasma* spp.	
	Excavata	*Trichomonas vaginalis* and Kinetoplastida	
Parasitism and pathogenicity	Opisthokonta	Various Microsporidia	12,50,149, 150
	Amoebozoa	*Entamoeba* spp.	
	SAR	*Cryptosporidium* spp., *Plasmodium* spp., *Toxoplasma* spp. and *Blastocystis* spp.	
	Excavata	*Trichomonas vaginalis*, *Giardia intestinalis*, *Leishmania* spp. and *Trypanosoma* spp.	

Some of the categories can overlap with others, and the categorization reflects a simplification. The table focuses on only horizontal gene transfer (HGT) events with an assigned functional categorization, so the list is not exhaustive. SAR, eukaryotic supergroup containing Stramenopiles, Alveolata and Rhizaria. *The *Nematostella* genome was suggested several times to be highly contaminated by bacterial data; thus, only one well-supported HGT event (transfer of the type VI secretion system amidase effector (*tae*) genes) is included.

Conjugation
Gene transfer from a donor to a recipient by direct cell-to-cell contact, such as plasmid transfer between two bacterial cells.

Transduction
Gene transfer carried out by a virus, such as a bacteriophage, transferring DNA from one bacterium to another.

Transformation
Direct acquisition of DNA from the environment through the cell membrane.

Gene transfer agents
Bacteriophage-like elements that package random DNA regions from a host cell and transfer them to a recipient cell.

Non-homologous end joining
(NHEJ). A pathway for the direct repair of double-strand DNA breaks without a homologous template.

Nuclear mitochondrial transfers
(numts). Transfers of mitochondrial DNA into the nuclear genome of a eukaryotic host; the encoded genes often become non-functional.

Nuclear plastid transfers
(nupts). Transfers of plastid DNA into the nuclear genome of a eukaryotic host; the encoded genes often become non-functional.

embryos) are likely to be recipient cells for foreign DNA that could be passed to offspring[56]. Bdelloid rotifers, which are microscopic freshwater animals that reproduce asexually, are the animals with the most reported HGT events from microorganisms, perhaps because of chromosomal instability formed by bouts of desiccation and rehydration[39]. In cases where early developmental stages are not exposed to the environment, the transfer, genome integration and maintenance of DNA is often promoted by parasites, symbionts or pathogens that infect germline cells[28,56,57], as exemplified by the numerous transfers of *Wolbachia* spp. DNA into arthropod genomes[37,58,59].

DNA acquisition and integration. Mechanisms of gene transfer[4,11] include conjugation, transduction, transformation and gene transfer agents. Non-homologous end joining (NHEJ) seems to be the major mechanism of incorporation of foreign DNA into the eukaryotic genome[60], although recombination with a homologue already present in the genome can also occur (for example, in plant mitochondria)[61]. As these mechanisms have been thoroughly discussed elsewhere[4,11], we focus mainly on the steps that enable transferred genes to be expressed and thus provide the host with new functions.

Whether a newly arrived foreign sequence attains functionality probably depends on its features (for example, length of the acquired DNA, GC content, codon usage, genetic code and epigenetic marks) and the position of where the sequence is integrated in the genome (heterochromatin or euchromatin). The size of the transferred DNA seems to be a major factor in whether the transferred sequence becomes functional (FIG. 2A). Several examples from diverse eukaryotes suggest that HGT events involving large DNA sequences are more likely to result in junk DNA than those involving small fragments[38,62,63]. For example, large DNA fragments or even entire genomes of *Wolbachia* spp. that have been transferred to arthropod and nematode genomes are fairly common[36,58,59,63,64]. In most cases, such transferred fragments undergo rapid non-functionalization through pseudogene formation and DNA deletion and exhibit very low levels of transcription[37,65]. However, functional genes could still be hidden in these large genome regions. Many of the functional bacteria-to-animal HGT events discussed below are also from *Wolbachia* spp., but in these cases, the transferred sequences seem to be either much smaller in size or from larger fragments that were reduced in size after transfer[25,32,34,51]. A fascinating exception to this rule comes from the pillbug *Armadillidium vulgare*, in which incorporation of an ~1.5 Mb fragment of *Wolbachia* spp. DNA into the genome of the pillbug (which subsequently duplicated to a final size of 3 Mb) seems to have been involved in creating a new female sex chromosome[64].

Eukaryotic nuclei are often likely to be exposed to DNA fragments from lysed mitochondria and plastids that can become incorporated into the host chromosomes, but they often become non-functional or are eliminated. Transferred sequences from organelle genomes are called nuclear mitochondrial transfers (numts) and nuclear plastid transfers (nupts) or organellar gene transfers[56]. Several studies have shown that numts and nupts are inserted via NHEJ at double-strand breaks

and that microhomology-mediated or blunt-end repair is involved in the incorporation of the DNA (reviewed in REFS 10,40). Incorporation is enriched at open chromatin regions and can occur in different genome regions in different lineages[40]. For example, numts are enriched at introns in the human genome[66] but not in the intron-poor genome of *Saccharomyces cerevisiae*[67]. Genomic regions flanking numts are often rich in retrotransposons, and the insertion often occurs immediately adjacent to AT oligomers in mammals[68]. Transfers of DNA fragments ranging from only several bp to several hundred kb have been reported[40], and numts and nupts can be further amplified after acquisition and give rise to tandem repeats[40].

In principle, foreign DNA could be incorporated anywhere in a recipient genome that does not disrupt an existing functional genomic element. Several examples suggest that, similar to numts, horizontally transferred sequences are often integrated into genome regions that are enriched in DNA transposons and retrotransposons[46,69–72] (FIG. 2A). In some genomes, sequences that were acquired long ago (in evolutionary terms) are found in gene-rich regions, whereas more recent horizontally transferred sequences are integrated in less conserved and less gene-rich locations, such as telomeric regions, or within or around transposable elements[28,72]. For example, horizontally transferred sequences that have been acquired by mealybugs tens of millions of years ago are found in gene-rich, possibly euchromatic, genomic regions (FIG. 2B), whereas sequences that were transferred more recently are found in less gene-rich and more

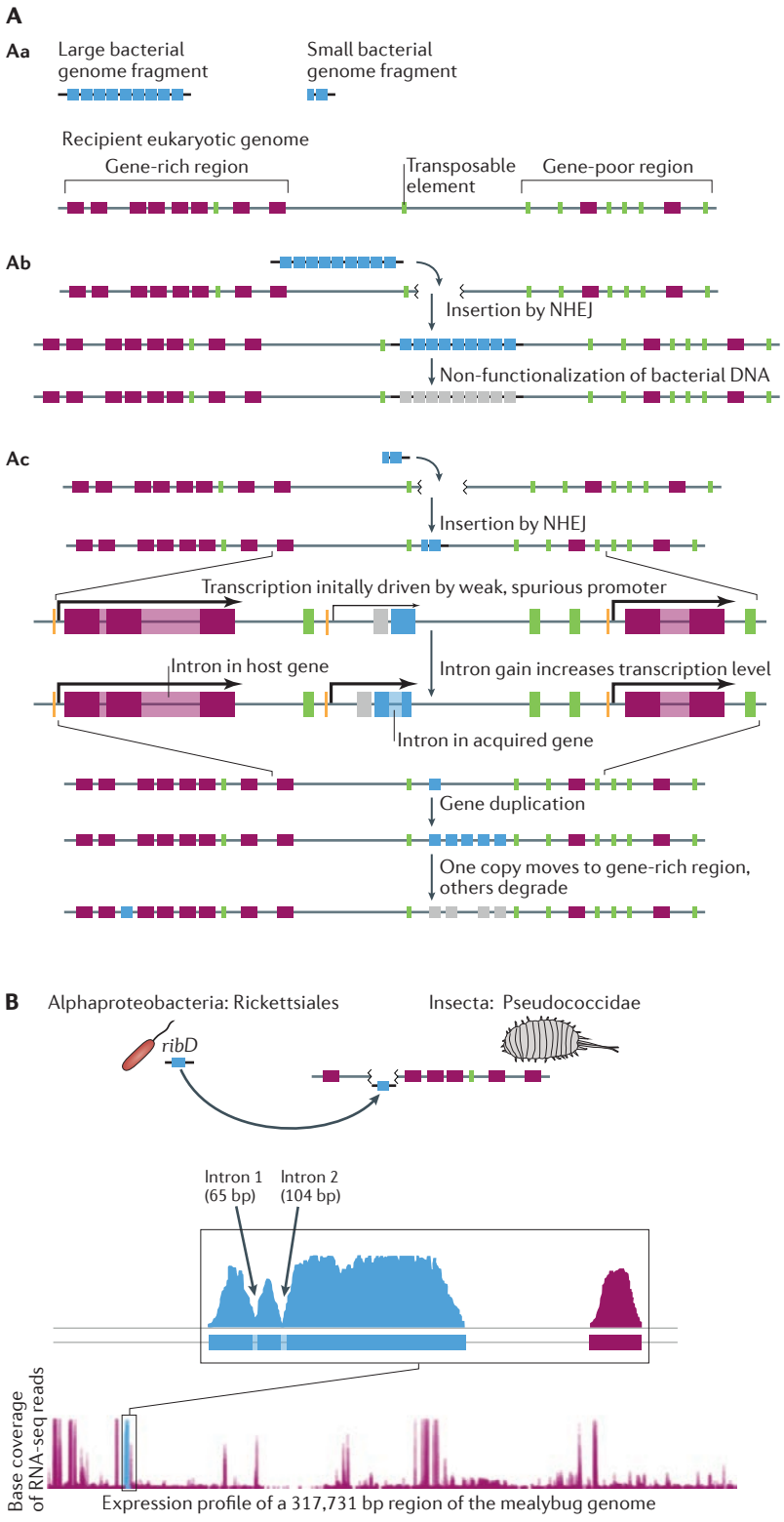

Figure 2 | **How foreign DNA gains functionality in eukaryotes. A** | The schematic shows the various steps a foreign bacterial sequence may take when becoming functional in a eukaryotic genome. **Aa** | The large and small bacterial genomic fragments are shown in blue, a section of the recipient eukaryotic genome with a gene-rich region on the left and a gene-poor region on the right (eukaryotic genes are shown in purple; transposable elements, which are common in dynamic regions of the genome, are shown in green). **Ab** | Acquisition of a large fragment of bacterial genome by a eukaryote (by non-homologous end joining (NHEJ)) often results in non-functionalization of the bacterial gene (shown in grey) and the formation of junk DNA in a manner dependent on the host genome. **Ac** | Acquisition of a small fragment of bacterial genome is more likely to result in a functional gene. The inset shows the insertion site. Intron gain might promote the increased expression (thick black arrow) of a transferred gene. Duplication of the transferred gene may help it become functional. Finally, the functional copy of the newly acquired gene can move to a gene-rich region in the host genome, whereas the other copies become non-functional. Although this process is shown in a sequential manner, functional transfers can enter and exit at any part of this pathway and can move through the steps in any order. **B** | An example of a functional HGT event from bacteria to the mealybug genome is depicted, showing the intron structure of the inserted gene and high levels of gene expression in a gene-rich region of the mealybug genome. The riboflavin biosynthesis gene (*ribD*) was transferred horizontally from a bacterium in the class of Alphaproteobacteria to the ancestor of Pseudococcidae mealybugs. The gene now resides in the insect genome in a gene-rich region, co-diverged with mealybugs for millions of years, acquired two canonical eukaryotic introns and gained gene expression levels similar to those of native insect genes.

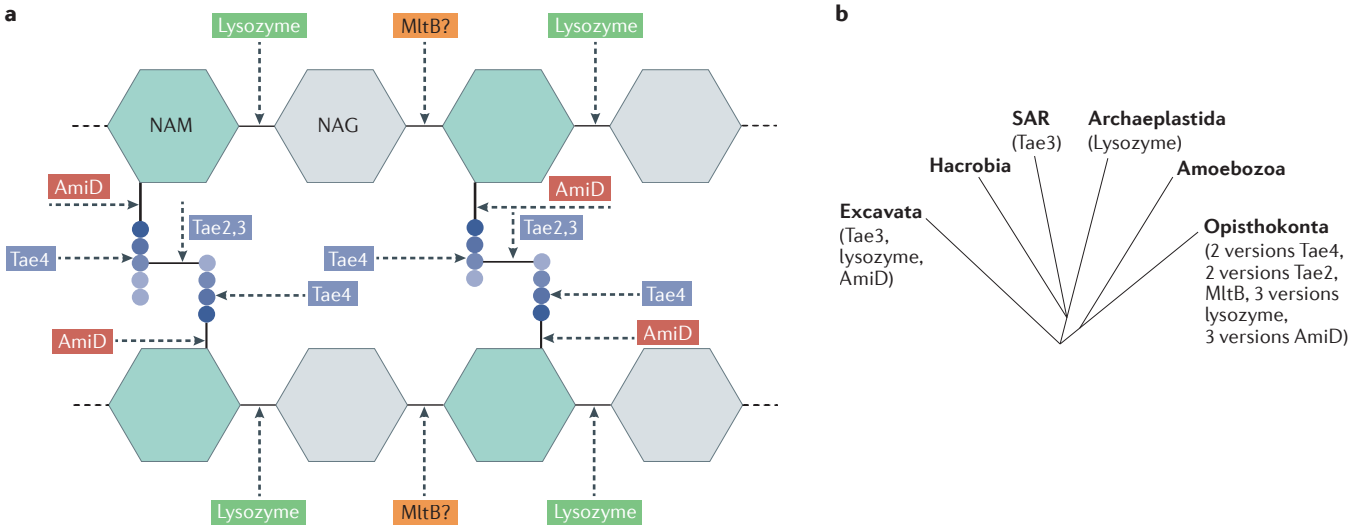

Figure 3 | **Horizontal gene transfer events targeting peptidoglycan bonds are common across eukaryotes.** Parallel adaptive horizontal gene transfer events lead to the acquisition of new functions in eukaryotes, including defence against bacterial pathogens by the ability to degrade bacterial cell envelopes. **a** | A schematic of a bacterial peptidoglycan layer is depicted, showing the sites of action of several peptidoglycan-degrading enzymes including *N*-acetylmuramoyl-L-alanine amidase (AmiD) and type IV secretion system amidase effector proteins (Tae proteins, which cleave the amide linkages in the pentapeptides of peptidoglycan), lysozymes (which cleave peptidoglycans between *N*-acetylglucosamine (NAG) and the peptidyl *N*-acetyl muramic acid (NAM)) and membrane-bound lytic murein transglycosylase B (MltB; an enzyme that likely also cleaves the bond between NAG and NAM moieties). **b** | Peptidoglycan-degrading enzymes have been acquired by diverse eukaryotes. In addition to peptidoglycan-degrading enzymes found in Excavata, 20 putative cell-wall hydrolases were reported from Trepomonas sp. PC1. SAR, eukaryotic supergroup containing Stramenopiles, Alveolata and Rhizaria.

poorly assembled genomic regions[51]. However, owing to a scarcity of data and the poor assembly quality of many eukaryotic genomes, it is difficult to draw firm conclusions with regards to the importance of the position at which the transferred gene is integrated.

Gaining functionality.
Transfer events that become functional often seem to involve short foreign DNA fragments, which are mostly integrated in gene-poor but transposable element-rich and dynamic parts of the genome. Tandem duplications of horizontally transferred genes are frequently observed[12,25,28,33,49,64,71,73] (FIG. 2A). For example, out of the 48 transferred sequences that are present in oomycete genomes, 38 exist in at least two copies[49]. Although these duplicates have not been studied in detail, it seems plausible that some copies might maintain the original function, some might gain new functions and some might become non-functional (FIG. 2A).

Intron gain might promote the increased expression of a transferred gene, as the presence of introns has been shown to increase gene expression in many eukaryotes[74]. Indeed, introns are commonly found in functional horizontally transferred genes in many eukaryotes[26,28,29,46,53,72,75–77] (FIG. 2A), although these introns are sometimes found in the 5′-UTR or the 3′-UTR of the gene[51]. However, regardless of the location of the integrated transferred gene or whether it gains introns, eventually all functional transferred genes will evolve as does any eukaryotic gene: their GC content and codon usage will gradually adjust to the host, and they will be expressed in a cell-compartment-specific and/or tissue-specific manner.

Functions of transferred DNA

We classify horizontally transferred genes into two broad types: those that provide a new function in the recipient organism (innovative transfers) and those that replace or maintain a functional loss in the recipient organism (maintenance transfers) (FIGS 3,4). Innovative transfers commonly enable recipient organisms to feed on nutritionally poor or toxic diets, parasitize other eukaryotes, survive in cold, hot, acidic, anaerobic or toxic environments or protect themselves from other organisms[11,13,17,78] (TABLE 1). Maintenance transfers are most common in recipients that also house a bacterial endosymbiont that is required for normal host function and can often be explained as a mechanism to maintain the function initially encoded in a degenerating symbiont or organelle genome (TABLE 1).

Maintenance transfers: enabling the loss of endosymbiont function. All (or most; see below) eukaryotes rely on at least one bacterial endosymbiont, now called the mitochondrion, whereas other eukaryotes rely on an additional bacterial endosymbiont now called the plastid or chloroplast[79]. Extant mitochondrial and plastid genomes are small and encode only a few genes, and organelle function thus requires extensive participation from the host. Many of these host-encoded genes have

Transposable elements
DNA sequences (also known as jumping genes) that can move within a genome (and sometimes also between genomes) by a 'cut and paste' (DNA transposons) or a 'copy and paste' (retrotransposons) mechanism.

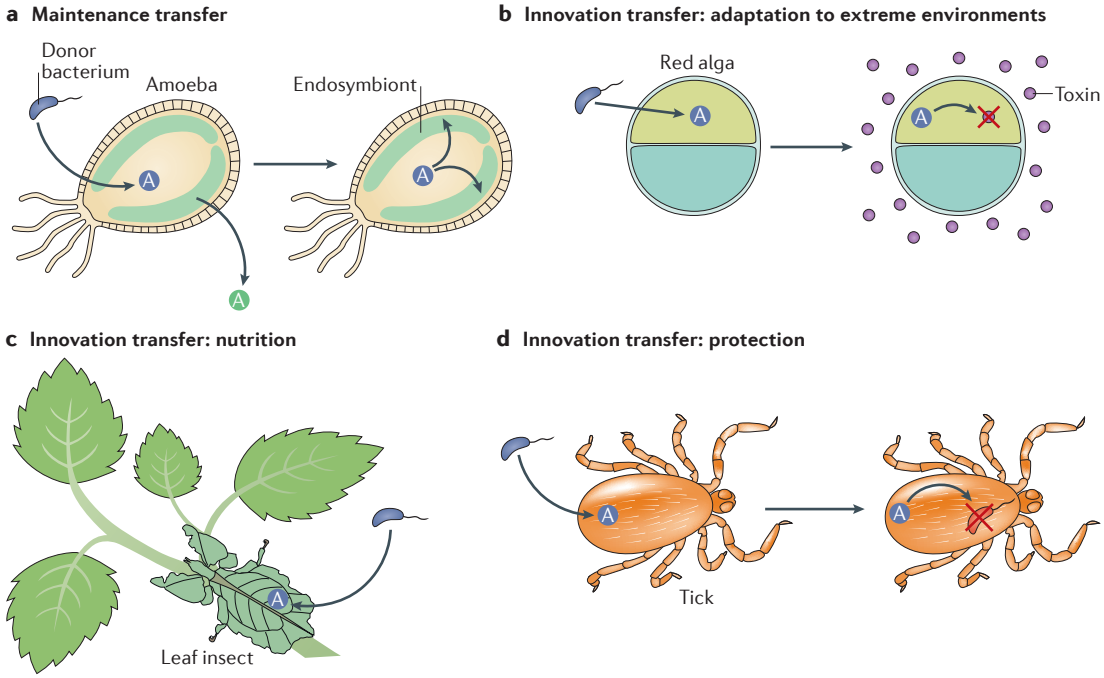

Figure 4 | **Schematic diagrams of maintenance transfers and innovation transfers.** In all cases, a horizontally transferred gene (generically shown as 'A') is transferred from a generic bacterium. **a** | *Paulinella chromatophora* contains a cyanobacterium as an endosymbiont. *P. chromatophora* has horizontally acquired nutrition-related genes that have enabled the loss of genes with overlapping function from its endosymbiont (maintenance transfer). **b** | A horizontally transferred gene has enable the red alga *Galdieria sulphuraria* to survive hot, metal-rich and acidic environments (innovation transfer). The protein encoded by the transferred gene detoxifies a generic toxin. **c** | An innovation transfer has enabled leaf and stick insects to diversify and feed on plant tissue. **d** | The innovation transfer of a gene into ticks has enabled them to protect themselves from pathogenic bacteria (shown in red) by targeting their peptidoglycan layer.

been transferred from bacteria, the protein products of which are transported back into the organelle. As such, maintenance transfer of genes from bacteria has shaped the content of all eukaryotic genomes[11,40,80,81].

However, the origins of these endosymbiont-related horizontally transferred genes are difficult to establish. Although it is not disputed that the endosymbiont that became the mitochondrion was an alphaproteobacterium (and the plastid a cyanobacterium[82,83]), controversy arises because the bacterial horizontally transferred genes that have been found in eukaryotic genomes affiliate with numerous other bacterial groups and not only the Alphaproteobacteria and Cyanobacteria in phylogenetic trees[40,84]. This taxonomic diversity has motivated several hypotheses to explain the data[41,85–90], which fall roughly into two groups. The first group hypothesizes that the endosymbionts that eventually became organelles had highly mosaic genomes owing to HGT among bacteria[40,41]. The second group hypothesizes that the cell that became host to the organelle already had previously acquired genes by HGT before the endosymbiont became fixed[91–94]. These (not mutually exclusive) hypotheses will likely always be difficult to differentiate owing to the antiquity of organellogenesis. Might more recent endosymbioses provide some insight into the possible general mechanisms used to build mosaic metabolic pathways? The advantage of more recent endosymbioses, such as those found in sap-feeding insects[25,32,34,51], is that inferring the origin of transferred genes is much more straightforward because the genes often come from bacteria, such as *Wolbachia* spp., that have extensive germ cell interactions with insects. If phylogenetic analyses of an HGT in an insect genome suggest that the gene came from *Wolbachia* spp., the most parsimonious explanation is that the transfer came from infections with the bacteria sometime in the history of the insect lineage. The disadvantage is that the cell biological and genetic contexts of these symbioses are different than those encountered at organellogenesis.

Paulinella chromatophora (FIG. 4a) is an amoeboid protist that acquired an organelle-like cyanobacterial endosymbiont ~100 million years ago[95]. In this case, only ~25% of its 229 nuclear genes of bacterial origin seem to result from transfer from the cyanobacterial endosymbiont[44]. The remaining 75% taxonomically affiliate with other bacterial groups in phylogenetic tress. Similarly, the incomplete nutritional pathways in some trypanosomatid bacterial endosymbionts are complemented by genes that were horizontally transferred from diverse bacteria to the protist genome[96,97]. Finally, analyses of insect nutritional endosymbioses in aphids, psyllids, whiteflies and mealybugs also show similar patterns of mosaic nutritional pathways that are constructed from multiple HGT events[25,32,34,51]. Many of these transferred genes seem to compensate for genes that have been lost in the genomes of the endosymbionts, but — as in

Organellogenesis
The process by which an endosymbiont becomes an organelle, in part by becoming (nearly) irreversibly integrated with its host cell at both a genetic and cell biological level.

the protist examples above— most of those genes have been transferred from bacteria other than the existing endosymbionts. Importantly, these transferred genes come from 'reproductive manipulators' such as *Wolbachia* spp. and *Candidatus* Cardinium spp., bacteria that are extremely common in insect germ cells. Thus, the transferred genes come not from the nutritional endosymbiont but from endosymbiotic bacteria with germline-cell tropism. Taken together, these results suggest that gene transfer from degenerate (or degenerating) extant endosymbionts to the host is not necessarily needed, or perhaps even common, and in some hosts, HGT events from other sources can compensate for gene loss in the endosymbiont[28]. We argue that these data support the idea that the taxonomic diversity of HGT events in eukaryotic genomes could have resulted from previously existing HGT from non-organelle sources[44,54,84,98–100], probably in combination with the 'inherited chimaerism' of organelle progenitors resulting from bacteria–bacteria HGT events that predated endosymbiosis[40,41].

A final example of a maintenance HGT event comes from the microbial eukaryote called *Monocercomonoides* sp. PS203 (REF. 101). As a member of the eukaryotic supergroup Excavata, *Monocercomonoides* sp. PS203 is related to several microaerophilic or anaerobic parasites, such as *Giardia intestinalis* and *Trichomonas vaginalis*. Both *G. intestinalis* and *T. vaginalis* have highly degenerate mitochondria-related organelles that no longer produce ATP through oxidative respiration but still exist in the cytoplasm as double-membrane-bound organelles. These mitochondria-related organelles provide the only function conserved in all mitochondria and related organelles across eukaryotes: iron–sulfur (Fe–S) cluster biogenesis[102]. However, in *Monocercomonoides* sp. PS203, none of the proteins related to mitochondrial function, including Fe–S cluster biogenesis, could be identified in its genome[101]. Instead, the authors found that a related set of genes involved in Fe–S cluster biogenesis had been transferred from bacteria to the *Monocercomonoides* sp. PS203 genome[101]. These HGT events thus enabled the loss not only of all mitochondria-related proteins but also of the organelle itself[101]. This example highlights an important point about maintenance HGTs: the transfer of a new bacterial gene to the eukaryotic host likely needs to occur before the loss of the ancestral gene.

Innovative transfers: detoxification and protection. The genetic repertoire of multicellular eukaryotes is relatively small compared with that of bacteria. Thus, it is not surprising that eukaryotes have exploited the complexity of bacterial metabolism by acquiring genes to help detoxify novel environments (FIG. 4b). For example, herbivorous eukaryotes must cope with complex mixtures of toxic compounds that are produced by plants[17]. Horizontally transferred genes that are involved in detoxifying cyanide were found in phytophagous mites and various lepidopterans[33]. The beetle *Hypothenemus hampei* can feed exclusively on coffee beans owing to a mannanase that has been acquired through HGT[70]. The silkworm *Bombyx mori* has acquired the ability to circumvent alkaloids in the latex of mulberry plants[103].

Fungi in the genus *Epichloe* are intercellular symbionts of grasses that protect their hosts from insect herbivores by use of a cocktail of fungal alkaloids. This anti-insect arsenal of *Epichloe* fungi seems to be supplemented by the *mcf* gene, which was acquired horizontally by the fungal genomes from a bacterium[104]. It was shown that *mcf* encodes a toxin produced by the endosymbiotic fungi to help kill insect larvae that feed on their host grass plants. Interestingly, the *mcf* gene is also found in the genome of the bacterial symbionts of entomopathogenic nematodes and was shown to be sufficient to kill insects[105]. Thus, HGT from one tripartite interaction (nematodes, bacteria and insects) has possibly altered the interactions in another tripartite interaction (grasses, fungi and insects)[104].

Innovative transfers: nutrition. Plant material represents an enormous amount of the biomass of Earth, and many eukaryotes have evolved to feed on both living and dead plants. Herbivores must not only avoid plant defences but also degrade complex plant carbohydrates, and the genes required for degrading these complex carbohydrates have been acquired in numerous eukaryotic lineages. HGT of bacterial genes involved in carbohydrate metabolism has been found in herbivorous insects[17], rumen ciliates[106], oomycetes and fungi[49,107], plant-parasitic nematodes[46,77,108], necromenic nematodes from the *Pristionchus* genus[109] and rotifers[52]. Conversely, tunicates can synthesize cellulose for their protective exoskeleton (the tunic) because of an ancient cellulose synthase gene that has been acquired horizontally[110].

HGT of the genes encoding biosynthetic enzymes has enabled many eukaryotes to live in extremely nutrient-poor environments (FIG. 4c). Genes involved in amino acid, vitamin and carbohydrate metabolism are perhaps most often transferred via HGT (of note, in cases in which the host has an endosymbiont that provides these nutrients to the host, such transfer events are probably maintenance transfers[28,32,34,73]). Most of the transferred genes found in 13 genomes of unicellular protists involve amino acid and carbohydrate metabolism[12]. For example, the genomes of *Cryptosporidium* spp. contain genes that were transferred horizontally, such as genes encoding tryptophan synthase, aspartate–ammonia ligase and glutamine synthetase[111], and trypanosomatids have acquired numerous horizontally transferred genes involved in arginine, tryptophan, threonine, methionine, cysteine, lysine and vitamin B_5 metabolism from diverse bacterial donors[96,97]. Marine environments can be notoriously poor in nitrogen and iron, and HGT events have had a role in enabling several distinct eukaryotic lineages to scavenge these growth-limiting compounds from their environments. Iron-binding proteins of bacterial origin were found in diatoms[112,113], an extremophilic red alga *Galdieria sulphuraria*[47] and a soil-dwelling amoeba *Dictyostelium discoideum*[114,115]. Nitrogen metabolism was influenced by HGT events, at least in diatoms[76] and green algae from the *Micromonas* genus[116]. In other environments, the limiting nutrient is not that obvious; for example, the acquisition of *N*-acetylneuraminate lyase probably

Reproductive manipulators
Bacteria, such as *Wolbachia* spp., that are transmitted in the egg cytoplasm of arthropods and nematodes and shift the sex ratio of the host population.

enables *T. vaginalis* to scavenge sialic acids from its host for nutrition[117]. The transfer of genes involved in the biosynthesis of amino acids and vitamins from bacteria to animals includes genes involved in the diaminopimelic acid pathway in the choanoflagellate *Monosiga brevicollis*[118], haeme biosynthesis pathway (aferrochelatase) in the nematode *Brugia malayi*[29] and vitamin B_1, vitamin B_5 and vitamin B_6 pathways in the plant nematode *Heterodera glycines*[75,119].

Innovative transfers: adaptation to extreme environments. HGT events have enabled eukaryotes to live in extremely hot, cold and otherwise toxic environments. The transfer of bacterial genes that encode ice-binding proteins, which can enable recipients to survive in extremely cold environments, was found in the diatom *Fragilariopsis cylindrus*[120], the haptophyte *Phaeocystis antarctica*[121] and the green algae *Pyramimonas gelidicola*[121] and *Coccomyxa subellipsoidea*[122]. HGT events that enable recipients to survive hot, metal-rich and acidic environments were detected in the genome of red alga *G. sulphuraria*[47] (FIG. 4b), and transferred genes that enable survival in hypersaline conditions were detected in the genome of *Halocafeteria seosinensis*[123]. HGT events that protect hosts from other sources of environmental stress, such as desiccation and oxidative and osmotic stress, seem to be frequent in rotifer species from desiccating habitats[39]. Likewise, two HGT events seem to protect the microsporidium *Antonospora locustae* from oxidative and ultraviolet light-induced damage[124,125].

Innovation transfers: bactericidal activities. Genes of bacterial origin that target the bacterial cell envelope are commonly found as functional transferred genes in eukaryotic genomes[30,31]. The phenomenon of eukaryotes using bacterial genes to defend themselves against other bacteria has been called 'the eukaryotes strike back' (REF. 126). Bacterial cell envelopes can be disrupted by several mechanisms, but peptidoglycan is a common target (FIG. 3).

An example of eukaryotes acquiring bacterial genes to protect themselves from other bacteria involves a family of amide-bond-breaking genes called type IV secretion system amidase effectors (*tae*)[30]. Tae proteins cleave the amide linkages of peptidoglycan and were domesticated in at least three eukaryotic supergroups (FIG. 3; TABLE 1). Some of these transfers date back to more than 800 million years ago[30]. Importantly, the functional role of these HGT events was experimentally verified, and it was demonstrated that they limit the proliferation of *Borrelia burgdorferi* in the deer tick *Ixodes scapularis*[30] (FIG. 4d). Other examples show that bacterial genes that encode lysozymes, which cleave peptidoglycan between *N*-acetylglucosamine (NAG) and *N*-acetyl muramic acid (NAM) moieties, have also been acquired by eukaryotes such as plants, fungi and insects multiple times independently[25,31,127] (FIG. 3). The antibacterial effect of the horizontally transferred genes was also verified experimentally with a recombinant lysozyme, and the proteins encoded by the genes exhibited broad-spectrum antibacterial action[31]. Perhaps the most astounding toolkit for the disruption of the bacterial cell envelope acquired through HGT was reported for the marine protist *Trepomonas* sp. PC1 (Diplomonadida)[45]. This species clusters deeply within a clade of parasites but seems to have transitioned to a free-living and bacterivorous lifestyle. This nutritional transition is hypothesized to have been enabled by the acquisition of forty bactericidal, permeability-increasing proteins (which bind to lipopolysaccharides), twenty cell wall hydrolases, four *N*-acetylmuramoyl-L-alanine amidases and five lysozymes that now enable the species to feed on bacteria.

Innovation or maintenance: peptidoglycan production. Bacteria-to-eukaryote HGT events include not only genes for peptidoglycan degradation but also genes for peptidoglycan synthesis. Although the functional role of these transfers remains unclear (and thus their classification in the system we use in this Review remains unclear), putative horizontally transferred genes involved in peptidoglycan synthesis have been found to be expressed in bdelloid rotifers[72], aphids[25] and mealybugs[28,51]. Interestingly, in some Archaeplastida chloroplasts, the generation of peptidoglycan seems to also require numerous bacterial genes that were horizontally transferred to the host[128,129]. These genes have been shown to be essential for chloroplast division in both mosses[130] and *Arabidopsis thaliana*[131]. Although a peptidoglycan layer has been found surrounding the chloroplasts of mosses[120], no detectable peptidoglycan layer seems to exist in *A. thaliana*.

A similarly unclear situation exists for animals with obligate endosymbionts that have horizontally acquired amidases[25,28,51]. Amidases are related to peptidoglycan-recognition proteins (PGRPs), which are normally used by the innate immune system of animals as bacterial sensors[132]. Some PGRPs possess amidase activity that can cleave peptidoglycan at positions distinct from Tae proteins. Interestingly, PGRPs have been shown to be involved in endosymbiont maintenance rather than pathogen defense[133]. For example, native amidase-active PGRPs are used by tsetse flies to shield their nutritional endosymbionts from the host immune system by recycling peptidoglycan from lysed endosymbiont cells[134]. Some sap-feeding insects with obligate bacterial endosymbionts have acquired bacterial amidases by HGT[25,28,51], raising the prospect that some of these horizontally acquired amidases are used not as antiacterial control measures by the host but rather for endosymbiont maintenance. In a potentially parallel process, bacterial β-lactamases have been found to have been transferred to the genomes of mealybugs and the slime mould *D. discoideum*[28,114]; β-lactamases are enzymes that provide resistance to β-lactam antibiotics, such as penicillin. Why would eukaryotes need genes targeting bacterial antibiotics? It seems plausible that, similar to PGRPs, these enzymes could be used for symbiont protection rather than defence from pathogens, as both slime moulds and mealybugs are tightly associated with beneficial bacteria[51,135].

Peptidoglycan
A structural matrix in bacterial cell walls formed by alternating *N*-acetylglucosamine (NAG) and *N*-acetylmuramic acid (NAM) residues, where peptide chains of up to five amino acids link NAM to other NAM-connected peptides.

Concluding remarks

Both early and recent misattributions of HGT events in animal genomes have made it clear that care is required when hypothesizing that a gene has been transferred to a genome from an unrelated organism. However, numerous papers reporting HGT events from bacteria to eukaryotes stand on solid conceptual and methodological ground (TABLE 1). Although the field of HGT research is vibrant, there is clearly room for improvements in both the methods used to screen for contamination in genome projects and the methods used to verify putative HGT events (BOX 1). As discussed in this Review, HGT events are used to maintain functionality — often in situations involving an endosymbiont that is subject to genomic erosion — but are also used to acquire new functions, such as altered nutrient acquisition and protection, and to explore new environments. To better understand the true phylogenetic and functional scope of bacterial genes in eukaryotes, a focus on phylogenetically important but neglected groups such as protists is warranted, as the majority of eukaryotic diversity resides in these understudied organisms.

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This comprehensive analysis of HGT events in Microsporidia and Cryptomycota fungi detects dozens of novel HGT candidates, including parallel acquisitions that enable these pathogenic fungi to scavenge nucleosides and nucleotides from their hosts.

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