

Recapitulation of plastid origin: insight from *Paulinella micropora* genome analysis

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Eukaryotic photosynthetic organelles, plastids, are the powerhouses of many aquatic and terrestrial ecosystems. The canonical plastid in algae and plants originated >1 billion years ago and therefore offers limited insights into the initial stages of organelle evolution. To address this issue, we focus here on the photosynthetic amoeba *Paulinella micropora* strain KR01 (hereafter, KR01) that underwent a more recent (ca. 124 Mya) primary endosymbiosis of a photosynthetic organelle, termed the chromatophore. Phylogenetic analyses using four gene markers revealed three distinct lineages of photosynthetic *Paulinella* species. We generated the complete chromatophore genome sequences from *P. longichromatophora* and *P. micropora* KR01/NZ27. Our analysis suggests that when a basal split occurred among photosynthetic *Paulinella* species ca. 60 Mya, only 35% of the ancestral orthologous gene families from the cyanobacterial endosymbiont remained in chromatophore DNA. Analysis of genomic and transcriptomic data resulted in a high-quality draft assembly of size 707 Mbp and 32,358 predicted gene models. A total of 287 chromatophore targeted long-proteins were predicted in silico, 206 of which comprise the ancestral organelle proteome in photosynthetic *Paulinella* species with functions in nucleotide metabolism and oxidative stress response. Gene co-expression analysis identified networks containing known high light stress response genes as well as a variety of putative “dark” genes of unknown function. We characterized diurnally rhythmic genes in this species and

found that over 51% are dark. Our results demonstrate the massive amount of genetic innovation needed to domesticate a photosynthetic organelle and identify a storehouse of novel genes implicated in the transition from a heterotrophic lifestyle to photoautotrophy.