Evaluation of the MTA doctoral dissertation of Dr. Christos Chinopoulos entitled „Mitochondrial substrate-level phosphorylation: from salvaging hypoxic cells to a promising cancer target".

Mitochondria play a critical role in the energy production of eukaryotic cells. The typical metabolic process is the citric acid cycle-coupled oxidative phosphorylation in the presence of adequate oxygen amount. However, in hypoxia or anoxia another important task is to provide ATP using mitochondrial substrate level phosphorylation, to prevent the use of cytosolic ATP by the mitochondria. Many aspects of this second pathway were studied and characterized by the group of Chinopoulos, including the characterization of the modulators of mitochondrial substrate level phosphorylation using both isolated and *in situ* mitochondria as well as animal models. The applicant started his scientific carrier in the research group of Veronika Ádám at the Semmelweis University, then he established his own research group which – among other sources – was also awarded a Momentum grant by the HAS.

The almost 300 page long dissertation by Christos Chinopoulos was written in a traditional structure, based on 33 publications, 15 of which is his first authorship paper, and in another 15 he is the last author. Most of these publications appeared in prestigious journals. The applicant' scientific achievement is very impressive and substantially above the achievement of a typical applicant of the same field.

While the dissertation was written in English (therefore I wrote the evaluation also in English), the thesis was written in Hungarian. The structure of the dissertation is mostly conventional, although instead of the overview of the general relevant literature and state of the field it rather relates only to the work of the author and seems to be rather a summary of the achieved results. This chapter is followed by a detailed description of the utilized methods, followed by the results section then by the discussion. Discussion is followed by a far from complete list of abbreviations, acknowledgement, and about 850 cited references. The list of the relevant own publications is not provided separately, only the thesis contains it. One reason of the quite extraordinary length of the dissertation is that the author copy-pasted large section of the respective materials and methods, results, figures and discussion part of the original manuscripts that caused a large amount of unnecessary redundancy. Furthermore, the use of copy-pasting did not occur in a coherent way. For example, the introduction properly starts with the adenine nucleotide translocase assay, the materials and methods rather with the brain tissues that are discussed last. It would have been much more reader (and reviewer) friendly to provide a thesis-like dissertation, highlighting only the important findings in a coherent way.

Novel findings described in the dissertation:

- Development of a continuous fluorescent assay for measuring the adenine nucleotide translocase (ANT)-mediated ADP-ATP exchange than can be utilized not just for isolated mitochondria but also for permeabilized cells and tissue homogenates.
- Development of a biosensor test to detect the directionality of ANT and hence the directionality of Succinyl-CoA ligase (SUCL).
- Using the developed methods, discovery the fact that the mitochondrial F0-F1 ATPase and the ANT are not necessarily synchronized in action, the SUCL-generated ATP can be used by the ATPase and/or by ANT, depending on the circumstances.
- Discovery of the important role of alpha-ketoglutarate dehydrogenase complex in the SUCL-mediated mitochondrial substrate-level phosphorylation (mSLP) and diaphorases to provide the required NAD^+.
- Discovery of the dependence of mSLP on alpha-ketobutyrate and GABA metabolisms
- Mapping of the expression of mSLP-relevant enzymes in human brain sections.

Comments and questions related to the dissertation:

Some of the figures are in much worse quality than the others (e.g. Fig 5.1 is fairly blurred, text in Fig 5.2C or Fig 6.4.A is unreadable.)

Glutamine appears to be a very important fuel for supporting mSLP. However, its conversion to alpha-ketoglutarate releases ammonia which is known to cause ROS production and damage of mitochondria. What is the fate of this ammonia in heavily glutamine-utilizing mitochondria?

Two forms of SUCL is known, and they either produce GTP of ATP directly. Although these forms are interconvertible, are there any metabolic basis of their differential expression, for example in the light of GLUD1 inhibition by GTP which also may influence the glutamine-use.

Diaphorases appear to be important to provide NAD for mSLP. Vitamin K reductase also belongs to the diaphorases. Is there any relevance of the Vitamin K status of the cells regarding the mSLP capacity. Could this be medically relevant?

In summary, Christos Chinopoulos provides substantial results in the field of mitochondrial respiration. His scientific achievements, quality of publication, citations of his papers all suggest that he is eligible to obtain the Doctor of Science title, which aspiration I fully support.

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