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# Structural and Functional Interactions of Mitochondrial ADP-Phosphorylating Apparatus

# (Strukturní a funkční interakce mitochondriálního systému fosforylace ADP)

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# ABSTRACT

The complexes of the oxidative phosphorylation (OXPHOS) system in the inner mitochondrial membrane are organised into structural and functional super-assemblies, so-called supercomplexes. This type of organisation enables substrate channelling and hence improves the overall OXPHOS efficiency. ATP synthase associates into dimers and higher oligomers. Within the supercomplex of ATP synthasome, it interacts with ADP/ATP translocase (ANT), which exchanges synthesised ATP for cytosolic ADP, and inorganic phosphate carrier (PiC), which imports phosphate into the mitochondrial matrix. The existence of this supercomplex is generally accepted. Experimental evidence is however still lacking.

In this thesis, structural interactions between ATP synthase, ANT and PiC were studied in detail. In addition, the interdependence of their expression was examined either under physiological conditions in rat tissues or using model cell lines with ATP synthase deficiencies of different origin. Specifically, they included mutations in the nuclear genes *ATP5E* and *TMEM70* that code for subunit *ε* and the ancillary factor of ATP synthase biogenesis TMEM70, respectively, and a microdeletion at the interface of genes *MT-ATP6* and *MT-COX3* that impairs the mitochondrial translation of both subunit *a* of ATP synthase and subunit Cox3 of cytochrome *c* oxidase.

Functional and structural characterisation of the cell lines with ATP synthase defects revealed that nuclear mutations in the genes *TMEM70* and *ATP5E* (the first reported mutation in a nuclear gene coding for a structural subunit of ATP synthase) lead to a reduced content of fully functional ATP synthase. In contrast, a mutation in *MT-ATP6* is accompanied by a normal amount of incomplete ATP synthase that is non-functional due to the lack of subunit *a*. In this case, the pathological phenotype manifests itself above 90 % heteroplasmy of mutated mtDNA. At all the studied defects, a compensatory up-regulation of ANT and PiC was found, likely due to an adaptive mechanism at the post-transcriptional level. Under physiological conditions, however, the expression of ATP synthase, ANT and PiC appears to be co-regulated at the level of transcription.

Although structural analyses revealed the existence of ATP synthasome in rat heart mitochondria, the majority of ATP synthase, ANT and PiC were found as separate entities. The functional significance of ATP synthasome therefore still remains controversial. The analyses also detected an association of ATP synthase with succinate dehydrogenase that had been previously reported as the so-called mitochondrial ATP-sensitive $K^+$ channel.

**KEYWORDS:** Mitochondria; oxidative phosphorylation; ATP synthase; ADP/ATP translocase; phosphate carrier; mitochondrial supercomplexes; mitochondrial disorders.

# ABSTRAKT

Komplexy oxidativní fosforylace (OXPHOS) se ve vnitřní mitochondriální membráně sdružují ve vyšší strukturní a funkční celky, tzv. superkomplexy. Jejich význam spočívá v cíleném směrování substrátu z jednoho komplexu na druhý. V případě ATP syntázy byly popsány její dimery i vyšší oligomery a také ATP syntazom, v rámci nějž dochází k seskupení ATP syntázy s přenašečem adeninových nukleotidů (ANT), zajišťujícím výměnu syntetizovaného ATP za cytosolické ADP, a fosfátovým přenašečem (PiC), umožňujícím import fosfátu do matrix mitochondrie. I když je existence tohoto superkomplexu obecně přijímána, experimentální důkazy nejsou dostatečné.

V rámci této práce byly detailně zkoumány strukturní interakce ATP syntázy, ANT, a PiC. Jejich vzájemné vztahy byly sledovány nejprve na úrovni exprese jednotlivých komponent ATP syntazomu, ať již za fyziologických podmínek v různých tkáních potkana, nebo na modelu deficiencí ATP syntázy v buňkách pacientů s různými genetickými defekty ATP syntázy. Konkrétně se jednalo o mutace v jaderných genech *ATP5E* a *TMEM70*, které kódují podjednotku ε, respektive pomocný faktor v biogenezi ATP syntázy TMEM70, a o mikrodeleci na rozhraní genů *MT-ATP6* a *MT-COX3*, která negativně ovlivňuje mitochondriální translaci podjednotek *a* ATP syntázy a Cox3 cytochrom *c* oxidázy.

Funkční a strukturní charakterizace buněčných linií s defekty ATP syntázy ukázala, že jaderné mutace v genech *TMEM70* a *ATP5E* (první jaderný gen kódující podjednotku ATP syntázy, v němž byla objevena mutace) mají za následek snížené množství jinak plně funkční ATP syntázy, kdežto v případě mitochondriální mutace v *MT-ATP6* je přítomno normální množství neúplné ATP syntázy, u níž chybějící podjednotka *a* vede k její nefunkčnosti. Patologický fenotyp této mutace se projeví až při překročení 90% heteroplazmie mutované mtDNA. U všech zkoumaných defektů bylo pozorováno kompenzační zvýšení přenašečů ANT a PiC, které je pravděpodobně způsobeno zatím neznámým adaptivním posttranskripčním mechanismem. Za fyziologických podmínek se ovšem zdá, že ATP syntáza, ANT a PiC jsou společně regulovány na úrovni transkripce.

Strukturní analýzy ukázaly přítomnost ATP syntazomu v mitochondriích izolovaných ze srdce potkana. Většina ATP syntázy, ANT a PiC ale vzájemně neasociuje, což ještě více podtrhuje otázku funkčního významu ATP syntazomu. Analýzy odhalily také interakci ATP syntázy se sukcinát dehydrogenázou. Seskupení těchto dvou komplexů už dříve byla přisouzena funkce tzv. mitochondriálního ATP-senzitivního $K^+$ kanálu.

**KLÍČOVÁ SLOVA:** Mitochondrie; oxidativní fosforylace; ATP syntáza; přenašeč adeninových nukleotidů; fosfátový přenašeč; mitochondriální superkomplexy; mitochondriální onemocnění.

# INTRODUCTION

## 1. Mitochondrial ADP-phosphorylating apparatus

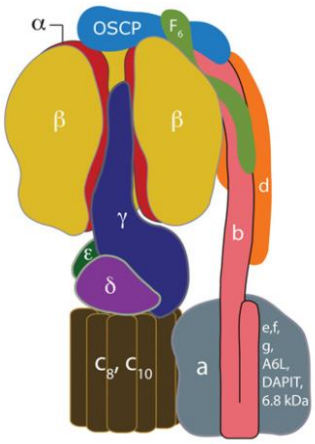
The electrochemical proton gradient generated by the respiratory chain (RC) enzyme complexes is utilised by $F_1F_o$-ATP synthase in the key process of energy metabolism in the cell, *i.e.* mitochondrial ATP synthesis (*1, 2*). ATP synthase catalyses the actual phosphorylation of ADP. To fulfil this function, it requires supply of the substrates, ADP and inorganic phosphate, which is mediated by two specialised carriers in the inner mitochondrial membrane (IMM). ADP/ATP translocase (also referred to as adenine nucleotide translocator, ANT) transports ADP into the mitochondrial matrix in exchange for newly synthesised ATP (*3*). Phosphate carrier (PiC) ensures supply of phosphate from the cytosol, utilising symport with protons or antiport with hydroxyl ions (*4*). Together, they form the mitochondrial ADP-phosphorylating apparatus.

### 1.1. $F_1F_o$-ATP synthase

The mammalian mitochondrial $F_1F_o$-ATP synthase is a large multisubunit enzyme complex with a molecular weight of ~650 kDa (**Fig. 1**). Two large subcomplexes can be distinguished – the globular catalytic $F_1$ domain and the proton-pumping membrane-embedded $F_o$ domain that are connected via two stalks (*2*). The mammalian ATP synthase consists of about 30 of 18 different types (17 structural subunits – *α*, *β*, *γ*, *δ*, *ε*, *a*, *b*, *c*, *d*, *e*, *f*, *g*, A6L, DAPIT, F6, MLQ, OSCP, and one regulatory subunit – $IF_1$) (*5*). Only two of them (A6L and *a*) are encoded in the mtDNA

The biogenesis of ATP synthase complex requires the assistance of several ancillary factors. ATP11 and ATP12 are conserved among eukaryotes (*6-8*). ATP10 and ATP23 are specific for yeast (*9*) whereas TMEM70 is found only in higher eukaryotes (*10, 11*). Furthermore, the content of subunit *c* seems to be indicative of the total content of ATP synthase complex in mammals. Brown adipose tissue (BAT), a specialised thermogenic organ that utilises a high oxidative capacity to produce heat instead of ATP, is characterised by a low content of ATP synthase (*12*), which correlates with a down-regulated expression of subunit *c* (*13*).

***Fig. 1 Structure of mammalian $F_1F_o$-ATP synthase.***




*The upper part of the model contains the subunits (*α, β, γ, δ, ε*) of the $F_1$ catalytic domain that protrudes into the mitochondrial matrix. Subunit* γ *is in contact with the $F_o$ membrane domain that contains the* c*-ring and the associated subunit* a*. The number of* c *subunits in the* c*-ring differs between species. The $F_o$ domain contains a number of supernumerary subunits with single transmembrane α-helices (*e, f, g, *A6L, DAPIT, 6.8PL). The peripheral stalk is on the right (subunits OSCP,* b, d, *and $F_6$). One of the three subunits* α *has been removed to expose the elongated α-helical structure of subunit* γ*. Adapted from Walker et al. (2013) (2).*

ATP synthase is able to perform both ATP synthesis and ATP hydrolysis. As a molecular motor, it is composed of both a stator – the peripheral stalk, and a rotor – the central stalk associated with the *c*-ring. Proton translocation in the direction of proton gradient generates rotation of the central stalk, which, in turn, generates conformational changes of the heterohexamer $\alpha_3\beta_3$ that catalyses ATP synthesis (*14*). On the contrary, ATP hydrolysis triggers rotation of the central stalk that drives proton pumping from the matrix to the intermembrane space against the proton gradient.

Inherited isolated defects of ATP synthase belong to the most severe early-onset mitochondrial diseases. They can be classified into two groups with different pathogenic mechanisms, biochemical phenotypes and clinical presentations. On the one hand, qualitative defects are characterised by normal levels of incomplete and non-functional ATP synthase and have been described for mtDNA mutations. The pathogenesis of mtDNA mutations does not follow the rules of Mendelian genetics. Instead, they are maternally inherited and the resulting phenotype depends on the mutational load (the level of heteroplasmy) since mtDNA is usually present in multiple copies (*15*). Quantitative defects, on the other hand, underlie mitochondrial disorders of nuclear genetic origin and are characterised by a reduction in the content of fully assembled and functional ATP synthase (*16*). In both cases, energy deprivation and reactive oxygen species (ROS) production represent important factors in the pathogenesis (*17*).

Point mutations in the mtDNA gene *MT-ATP6* encoding subunit *a* are a predominant cause of maternally inherited ATP synthase defects. The clinical phenotype manifests as NARP (neuropathy, ataxia, retinitis pigmentosa) or the more severe MILS (maternally inherited Leigh's syndrome), depending on the level of heteroplasmy (*18*). In addition, two cases with a unique microdeletion of two base pairs (*m.9205delTA*) at the interface of *MT-ATP6* and *MT-COX3* genes in the polycistronic mitochondrial transcript *MT-ATP8*/*MT-ATP6*/*MT-COX3* have been reported (*19, 20*).

Mutations in four nuclear genes have been associated with an isolated deficiency of ATP synthase so far. Two of them, *ATP5A1* and *ATP5E*, code for the structural subunits α and ε, respectively (*21, 22*). The other two, *ATPAF2* and *TMEM70*, encode ancillary factors that play specific roles in the biogenesis of ATP synthase. Mutations in *TMEM70* represent one of the most frequent causes of ATP synthase deficiencies (*23*). Lactic acidosis and methyl glutaconic aciduria are major clues in the diagnosis (*24*).

## 1.2. ADP/ATP translocase (ANT)

ANT is one of the most abundant mitochondrial proteins and represents up to 12 % of the total mitochondrial protein mass (*25-28*). It belongs to the mitochondrial carrier family (MCF) encoded by the *SLC25* genes (*29-31*). The size (30–34 kDa) and structure of all SLC25 transporters are very similar. Whether they function as monomers or dimers is still a matter of debate (*32, 33*). They contain three tandem-repeated homologous domains, and each of these consists of two transmembrane helices (*29, 34*). The members of SLC25 family are extremely hydrophobic

proteins with several tissue-specific isoforms. In mammals, each isoform is usually encoded by a unique nuclear gene (*35*).

Specifically in rodents, three genes coding for tissue-specific ANT isoforms have been described whereas four genes have been identified in humans (*36*) – *SLC25A4* (ANT1, a heart-type isoform), *SLC25A5* (ANT2, a liver-type isoform, expressed ubiquitously), *SLC25A6* (ANT3, expressed in highly proliferative cells, present only as a pseudogene in rodents), and *SLC25A31* (ANT4, a testes-specific isoform) (*37, 38*). Each ANT isoform has a tissue-specific expression pattern that may be related to specific energy requirements (*39*). Moreover, expression of the same isoform may be regulated by different transcriptional mechanisms in different tissues (*40*).

### 1.3. Phosphate carrier (PiC)

PiC, as well as ANT, belongs to the *SLC25* gene family (*41*), but unlike ANT, its two tissue-specific isoforms, PiC-A (a heart-type isoform) and PiC-B (a liver-type isoform that is expressed ubiquitously), originates from alternative splicing of a single gene transcript (*SLC25A3*) (*29, 42*). Their catalytic Michaelis constants $K_m$ are different and most likely reflect energy demands of the tissues that they are expressed in (*42, 43*).

## 2. Mitochondrial supercomplexes

The RC complexes associate into larger structures that are called RC supercomplexes (respirasomes) (*44*). The existence of supercomplexes significantly reduces the distances between the consecutive RC complexes that their substrates, *i.e.* coenzyme *Q* and cytochrome *c*, must surpass using only diffusion. Consequently, the efficiency of the RC is increased due to substrate channelling (*45*) and the formation of reactive oxygen species is attenuated (*46, 47*). Similarly, the key OXPHOS component, ATP synthase, is also able to organise itself into more complex structures, such as dimers and higher oligomers (*48-50*). Furthermore, substrate channelling was suggested to favour the assembly of ATP synthase, ANT and PiC into a supercomplex called ATP synthasome. Supercomplexes of ATP synthase with other mitochondrial proteins have been reported to perform an array of mitochondrial functions, including mitochondrial permeability transition (*51, 52*) or mitochondrial ATP-sensitive $K^+$ import (*53*).

### 2.1. Supramolecular structures within the respiratory chain

To describe the organisation of RC enzymes in the IMM, two models have been suggested – the so-called solid state model (*54*) and fluid state model (*55*). The fluid state model is defined by free movement of individual OXPHOS components in the IMM. It regards electron transport as a diffusion-based random collision process. On the contrary, the solid state model suggests a stable, at least for a limited period of time, organisation of RC complexes into higher molecular structures that ensure substrate channelling from one complex to another. In reality, mutual co-existence of isolated RC complexes and supercomplexes is expected with dynamic conversions between each other in order to optimize OXPHOS performance (*56*). Therefore, the plasticity model has been developed (*57*).

Supercomplexes are composed of the RC complexes I, III, and IV. The RC complex II (succinate dehydrogenase) that represents an alternative input of electrons into the RC is believed not to be involved in the formation of RC supercomplexes (*58*), possibly because of its concomitant involvement in the tricarboxylic acid cycle (*59*).

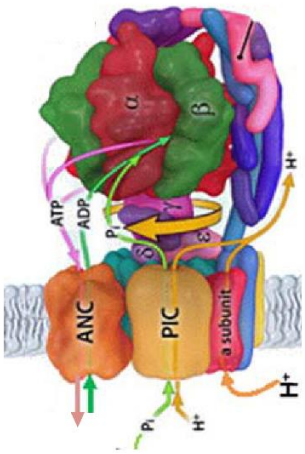
Characterisation of supercomplexes relies on their solubilisation as an intact structure from the IMM. The efficiency of solubilisation depends on the type of detergent used. To study supercomplexes, digitonin and Triton X-100 are usually applied because they are mild enough not to disrupt the non-covalent protein-protein interactions inside supercomplexes (*60, 61*). The composition of solubilised supercomplexes can then be revealed by using a type of native electrophoresis (*60, 61*), immunoprecipitation (*62*), or sucrose density ultracentrifugation (*63*).

## 2.2. Supramolecular organisation of ATP synthase

### 2.2.1. ATP synthasome

While the association of ATP synthase with a 29 kDa protein (likely ANT) in bovine heart was already reported at the end of the 1970's (*64, 65*), ATP synthasome, a putative supercomplex of ATP synthase, ANT, and PiC (**Fig. 2**), was first described by Pedersen and his co-workers in 2003. They detected co-localisation of its components in highly purified vesicles of mitochondrial cristae membrane isolated from rat liver (*66, 67*). Since then, ATP synthasome has been reported in digitonin-solubilised bovine heart mitochondria on clear native (*68*) or blue native gels (*49*) as well as in the protozoan *Leishmania (69)*. This would suggest that the ATP synthasome is an evolutionarily conserved structure that would form a single catalytic unit responsible for mitochondrial ATP production. However, a targeted search for ATP synthasome in a close relative of *Leishmania*, *Trypanosoma brucei*, was unsuccessful (*70*). The results of complexome profiling, a recently developed approach to detect novel mitochondrial supercomplexes, are also heterogeneous (*71, 72*). The existence of this supercomplex therefore remains controversial.

***Fig. 2 ATP synthasome.***
*ATP synthasome, a supercomplex composed of ATP synthase, ANT (also known as ANC, adenine nucleotide carrier), and PiC (inorganic phosphate carrier). Adapted from Timohhina et al. (2009) (73).*



### 2.2.2. Mitochondrial $K_{ATP}$ channel

For many years, a mitochondrial ATP-sensitive $K^+$ (mito$K_{ATP}$) channel, especially its opening, has been supposed to play an important role in cardioprotection and ischemic pre-conditioning, without its precise molecular identity being known (*74, 75*). Among others, a large multiprotein complex whose components included ATP synthasome, succinate dehydrogenase (RC complex II) and one of ABC transporters (mitochondrial ATP-binding cassette protein 1, mABC1) has been proposed to confer the mito$K_{ATP}$ channel activity (*53*). However, it was never validated. The pursuit of structural information on the mito$K_{ATP}$ channel, nevertheless, continues even though its existence is still controversial (*74-76*).

### 2.2.3. Mitochondrial permeability transition pore

Mitochondrial permeability transition is defined as a non-selective increase in the permeability of the IMM to solutes with molecular masses up to 1.5 kDa that is usually induced by intramitochondrial $Ca^{2+}$ accumulation (*51*). However, the molecular nature of mitochondrial permeability transition pore (mPTP) has been a mystery. As the channel-forming component of mPTP, the ATP synthase complex was identified most recently (*77*). However, the molecular identity of mPTP has not been completely elucidated.

# AIMS OF THE THESIS

The first objective of my thesis was to characterise structural-functional relationships accompanying ATP synthase defects of a different genetic origin. The investigated cell lines were used to achieve my second objective, *i.e.* to study the association of ATP synthase, ANT and PiC into the ATP synthasome.

The specific aims of my thesis were:

- To examine in detail structural interactions in the putative supercomplex of ATP synthasome in mammalian mitochondria
- To analyse the expression of ATP synthasome components in rat tissues and to test whether their expression is affected by different genetic defects of ATP synthase in patient cells
- To characterise molecular pathogenic mechanisms underlying the genetic defects of ATP synthase due to mutations in the following genes:
    - → *ATP5E* (coding for subunit ε)
    - → *MT-ATP6* (coding for subunit *a*)
    - → *TMEM70* (coding for the ancillary factor of ATP synthase biogenesis TMEM70)

# SUMMARY OF THE RESULTS

My first-author publication focuses on the components of mitochondrial ADP-phosphorylating apparatus, their expression and structural interactions (**Article 1**). We showed limited association of ATP synthase not only with ANT and PiC (into a supercomplex called ATP synthasome) but also with succinate dehydrogenase (**Article 2**). To study expression of ATP synthasome components and their interdependence, we used several cell lines with different genetic defects of ATP synthase resulting in a low content of the enzyme or an alteration of its structure. In the other four articles, these model cell lines are characterised with regard to impacts of the defect they harbour on mitochondrial functions. Specifically, we described structural and functional consequences of genetic defect of subunit ε in cells of a patient (**Article 3**) as well as in model cells with down-regulated expression of this subunit (**Article 4**). Compared to defects in nuclear-encoded subunits of ATP synthase, mutations in the mitochondrial gene *MT-ATP6* coding for subunit *a* are reported more regularly. We also characterised one mutation, specifically *m.9205delTA* that does not only affect ATP synthase but also cytochrome *c* oxidase (**Article 5**). In the last article, we investigated adaptive changes in the expression of mitochondrial proteins in patients with a mutation in *TMEM70* coding for an ancillary factor of ATP synthase biogenesis (**Article 6**).

1. **Mitochondrial ATP synthasome: expression and structural interaction of its components**
   **Nůsková, H.**, Mráček, T., Mikulová, T., Vrbacký, M., Kovářová, N., Kovalčíková, J., Pecina, P., Houštěk, J. (2015). Biochem Biophys Res Commun. doi: 10.1016/j.bbrc.2015.07.034
   IF 2.297 (2014)

To characterise the relationships in the expression of ATP synthase, ANT, and PiC in mammalian cells, we used two models of isolated ATP synthase deficiency – rat brown adipose tissue with a physiological down-regulation of ATP synthase, and fibroblast cultures of patients with different types of ATP synthase deficiency. In rat tissues, both transcript and protein levels of ANT and PiC correlate with the content of ATP synthase. Therefore, ANT and PiC levels appear to be transcriptionally controlled in accordance with the biogenesis of ATP synthase. In contrast, the content of ANT and PiC is increased in the ATP synthase deficient patient fibroblasts compared to control cells. Since there is no significant change in the transcript levels of ANT and PiC, the observed adaptive responses are likely regulated post-transcriptionally, possibly at the level of protein synthesis or stability.

To describe further the structural interactions of ANT, PiC, and ATP synthase, we analysed rat heart mitochondria solubilised with different mild non-ionic detergents. We characterised the association of ATP synthasome components by immunoprecipitation, blue native and SDS polyacrylamide gel electrophoresis combined with immunodetection and MS analysis. Our results indicate that both carriers can be found attached to monomeric and dimeric forms of ATP synthase. However, the majority of immunodetected PiC and especially ANT did not associate

with the ATP synthase, suggesting that while ATP synthasome is present in heart mitochondria, most of the PiC, ANT, and also ATP synthase probably exist as separate entities.

2. **High molecular weight forms of mammalian respiratory chain complex II.**
   Kovářová, N., Mráček, T., **Nůsková, H.**, Holzerová, E., Vrbacký, M., Pecina, P., Hejzlarová, K., Kl'učková, K., Rohlena, J., Neužil, J., Houštěk, J. (2013). PLoS One *8*, e71869.
   IF 3.534

While studying the association of ATP synthase, ANT and PiC, we uncovered an unexpected connection between ATP synthase and succinate dehydrogenase (RC complex II).

In this publication, we reported the existence of structures of high molecular weight that contained complex II ($CII_{hmw}$) and were preserved only under specific conditions, i.e. under the combination of solubilisation by digitonin and resolution by clear native electrophoresis (CNE). We showed that $CII_{hmw}$ structures are enzymatically active and differ in the electrophoretic mobility between rat tissues (500–1,000 kDa) and cultured human cells (400–670 kDa). Furthermore, they are destabilised in mtDNA-depleted $rho^0$ cells whereas their formation is unaffected by isolated defects in the other OXPHOS complexes. Electrophoretic studies and immunoprecipitation experiments of $CII_{hmw}$ did not reveal any specific interactions with the RC complexes I, III or IV or enzymes of the tricarboxylic acid cycle. However, they suggest a specific interaction between complex II and ATP synthase. Their association has been previously reported in a supercomplex that was suggested to confer the activity of mitochondrial ATP-sensitive $K^+$ channel (*53*).

3. **Mitochondrial ATP synthase deficiency due to a mutation in the *ATP5E* gene for the $F_1$ epsilon subunit.**

   Mayr, J.A., Havlíčková, V., Zimmermann, F., Magler, I., Kaplanová, V., Ješina, P., Pecinová, A., **Nůsková, H.**, Koch, J., Sperl, W., Houštěk, J. (2010). Hum Mol Genet *19*, 3430-3439.
   IF 8.058

This publication was a result of our collaboration with the Paediatric Department of Prof. Wolfgang Sperl at the Paracelsus Medical University in Salzburg, Austria. Similarly to the next publication, it demonstrates the importance of subunit ε for the biogenesis of ATP synthase.

In 2008, a common mutation in the gene *TMEM70* coding for an ancillary factor of ATP synthase biogenesis was identified in a group of patients with an isolated ATP synthase defect (*10*). However, there was a single patient with a distinct clinical phenotype and no detected mutation in *TMEM70*. In this publication, we reported that the underlying genetic cause of ATP synthase defect in this patient was a homozygous mutation *c.35A>G* in the nuclear gene *ATP5E* coding for subunit ε. The identified mutation was the first mutation reported in a nuclear encoded structural

subunit of ATP synthase. It leads to an amino acid substitution (p.Tyr12Cys) that affects a tyrosine residue at the N-terminus highly conserved among eukaryotes.

Biochemical analysis of the patient fibroblasts showed a reduction of both oligomycin-sensitive ATP-hydrolytic and ATP-synthesising activity to ~30 % when compared to controls. The mitochondrial content of fully assembled ATP synthase was equally reduced. However, its molecular weight is unchanged due to incorporation of the mutated subunit *ε* and its catalytic activity seems to be preserved. The insufficient capacity of ATP synthase then leads to a decrease in respiration and to an increase in mitochondrial membrane potential under ADP stimulation, compared to control cells. Similarly to the down-regulation of subunit *ε* in the HEK293 cells, a detailed protein analysis revealed a decrease in the content of both $F_1$ and $F_o$ subunits of ATP synthase with the only exception of subunit *c* that was accumulated in a detergent-insoluble form. Furthermore, the content of RC complexes I, II, III, and IV were unchanged or slightly increased compared to controls.

4. **Knockdown of $F_1$ epsilon subunit decreases mitochondrial content of ATP synthase and leads to accumulation of subunit c.**
   Havlíčková, V., Kaplanová, V., **Nůsková, H.**, Drahota, Z., and Houštěk, J. (2010). Biochim Biophys Acta *1797*, 1124-1129.
   IF 5.132

Subunit *ε* is the smallest and functionally least characterised subunit of the $F_1$ domain of mammalian ATP synthase. It lacks the N-terminal targeting sequence and there are no known homologues in bacteria and chloroplasts (*14*).

To characterise the function of mammalian subunit ε, we knocked down the expression of the respective nuclear gene *ATP5E* in the HEK293 cell line, using the technique of RNA interference. As a consequence, the content and activity of ATP synthase dropped to ~40 % of controls, which was accompanied by a decrease in the ADP-stimulated respiration and by an increase in the mitochondrial membrane potential.

A more detailed investigation of changes at the protein level revealed that the decrease in subunit ε was followed by a decrease in other ATP synthase subunits, except subunit c. The accumulated subunit c was incorporated into the fully assembled ATP synthase and also into other subcomplexes with the molecular weight of 200–400 kDa that contained neither $F_1$ subunits (*α*, *β*) nor $F_o$ subunits (*a*, *b*, *d*). Subunit *ε* seems to play an important role in the biosynthesis and assembly of the $F_1$ domain of ATP synthase and to be involved in the incorporation of hydrophobic subunit *c* to the rotor structure ($F_1$-*c*-ring) of the mammalian enzyme.

5. **Alteration of structure and function of ATP synthase and cytochrome *c* oxidase by lack of $F_o$-a and Cox3 subunits caused by mitochondrial DNA 9205delTA mutation.**
   Hejzlarová, K., Kaplanová, V., **Nůsková, H.**, Kovářová, N., Ješina, P., Drahota, Z., Mráček, T., Seneca, S., Houštěk, J. (2015). Biochem J *466*, 601-611.
   IF 4.396 (2014)

Severe mitochondrial disorders are frequently caused by mutations in the *MT-ATP6* gene coding for subunit *a* of ATP synthase. The majority of them are missense mutations (*23*). In this publication, we studied a unique microdeletion (*m.9205delTA*) in the polycistronic mitochondrial transcript *MT-ATP8*/*MT-ATP6*/*MT-COX3*. This microdeletion removes the stop codon in the *MT-ATP6* gene, which interferes with the processing of this mRNA and negatively affects the cleavage site between *MT-ATP6* and *MT-COX3* and as a result also the translation of both subunit *a* of ATP synthase and subunit Cox3 of cytochrome *c* oxidase (COX) (*20*).

So far, this rare mutation has been found in two unrelated patients whose clinical phenotypes differed strikingly. While the first patient was characterised by mild transient lactic acidosis (*19*), the other one suffered from fatal encephalopathy (*20*). Nevertheless, both patients were reported as homoplasmic. Therefore, we set to compare the cells of both patients to search for another factor that could modulate the outcome of the *m.9205delTA* microdeletion. In the fibroblasts obtained from the first patient with a milder phenotype, a heteroplasmy of this mutation was revealed after a prolonged time of cultivation, most likely due to negative segregation of the mutation. To gain more insight into the effect of *m.9205delTA* heteroplasmy, we prepared transmitochondrial cybrids with a varying mutation load (52–99 %). All parameters that were determined, i.e. the content of subunits *a* and Cox3, ADP-stimulated respiration, mitochondrial ATP production, and COX activity, were found to be strongly dependent on the mutation load with a heteroplasmy threshold at ~90 % mutation. Therefore, the distinct phenotypes of the two reported patients most likely resulted from a different mutation load with a critical threshold for the severity of disease manifestation at a very high heteroplasmy level.

While comparing the control and *m.9205delTA* homoplasmic cybrid lines, we found that a lack of subunit *a* alters the structure but not the content of ATP synthase, which assembles into a labile ~60 kDa smaller complex retaining the ATP-hydrolytic but not ATP-synthesising activity. On the contrary, a lack of Cox3 limits the biosynthesis of COX but does not alter the structure of the enzyme. The reduced content of COX leads to a decrease in the respiratory rates and the total $H^+$-pumping activity of the RC as evidenced by a reduced mitochondrial membrane potential.

6. **Compensatory upregulation of respiratory chain complexes III and IV in isolated deficiency of ATP synthase due to TMEM70 mutation.**
   Havlíčková Karbanová, V., Čížková Vrbacká, A., Hejzlarová, K., **Nůsková, H.**, Stránecký, V., Potocká, A., Kmoch, S., Houštěk, J. (2012). Biochim Biophys Acta *1817*, 1037-1043.
   IF 4.624

Mutations in *TMEM70* that codes for an ancillary factor of ATP synthase biogenesis are the most frequent genetic cause of isolated ATP synthase deficiencies. Medical symptoms usually fall into the category of early-onset mitochondrial encephalo-cardiomyopathies. As a consequence of decreased content of functional ATP synthase, patient cells are characterised by a lower ATP production and an elevated mitochondrial membrane potential (*10*) that often leads to an increase in ROS production. To investigate adaptive mechanisms of patient cells under such metabolic imbalance, we analysed the expression of OXPHOS complexes and intramitochondrial proteases that are involved in their turnover.

We investigated primary fibroblasts derived from skin biopsies of 10 patients with the common homozygous mutation *c.317-2A>G* in *TMEM70.* In patient fibroblasts, the content of fully assembled ATP synthase was reduced to 11 % of controls on average. On the other hand, we found an increase in the content of complex III and IV to 153 % and 184 % of controls, respectively. The absolute content of individual OXPHOS subunits that was analysed under denaturing conditions followed this pattern. The reduced content of fully assembled ATP synthase that was accompanied by a decrease in the content of individual ATP synthase subunits indicated that the synthesised but not assembled ATP synthase subunits are subject to degradation. Therefore, we analysed the protein levels of mitochondrial proteases Lon, paraplegin, and prohibitin 1 and 2, in which we did not find any significant change.

Whole-genome expression profiling revealed generalized up-regulation of transcriptional activity in patient fibroblasts but did not show any consistent changes in mRNA levels of structural subunits or specific assembly factors of OXPHOS complexes that would correspond to the protein data. Therefore, the reported compensatory increase in the RC complexes III and IV (as well as ANT and PiC as reported in Article 1) in response to the ATP synthase deficiency is most likely a result of an adaptive mechanism at the post-transcriptional level.

**MY CONTRIBUTION TO THESE PUBLICATIONS:**

The presented data resulted from a team effort of all co-authors detailed in the above mentioned publications. Here, my experimental involvement in the individual publications is summarised:

1. Characterisation of structural interactions of ATP synthase, ANT, and PiC using blue-native and multidimensional electrophoreses and immunoprecipitation; quantitative analysis of ATP synthasome components at the protein level using Western blots; preparation of samples for MS analysis; isolation of mitochondria from rat tissues and patient fibroblasts; cell cultivation

2. Detection of structural interactions between ATP synthase and succinate dehydrogenase by means of immunoprecipitation; isolation of mitochondria from rat tissues

3. Evaluation of mitochondrial functions, specifically cell respiration (oxygraph measurements)

4. + 5. Evaluation of mitochondrial functions, specifically mitochondrial membrane potential ($TPP^+$-selective electrode)

6. Quantitative analysis of the protein content of OXPHOS components using the combination of SDS-PAGE and immunodetection on Western blots; cell cultivation

# DISCUSSION

Cell lines obtained from ATP synthase-deficient patients represent a valuable model to study the biogenesis, function and regulation of ATP synthase as well as of the whole ADP-phosphorylating apparatus. Specifically, we studied nuclear mutations in *ATP5E* (**Article 3** and **4**) and *TMEM70* (**Article 6**) that affect subunit *ε* of ATP synthase and the ancillary factor of its biogenesis TMEM70, respectively. These genetic defects of ATP synthase result in a markedly reduced content of fully assembled and functional ATP synthase. On the other hand, a normal amount of incomplete and non-functional ATP synthase complex lacking subunit *α* is a result of the unique mitochondrial microdeletion *m.9205delTA* that impairs the translation of both subunit a of ATP synthase and subunit Cox3 of cytochrome *c* oxidase (**Article 5**). Unlike the nuclear mutations, the pathological outcome of this mutation depends on the level of heteroplasmy (mutational load).

When we described a pathogenic homozygous mutation in the *ATP5E* gene in 2010 (**Article 3**), it was the very first reported patient with a mutation in a nuclear encoded structural subunit of ATP synthase (*21*). Three years later, a mutation in *ATP5A1* coding for subunit *α* was reported in two siblings (*22*). Currently, no other mutations in the nuclear genes encoding ATP synthase subunits have been described. The two cases that were published indicate that pathogenic nuclear mutations are usually recessive and likely very rare. As a consequence, the frequency of ATP synthase defects of this kind is extremely low. Furthermore, the functional outcomes of mutations in the structural subunits of ATP synthase may be so severe that they are embryonically lethal for their homozygous carriers, which would also contribute to the fact that more cases are not recorded. Therefore, the prevalence of all pathogenic mutations in mitochondrial components may be significantly underestimated (*4*).

The yeast subunit *ε* is required for the functional coupling of proton translocation and ATP synthesis (*78*). We have shown in the patient with a mutation in *ATP5E* (**Article 3**) (*21*) and also in our model of *ATP5E* knock-down (**Article 4**) (*79*) that the mammalian and yeast ATP synthase differs in this regard. Whereas a reduction in the content of subunit ε results in uncoupling of proton translocation from ATP synthesis in yeast, it leads to a decrease in the total amount of ATP synthase that is otherwise fully functional and coupled in humans. As a consequence, the mitochondrial membrane potential is partially depleted in yeast but increased in humans due to a decreased capacity of ATP synthase to consume it. The pathogenic mutation p.Tyr12Cys in *ATP5E* that we had reported was later studied in detail in *Saccharomyces cerevisiae* (*80*). A yeast equivalent of the reported mutation did not affect the assembly or stability of ATP synthase complex, as opposed to the patient, which suggests that the biogenesis of ATP synthase differs substantially between humans and yeast.

Another example of this difference is the importance of TMEM70, an ancillary factor of ATP synthase biogenesis in mammals, whose homologue has not been found in yeast. We found a compensatory up-regulation of CoIII and CoIV in patient fibroblasts (**Article 6**) (*81*) and the same was later observed also by (*82*). A similar adaptive response was found in tissues (*83*) and fibroblasts of the *ATP5E* patient (**Article 3**) (*21*). However, these changes obviously cannot

compensate for the energetic dysfunction originating from the lack of functional ATP synthase. The same applies for the compensatory increase in the content of other components of mitochondrial phosphorylation apparatus, ANT and PiC, that we observed in *TMEM70*, *ATP5E*, and *MT-ATP6* patients (**Article 1**) (*84*). A compensatory up-regulation in the total mitochondrial content, another adaptive mechanism reported in mice lacking PiC (*85*) and also in hearts of patients lacking PiC-A (*86*), was not associated with any of the studied mutations. Since the transcript levels of up-regulated proteins were not changed, a post-transcriptional adaptive mechanism is likely involved. Under physiological conditions in rat tissues, in contrast, the protein content of ATP synthase, ANT, and PiC appears to be co-regulated at the level of transcription.

The supercomplex of ATP synthasome is generally accepted although the interaction of its components has not been studied as thoroughly as other mitochondrial higher molecular structures. Two key publications from Pedersen's laboratory that established ATP synthasome as a mitochondrial supercomplex are based on the fact that all its components co-localized in vesicles of enriched mitochondrial cristae membrane from rat liver (*66, 67*). The evidence for its existence is, therefore, lacking and the functional advantages that it would provide for ADP phosphorylation are also questionable. We were able to detect this supercomplex in rat heart mitochondria by BNE and immunoprecipitation experiments but its prevalence was strikingly low (**Article 1**) (*84*). Reasons for this observation may be found in the recent plasticity model of protein organisation in the IMM (*57*). The ratio between ATP synthasome and its separate components may be indeed very low. ATP synthasome could also represent an intermolecular association that is extremely short-lived and thus difficult to capture by biochemical methods. Nevertheless, its functional significance still remains elusive.

While we were studying the putative supercomplex of ATP synthasome, we found an association of ATP synthase with another mitochondrial protein – succinate dehydrogenase (**Article 2**) (*87*). The interaction of the entire ATP synthasome with succinate dehydrogenase had been reported and assigned the function of mitoK$_{ATP}$ channel (see chapter 3.2.4.). Despite all controversies, strong evidence links the functional properties of mitoK$_{ATP}$ channel with succinate dehydrogenase (*76*). Diazoxide, an inhibitor of succinate dehydrogenase, has been used by many as an opener of mitoK$_{ATP}$ channel, which is proposed to mediate its cardioprotective effects (*74*). Taking into account recently published findings (*88*), the cardioprotective effect might result not from the mitoK$_{ATP}$ channel opening but from a reduction of ROS production in the ischemic heart. After reperfusion, rapid oxidation of succinate accumulated under hypoxia saturates the RC with electrons, which leads to reverse electron flow to complex I. ROS production by this enzyme is then responsible for the ischemia/reperfusion injury. The complex II inhibitors might therefore protect the heart from oxidative damage without any involvement of mitoK$_{ATP}$ channel.

In conclusion, my thesis shows that studying the phenotype of patients and molecular and biochemical processes in their cells can not only shed light on the pathogenic mechanism of their disease but also contribute to elucidating the function of the affected gene and its protein product. In a broader picture, we can learn more about associations of the affected protein with other partners and the interdependence of their regulation.

# CONSLUSIONS

- Structural analyses indicate presence of ATP synthasome in rat heart mitochondria. However, the majority of PiC, ANT, and ATP synthase exist as separate entities. In addition, ATP synthase was found to interact with succinate dehydrogenase. This association had been suggested to confer the activity of mitochondrial ATP-sensitive $K^+$ channel.

- In rat tissues, levels of ATP synthase correlate with those of ANT and PiC. On the other hand, human ATP synthase deficiencies lead to a compensatory increase in the content of ANT and PiC, likely due to a post-transcriptional adaptive mechanism. Similarly, complex III and IV are also up-regulated in the patients harbouring a mutation in *TMEM70*.

- The *ATP5E* gene was identified as the first nuclear gene coding for a structural subunit of ATP synthase responsible for deficiency of this enzyme in human patients. The respective subunit $\varepsilon$ is required for assembly and/or stability of the $F_1$ catalytic domain of the mammalian ATP synthase and plays a role in the incorporation of the hydrophobic subunit *c* into the $F_1$-*c* oligomer during the process of ATP synthase assembly.

- The unique microdeletion *m.9205delTA* in the mitochondrial gene *MT-ATP6*, affecting the function of both ATP synthase and cytochrome *c* oxidase, only leads to a mitochondrial disease phenotype when heteroplasmy is over 90 %. The lack of subunit *a* does not affect the amount of ATP synthase. The incomplete enzyme is, however, unstable and unable to produce ATP.

# REFERENCES


1. P. D. Boyer, *Annual review of biochemistry* **66**, 717-749 (1997).
2. J. E. Walker, *Biochem Soc Trans* **41**, 1-16 (2013).
3. H. Itoh *et al.*, *Nature* **427**, 465-468 (2004).
4. E. L. Seifert, E. Ligeti, J. A. Mayr, N. Sondheimer, G. Hajnoczky, *Biochemical and biophysical research communications* **464**, 369-375 (2015).
5. J. Lee *et al.*, *J Biol Chem* **290**, 13308-13320 (2015).
6. S. H. Ackerman, A. Tzagoloff, *Proc Natl Acad Sci U S A* **87**, 4986-4990 (1990).
7. S. Bowman, S. H. Ackerman, D. E. Griffiths, A. Tzagoloff, *J Biol Chem* **266**, 7517-7523 (1991).
8. S. H. Ackerman, J. Martin, A. Tzagoloff, *J Biol Chem* **267**, 7386-7394 (1992).
9. M. Rak, X. Zeng, J. J. Briere, A. Tzagoloff, *Biochim Biophys Acta* **1793**, 108-116 (2009).
10. A. Cizkova *et al.*, *Nature genetics* **40**, 1288-1290 (2008).
11. J. Houstek, S. Kmoch, J. Zeman, *Biochim Biophys Acta* **1787**, 529-532 (2009).
12. B. Cannon, J. Nedergaard, *Physiol Rev* **84**, 277-359 (2004).
13. J. Houstek, U. Andersson, P. Tvrdik, J. Nedergaard, B. Cannon, *J Biol Chem* **270**, 7689-7694 (1995).
14. T. Xu, V. Pagadala, D. M. Mueller, *Microbial cell* **2**, 105-125 (2015).
15. S. DiMauro, *JAMA neurology* **70**, 1484-1485 (2013).
16. J. Houstek *et al.*, *Biochim Biophys Acta* **1757**, 1400-1405 (2006).
17. T. Mracek *et al.*, *Exp Gerontol* **41**, 683-687 (2006).
18. A. I. Jonckheere, J. A. Smeitink, R. J. Rodenburg, *Journal of inherited metabolic disease* **35**, 211-225 (2012).
19. S. Seneca *et al.*, *Journal of inherited metabolic disease* **19**, 115-118 (1996).
20. P. Jesina *et al.*, *The Biochemical journal* **383**, 561-571 (2004).
21. J. A. Mayr *et al.*, *Hum Mol Genet* **19**, 3430-3439 (2010).
22. A. I. Jonckheere *et al.*, *Brain : a journal of neurology* **136**, 1544-1554 (2013).
23. K. Hejzlarova *et al.*, *Physiological research / Academia Scientiarum Bohemoslovaca* **63 Suppl 1**, S57-71 (2014).
24. L. De Meirleir *et al.*, *Journal of medical genetics* **41**, 120-124 (2004).
25. P. Riccio, H. Aquila, M. Klingenberg, *FEBS Lett* **56**, 133-138 (1975).
26. D. H. Boxer, *FEBS Lett* **59**, 149-152 (1975).
27. M. Klingenberg, P. Riccio, H. Aquila, *Biochim Biophys Acta* **503**, 193-210 (1978).
28. H. Hackenberg, M. Klingenberg, *Biochemistry* **19**, 548-555 (1980).
29. F. Palmieri, *Pflugers Archiv : European journal of physiology* **447**, 689-709 (2004).
30. F. Palmieri, C. L. Pierri, A. De Grassi, A. Nunes-Nesi, A. R. Fernie, *The Plant journal : for cell and molecular biology* **66**, 161-181 (2011).
31. M. Gutierrez-Aguilar, C. P. Baines, *The Biochemical journal* **454**, 371-386 (2013).
32. M. Monne, F. Palmieri, *Curr Top Membr* **73**, 289-320 (2014).
33. V. Trezeguet, L. Pelosi, G. J. Lauquin, G. Brandolin, *Journal of bioenergetics and biomembranes* **40**, 435-443 (2008).
34. E. Pebay-Peyroula *et al.*, *Nature* **426**, 39-44 (2003).
35. Y. Rondelez *et al.*, *Nature* **433**, 773-777 (2005).
36. C. Dahout-Gonzalez *et al.*, *Physiology* **21**, 242-249 (2006).
37. J. V. Brower *et al.*, *J Biol Chem* **282**, 29658-29666 (2007).
38. V. Dolce, P. Scarcia, D. Iacopetta, F. Palmieri, *FEBS Lett* **579**, 633-637 (2005).
39. G. Stepien, A. Torroni, A. B. Chung, J. A. Hodge, D. C. Wallace, *J Biol Chem* **267**, 14592-14597 (1992).
40. K. Dummler, S. Muller, H. J. Seitz, *The Biochemical journal* **317 ( Pt 3)**, 913-918 (1996).
41. M. J. Runswick, S. J. Powell, P. Nyren, J. E. Walker, *The EMBO journal* **6**, 1367-1373 (1987).
42. V. Dolce, G. Fiermonte, F. Palmieri, *FEBS Lett* **399**, 95-98 (1996).
43. G. Fiermonte, V. Dolce, F. Palmieri, *J Biol Chem* **273**, 22782-22787 (1998).
44. N. V. Dudkina, I. M. Folea, E. J. Boekema, *Micron* **72**, 39-51 (2015).
45. C. Bianchi, M. L. Genova, G. Parenti Castelli, G. Lenaz, *J Biol Chem* **279**, 36562-36569 (2004).
46. R. Acin-Perez, P. Fernandez-Silva, M. L. Peleato, A. Perez-Martos, J. A. Enriquez, *Molecular cell* **32**, 529-539 (2008).
47. G. Lenaz, M. L. Genova, *The international journal of biochemistry & cell biology* **41**, 1750-1772 (2009).
48. I. Wittig, H. Schagger, *Biochim Biophys Acta* **1787**, 672-680 (2009).
49. H. Seelert, N. A. Dencher, *Biochim Biophys Acta* **1807**, 1185-1197 (2011).
50. J. Habersetzer *et al.*, *The international journal of biochemistry & cell biology* **45**, 99-105 (2013).
51. P. Bernardi, F. Di Lisa, F. Fogolari, G. Lippe, *Circulation research* **116**, 1850-1862 (2015).
52. P. Bernardi, F. Di Lisa, *Journal of molecular and cellular cardiology* **78**, 100-106 (2015).
53. H. Ardehali, Z. Chen, Y. Ko, R. Mejia-Alvarez, E. Marban, *Proc Natl Acad Sci U S A* **101**, 11880-11885 (2004).
54. B. Chance, G. R. Williams, *Nature* **176**, 250-254 (1955).

55. C. R. Hackenbrock, B. Chazotte, S. S. Gupte, *Journal of bioenergetics and biomembranes* **18**, 331-368 (1986).
56. E. J. Boekema, H. P. Braun, *J Biol Chem* **282**, 1-4 (2007).
57. J. A. Enriquez, G. Lenaz, *Molecular syndromology* **5**, 119-140 (2014).
58. H. Schagger, K. Pfeiffer, *J Biol Chem* **276**, 37861-37867 (2001).
59. Y. Chaban, E. J. Boekema, N. V. Dudkina, *Biochim Biophys Acta* **1837**, 418-426 (2014).
60. H. Schagger, K. Pfeiffer, *The EMBO journal* **19**, 1777-1783 (2000).
61. H. Eubel, J. Heinemeyer, S. Sunderhaus, H. P. Braun, *Plant physiology and biochemistry : PPB / Societe francaise de physiologie vegetale* **42**, 937-942 (2004).
62. C. M. Cruciat, S. Brunner, F. Baumann, W. Neupert, R. A. Stuart, *J Biol Chem* **275**, 18093-18098 (2000).
63. N. V. Dudkina, H. Eubel, W. Keegstra, E. J. Boekema, H. P. Braun, *Proc Natl Acad Sci U S A* **102**, 3225-3229 (2005).
64. C. Montecucco, R. Bisson, F. Dabbeni-Sala, A. Pitotti, H. Gutweniger, *J Biol Chem* **255**, 10040-10041 (1980).
65. Y. M. Galante, S. Y. Wong, Y. Hatefi, *J Biol Chem* **254**, 12372-12378 (1979).
66. C. Chen *et al.*, *J Biol Chem* **279**, 31761-31768 (2004).
67. Y. H. Ko, M. Delannoy, J. Hullihen, W. Chiu, P. L. Pedersen, *J Biol Chem* **278**, 12305-12309 (2003).
68. I. Wittig, H. Schagger, *Biochim Biophys Acta* **1777**, 592-598 (2008).
69. S. Detke, R. Elsabrouty, *Acta tropica* **105**, 16-20 (2008).
70. A. Gnipova *et al.*, *Eukaryotic cell* **14**, 297-310 (2015).
71. H. J. Wessels *et al.*, *PloS one* **8**, e68340 (2013).
72. H. Heide *et al.*, *Cell metabolism* **16**, 538-549 (2012).
73. N. Timohhina *et al.*, *Journal of bioenergetics and biomembranes* **41**, 259-275 (2009).
74. B. O'Rourke, *Circulation research* **94**, 420-432 (2004).
75. I. Szabo, M. Zoratti, *Physiol Rev* **94**, 519-608 (2014).
76. K. D. Garlid, A. P. Halestrap, *Journal of molecular and cellular cardiology* **52**, 578-583 (2012).
77. V. Giorgio *et al.*, *J Biol Chem* **284**, 33982-33988 (2009).
78. E. Tetaud, F. Godard, M. F. Giraud, S. H. Ackerman, J. P. di Rago, *Molecular biology of the cell* **25**, 791-799 (2014).
79. V. Havlickova, V. Kaplanova, H. Nuskova, Z. Drahota, J. Houstek, *Biochim Biophys Acta* **1797**, 1124-1129 (2010).
80. E. Sardin, S. Donadello, J. P. di Rago, E. Tetaud, *Frontiers in genetics* **6**, 159 (2015).
81. V. Havlickova Karbanova *et al.*, *Biochim Biophys Acta* **1817**, 1037-1043 (2012).
82. A. Torraco *et al.*, *Neurogenetics* **13**, 375-386 (2012).
83. J. A. Mayr *et al.*, *Pediatric research* **55**, 988-994 (2004).
84. H. Nuskova *et al.*, *Biochemical and biophysical research communications* **464**, 787-793 (2015).
85. J. Q. Kwong *et al.*, *Cell death and differentiation* **21**, 1209-1217 (2014).
86. J. A. Mayr *et al.*, *Neuromuscular disorders : NMD* **21**, 803-808 (2011).
87. N. Kovarova *et al.*, *PloS one* **8**, e71869 (2013).
88. E. T. Chouchani *et al.*, *Nature* **515**, 431-435 (2014).

# LIST OF MY PUBLICATIONS

## PUBLICATIONS INCLUDED IN MY PHD THESIS:

**Nuskova, H.**, Mracek, T., Mikulova, T., Vrbacky, M., Kovarova, N., Kovalcikova, J., Pecina, P., and Houstek, J. (2015) Mitochondrial ATP synthasome: Expression and structural interaction of its components. *Biochem Biophys Res Commun* **464**, 787-793 (IF 2.297, 2014)

Hejzlarova, K., Kaplanova, V., **Nuskova, H.**, Kovarova, N., Jesina, P., Drahota, Z., Mracek, T., Seneca, S., and Houstek, J. (2015) Alteration of structure and function of ATP synthase and cytochrome c oxidase by lack of Fo-a and Cox3 subunits caused by mitochondrial DNA 9205delTA mutation. *Biochemical J* **466**, 601-611 (IF 4.396, 2014)

Kovarova, N., Mracek, T., **Nuskova, H.**, Holzerova, E., Vrbacky, M., Pecina, P., Hejzlarova, K., Kluckova, K., Rohlena, J., Neuzil, J., and Houstek, J. (2013) High molecular weight forms of mammalian respiratory chain complex II. *PLoS One* **8**, e71869 (IF 3.534)

Havlickova Karbanova, V., Cizkova Vrbacka, A., Hejzlarova, K., **Nuskova, H.**, Stranecky, V., Potocka, A., Kmoch, S., and Houstek, J. (2012) Compensatory upregulation of respiratory chain complexes III and IV in isolated deficiency of ATP synthase due to TMEM70 mutation. *Biochim Biophys Acta* **1817**, 1037-1043 (IF 4.624)

Mayr, J. A., Havlickova, V., Zimmermann, F., Magler, I., Kaplanova, V., Jesina, P., Pecinova, A., **Nuskova, H.**, Koch, J., Sperl, W., and Houstek, J. (2010) Mitochondrial ATP synthase deficiency due to a mutation in the ATP5E gene for the F1 epsilon subunit. *Hum Mol Genet* **19**, 3430-3439 (IF 8.058)

Havlickova, V., Kaplanova, V., **Nuskova, H.**, Drahota, Z., and Houstek, J. (2010) Knockdown of F1 epsilon subunit decreases mitochondrial content of ATP synthase and leads to accumulation of subunit c. *Biochim Biophys Acta* **1797**, 1124-1129 (IF 5.132)

## PUBLICATIONS NOT INCLUDED IN MY PHD THESIS:

Hermanova, I., Arruabarrena-Aristorena, A., Valis, K., **Nuskova, H.**, Jorda, M. A., Fiser, K., Fernandez-Ruiz, S., Kavan, D., Pecinova, A., Niso-Santano, M., Zaliova, M., Novak, P., Houstek, J., Mracek, T., Kroemer, G., Carracedo, A., Trka, J., and Starkova, J. (2015) Pharmacological inhibition of fatty-acid oxidation synergistically enhances the effect of l-asparaginase in childhood ALL cells. *Leukemia* – in press (IF 10.431, 2014)

Pecina, P., Houstkova, H., Mracek, T., Pecinova, A., **Nuskova, H.**, Tesarova, M., Hansikova, H., Janota, J., Zeman, J., and Houstek, J. (2014) Noninvasive diagnostics of mitochondrial disorders in isolated lymphocytes with high resolution respirometry. *Biochim Biophys Acta* **2**, 62-71 (IF pending)

Hejzlarova, K., Mracek, T., Vrbacky, M., Kaplanova, V., Karbanova, V., **Nuskova, H.**, Pecina, P., and Houstek, J. (2014) Nuclear genetic defects of mitochondrial ATP synthase. *Physiol Res* **63 Suppl 1**, S57-71 (IF 1.487)

Folbergrova, J., Jesina, P., **Nuskova, H.**, and Houstek, J. (2013) Antioxidant enzymes in cerebral cortex of immature rats following experimentally-induced seizures: upregulation of mitochondrial MnSOD (SOD2). *Int J Dev Neurosci* **31**, 123-130 (IF 2.918)

Pecinova, A., Drahota, Z., **Nuskova, H.**, Pecina, P., and Houstek, J. (2011) Evaluation of basic mitochondrial functions using rat tissue homogenates. *Mitochondrion* **11**, 722-728 (IF 3.615)

**Nuskova, H.**, Vrbacky, M., Drahota, Z., and Houstek, J. (2010) Cyanide inhibition and pyruvate-induced recovery of cytochrome c oxidase. *J Bioenerg Biomembr* **42**, 395-403 (IF 3.637)

Drahota, Z., Vrbacky, M., **Nuskova, H.**, Kazdova, L., Zidek, V., Landa, V., Pravenec, M., and Houstek, J. (2010) Succinimidyl oleate, established inhibitor of CD36/FAT translocase inhibits complex III of mitochondrial respiratory chain. *Biochem Biophys Res Commun* **391**, 1348-1351 (IF 2.595)

Endlicher, R., Krivakova, P., Rauchova, H., **Nuskova, H.**, Cervinkova, Z., and Drahota, Z. (2009) Peroxidative damage of mitochondrial respiration is substrate-dependent. *Physiol Res* **58**, 685-692 (IF 1.430)