Mitochondria, besides a key role in the generation of energy by ATP synthase, carry out a lot of functions essential for cell physiology and viability, thus impairment of any of them can result in a wide spectrum of severe abnormalities, which in humans are known as mitochondrial diseases. The mitochondrial diseases encompass two main categories: inherited maternally mutations in mitochondrial DNA (mtDNA) and mutations in nuclear DNA (nDNA). Mutations in mtDNA include deletions and rearrangements of mtDNA, point mutations in mitochondrial rRNA, tRNA and in genes encoding proteins (13 in human mtDNA). Even though there are numerous symptoms classified as associated with impaired mitochondria, the diagnosis is difficult not only due to multiplicity of clinical manifestation depending on involved functions, but it depends also on affected tissues and additionally is complicated by heteroplasmy of mtDNA - it means that only a fraction of mtDNA copies in one cell may be mutated. Another difficulty results from the impact of nuclear genetic background.

To uncover cellular and molecular mechanisms underlying mitochondrial diseases, the use of model organisms became vital and without any doubt the yeast *S. cerevisiae* is the organism of choice. As a model of fundamental cellular processes and metabolic pathways of human, yeast have improved the understanding and facilitated the molecular analysis of many diseases and this organism is particularly suited to study mitochondrial impairment. Considering the investigation of mitochondrial diseases, the most important is the capability to use fermentable carbon substrates as energy source, resulting in ability to survive even when mtDNA has been completely depleted. It is the unique organism in which site-direct mutagenesis of mitochondrial genome is possible. The population of yeast mtDNA is 100% homoplasmic what permit study the effects of particular mutation.

Thanks to my knowledge of the yeast genetic and genetic engineering, my laboratory is one of two/three in the world, able to introduce mutations into the mtDNA of this organism. So far we have concentrated on mtDNA nine point mutations in gene encoding subunit Atp6 of mitochondrial ATP synthase. This is a multi-subunit enzyme located in the inner mitochondrial membrane, which uses the energy provided by the proton electrochemical gradient as a force to drive ATP synthesis. We have proved the pathogenic character of those mutations and shown the mechanism leading to diseases. Our results proved the rationale to exploit potential of yeast to investigate human mitochondrial diseases. Now we want to expand our research to subunit Atp8. In human, 55 point mutations in MT-ATP6 and MT-ATP8 genes were identified mainly in patients suffering from the neurological defects. With the development of sequencing techniques and their availability for diagnosis, mtDNA mutations become now routinely screened and the number of case-reports increased during last year's and will grow. The main research question remains: are they pathogenic or polymorphic?

In this project we will undertake the systematic investigation of molecular, physiological and cellular effects of fourteen myopathy associated mutations in human MT-ATP6 and MT-ATP8 genes in *S. cerevisiae* in the goal to define i) their pathogenicity and mechanism of diseases caused by these mutations and ii) find out the not known role of Atp8 subunit in ATP synthase function. Besides, we will use the model strains to study effects of new drugs, isolated from Prestwick library by our team, on ATP synthase functioning in the goal to know their mechanism of action.

To study the impact of mutations on ATP synthase functioning we are using a set of methods including the growth test on non fermentable carbon source, determination of stability of mtDNA, reactive oxygen species level in cells, morphology of mitochondria, oxygen consumption by yeast cells and isolated mitochondria *in vitro*, mtATP synthesis and hydrolysis, ATP-driven translocation of protons across mitochondrial inner membrane, assembly and stability of ATP synthase. Last year the structure of membranous domain of ATP synthase was finally obtained. We will also introduce the chosen mutations into structural model of Atp6 and Atp8 proteins in the goal to analyze their positions and possible impact on the protein structure. This part of the work will be done by Dr Alain Dautant, specialist in ATP synthase structure, from constantly collaborating with us team of prof. di Rago from Bordeaux.