68 genes for Yeast Flavoproteoma: updates of Flavin compounds Biosynthesis, Transport and Catabolism in *Saccharomyces cerevisiae* mitochondria

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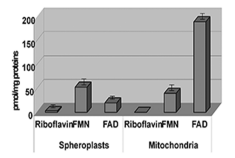
Introduction

Otto Warburg and his collaborators were the first to isolate a "yellow ferment" from yeast cells (Warburg and Christian 1933). The yeast genome contains 68 genes encoding for a flavin-dependent protein and thus 1.1% of all yeast protein (5885 protein-encoding genes, Goffeau et al. 1996) have a requirement for either FMN or FAD (Macheroux and coworkers, 2014). Saccharomyces cerevisiae cells are known to be an excellent dietary source of riboflavin (Bässler et al. 2002) and to possess all enzymes required for riboflavin biosynthesis, which are encoded by the RIB1-7 genes, as reported in Reihl and Stolz (2005).

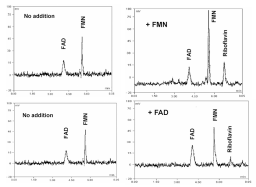
We know little about the enzymes responsible for turnover of FMN and FAD and their subcellular localization, despite the crucial roles of flavin cofactors in metabolism.

The formation of holo-flavoproteins, by binding of a flavin prosthetic group to an apoflavoprotein, depends on the availability of FMN and FAD. The homeostasis of riboflavin and flavin prosthetic groups may be altered by some factors, such as defective FMN and/or FAD synthesis, increased FMN and/or FAD catabolism, by different susceptibility of holo- and apo-flavoproteins to proteolytic digestion (Pallotta et al. 1998; Pallotta 2011) and altered mitochondrial transport as was studied by Alex Tzagoloff's lab (see references).

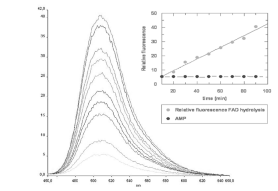
Mitochondria competence to metabolize externally added and endogenous FAD and FMN was investigated by spectroscopically and via HPLC. Thus, two novel yeast mitochondrial enzymatic activities, i.e. FAD pyrophosphatase (diphosphatase; EC 3.6.1.18) and FMN phosphohydrolase (EC 3.1.3.2), which catalyse the reactions FAD +H_2O→FMN+AMP and FMN+H_2O→ riboflavin + Pi conversion, respectively, were reported (Pallotta 2011).



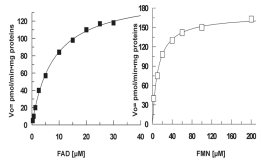
Endogenous Riboflavin, FMN and FAD content in Saccharomyces cerevisiae.



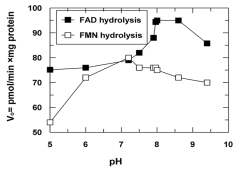
HPLC evidences for FAD and FMN uptake and metabolism in SCM.



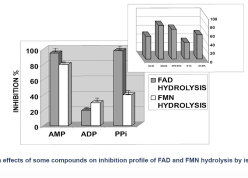
Externally added FAD hydrolysis by isolated Saccharomyces cerevisiae mitochondria; inhibition by AMP.



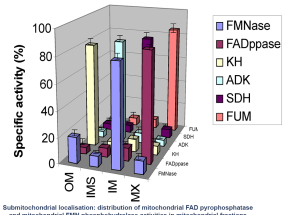
The dependence of the rate of FAD and FMN hydrolysis on the substrate concentration in SCM.



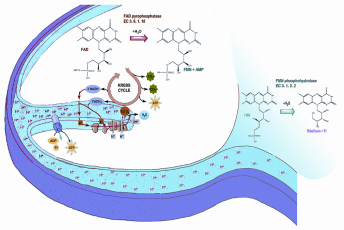
pH profile of the rate of FAD and FMN hydrolysis in SCM



The inhibition effects of some compounds on inhibition profile of FAD and FMN hydrolysis by isolated SCM



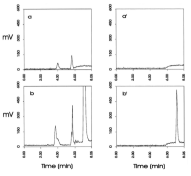
Submitochondrial localisation: distribution of mitochondrial FAD pyrophosphatase and mitochondrial FMN phosphohydrolase activities in mitochondrial fractions.



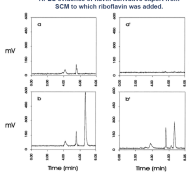
FAD and FMN metabolism in Saccaromyces cerevisiae mitochondria.

Results

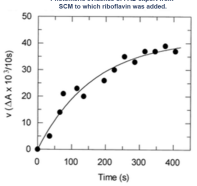
HPLC evidence for FMN and FAD synthesis in SCM to which riboflavin was added.



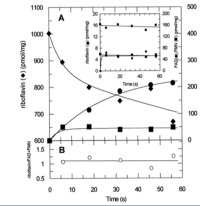
HPLC evidence of flavin derivative export from SCM to which riboflavin was added.



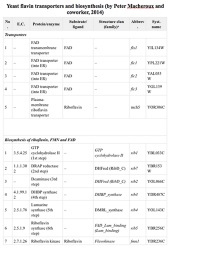
Photometric evidence of FAD export from SCM to which riboflavin was added.



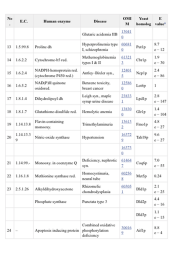
Time course of flavin derivative export from SCM to which riboflavin was added.



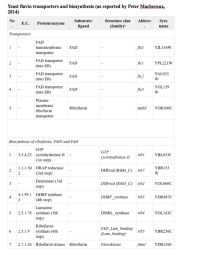
Yeast flavin transporters and biosynthesis (by Peter Macheroux and coworker, 2014)

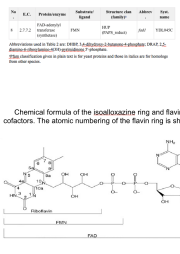


Yeast flavoproteins as human disease models.

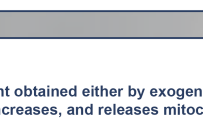


Yeast flavin transporters and biosynthesis (as reported by Peter Macheroux, 2014)

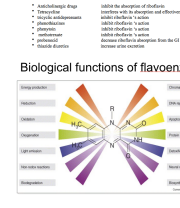




Chemical formula of the isoalloxazine ring and flavin cofactors. The atomic numbering of the flavin ring is shown



CHARACTERISTICS OF WATER SOLUBLE RIBOFLAVIN-vitamin B2



Biological functions of flavoenzymes

Conclusions

1) These researches provide a functional framework that could help to elucidate the role that an increased cellular riboflavin content obtained either by exogenous riboflavin uptake or from FAD/FMN turnover via novel mitochondrial enzyme activities or during apoptosis, in which mitochondrial membrane permeability increases, and releases mitochondrial proteins and compounds into the cytosol (Fleury et al. 2002) where mitochondria can accomplished flavoproteins turnover.

2) Riboflavin, FMN and FAD concentrations in yeast spheroplast cytosol were about 0.5, 4 and 8 μM, respectively, as calculated via HPLC measurement of the spheroplast neutralised perchloric extracts, by assuming both the spheroplast volume to be equal to 3 μl/mg protein and mitochondrial protein to be equal to 10% of spheroplast protein (Pallotta et al.1998; 2011).

3) Consequently, besides being the major reservoir of the cellular FMN and FAD, this work also showed that mitochondria represent a site of active metabolism.
Therefore yeast seems suitable as model organism to expand our knowledge in this issue and might be used to design novel drugs/therapies as new metabolic targeting strategies in human diseases.

References

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