



COMENIUS UNIVERSITY IN BRATISLAVA
FACULTY OF NATURAL SCIENCES
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Reference to the Ph.D. Thesis: Mgr. Jana Szabová, The complicated evolution of methionine adenosyltransferase in euglenids and eukaryotes in general

Jana Szabová Ph.D Thesis is focused on the evolution of Methionine AdenosylTransferase (MAT) primarily in euglenids. Original results are presented and interpreted within a broad frame of quite complex and complicated evolutionary history of this enzyme (or its genes) among all organisms especially eukaryotic ones.

From the formal point of view thesis is composed from a relatively short Overview (pp. 7-23) and Conclusions (pp. 26-30) complemented by Abstract, The main aims of the thesis and References. The essential part of the work constitute reprints of three articles published in well impacted journals. In two publications particularly those ones which are crucially connected with the main thesis topic Jana is the first author. I have two small objections to the formal composition of submitted thesis.

(i) As a reviewer I would have appreciated if the Supplementary materials especially all the figures had been included into the printed thesis version. There is a lot of space for them in such a document; it is very inconvenient to search for those additional data/files on the web.

(ii) Concerning the Overview and Conclusions let me say that in Ph.D. thesis again there is enough space (for sure much more than in journals) to extend some ideas, forms, hypotheses, maybe to mention some unpublished results as well, work in progress and plans for the future etc. (of course this is not obligatory).

Since all three articles where Jana is a co-author were peer reviewed there is no reason to relativise the published results or their interpretation. Apart from this there is any time a space for some questions or comments especially when the topic is close to me and the conclusions underline that studied enzymes and their genes demonstrate complicated evolutionary history which is not completely understood.

I can see the main conclusion of the presented work in two following sentences: „(i) Our results suggest that MATX entered the euglenid lineage in a single horizontal gene transfer event that took place after the secondary endosymbiotic origin of the euglenid chloroplast. (ii) The origin of the MATX paralogue is unclear, and it cannot be excluded that it arose by a gene duplication event before the most recent common ancestor of eukaryotes“.

Would it be possible to interpret those contradictory conclusions also in an absolute time scale? According to a recent review (Stiller JW, *J. Phycol.* 2014) Euglenophyta plastids are very young (100 Million years) not only in general but in respect to other representatives of algae having complex plastids as well. On the other hand euglenids are considered to be ones of the oldest eukaryotes.

Your statement is: „... the original source of MATX is not yet known and it could originate by gene duplication from MAT in the last eukaryotic common ancestor“. Would it be possible to be more specific in this respect, i.e. what evolutionary line (even domain?) can be excluded? It looks that Archaea. In respect to Bacteria especially cyanobacteria and/or alpha-proteobacteria can we exclude that the early eukaryotic MAT gene is derived from a bacterial gene as a product of endosymbiotic gene transfer?

Are there any introns in MAT/MATX genes?



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You have found both MAT/MATX paralogues in two euglenid species (*Monomorpha pyrnum* and *Phacus orbicularis*). Could the enzymes exist as heterotetramers?

Remarks to the terminology.

(i) Names of the genes. In the thesis and both articles focused on the evolution of Methionine AdenosylTransferase (MAT/MATX), primarily genes/nucleotide sequences encoding those enzymes, the authors are not using "direct" gene names. I can see only two exceptions from this rule, i.e. on the page 11 of the thesis where *Escherichia coli* MAT gene is labeled as *metK* gene and on the page 12 where three human and rat MAT isozymes are described (genes *MAT1A* and *MAT2A/B*). Why the "indirect" terminology, i.e. MAT gene is preferred? Quite often only the enzyme acronym MAT is used (not MAT gene) but it is clear that they are talking about the gene/s and not the enzyme/s. Contrary to this in the article on the plastid genome of *Eutreptiella provides* real names of the genes are used (generally accepted abbreviations/labels for them). As a curiosity you can find in this article also the gene named *mat* which is encoding a maturase, i.e. a completely different enzyme. To avoid any confusion it should be mentioned that MAT genes (*MAT* as well as *met*?) are not localized in plastid or mitochondrial genomes.

(ii) Mixotrophy in Euglenida. On the page 2 of the second article (Szabová et al., *BMC Evol. Biol.* 2014) there is a paragraph on the diverse modes of nutrition of euglenids. Mentioned are phagotrophy, osmotrophy, photoautotrophy, and a recently discovered example of mixotrophy (a euglenid capable of both phagotrophy and photosynthesis - *Rapaza viridis*). I have to mention that in many even old Biology textbook *Euglena gracilis* is presented as a mixotrophic organism since it is able to live both (photo)autotrophically and heterotrophically, i.e. mixotrophy in Euglenida can be defined also in a different way and more general (and not so recently discovered).

(iii) On some places in the text there are comparisons "eukaryotes versus prokaryotes" (e.g. pages 11 and 15); it would be more precise to compare eukaryotes with bacteria or archaeons (since the work clearly distinguishes those two domains).

(iv) Citation Soukal 2013 is incomplete.

In my capacity as a referee of the Mgr. Jana Szabová Ph.D. Thesis I can summarize.

All my questions and comments that had arisen reading the thesis do not decrease the scientific level or impact of the documented work. I just wanted to learn more since the topic is very close to my scientific interest.

The submitted thesis and presented work clearly indicate that Jana is able to form working hypotheses and prepare original research projects, to organize the experiments, to analyze and evaluate the results and discuss them in a broad frame of related scientific topics. According to my opinion the submitted work fit well into the frame of Charles University policy for Ph.D. Thesis and I can recommend it for a "philosophiae doctor" ("PhD.") defense process.

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