



## PLASMID-ASSOCIATED ORGANELLE GENOME EVOLUTION IN RED ALGAE

Mitochondria and plastids are derived through endosymbiosis events from  $\alpha$ -proteobacteria and cyanobacteria, respectively. In the case of plastids, a heterotrophic eukaryote engulfed the prokaryotic symbiont, followed by the many complex processes associated with organellogenesis (Brodie et al. 2017). In general, eukaryotic organelles are maternally inherited and lack genetic recombination (Bhattacharya et al. 2004, Timmis et al. 2004), thereby providing powerful phylogenetic markers (e.g., Yang et al. 2015, Costa et al. 2016, Lee et al. 2016a, Díaz-Tapia et al. 2017). Since the rise of high-throughput sequencing (HTS) technologies, massive organelle genome databases have become available to better understand the evolutionary history of eukaryotes. For example, based on HTS data, it was suggested that the primary plastid-containing Archaeplastida (i.e., Rhodophyta, Glaucophyta, and Viridiplantae) underwent differential gene gains and losses in their organelle genomes after the endosymbiosis event. As a consequence, Rhodophyta (red algae) contains the largest number of plastid genes but the smallest mitochondrial gene inventory is found among Archaeplastida (Lee et al. 2016a, Qiu et al. 2017). Exceptions to genome size conservation are provided by the plastid of the green alga *Haematococcus lacustris* (~1.3 million base pairs [Mbp]; Bauman et al. 2018) and red algal Rhodellophyceae species (~0.5–1.1 Mbp; Muñoz-Gómez et al. 2017), which however, contain conserved gene inventories. Additional organellar genome data will undoubtedly bring new insights into evolutionary trends as well as further resolve algal and other eukaryotic phylogenetic relationships.

In red algae, there have been several interesting studies in recent decades about plasmid-associated organelle genome evolution (Hagopian et al. 2004, Janoušková et al. 2013, Campbell et al. 2014, Lee et al. 2015, 2016a,b, Du et al. 2016, Ng et al. 2017). Plasmid-derived sequences (PDS) were frequently reported in red algal organellar genomes that were likely derived from viruses, bacterial plasmids, as well as nuclear and mitochondrial genomes of diverse eukaryotic lineages (i.e., land plants, stramenopiles, Rhizaria). For instance, plasmid-derived

horizontal gene transfers (HGTs) of cyanobacterial hypothetical proteins containing the novel DNA polymerase type-B family catalytic domain superfamily was found in red algal organellar genomes, analogous to the proteobacterial-derived leucine biosynthesis operon (*leuC/D* genes; Janoušková et al. 2013, Lee et al. 2016b).

Interestingly, the PDS regions are present in all reported organellar genomes in the Gracilariaceae. Iha et al. (2018) summarized these organellar PDS in the Gracilariaceae with the addition of nine plastid and 10 mitochondrial genomes from 10 species (*Gracilaria caudata*, *G. ferox*, *G. gracilis*, *G. rangiferina*, *G. tenuistipitata*, *G. vermiculophylla*, *Gracilariopsis longissima*, *Gp. mclachlanii*, *Gp. tenuifrons*, and *Melanthalia intermedia*) as well as three new plasmid sequences from two species (*G. ferox* [Gfe3115], and *G. vermiculophylla* [Gve4548 and Gve7464]). This study found the leucine biosynthesis genes (*leuC/D*) not only in plastid genomes but also in plasmids of the Gracilariaceae (i.e., the *leuC/D* homologous proteins in *G. vermiculophylla* plasmids) and suggested plasmid-mediated HGTs to the plastid genome. These three novel plasmids in the Gracilariaceae could provide the opportunity to study the intertwined evolutionary history of red algal organelles and plasmids within a species. Because abundant plasmid gene pools and their PDS regions are available, this study suggested that the family Gracilariaceae is a useful taxonomic group to study the impacts of PDS in genome evolution. However, to trace the evolutionary trajectory of PDSs, it is essential to study nuclear genomes. There are currently two available nuclear genomes from Gracilariaceae species (*Gp. chorda* [92 Mbp, N50 = 220 Kbp, 1,211 contigs; Lee et al. 2018], and *Gp. lemneiformis* [88 Mbp, N50 = 30 Kbp, 13,825 contigs; Sun et al. 2018]); therefore, further study should focus on how genes are transferred among different genetic compartments of plasmid, organellar, and nuclear genomes. Such data might prove useful in testing hypotheses about plasmid-associated genome evolution.

What is the role of PDS insertions? The possible role of plasmids as mediators of HGTs is intriguing

because the plasmid sequences frequently deliver foreign genetic material to their host genome (Janoušková et al. 2013, Ruck et al. 2014, Lee et al. 2016b, Ng et al. 2017, the Iha et al. 2018). Originally, plasmids were defined as extrachromosomal genetic elements containing backbone (i.e., plasmid function) and accessory (i.e., beneficial to the host) genes. Plasmid-mediated HGT is a key process in the coevolution of prokaryotes (Harrison and Brockhurst 2012). Similarly, this evolutionary process in eukaryotes enables gene transfers between broad taxonomic ranges mediated by plasmids (Lee et al. 2016b). Plasmids deliver genes, and have self-replicating mechanism such as transposable elements (TEs), which contribute to genome structure and gene evolution in eukaryotes (Kazazian 2004, Bennetzen and Wang 2014, Bhattacharya et al. 2018, Lee et al. 2018). Therefore, plasmids and their PDS have a potential function in the modification of gene and genome structure similar to TEs (e.g., DNA rearrangement and gene mutation, modification, movement; Bennetzen and Wang 2014). The pseudogenization of a conserved plastid gene near the insertion site of a red algal PDS has been reported (Lee et al. 2016b). Genetic material in such cases can be more efficiently co-opted from plasmid to organelle DNA due to several endosymbiont-like features similar to prokaryotic genomes such as multiple copies within the host cell and vertical inheritance (Harrison and Brockhurst 2012). As another potential role for plasmids, copy number and AT content can impact mutational bias and genetic drift in organelle genomes, as shown for bacterial endosymbionts and their hosts (Moran 1996, Wernegreen and Moran 1999, Dietel et al. 2018).

Relying on the distinguishing feature of plasmids as a potential mediator of HGT, a plasmid transformation system was recently established as a genetic engineering tool in the unicellular red alga *Porphyridium purpureum*, in which transgenic plasmids were maintained episomally (Li and Bock 2018). This new transformation system was inspired by the high frequency of plasmid-like elements and abundant bacterial genes in red algal genomes. Given this system, it will be very interesting to develop new plasmid transformation systems in plasmid-rich multicellular red algal species (e.g., Gracilariaceae). If successful, this system could be applied to reestablish plasmid-mediated HGTs in organellar genomes as well as other plasmid-associated evolutionary changes.

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