ANNUAL REVIEWS

Annual Review of Genetics

Witnessing Genome Evolution: Experimental Reconstruction of Endosymbiotic and Horizontal Gene Transfer

Ralph Bock

Max-Planck-Institut für Molekulare Pflanzenphysiologie, D-14476 Potsdam-Golm, Germany; email: rbock@mpimp-golm.mpg.de

Annu. Rev. Genet. 2017. 51:1–22

First published as a Review in Advance on August 28, 2017

The *Annual Review of Genetics* is online at genet.annualreviews.org

https://doi.org/10.1146/annurev-genet-120215-035329

Keywords

endosymbiosis, chloroplast, mitochondrion, endosymbiotic gene transfer, horizontal gene transfer, horizontal genome transfer, experimental evolution, speciation

Abstract

Present day mitochondria and plastids (chloroplasts) evolved from formerly free-living bacteria that were acquired through endosymbiosis more than a billion years ago. Conversion of the bacterial endosymbionts into cell organelles involved the massive translocation of genetic material from the organellar genomes to the nucleus. The development of transformation technologies for organellar genomes has made it possible to reconstruct this endosymbiotic gene transfer in laboratory experiments and study the mechanisms involved. Recently, the horizontal transfer of genetic information between organisms has also become amenable to experimental investigation. It led to the discovery of horizontal genome transfer as an asexual process generating new species and new combinations of nuclear and organellar genomes. This review describes experimental approaches towards studying endosymbiotic and horizontal gene transfer processes, discusses the new knowledge gained from these approaches about both the evolutionary significance of gene transfer and the underlying molecular mechanisms, and highlights exciting possibilities to exploit gene and genome transfer in biotechnology and synthetic biology.

INTRODUCTION

The rise of eukaryotes began more than 1.5 billion years ago with the establishment of an endosymbiotic association between an archaeon presumably related to the Lokiarchaeota, a recently recognized phylum of archaea (103), and an α-proteobacterial endosymbiont (82). By providing its host cell with additional energy through oxidative phosphorylation, the bacterial endosymbiont and its conversion into an intracellular organelle now known as the mitochondrion conferred an enormous selective advantage that sparked the rapid subsequent diversification of the eukaryotic lineage (130). Probably only a few hundred million years after the engulfment of the α-proteobacterial endosymbiont, a eukaryotic (mitochondria-possessing) cell engaged in another endosymbiosis by swallowing up a cyanobacterium and converting it into a second DNA-containing organelle, the plastid (chloroplast). By founding a lineage of eukaryotes that was capable of photoautotrophic growth, this event triggered another major evolutionary transition and decisively changed the life on our planet.

For a long time, it was thought that the primary endosymbioses leading to the establishment of mitochondria and plastids were singular events. Although this is still the current thinking for mitochondria in that all eukaryotes are believed to go back to the same α-proteobacterial endosymbiont and the same archaeon host cell (a hypothetical organism also referred to as the last eukaryotic common ancestor, or LECA), a recent study suggests that the host was not a simple archaeon but a more complex, genetically chimeric organism that had received a substantial number of genes from different bacteria by either earlier endosymbiotic interactions or massive horizontal gene transfer (HGT) (80). For plastids, it has become clear that evolution is repeatable: The photosynthetic freshwater amoeboid *Paulinella chromatophora* harbors a chloroplast-like organelle, originally termed the chromatophore, that also derives from a cyanobacterium but is of much more recent evolutionary origin (and was established only approximately 60 Mya) (63, 72, 73). Adding to the complexity of the picture of plastid evolution, plastids can also be acquired through secondary endosymbioses, a process in which a eukaryotic cell engulfs a plastid-harboring eukaryotic cell and either eliminates the nucleus of the endosymbiont or reduces it to a vestigial genome called the nucleomorph (2, 31).

The endosymbioses that led to the establishment of the DNA-containing cell organelles can be viewed as the most extreme form of HGT: the transfer of the genetic information of an entire organism into a recipient cell through an asexual process. The transfer event was followed by a long-lasting phase of evolutionary streamlining that led to the gradual optimization of the relationship between the host genome and the genome of the acquired endosymbiont. Co-evolution of the two genomes involved (*a*) the loss of dispensable genetic information (e.g., genes for bacterial cell wall biosynthesis), (*b*) the elimination of redundant genetic information (e.g., genes for metabolic pathways present in both the nuclear and the organellar genomes), (*c*) the establishment of protein transport and metabolite exchange systems between the cytosol and the organelles, (*d*) the installation of communication pathways from the nucleus to the organelle (anterograde signaling), and (*e*) the establishment of communication pathways from the organelle to the nucleus (retrograde signaling). In addition to genome reduction by loss of superfluous and redundant genes, a massive reshuffling of genetic information between compartments also occurred. Presumably driven by the advantage of being able to participate in sexual recombination (35), thousands of mitochondrial and plastid genes emigrated to the nucleus and evolved from prokaryotic genes into eukaryotic genes (18, 65), a process also known as endosymbiotic gene transfer (EGT). Although this process may have reached an end point in some lineages of evolution, e.g., with the highly reduced and gene-dense mitochondrial genomes of many animals, or the genome-free mitochondrion-derived hydrogenosomes in some protists (37), it is clearly a still ongoing process in many lineages of

evolution. The latter is supported by two lines of evidence: (*a*) the occurrence of recent transfers of plastid and mitochondrial genes to the nucleus as concluded from the absence of typical organellar genes from the plastid or mitochondrial genome of certain taxa, with remnants of the organellar genes sometimes still being present as pseudogenes (23, 67, 75), and (*b*) the presence of organellar DNA fragments in the nuclear genome, some of which originate from relatively recent DNA transfer events: nuclear integrants of plastid DNA (NUPTs) and nuclear integrants of mitochondrial DNA (NUMTs) (43, 77, 87). These so-called promiscuous DNA fragments are often nonfunctional, but may provide intermediates on the way to functional gene transfers (104) or raw material for the construction of unrelated gene loci in the nucleus (52).

In addition to EGT, which can be viewed as intracellular gene transfer, genetic material can also be transferred between cells and organisms in HGT, which describes an intercellular gene transfer. HGT, which is sometimes called lateral gene transfer, refers to the asexual movement of genetic material by nonvertical transmission (i.e., not from parent to progeny). It is best known from prokaryotes, where, for example, antibiotic resistance genes can be transferred between bacterial species and confer a dramatic selectable advantage to the recipient organism. Known mechanisms of HGT in bacteria include conjugation, cell fusion, transduction by phages, and natural transformation with naked DNA (as, for example, released upon decomposition of dead cells) (102). The contribution of HGT to eukaryotic genome evolution is only beginning to be appreciated (14, 49). However, the rapid progress in genome sequencing has led to the discovery of an increasing number of HGT events that are likely to have provided the recipient organism with a strong fitness benefit in either natural selection or artificial selection (e.g., domestication by humans). Recently discovered, particularly spectacular examples include, for example, the transfer of a chimeric photoreceptor gene from a bryophyte to ferns (54), the transfer of a light-harvesting photosynthetic antenna protein from a green alga to mosses (44), the acquisition of the iron-sequestering protein ferritin by marine bloom-forming diatoms (61), the acquisition of useful fermentation-related traits by wine yeasts (64) and the exchange of metabolic genes between cheese-making fungi (89).

Known mechanisms of EGT and HGT in eukaryotes are only beginning to emerge. Recently, experimental evolution approaches have made it feasible to visualize both EGT and HGT events in the laboratory. This opens up the exciting opportunity to watch key processes in genome evolution in real time and study the underlying molecular mechanisms. In this article, I discuss evolutionary and mechanistic aspects of EGT and HGT in eukaryotes. I focus on systems where experimental reconstruction of gene transfer has become possible and has yielded valuable insights into transfer pathways and mechanisms of gene and genome evolution.

ENDOSYMBIOTIC GENE TRANSFER AND ITS EXPERIMENTAL RECONSTRUCTION

Based on phylogenetic data, it seems reasonable to assume that EGT events are relatively rare. The low frequency of DNA mobilization from the DNA-containing organelles to the nucleus, therefore, requires stringent selection schemes to detect EGT events in laboratory experiments. Typically, this requires introduction of a silent selectable marker gene into the organellar genome whose escape to the nucleus can be detected by gain of function (i.e., transcriptional activation) (**Figure 1**). This approach limits the available experimental systems to species in which the organellar genome(s) are transformable (**Figure 1**). For mitochondria, these species are the yeast *Saccharomyces cerevisiae* and the unicellular green alga *Chlamydomonas reinhardtii* (19, 47, 81). Chloroplast genomes currently can be engineered in *Chlamydomonas* and a few multicellular plants, with tobacco being the most frequently used seed plant model (for review, see, e.g., 16).

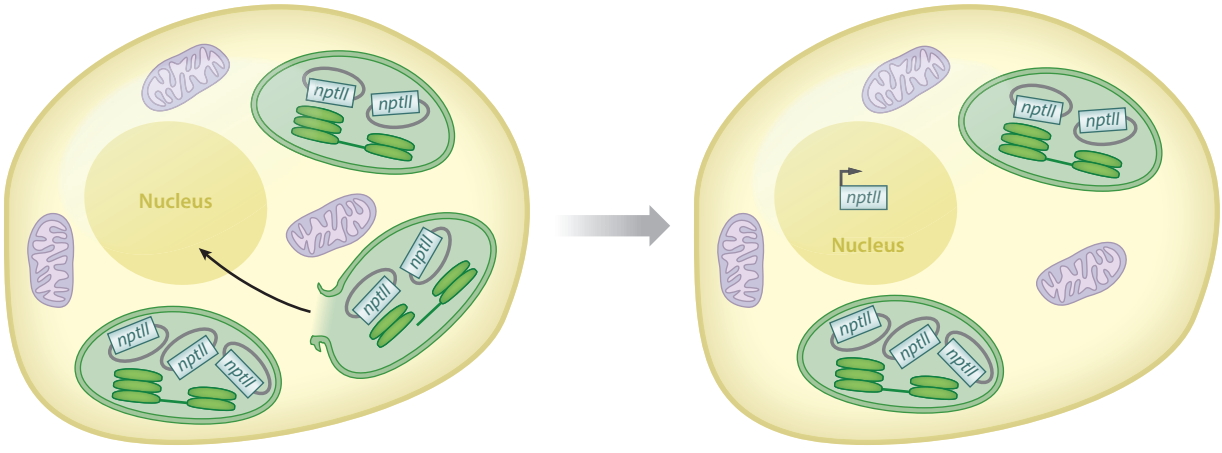

Figure 1

Genetic screen for the escape of plastid DNA to the nucleus by intracellular gene transfer. A selectable marker gene, here the kanamycin resistance gene *nptII*, is tethered to nuclear expression signals (promoter and terminator) and incorporated into the chloroplast genome by stable transformation (16, 109). The nuclear (eukaryotic-type) expression signals of the antibiotic resistance gene are not recognized by the prokaryotic gene expression machinery of the plastid, which still largely resembles that of its cyanobacterial ancestor. Chloroplast rupture, turnover, or perhaps spontaneous release of one of the many copies of the plastid genome from an organelle that otherwise remains intact can transfer plastid DNA to the cytosol. During the next round of cell division, when the nuclear envelope will be dissolved, this DNA can get in contact with nuclear DNA and be ligated into pre-existing double-strand breaks in chromosomes (53, 84, 119, 129). Upon arrival to the nucleus, the eukaryotic expression signals of the *nptII* gene are recognized and the gene is expressed, resulting in phenotypic resistance to kanamycin.

DNA Transfer from Mitochondria to the Nucleus

Thorsness & Fox (110) developed the first genetic assay for the detection of DNA escaping from mitochondria to the nucleus in brewer's yeast (*S. cerevisiae*). When introduced into mitochondria by stable genetic transformation, the nuclear genetic marker *URA3* failed to complement the uracil auxotrophy caused by loss-of-function mutations in the nucleus-encoded *URA3* gene. Subsequent selection for uracil prototrophy led to the isolation of yeast strains, in which the *URA3* gene had moved to the nucleus and stably integrated into the nuclear genome. The measured frequency of gene escape was much higher than what reasonably could have been expected: approximately 2×10^{-5} transfer events per cell and generation. This high rate of DNA migration from the mitochondrion to the nucleus suggested that mitochondrial EGT is a still ongoing process and eukaryotic nuclei repeatedly capture mitochondrial DNA fragments. The consequences of these incorporations may be twofold. First, they allow mitochondrial genes to evolve into functional nuclear genes, which, if successful, can make the mitochondrial gene copy dispensable and provide the newly nuclear gene with the advantage of participating in sexual recombination. However, for many genes in the conserved gene set retained in present-day mitochondrial genomes, this potential advantage may be offset or even overcompensated by the disadvantage of having to import highly hydrophobic membrane proteins into the mitochondrial compartment after their synthesis in the cytosol (12). A second possible use of escaped mitochondrial DNA fragments is in double-strand break repair in the nucleus. A number of studies have shown that broken chromosomes can be patched by pasting pieces of mitochondrial DNA into the double-strand breaks (53, 84, 129),

thus also suggesting a likely explanation for the origin of promiscuous mitochondrial DNA in the nucleus (113).

In an attempt to identify genetic factors that influence the escape rate of DNA from mitochondria, genetic screens for increased rates of gene transfer to the nucleus were conducted in yeast. Isolation and genetic analysis of 21 nuclear mutants revealed that mutants fall into six complementation groups. The six nuclear genes were termed *YME1–6* (for yeast mitochondrial DNA escape), and the mutant alleles all turned out to be recessive (111). Characterization of some of the encoded gene products revealed that they represent integral mitochondrial membrane proteins (e.g., 38). This finding suggests a simple model in which loss of certain protein components of the inner or outer mitochondrial membrane compromises mitochondrial membrane integrity, and in this way, allows for the more frequent escape of mitochondrial DNA or more often results in mitochondrial rupture and release of mitochondrial nucleoids into the cytosol.

DNA Transfer from Plastids to the Nucleus

Similar to mitochondrial DNA sequences found in the nuclear genomes of fungi, plants, and animals, the presence of promiscuous plastid DNA integrants of different ages had suggested that there was a continuous influx of chloroplast DNA into the plant nucleus (43, 66, 77, 87). With the development of a technology for stable transformation of the chloroplast genome in tobacco (109), it became possible to develop genetic screens for the escape of plastid DNA to the nucleus (18, 41, 106) (**Figure 1**). The screens are based on the incorporation of an antibiotic resistance gene (typically the kanamycin resistance gene *nptII*) into the plastid genome (plastome). If tethered to nuclear expression signals (i.e., a nuclear gene-derived promoter and transcription terminator), the resistance gene cannot be expressed in the chloroplast. This is because the mechanisms of gene expression in plastids are still largely prokaryotic in nature and resemble those of the cyanobacterial ancestor of chloroplasts. Consequently, the plastid gene expression machinery does not recognize nuclear (eukaryotic-type) expression signals. The kanamycin resistance gene, therefore, remains silent, and the plastid-transformed (transplastomic) tobacco plants are sensitive to kanamycin, even though they harbor the *nptII* resistance gene in their chloroplast genome (**Figure 1**). Movement of the gene to the nucleus leads to transcriptional activation, because the eukaryotic expression signals are now recognized by the nuclear gene expression machinery. In this way, DNA escape events become detectable as plantlets capable of growing in the presence of kanamycin in the culture medium (**Figure 1**).

Based on this straightforward selection principle (**Figure 1**), genetic screens for DNA transfer from the plastid to the nuclear genome were developed using two different experimental setups: (*a*) selection for kanamycin-resistant seedlings by germinating seeds from a cross between a wild-type plant and a transplastomic pollen donor (41) and (*b*) selection for kanamycin-resistant regenerants from leaf explants exposed to a kanamycin-containing plant regeneration medium (106). Whereas the former determines the DNA escape frequency in pollen cells, which do not normally transmit plastid genes due to the maternal mode of plastid inheritance (91), the latter approach measures the DNA transfer rate in somatic cells. Interestingly, although the rates are astonishingly high in both systems (1 transfer event per 16,000 pollen grains and 1 event per 5 million somatic cells), the DNA escape frequency was substantially higher in pollen cells, a finding further confirmed in subsequent studies (98). A possible explanation for the greatly elevated transfer rate in pollen lies in the maternal mode of chloroplast inheritance that involves plastid elimination during male gametogenesis. It is conceivable that, in this elimination process, plastid degradation occurs and results in the frequent release of large amounts of chloroplast DNA into the cytosol (**Figure 1**).

An important finding that emerged from the early screens for chloroplast DNA escape was that the plastid DNA pieces found to be integrated into the nuclear genome were not confined to the *nptII* locus selected for. Instead, large chloroplast genome fragments (up to 20 kb and more) were detected in the nuclear genomes, and often, the integrants comprised multiple fragments inserted into the same nuclear locus (42), a situation very much reminiscent of the complex loci generated in evolution by promiscuous DNA insertion into the nucleus.

The use of reporter genes that become activated upon transfer to the nucleus has increased the throughput of screens for gene transfer events in that it eliminated the need to select for antibiotic-resistant plant lines. Instead, cells (or groups of cells) expressing the reporter gene can be identified by visual or microscopic inspection of leaves, and their number per leaf or per plant can simply be counted. This approach has facilitated the quantitative analysis of gene transfer rates dependent upon environmental conditions. In tests of conditions of mild heat stress, the rate of DNA escape to the nucleus increased 2.5- to 3.5-fold (119). It seems conceivable that heat and perhaps also other stresses lead to increased chloroplast damage, which in turn may increase the release of plastid DNA from damaged or broken organelles.

An intriguing question immediately resulting from the massive ingress of plastid DNA into the nucleus concerns the stability of the transferred chloroplast sequences in the nuclear genome. Considering the high frequency of events and the large size of the transferred DNA fragments (18, 41, 42, 106), it is hard to imagine that no mechanisms exist in the nucleus that counteract the genomic enlargement that would result from the constant bombardment with organellar DNA. Indeed, following the fate of escaped plastid DNA fragments over the next generations revealed substantial instability (99), as initially evidenced by frequent loss of the kanamycin-resistant phenotype in part of the progeny. This was subsequently confirmed by the physical loss of the *nptII* gene from the nuclear genome due to the occurrence of deletions in the transferred plastid DNA segment. Interestingly, stability of the escaped plastid DNA sequences appears to depend on their integration site in the genome. Whereas some integrants are stable over generations, others suffer from frequent deletions occurring during mitosis and/or meiosis (99). The integration site properties underlying these differences and the molecular mechanisms involved in recognition of invading plastid DNA sequences are currently unclear, but it is tempting to speculate that the lack of cytosine methylation and/or the high AT content of chloroplast DNA sequences may help the nuclear genome surveillance machinery to distinguish invading plastid sequences from resident nuclear DNA.

The microalga *C. reinhardtii* is currently the only alga whose chloroplast genome is routinely transformable. Stimulated by the success with reconstructing plastid-to-nucleus gene transfer in tobacco, attempts were made to establish *Chlamydomonas* as a green algal model to study EGT in the laboratory. However, incorporation of a nuclear selectable marker gene into the plastid genome of *Chlamydomonas* and subsequent selection for gene escape to the nucleus did not lead to the recovery of gene transfer events (55). A possible explanation could lie in a key difference in the structure of the chloroplast compartment between *Chlamydomonas* and seed plants. Whereas seed plants have many small chloroplasts per cell, the algal cell harbors a single large chloroplast that occupies more than half of the cell volume. Assuming that the escape of plastid DNA to the nucleocytosolic compartment involves chloroplast decay (**Figure 1**), this might be a lethal event in *Chlamydomonas*, whereas the loss of a single chloroplast can easily be tolerated by a plant cell possessing a hundred chloroplasts. However, more extensive screens are needed to confirm this hypothesis and to determine whether this completely prevents DNA escape or just reduces it to levels that are orders of magnitude lower than those in seed plants.

Reconstruction of Functional Gene Transfer from Plastids to the Nucleus

It is important to note that, although they revealed some intriguing properties of intracellular DNA transfer, the genetic screens that detected DNA escape from mitochondria and chloroplasts (**Figure 1**) did not faithfully reconstitute EGT events as they presumably occurred in evolution. To visualize gene transfer, researchers equipped the selectable marker gene incorporated into the organellar genome with nuclear expression signals that would immediately function after successful transfer of the gene into the nuclear genome (41, 106, 110). In real-world EGT, this would not be the case. Instead, the organellar gene would arrive in the nuclear genome with its prokaryotic, bacterial-type expression signals that do not function in the eukaryotic environment of the nucleus. Consequently, if the DNA transfer is to result in EGT and ultimate replacement of the organellar gene by a functional nuclear gene copy, the transferred organellar gene needs to evolve from a prokaryotic into a eukaryotic gene.

Screens for functional gene transfer were designed in tobacco plants to test whether this process can be observed in real time and to study the molecular mechanisms involved (104). They take advantage of the observation that gene transfer is not restricted to the selected *nptII* gene (**Figure 1**), but most transfer events include large flanking regions of plastid DNA. In most cases, the cotransferred flanking regions include the *aadA* gene, the spectinomycin resistance gene used as a selectable marker for stable transformation of the plastid genome (106). Although cotransferred with *nptII*, *aadA* is not functional in the nucleus, because its plastid-specific (prokaryotic-type) expression signals are not recognized by the eukaryotic gene expression machinery of the nucleus. Therefore, the cotransferred *aadA* gene provides a suitable model gene to select for functional gene transfer: It was freshly transferred to the nucleus (and is still embedded in a large fragment of chloroplast DNA), and because it still carries its plastid expression signals, it is not functional in the nucleus. To test whether *aadA* can evolve into a functional nuclear gene, the transgenic chloroplasts (and thus the spectinomycin resistance) were crossed out from the gene transfer lines, and this facilitated genetic screens for activation of the transferred *aadA* gene in the nucleus by selection for gain of spectinomycin resistance. Indeed, spectinomycin-resistant lines could be recovered in which the transferred *aadA* gene had become transcriptionally activated inside the nucleus (104). Mapping of the genomic rearrangements responsible for gene activation revealed that, in all cases studied so far, transcriptional activation was caused by upstream deletions that allowed the *aadA* gene to capture the promoter of a neighboring nuclear gene. This mechanism inactivates the gene from which the promoter is snagged, which, however, does not necessarily represent a deleterious event. In fact, one potential benefit of whole-genome duplications, which have occurred multiple times in the evolution of fungi and plants (101, 124), could be that they provide ample opportunities for promoter capture in one of two resulting genomes that, by remaining phenotypically neutral, facilitates functional EGT.

If functional EGT requires rerouting of the encoded gene product into the organellar compartment, a transit peptide-encoding sequence needs to be acquired in addition to the nuclear promoter. It is important to note that, in evolution, a substantial fraction of the organellar genes that transferred to become functional nuclear genes now operate in cellular compartments that differ from those they came from (8, 65). This indicates that transit peptide acquisition, although clearly needed for many transferred genes, is not a strict requirement for functional EGT to occur. In those cases where it is necessary, it is conceivable that a transit peptide-encoding sequence could be coacquired with the captured promoter (104) in a one-step process. Phylogenetic evidence supports the utilization of preexisting or duplicated transit peptide-encoding sequences for functional EGT (48, 114, 115). However, given that transit peptides are short and the sequence requirements

for functional transit peptides are not very stringent, it also seems possible that suitable sequence stretches can be present (or be generated) by chance (5, 116).

The presence of large fragments of transferred plastid DNA in the nuclear genome (42) suggested a direct DNA-mediated transfer mechanism. This was an intriguing observation, because earlier phylogenetic studies had also provided evidence of EGT events likely involving an RNA and/or complementary DNA (cDNA) intermediate (23, 74). Especially organellar genes harboring introns and genes encoding multiple mRNA editing sites are conceptually easier to transfer as fully processed mRNA rather than as plain DNA sequence. This is because both intron excision and RNA editing, which is a post-transcriptional process resulting in C-to-U modifications in plant organellar mRNA sequences at highly specific sites (13, 51), are usually required for synthesis of a functional gene product (17, 46), and organellar introns and RNA editing sites are unlikely to be faithfully processed in the nucleus. A chloroplast group II intron was built into the *nptII* gene to investigate how an intron-containing gene can end up in the nucleus. The construct was introduced into the plastid genome by transformation, and the resulting transplastomic plants were used in genetic screens for functional EGT thought to require intron removal (29). Interestingly, all events that were recovered turned out to represent direct DNA-mediated gene transfers to the nuclear genome, as evidenced by retention of the group II intron in the now nuclear *nptII*. Gene activity in the nucleus (and, hence, kanamycin resistance) was brought about by cryptic splice sites within the chloroplast intron that were recognized by the splicing machinery in the nucleus. Excision of these intronic sequences, although relatively inefficient and not occurring precisely at the native splice sites of the intron in the chloroplast, can produce a contiguous reading frame and thus result in synthesis of a functional neomycin phosphotransferase (NptII) enzyme. This finding demonstrated that even interruption of the reading frame by a bacterial-type intron does not present an insurmountable obstacle to DNA-mediated EGT. However, although there is currently no direct experimental evidence for RNA/cDNA-mediated gene transfer, especially in the case of fungal and plant mitochondria that are known to possess reverse transcriptase activities (21, 27, 68, 95, 122), it cannot be excluded that such a pathway exists and was at least occasionally utilized for EGT in eukaryotic genome evolution.

Taken together, the evidence obtained from experimental approaches toward reconstruction of EGT suggests a model in which organellar genes are frequently transferred to the nuclear genome, where they can experience one of two alternative fates. The presumably relatively rare fate is acquisition of functionality, initiated by promoter capture and likely followed by a longer phase of evolutionary optimization to complete the conversion from a prokaryotic to a full-fledged eukaryotic gene that can replace the organellar gene copy from which it originated. If functionalization does not occur within the timeframe in which the coding sequence of the transferred gene stays intact, it will be condemned to gradual deterioration through the accumulation of mutations, a process that, with the appearance of the first missense or nonsense mutation, likely becomes irreversible.

HORIZONTAL GENE TRANSFER AND ITS EXPERIMENTAL RECONSTRUCTION

HGT events are typically inferred from phylogenetic and taxonomic studies that, upon using gene sequences for the construction of phylogenetic trees, detect striking incongruences in the trees (14, 49). HGT appears to be particularly prevalent in unicellular organisms and in those groups of multicellular organisms that show an incomplete separation of soma and germ line and, therefore, an inefficient protection of their germ line from interaction with other organisms or exposure to DNA present in the environment.

In plants, all three genetic compartments can participate in the horizontal exchange of genetic material. The horizontal transfer of sequences from microbes interacting with plants (and algae) into the nuclear genome has been known, studied, and exploited for a long time, with *Agrobacterium*-mediated plant transformation being the most prominent example. By contrast, it was recognized only relatively recently that horizontal DNA transfer can occur also between plants and, particularly frequently, involves the two DNA-containing cell organelles, plastids and mitochondria. For mitochondria, the first evidence came from interactions between parasitic plants and their host plants, which apparently can result in the transfer of mitochondrial DNA fragments (26, 70; reviewed, e.g., in 14, 86). The subsequent discovery of events that obviously do not involve parasitic interactions (10, 125) suggested plant-to-plant mitochondrial HGT as a more general phenomenon in plant evolution and raised intriguing questions about the DNA transfer pathways involved. Similarly, phylogenetic studies had provided evidence for the exchange of chloroplast genomes between species (often without detectable traces of nuclear introgression), a phenomenon dubbed chloroplast capture (1, 88). This phylogenetic evidence for HGT and the unexplained mechanistic basis stimulated experimental approaches towards the visualization of HGT in the laboratory and the identification of potential paths for the transfer of (organellar) DNA from plant to plant.

Horizontal Transfer of Plastid DNA

The phylogenetic evidence for HGT between plants and, especially, the evidence for horizontal DNA transfer between parasitic plants and their hosts (**Figure 2*a***) had suggested that close cell-to-cell interactions between donor and recipient plant are required to facilitate the horizontal exchange of genetic information between plants. The most common form of cell-to-cell interactions in which plants engage is natural grafting (**Figure 2*b***). Interlocking stems, branches, or roots

Figure 2

Avenues for horizontal gene transfer between plants. (*a*) Plant–plant parasitism. Shown here is a mistletoe (*Viscum album*), a hemiparasitic seed plant, growing on a flowering apple tree on the Max Planck campus in Potsdam-Golm, Germany. (*b*) Natural grafts. Growing closely together, the birch (*left*) and the oak (*right*) have formed two graft junctions between their stems. The photograph was taken near Wildpark-West, Germany. (*c*) An experimental graft between two Solanaceous species mimicking the kissing trees in panel *b*. Stem grafting between a cigarette tobacco (*Nicotiana tabacum*; *left*) and a tree tobacco (*Nicotiana glauca*; *right*) was induced by temporarily tying together the two stems. The two plants were grown together in the same pot in the greenhouse. (*d*) Splice graft of the same two species in sterile culture. Stock (*N. tabacum*) and scion (*N. glauca*) are held together by a silicon sleeve that can be removed once the graft junction is established (typically after 2–3 days).

can exert mutual mechanical pressure that is strong enough to cause local wounding followed by tissue fusion and establishment of new vascular connections between neighboring plants.

Experimental grafting (**Figure 2*c,d***) was employed to test the idea that the graft partners exchange genetic information. A transgenic tobacco plant equipped with a kanamycin resistance gene in its nuclear genome was grafted to a transplastomic tobacco plant harboring a spectinomycin resistance gene in its chloroplast genome to facilitate the detection of HGT events across the graft junction and to specifically explore the possibility that organellar DNA is mobile (**Figure 3**) (105). Following graft fusion, the graft site was excised and subjected to double selection for kanamycin and spectinomycin resistance. Doubly resistant plants can regenerate only if one of the two antibiotic resistance genes has traveled across the graft junction, resulting in a cell equipped with both resistances (**Figure 3**). This straightforward selection scheme resulted in the frequent detection of horizontal DNA transfer events that were additionally visualized with built-in fluorescent reporter genes and also confirmed molecularly by employing molecular markers, which was possible because different tobacco cultivars had been used as graft partners (105). The molecular data strongly suggested horizontal transfer of plastid DNA rather than nuclear DNA, a finding that was subsequently confirmed by using different species for the grafting experiments (**Figure 3**) (107, 112). Plastid genome sequencing in several events revealed complete replacement of the resident plastid genome by the plastid genome of the donor plant. No evidence of plastome recombination was seen, indicating horizontal transfer at the level of the genome rather than individual genes (107).

Currently, the horizontal transfer of naked plastid genomes cannot be distinguished from the horizontal transfer of entire organelles. Although it seems hard to envision that naked plastid genomes, after their transfer from cell to cell, would reenter an organelle to function in the recipient cell, it is also difficult to imagine how a cellular structure as large as the chloroplast can travel across the cell membrane and the cell wall. The experiments performed to date indicate that wounding, an inherent part of both natural and experimental grafting (**Figure 2**), and physical contact between scion and stock are insufficient for genome transfer to occur. Instead, tissue fusion appears to be an absolute requirement for the transfer (105), suggesting that it is mediated by interactions between two intact cells. Although the cytoplasms of neighboring plant cells are connected through thin plasmatic channels called plasmodesmata, the diameter of these channels (and their so-called size exclusion limit) is far too small to allow for the passage of structures as large as even the smallest plastids (50, 57, 58). However, upon graft fusion, plasmodesmata are formed de novo at the graft junction (45, 79) in an understudied process that perhaps provides opportunities for the transfer of larger macromolecular structures, including entire organelles. It is also known that, under certain conditions, the diameter of plasmodesmata increases. For example, plasmodesma-binding proteins encoded in the genomes of many plant viruses (dubbed movement proteins) can widen the plasmodesmata to allow the passage of entire viral particles, thus facilitating cell-to-cell spread of the virus (123). Interestingly, related proteins are also found encoded in plant nuclear genomes (128), but whether they are involved in horizontal gene and/or genome transfer needs to be investigated.

Are there any boundaries to the horizontal transfer of plastid genomes between species by grafting or other asexual processes? Two limitations seem apparent. First, plastid and nuclear genomes have coevolved over millions of years. Thus, introducing an alien plastid genotype that has not properly coevolved with the resident nuclear genome bears the risk of genetic incompatibilities that are analogous to the Dobzhansky-Muller model of hybrid incompatibility and are referred to as plastome-genome incompatibilities (34). Although the gene set present in the plastid genome of seed plants is generally highly conserved, the expression of plastid genes and the functioning of the large multiprotein complexes operating in plastids (e.g., photosystems, ribosomes) require intricate

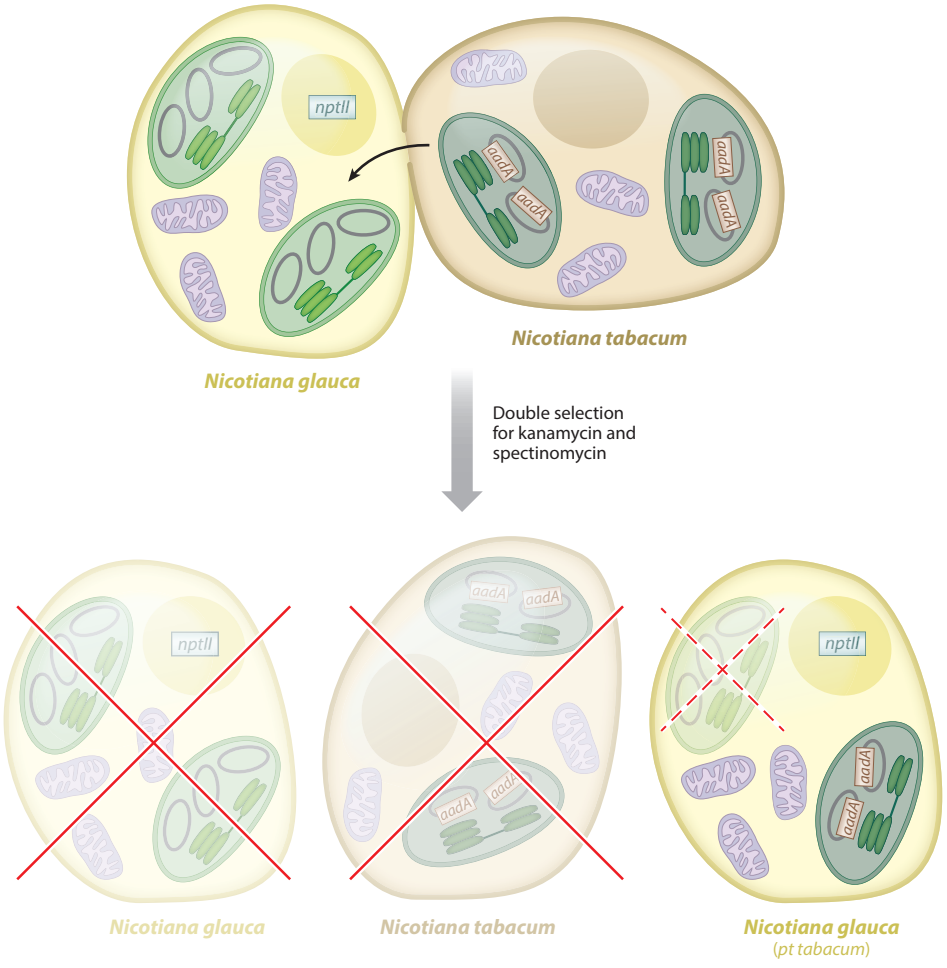

Figure 3

Experimental reconstruction of horizontal transfer of chloroplast DNA between plant species. Chloroplast DNA transfer across the graft junction from the cigarette tobacco (*Nicotiana tabacum*) to the tree tobacco (*Nicotiana glauca*; cf. **Figure 2**) is detected by following antibiotic resistance encoded in the plastid genome. The cigarette tobacco is transplastomic and carries the spectinomycin-resistance gene *aadA* in its chloroplast genome, whereas the tree tobacco is nuclear-transgenic and harbors the kanamycin resistance gene *nptII* in its nuclear genome. Following grafting (**Figure 2**), double selection for kanamycin and spectinomycin visualizes horizontal gene transfer events in which the chloroplast *aadA* gene has entered an *N. glauca* cell (*black arrow*), thus generating doubly resistant cells that can grow and regenerate in the presence of both drugs (105, 107). The cells of the two graft partners die under double selection (*solid red crosses*), because they are only resistant to one of the two antibiotics. Plastid genome analyses support cell-to-cell transfer of entire plastid genomes or entire plastids (107, 112). Initially, the recipient cell will be heteroplasmic and contain a mix of resident *N. glauca* plastids and new plastids harboring the *N. tabacum* plastome. However, because the resident *N. glauca* plastids are sensitive to spectinomycin, they will quickly disappear during subsequent rounds of cell and organelle division under selective pressure (*dashed red cross*). Abbreviation: pt, plastid.

interactions of nucleus-encoded and plastid-encoded factors. These interactions can be disturbed by very small sequence changes in one of the two compartments (93). Second, although grafting is not as much limited by species boundaries as sexual hybridization and widely different species can be grafted, not all combinations of species are compatible. As phylogenetic distance increases so too does the probability of graft rejection (graft incompatibility) (33, 79). Interfamily grafts are less likely to succeed and result in the establishment of a graft union than grafts between closely related species. The molecular causes of graft incompatibility are currently poorly understood, but it seems conceivable that both chemical (e.g., metabolic incompatibilities) and developmental factors can contribute. Thus, more systematic grafting experiments and tests of different plastid genotypes that are horizontally transferred into a given nuclear background are needed to obtain a better picture of the genetic and physiological limitations to the horizontal transfer of chloroplast genomes between plant species.

Nonetheless, the discovery that plastid genomes can move asexually between species raises the attractive possibility that this process generates new combinations of nuclear and plastid genomes in nature and, in this way, contributes to environmental adaptation (1). Moreover, it opens up exciting new applications in plant breeding and biotechnology. Although it is known that the genotype of the plastid, in addition to determining the efficiency of photosynthesis, also influences a number of other important agricultural traits (90), plastids are often ignored in breeding programs. The possibility to systematically replace plastid genotypes by horizontal genome transfer and test many plastomes against a constant nuclear background may allow breeders to identify superior combinations of plastid and nuclear genomes, thus offering significant potential for crop improvement.

Finally, the horizontal transfer of plastid genomes also provides researchers with a novel tool to address the current technological limitations in plastid genome engineering. Chloroplast transformation technology is still restricted to a handful of species, and most species (including nearly all major crops) are currently recalcitrant to plastid engineering. The feasibility of moving transgenic chloroplast genomes horizontally between species offers the exciting possibility to conduct plastid engineering in an easy-to-transform species and then transfer the transgenic chloroplast genome via grafting into the recalcitrant species, thus obtaining transplastomic plants without having to develop a plastid transformation protocol for the recalcitrant species (15, 16).

Horizontal Transfer of Mitochondrial DNA

Because horizontal transfer of mitochondrial DNA is frequently observed in nature (7, 9, 14, 25, 85, 86, 125, 127), it would be of great interest to conduct similar screens for mitochondrial HGT as have been developed for plastids (105). Unfortunately, our inability to genetically transform the mitochondrial genomes of plants currently does not permit the development of experimental systems for the direct reconstruction of mitochondrial HGT events in the laboratory. However, a recent report suggests that, occasionally, the horizontal transfer of plastid genomes can be accompanied by the horizontal transfer of mitochondrial DNA (36). In an experiment designed to study the horizontal movement of chloroplast DNA from a transplastomic *Nicotiana sylvestris* line to a cytoplasmically male sterile line of *Nicotiana tabacum* (a so-called alloplasmic line that carries the cytoplasmic genomes of another tobacco species, *Nicotiana undulata*), restoration of male fertility was observed in a single plant. Because cytoplasmic male sterility is a mitochondrially encoded trait, the mitochondrial genome of the *N. tabacum* plant with restored male fertility was investigated. Extensive recombination between the resident *N. undulata*–derived mitochondrial genome of *N. tabacum* and the mitochondrial genome of *N. sylvestris* was detected. The resulting chimeric mitochondrial genome represents a mosaic of segments of the *N. sylvestris* and *N. undulata*

mitochondrial DNA, suggesting horizontal movement of mitochondrial DNA followed by multiple homologous recombination events between the resident genome and the transferred DNA. Although a singular event, its observation is remarkable in that it was detected in the absence of selection for mitochondrial HGT. This may suggest that cell-to-cell transfer of mitochondrial DNA across graft sites occurs rather frequently, in line with its frequent detection in phylogenetic studies.

The most striking difference between the horizontal transfer of chloroplast DNA and that of mitochondrial DNA is that, in the case of chloroplast DNA, the entire genome is transferred, whereas, in the case of mitochondrial DNA, genome pieces are transferred. The most likely explanation for this difference is that chloroplasts do not normally recombine with each other, whereas mitochondria are much more dynamic and constantly engage in fusion and fission events (4, 56). Thus, the most plausible model for organellar HGT is that entire organelles move from cell to cell with different consequences for plastids and mitochondria. Because plastids do not fuse and recombine, the transferred plastid either displaces the resident plastids during the subsequent rounds of cell division (through selection or in a stochastic process by random sorting out) or is lost again. By contrast, a horizontally transferred mitochondrion will very likely fuse with one or more resident mitochondria, thereby allowing for (homologous) recombination and gene conversion (39, 69, 78) between the two mitochondrial genomes, which results in the generation of novel mitochondrial genotypes that are genetic mosaics. It thus appears likely that the same mechanism underlies chloroplast and mitochondrial HGT in that, initially, entire genomes (presumably within intact organelles) are transferred. The outcome, however, is different. Whereas the complete genome transfer remains visible in plastids, intergenomic recombination masks the genome transfer events in mitochondria and lets them appear as transfer or exchange of subgenomic DNA fragments.

It is noteworthy that these two different outcomes are also seen in nonplant systems. Yeast mitochondria, for example, also readily fuse and recombine and have shown HGT involving the formation of highly chimeric mitochondrial genomes (126). By contrast, animal mitochondria do not normally undergo recombination, and therefore, horizontal transfer events remain detectable as genome transfers, also referred to as mitochondrial capture (40, 83, 108).

Because mitochondrial transfer between animal cells was recognized only after horizontal transfer of organellar genomes had been discovered in plants (105), the roles of mitochondrial genome transfer in the development of cancers (83, 108) and other human and animal diseases (40) are only beginning to emerge. However, it is interesting to note that the first examples of mitochondrial genome transfer in animals were detected in transmissible cancers (83, 108), and like tissue transplantation and organ transplantation, cancer transmission between animals is analogous to grafting in plants. It will be interesting to investigate whether mitochondrial genome transfer occurs regularly upon cell, tissue, and organ transplantation in humans.

Horizontal Transfer of Nuclear DNA

The discovery of horizontal transfer of whole organellar genomes, presumably within intact organelles (105, 107) (**Figure 3**), raised the question of whether nuclear DNA fragments, chromosomes, or even entire nuclei can be mobilized in a similar manner and travel from cell to cell and between species. This idea was directly amenable to experimental investigation by introducing a simple modification into the selection scheme applied previously to visualize horizontal movement of chloroplast DNA (**Figure 3**). If both selectable marker genes are placed into the nuclear genomes of the two grafted species, mobilization of nuclear DNA should become detectable as

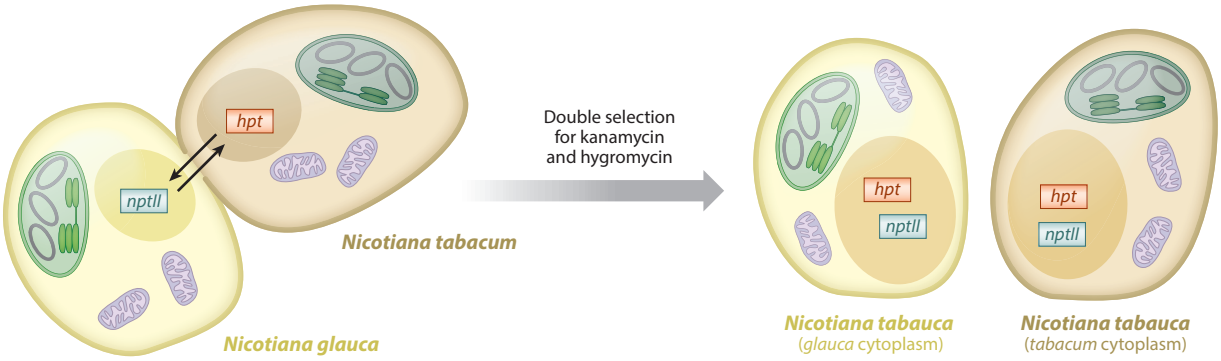

Figure 4

Experimental reconstruction of horizontal transfer of nuclear DNA between plant species. To visualize transfer of nuclear DNA across graft junctions, the researcher inserts two different antibiotic resistance genes into the nuclear genomes of the two species grafted together. In the example illustrated here, the hygromycin resistance gene *hpt* is integrated into the genome of the cigarette tobacco (*Nicotiana tabacum*), and the kanamycin resistance gene *nptII* is built into the genome of the tree tobacco (*Nicotiana glauca*). Grafting (cf. **Figure 2**) followed by double selection for both kanamycin and hygromycin resistance detects events in which either the *hpt* gene has moved from *N. tabacum* into an *N. glauca* cell or the *nptII* gene traveled from an *N. glauca* cell into an *N. tabacum* cell. Karyotyping of the events obtained from the screen revealed horizontal transfer of entire nuclear genomes resulting in the formation of a new allopolyploid species, *Nicotiana tabauca* (30). Depending on which nuclear genome was mobilized, two different subspecies of *N. tabauca* are obtained: *N. tabauca* with *glauca* cytoplasm and *N. tabauca* with *tabacum* cytoplasm. Although, for simplicity, only the plastids are indicated as genetically different here (*different shades of green*), it is important to note that also the mitochondrial genomes differ between the cytoplasms of the two species and thus can potentially contribute to phenotypic differences between the two *N. tabauca* genotypes resulting from horizontal nuclear genome transfer (30, 34).

double resistance to both selection agents (**Figure 4**). To determine whether this occurs, researchers equipped two species in the nightshade family, the cigarette tobacco (*N. tabacum*) and the tree tobacco (*Nicotiana glauca*) (**Figures 2** and **4**), with two different antibiotic resistance genes (conferring kanamycin resistance or hygromycin resistance) and used these in grafting experiments. Following establishment of the graft union, the graft sites were subjected to double selection for kanamycin and hygromycin resistance to reveal events in which one of the antibiotic resistance genes had migrated into cells of the graft partner, thus giving rise to cells harboring both *trans*-genes (**Figure 4**). Events were readily detected and all regenerating plants were shown to be the product of horizontal transfer of entire nuclear genomes (30). Consequently, the resulting plants were allopolyploid, combining within their nuclei the complete nuclear genomes of *N. tabacum* and *N. glauca* (**Figure 4**). Because these new allopolyploid plants are fertile, produce fertile progeny, and are morphologically distinct from both progenitor species, they meet all the criteria of a new species (which was dubbed *Nicotiana tabauca*). As expected, *N. tabauca* can harbor either the cytoplasmic genomes of *N. tabacum* or those of *N. glauca*, depending on which nucleus was mobilized and transferred across the graft junction (30) (**Figure 4**).

In plants, polyploidization represents a common mechanism of speciation. A recent survey across 47 genera of vascular plants found that at least 24% of all species are polyploid (13% autopolyploid and 11% allopolyploid) (6). Also, many of our modern crops are polyploid (92). The evolutionary success of polyploids is commonly associated with a heterosis-like growth vigor (22, 60, 71), improved stress tolerance (20, 101), and accelerated evolutionary adaptation (97). The apparent ease with which new allopolyploid species can arise through horizontal transfer of nuclear genomes (30) raises the attractive possibility that natural grafting (**Figure 2**) provides an asexual avenue to speciation. Its potential advantage over sexual mechanisms leading to speciation

Figure 5

Massive shoot initiation from the cambium on a cut poplar tree. Shoot formation from the cambium is induced by wounding, a process involved in natural and experimental grafting. Lateral shoot formation from the graft site provides a potential path for how horizontal genome transfer events can ultimately enter germ cells and become fixed.

by polyploidization (i.e., interspecies hybridization followed by genome doubling) lies in the fact that, compared to sexual hybridization, grafting is much less restricted by species boundaries, thus potentially creating much greater evolutionary opportunities for genome mergers.

It is important to note that, in natural grafts, horizontal genome transfers will initially be somatic events that do not immediately become heritable. In fact, given the high frequency of horizontal genome transfer observed in experimental grafts (30, 105, 107), it seems reasonable to assume that restricted entry into germ cells represents the bottleneck in speciation through horizontal genome transfer. Entry into the germ cells requires lateral shoot formation from the graft site, allowing the newly formed allopolyploid genome to become part of an apical meristem, which ultimately will give rise to flowers and seeds. Lateral shoot formation from the graft site is quite common and typically originates from the cambium, a meristematic ring surrounding the stem of vascular plants. Shoot outgrowth from the cambium is readily induced by wounding (**Figure 5**), a process also involved in graft formation (**Figure 2**). It seems conceivable that, in nature, the majority of horizontal genome transfer events remains somatic and does not become fixed, and that only in those rare cases when the recipient cell enters a newly formed shoot apical meristem does the event become heritable for the next generation.

In addition to suggesting an attractive asexual path towards the formation of new species, the horizontal transfer of nuclear genomes also opens up exciting new opportunities in plant breeding and crop improvement. Grafting can now be used as a tool to generate new allopolyploid crops

by combining the genomes of even relatively distantly related species. The phylogenetic distances that can be overcome with this approach and the novel properties of the synthetic species that can be created will be interesting to explore.

OUTLOOK

Recently developed experimental approaches allow researchers to direct the evolution of new genes, genomes, and genome combinations in the laboratory, and to experimentally reconstruct major processes in eukaryotic genome evolution, including EGT and horizontal DNA transfer. The development of facile transformation methods for nuclear and organellar genomes and the design of stringent selection schemes have made it possible to visualize even rare events in genome evolution that normally can only be detected on large evolutionary timescales and, previously, could only be inferred from phylogenetic evidence. The possibility to watch the evolution of genes and genomes in real time, by performing laboratory experiments, has opened up entirely new possibilities for the study of underlying molecular mechanisms.

Although great strides forward have been made with the development and improvement of genetic transformation methods, several technical limitations remain. The inability to engineer the mitochondrial genomes of most organisms (with the notable exception of yeast) severely limits our current capacity to study both EGT and HGT events involving the mitochondrial genome as either donor or recipient genome. This is unfortunate, because mitochondrial genomes not only send genes to the nucleus by EGT, but also receive DNA sequences and active genes from the chloroplast and nuclear genomes (62, 96, 117, 121). For example, a substantial fraction of plant mitochondrial tRNA genes is of chloroplast origin and presumably was acquired through intracellular gene transfer events (118, 120, 121). Recent work suggests that there are also rare cases of mitochondrion-to-plastid gene transfer (59, 100). With the development of workable mitochondrial transformation technologies, all these evolutionary processes would become amenable to experimental investigation.

HGT is particularly rampant in some groups of aquatic organisms, including, for example, rotifers (32, 76) and some algae (3, 11, 28, 94). Most of these organisms are currently not amenable to transgenic manipulations, a significant experimental limitation that hampers the elucidation of how they take up foreign genetic material. Given the prevalence of HGT in the nuclear genomes of these organisms, it seems conceivable that some of them exhibit natural competence for nucleic acids present in the environment. The only requirement for testing this hypothesis would be suitable selectable marker genes. Those should be easily identifiable, for example, by systematically testing antibiotic sensitivities or developing auxotrophic markers. It is important to note that, with the exception of gene transfer from pathogens (14), the experimental reconstruction of horizontal single-gene transfer has not yet been achieved in eukaryotes: Selection for horizontal DNA transfer in plants so far has only detected genome transfer events. Elucidating the pathways of how single genes are transferred horizontally into eukaryotic genomes represents a major challenge for the field. At present, we also know next to nothing about the genetic, physiological, and environmental factors that stimulate the occurrence of gene transfer and thus may have facilitated EGT and HGT in evolution. Heat stress has been proposed to result in elevated rates of DNA transfer from chloroplasts to the nucleus (119). Likewise, adoption of a parasitic lifestyle and the associated loss of photosynthesis appear to trigger higher rates of DNA escape from plastids during reductive genome evolution (24). However, much more systematic investigations are needed to identify the key factors that trigger nucleic acid release, migration between cells and compartments, and uptake and integration into the recipient genome. Clearly, with the availability of experimental systems that allow researchers to accurately measure gene transfer frequencies under highly controlled

conditions, our understanding of how, when, and why gene transfer has happened and shaped eukaryotic genomes should increase rapidly in the near future.

DISCLOSURE STATEMENT

The author is not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

Research on experimental genome evolution in the author's laboratory receives funding from the Max Planck Society and the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation program (ERC-ADG-2014; grant agreement 669982).

LITERATURE CITED

1. Acosta MC, Premoli AC. 2010. Evidence of chloroplast capture in South American *Nothofagus* (subgenus *Nothofagus*, Nothofagaceae). *Mol. Phylogenet. Evol.* 54:235–42
2. Archibald JM. 2015. Endosymbiosis and eukaryotic cell evolution. *Curr. Biol.* 25:R911–21
3. Archibald JM, Rogers MB, Toop M, Ishida K, Keeling PJ. 2003. Lateral gene transfer and the evolution of plastid-targeted proteins in the secondary plastid-containing alga *Bigelowiella natans*. *PNAS* 100:7678–83
4. Arimura S, Yamamoto J, Aida GP, Nakazono M, Tsutsumi N. 2004. Frequent fusion and fission of plant mitochondria with unequal nucleoid distribution. *PNAS* 101:7805–8
5. Baker A, Schatz G. 1987. Sequences from a prokaryotic genome or the mouse dihydrofolate reductase gene can restore the import of a truncated precursor protein into yeast mitochondria. *PNAS* 84:3117–21
6. Barker MS, Arrigo N, Baniaga AE, Li Z, Levin DA. 2016. On the relative abundance of autopolyploids and allopolyploids. *New Phytol.* 210:391–98
7. Barkman TJ, McNeal JR, Lim S-H, Coat G, Croom HB, et al. 2007. Mitochondrial DNA suggests at least 11 origins of parasitism in angiosperms and reveals genomic chimerism in parasitic plants. *BMC Evol. Biol.* 7:248
8. Bayer RG, Köstler T, Jain A, Stael S, Ebersberger I, Teige M. 2014. Higher plant proteins of cyanobacterial origin: Are they or are they not preferentially targeted to chloroplasts? *Mol. Plant* 7:1797–800
9. Bergthorsson U, Adams KL, Thomason B, Palmer JD. 2003. Widespread horizontal transfer of mitochondrial genes in flowering plants. *Nature* 424:197–201
10. Bergthorsson U, Richardson AO, Young GJ, Goertzen LR, Palmer JD. 2004. Massive horizontal transfer of mitochondrial genes from diverse land plant donors to the basal angiosperm *Amborella*. *PNAS* 101:17747–52
11. Bhattacharya D, Price DC, Chan CX, Qiu H, Rose N, et al. 2013. Genome of the red alga *Porphyridium purpureum*. *Nat. Commun.* 4:1941
12. Björkholm P, Harish A, Hagström E, Ernst AM, Andersson SGE. 2015. Mitochondrial genomes are retained by selective constraints on protein targeting. *PNAS* 112:10154–61
13. Bock R. 2000. Sense from nonsense: how the genetic information of chloroplasts is altered by RNA editing. *Biochimie* 82:549–57
14. Bock R. 2010. The give-and-take of DNA: horizontal gene transfer in plants. *Trends Plant Sci.* 15:11–22
15. Bock R. 2014. Genetic engineering of the chloroplast: novel tools and new applications. *Curr. Opin. Biotechnol.* 26:7–13
16. Bock R. 2015. Engineering plastid genomes: methods, tools, and applications in basic research and biotechnology. *Annu. Rev. Plant Biol.* 66:211–41
17. Bock R, Kössel H, Maliga P. 1994. Introduction of a heterologous editing site into the tobacco plastid genome: The lack of RNA editing leads to a mutant phenotype. *EMBO J.* 13:4623–28

18. Bock R, Timmis JN. 2008. Reconstructing evolution: gene transfer from plastids to the nucleus. *BioEssays* 30:556–66
19. Bonnefoy N, Remacle C, Fox TD. 2007. Genetic transformation of *Saccharomyces cerevisiae* and *Chlamydomonas reinhardtii* mitochondria. *Methods Cell Biol.* 80:525–48
20. Chao D-Y, Dilkes B, Luo H, Douglas A, Yakubova E, et al. 2013. Polyploids exhibit higher potassium uptake and salinity tolerance in *Arabidopsis*. *Science* 341:658–59
21. Chiang C-C, Kennell JC, Wanner LA, Lambowitz AM. 1994. A mitochondrial retroplasmid integrates into mitochondrial DNA by a novel mechanism involving the synthesis of a hybrid cDNA and homologous recombination. *Mol. Cell. Biol.* 14:6419–32
22. Comai L. 2005. The advantages and disadvantages of being polyploid. *Nat. Rev. Genet.* 6:836–46
23. Covello PS, Gray MW. 1992. Silent mitochondrial and active nuclear genes for subunit 2 of cytochrome *c* oxidase (*cox2*) in soybean: evidence for RNA-mediated gene transfer. *EMBO J.* 11:3815–20
24. Cusimano N, Wicke S. 2016. Massive intracellular gene transfer during plastid genome reduction in nongreen Orobanchaceae. *New Phytol.* 210:680–93
25. Davis CC, Anderson WR, Wurdack KJ. 2005. Gene transfer from a parasitic flowering plant to a fern. *Proc. R. Soc. B.* 272:2237–42
26. Davis CC, Wurdack KJ. 2004. Host-to-parasite gene transfer in flowering plants: phylogenetic evidence from Malpighiales. *Science* 305:676–78
27. Faßbender S, Brühl K-H, Ciriacy M, Kück U. 1994. Reverse transcriptase activity of an intron encoded polypeptide. *EMBO J.* 13:2075–83
28. Foflonker F, Price DC, Qiu H, Palenik B, Wang S, Bhattacharya D. 2015. Genome of the halotolerant green alga *Picochlorum* sp. reveals strategies for thriving under fluctuating environmental conditions. *Environ. Microbiol.* 17:412–26
29. Fuentes I, Karcher D, Bock R. 2012. Experimental reconstruction of the functional transfer of intron-containing plastid genes to the nucleus. *Curr. Biol.* 22:763–71
30. Fuentes I, Stegemann S, Golczyk H, Karcher D, Bock R. 2014. Horizontal genome transfer as an asexual path to the formation of new species. *Nature* 511:232–35
31. Gilson PR, Su V, Slamovits CH, Reith ME, Keeling PJ, McFadden GI. 2006. Complete nucleotide sequence of the chlorarachniophyte nucleomorph: nature's smallest nucleus. *PNAS* 103:9566–71
32. Gladyshev EA, Meselson M, Arkhipova IR. 2008. Massive horizontal gene transfer in bdelloid rotifers. *Science* 320:1210–13
33. Goldschmidt EE. 2014. Plant grafting: new mechanisms, evolutionary implications. *Front. Plant Sci.* 5:727
34. Greiner S, Bock R. 2013. Tuning a ménage à trois: co-evolution and co-adaptation of nuclear and organellar genomes in plants. *BioEssays* 35:354–65
35. Greiner S, Sobanski J, Bock R. 2014. Why are most organelle genomes transmitted maternally? *Bioessays* 37:80–94
36. Gurdon C, Svab Z, Feng Y, Kumar D, Maliga P. 2016. Cell-to-cell movement of mitochondria in plants. *PNAS* 113:3395–400
37. Hackstein JHP, Tjaden J, Huynen M. 2006. Mitochondria, hydrogenosomes and mitosomes: products of evolutionary tinkering! *Curr. Genet.* 50:225–45
38. Hanekamp T, Thorsness PE. 1996. Inactivation of *YME2/RNA12*, which encodes an integral inner mitochondrial membrane protein, causes increased escape of DNA from mitochondria to the nucleus in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* 16:2764–71
39. Hao W, Richardson AO, Zheng Y, Palmer JD. 2010. Gorgeous mosaic of mitochondrial genes created by horizontal transfer and gene conversion. *PNAS* 107:21576–81
40. Hayakawa K, Esposito E, Wang X, Terasaki Y, Liu Y, et al. 2016. Transfer of mitochondria from astrocytes to neurons after stroke. *Nature* 535:551–55
41. Huang CY, Ayliffe MA, Timmis JN. 2003. Direct measurement of the transfer rate of chloroplast DNA into the nucleus. *Nature* 422:72–76
42. Huang CY, Ayliffe MA, Timmis JN. 2004. Simple and complex nuclear loci created by newly transferred chloroplast DNA in tobacco. *PNAS* 101:9710–15

43. Huang CY, Grünheit N, Ahmadinejad N, Timmis JN, Martin W. 2005. Mutational decay and age of chloroplast and mitochondrial genomes transferred recently to angiosperm nuclear chromosomes. *Plant Physiol*. 138:1723–33
44. Iwai M, Yokono M, Kono M, Noguchi K, Akimoto S, Nakano A. 2015. Light-harvesting complex Lhcb9 confers a green alga-type photosystem I supercomplex to the moss *Physcomitrella patens*. *Nat. Plants* 1:14008
45. Jeffree CE, Yeoman MM. 1983. Development of intercellular connections between opposing cells in a graft union. *New Phytol*. 93:491–509
46. Jenkins BD, Kulhanek DJ, Barkan A. 1997. Nuclear mutations that block group II RNA splicing in maize chloroplasts reveal several intron classes with distinct requirements for splicing factors. *Plant Cell* 9:283–96
47. Johnston SA, Anziano PQ, Shark K, Sanford JC, Butow RA. 1988. Mitochondrial transformation in yeast by bombardment with microprojectiles. *Science* 240:1538–41
48. Kadowaki K, Kubo N, Ozawa K, Hirai A. 1996. Targeting presequence acquisition after mitochondrial gene transfer to the nucleus occurs by duplication of existing targeting signals. *EMBO J*. 15:6652–61
49. Keeling PJ, Palmer JD. 2008. Horizontal gene transfer in eukaryotic evolution. *Nat. Rev. Genet*. 9:605–18
50. Kim I, Cho E, Crawford K, Hempel FD, Zambryski PC. 2005. Cell-to-cell movement of GFP during embryogenesis and early seedling development in *Arabidopsis*. *PNAS* 102:2227–31
51. Knoop V. 2004. The mitochondrial DNA of land plants: peculiarities in phylogenetic perspective. *Curr. Genet*. 46:123–39
52. Kudla J, Albertazzi FJ, Blazević D, Hermann M, Bock R. 2002. Loss of the mitochondrial *cox2* intron 1 in a family of monocotyledonous plants and utilization of mitochondrial intron sequences for the construction of a nuclear intron. *Mol. Genet. Genom*. 267:223–30
53. Lenglez S, Hermand D, Decottignies A. 2010. Genome-wide mapping of nuclear mitochondrial DNA sequences links DNA replication origins to chromosomal double-strand break formation in *Schizosaccharomyces pombe*. *Genome Res*. 20:1250–61
54. Li F-W, Villarreal JC, Kelly S, Rothfels CJ, Melkonian M, et al. 2014. Horizontal transfer of an adaptive chimeric photoreceptor from bryophytes to ferns. *PNAS* 111:6672–77
55. Lister DL, Bateman JM, Purton S, Howe CJ. 2003. DNA transfer from chloroplast to nucleus is much rarer in *Chlamydomonas* than in tobacco. *Gene* 316:33–38
56. Logan DC. 2003. Mitochondrial dynamics. *New Phytol*. 160:463–78
57. Lucas WJ, Ham B-K, Kim J-Y. 2009. Plasmodesmata—bridging the gap between neighboring plant cells. *Trends Cell Biol*. 19:495–503
58. Lucas WJ, Lee JY. 2004. Plasmodesmata as a supracellular control network in plants. *Nat. Rev. Mol. Cell Biol.* 5:712–26
59. Ma P-F, Zhang Y-X, Guo Z-H, Li D-Z. 2015. Evidence for horizontal transfer of mitochondrial DNA to the plastid genome in a bamboo genus. *Sci. Rep.* 5:11608
60. Madlung A. 2013. Polyploidy and its effect on evolutionary success: old questions revisited with new tools. *Heredity* 110:99–104
61. Marchetti A, Parker MS, Moccia LP, Lin EO, Arrieta AL, et al. 2009. Ferritin is used for iron storage in bloom-forming marine pennate diatoms. *Nature* 457:467–70
62. Marienfeld J, Unseld M, Brennicke A. 1999. The mitochondrial genome of *Arabidopsis* is composed of both native and immigrant information. *Trends Plant Sci*. 4:495–502
63. Marin B, Nowack ECM, Melkonian M. 2005. A plastid in the making: evidence for a second primary endosymbiosis. *Protist* 156:425–32
64. Marsit S, Mena A, Bigey F, Sauvage F-X, Couloux A, et al. 2015. Evolutionary advantage conferred by an eukaryote-to-eukaryote gene transfer event in wine yeasts. *Mol. Biol. Evol.* 32:1695–707
65. Martin W, Rujan T, Richly E, Hansen A, Cornelsen S, et al. 2002. Evolutionary analysis of *Arabidopsis*, cyanobacterial, and chloroplast genomes reveals plastid phylogeny and thousands of cyanobacterial genes in the nucleus. *PNAS* 99:12246–51
66. Michalovova M, Vyskot B, Kejnovsky E. 2013. Analysis of plastid and mitochondrial DNA insertions in the nucleus (NUPTs and NUMTs) of six plant species: size, relative age and chromosomal localization. *Heredity* 111:314–20

67. Millen RS, Olmstead RG, Adams KL, Palmer JD, Lao NT, et al. 2001. Many parallel losses of *infA* from chloroplast DNA during angiosperm evolution with multiple independent transfers to the nucleus. *Plant Cell* 13:645–58
68. Moenne A, Bégu D, Jordana X. 1996. A reverse transcriptase activity in potato mitochondria. *Plant Mol. Biol.* 31:365–72
69. Mower JP, Stefanović S, Hao W, Gummow JS, Jain K, et al. 2010. Horizontal acquisition of multiple mitochondrial genes from a parasitic plant followed by gene conversion with host mitochondrial genes. *BMC Biol*. 8:150
70. Mower JP, Stefanović S, Young GJ, Palmer JD. 2004. Gene transfer from parasitic to host plants. *Nature* 432:165–66
71. Ni Z, Kim E-D, Ha M, Lackey E, Liu J, et al. 2009. Altered circadian rhythms regulate growth vigour in hybrids and allopolyploids. *Nature* 457:327–31
72. Nowack ECM, Grossman AR. 2012. Trafficking of protein into the recently established photosynthetic organelles of *Paulinella chromatophora*. *PNAS* 109:5340–45
73. Nowack ECM, Melkonian M, Glöckner G. 2008. Chromatophore genome sequence of *Paulinella* sheds light on acquisition of photosynthesis by eukaryotes. *Curr. Biol.* 18:410–18
74. Nugent JM, Palmer JD. 1991. RNA-mediated transfer of the gene *coxII* from the mitochondrion to the nucleus during flowering plant evolution. *Cell* 66:473–81
75. Ong HC, Palmer JD. 2006. Pervasive survival of expressed mitochondrial *rps14* pseudogenes in grasses and their relatives for 80 million years following three functional transfers to the nucleus. *BMC Evol. Biol.* 6:55
76. Overballe-Petersen S, Willerslev E. 2014. Horizontal transfer of short and degraded DNA has evolutionary implications for microbes and eukaryotic sexual reproduction. *BioEssays* 36:1005–10
77. Pamilo P, Viljakainen L, Vihavainen A. 2007. Exceptionally high density of NUMTs in the honeybee genome. *Mol. Biol. Evol.* 24:1340–46
78. Park S, Grewe F, Zhu A, Ruhlman TA, Sabir J, et al. 2015. Dynamic evolution of *Geranium* mitochondrial genomes through multiple horizontal and intracellular gene transfers. *New Phytol*. 208:570–83
79. Parkinson M, Jeffree CE, Yeoman MM. 1987. Incompatibility in cultured explant-grafts between members of the Solanaceae. *New Phytol*. 107:489–98
80. Pittis AA, Gabaldón T. 2016. Late acquisition of mitochondria by a host with chimaeric prokaryotic ancestry. *Nature* 531:101–4
81. Randolph-Anderson BL, Boynton JE, Gillham NW, Harris EH, Johnson AM, et al. 1993. Further characterization of the respiratory deficient *dum-1* mutation of *Chlamydomonas reinhardtii* and its use as a recipient for mitochondrial transformation. *Mol. Gen. Genet.* 236:235–44
82. Rasmussen B, Fletcher IR, Brocks JJ, Kilburn MR. 2008. Reassessing the first appearance of eukaryotes and cyanobacteria. *Nature* 455:1101–4
83. Rebbeck CA, Leroi AM, Burt A. 2011. Mitochondrial capture by a transmissible cancer. *Science* 331:303
84. Ricchetti M, Fairhead C, Dujon B. 1999. Mitochondrial DNA repairs double-strand breaks in yeast chromosomes. *Nature* 402:96–100
85. Rice DW, Alverson AJ, Richardson AO, Young GJ, Sanchez-Puerta MV, et al. 2013. Horizontal transfer of entire genomes via mitochondrial fusion in the angiosperm *Amborella*. *Science* 342:1468–73
86. Richardson AO, Palmer JD. 2007. Horizontal gene transfer in plants. *J. Exp. Bot.* 58:1–9
87. Richly E, Leister D. 2004. NUMTs in sequenced eukaryotic genomes. *Mol. Biol. Evol.* 21:1081–84
88. Rieseberg LH, Soltis DE. 1991. Phylogenetic consequences of cytoplasmic gene flow in plants. *Evol. Trends Plants* 5:65–84
89. Ropars J, de la Vega RCR, López-Villavicencio M, Gouzy J, Sallet E, et al. 2015. Adaptive horizontal gene transfers between multiple cheese-associated fungi. *Curr. Biol.* 25:2562–69
90. Roux F, Mary-Huard T, Barillot E, Wenes E, Botran L, et al. 2016. Cytonuclear interactions affect adaptive traits of the annual plant *Arabidopsis thaliana* in the field. *PNAS* 113:3687–92
91. Ruf S, Karcher D, Bock R. 2007. Determining the transgene containment level provided by chloroplast transformation. *PNAS* 104:6998–7002
92. Sattler MC, Carvalho CR, Clarindo WR. 2016. The polyploidy and its key role in plant breeding. *Planta* 243:281–96

93. Schmitz-Linneweber C, Kushnir S, Babiychuk E, Poltnigg P, Herrmann RG, Maier RM. 2005. Pigment deficiency in nightshade/tobacco cybrids is caused by the failure to edit the plastid ATPase α-subunit mRNA. *Plant Cell* 17:1815–28
94. Schönknecht G, Chen W-H, Ternes CM, Barbier GG, Shrestha RP, et al. 2013. Gene transfer from bacteria and Archaea facilitated evolution of an extremophilic eukaryote. *Science* 339:1207–10
95. Schuster W, Brennicke A. 1987. Plastid, nuclear and reverse transcriptase sequences in the mitochondrial genome of *Oenothera*: Is genetic information transferred between organelles via RNA? *EMBO J.* 6:2857–63
96. Schuster W, Brennicke A. 1988. Interorganellar sequence transfer: Plant mitochondrial DNA is nuclear, is plastid, is mitochondrial. *Plant Sci.* 54:1–10
97. Selmecki AM, Maruvka YE, Richmond PA, Guillet M, Shoresh N, et al. 2015. Polyploidy can drive rapid adaptation in yeast. *Nature* 519:349–52
98. Sheppard AE, Ayliffe MA, Blatch L, Day A, Delaney SK, et al. 2008. Transfer of plastid DNA to the nucleus is elevated during male gametogenesis in tobacco. *Plant Physiol.* 148:328–36
99. Sheppard AE, Timmis JN. 2009. Instability of plastid DNA in the nuclear genome. *PLOS Genet.* 5:e1000323
100. Smith DR. 2014. Mitochondrion-to-plastid DNA transfer: It happens. *New Phytol.* 202:736–38
101. Soltis PS, Marchant DB, Van de Peer Y, Soltis DE. 2015. Polyploidy and genome evolution in plants. *Curr. Op. Genet. Dev.* 35:119–25
102. Soucy SM, Huang J, Gogarten JP. 2015. Horizontal gene transfer: building the web of life. *Nat. Rev. Genet.* 16:472–82
103. Spang A, Saw JH, Jørgensen SL, Zaremba-Niedzwiedzka K, Martijn J, et al. 2015. Complex archaea that bridge the gap between prokaryotes and eukaryotes. *Nature* 521:173–79
104. Stegemann S, Bock R. 2006. Experimental reconstruction of functional gene transfer from the tobacco plastid genome to the nucleus. *Plant Cell* 18:2869–78
105. Stegemann S, Bock R. 2009. Exchange of genetic material between cells in plant tissue grafts. *Science* 324:649–51
106. Stegemann S, Hartmann S, Ruf S, Bock R. 2003. High-frequency gene transfer from the chloroplast genome to the nucleus. *PNAS* 100:8828–33
107. Stegemann S, Keuthe M, Greiner S, Bock R. 2012. Horizontal transfer of chloroplast genomes between plant species. *PNAS* 109:2434–38
108. Strakova A, Leathlobhair MN, Wang G-D, Yin T-T, Airikkala-Otter I, et al. 2016. Mitochondrial genetic diversity, selection and recombination in a canine transmissible cancer. *eLife* 5:14552
109. Svab Z, Maliga P. 1993. High-frequency plastid transformation in tobacco by selection for a chimeric *aadA* gene. *PNAS* 90:913–17
110. Thorsness PE, Fox TD. 1990. Escape of DNA from mitochondria to the nucleus in *Saccharomyces cerevisiae*. *Nature* 346:376–79
111. Thorsness PE, Fox TD. 1993. Nuclear mutations in *Saccharomyces cerevisiae* that affect the escape of DNA from mitochondria to the nucleus. *Genetics* 134:21–28
112. Thyssen G, Svab Z, Maliga P. 2012. Cell-to-cell movement of plastids in plants. *PNAS* 109:2439–43
113. Timmis JN, Ayliffe MA, Huang CY, Martin W. 2004. Endosymbiotic gene transfer: Organelle genomes forge eukaryotic chromosomes. *Nat. Rev. Genet.* 5:123–35
114. Ueda M, Fujimoto M, Arimura S, Murata J, Tsutsumi N, Kadowaki K. 2007. Loss of *rpl32* gene from the chloroplast genome and subsequent acquisition of a preexisting transit peptide within the nuclear gene in *Populus*. *Gene* 402:51–56
115. Ueda M, Fujimoto M, Arimura S, Tsutsumi N, Kadowaki K. 2006. Evidence for transit peptide acquisition through duplication and subsequent frameshift mutation of a preexisting protein gene in rice. *Mol. Biol. Evol.* 23:2405–12
116. Ueda M, Fujimoto M, Arimura S, Tsutsumi N, Kadowaki K. 2008. Presence of a latent mitochondrial targeting signal in gene on mitochondrial genome. *Mol. Biol. Evol.* 25:1791–93
117. Unseld M, Marienfeld JR, Brandt P, Brennicke A. 1997. The mitochondrial genome of *Arabidopsis thaliana* contains 57 genes in 366,924 nucleotides. *Nat. Genet.* 15:57–61

118. Veronico P, Gallerani R, Ceci LR. 1996. Compilation and classification of higher plant mitochondrial tRNA genes. *Nucleic Acids Res*. 24:2199–203
119. Wang D, Lloyd AH, Timmis JN. 2012. Environmental stress increases the entry of cytoplasmic organellar DNA into the nucleus in plants. *PNAS* 109:2444–48
120. Wang D, Rousseau-Gueutin M, Timmis JN. 2012. Plastid sequences contribute to some plant mitochondrial genes. *Mol. Biol. Evol.* 29:1707–11
121. Wang D, Wu Y-W, Shih AC-C, Wu C-S, Wang Y-N, Chaw S-M. 2007. Transfer of chloroplast genomic DNA to mitochondrial genome occurred at least 300 MYA. *Mol. Biol. Evol.* 24:2040–48
122. Wang H, Lambowitz AM. 1993. The Mauriceville plasmid reverse transcriptase can initiate cDNA synthesis de novo and may be related to reverse transcriptase and DNA polymerase progenitor. *Cell* 75:1071–81
123. Wolf S, Deom CM, Beachy RN, Lucas WJ. 1989. Movement protein of tobacco mosaic virus modifies plasmodesmatal size exclusion limit. *Science* 246:377–79
124. Wolfe KH, Shields DC. 1997. Molecular evidence for an ancient duplication of the entire yeast genome. *Nature* 387:708–13
125. Won H, Renner SS. 2003. Horizontal gene transfer from flowering plants to Gnetum. *PNAS* 100:10824–29
126. Wu B, Buljic A, Hao W. 2015. Extensive horizontal transfer and homologous recombination generate highly chimeric mitochondrial genomes in yeast. *Mol. Biol. Evol.* 32:2559–70
127. Xi Z, Wang Y, Bradley RK, Sugumaran M, Marx CJ, et al. 2013. Massive mitochondrial gene transfer in a parasitic flowering plant clade. *PLOS Genet*. 9:e1003265
128. Xoconostle-Cázares B, Xiang Y, Ruiz-Medrano R, Wang H-L, Monzer J, et al. 1999. Plant paralog to viral movement protein that potentiates transport of mRNA into the phloem. *Science* 283:94–98
129. Yu X, Gabriel A. 1999. Patching broken chromosomes with extranuclear cellular DNA. *Mol. Cell* 4:873–81
130. Zhu S, Zhu M, Knoll AH, Yin Z, Zhao F, et al. 2016. Decimetre-scale multicellular eukaryotes from the 1.56-billion-year-old Gaoyuzhuang formation in North China. *Nat. Commun.* 7:11500