Letter

Mitochondrion-to-plastid DNA transfer: it happens

Introduction

When different genetic compartments exist in close proximity to one another there is the potential for intercompartmental DNA transfer. Land plants and eukaryotic algae exemplify this point. Within each of their cells there are three distinct genomes: nuclear, mitochondrial, and plastid, which are made up of nucDNA, mtDNA, and ptDNA, respectively. The sequencing of these different DNAs has uncovered staggering amounts of intracellular gene transfer (Timmis *et al.*, 2004; Kleine *et al.*, 2009). For example, the nuclear genomes of many seed plants, including rice and corn, contain thousands of nucleotides of recent mitochondrial and plastid origin, most of which are nonfunctional (Hazkani-Covo *et al.*, 2010; Smith *et al.*, 2011). Similarly, seed plant mitochondrial genomes have accumulated large amounts of DNA from the neighboring plastid and nuclear genomes (Wang *et al.*, 2007; Smith, 2011). More than 5% of the cumber (*Cucumis sativus*) and zucchini (*Cucurbita pepo*) mitochondrial genomes, for instance, are represented by plastid- and nuclear-derived sequences (Alverson *et al.*, 2010, 2011).

Given the prevalence of intracellular DNA migration into the nuclear and mitochondrial genomes of seed plants, one might expect their plastid genomes to also harbor large amounts of horizontally acquired DNA. But the opposite is true. Seed plant plastid genomes, and ptDNAs as a whole, are surprisingly devoid of either mitochondrial- or nuclear-derived DNA (Richardson & Palmer, 2007; Smith, 2011). This observation has led to the assumption that the intracellular transfer of mtDNA or nucDNA to plastids does not occur – a theory backed by the observed lack of an efficient DNA uptake system within plastids (Bock, 2010), but see Cerutti & Jagendorf (1995). Moreover, plastids, unlike mitochondria, do not regularly undergo fusion event, thus, limiting the potential for foreign DNA to sneak in. Recent data from the organelle genomes of carrots and milkweeds, however, are changing this view, proving that mitochondrion-to-chloroplast genetic transfer does occur and that plastids are not as impenetrable to foreign DNA as previously thought.

Clues from the carrot chloroplast

Three years after it was sequenced, the plastid genome of carrot, *Daucus carota*, was unexpectedly found (during a cursory blast analysis) to have a *c.* 1.5 kb region of potential mitochondrial origin (Goremykin *et al.*, 2009). This region, which is dubbed the *D. carota* Mitochondrial-like Plastid (*Dc*MP) sequence and located in the *rps12-trnV* intergenic spacer, is similar to conserved coding and noncoding segments of seed plant mtDNA, including *cox1*, suggesting it arose through mtDNA transfer to the plastid (Fig. 1). Support for this hypothesis came from the sequencing of the carrot mitochondrial genome and the discovery that it too contains a complete, but fragmented, *Dc*MP sequence (Iorizzo *et al.*, 2012a). Further analyses revealed that diverse members of the carrot family (Apiaceae) contain a large piece of *Dc*MP within their mtDNA, but that only *Daucus* species and their close relative cumin (*Cuminum cyminum*) have a plastid *Dc*MP. Together, these observations were taken as proof that a mitochondrial-located *Dc*MP was present in the ancestor of the Apiaceae and subsequently spread to the plastid genome in the common ancestor of *Daucus* and *Cuminum* (Iorizzo *et al.*, 2012b) (Fig. 1).

Complicating the story is that *Dc*MP, apart from having a short *cox1*-like segment, shows little similarity to other sequences in GenBank, making it difficult to trace its origin within the Apiaceae, and leaving open the possibility that it began in the nuclear compartment and was then transferred independently to the mitochondrial and plastid genomes (Iorizzo *et al.*, 2012a). Moreover, within the carrot mitochondrial genome, *Dc*MP is fragmented and scrambled into three pieces, two of which are 80 kb apart from one another (Fig. 1). Assuming that *Dc*MP migrated from the mtDNA to the ptDNA as an intact element and in a single event, its fragmentation within the mitochondrial genome must have occurred post-migration, most likely through mtDNA recombination (Iorizzo *et al.*, 2012a), which frequently occurs in seed plants (Alverson *et al.*, 2011). *Dc*MP has some characteristics resembling those of non-LTR retrotransposons, including an open reading frame for a putative reverse transcriptase (Iorizzo *et al.*, 2012b), and it is speculated that *Dc*MP retrotransposed from the mitochondrial to the plastid genome – a scenario that could explain the short direct repeats bookending its ptDNA sequence. Some non-LTR elements target tRNA-coding regions, which may be why *Dc*MP is located upstream of *trnV* within the plastid genome. There is also reason to believe that *Dc*MP is acting as a surrogate promoter for *trnV*, having replaced the original promoter upon insertion (Iorizzo *et al.*, 2012b). If true, it would mean that a mitochondrial-derived sequence is playing a functional role within a plastid genome.

Milkweeds: mitochondrial proof in the ptDNA pudding

A separate example of a DNA transfer from a mitochondrial to a plastid genome has come from the common milkweed, *Asclepias syriaca,* which belongs to a well-studied and large family of flowering plants called the Apocynaceae. Similar to the situation for *Daucus carota*, the *A. syriaca* plastid genome sequence was published and available in GenBank for over a year (Straub *et al.*, 2011) before it was discovered to have a 2.4 kb mtDNA-like insert,

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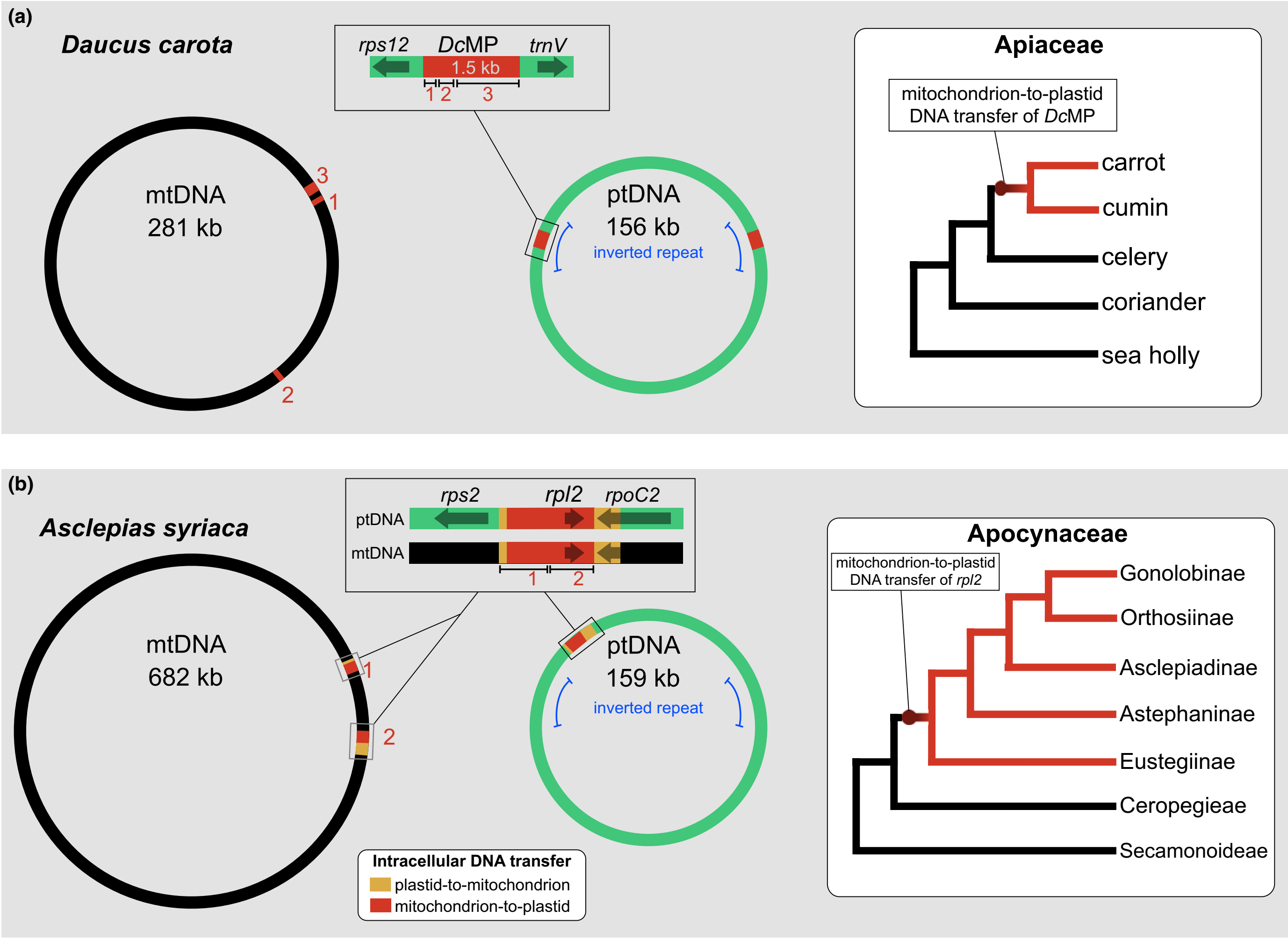

Fig. 1 Examples of mitochondrion-to-plastid DNA transfer from (A) the carrot, *Daucus carota*, which belongs to the Apiaceae, and (B) the common milkweed, *Asclepias syriaca*, which belongs to the Apocynaceae. Genome maps (not to scale) and trees are based on the results of Iorizzo *et al.* (2012a,b) and Straub *et al.* (2013).

located in the *rps2*-*rpoC2* intergenic spacer (Ku *et al.*, 2013) (Fig. 1). But unlike the case for carrot, the putative transferred element contains an intact exon for a mitochondrial ribosomal protein (*rpl2*), leaving little doubt about its mtDNA origin (Straub *et al.*, 2013). Assembly of the *A. syriaca* mitochondrial genome confirmed that the *rpl2*-containing segment of the plastid genome does indeed match to milkweed mtDNA, specifically to the second exon of *rpl2* and its flanking noncoding sequence, as well as to a piece of mtDNA from a completely different region (Straub *et al.*, 2013). The sequence identity between the mitochondrial and plastid versions of *rpl2* is high (*c.* 92%), but the plastid copy has a nonsense mutation, resulting in a premature stop codon and implying that it is a pseudogene, which is consistent with its observed low levels of transcription relative to other plastid-located genes (Straub *et al.*, 2013). Remarkably, the sequence bordering the *rpl2* insert in the *A. syriaca* plastid genome, including a portion of *rpoC2*, is present in the milkweed mitochondrial genome immediately adjacent to the genuine *rpl2*, meaning that the fragment of mtDNA that migrated to the plastid (*rpl2*) sits beside a section of ptDNA that migrated to the mitochondrion (*rpoC2*), and *vice versa* (Fig. 1).

Using PCR and next-generation sequencing, Straub *et al.* (2013) uncovered an *rpl2* insert in the ptDNAs from other members of the Apocynaceae, and ultimately traced its origin back to a single mitochondrion-to-plastid DNA transfer event in the common ancestor of the Asclepiadeae and *Eustegia* (Fig. 1). They also found within various apocynacean species a mitochondrial-located *rpoC2*, which, as it turns out, is more broadly distributed within the group than the *rpl2* insert, suggesting that the transfer of *rpoC2* from the plastid to the mitochondrion predates the mtDNA-to-ptDNA *rpl2* migration. In fact, the close proximity of the mitochondrial *rpl2* to a ptDNA-derived sequence might have facilitated its eventual integration into the plastid genome via repair of a double-stranded break by homologous recombination between mtDNA and ptDNA (Straub *et al.*, 2013).

More examples on the way?

In light of these new data from carrots and milkweeds, it is safe to say that mitochondrion-to-plastid DNA transfer does occur (but the reverse process occurs much more frequently). It is still not

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known in either of these cases, however, how the mtDNA entered the plastid or how it integrated into the plastid genome, be it by retrotransposition, homologous recombination, or some other process. The complete plastid genome sequences of *Daucus carota* and *Asclepias syriaca* were both available for some time before the proposed mtDNA insert was discovered, hinting that there are likely other published ptDNA sequences with undiscovered mitochondrial-derived regions.

Of the six possible directions of intracellular genetic transfer within a plastid-containing species only one – nuclear-to-plastid DNA migration – remains to be described. But if the study of organelle genetics has taught us anything, it is to expect the unexpected. Next-generation sequencing techniques have greatly increased the rate at which plastid, mitochondrial, and nuclear genomes are becoming available. Soon we will be inundated with unprecedented amounts of organelle genomic data, some of which will most certainly contain more examples of foreign DNA within plastid genomes.

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