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Review article

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Horizontal gene transfer in eukaryotes: aligning theory with data

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Abstract

Horizontal gene transfer (HGT), or lateral gene transfer, is the non-sexual movement of genetic information between genomes. It has played a pronounced part in bacterial and archaeal evolution, but its role in eukaryotes is less clear. Behaviours unique to eukaryotic cells — phagocytosis and endosymbiosis — have been proposed to increase the frequency of HGT, but nuclear genomes encode fewer HGTs than bacteria and archaea. Here, I review the existing theory in the context of the growing body of data on HGT in eukaryotes, which suggests that any increased chance of acquiring new genes through phagocytosis and endosymbiosis is offset by a reduced need for these genes in eukaryotes, because selection in most eukaryotes operates on variation not readily generated by HGT.

Sections

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Introduction

Horizontal gene transfer (HGT), or lateral gene transfer, refers to the movement of genetic information between organisms outside the context of sexual recombination, including transfers between distantly related species. The process has been known for decades[1], and it has long been understood to have played a major part in genome evolution[2–4]. Indeed, the degree to which HGT has impacted many bacterial and archaeal genomes is so substantial that some researchers have questioned if a tree of life is even an appropriate metaphor for evolutionary relationships, suggesting a tangled web might better represent how organisms are related[2,5].

Most of what we know about the prevalence, mechanisms and functional and evolutionary impacts of HGT comes from bacterial and archaeal genomes[6–11]; how eukaryotic genomes are affected by HGT has been more of a debate[12–16]. This lack of clarity is partly down to a scarcity of data: despite the fact that the first nuclear genome was published only 1 year after the first bacterial genome[17,18], nuclear genomics has lagged behind that of bacteria and archaea. The reasons for this are obvious: eukaryotic nuclear genomes are relatively large and complex, making their sequencing, and especially their bioinformatic analysis, much more difficult. Moreover, understanding patterns of HGT requires many genomes from diverse taxa, but eukaryotic genomics has been severely biased towards a few lineages (animals, fungi and plants), and even among the vast diversity of 'other' eukaryotes, the biases extend to strongly favour the relatively small genomes of parasites and algae[19,20].

Despite these obstacles, the body of genomic data from diverse eukaryotes has grown substantially in recent years owing to advances in sequencing methodologies, particularly culture-free genomics methods, and a greater appreciation of the evolutionary diversity of eukaryotes. It is now clear that HGT in nuclear genomes is not only different from what we see in bacteria and archaea but perhaps also different from what we might have predicted based on influential ideas about eukaryotic genome evolution. Eukaryotes differ from bacteria and archaea in many ways, but one particularly important difference is that they 'eat' other cells by phagocytosis and sometimes retain them as endosymbionts. It has been argued that both these characteristics should increase the frequency of HGT and, as I will show throughout this Review, these ideas have had a deep impact on how nuclear genomes have been interpreted. Although these hypotheses are intuitively appealing, it now seems that they do not actually fit observations from nuclear genomes and that some commonly held assumptions are likely false.

This Review will frame what we know about HGT in eukaryotes in the context of their biology, beginning with a reminder of the major differences in eukaryotic biology that might have affected HGT, and summarizing the data on the effects of HGT on nuclear genomes and eukaryotic biology. I will then put this into context by arguing that we have put too much emphasis on aspects of this biology that affect the exposure of a genome to foreign genes and not enough on whether selection is likely to favour acquiring such genes. The same argument also applies to how endosymbiosis-affected HGT, which I will argue to be similarly misinterpreted.

HGT in eukaryotes

Foundational ideas about the effects of HGT on genome evolution have been based almost entirely on data from archaea and bacteria[2], which saddles our interpretation of eukaryotic genomics with theoretical baggage that was developed to explain data from organisms with very different genetics, cell biology, evolution and ecology. Genomic sampling of the wider diversity of eukaryotes is still poor, but it has improved to the point that some clear patterns have emerged and, perhaps not surprisingly, HGT in eukaryotes is somewhat different from what we see in bacteria and archaea.

Eukaryotic cells and genomes are different from those of archaea and bacteria

That eukaryotic cells and genomes are organized very differently from those of archaea and bacteria is textbook biology. But, like most textbook biology, the actual situation is more complex and nuanced than it might first appear (Fig. 1), so it is worth reviewing a few of these differences, because genomic and cellular characteristics impact one another in complex ways.

At the genomic level, eukaryotes are distinguished from bacteria and archaea by their compartmentalized genomes, with major differences in how both chromosomes and genes function. The principal genome in all eukaryotes is the nuclear genome, and it is characterized by both its structure and content, with large numbers of genes and gene family expansions, all organized on multiple linear chromosomes. The mitochondrion and plastid contain their own genomes derived from the bacterial endosymbionts that gave rise to these organelles[21]. As will be discussed in detail later in this Review, these endosymbioses are a major focus of our thinking about HGT in eukaryotes, but the organelle genomes themselves are, with some exceptions[22–27], HGT deserts and will not be discussed in much detail here.

At the cellular level, the nucleus is 'officially' the defining feature of eukaryotes, but their dynamic cytoskeleton and endomembrane systems are probably better distinguishing features; indeed, the nucleus is really just one small extension of the endomembrane system (Fig. 1). Distant homologues of several key cytoskeletal proteins are present in both bacteria and archaea[28–32], but whether structural or functional equivalents of the cytoskeletal system as a whole exist outside eukaryotes is another question. By contrast, all eukaryotes use the cytoskeleton and endomembrane systems to actively control and change the shape of the cell, whether or not they have secondarily re-adopted the use of a wall for added structural stability. However, perhaps the most important emergent property of these systems is their ability to change the cell shape sufficiently to engulf other cells, an action known as phagocytosis. Among bacteria, a single planctomycete has been proposed to engulf other cells, but not through a process homologous to phagocytosis, and this does not represent a general property of bacteria[33]. The Asgard archaea might represent a more important exception: their genomes include larger numbers of cytoskeletal and endomembrane protein-coding genes than bacteria, and the one cultured representative of this group has a complex morphology consistent with an active cytoskeleton[30–32]. If Asgard archaea also prove to use a homologous (if rudimentary) form of phagocytosis, it substantially changes how we view the evolution of this process, given that this lineage is also one of the closest known relatives of eukaryotes[34]. Even so, the last common ancestor of eukaryotes was clearly already quite distinct from these or any other bacteria or archaea in possessing a very complex and dynamic cytoskeleton capable of highly coordinated and controlled phagocytosis. It most likely relied on phagocytosis for its main sources of energy and nutrients (that is, it was likely a heterotrophic phagotroph), and this property remains conserved in the vast majority of eukaryotes today and defines the trophic mode of much of eukaryotic diversity[35].

This ability to 'eat' other cells by phagocytosis would also would have facilitated endosymbiosis: the uptake and retention of an

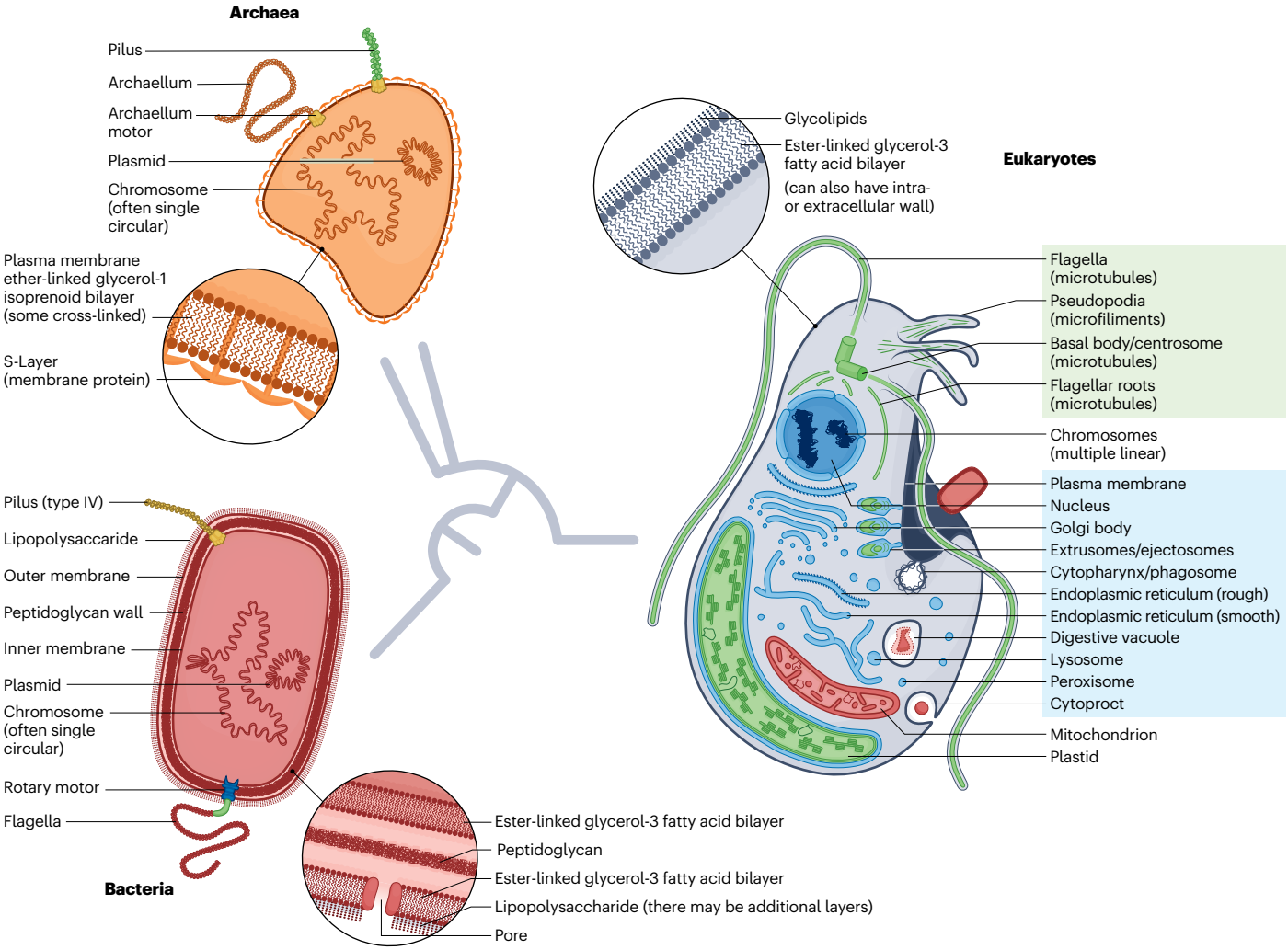


Fig. 1 | Differences between eukaryotic, bacterial and archaeal cells. Schematic diagrams of cells representing the three domains of life, bacteria, archaea and eukaryotes, reflecting their fundamental differences in cellular structure. In the centre of the figure is a tree showing the relationships of these three cell types (the archaea are shown as paraphyletic, and the Asgard archaea are not represented in the cell diagrams, because we still know relatively little about their structure). For each group, distinguishing cellular features common to most members of each group are shown, with the cell envelope blown up to show detail. Whereas bacterial and archaeal cells typically rely on an external skeleton (that is, a rigid layer based on proteins or peptidoglycans) for cellular integrity, eukaryotes typically have a flexible envelope supported by an internal cytoskeleton (component names clustered and boxed in green). Some eukaryotic lineages augment this envelope with a rigid wall, but most do not, and those that do retain the cytoskeleton. This flexibility allows the cytoskeleton to work with the endomembrane system (component names boxed in blue), a complex machinery that sorts and traffics membranes, to give eukaryotic cells a degree of controlled morphological plasticity that is not possible in bacteria or archaea. The cytoskeleton and endomembrane systems together account for most of the important structural elaboration within eukaryotic cells, and components of both (often working together) have been repurposed in many ways to create a multitude of diverse structures and functions. One overarching outcome of these systems is the ability to surround and engulf (phagocytose) other cells, on which eukaryotes can feed, by controlling the trafficking of these cells and of digestive vacuoles inside the cell. Sometimes cells inside a eukaryote are not digested immediately, becoming endosymbionts instead. The mitochondrion and plastid are the most famous of these endosymbionts, but many others exist.

'endosymbiont' cell within a 'host' cell. This is a time-dependent definition, because the ultimate fate of many endosymbionts is digestion, so the line between 'food' and 'endosymbiont' can be blurry, but many endosymbionts are long-term inhabitants that undergo cell division and growth within the host. They can be bacteria, archaea or eukaryotes, but the host is virtually always a eukaryote[36]. Again, there are proposed exceptions[37], but these are not only rare but also questionable comparisons, because the bacterial 'host' also lives in a eukaryote and likely cannot make its own membrane, instead using one from its eukaryotic host[38].

Of course, the most famous and well-studied endosymbionts are the mitochondrion and plastid organelles, both of which are ancient and genetically integrated with their host through massive gene transfer and protein targeting[39]. This genetic integration has given

these organelles an outsized role in our thinking about HGT and how it affected the origin of eukaryotes. Indeed, phagocytosis, endosymbiosis and HGT collectively dominate an ongoing and lively debate about how eukaryotes arose[40–50]. This Review will focus on processes whose presence in the last common ancestor of all eukaryotes is not disputed, making it equally consistent with different views of the debate.

Evidence for HGT to the nucleus

A disproportionate amount of genome data currently comes from animals, and progress towards a clear understanding of how HGT has affected their genomes has been side-tracked by two widely publicized cases in which HGT in animals was massively overestimated[51,52] (Box 1), overshadowing more moderate claims. Nevertheless, over time, a great number of cases of HGT backed by solid evidence has accumulated across the tree of eukaryotes, including animals[16,53–63]. The proportion of HGTs observed to date in eukaryotes is, however, many times lower than that inferred in bacteria and archaea. Most analyses, meta-analyses and studies across diverse groups consistently conclude that, at most, only a few percent of genes in a nuclear genome are detectably derived from HGT, which is several-fold lower than in most bacterial and archaeal genomes. These studies mostly focus exclusively on transfers from bacteria, because they are more easily identified (too few diverse nuclear genomes are available to be as certain of eukaryote-to-eukaryote transfers), but this means they underestimate the true number of total HGTs. These studies also tend to focus on relatively recent transfers, which are easier to identify but also potentially more frequent[64]. The lack of ancient transfers suggests that HGTs have not massively accumulated over evolutionary time, and it has been offered as evidence that HGT does not happen in eukaryotes at all[13,65]. This argument has been countered on methodological grounds[14], but it is also possible that this observation should have been the expected outcome given constant, low-frequency HGT, because most acquired genes would convey transient benefits and would later be lost, once conditions had changed, as has long been observed in bacteria[66,67].

Many cases of eukaryotic HGT come from lineages that have adapted to a new niche and acquired genes that have an obvious functional link to that ecological transition. For example, anaerobic protists acquired new metabolic pathways, often seemingly from bacteria that had already adapted to these environments[68–75]. Other adaptive acquisitions include denitritrification[76], carbohydrate degradation[77] and thermal adaptation[56,78]. The adaptation to parasitism has also been linked to HGT[54,79–84], as has defence against parasites and predators[85,86]. In these examples, the genomes are not particularly rich in HGTs overall but instead contain a small number of functionally important HGTs that impact key pathways. These events can be seen to represent short but functionally important pulses of HGT that occurred as a eukaryote made a major ecological transition, often involving altered metabolism.

These data collectively offer compelling evidence that HGT affects nuclear genomes in important ways, but at a low frequency. However, the data remain frustratingly anecdotal, because different methods are used to examine each genome. The poor sampling of eukaryotic genomic diversity restricts many studies to looking for the most obvious bacterial genes in the nucleus, and even then the use of different methods makes it hard to compare data across studies: more studies that compare HGT with consistent parameters across many lineages are needed[16,53,62,64]. Another major gap in our knowledge is mechanism: we conclude HGT happened, and sometimes plausibly explain why, but we almost never know how it happened. There are some exciting new insights into mechanisms, including agents that actively transform eukaryotic hosts[58], transposable elements that may mediate transfers[87], extracellular membrane vesicles that transfer genes between cells[88,89], potential contact mechanisms such as tunnelling nanotubes[90], a variety of plausible mechanisms specific to plants[91] and, of course, viral transduction[92–95]. These mechanisms are potentially important conduits for gene flow to the nucleus, but they have not been examined very thoroughly. By contrast, one mechanism has been repeatedly evoked and has greatly impacted theory and interpretation: eating, and, by extension, endosymbiosis.

Chance and necessity

Even in bacteria and archaea, in which HGT is said to be common, it is actually rare relative to vertical inheritance of ancestral genes, because a chain of unlikely events has to take place in the correct order for a transfer to be successful. The gene has to get into the new cell, integrate into the genome and be expressed; the protein has to be folded correctly and may have to be targeted to a specific location in the cell. We often intuitively think of the likelihood of gene transfer as dependent on factors that might increase or decrease the frequency of these chance events. For example, increasing the exposure to foreign genes by eating other cells or taking them up to reside in the cytoplasm may indeed increase the likelihood of acquiring some of those genes, as compared with an otherwise identical situation in which the cell is not exposed to them at all. Similarly, organisms with a high rate of non-homologous recombination and/or genomes with a large fraction of non-essential DNA may be more likely to have foreign DNA inserted into their chromosomes with higher frequency or without deleterious effect, as seems

Box 1

HGT and animals

Although animals are disproportionally represented in currently available data from nuclear genomes, our view of horizontal gene transfer (HGT) in animals is particularly unclear, in part owing to dramatic reports of high levels of HGT that proved to be wrong. The first example comes from the human genome, which was remarkably twice claimed to encode scores of foreign genes[51,210], and in both cases this conclusion was quickly demonstrated to be the result of methodological artefacts[211,212]. The second case comes from tardigrades, famously cute microbial animals that were reported to have one-fifth of their genes derived from HGT[52], again owing to methodological errors[213–215]. These high-profile mistakes cast a shadow on all reports of HGT in eukaryotes[13], deservedly or not. Certainly, they are a call to examine the methodology carefully and err on the side of caution, but they are not evidence against any other cases reported using more stringent methods. Indeed, a third animal lineage also stands out among eukaryotes: the genomes of bdelloid rotifers, an unusual group of microscopic asexual animals, were also reported to contain an exceptionally high proportion of HGTs[216]. This claim, however, has been shown to be true for successive genomes and has stood up to re-analysis[217,218], and the debate has moved on to examine why this is the case, with disputed suggestions that HGT might substitute for genetic variation normally supplied by sex or aid the adaptation to desiccation[219–222].

to be the case with dinoflagellate protists, for example, in which large numbers of both reverse-transcribed mRNAs and fragments of viruses have been inserted into chromosomes[95,96]. However, increasing the frequency of these chance events that are requirements for successful HGT only leads to a higher frequency of successful transfers over evolutionary time if a last, critical condition is also met: the genes must be beneficial and spread through the population, ultimately leading to fixation. Focusing on chance alone misses the role of necessity, with potentially misleading results.

For some gene transfers, this unlikely series of events can be driven forward by an evolutionary ratchet: given the vast number of opportunities over long periods of evolutionary time, even unlikely events become inevitable, if they are a one-way street[97]. Although this is theoretically true, it probably has vastly different effects on different kinds of genes, because the conditions most likely to evolve in a ratchet-like way are not always those most likely to be driven by selection. For example, horizontal transfers that replace essential genes with a functional equivalent are the most likely to evolve in a ratchet-like manner, because they are the least prone to reversion to the ancestral state: the original gene is lost but the function remains essential. These cases are also the most dependent on drift rather than selection for fixation, because the genes are probably at best functionally equivalent rather than beneficial. Most transfers that introduce new functions are unlikely to be beneficial, but if they are, they will be more likely to be fixed by selection. However, they are also more reversible, given that the new function cannot be essential in all contexts or it would have been ancestrally present. Although selection may push these to fixation, they will not necessarily accumulate in a ratchet-like process, because most non-essential functions are transient in evolutionary time: when conditions change and selection drops off, they are more likely to be lost without consequence, so even successful transfers are often transient over evolutionary timescales[66,67]. The number of genes that fulfil both conditions must be very small: they must represent non-essential but extremely beneficial functions that are difficult to lose once gained. One avenue that could make successful transfers more likely is hitchhiking of genes with neutral or redundant functions with linked genes that have highly beneficial functions. Examples would include several biosynthetic functions, beyond respiration and photosynthesis, which came into eukaryotic cells with mitochondria and plastids. They were likely redundant but retained owing to selection on other functions of the organelle and its compartmentalization, and over time might have allowed the loss of ancestral host functions, making them essential. It has been shown in plants that functionally neutral genes derived from HGT have hitchhiked through a population owing to linkage to a beneficial gene acquired in the same transfer, and later acquired a beneficial function in a new context, leading to their further spread[98].

For most HGTs in the nuclear genome, however, the role of chance has dominated the discussion, and overall the ways that phagocytosis and endosymbiosis might increase HGT have been dominant themes. However, considering necessity turns this picture upside down.

You are what you eat

That the ability to eat other cells and take up endosymbionts both increase exposure to foreign genes is beyond dispute: foreign genes are probably taken up into the endomembrane system of eukaryotes billions of times per millisecond in modern eukaryotes. In endosymbiosis, eukaryotes take up and keep whole cells: populations of these endosymbionts are growing, dividing and occasionally lysing in millions of hosts across the tree of eukaryotes[36], presumably almost constantly bathing the host genomes in endosymbiont DNA.

A model for how this might affect HGT in eukaryotes was formally elaborated in the 'you are what you eat hypothesis'[97]. Briefly, the constant exposure of eukaryotic genomes to foreign genes acquired though phagocytosis and endosymbiosis sets up an evolutionary ratchet that was proposed to lead to an inevitable replacement of genes and addition of new functions from their food. This idea has been highly influential, probably because it is so intuitive: bathing the inside of your cell with foreign genes must increase the frequency of HGT. Indeed, we can actually measure the frequency of organelle DNA transfer to the nucleus in laboratory timescales[39,99–102], and massive amounts, including large fragments and even whole genomes of both organelles and endosymbionts, have been found integrated into host nuclear chromosomes[103–105]. However, nearly all of this DNA is non-functional[59]: the actual frequency of functional transfers to nuclear genomes is lower in most eukaryotes than it is in bacteria and archaea, which do not engulf and internalize foreign cells.

There is a disconnect between the intuitive expectation and the data: genomics do not support the idea that any potential increased opportunity to acquire new genes through feeding or endosymbiosis led to an actual increase in the frequency of HGT. One possible explanation is that phagocytosis increased the chance of HGT but obviated its necessity.

Why phagocytosis should reduce HGT

The key to understanding the disconnect between what is observed and what was predicted with respect to feeding and HGT is to consider that eating other cells changes the kinds of genetic variation that selection operates on most effectively. Bacterial ecology is frequently described in terms of metabolic networks and how individual species fit into those networks by virtue of the enzymes they express and how they can make use of resources within the network[106–109]. Metabolic enzymes are highly modular in nature; carrying out simple reactions on specific substrates, their core function is relatively independent of their cellular context. Promiscuous enzymes can mediate 'underground metabolism' that makes enzyme acquisition more advantageous and increases connections in these networks[110]. Metabolic pathways are themselves also relatively modular; the ecological impact of a metabolic process within a network is more important than which cell or species is carrying out that process, and metabolic reactions and pathways can be plugged into networks in different ways[108,109]. Overall, the cellular context is not as important as the broader metabolic context for whether the possession of a certain enzyme will be beneficial to a cell or not. As a result, selection on bacteria and archaea would be expected to operate on variation in the form of the presence or absence of enzymes and pathways, and this kind of variation is easily acquired by HGT: genes for enzymes or operons with whole pathways can be readily acquired from neighbouring cells and can be immediately beneficial if they give access to new sources of energy or nutrients (Fig. 2). This is not to say that selection does not work on other kinds of variation (such as structural or sensory variation) or that enzymes do not evolve by other means (such as gene duplication or mutation), but it is a major factor in bacterial ecology and evolution, and one that is extremely well-suited to HGT.

Most eukaryotes do not work this way. The advent of phagotrophy allowed the cell to internalize and use diverse packets of food, which to some extent unplugged them from the metabolic network surrounding them by turning what were formerly competitors into food. This also changed the kind of variants that would be most favoured

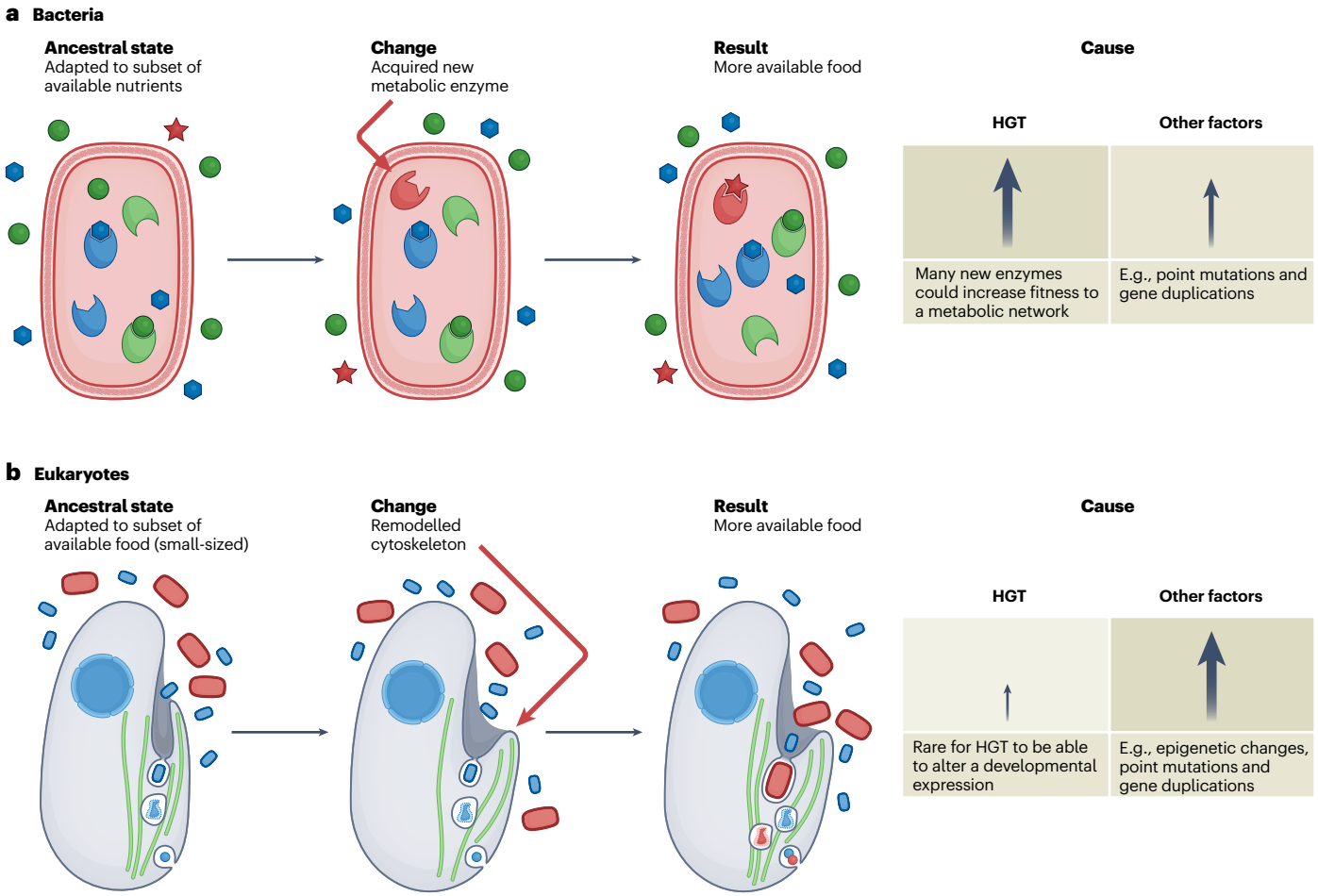


Fig. 2 | Ecological strategies that promote or suppress horizontal gene transfer. The kinds of variations most likely to be selectively advantageous in bacteria are different from those of eukaryotes, because these organisms rely on completely different feeding strategies. **a**, Bacteria feed primarily by extracellular heterotrophy, and the success or failure of cells in a population to reproduce is greatly affected by the metabolic enzymes and transporters they express, and how they fit into the metabolic network of their environment. The acquisition of a new metabolic capacity that allows them to access a new pool of energy or nutrients will be advantageous (in this case, acquiring an enzyme to digest the red, star-shaped molecules), and this kind of variation is readily available by horizontal gene transfer (HGT) in the form of genes for metabolic enzymes or transporters that already evolved to serve the same function in another cell. **b**, In eukaryotes, feeding by phagocytosis can afford access to new sources of energy or nutrients, primarily via changes to the eukaryotes' cellular shape and behaviour (in this example, by evolving a larger mouth to feed on larger prey). These kinds of variations are not readily available by HGT and arise instead through changes such as modified expression networks that affect developmental pathways.

by selection, because a cell's ability to hunt down, capture and engulf prey is not mediated by metabolic enzymes. Instead, it is mediated by cellular structure and the active behaviours those structures impart[111]. We would never believe that a cheetah evolved to run fast by eating other fast animals and acquiring their 'fast' genes: we know that cheetahs evolved speed by modifying developmental pathways that altered their body plan and the behaviours imparted by their physical characteristics. The same is fundamentally true for other eukaryotes at the cellular level: a heterotrophic flagellate does not evolve the ability to eat larger cells by acquiring 'big mouth' genes through HGT. Such variation comes from changes to regulatory cascades that affect cellular development pathways, and these changes come in the form of gene family expansion, mutations to coding or regulatory regions or epigenetic effects (Fig. 2).

When eukaryotes evolved the ability to eat other cells, it may have increased the chances of acquiring new genes in their genome, but because it fundamentally changed the kind of variation required by selection to drive adaptation, it also undercut the necessity for most such genes. Without a beneficial function driving selection, most genes acquired from food would simply be lost, resulting in a lower frequency of functional HGTs.

Exceptions prove the rule?

Just as bacterial and archaeal evolution is not solely driven by HGT, eukaryotic evolution has still been affected by HGT to a point, just not as extensively as bacterial and archaeal evolution. That for many eukaryotes selection operates on variation not readily available through HGT is evident in the many case studies mentioned earlier (see Evidence

for HGT to the nucleus), which often reveal that a small number of key genes are acquired during an ecological transition, such as adaptation to some new environment. However, it is also evident from the variation between different lineages in their tendencies to acquire genes.

This is most obvious in fungi, for which comparative genomics has shown HGT to be particularly prevalent[112–115]. This is consistent with the idea that the typically eukaryotic dependence on structure and behaviour downplays the need for HGT, because, in the context of metabolism and feeding, fungi are very 'bacterial-like' eukaryotes. They are incapable of phagocytosis and instead feed by extracellular heterotrophy (such as saprotrophy), and, similarly to bacteria, they are amazingly versatile metabolically and often fit into ecological metabolic networks in ways akin to bacteria and archaea. As a result, the kinds of variation most favoured by selection in fungi also seem to be easily acquired by HGT, and indeed many fungal HGTs affect metabolism and metabolite transport[71,114,116–119]. Consistent with this idea, a distantly related protist group, the oomycetes, are so fungus-like in their appearance and dependence on extracellular heterotrophy that they were once thought to actually be fungi, and they too have been reported to have acquired a substantial number of HGTs related to saprotrophic feeding, and many of these genes were acquired from fungi[120–124].

Another interesting case is plants[91,125–130]. These organisms generally do not feed by phagocytosis and are instead autotrophs that acquire energy and nutrients by photosynthesis and absorption. HGT in plants was initially under-appreciated, but evidence has steadily accumulated that a substantial number of foreign genes affecting several key systems have been acquired during plant evolution. The scale was first clear in organelle genomes, which normally do not acquire genes from HGT at a high frequency, but they do so in some plants[22,131–135]. In the nuclear genome, HGT has also been observed at a relatively high frequency, and some ancient events persist across a wide diversity of plants[136–138]. Acquired genes have been found to affect many core physiological systems, including photosynthesis[128–130,138–146], as well as key structures, signalling and developmental pathways[138,145,147–150]. HGT is also particularly prevalent in conflict management: HGT has impacted functions of both pathogenic plants and plant pathogens, as well as defence mechanisms against pathogens and consumers, in which both plants and their many enemies have used HGT in an endless cycle to gain the upper hand[84,127,151–157].

Overall, these exceptions are consistent with the idea that the frequency of HGT in eukaryotes is a function of sometimes duelling likelihoods of getting new genes versus needing them, and that in most lineages need plays the more important role.

Endosymbiosis is not a special case

Endosymbiosis famously gave rise to mitochondria and plastids in the ancient past (the 'endosymbiont hypothesis')[158,159], but it less famously is ongoing today: a huge variety of eukaryotes harbour an even greater variety of endosymbionts with functional and evolutionary impacts beyond our current knowledge[36]. The uptake and retention of a cell within a cell can be viewed as a major adaptive shift for both partners, and, not surprisingly, HGT plays a part, as it does with other adaptive shifts in eukaryotes described above. However, theory has outstripped data in explaining the effects of HGT on organelle and endosymbiont biology, and, as a result, we currently rely on untested or unreliable but deeply held assumptions to ground many expectations. Moreover, the concept that HGT frequently does not provide the kind of variation for selection to act on most effectively in eukaryotic cells also applies to genes acquired through endosymbiosis, but it is complicated by the genetic integration of endosymbionts and their hosts. Below, two common characteristics of this genetic integration are examined in light of existing nuclear genomic data: the quantity of nuclear genes transferred from endosymbionts and the origin of genes encoding endosymbiont-targeted proteins.

Endosymbiotic gene transfer

A critical corollary to the endosymbiont hypothesis for the origin of mitochondria and plastids[21,160] is that genes must have moved from the endosymbiont to the host nucleus, and their products were targeted back to the organelle[39,161,162]. This has been repeatedly demonstrated to be true for both plastid and mitochondrial genes, and these cases of HGT are called endosymbiotic gene transfers (EGTs).

The evidence for EGT is incredibly strong. In the case of plant mitochondria, for example, many plant species have been found to have some nucleus-encoded, mitochondrion-targeted protein that is highly similar to homologues still encoded in the organelle genome of closely related species[163–165]. Recently transferred genes also reveal details about how proteins adapt to existing trafficking machinery, and, in some experimentally tractable systems, these events can be dissected in the lab[166–169]. Taken together, such studies provide compelling evidence for recent gene transfer to the nucleus and offer glimpses into possible mechanisms to overcome the many hurdles a successful transfer faces.

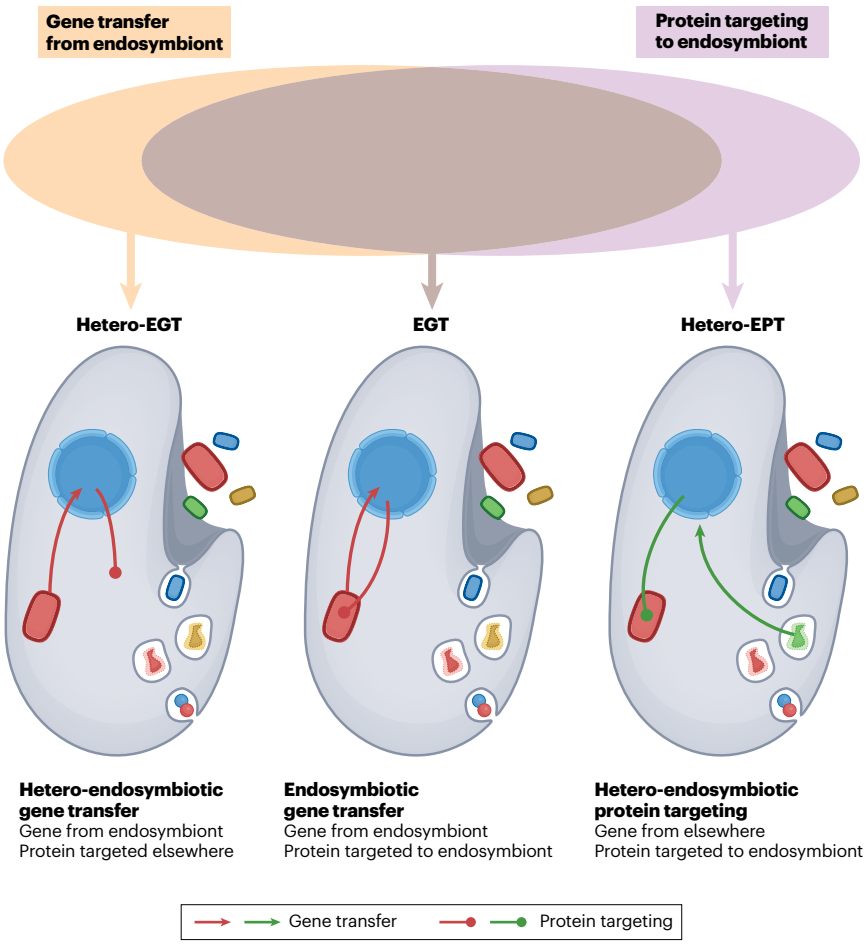
The demonstration that the nucleus acquired many such genes from the organellar endosymbionts reinforces the intuitively appealing idea that the host had plenty of opportunity to acquire other genes as well. It has long been assumed that this increased chance translated into a major impact on the gene content of the nuclear genome, with endosymbiosis opening the door to a major influx of genes encoding endosymbiont-derived proteins that found some new function in the cytosol[39,170]. The protein products of these gene transfers do not, by definition, function in the same compartment from which they originated, but they are also referred to as EGT, which obscures the fact that they are quite different from the canonical EGTs originally envisioned as proteins that are targeted back to their source endosymbiont[162]. This vague terminology has long confused discussion of EGT, so here three different terms are proposed to distinguish three different processes and their outcomes (Box 2): EGT is retained for its original meaning, in which an endosymbiont-derived protein is targeted back to the same endosymbiont; hetero-EGT is used when an endosymbiont-derived protein is acquired by the host but not targeted back to the endosymbiont; and hetero-EPT (for hetero-endosymbiont protein targeting) is used when proteins are targeted to an endosymbiont, but they are derived from somewhere else.

Early analyses of nuclear genomes from plants and yeast concluded that a large proportion of genes was derived from the cyanobacteria plastid endosymbiont and alphaproteobacterial mitochondrial endosymbiont, respectively[171–173]. Many of these genes corresponded to organelle-targeted proteins (EGT), as expected, but a substantial number were proposed to encode proteins now functioning in the cytosol (hetero-EGT). Probably because this idea is so intuitively appealing, the notion that hetero-EGT greatly impacted nuclear genomes became a deeply held assumption within the field and guided the formulation of further ideas without being repeatedly re-tested as more genomes and better analytical methods became available. For example, the assumption that endosymbiosis should lead the host to possess substantial numbers of hetero-EGTs is used to argue against the existence

Box 2

Clarifying the concept of endosymbiotic gene transfer

The concept of endosymbiotic gene transfer (EGT) originally referred to genes that moved from an endosymbiont to the host genome and whose products were targeted back to the same endosymbiont; this term was coined to explain the presence in the nucleus of genes encoding proteins that seemed to be from the organelle and function there[162]. Since then, the meaning of the term EGT has been expanded to refer to two other situations: genes from the endosymbiont whose proteins are targeted elsewhere and genes from elsewhere whose products are targeted to the endosymbiont. These genes have very different histories and implications, so there is growing need to clarify the terminology. The problem stems from EGT relating to two different processes: gene transfer and protein targeting. A Venn diagram of these processes (see figure) shows a massive overlap corresponding to the original concept of EGT, and here I propose to restrict this term to this concept: genes derived from an endosymbiont whose products are targeted back to that same endosymbiont (intersection of the Venn diagram, coloured grey in the figure). I propose to call hetero-EGT the transfer of genes from an endosymbiont whose products are targeted elsewhere in the cell, because they are still gene transfers from the endosymbiont, but they are heterologously targeted (coloured orange in the figure). I propose to call hetero-EPT (for hetero-endosymbiont protein targeting; coloured purple in the figure) the process in which proteins encoded by genes that are not from the endosymbiont are targeted to it, because they represent endosymbiont protein targeting, but the relevant genes are heterologously derived (for example, from the host nucleus, some other endosymbiont or some food). With these terms, we can distinguish very different evolutionary histories and effects that are currently vaguely referred to using the same term.



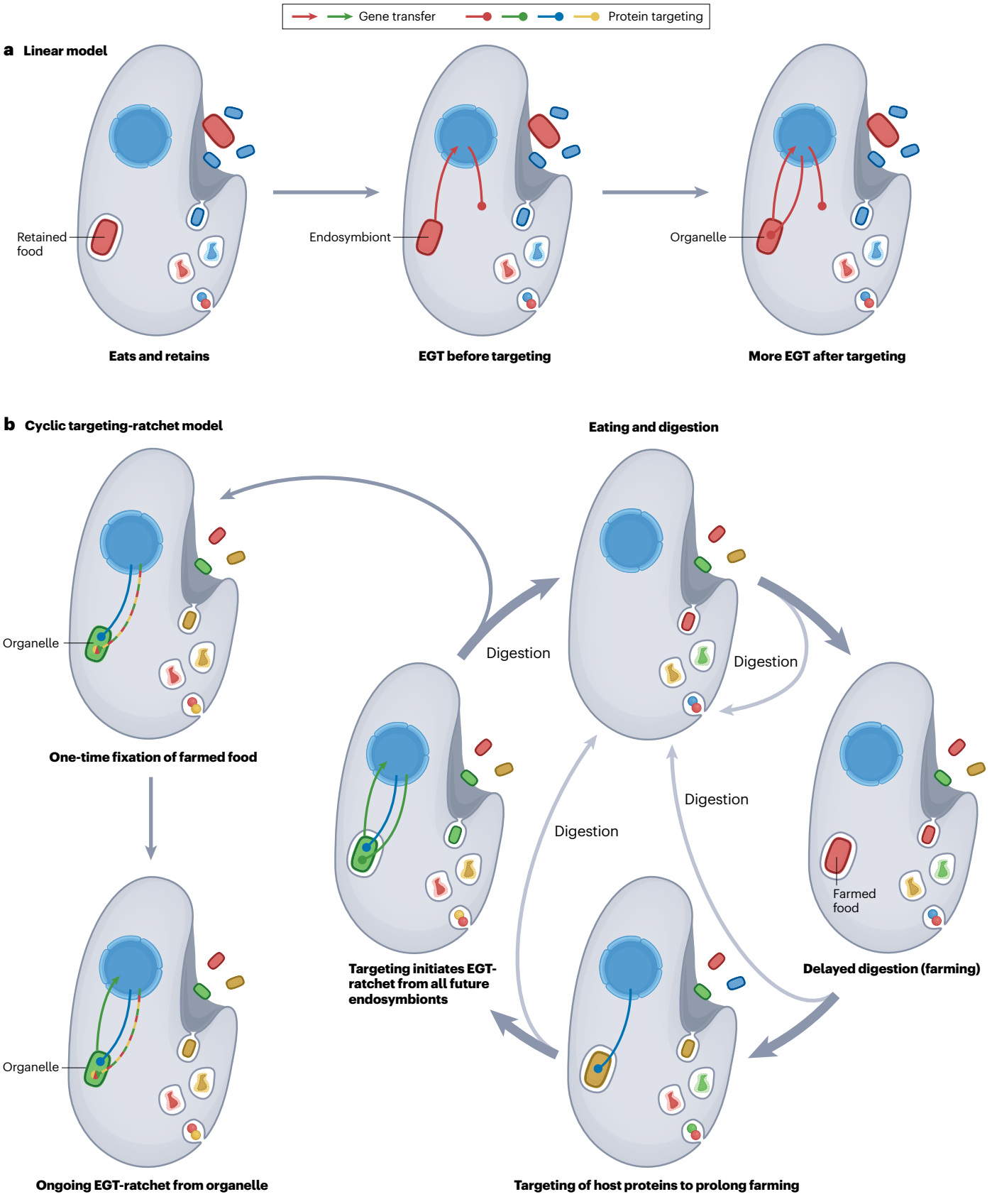

Gene transfer
Protein targeting
a Linear model
Retained food
Endosymbiont
Organelle
Eats and retains
EGT before targeting
More EGT after targeting
b Cyclic targeting-ratchet model
Eating and digestion
Organelle
One-time fixation of farmed food
Digestion
Digestion
Digestion
Digestion
Farmed food
Targeting initiates EGT-ratchet from all future endosymbionts
Delayed digestion (farming)
Organelle
Ongoing EGT-ratchet from organelle
Targeting of host proteins to prolong farming

Fig. 3 | Endosymbiotic gene transfer in early organelle evolution. Genetic integration and fixation in the cell are hallmarks of the transition from endosymbiont to organelle, but how the order and nature of these events is modelled frequently does not fit the data. **a**, Endosymbiotic organelles are typically portrayed as first being taken up and retained (by eating or through some close physical association), and that genetic integration follows as a result of this intracellular association[39,204]. Because the endosymbiont is fixed in the cell from the outset, this model predicts organelle-targeted proteins should virtually all be derived from the endosymbiont itself by endosymbiotic gene transfer (EGT). In this model, EGT to the host is possible before their protein products can be targeted back to the organelle, which has been used to suggest that the nucleus should contain a large number of genes derived from the endosymbiont, which are not targeted to the same endosymbiont (hetero-EGT)[39]. **b**, A contrasting model reverses this order of events, so that genetic integration precedes the fixation of the actual endosymbiont in the cell[206,207]. In the first stage, an ordinary cycle of uptake and digestion is prolonged by delayed digestion or 'farming' by the host. Selection on the host to exert greater control over the relationship leads it to target proteins to the farmed cell: for instance, transporters to extract more nutrients or proteins to suppress and control its division (hetero-endosymbiont protein targeting, or hetero-EPT). Once this targeting is established, genes that move from the farmed food to the host can also be targeted to future farmed cells (more hetero-EPT). This cycle could proceed indefinitely (as it happens for example in modern kleptoplastic species, which keep 'stolen' plastids for long periods of time but eventually have to replace them), but the farmed food could also be fixed more permanently, if digestion were completely halted. At this point, EGT from this nascent organelle would initiate, but the host would still target older proteins derived from hetero-EPT to it. Data from relatively recent secondary and tertiary endosymbioses are consistent with the second model, as they show that protein targeting evolved prior to the fixation of the organelle in the cell[182,205], and most endosymbiotic systems show evidence of hetero-EPT[37,38,59,173,201].

of any other kind of HGT, by suggesting that most or even all foreign genes in a genome are due to artefact or have an organelle origin[13,39]. The same logic underpins searching for an organelle 'footprint'. For example, the first nuclear genomes of non-photosynthetic relatives of secondary plastid-containing algae were intensively searched for 'relict' genes that would demonstrate that their ancestor contained a plastid that had subsequently been lost[174–176]. Similarly, genomes from algae with one kind of secondary plastid were searched for genes from other kinds of plastids in pursuit of the idea that there had been a series of repeated plastid endosymbioses[176,177]. These tests all rest on the assumption that an ancient endosymbiosis would leave a distinct genetic footprint on the host genome that would persist even after the loss of the organelle[175], and this assumption is based on the assumption that the host acquired a lot of genes by hetero-EGT in the first place. But if either of these largely unquestioned assumptions were false then the test would be meaningless.

The impact of hetero-EGT from the original mitochondrial and plastid endosymbioses has still not been revisited systematically, but a great deal of genomic data are now available from eukaryotes with a variety of more recent endosymbioses. Analyses of nuclear genomes of various lineages of eukaryotes with genetically integrated secondary and tertiary plastids have shown no evidence for an 'excess' of genes from the plastid lineage, beyond the genes encoding proteins that still function in the organelle[178–182]. Similarly, several claims for a footprint of an ancient endosymbiont were re-tested directly and found to be the result of artefacts[183–185]. None of this is to say that a host cannot acquire any genes from an endosymbiont, but we do lack evidence for large numbers or high frequency.

Lastly, genomic data have recently become available from several lineages in which the complete loss of an organelle can be demonstrated with high confidence. This is a very rare process and currently only known in one lineage for mitochondria[186] and a handful of lineages for plastids[187–189]. In none of these lineages is there strong evidence for a genetic footprint of the now-lost organelle[186–189]. Many of these cases are parasites with generally reduced genomes, so it might be argued that they are poor representatives. However, complete organelle loss in free-living species shows the same lack of a hetero-EGT footprint in the nuclear genomes[189], and, in other cases, genomes from free-living species with massively functionally reduced organelles have only a tiny number of genes for the few proteins still functioning in the organelle[190].

The explanation for the lack of hetero-EGT is simply an extension of why eukaryotes do not acquire excessive numbers of HGTs from food: beyond those genes relating to organelle function, endosymbionts have few genes of use to a eukaryotic host. Every independent case of endosymbiosis is potentially different, so none of these cases argues that acquiring a bolus of new genes by hetero-EGT from an endosymbiont is impossible, but they do show that, in every case that has been examined, this is not the outcome. This has important implications. First, we must abandon the assumption that endosymbiosis automatically leads to substantial levels of hetero-EGT as our null hypothesis when interpreting the evolution of a genome. Second, and by extension, we cannot use this assumption as a test for the past possession of an organelle: we must acknowledge that we cannot genetically distinguish between the genome of an organism that once had an endosymbiont or organelle and lost it, from one that never had it in the first place.

Where do endosymbiont-targeted proteins come from?

An even more widely held assumption about EGT is that all genes encoding proteins that are targeted to an endosymbiont came from that endosymbiont's genome[161,162]. This is also very intuitive as the endosymbiont seems the likely source for proteins that are targeted to it. Once again, however, recent data show that this is not always the case, particularly early in the integration of an endosymbiont.

The strongest examples of EGT noted in the previous section are all very recent transfers, which provide an unambiguous phylogenetic signal and occasional glimpses of informative but short-lived intermediates. However, going further back in time, the situation becomes murkier, partially because the noise obscuring phylogenetic signal increases, but also because there is growing evidence that a lot of endosymbiont-targeted proteins did not come from the endosymbiont. Ironically, some of the first evidence for this phenomenon came from examining HGT using organelle-targeted proteins, because they had the clearest phylogenetic signals[191]. Large-scale studies of mitochondrial and plastid proteomes soon revealed a similar pattern: many organellar proteins are non-alphaproteobacterial or non-cyanobacterial[173,192–195]. However, it was the examination of some of the most recently integrated endosymbionts that really led to a new interpretation of this pattern. The rhizarian amoeba *Paulinella* has a genetically integrated cyanobacteria photosymbiont (or chromatophore) that is distantly related to the plastid[196,197]. Analysis of the host and endosymbiont genomes and proteomes revealed host-encoded

proteins targeted to the chromatophore, but most of these were not related to the cyanobacterial lineage to which the chromatophore belongs[198–202]. A similar observation was described in insects with obligate endosymbionts[37,38] and most recently in the trypanosome *Angomonas*[203]: both hosts encode a small number of genes encoding proteins targeted to their bacterial symbionts, but none of these are derived from the same lineage of the endosymbiont[59].

Indeed, classical EGT has proven to be a relatively minor factor in all studied cases of early-stage endosymbiosis; instead, all evidence points to a more counter-intuitive picture in which the first steps of genetically integrated endosymbiosis do not involve acquiring genes from the endosymbiont but rather targeting proteins acquired from somewhere else. These genes encoding the targeted proteins should not be confused with genes arising from EGT, given that they are not gene transfers from the endosymbiont; instead, they are cases of hetero-EPT (Box 2).

This is one part of an entirely new way to look at the process of organelle genetic integration. The classical, textbook view of this process is something similar to 'cellular indigestion' (Fig. 3a): one cell eats another cell, but the former fails to digest its prey, which instead takes up residence inside its new host, eventually transferring genes to the host nucleus and subsequently having those proteins targeted back in an increasingly mutualistic integration[204]. Indeed, this order of events — with the transfer of genes preceding the establishment of a protein-targeting system — has been specifically suggested to have led to large-scale hetero-EGT, as the protein products of early transfers could not be targeted back to their original cell[39]. However, this order of events is not consistent with early-stage protein targeting being dominated by hetero-EPT or with the lack of evidence for large-scale hetero-EGT, and there is also now evidence that protein-targeting systems evolved before the endosymbiont was established. Early cases of hetero-EPT have been described in two different plastid-acquisition events: a tertiary plastid in dinoflagellates and a secondary plastid in euglenophytes; more importantly, however, in both systems protein targeting was shown to predate the current organelles[182,205].

The heterogeneity of organelle-targeted proteins, the prevalence of hetero-EPT in early-stage endosymbioses and the early establishment of protein targeting all support a completely different model for the genetic integration of organelles[206–208] (Fig. 3b). This more gradualist model begins with a long cyclic phase during which cells engulfing prey begin to delay digestion and 'farm' prey. Targeting evolves early, not as a way to move endosymbiont-derived proteins back to the endosymbiont but to allow the host greater control over its farmed food, for instance by moderating its replication and/or extracting nutrients more efficiently by inserting transporters into the membranes surrounding it. Once targeting evolves, the potential impact of HGT changes: the host can now acquire genes from any of these temporarily enslaved and doomed cells and target their proteins to future farmed cells (these are hetero-EPTs). At some point, after protein targeting has been established and many genes encoding targeted proteins have perhaps already been acquired, one of these endosymbionts is maintained permanently instead of being digested. This endosymbiont/organelle is already genetically integrated in the sense that proteins are already targeted to it, but over time a large number of its own genes can also move to the nucleus to encode proteins targeted back to the organelle (these are EGTs).

A lot about the concept of EGT has stood the test of time, but current data suggest it played its part slightly later in the genetic integration of organelles than originally thought. Before EGT could take place, a targeting system had to have evolved, and the first proteins targeted to the endosymbiont were derived from a mix of host, other endosymbionts, prey or other HGTs. This model of genetic integration also does not predict any hetero-EGT: with targeting taking place early, there is no mechanism to encourage hetero-EGT, and current data seem to show that it might not be important. This too makes sense against the backdrop of the relatively few HGTs in eukaryotic genomes in general; if this is because selection does not operate on this kind of variation in most eukaryotes, the host genome might have been inundated with thousands of endosymbiont genes, but it did not keep them because they had no useful function and were not maintained by selection. It is important to restate that each independent genetic integration of a host–endosymbiont pair may have unfolded differently, so any model may not explain them all equally well. Additionally, this does not preclude the possibility that endosymbiosis led to a few useful genes being acquired through hetero-EGT, just as HGT has led to a small number of useful acquisitions in eukaryotes more generally. However, current data all support a lack of large-scale hetero-EGT leading to large numbers of endosymbiont-derived proteins now unrelated to organelle function. Instead, current data support the early establishment of a protein-targeting system and the early importance of hetero-EPT in the genetic integration of organelles.

Conclusion

It is an interesting time in our understanding of eukaryotic HGT and endosymbiosis, with data and theory all in flux at once. In general, it is now mostly accepted that HGT plays a part in eukaryotic genome evolution and mediated specific adaptations in many lineages. Overall, however, the magnitude of HGT in eukaryotes is markedly inferior to that in bacteria and archaea. This might seem surprising when the uniquely eukaryotic facilities to engulf other cells and sometimes keep them as endosymbionts were both widely believed to increase the likelihood of HGT. It may indeed be true that these factors do expose the cell to more foreign DNA, but there is no sign eukaryotic genomes have been continuously replaced or augmented by genes from food. Even long-term endosymbiotic organelles have seemingly not donated substantial numbers of genes to their hosts, beyond those required for organelle function. To clearly see how HGT in eukaryotes differs from that of bacteria and archaea, we have to step back and think about how eukaryotic cells function at the levels of cell biology, genetics, ecology and evolution. From this perspective, when eukaryotic cells began eating other cells, it may have exposed them to more foreign DNA, but it also made that DNA less likely to be retained as a functional HGT, because selection in eukaryotes operates most effectively on kinds of genetic variation that are not generally acquired through HGT.

Moving forward, some of the remaining gaps in our understanding of HGT in eukaryotes will simply be filled by the ongoing and inevitable growth in genomics: broader coverage of eukaryotic diversity coupled with deeper coverage of more closely related species will enable quantitative analysis of some basic questions, such as how much transfer takes place between two eukaryotes or exactly how much variability there is in the effects of HGT between major lineages. Although the amount of data from diverse eukaryotes is increasing quickly, it is worth noting that these questions will remain out of reach if we fail to embrace the concept of just how diverse eukaryotes really are[209]. The current tendency is to favour breadth, and with this comes the idea that one member of a lineage sufficiently represents that whole group. However, when a group of protists is itself as diverse as all animals combined (of which there are many), we need to stop and ask, would a single animal genome have adequately represented all animal diversity? Similarly, major

questions surrounding endosymbiosis and gene transfer have been investigated by a variety of analyses on different lineages using different methods, which has made it difficult to meaningfully compare data across studies. Given the massive growth in relevant data and equally important improvements in our ability to analyse these data, comprehensively revisiting these questions is an obvious requirement. And beyond these data-driven points is the overarching need to pause occasionally and consider when we need to realign our aging theoretical models with ones that actually fit the experimental observations.

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Competing interests

The author declares no competing interests.

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