

Abstract

Many eukaryotic genes do not follow vertical inheritance pattern. In the present work, we have chosen as a model the gene for methionine adenosyltransferase (MAT), in which we have decided to examine in detail the evolutionary history. MAT is a ubiquitous essential enzyme that, in eukaryotes, occurs in two relatively divergent paralogs: MAT and MATX. Both paralogs have punctate distributions across the tree of eukaryotes and, except for a few cases, they are mutually exclusive. This points to the complicated evolutionary history of this gene couple, which may be caused by either differential loss of old paralogs or the spread of one of these paralogs by horizontal gene transfer (HGT). We have focused on the evolution of this enzyme particularly within one of the best-known groups of flagellates, the euglenids, because it was hypothesized that MATX evolved in photosynthetic euglenids before it spread to other lineages.

We gained 26 new sequences from 23 euglenid lineages and one prasinophyte alga *Pyramimonas parkeae*. MATX was found only in photoautotrophic euglenids. Both, mixotroph *Rapaza viridis* and the prasinophyte alga *Pyramimonas parkeae*, the closest known relative of the euglenid plastid ancestor, only displayed the MAT paralog. In contrast, both paralogues were found in two euglenid species (*Monomorpha pyrux* and *Phacus orbicularis*). However, these two MAT genes were not related to any ancestral-euglenid MATs. The distribution of MAT/MATX in euglenids can be explained by three events: a single HGT of MATX that happened after the origin of euglenid secondary plastid, and two HGTs of MAT into two photoautotrophic species.

The plausibility of processes putatively involved in MAT and MATX evolution was investigated using the *Trypanosoma brucei/Euglena gracilis in vivo* experimental model. This confirmed that MATX is able of both, a long-term coexistence with its MAT counterpart, and immediate replacement of MAT function. The conflict between species phylogeny and phylogeny inferred from MATX sequences suggests that MATX paralog has undergone HGT across the eukaryotic tree. Since phylogenetic analyzes do not exclude the presence of MATX in a common ancestor of eukaryotes, we assume that MATX originated in very ancient gene duplication, possibly in a common ancestor of all extant eukaryotes. This duplication was followed by more or less long period of coexistence of both paralogs until individual eukaryotic lines lost one of them. In addition, both paralogs have undergone HGTs. During one of the HGTs MATX was introduced into the lineage of photosynthetic euglenids, where it replaced the original MAT. The initial idea that euglenids was the group in which MATX originated proved to be very unlikely.