Is mRNA decapping activity of ApaH like phosphatases (ALPH's) the reason for the loss of cytoplasmic ALPH's in all eukaryotes but Kinetoplastida?

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Research article

Keywords: ApaH like phosphatase, ApaH, ALPH, Trypanosoma brucei, mRNA decapping, m7G cap, mRNA cap, ALPH1, Kinetoplastida

Posted Date: February 5th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-192222/v1

Abstract

Background: ApaH like phosphatases (ALPHs) originate from the bacterial ApaH protein and are present in eukaryotes of all eukaryotic super-groups; still, only two proteins have been functionally characterised. One is ALPH1 from the Kinetoplastid Trypanosoma brucei that we recently found to be the mRNA decapping enzyme of the parasite . mRNA decapping by ALPHs is unprecedented in eukaryotes, which usually use nudix hydrolases, but the bacterial ancestor protein ApaH was recently found to decap non-conventional caps of bacterial mRNAs. These findings prompted us to explore whether mRNA decapping by ALPHs is restricted to Kinetoplastida or more widespread among eukaryotes.

Results: We screened 824 eukaryotic proteomes with a newly developed Python-based algorithm for the presence of ALPHs and used the data to refine phylogenetic distribution, conserved features, additional domains and predicted intracellular localisation of ALPHs. We found that most eukaryotes have either no ALPH (500/824) or very short ALPHs, consisting almost exclusively of the catalytic domain. These ALPHs had mostly predicted non-cytoplasmic localisations, often supported by the presence of transmembrane helices and signal peptides and in two cases (one in this study) by experimental data. The only exceptions were ALPH1 homologues from Kinetoplastida, that all have unique C-terminal and mostly unique N-terminal extension, and at least the T. brucei enzyme localises to the cytoplasm. Surprisingly, despite of these non-cytoplasmic localisations, ALPHs from all eukaryotic super-groups had in vitro mRNA decapping activity.

Conclusions: ALPH was present in the last common ancestor of eukaryotes, but most eukaryotes have either lost the enzyme since, or use it exclusively outside the cytoplasm in organelles in a version consisting of the catalytic domain only. While our data provide no evidence for the presence of further mRNA decapping enzymes among eukaryotic ALPHs, the broad substrate range of ALPHs that includes mRNA caps provides an explanation for the selection against the presence of a cytoplasmic ALPH protein as a mean to protect mRNAs from unregulated degradation. Kinetoplastida succeeded to exploit ALPH as their mRNA decapping enzyme, likely using the Kinetoplastida-unique N- and C-terminal extensions for regulation.

Full Text

Due to technical limitations, full-text HTML conversion of this manuscript could not be completed. However, the manuscript can be downloaded and accessed as a PDF.

Figures

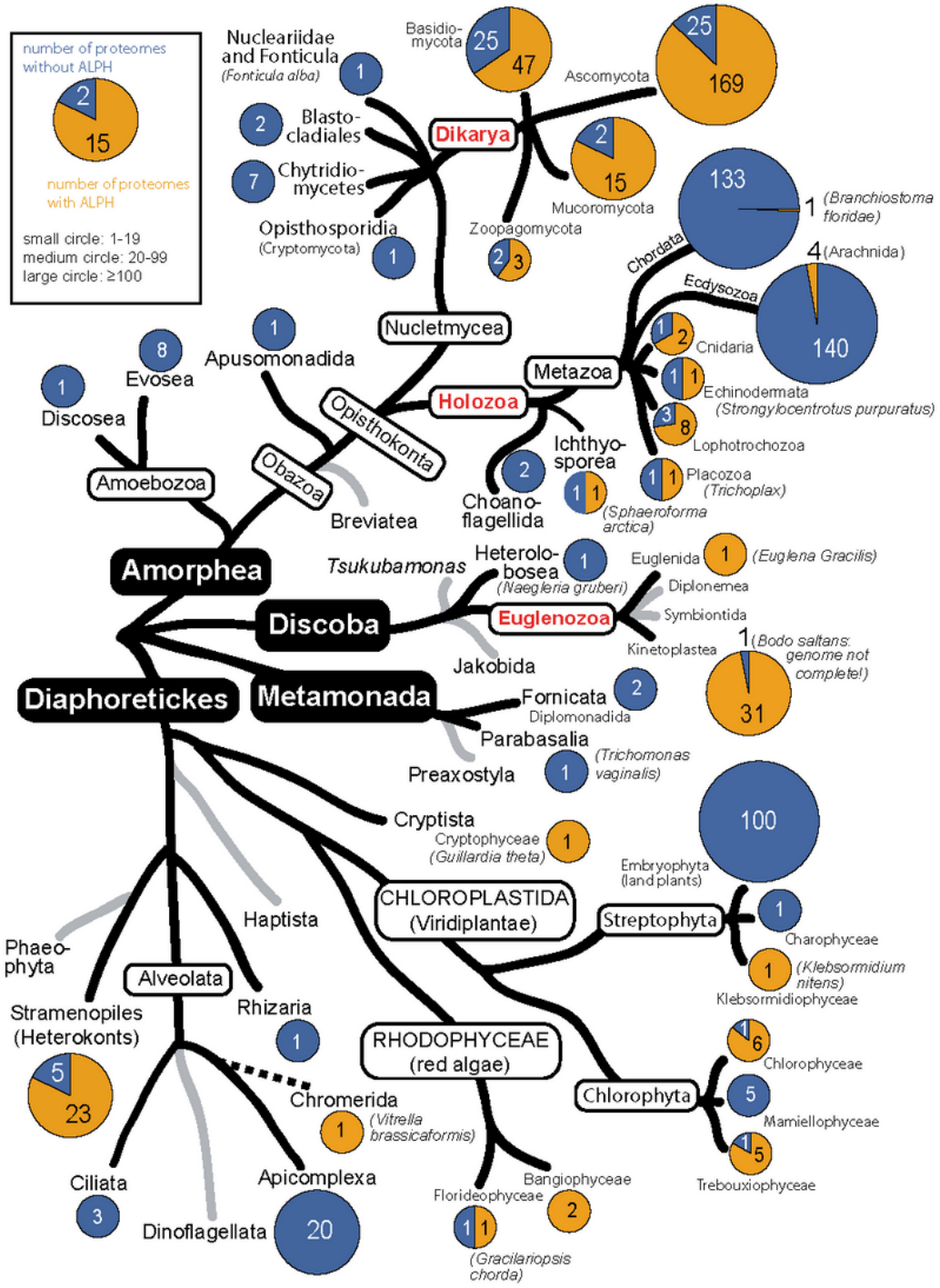

Figure 1

Presence and absence of ALPH in the different eukaryotic subgroups eukaryotic proteomes were screened for the presence of ALPH. Absence or presence of ALPH is shown in pie diagrams in blue and orange, respectively, for each phylogenetic group as indicated. The diameter of a circle roughly reflects the number of available proteomes. Information for the phylogenetic tree was taken from [30]. All organisms can be found in Supplementary Table S1.

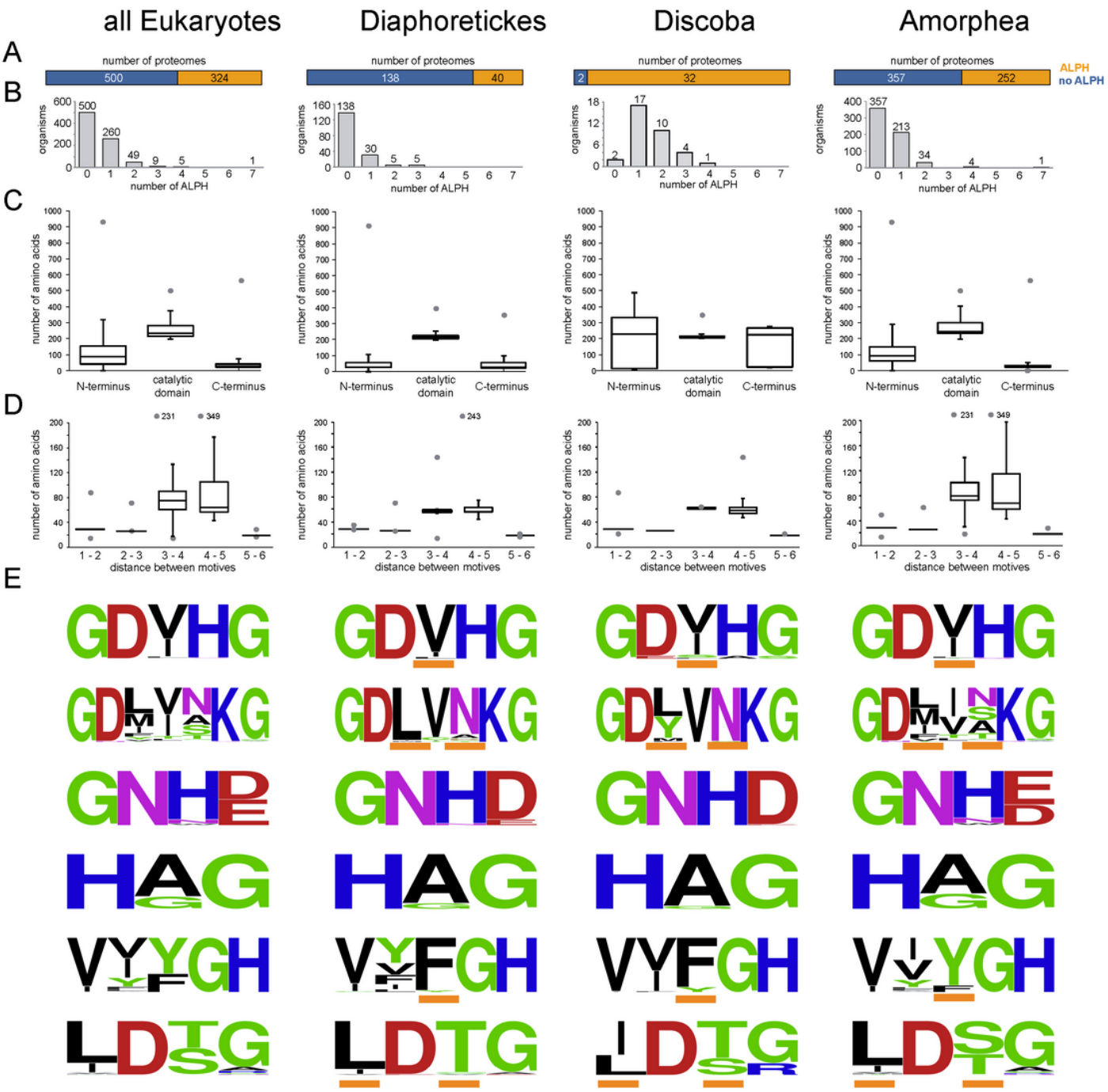

Figure 2

General Features of ALPHs (A) Proteomes with (orange) and without (blue) ALPHs. (B) Number of ALPH proteins per organism. (C) Sizes of the different ALPH 'domains' (N-terminus, catalytic domain, C-terminus) are presented as box plot (waist is median; box is IQR; whiskers are ±1.5 IQR; only the smallest and largest outliers are shown). The catalytic domain is defined as the range between the first and last motif, with an additional six N-terminal amino acids and an additional eight C-terminal amino acids. Eukaryotic ALPHs (D) Distances between the six different motifs (in amino 838 acids) are presented as

box plot. (E) Sequence motifs were created with WebLogo [31]. The most obvious differences between the three groups are marked with orange bars.

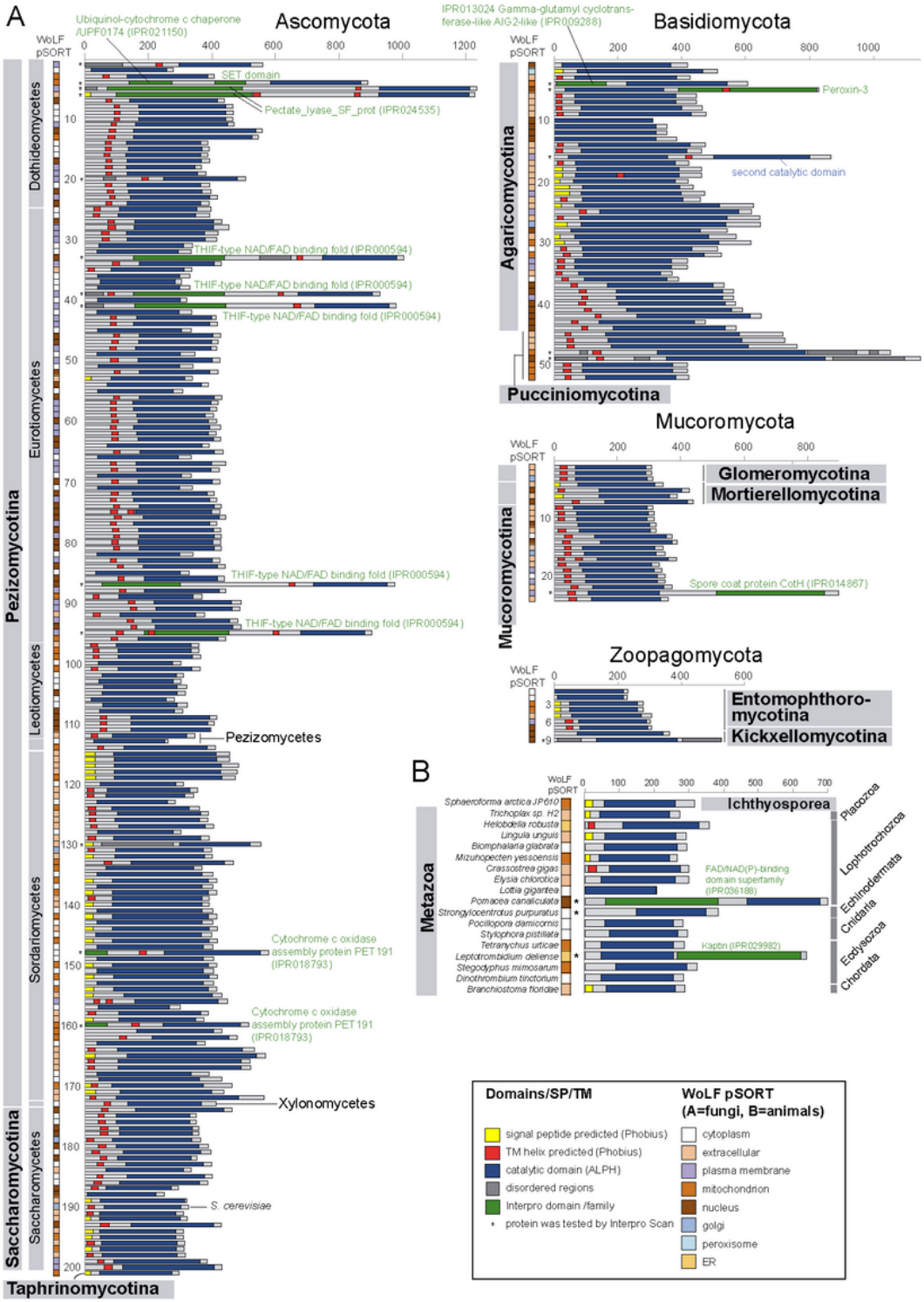

Figure 3

ALPHs of Opisthokonts ALPHs of Dikarya (A) and ALPHs of Holozoa (B) are presented with their catalytic domain (dark blue) as well as further predicted sequence features, domains and localisation predictions.

The organism names for the Dikarya ALPHs can be found in Supplementary Table S1c (number). More details on both Dikarya and Holozoa ALPHs are listed in Supplementary Table S1c and S1d, respectively.

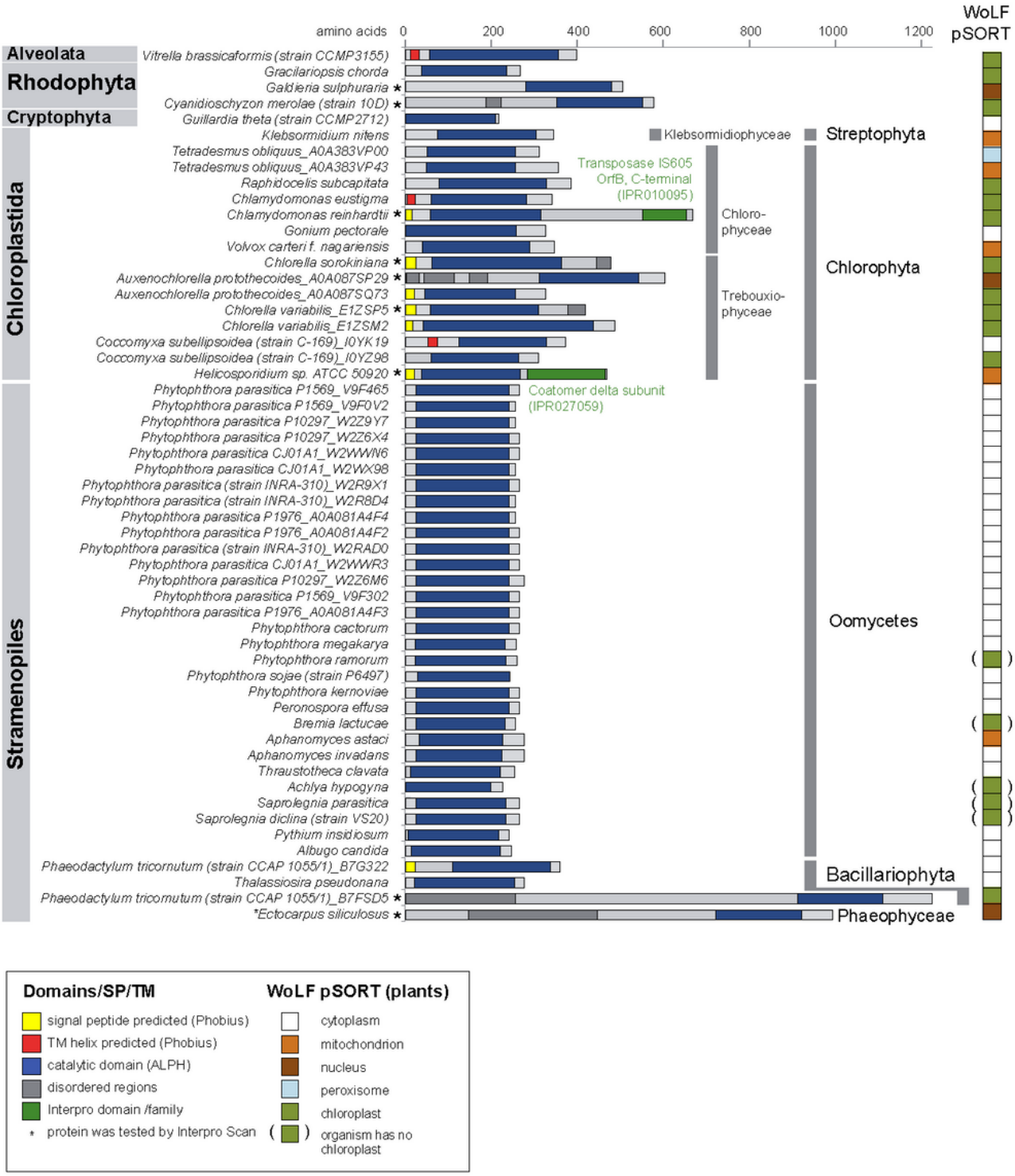

Figure 4

ALPHs of Diaphoretickes ALPHs of Diaphoretickes are presented with their catalytic domain (dark blue), further predicted sequence features and domains and localisation predictions. More details are listed in Supplementary Table S1e.

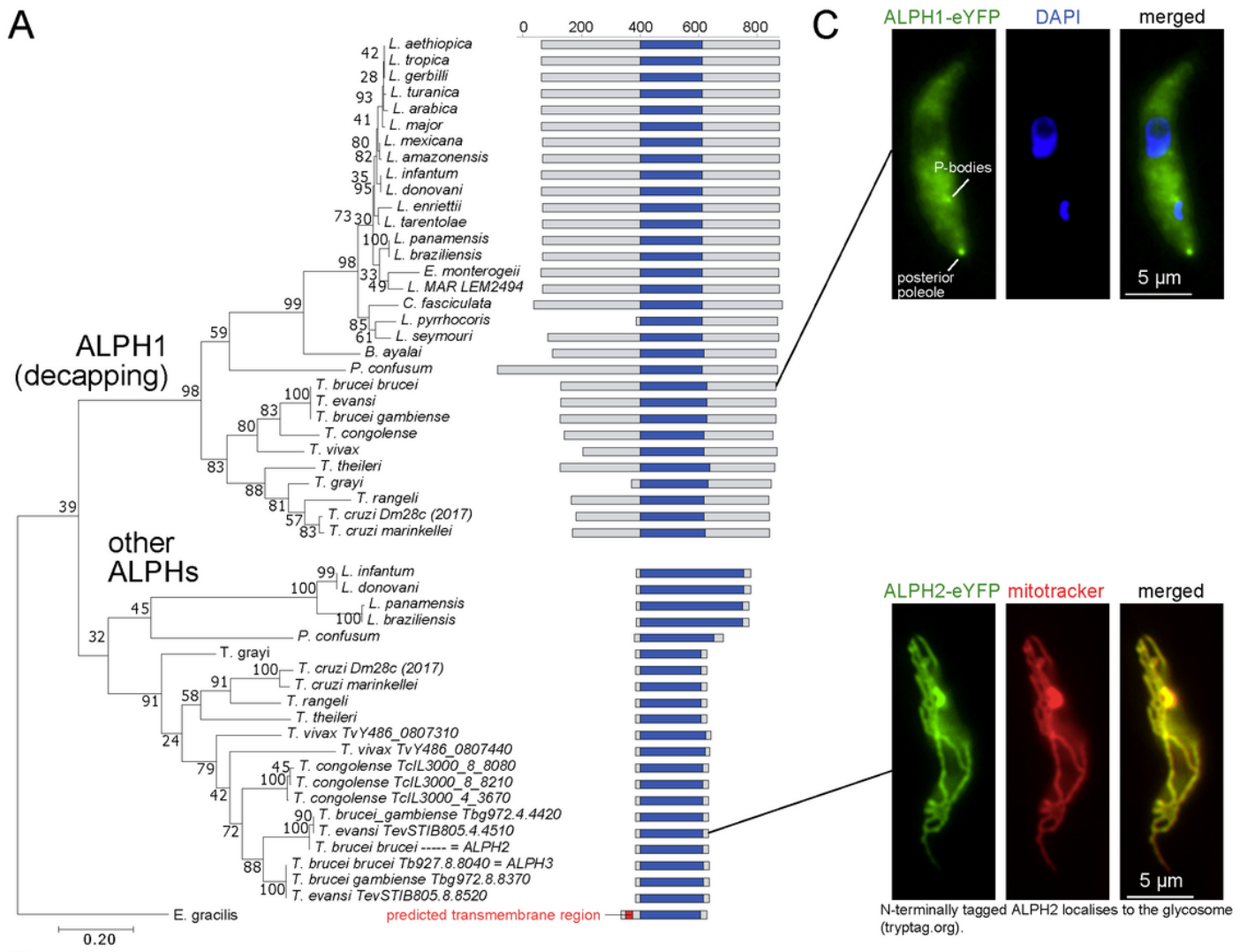

Figure 5

ALPHs of Euglenozoa (A) ALPHs of Euglenozoa are presented with their catalytic domain (dark blue) along a maximum likelihood phylogenetic tree based on an alignment of gap corrected sequences of ALPH catalytic domains. Different methods (minimal evolution, neighbour joining) gave slightly different trees, but with all methods, the ALPH1 isoforms group together in one clade that never contains non-ALPH1 isoforms. All details on Euglenozoa ALPHs are listed in Table S1f. The localisation of T. brucei ALPH1 and ALPH2 was determined by expressing eYFP fusion proteins. One Eukaryotic ALPHs representative fluorescent image of each cell line is shown. At 863 least three different clonal cell lines

showed identical localisation. (B) Sequence motifs of the ALPH1 orthologues and the Kinetoplastida orthologues to other ALPHs. Major differences are marked by asterisks.

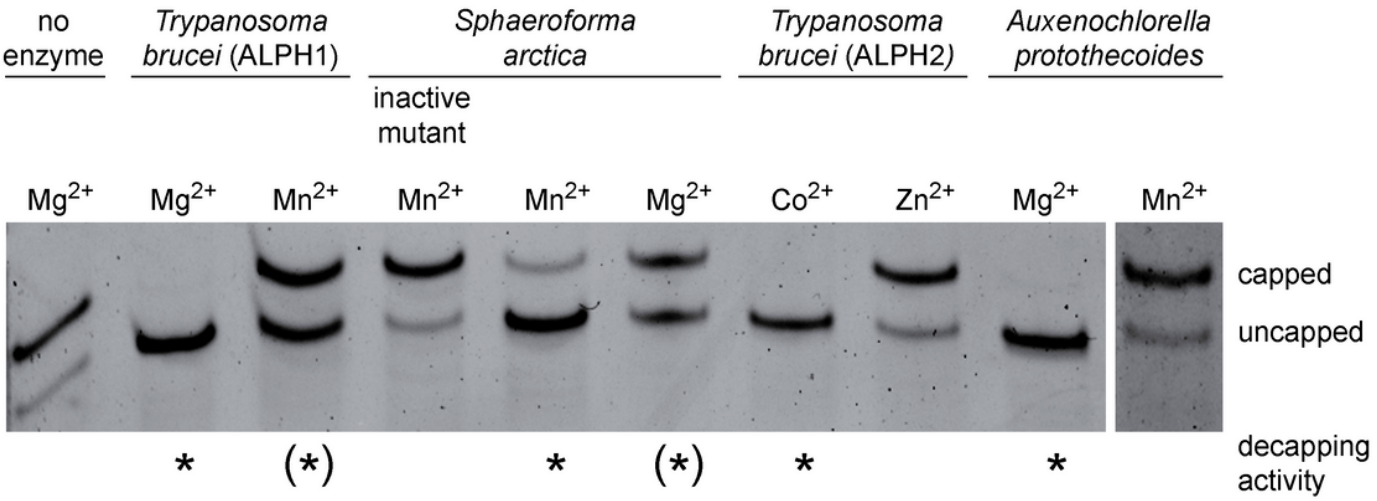

Figure 6

ALPH proteins have mRNA decapping activity In vitro mRNA decapping assays were done with recombinant ALPH enzymes from T. brucei (the decapping enzyme ALPH1 and the mitochondrial localised enzyme ALPH2), from Sphaeroforma arctica (both the wild type and a catalytically inactive mutant) and from Auxenochlorella protothecoides, using an m7G-capped RNA oligo as a substrate. Note that a small portion of this oligo is uncapped even in the absence of enzyme due to production issues (there is no auto-decapping activity during the assay, data not shown). mRNA decapping activity was observed, as expected, with T. brucei ALPH1 [10] and was fully absent in the catalytically inactive mutant of ALPH from Sphaeroforma arctica. All three previously untested ALPH proteins had in vitro decapping activity, albeit the ions requirements differed between the enzymes.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupFigures.pdf
- TableS1.xlsx
- TableS2.xlsx