

## Abstract

Approximately one-fourth of all cellular proteins represent integral membrane proteins (IMPs) that are transported through the cytosol across or into the organellar or plasma membrane. Transport of IMPs requires precise timing which needs to be precisely regulated for them to reach their final destination. Tail-anchored (TA) proteins represent specific class of membrane proteins that lack the N-terminal signal peptide, which targets the nascent polypeptide to the endoplasmic reticulum (ER) membrane for the co-translational transport. Instead, they possess single C-terminal transmembrane domain (TMD) that serves as their targeting signal. Therefore, TA proteins are transported only post-translationally when the C-terminal TMD appears from the ribosome. The Guided Entry of Tail-anchored proteins (GET) pathway is the dominant way of how TA proteins find their way into the ER membrane. It is a multistep process that is mediated by six (Sgt2, Get1-Get5) proteins in yeast and seven (plus Bag6) proteins in human, which involves recognition of a TA protein, its targeting to the ER membrane and the actual membrane insertion. In addition to the model cell systems, some of GET pathway components were studied in plants and recently in *Plasmodium falciparum*, which makes our knowledge on the distribution and the general mechanism of the pathway very limited.

We used parasitic protist *Giardia intestinalis* as model organism to study the general aspects of GET pathway and also to understand the evolution of this eukaryote-specific machinery. Detailed characterization and crystal structures of *G. intestinalis* Get3 greatly contributed to our understanding of the Get3 catalytic cycle during the transport of TA proteins. In addition, the identification of the GET pathway components in *G. intestinalis*, showed the involvement of a Bag6 homologue, which was so far known only in metazoan species. Our data show that last eukaryotic common ancestor (LECA) possessed all the GET pathway components (Sgt2, Get1-Get5, Bag6) and therefore Bag6 was secondarily lost in some lineages of eukaryotes including yeast. Our results from both the experimental and bioinformatic studies suggest that the GET pathway is an ancestral eukaryotic pathway with critical role for eukaryotes.