Metabolic Role of the Mitochondrial Electron Transfer Proteins ETF and ETF-DH in *Saccharomyces cerevisiae*

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**Saccharomyces cerevisiae** ORF5a YPR004c, YGR207c and YCR250w encodes uncharacterized proteins with high homology sequence to the human electron transfer flavoprotein (ETF) subunits α and β, and to the ETF-dehydrogenase (ETF-DH), respectively. The human ETF is a mitochondrial heterodimer (ETFα and ETFβ), that functions as the physiological electron acceptor for several mitochondrial matrix dehydrogenases. The reducing equivalents from these dehydrogenases are transferred via ETF to ETF-DH, a mitochondrial inner membrane trans-striptidinio flavoprotein, also named ETF:ubiquinone oxidoreductase (ETF-QQ). The electrons are further passed from ETF-DH into the electron transport system (ETS) via coenzyme Q (CoQ) to the CoQH₂-cytochrome c oxidoreductase. Metabolic detoxification reactions coupled to ETS and ETF-DH catalyzed by α-CoA dehydrogenases involved in mitochondrial fatty acids β-oxidation, and also in nitrogen metabolism, namely the detoxification of six different amino acids (leucine, valine, isoleucine, lysine, hydroxylysine and hydroxyproline) and also of oxalate (Figure 1). Impairment of electron transfer activity due to mutations in ETF or ETF-DH causes the pathological condition Multiple Acyl-CoA Dehydrogenation Deficiency (MADD), also known as glutaric aciduria II. *S. cerevisiae* YPR004c, YGR207c and YCR250w mitochondrial localization was already determined, but their function in the mitochondria is still unknown. In yeast cells, β-oxidation takes place exclusively in peroxisomes, which, presumably, excludes the involvement of ETF and ETF-DH in the metabolic pathway. Considering that orthologous genes encoding for ETF and ETF-DH are present in non-mammalian eukaryotic cells, it is conceivable that their function might be involved, at least, in the catabolism of amino acids and choline. This is further supported by microarrays in global transcriptome analysis under amino acid starvation and nitrogen depletion. Genome-wide approaches by microarrays for the determination of the transcriptional profile of *S. cerevisiae* under stress condition also yielded data suggesting an involvement of ETF and ETF-DH in stress responses to oxidative conditions and temperature, confirmed by in silico promoter analysis (Table 1).

**Drop Screening with Different Carbon Sources**

All mutant strains were grown on fermentable and non-fermentable carbon sources. None of the mutants showed growth deficiency on glucose, ethanol, glycerol, maltose, glycerate, succinate nor methanol, when compared to the parental strain. Growth on palmitic acid (C₁₆₇), saturated fatty acid, was unaffected by these mutations (similar clearing zones), however, on oleic acid (C₁₆:1) the mutants ypr004c and ygr207c displayed growth deficiency. Surprisingly, on linoleic acid (C₁₆:2,ω₆) all mutants had better fitness than the parental strain. Since β-oxidation in yeast is exclusively peroxisomal, this unexpected result suggests an involvement of ETF and ETF-DH on metabolism of unsaturated fatty acids. This implication will be further investigated by testing other fatty acids.

**Drop Screening with Different Nitrogen Sources**

All mutant strains were grown with different nitrogen sources, whose catalytic pathways involve FAD containing dehydrogenases that pass electrons to the ETS through ETF and ETF-DH. With glucose as a carbon source, all strains showed normal growth. On ethanol, the mutant yor356w grew better than all the other strains.

**Drop Screening with Oxidative and Temperature Stresses**

All mutant strains were tested under oxidative stress conditions with menadione and hydrogen peroxide. The three mutants were affected in all media tested, with severe phenotypes on non-fermentable carbon sources. All strains were more sensitive to oxidative stress induced by menadione and none grow with ethanol as a carbon source. Temperature stress only affected the mutant ypr004c when grown on glucose and on ethanol at 37°C.