Horizontal Gene Transfer of Chlamydial-Like tRNA Genes into Early Vascular Plant Mitochondria

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Abstract

Mitochondrial genomes of lycophytes are surprisingly diverse, including strikingly different transfer RNA (tRNA) gene complements: No mitochondrial tRNA genes are present in the spikemoss Selaginella moellendorffii, whereas 26 tRNAs are encoded in the chondrome of the clubmoss Huperzia squarrosa. Reinvestigating the latter we found that trnL(gag) and trnS(gga) had never before been identified in any other land plant mitochondrial DNA. Sensitive sequence comparisons showed these two tRNAs as well as trnN(guu) and trnS(gcu) to be very similar to their respective counterparts in chlamydial bacteria. We identified homologs of these chlamydial-type tRNAs also in other lycophyte, fern, and gymnosperm DNAs, suggesting horizontal gene transfer (HGT) into mitochondria in the early vascular plant stem lineages. These findings extend plant mitochondrial HGT to affect individual tRNA genes, to include bacterial donors, and suggest that Chlamydiae on top of their recently proposed key role in primary chloroplast establishment may also have participated in early tracheophyte genome evolution.

Key words: Chlamydiae, horizontal gene transfer (HGT), mitochondrial tRNA genes, lycophytes, monilophytes, gymnosperms, Class II tRNAs.

Extant lycophytes (club mosses, quillworts, and spike mosses) represent the most ancient surviving lineage of early vascular plants with an origin dating back to Devonian times more than 400 Ma. Their common names are misleading given that lycophytes do not belong to bryophytes, or mosses in particular, but are true vascular plants, tracheophytes. The evolutionary innovation of long-distance water-conducting tissue in tracheophytes gave rise to one of the most dramatic changes of the biosphere on our planet. Concomitantly, the ancestral bryophyte lifestyle dominated by a haploid gametophyte growth-phase changed to a plant life-cycle dominated by the diploid sporophyte generation. Surprisingly, these evolutionary transitions were accompanied by major changes in the mitochondrial DNA (mtDNA) as recently documented with the first completely determined mitochondrial genome sequences of the quillwort Isoetes engelmannii (Grewe et al. 2009), the spikemoss Selaginella moellendorffii (Hecht et al. 2011), and the club moss Huperzia squarrosa (Liu et al. 2012). In essence, the Isoetes and Selaginella mtDNAs are characterized by extensive mitochondrial genome recombination, trans-splicing introns and frequent endosymbiotic gene transfer also characteristic of flowering plants. In contrast, the Huperzia mtDNA turned out to be much more conservative in evolution, even retaining numerous ancient bryophyte mitochondrial gene syntenies (Liu et al. 2012; Knoop 2013). One of the most surprising discrepancies among the lycophyte mtDNAs is the particularly large set of 26 transfer RNA (tRNA) genes in H. squarrosa, which contrasts their complete absence in S. moellendorffii. Notably, to account for some of the tRNA genes present in the H. squarrosa mtDNA one would have to postulate numerous independent tRNA gene losses in other lineages in the light of a modern understanding of plant phylogeny (Qiu et al. 2006), a scenario which appeared very unlikely to us.

Initially, two tRNA genes in the H. squarrosa mtDNA caught our attention in particular: trnL(gag) and trnS(gga). A mitochondrial trnL(gag) gene had never before been reported for the land plant (embryophyte) lineage but had only sporadically been observed in distant algae (fig. 1). A mitochondrial trnS(gga) gene tracing back to the original $\alpha$-proteobacterial endosymbiont that gave rise to mitochondria has, to our knowledge, in fact never before been reported for any plant sensu lato (Viridiplantae) mitochondrial genome. In fact, trnS(gga) homologs are also rare among pro-tist mtDNAs. The orphan trnS(gga) in the mtDNA of the protist Naegleria gruberi (Gray et al. 2004) is a unique conversion of a cognate trnS(uga), whereas the trnS(gga) genes in Andalucia godoyi and Seculamonas ecuadoriensis (Burger et al. 2013) and in Tsukubamonas globosa (Kamikawa et al. 2014) are as yet of unclear origin. The trnS(gga) genes present in flowering plant mtDNAs in contrast are promiscuous copies of their chloroplast counterparts (see Knoop 2012).

To exclude potential artifacts of the H. squarrosa mitochondrial genome assembly, we first strived to retrieve homologous sequences from independent biological material of this and three further related species of the Lycopodiaceae available in our laboratory (H. selago, H. megastachys, and H. hippocus) through polymerase chain reaction (PCR). We easily obtained the corresponding trnL(gag) and trnS(gga) loci from these taxa, whereas control amplification attempts failed for other plant nucleic acids included as negative controls. Cloning and sequencing perfectly confirmed conservation.
of both tRNA identities among Lycopodiaceae (fig. 2 and supplementary fig. S1, Supplementary Material online).

Based on the current insights on plant phylogeny (fig. 1) and assuming that these tRNA genes trace back to the original endosymbiont, the presence of trnL(gag) and trnS(gga) tRNA genes in Lycopodiaceae would imply numerous independent losses from mtDNAs among Viridiplantae alone (in several Chlorophytes as well as in Mesostigma, Chlorokybus, Chaetosphaeridium, Charales, Liverworts, Mosses and Hornworts, Isoetales, and Euphyllophytes), obviously a nonparsimonious scenario. We used the Lycopodiaceae tRNA sequences for sensitive BLASTN searches (word size = 7 and experimentally variable matching and gapping parameters). These searches conclusively revealed top similarity hits (i.e., random similarity probabilities consistently below expectancies of $10^{-10}$) for trnL(gag) and trnS(gga) among the homologous genes of Chlamydiae, with top scores most frequently in the recently determined genomes of three chlamydial bacteria genera (Parachlamydia, Simkania, and Waddlia), here further referred to as “PSW-Chlamydiae,” which are distinctly set apart from the well-known Chlamydia/Chlamydophila clade of human and
animal pathogens (Collingro et al. 2011). Only few positions in the core tRNA structures of trnS(gga) and trnL(gag) are conserved differently among the PSW-Chlamydiae aside from the sequences of the extra arms, which are highly variable among the Chlamydiae (fig. 2 and supplementary fig. S1, Supplementary Material online). Like their Lycopodiaceae pendants, the chlamydial tRNA genes also lack a genomically encoded CCA aminoacyl acceptor end.

The above findings, indicating that an ancestral Lycopodiaceae mitochondrial genome may have functionally integrated chlamydial DNA sequences after horizontal gene transfer (HGT), made us reinvestigate the entire H. squarrosa mtDNA sequence in detail. We used sensitive sequence comparisons in small sequence intervals to detect further evidence for transfer of chlamydial (or any other) foreign sequence integrations. Nearly all coding regions expectedly shared highest similarity with homologs in the plant lineage or, when restricted to bacterial sequences, to proteobacterial sequences supporting their mitochondrial genealogy. However, there were two further striking exceptions: The tRNA genes for trnN(guu) and trnS(gcu) again showed highest similarities to chlamydial instead of other mitochondrial or proteobacterial counterparts. Significantly, both these tRNA genes are present in algal and liverwort but absent in moss and hornwort mitochondrial genomes (fig. 1 and supplementary fig. S2, Supplementary Material online). Most notably, we now identified a homolog of the Huperzia trnS(gcu) also in the I. engelmannii mtDNA, which had previously been overlooked (Grewe et al. 2009), very likely due to its unique chlamydial nature (supplementary fig. S2, Supplementary Material online).

Aspleniun nidus (accession AM600641, Panarese S, Rainaldi G, De Benedetto C and Gallarini R unpublished data). Interestingly, trnN(guu) is functionally replaced by its chloroplast homolog promiscuously copied into the mitochondrial genomes in angiosperms (fig. 1). Although naturally very limited in phylogenetic resolution owing to their short sequences, the different plant mitochondrial trnN gene sequences very clearly reveal their three different genetic origins (fig. 3 and supplementary fig. S2, Supplementary Material online). The lycophyte, fern, and gymnosperm chlamydial trnN xenologs cluster within the PSW-Chlamydiae clade to the exclusion of the native mitochondrial homologs in liverworts and streptophyte algae and the previously...
known angiosperm homologs which are nearly identical to their chloroplast source loci (fig. 3).

The chlamydial-type trnN(guu) genes in *C. taitungensis* and *A. nidus* suggested an early gain of this xenolog through HGT in the tracheophyte stem lineage (fig. 1). We hence wished to investigate this evolutionary scenario using primers targeting the four chlamydial tRNA genes in a sampling of monilophytes and gymnosperms. Although we were unable to obtain any PCR products for trnS(gca) and consistently only obtained the native mitochondrial trnS(gcu) gene in ferns, we retrieved the chlamydial-type sequences of trnN(guu) in two further gymnosperms and six fern taxa covering most of the extant monilophyte diversity (fig. 1). Likewise, we retrieved chlamydial-type PCR products and sequences also for trnL(gag) in the early branching monilophyte genera *Angiopteris* and *Equisetum*. Hence, at least trnN(guu) and trnL(gag) indeed seem to be early chlamydial gains in mitochondria of the tracheophyte stem lineage (fig. 1).

The likelihood of HGT early in the tracheophyte mtDNAs is supported by a noteworthy additional finding. Searches with the *Huperzia* trnS(gga) among plant sequences identified a stretch of 91% identity corresponding to tRNA positions 13–55 as a single significant additional hit in an intergenic region of the *C. taitungensis* mtDNA (supplementary fig. S3, Supplementary Material online), strongly indicative of a pseudogene leftover in the gymnosperm after a chlamydial HGT in the tracheophyte stem lineage. An additional intriguing observation along similar lines is that three of the chlamydial tRNA xenologs, trnL(gag), trnS(gcu), and trnS(gga), are all located within 20 kb of the *H. squarrosa* mitochondrial genome, devoid of other functional coding sequences (supplementary fig. S4, Supplementary Material online). This observation might suggest a joint gain of at least those three tRNA genes in a single HGT event. In fact, a gain of all four chlamydial tRNA genes in a single event of HGT at the origin of tracheophytes (Label 1 in fig. 1), followed by differential retention of the xenologs would be a parsimonious scenario. Indeed, trnS(gcu) and trnS(gga) are encoded in the same orientation and in a similar distance only 3.8 kb apart in the *Waddlia chondrophila* genome. In contrast, however, the other genes for the tRNAs under consideration here are separated by large distances in the genomes of the extant PSW-Chlamydiae, possibly reflecting millions of years of bacterial genome recombination. Interestingly, however, two sequence stretches of approximately 260 bp downstream of trnS(gga) show additional significant similarities with intergenic regions in the genome of *Parachlamydia acanthamoebae* strain UV-7, an extant species that may be closely related to the ancestral PSW-chlamydial taxon that donated the plant tRNA gene xenologs (fig. 3 and supplementary fig. S4, Supplementary Material online).

The four plant mitochondrial tRNAs under consideration here were consistently found to be most similar to their chlamydial counterparts setting them apart from more distant homologs (supplementary table S1 and fig. S5, Supplementary Material online). The short lengths of tRNA genes necessarily restrict individual statistical supports. A concatenated alignment of the four tRNA loci including their respective top-scoring homologs in gram-positive bacteria, *Escherichia coli*, *Azopirillum* spp., Rickettiales, mitochondria and chloroplasts or cyanobacteria, respectively, in addition to the chlamydial homologs rather clearly demonstrates the distance to the former and their being nested among the latter, however (fig. 4). Taken together, we here report the HGT of four tRNA genes, very likely originating from *Chlamydiae* (and most likely of the “PSW clade” in particular) into mitochondria of the vascular plant stem lineage.

The import of tRNAs into mitochondria is particularly prominent in plants where many tRNA genes are lacking from the mtDNAs (Duchêne et al. 2009; Rubio and Hopper 2011; Schneider 2011; Sieber et al. 2011; Knoop 2012). In fact, no tRNA genes at all were found in the mitochondrion of *S. moellendorffii* (Hecht et al. 2011), suggesting that the entire tRNA set has to be imported from the cytosol. It is all the more surprising to now identify the four chlamydial tRNA xenologs in the mtDNA of *H. squarrosa* where they coexist with 22 native tRNA genes addressing other codons. A general propensity for more flexible and promiscuous tRNA import into mitochondria of the one versus the other lycophyte may be a possible explanation for this peculiar evolutionary outcome. Other than import from the cytosol or acquiring the homologous chloroplast tRNA genes through lateral gene transfer between the endosymbiont genomes within the same cell, the gain of bacterial xenolog tRNA genes through HGT obviously adds a third option for plants to complete their set of mitochondrial tRNAs.

After initial reports of HGT between seed plant mitochondria (Bergthorsson et al. 2003; Won and Renner 2003) several further examples of plant-to-plant mitochondrial HGT have been documented (e.g., Davis and Wurdack 2004; Mower et al. 2004; Davis et al. 2005; Barkman et al. 2007; Mower et al. 2010; Hao and Palmer 2011; Sanchez-Puerta et al. 2011; Hepburn et al. 2012; Renner and Bellot 2012). The most prominent example for extensive mitochondrial HGT is the case of the basal angiosperm *Amborella trichopoda* with an enormously expanded mtDNA featuring extensive sequences originating through HGT from mitochondria of other angiosperms, a moss and even algae (Bergthorsson et al. 2004; Rice et al. 2013). Fewer reports have documented HGT affecting plant nuclear DNA. As in the examples of mitochondrial HGT, intimate plant–plant contacts such as host–parasite relationships are likely key to interpretation of the known examples of plant nuclear HGT (Yoshida et al. 2010; Xi et al. 2012).

The possibly most significant example for plant-to-plant HGT on a much deeper evolutionary level is the recently reported acquisition of a neochrome gene in ferns originating from hornworts (Cooper 2014; Li et al. 2014) adding a significant item to the list of novel functionalities acquired through HGT in eukaryotes (recently summarized by Schönknecht et al. 2014). Microorganisms have likely served as donors of HGT into plants much more frequently than other plants (Broothaerts et al. 2005; Emiliani et al. 2009; Yue et al. 2012). Most interestingly, Chlamydiae have already been identified earlier as likely sources for HGT of more than 50 protein-encoding genes into plant nuclear genomes.
Mitochondrial tRNA genes of gram-positive bacterial homologs, respectively. Node support from 1,000 bootstrap replicates is indicated on branches where exceeding 50%.

The respective top-scoring most similar homologs for each Huperzia squarrosa tRNA have been selected for inclusion in the alignment of the compound taxon homologs (i.e., the respective most similar mitochondrial, chloroplast or cyanobacterial, and gram-positive bacterial homologs, respectively). Node support from 1,000 bootstrap replicates is indicated on branches where exceeding 50%. Phylogenetic tree was constructed using MEGA 5 (Tamura et al. 2011).

The here reported gain of four mitochondrial tRNA genes from chlamydial bacteria, two of which had never entered the land plant lineage in their native mitochondrial form, now demonstrates that at least one further major event later in the evolution of plant life, the origin of vascular plants, was associated with a close contact and the uptake of genetic material from Chlamydiae, in this case targeting the mitochondrial genome. The ancient chlamydial donor appears to be most closely related to modern chlamydial taxa that have originally been shown for HGT of a tRNA guanine methyl-transferase protein domain into the slime mould Dictyostelium discoideum (Manna and Barth 2013).

Summary Material

Supplementary figures S1–S5 and table S1 are available at Molecular Biology and Evolution online (http://www.oxfordjournals.org/).

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