Against the traffic
The first evidence for mitochondrial DNA transfer into the plastid genome

Massimo Iorizzo,1 Dariusz Grzebelus,2 Douglas Senalik,1,3 Marek Szklarczyk,2 David Spooner1,3* and Philipp Simon1,3,*

1Department of Horticulture; University of Wisconsin; Madison, WI USA; 2Department of Genetics; Plant Breeding and Seed Science; University of Agriculture in Krakow; Krakow, Poland; 3USDA-Agricultural Research Service; Vegetable Crops Research Unit; University of Wisconsin; Madison, WI USA

Keywords: Daucus carota, inter-compartmental DNA migration, plastid and mitochondrial genome, retrotransposon

Transfer of DNA between different compartments of the plant cell, i.e., plastid, mitochondrion and nucleus, is a well-known phenomenon in plant evolution. Six directions of inter-compartmental DNA migration are possible in theory, however only four of them have been previously reported. These include frequent cases of mitochondrion and plastid to nucleus transfer, plastid to mitochondrion transfer, and rare nucleus to mitochondrial migrations. The connection between the plastid and mitochondrial genomes in flowering plants has been viewed as a one way road. Contrary to these observations we found that a sequence widespread in the carrot mitochondrial genome, designated as DcMP, was transferred to the plastid genome of a carrot ancestor. Interestingly, DcMP was integrated into a tRNA promoter of the plastid trnV gene, replacing the original promoter sequence. The rearrangement of the plastid genome is specific for carrot and closely related species belonging Scandiceae clade. The structure of the sequence and the presence of a 6 nt target site duplication led us to speculate that the transfer was a result of a transposition event of a non-LTR retrotransposon. These findings open interesting questions about the evolution of organellar genomes and mobile genetic elements and provide a useful plastid marker to phylogenetically delineate species relationships within the Scandiceae clade.

Introduction

Inter-compartmental DNA migration is a well-known phenomenon in plant cell evolution and has contributed significantly to genome evolution by relocating and refashioning genes and consequently contributing to genetic diversity. DNA transfer among the plastid, mitochondrial and nuclear genomes resulted in a functional relocation of organellar genes early in organelle evolution.1,8 In contrast, almost all reported recent inter-compartmental DNA transfers gave rise to non-coding sequences or pseudogenes.1 Six directions of the inter-compartmental DNA migration are possible in theory, and four of them have been previously reported in angiosperms. Transfers of DNA from the mitochondria and from the plastid to the nucleus are the most common reported transfers.7,8 Transfers of DNA from the plastid to the mitochondrion have also been frequently observed. Cases of nuclear DNA transfer to the exceptionally large mitochondrial genomes of Fabaceae and Cucurbitaceae were also reported.9,10 In contrast, no evidence of DNA transfer from the nucleus or the mitochondrion to the plastid has been reported in angiosperms. Smith11 investigated evidence for the presence of mitochondrial DNA in the plastid genome of 42 species including 11 angiosperms and did not find any mtDNA-like sequences. Forces driving inter-comparmental DNA transfer are still unclear. Plant mitochondria actively import DNA via a permeability transition pore complex.12 In contrast, the relative absence of nuclear and mitochondrial DNA in the plastome could reflect the lack of a DNA uptake system, due to the integrity of the plastid membrane. Escape of DNA from organellar genomes to the nuclear genome seems to coincide with the degradation of organelle DNA.1 Both mobile elements and integration of DNA by nonhomologous end-joining (NHEJ) repair of double-strand breaks (DBSs) are important components of the DNA transfer machinery. In our recent paper13 we provided evidence that a sequence designated DcMP (D. carota mitochondrial-plastid), was transferred from the mitochondrial genome to the plastid genome of the carrot ancestor. Here we discuss a hypothetical mechanism explaining this event. To date, this represents the first evidence for this direction of DNA transfer. Given the large amount of available angiosperm sequence data, this strongly suggests that this is a rare event. However, the increasing availability of new organellar and nuclear genome sequences could reveal similar events in other species, and this could elucidate the mechanism that makes the uptake of DNA by the plastid genome such a rare event.

*Correspondence to: Philipp Simon; Email: philipp.simon@ars.usda.gov
Submitted: 10/13/12; Revised: 11/27/12; Accepted: 12/02/12
http://dx.doi.org/10.4161/mge.23088
**Daucus carota**  
**Mitochondrial-Plastid (DcMP) Region**

Following the evidence provided by Goremykin and colleagues\(^{14}\) that a region in the *Vitis vinifera* mitochondrial genome was similar to a fragment of the carrot plastid genome, we identified that this region was part of a DNA segment designated as DcMP that was likely transferred from the mitochondrial to the plastid genome of carrot.\(^{13}\) This was the first time that DNA transfer from the mitochondrion to the plastid was reported in flowering plants. Our evidence indicated that the transferred segment was at least 1,452 nt-long. Currently, it resides in mitochondrial genomes of a wide range of Apiaceae species, but its presence in the plastid genome is restricted to the genus *Daucus* and their close relatives including cumin (*Cuminum cyminum*), indicating that the transfer took place in their common ancestor. This observation suggests that DcMP could be a characteristic of species belonging to the tribe Scandiceae\(^{15,16}\) (Fig. 1A). The insertion into the plastid genome was accompanied by a deletion of 339 nt sequence (Fig. 1A, segment C), which was present in the empty insertion site of all examined non-Scandiceae species. The DcMP region was apparently transferred as a contiguous sequence from the mitochondria to the plastid, but at present it is highly rearranged in the investigated mitochondrial genomes (Fig. 1B). The position of the DcMP segments 3 and 4 is conserved across the mitochondrial genomes of all investigated Apiaceae species. In the carrot mitochondrial genome segments 1 and 2 are physically separated from 3 and 4 and located ca. 5 kb and over 80 kb downstream, respectively, relative to DcMP 3 and 4 (Fig. 1B). Notably, only DcMP segment 2 has homology to other plant mitochondrial genomes—it is a fragment of the *cox1* gene. It is therefore likely that the presence of the DcMP segments 1, 3 and 4 in the carrot mitochondrial genome resulted from a DNA transfer of unknown origin, common to Apiaceae, which predated the transfer from the mitochondria to the plastid. The observed fragmentation of the DcMP region in mtDNA can be explained by a high level of recombination activity often observed in plant mitochondrial genomes, and resulting in the intragenomic reshuffling of DNA segments.

---

**Functional Replacement of a tRNA\(^{\text{Val}}\) Initiation Site**

The DcMP sequence integrated into the plastid genome just upstream of the tRNA\(^{\text{Val}}\) gene (trnV), located in the large inverted repeat. Interestingly, Manna et al.\(^{17}\) investigated the expression level of *trnV* during carrot embryogenesis and identified three putative plastid *trnV* promoters: P1, P2 and P3 (Fig. 2). They observed that the three promoters mapped to a region of minor conservation when compared with the tobacco and maize plastid genomes, where two putative *trnV* promoters P4 and P5 were previously identified\(^{18}\) (Fig. 2). Promoters P1, P2 and P3 are located 105, 41 and 16 nt upstream of *trnV*, respectively. They suggested that these three promoters were responsible for the differential expression of *trnV* during embryogenesis. Our findings revealed that all three promoters were in fact a part of the integrated DcMP sequence (Fig. 2, shaded sequence), while P4 and P5 were located at the 3’ end of the 339 nt DNA fragment that was replaced by DcMP in the carrot plastid genome (Fig. 2, rectangular box; Fig. 1A, segment C). A comparison of the carrot, tobacco (*Nicotiana tabacum*), and parsley (*Petroselium crispum*) plastid genomes in...
this region revealed that P4 and P5 are conserved in parsley. This strongly suggests that with the integration of DcMP into the plastid genome, the conserved promoter region upstream of a trnV was replaced with a functional substitute, resulting in the modified expression pattern of trnV.

**Sequence Analysis of the Plastid DcMP Variant and a Possible Mechanism for the DcMP Transfer**

Analysis of the plastid DcMP sequence, the structure of which likely represents the organization of the transferred contiguous sequence, could suggest its possible mode of integration. Annotation of DcMP sequence was performed using Open Reading Frame Finder (http://www.ncbi.nlm.nih.gov/gorf/gorf.html) website. Investigation of repeat sequences was carried using blastn. In order to detect sequences flanking the plastid DcMP region with homology to sequences flanking the mitochondrial DcMP region, the sequence of the plastid genome was queried against a local database containing the carrot mitochondrial genome.

Sequence annotation identified three putative ORFs (Open Reading Frame), ORFa and ORFb of 153 and 408 nt respectively (Fig. 3) both with similarity to a gag domain annotated in Vitis vinifera, and the third ORF, ORFc of 144 nt (Fig. 3) with moderate similarity to a reverse transcriptase annotated in Medicago truncatula. Further characterization of the DcMP region in the plastid revealed the presence of a 6 nt direct repeat (CTTGAC), immediately flanking DcMP (Fig. 3, blue vertical lines), i.e., the repeats were present directly upstream of DcMP1 and directly downstream of DcMP4. These characteristics suggest that the DcMP might be a non-LTR retrotransposon and the direct repeats represent target site duplication (TSD) created as a result of the DcMP integration following its mobilization from a donor site localized in the mitochondrial genome. It has been shown that some non-LTR elements may specifically target RNA polymerase III dependent genes, such as tRNA genes. Recently, Wenke et al. described a group of non-LTR retrotransposons targeting tRNA genes in Dictyostelium discoideum. Integration of non-LTR elements can alter genome structure and function and has been associated with genome rearrangements, deletions and in some cases it resulted in modified expression of adjacent genes. Alternative mechanisms could have involved homologous recombination by microhomology or transfer of a group I intron. Homologous recombination would require that regions of plastome have homology to the recombinant DNA fragment. However, since we found no
homology between sequences flanking the mitochondrial DcMP and the plastid genome\textsuperscript{13} we find this possibility unlikely. In addition, homologous recombination is an unlikely mechanism for replacing a functional promoter. A second alternative mechanism could have involved group I introns which catalyze their own splicing and function as mobile elements, such as those found in the tRNA\textsubscript{Leu}\textsuperscript{26} genes of ptDNA.\textsuperscript{26} Also group I introns are present in the mitochondrial \textit{cox1} gene and are involved in horizontal gene transfer. The structure of DcMP does not support this hypothesis. In fact DcMP has almost no internal homology (11 nt maximum), excluding the possibility of the characteristic folding exhibited by group I introns. Interestingly, the \textit{D. carota} mt genome lacks the \textit{cox1} group I intron as reported by Sanchez-Puerta et al.\textsuperscript{27} for many other angiosperm species.

Considering the structure of the DcMP region and the functionality of its sequence proposed by Manna et al.\textsuperscript{17} we speculate that it represents a non-LTR element, and that a retrotransposition event likely caused its integration into the mitochondrial genome from an unknown source, which was then followed by the reported transfer to the plastid genome. At present, it is difficult to speculate about the exact mechanism accounting for the transfer and integration of DcMP into the organellar genomes. If the current non-contiguous organization of the DcMP region in the carrot mitochondrial genome is shared by many Apiaceae, this migration event likely was much older than the transfer to the plastid genome, which has been restricted to a much narrower group of closely related species. Moreover, the fragmentation of DcMP in the mitochondria likely predated the transfer into plastids based upon its apparent rearrangements observed in the mitochondrial genome and described above. However, the presence of the \textit{cox1} gene fragment in the region that migrated into the plastid genome suggests that the donor sequence must have originated in the mitochondrial genome. A possible scenario could have involved retrotransposition into the mitochondrial genome, mtDNA re-arrangements and acquisition of the \textit{cox1} sequence transcription from a chimeric mitochondrial donor sequence and reverse transcription, and finally transfer into the plastid and integration of the new copy comprising both retroelement-specific and mitochondrial

---

**Figure 4.** A schematic representation of the hypothetical mitochondrial to plastid genome transfer of DcMP in the carrot ancestor. (I) Transcription of a chimeric segment (DcMP) comprising non-LTR fragments and Mt-specific regions (including \textit{cox1}, red segments); (II) Migration of DcMP transcript to the cytoplasm and into the plastid; (III) Integration of DcMP and deletion of the ancestral Pt-specific segment (light green segment) including creation of a target site duplication (blue vertical lines) and replacement of the conserved promoters P4 and P5 (yellow vertical lines) with P1, P2 and P3 (red vertical lines) upstream of \textit{trnV} (violet segment), the reverse transcription step is not shown; (IV) Copy correction in the inverted repeat (IR, dark green segments); (V) Mt genome reshuffling and deletions of DcMP; (VI) Accumulation of lineage-specific deletions in the DcMP region. The scheme is not drawn to scale.
sequences (Fig. 4). The scenario involving retrotransposition gains some support from the fact that a retroelement-related ORF coding for reverse transcriptase had already been found in the plastome of green algae.28

In our scenario, following transcription, retrotransposon RNA migrates into the cytoplasm and proliferates via a ‘copy and paste’ mechanism requiring an RNA intermediate. Here the proteins required for transposition are produced (Fig. 4I). Although the mechanism of this migration is not clear, it is worth mentioning that cases of out-of-mitochondria transcript translocation have been well evidenced for animal cells.29,31 A horizontal transfer scenario would require that the RNA copy was transported into the plastid (Fig. 4II). The mechanism leading to this event is unknown, but previous studies revealed that stress conditions such as heat shock could cause pore formation in the plastid envelope and allow entry of DNA.32 Related research also demonstrated that plastids were actively able to import mRNA.33 We observed that, in the plastid genome DcMP integrated upstream of the trnV creating a deletion of the ancestral plastid sequence and a target site duplication (TSD) (Fig. 4III). It was not possible to identify the integration region in the mitochondrial genome, due to extensive rearrangements it underwent subsequent to insertion (Fig. 4V). The functional replacement of promoter activity upstream of the trnV avoided negative selection and we speculate that its transfer into the inverted repeat (IR), followed by a copy correction mechanism, that duplicated the introduced DcMP into the other inverted repeat (Fig. 4IV), resulted in a stable integration into the plastid genome. After the integration, deletion events could have occurred within other species of Daucus or Cuminum, both members of the Scandiceae clade (Figs. 1A and 4VI).

**Significance and Applications**

The data discussed here provide new insights in the dynamics of inter-compartmental DNA migration. The structure of the DcMP sequence, along with its functional aspects, provides a plausible scenario for the evolution of organelar genomes, including integration of mobile genetic elements, and suggests a mechanism for that process. As revealed by studies in plants and other organisms, transposons are very powerful markers to trace ancestor history. Considering the stability of the plastid genome, the presence of the DcMP region in members of the Scandiceae makes it a candidate marker to delineate relationships in this clade.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

**References**

1. Kleine T, Maier UG, Leister D. DNA transfer from organelles to the nucleus: the idiosyncratic genomics of endosymbiosis. Annu Rev Plant Biol 2009; 60:115-38; PMID:19014347; http://dx.doi.org/10.1146/annurev.arplant.030408.092119.

2. Woloszyńska M, Heteroplasmy and stoichiometric complexity of plant mitochondrial genomes—though this be madness, yet there’s method in’t. J Exp Bot 2010; 61:657-71; PMID:19958526; http://dx.doi.org/10.1093/jxb/erp361.

3. Davila HI, Arietia-Montiel MP, Wamboldt Y, Cao J, Hagmann J, Sledge V, et al. Double-strand break repair processes drive evolution of the mitochondrial genome in Arabidopsis. BMC Biol 2011; 9:64; PMID:21951689; http://dx.doi.org/10.1186/1741-7007-9-64.

4. Gray MW, Burger G, Lang BF. Mitochondrial evolution. Science 1999; 283:1476-81; PMID:10086161; http://dx.doi.org/10.1126/science.283.5407.1476.

5. Timmis JN, Ayliffe MA, Huang CY, Martin W. Endosymbiotic gene transfer: organellar genomes forge eukaryotic chromosomes. Nat Rev Genet 2004; 5:123-35; PMID:14757123; http://dx.doi.org/10.1038/nrg1271.

6. Archibald JM. The puzzle of plastid evolution.Curr Biol 2009; 19:R81-8; PMID:19174147; http://dx.doi.org/10.1016/j.cub.2008.11.067.

7. Ganit JS, Baldauf SL, Cao J, Lang BF, Ao NT, He X, et al. Mitochondrial evolution. Science 1999; 283:1476-81; PMID:10086161; http://dx.doi.org/10.1126/science.283.5407.1476.

8. Timmis JN, Ayliffe MA, Huang CY, Martin W. Endosymbiotic gene transfer: organellar genomes forge eukaryotic chromosomes. Nat Rev Genet 2004; 5:123-35; PMID:14757123; http://dx.doi.org/10.1038/nrg1271.

9. Archibald JM. The puzzle of plastid evolution. Curr Biol 2009; 19:R81-8; PMID:19174147; http://dx.doi.org/10.1016/j.cub.2008.11.067.

10. Ganit JS, Baldauf SL, Cao J, Lang BF, Ao NT, He X, et al. Mitochondrial evolution. Science 1999; 283:1476-81; PMID:10086161; http://dx.doi.org/10.1126/science.283.5407.1476.

11. Timmis JN, Ayliffe MA, Huang CY, Martin W. Endosymbiotic gene transfer: organellar genomes forge eukaryotic chromosomes. Nat Rev Genet 2004; 5:123-35; PMID:14757123; http://dx.doi.org/10.1038/nrg1271.

12. Archibald JM. The puzzle of plastid evolution. Curr Biol 2009; 19:R81-8; PMID:19174147; http://dx.doi.org/10.1016/j.cub.2008.11.067.

13. Ganit JS, Baldauf SL, Cao J, Lang BF, Ao NT, He X, et al. Mitochondrial evolution. Science 1999; 283:1476-81; PMID:10086161; http://dx.doi.org/10.1126/science.283.5407.1476.

14. Goremykin V, V Salamini F, Velira M. Mitochondrial DNA of Vitis vinifera and the issue of rampant horizontal gene transfer. Mol Biol Evol 2009; 26:99-110; PMID:18922764; http://dx.doi.org/10.1093/molbev/msn126.

15. Spalk K, Downie SR. Intergeneric disjunctions in Cryptotaenia (Apiaceae, Oenantheae): an appraisal using molecular data. J Biogeogr 2007; 34:2039-54; http://dx.doi.org/10.1111/j.1365-2699.2007.01752.x.

16. Greshebel D, Bara ski R, Spalk K, Allender C, Simon PW. Daucus In: Cole K, ed. Wild Crop Relatives: Genetic and Breeding Resources. Berlin-Heidelberg, Springer-Verlag, 2011:91-113.

17. Manna F, Massaro DR, Wolf K, Luccarini G, Carlomagno MD, Revelini F, et al. A rRNA gene mapping within the chloroplast DNA cluster is differential- ly expressed during the development of Daucus carota. Nucleic Acids Res 1994; 22:1712-8; PMID:8203276; http://dx.doi.org/10.1093/nar/22.9.1712.

18. Todoh N, Shimozaki K, Sugii M. Sequence of a putative promoter region for the rRNA genes of tobacco chloroplast DNA. Nucleic Acids Res 1981; 9:5399-406; PMID:7029469; http://dx.doi.org/10.1093/nar/20.5.5399.

19. Han JS. Non-long terminal repeat (non-LTR) retrotransposons: mechanisms, recent developments, and unanswered questions. Mol DNA 2010; 1:15; PMID:20462415; http://dx.doi.org/10.1016/j.molbd.2011.05.002.

20. Burwinkel B, Kiliann M. Unequal homologous recombination between LINE-1 elements as a mutational mechanism in human genetic disease. J Mol Biol 1998; 277:513-7; PMID:9533876; http://dx.doi.org/10.1006/jmbi.1998.1604.

21. Gilbert N, Lutz-Pruge S, Molan J. Genomic deletions created upon LINE-1 retrotransposition. Cell 2002; 110:315-25; PMID:12176320; http://dx.doi.org/10.1016/S0092-8674(02)00828-0.

22. Symer DE, Connelly C, Saik ST, Caputo EM, Cost GJ, Parmigiani G, et al. Human L1 retrotransposition is associated with genetic instability in vivo. Cell 2002; 110:327-38; PMID:12176320; http://dx.doi.org/10.1016/S0092-8674(02)00839-5.

23. Speck M. Antisense promoter of human L1 retrotransposon drives transcription of adjacent cellular genes. Mol Cell Biol 2001; 21:1973-85; PMID:11389833; http://dx.doi.org/10.1128/MCB.21.6.1973-1985.2001.

24. Cordaux R, Barter MA. The impact of retrotransposition on human genome evolution. Nature 2009; 10:691-703.

25. Sanchez-Puerta MV, Cho Y, Mower JP, Alverson AJ, Palmer JD. Frequent, phylogenetically local horizontal transfer of the cslx group I intron in flowering plant mitochondria. Mol Biol Evol 2008; 25:1762-77; PMID:18524785; http://dx.doi.org/10.1093/molbev/mms129.

26. Saldanha R, Mohr G, Belfort M, Lambowitz AM. Group I and group II introns. FASEB J 1993; 7:15-24; PMID:8422962.
28. Kück U. The intron of a plastid gene from a green alga contains an open reading frame for a reverse transcriptase-like enzyme. Mol Gen Genet 1989; 218:257-65; PMID:2476655; http://dx.doi.org/10.1007/BF00351276.

29. Villegas J, Azaya P, Bustos-Obregon E, Burzio LO. Localization of the 16S mitochondrial rRNA in the nucleus of mammalian spermatogenic cells. Mol Hum Reprod 2002; 8:977-83; PMID:12397209; http://dx.doi.org/10.1093/molehr/8.11.977.

30. Ninomiya Y, Ichinose S. Subcellular distribution of mitochondrial ribosomal RNA in the mouse oocyte and zygote. PLoS One 2007; 2:e1241; PMID:18043748; http://dx.doi.org/10.1371/journal.pone.0001241.

31. Landerer E, Villegas J, Burzio VA, Oliveira L, Villota C, Lopez C, et al. Nuclear localization of the mitochondrial ncRNAs in normal and cancer cells. Cell Oncol (Dordr) 2011; 34:297-305; PMID:21347712; http://dx.doi.org/10.1007/s13402-011-0018-8.

32. Cerutti HD, Jagendorf A. Movement of DNA across the chloroplast envelope: implications for the transfer of promiscuous DNA. Photosynth Res 1995; 46:329-37; http://dx.doi.org/10.1007/BF00020448.

33. Gómez G, Pallás V. Noncoding RNA mediated traffic of foreign mRNA into chloroplasts reveals a novel signaling mechanism in plants. PLoS One 2010; 5:e12269; PMID:20888865; http://dx.doi.org/10.1371/journal.pone.0012269.

34. Hawkins JS, Hu G, Rapp RA, Grafenberg JL, Wendel JF. Phylogenetic determination of the pace of transposable element proliferation in plants: copia and LINE-like elements in Gossypium. Genome 2008; 51:11-8; PMID:18356935; http://dx.doi.org/10.1139/G07-099.