

**Przemyslaw Gagat**

DEPARTMENT OF GENOMICS, FACULTY OF BIOTECHNOLOGY, UNIVERSITY OF WROCLAW, UL. PRZYBYSZEWSKIEGO 63/77, 51-148 WROCLAW, POLAND

e-mail: gagat@smorfland.uni.wroc.pl

**Paweł Mackiewicz**

DEPARTMENT OF GENOMICS, FACULTY OF BIOTECHNOLOGY, UNIVERSITY OF WROCLAW, UL. PRZYBYSZEWSKIEGO 63/77, 51-148 WROCLAW, POLAND

**Andrzej Bodyl**

DEPARTMENT OF BIODIVERSITY AND EVOLUTIONARY TAXONOMY, ZOOLOGICAL INSTITUTE, UNIVERSITY OF WROCLAW, UL. PRZYBYSZEWSKIEGO 63/77, 51-148 WROCLAW, POLAND

## **Evolution of protein targeting via endomembrane system to primary plastids**

Before 1.5 billion years ago a heterotrophic eukaryotic ancestor of glaucophytes, red algae, and green plants engulfed cyanobacteria, which then were transformed into primary plastids with two envelope membranes. Gene transfer from the cyanobacterial genome to the host nucleus fostered the integration of the endosymbiont and the host but it is still not clear how protein products of the transferred genes were initially transported back into the ancestral primary plastid. At present, almost all proteins encoded by the host nucleus are imported into primary plastids post-translationally using N-terminal transit peptides and the Toc and Tic translocons. Because these translocons consist of many specialized protein subunits, it is hypothesized that the protein import into the ancestral plastid proceeded by a simpler pathway based on the host endomembrane system involving the endoplasmic reticulum (ER) and/or the Golgi apparatus (GA). In accordance with this hypothesis, five known proteins with N-terminal signal peptides, which are directed to primary plastids in vesicles derived from the endomembrane system, could be considered relics of this primordial import pathway. To test if it is true, we performed phylogenetic analyses as well as applied other bioinformatics tools specialized in the prediction of N-terminal targeting signals. Our analyses show that all nuclear-encoded plastid-targeted proteins with signal peptides are of the eukaryotic (not cyanobacterial) origin and that their homologs are equipped with signal peptides responsible for their co-translational import to the ER. This indicates that only a limited subset of host proteins, normally targeted to different secretory compartments, exploited their signal peptides to reach higher plant primary plastids via the endomembrane system. Thus, currently known plastid proteins with signal peptides cannot be considered a relic of the primordial plastid vesicular trafficking. The protein import into primary plastids was dominated right from the beginning by the gradually evolving Toc-Tic-based pathway while the vesicular trafficking to primary plastids evolved secondarily long after the primary endosymbiosis and probably only in the land plant lineage.