Letters

Plastid genomes hit the big time

Introduction

Plastid genomes have long stood in the shadow of their mitochondrial counterparts. Indeed, the first complete plastid DNA (ptDNA) sequences (liverwort and tobacco) (Ohyama *et al.*, 1986; Shinozaki *et al.*, 1986) arrived half a decade after the first mitochondrial DNAs (mtDNAs) (human and mouse) (Anderson *et al.*, 1981; Bibb *et al.*, 1981). Mind you, these early ptDNA sequences are more than 10-times larger than the human and mouse mtDNAs, and their completion represents an impressive feat in the days before automated Sanger sequencing. But even today, there are thousands more mtDNAs in GenBank than ptDNAs (Smith & Keeling, 2015). I guess this is to be expected. Plastids, unlike mitochondria, do not span the entire breadth of eukaryotic diversity, and plants and algae have regularly taken a backseat to model animal systems, perhaps partly because of the modest economic or medicinal value of most plastid-bearing protists as well as the paucity of available axenic cultures.

That said, the vast majority of sequenced mtDNAs come from a single major group (the Metazoa), and for some protist lineages, ptDNAs are as well or better sampled than mtDNAs (Smith & Keeling, 2015). Moreover, ptDNA has long been used as a key source for phylogenetic characters in research on plastid-bearing organisms, particularly work on angiosperms (Ruhfel *et al.*, 2014) where mtDNA was often avoided for phylogenetic purposes because of its structural variability and low substitution rate, at least within genes (Christensen, 2013).

Biases aside, plastid genomes are continually outshined by mtDNAs. Name a genomic architectural feature and you will likely find that mitochondria are more varied and extreme than plastids. For example, mitochondrial and plastid genomes tend to be biased in adenine and thymine, but mtDNAs are consistently more AT-rich than ptDNAs, and the same goes for the few examples of GC-rich organelle DNA (Smith, 2009; Hecht *et al.*, 2011). Similarly, both types of genome can exhibit complicated and convoluted forms of post-transcriptional editing, but mitochondrial RNA-editing and processing is nearly always more elaborate and eccentric than that in plastids (Knoop, 2011; Smith & Keeling, 2016).

Perhaps nothing has been as demeaning for ptDNAs as their poor showing in the category of genome size. Mitochondria, however, are truly remarkable in this regard. Take, for instance, the angiosperm *Silene conica*, which at 11.3 Mb and > 95% noncoding is larger than the largest published alphaproteobacterial genomes (Sloan *et al.*, 2012). Mitochondrial genomes can also be incredibly tiny, measuring < 6 kb in certain apicomplexan parasites (Hikosaka *et al.*, 2009) and just over 10 kb in some animals (Pett *et al.*, 2011). The causes for these extremes in size are, at the upper end, the accumulation of noncoding nucleotides (as intronic and/or intergenic DNA) and, at the lower end, the contraction of noncoding regions and gene loss (Lynch *et al.*, 2006).

The size range for plastid genomes is much narrower than that of mtDNA, with most available ptDNAs falling in the range 75–200 kb (Green, 2011). Why plastomes have not been pushed to the same extremes in size as mtDNAs is poorly understood, but recent explorations of understudied plastid-bearing lineages have shown that ptDNA size variation is not as narrow as once thought. Giant plastid genomes are popping up in disparate groups across the eukaryotic tree of life, sometimes in the most unsuspecting places, and so are very tiny ptDNAs. These plastomes have a lot to teach us about genome evolution, and before long ptDNAs may start competing with mtDNAs for genome-size bragging rights. Later, I summarize the various plants and algae gaining attention for their unconventional ptDNA sizes.

Plastid-bearing organisms encompass a significant proportion of the known eukaryotic diversity (Burki, 2017) and here I highlight only a handful of that diversity – species with very big or very small plastomes. It is important to stress, however, that plastid genome architecture is diverse and can vary significantly within and among lineages, including those not discussed here. Also, the ease and popularity of sequencing small metazoan mtDNAs has likely contributed to the observed genome size discrepancy between plastids and mitochondria, at least at the lower end of the spectrum. As researchers characterize eukaryotic life in greater and greater detail, they will surely uncover other impressive examples of extremes in ptDNA size.

Green algae

For the longest time, the unicellular marine ulvophyte *Acetabularia acetabulum* held the honor of being the species with the largest known plastid genome (Palmer, 1985). Initial ptDNA-size estimates for this alga (and its close relative *Acetabularia cliftonia*), based on electron microscopy (Green, 1976), kinetic-complexity analyses (Padmanabhan & Green, 1978), and restriction digest experiments (Tymms & Schweiger, 1985), suggested a length of up to 2 Mb. The problem is that the plastid genome has yet to be completely sequenced.

A 2013 study investigating kleptoplasty in the sacoglossan sea slug *Elysia timida*, which feeds on and sequesters the chloroplasts of *A. acetabulum*, partially assembled the *Acetabularia* ptDNA (using 454 sequencing data) into 63 contigs, totaling *c.* 350 kb and containing 39 full-length genes and a modest number of large introns (de Vries *et al.*, 2013). Unfortunately, the short-read data were not enough to bridge the prodigious intergenic regions, meaning large segments of the *A. acetabulum* ptDNA remain unaccounted for and its exact size is still unknown. The available

contigs combined with long-read PacBio sequencing information would likely be enough to complete the assembly and provide a precise size value for this genome.

In addition to its extraordinary ptDNA, *Acetabularia* is among the largest single-celled organisms (3–6 cm in height) and has an unusually large nucleus (50–120 μm in diameter) (Mandoli, 1998). This is noteworthy when considering that a strong positive relationship between cell size and genome size has been documented in a wide range of organisms (Gregory, 2001; Beaulieu *et al.*, 2008), but such a trend has not been explored with respect to plastomes (Smith, 2017).

More recently, another green algal group – the Chlamydomonadales – has been identified as a hotspot for ptDNA inflation (Smith *et al.*, 2013). This chlorophycean order of primarily freshwater flagellates harbors multiple species with ptDNAs in excess of 350 kb, including the unicellular *Dunaliella salina* (> 370 kb, > 70% noncoding) (Del Vasto *et al.*, 2015), the colonial *Tetrabaena socialis* (> 405 kb, > 75% noncoding) (Featherston *et al.*, 2016), and the multicellular *Volvox carteri* (> 525 kb, > 80% noncoding) (Smith & Lee, 2010). Chlamydomonadalean plastid genomes stand out for having large numbers of repeats and low levels of silent-site genetic diversity (Smith & Lee, 2010; Smith *et al.*, 2013), features that might be contributing to their expansion (Lynch *et al.*, 2006; Smith, 2016). A sister clade to Chlamydomonadales – the Chaetopeltidales – contains at least two taxa with enlarged (> 520 kb) plastid genomes – *Floydiella terrestris* (521 kb) (Brouard *et al.*, 2010) and *Koshicola spirodelophila* (385 kb) (Watanabe *et al.*, 2016) – suggesting that other large ptDNAs are waiting to be found within the Chlorophyceae.

Like the Chlamydomonadales, members of the ulvophyte order Cladophorales, such as *Boodlea composita*, *Dictyosphaeria cavernosa*, and *Valonia ventricosa*, might have distended ptDNAs. The plastomes of these macro, multicellular, and multi-nucleated algae are comprised of highly fragmented, single-stranded linear chromosomes, of which only a few dozen have been well characterized, with an accumulative length of 91 kb (Del Cortona *et al.*, 2017). However, data suggest that these ptDNAs might contain many more unidentified chromosomes, most of which are probably 'empty', containing no genes, and meaning that the overall plastome length could be quite massive (Del Cortona *et al.*, 2017).

Not so long ago, a 500 kb plastome was big enough to turn heads, but red algae have raised the bar on what qualifies as a large ptDNA.

Red algae

Traditionally, the ptDNAs of red algae have been considered evolutionarily stable with respect to content and organization, and are thought to resemble the last common ancestral genome of all plastids (Lang & Nedelcu, 2012; Janouškovec *et al.*, 2013). Red algae are also renowned for having the largest plastid gene contents among all eukaryotes (166–235), but their ptDNA sizes have generally been unimpressive (Lang & Nedelcu, 2012; Lee *et al.*, 2016). These trends, however, are based on a limited and biased sampling of red algae, which has mostly focused on mesophilic seaweeds and unicellular thermoacidophilic cyanidiophycean species (Janouškovec *et al.*, 2013; Tajima *et al.*, 2014).

Thus, when a group from Dalhousie University, Canada, sequenced six new ptDNAs from four phylogenetically distinct classes of mesophilic, nonseaweed red algae they were surprised to find an unprecedented degree of plastid genomic diversity, including massive intron contents, gigantic intergenic regions, and the rampant spread of transposable elements (Muñoz-Gómez *et al.*, 2017). The ptDNAs of the unicellular rhodellophyceans *Corynoplastis japonica* and *Bulboplastis apyrenoidosa* are especially exceptional with lengths of 1.13 and 0.61 Mb, respectively, and noncoding contents in excess of 80%. The former is the largest plastid genome sequenced to date and currently holds the record for the most introns found in an organelle genome (311), followed in second place by none other than *B. apyrenoidosa* (220). The unrivalled proliferation of introns in these two genomes contrasts the mode of expansion in the large ptDNAs of the green algae *A. acetabulum*, *V. carteri*, and *F. terrestris*, which are repeat rich but surprisingly depauperate of introns.

On top of introns, the *B. apyrenoidosa* plastid genome has been invaded by bacterial-derived insertion sequences, which have dispersed throughout the intergenic regions – a characteristic not shared by *C. japonica* (Muñoz-Gómez *et al.*, 2017). Plastid genome inflation in *C. japonica*, however, is associated with a large cell size (18–33 μm in diameter) (Yokoyama *et al.*, 2009), paralleling the situation in *A. acetabulum*. Again, whether cell size is a contributing factor in plastome expansion remains to be determined, but there is no denying that the loss of photosynthesis is a key factor in plastome reduction.

Nonphotosynthetic species

The surest ways to shrink a ptDNA is to ditch photoautotrophy (Figueroa-Martinez *et al.*, 2015; Braukmann *et al.*, 2017; Graham *et al.*, 2017). Such a switch immediately removes the necessity for maintaining photosynthesis-related genes in the plastid (and nuclear) genome, allowing them to be purged. The smallest ptDNAs described to date are all found in nonphotosynthetic (and often parasitic) species. The colorless parasitic green alga *Helicosporidium* sp. has among the most minute and compact ptDNAs of any protist (37.4 kb, *c.* 95% coding) (de Koning & Keeling, 2006). Even smaller are the plastomes of the apicomplexan parasites *Plasmodium falciparum* (34.2 kb) (Wilson *et al.*, 2009) and *Eimeria tenella* (34.7 kb) (Cai *et al.*, 2003), the causative agents of malaria and coccidiosis.

Plastid genomes in nonphotosynthetic species typically bottom out at *c.* 34 kb, but some heterotrophic land plants are taking ptDNA contraction to new lows (Braukmann *et al.*, 2017; Graham *et al.*, 2017). A Cameroonian isolate of the mycoheterotrophic orchid *Epipogium roseum* has a plastome measuring only 18 339 nt and containing 29 genes (Schelkunov *et al.*, 2015). Not to be outdone, the endoparasitic plants *Pilostyles aethiopica* and *Pilostyles hamiltonii* (Apodanthaceae) have whittled their ptDNAs down to a paltry 11 348 and 15 167 nucleotides, which each appear to encode just five functional genes, making them the smallest plastomes on record (Bellot & Renner, 2015). Work on other

nonphotosynthetic angiosperms, such as the mycoheterotrophic orchid *Corallorhiza striata*, is providing insights into the early stages of plastome reduction, including the occurrence of pseudogenizations and elevated nonsynonymous substitution rates in photosynthesis-related genes (Barrett & Davis, 2012).

Of course, the smallest plastid genome is no genome at all. The outright loss of ptDNA, and an associated gene expression system, is thought to have occurred in the parasitic angiosperm *Rafflesia lagascae* (Molina *et al.*, 2014), certain colpodellids, such as *Voromonas pontica* (Janouškovec *et al.*, 2015), and the colorless green algal genus *Polytomella* (Smith & Lee, 2014). The absence of ptDNA in these lineages means that they are entirely dependent on nuclear-encoded plastid-targeted proteins for maintaining the crucial metabolic functions that persist within their nonphotosynthetic plastids.

It is a well-accepted tenet that nonphotosynthetic species have smaller plastid genomes than their close photosynthetic relatives, but apparently the unicellular green alga *Polytoma uvella* did not get the memo. This colorless free-living osmotroph is closely related to *Polytomella* algae, but both lineages lost photosynthesis independently of one another, and unlike the latter, *P. uvella* has a plastid genome (Lang & Nedelcu, 2001; Figueroa-Martinez *et al.*, 2015). Given its close phylogenetic proximity to *Polytomella*, one might have expected *P. uvella* to have a very tiny ptDNA, conceivably one *en route* to complete loss, but the opposite is true. *Polytoma uvella* currently has the largest and most bloated plastid genome ever found in a nonphotosynthetic species: *c.* 230 kb and 75% noncoding (Figueroa-Martinez *et al.*, 2017). Even more impressive, the genome is tens of thousands of nucleotides larger than those of its closest known photosynthetic relatives *Chlamydomonas leiostraca* (167 kb) and *Chlamydomonas applanata* (*c.* 203 kb), a trend not previously observed in any other close photosynthetic–nonphotosynthetic duo.

Regardless of its large size, the *P. uvella* plastome has, like other nonphotosynthetic ptDNAs, undergone significant gene loss, shedding all coding regions for photosynthetic pathways. But unlike other nonphotosynthetic ptDNAs, that of *P. uvella* has highly expanded intergenic regions. Maybe the tightening of intergenic regions in heterotrophic ptDNAs has less to do with the loss of photosynthesis and more to do with another life-history feature common among many nonphotosynthetic lineages: parasitism. With some exceptions, the transition from a free-living to a parasitic existence (particularly an obligate one) is associated with widespread genomic compaction (McCutcheon & Moran, 2012; Poulin & Randhawa, 2015). *Polytoma uvella*, however, is free-living and there is no reason to believe that it had a recent parasitic ancestor. Thus, the lack of genomic compaction in this colourless alga might partly be a consequence of it not being a parasite.

The hunt for giant plastomes goes on

It is conspicuous that the largest available ptDNAs all belong to species with primary plastids, which descend directly from the cyanobacterial endosymbiont. Much of the known plastid-bearing eukaryotic diversity have 'complex' plastids, which derive from one eukaryote merging with another (Burki, 2017). To the best of my knowledge, the largest known ptDNA from a complex plastid is that of *Heterosigma akashiwo* (159 kb) (Cattolico *et al.*, 2008). The green-algal-derived plastid of *Euglena gracilis*, although not particularly large (143 kb), stands out for having *c.* 150 introns, including introns embedded within introns (Hallick *et al.*, 1993), but that is still hundreds fewer introns than *C. japonica*. Many groups with complex plastids are poorly studied, and some have only been recently identified (Burki, 2017). As researchers delve deeper and deeper into remote regions of the eukaryotic tree of life, they will certainly discover even bigger plastid genomes, possibly big enough to put mtDNAs to shame.

It will be interesting to see if some of the same patterns observed for inflated mtDNAs also hold for bloated ptDNAs. Work on land plant mitochondrial genomes has suggested a link between inefficient DNA repair and organelle genome enlargement. Specifically, studies have exposed what appear to be two different types of double-strand break repair occurring in plant mitochondria: (1) long homology-based repair, such as gene conversion, and (2) short- or nonhomology-based repair, such as nonhomologous end-joining or break-induced replication (Christensen, 2013; Smith, 2016). The latter mode of repair, which seems to be employed on plant intergenic mtDNA, is error-prone and can lead to genomic expansion and rearrangements (Christensen, 2013). Data on plastid genetic diversity in coding and noncoding regions between isolates or closely related species should help to show if similar processes are occurring in large ptDNAs.

On a separate note, researchers have uncovered pervasive genome-wide transcription in both small and very big mtDNAs, indicating that vast amounts of noncoding RNA are being generated in certain mitochondria (Lima & Smith, 2017). It remains to be seen if giant plastomes, like those of *C. japonica* and *A. acetabulum*, are also fully transcribed. Without a doubt, there is still a lot to learn about plastid genomes, and arguably the bigger the genome, the more to discover.

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