

ABSTRACT

The acquisition of photosynthesis by eukaryotes was undoubtedly a key step in the evolution of life, shaping the face of this planet like no other evolutionary event. It is commonly accepted that a single primary endosymbiosis, in which a cyanobacterium was engulfed by a heterotrophic host cell, gave rise to the photosynthetic organelles of plants, the plastids, probably more than 1 billion years ago. Recently, we presented evidence that the photosynthetic inclusions, termed 'chromatophores', present in the filose thecamoeba *Paulinella chromatophora* originated from an independent, more recent primary endosymbiotic event, involving a cyanobacterium from the *Prochlorococcus/Synechococcus* clade (=PS-clade).

A subclade of the PS-clade is known to have obtained a proteobacterial form 1A RubisCO by horizontal gene transfer (HGT) and its members are therefore referred to as α -cyanobacteria. In rDNA-phylogenies, the chromatophore diverged basal to the PS-clade, raising the question whether the HGT occurred before or after the split of the chromatophore ancestor from the PS-clade. By sequence determination and phylogenetic analysis of the RubisCO large subunit gene of *P. chromatophora* and several *Synechococcus* strains it was demonstrated in this thesis that the HGT predated the divergence of the chromatophore. Thus, the whole PS-clade plus the chromatophore have to be regarded as α -cyanobacteria. The γ -proteobacterium *Nitrococcus mobilis* was identified as closest known relative to the donor of the HGT.

To clarify the chromatophore's metabolic role and its state of integration into the host, the complete genome sequence of the chromatophore was determined. The data presented here reveal a fundamental reduction of the chromatophore genome. The single, circular chromosome of 1.02 Mb encodes 867 protein-coding genes and is, therewith, the smallest cyanobacterial genome reported to date. Although the chromatophore genome contains a complete set of photosynthesis genes, it lacks not only genes thought to be dispensable for an intracellular lifestyle but also genes of essential pathways for amino acid and cofactor biosynthesis, characterizing the chromatophore as a photosynthetic entity that is absolutely dependent on its host for growth and survival.

The genome data were complemented with gene expression data obtained from a shotgun proteome analysis as well as an RT-PCR approach. The proteome analysis yielded semi-quantitative expression data for more than 50% of the chromatophore-encoded genes. The physiological profile obtained highlights photosynthesis as the chromatophore's principal contribution to the symbiotic relationship, but also underlines the ability to provide fatty acids, isopentenyl diphosphate, several amino acids, and reduced sulfate compounds. Although genetic integration of the chromatophore by endosymbiotic gene transfer (EGT) is –due to the unicellularity of the host cell– plausible, no

conclusive evidence for large-scale EGT was obtained by examination of the proteome as well as Southern blot analysis.

In conclusion, the chromatophores of *P. chromatophora* are the only known cyanobacterial descendants besides plastids with a significantly reduced genome that confer photosynthesis to their eukaryotic host. The resemblance of the chromatophores to primary plastids, their independent evolution, and their earlier state of symbiotic integration places the *Paulinella* symbiosis in a pivotal position for understanding the evolution of phototrophic eukaryotes. Comparison with plastids and bacterial endosymbionts of invertebrates sheds light on early steps of the integration of a photosynthetic prokaryote into a eukaryotic cell.