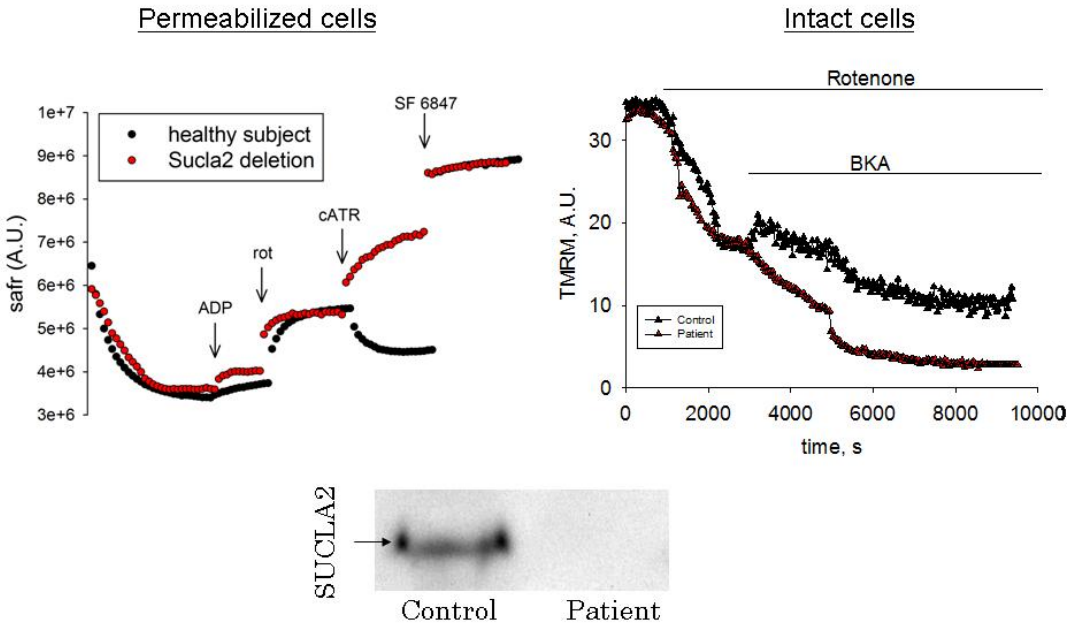
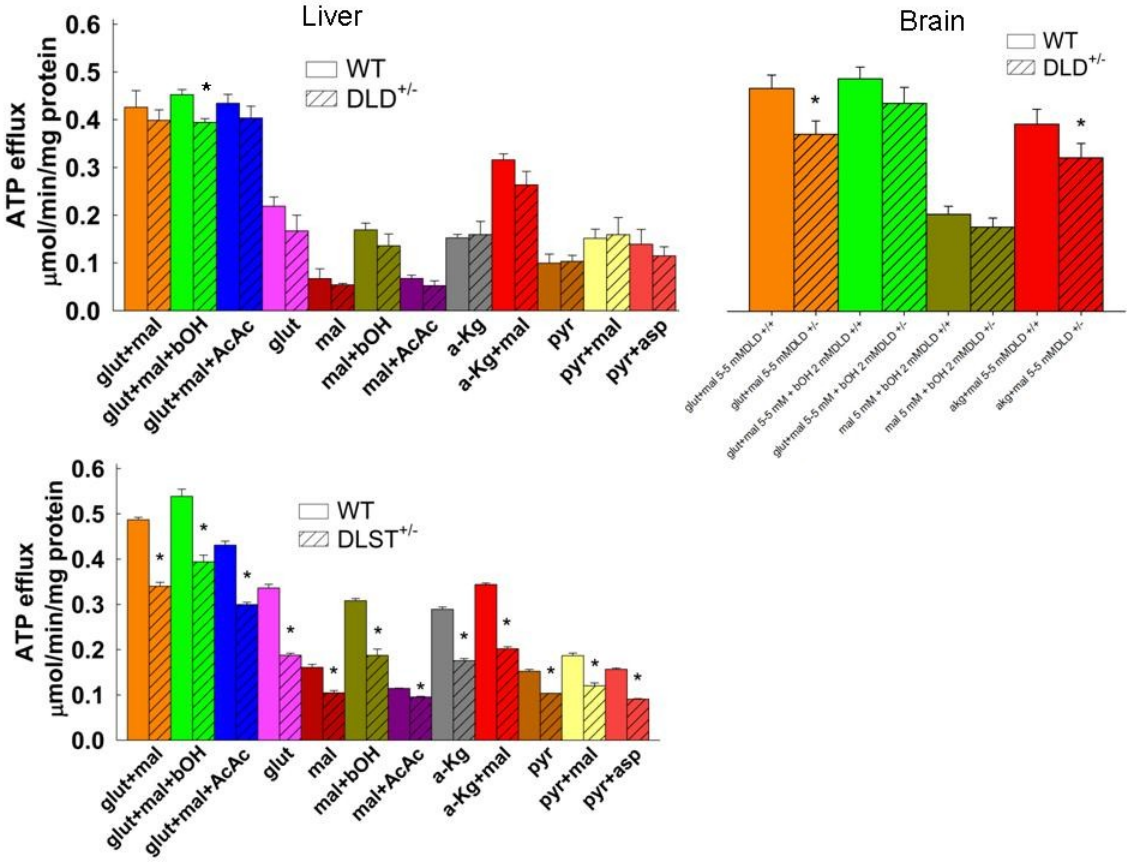
Work related to grant NNF2 85658 is closely connected to the experiments proposed in the previous grant NNF 78905, and a vast amount of data have been generated that have not been published yet. Furthermore, 5 papers have been published (amounting to a cumulative impact factor of 24.447, but only 4 of them are directly linked to the grant), one is under submission, and it is anticipated that there will be 4 more submissions (and hopefully publications) within the next 6-12 months, related to the results funded by both grants. The results obtained that were partially funded by both grants NNF 78905 and NNF2 85658 and have not been submitted for publication yet, are the following:
i) Experiments performed using fibroblasts obtained from patients with sucla2 mutations

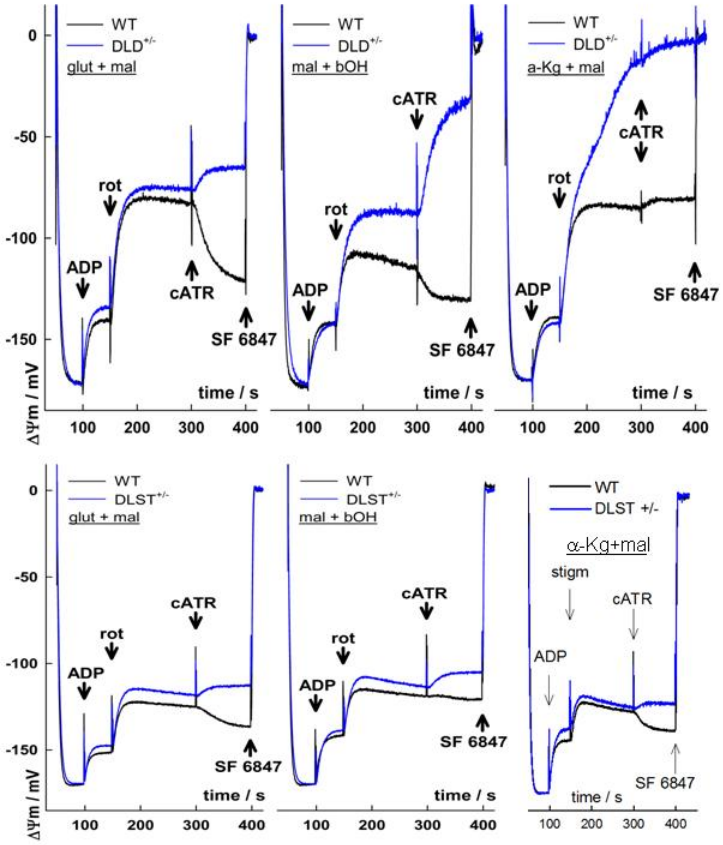
Effect of bongkrekic acid (BKA) or cATR on the rotenone-evoked depolarization of ΔΨm in fibroblasts from a patient with complete absence of Sucla2



clearly show premature reversals of the adenine nucleotide translocase. This is depicted in the figure above.
In the left panel the effect of carboxyatractyloside is shown on respiration-impaired permeabilized fibroblasts from a control subject and a patient suffering from a complete abscence of sucla2. In the right panel a similar experiment is performed but in intact cells, and using bongkrekic acid in lieu of carboxyatractyloside. The experimental paradigm used in these two panels is explained in FASEB J 24:2405-2416. Similar results were obtained from other patients exhibiting different mutations in the sucla2 gene.
To address this from a different point of view, we have tried to use cytosolic/nuclear ATP reporting plasmids that have been generated by a Japanese group, shown to report cytosolic, nuclear, or mitochondrial ATP levels, operating as FRET-based indicators, however, we found that their fluorescent properties significantly overlap to those from FADH2, so they are unusable. The results obtained from the patients with sucla2 mutations point to the necessity of characterizing the novel transgenic mouse with a targeted deletion in the sucla2 gene (see below).

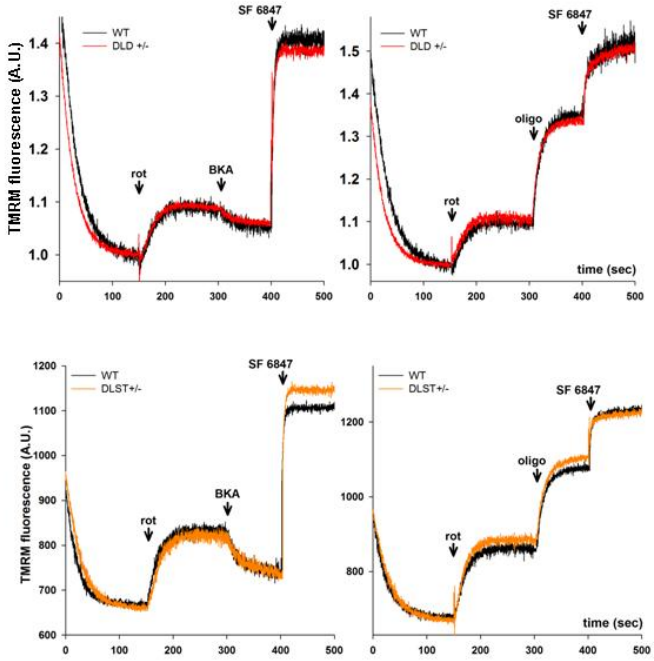
ii) Regarding work with the DLD+/- and DLST+/- mice, we found that a diminished provision of succinyl-CoA, affects succinyl-CoA ligase, so that ATP output is diminished, and the ANT also reverses prematurely. This is shown in the figures below:



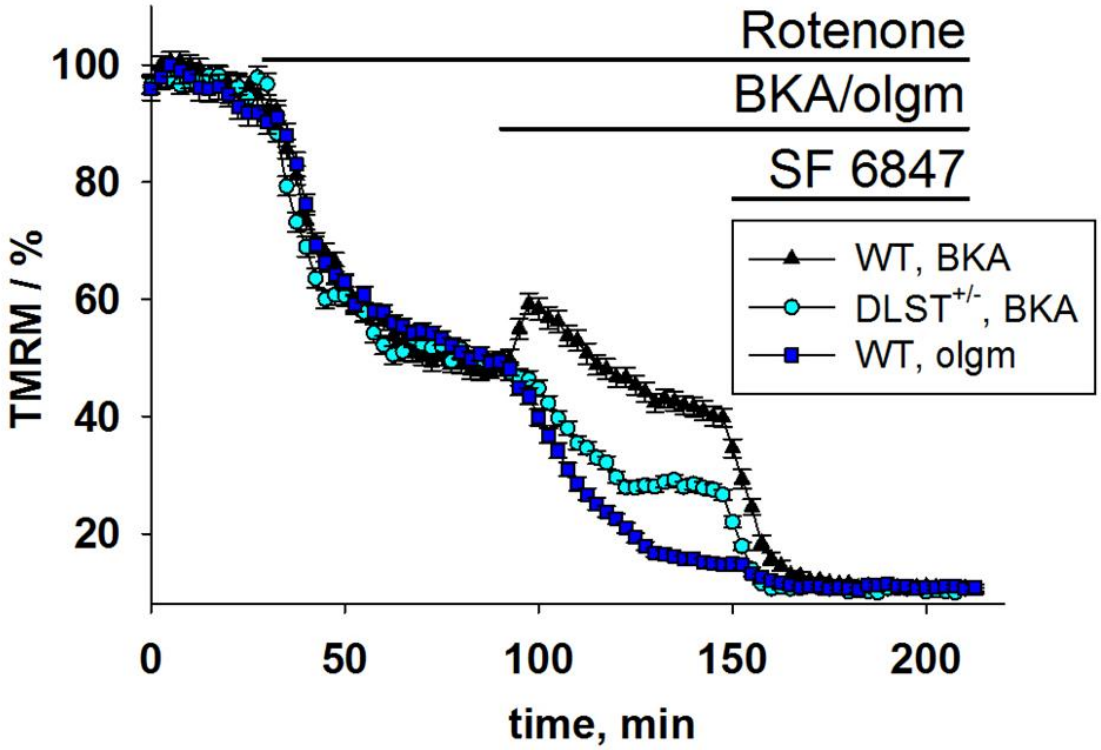


The experimental paradigm used in these two figures is explained in FASEB J 24:2405-2416. Regarding work on isolated nerve terminals and cultured neurons, results are depicted in the figures below:

Effect of bongkrekic acid (BKA) vs oligomycin (olgm) on the rotenone-evoked depolarization of ΔΨm in mouse WT, DLD +/- and DLST+/- synaptosomes

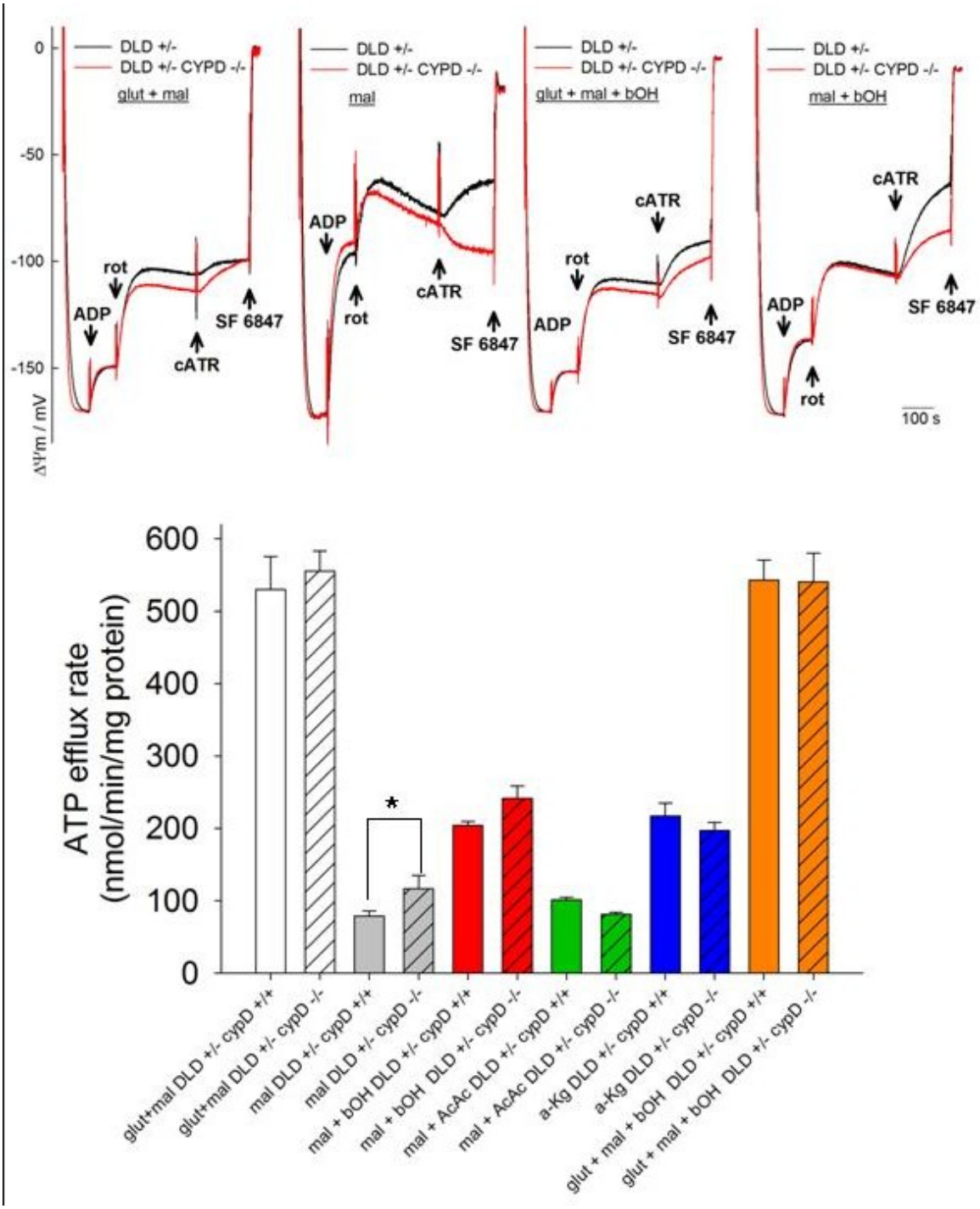


Effect of bongkrekic acid (BKA) vs oligomycin (olgm) on the rotenone-evoked depolarization of ΔΨm in cultured mouse WT and DLST+/- cortical neurons



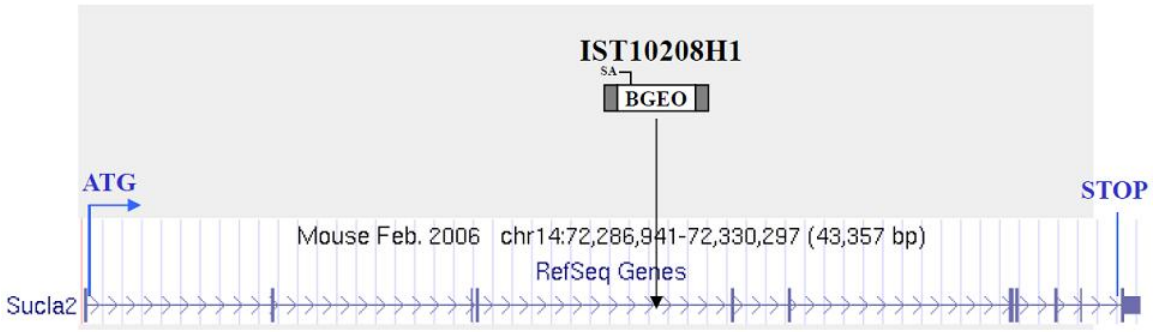
The experimental paradigm used in the above two figures is explained in FASEB J 24:2405-2416.

iii) Regarding work related to DLD+/- cypD-/- double KO, we found that acceleration of ATP synthesis or hydrolysis by removal of cypD from the F0-F1 ATP synthase compensates DLD+/- deficiency only if mitochondria respire on malate alone. This is depicted in the figure below:

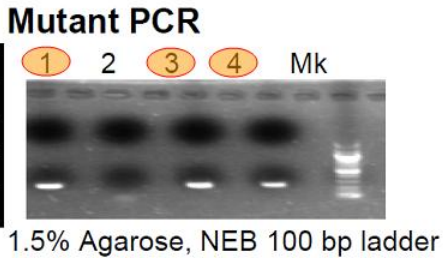


iv) Regarding the generation of the SUCLA2 transgenic mice, results are shown below for the first three +/- mice obtained:

Sucla2 knock-out mouse



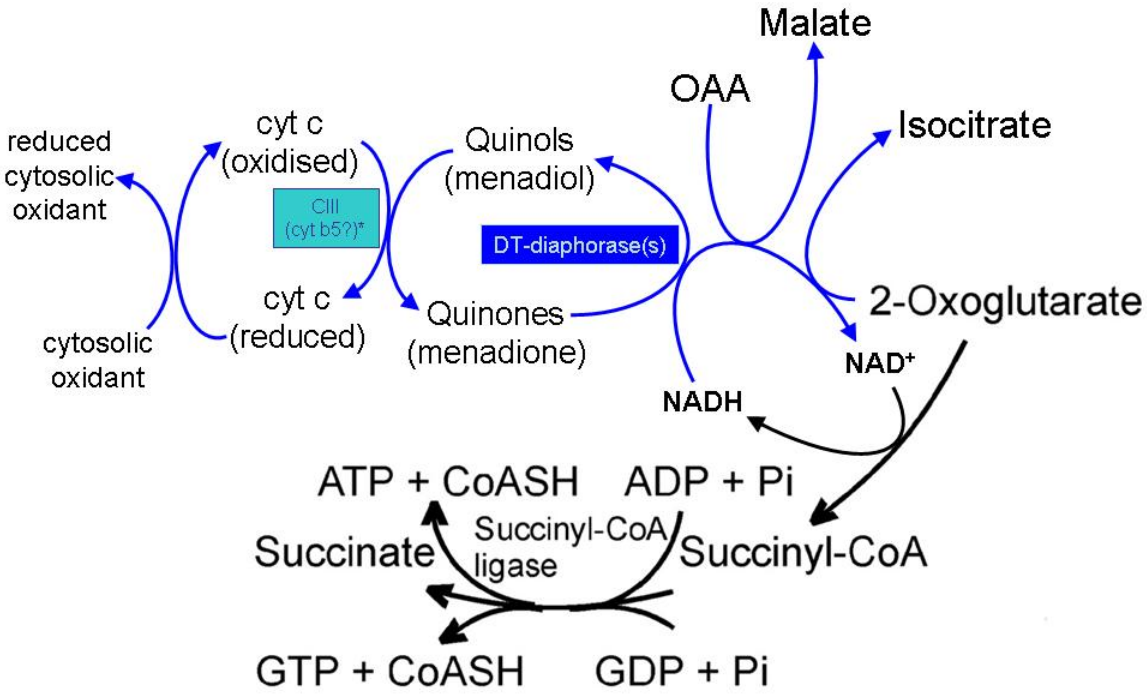
Well	Genotype	Primers
1	het	F+Downstream Rev
2	wt	F+Downstream Rev
3	het	F+Downstream Rev
4	het	F+Downstream Rev



We currently have only 4 +/- male and 4+/- female mice. These mice will be completely characterized in the near future.

v) Regarding the MDH2 (b variant) transgenic mouse colony, these mice did not exhibit a decreased enzymatic activity of malate dehydrogenase compared to WT mice, therefore this lineage has been abandoned.

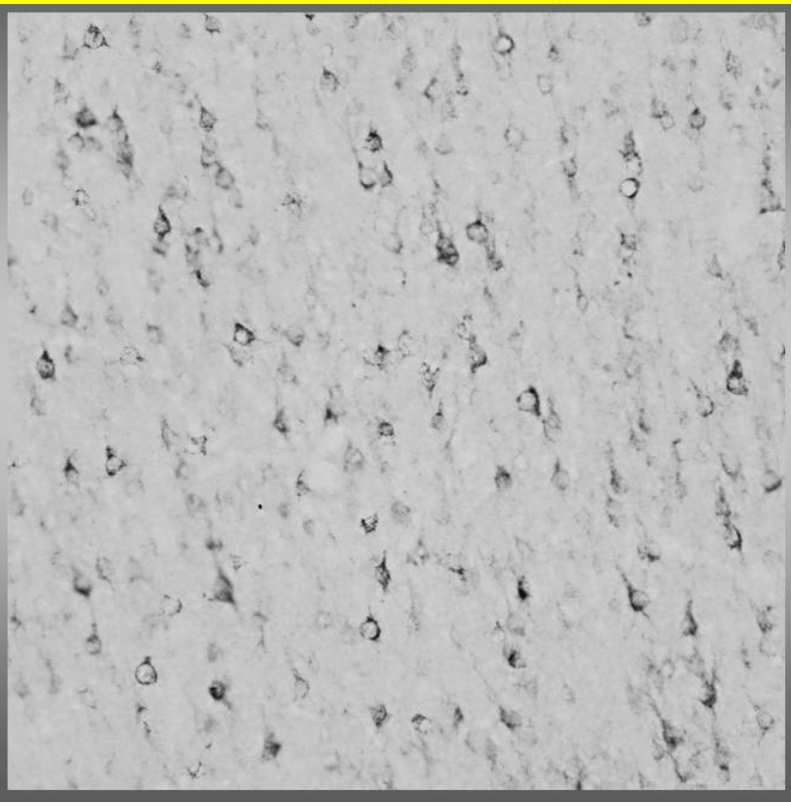
vi) Since KGDHC was active during impairment of the respiratory chain, we sought for alternative producers of NAD+ other than the respiratory chain, that led us to discover that mitochondrial diaphorases play a very important role in this respect. We also found that regeneration of oxidized substrates for the diaphorases is mediated by cytochrome b5 reductase of the complex III, in the absence of a functional complex IV. We have obtained cyb5r (isoform 2) knock out mice, and by using these mice we will verify the validity of the pathway depicted below:



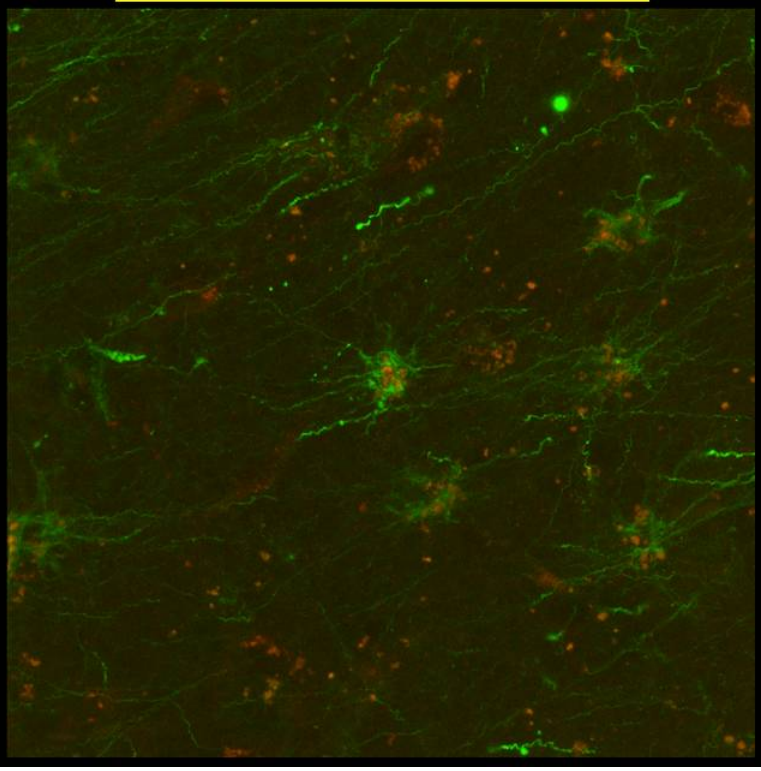
From our experiments, the importance of mitochondrial diaphorases also emerged. This will be actively pursued in the near future, and we plan to submit a new grant proposal on this matter.

There are a few more results obtained, that are indirectly related to the grant: vii) we obtained human cell lines (fibroblasts) with a complete absence of ANT1, and check phosphorylation potentials; viii) sucla2 activity has been traced almost exclusively in human neurons, being practically absent from astrocytes, shown below:

Sucla2 immunohistochemistry (Nickel-DAB) of a human brain



Sucla2 (red) and GFAP (green)
immunohistochemistry of a human brain



This finding will be investigated further. The project using the human tissues is with Dr. Arpad Dobolyi and Prof. Miklos Palkovits, who hold the required ethical permission for such experiments; ix) Finally, we obtained SOD2 overexpressing mice, in order to test if the altered NAD+/NADH in DLD+/- DLST+/- mice is due to altered ROS production, that may be compensated by an elevated SOD2 (mitochondrial superoxide dismutase) activity.