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Evolution of protein transport systems in primary plastids and *Paulinella* chromatophores

Primary plastids are photosynthetic organelles characteristic of glaucophytes, red algae and green plants. They evolved about 1.5 billion years ago from a β -cyanobacterium that was enslaved by a phagotrophic eukaryote in the process called primary endosymbiosis. During this endosymbiosis, the cyanobacterium underwent a dramatic transformation involving gene transfer and evolution of protein import machinery for nuclear-encoded proteins. At present, almost all nuclear-encoded, plastid-targeted proteins are imported into primary plastids using N-terminal transit peptides and Toc-Tic translocons. Because the translocons are quite complex, a simpler pathway based on vesicular trafficking, was hypothesized to operate in the ancestral primary plastid. The existence of several proteins with N-terminal signal peptides targeted to primary plastids in endomembrane vesicles has been considered evidence for such primordial transport. To test this hypothesis, we performed multi-method phylogenetic and bioinformatic analyses of these proteins and studied presequences of their homologues. Our results indicate that vesicular trafficking evolved relatively late in primary plastids, contrary to the Tic-Toc translocons, possibly only in higher plants, to permit glycosylation and/or transport to more than one cellular compartment.

Interestingly, primary endosymbiosis took place again, ~60 million years ago, between an α -cyanobacterium and a heterotrophic ancestor of amoeba *Paulinella chromatophora*, resulting in two photosynthetic bodies called chromatophores. We elaborated a model for protein import into *Paulinella* chromatophores based on analyses of the chromatophore genomes, *Paulinella* EST database and presequences of proteins imported to the

chromatophores. The model involves vesicular trafficking to the outer chromatophore membrane, while the inner membrane is crossed by a simplified Tic-like complex. Proteins following this pathway are characterised by low molecular weight and nearly neutral charge, which constitute good adaptations to their passage through the peptidoglycan wall still present in chromatophores. Results of this dissertation indicate that although *Paulinella* chromatophores represent true photosynthetic organelles like primary plastids, they evolved independently from distinct cyanobacteria, which resulted in their distinct integration with the host cells. Therefore mutual modelling of their evolution should be carried out with caution. This is, especially, well exemplified by the protein transport systems we have investigated.