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Role of the *Saccharomyces cerevisiae SYM1* deletion on cellular dNTPs pool

The human *MPV17* gene encodes a small protein of 176 aminoacids located in the inner mitochondrial membrane (Spinazzola et al., 2006). Mutations in the *MPV17* gene were described as cause of the hepatocerebral form of Mitochondrial DNA depletion syndromes (MDDS) and Navajo neuro-hepathopathy (Karadimas et al., 2006) both characterized by multiple mtDNA depletions. Thanks to the high degree of conservation observed between MPV17 and its yeast homolog SYM1, it was possible to elucidate the molecular consequences of MPV17 variants identified in MDDS patients. It was demonstrated that Sym1 takes part in an high molecular weight complex to form a membrane pore capable of allowing the transport of large molecules, such as metabolites, across the inner mitochondrial membrane. However, the specific role of the complex and the nature of molecules transported by the Sym1-Mpv17 channel remain elusive. Recently it has been observed, in mouse and patient-derived cells characterized by MPV17 deficiency, a decrease in the mitochondrial dNTPs pool suggesting that loss of function of Mpv17 causes nucleotide insufficiency in the mitochondria that slows rate of mtDNA replication and induce mtDNA depletion. So it has been suggested that Mpv17 forms a channel in the inner mitochondrial membrane, supplying the matrix with deoxynucleotide phosphates and/or nucleotide precursors.

Here, I used *Saccharomyces cerevisiae* to test this hypothesis and to investigate whether beneficial molecules previously identified for sym1 mutants could act by restoring dNTP pool. Firstly, I have set up an enzymatic assay that allows the simultaneous determination of two pyrimidine or two purine deoxyribonucleoside triphosphates. This method is based on elongation of 5'-end-labeled oligonucleotide primers annealed to complementary oligonucleotyde. Incorporation within the primer/template is catalyzed by the Klenow fragment of *Escherichia coli* DNA polymerase I (Roy et al., 1999). Since the elongated products are clearly separated on a 12,5% polyacrylamide-urea gel, the two dNTP can be quantified in a single lane.

To validate the nucleotide extraction method two control mutants are used, the first corresponding to a mutant lacking *SML1*, the ribonucleotide reductase inhibitor so it shows a higher cytosolic dNTPs quantity than a wild type strain. I used this mutant to choose the right amount of cells to extract and the optimal extraction time. For what concerns mithocondrial dNTPs extraction, there are two main issues: to avoid cytosolic dNTPs contamination and to obtain enough material to extract dNTPs. Therefore I used an adaption of zymoliase extraction method with some advices found in literature (Giovanna Pontarin et al., 2003; Marti *et al.,*2012). To validate this protocol I used a mutant lacking *POS5,* a mitochondrial NADH kinase that displays a higher mitochondrial dNTP pool.

Once set up, I have applied the method on mutant lacking *SYM1. SYM1* deletion leads not only to a reduction of mitochondrial dNTPs pool but also of the cytosolic dNTPs pool suggesting that the ablation of Sym1 leads not only to nucleotide insufficiency in the mitochondria but also to a general impairment of the exchange between mitochondria and cytosol. Given these results, I decided to test the effect of beneficial molecules previously identified, on cytosolic and mitochondrial dNTPs pool. The results shows that four of the eleven tested molecules restore cytosolic dNTPs pool. Analysis on mitochondrial dNTPs pool are still ongoing. Anyway the demonstration that not all the beneficial molecules act by increasing the dNTPs pool support the hypothesis that the reduction of dNTPs pool is not the only cause of the disease and we suggest that other metabolites are also transported by the Sym1-Mpv17 channel.