ABSTRACT OF DOCTORAL THESIS

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Assembly factors in the biogenesis of mitochondrial ATP synthase

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CONTENTS

1. INTRODUCTION

The F_1F_O-ATP synthase, which Boyer once pertinently called „A Splendid Molecular Machine“, represents the central enzyme of energy conversion in living organisms. It couples H^+ or Na^+ transport across the bacterial plasma membrane, thylakoid or mitochondrial membrane to the synthesis or hydrolysis of ATP. The aerobic organisms synthesise vast majority of ATP in the process of oxidative phosphorylation (OXPHOS). The OXPHOS apparatus resides in the mitochondrial inner membrane and consists of respiratory chain (RC) enzymes together with glycerophosphate dehydrogenase and ATP synthase. The RC is represented by a set of oxidoreductases (complex I-IV) that trasport electrons from reduced cofactors (NADH, $FADH_2$) to molecular oxygen and simultaneously pump protons from the matrix to the inter-membrane space. The resultant electrochemical proton gradient ($\Delta\mu_{H^+}$) fuels the formation of ATP from ADP and inorganic phosphate by ATP synthase.

1.1. ATP Synthase Structure and Function

The ATP synthase is a multisubunit enzyme, originally described in terms of two sectors: a hydrophilic F_1 part, which is capable of ATP hydrolysis but not synthesis when detached form the membraneous part, and a hydrophobic F_O domain possessing the proton-transporting activity. Electron microscopy and biochemical studies brought the current view about the structural features of the ATP synthases [1]. These include a headpiece, two stalks, a basepiece and a cap. The mitochondrial enzyme contains an extra collar above the basepiece. The ATP synthase consists of 8 types of subunits in bacteria or has about two times more subunits in mitochondria (Table I.; Figure 1). The composition of the F_O part is more complex and heterogeneous than that of the F_1 domain.

The primary function of the electrochemical gradient of protons or sodium ions across the membrane is to provide the torque required for subunits rotation (*c*-ring plus central stalk) in the direction of ATP synthesis. The direction of γ subunit rotation during sythesis (clockwise as viewed from the membrane) is opposing to that of hydrolysis [2]. Either ATP synthesis or hydrolysis occurs at three catalytic sites located primarily on the β subunits at the α/β interfaces [3,4]. Models considering conformation changes of the catalytic sites have been proposed [5-7].

Table I. Subunits and genes of ATP synthase: comparison among human, yeast and bacterial model organisms.

		H. sapiens		*S. cerevisiae*		*E. coli*	
		subunit	gene	subunit	gene	subunit	gene
F_1	catalysis	α_3	*ATP5A1*	α_3 (1)	*ATP1*	α_3	*uncA*
		β_3	*ATP5B*	β_3 (2)	*ATP2*	β_3	*uncD*
	central stalk	γ	*ATP5C1-2*	γ (3)	*ATP3*	γ	*uncG*
		δ	*ATP5D*	δ	*ATP16*	ε	*uncC*
		ε	*ATP5E*	ε	*ATP15*		
	regulatory protein	IF1	*ATPIF1*	Inh1p	*INH1*		
				Stf1p	*STF1*		
				Stf2p	*STF2*		
				Stf3p	*STF3*		
F_O	proton channel	*a*	*ATP6*[mt]	6	*ATP6/oli2*[mt]	*a*	*uncB*
		$c_{9\text{-}12}$	*ATP5G1-3*	9_{10}	*ATP9/oli1*[mt]	c_{10}	*uncE*
	peripheral stalk	A6L	*ATP8*[mt]	8	*ATP8/aap1*[mt]		
		OSCP	*ATP5O*	OSCP (5)	*ATP5*	δ*	*uncH*
		b	*ATP5F1*	*b* (4)	*ATP4*	b_2	*uncF*
		d	*ATP5H*	*d* (7)	*ATP7*		
		F_6	*ATP5J*	*h*	*ATP14*		
		f	*ATP5J2*	*f*	*ATP17*		
	inter-complex interaction	*e*	*ATP5I*	*e* (Tim11)	*ATP21/TIM11*		
		g	*ATP5L*, *ATP5L2*	*g*	*ATP20*		
				i (*j*)	*ATP18*		
				k	*ATP19*		
	regulatory protein	Factor B (*s*)	*ATP5S*				

The numbers in subscript indicate the subunit stoichiometry. * The *E. coli* δ subunit is a part of the F_1 domain. [mt] Encoded in mtDNA. Data for human ATP synthase come from http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene [8] . For citations to the yeast and bacterial enzyme see [9] and [10], respectively.

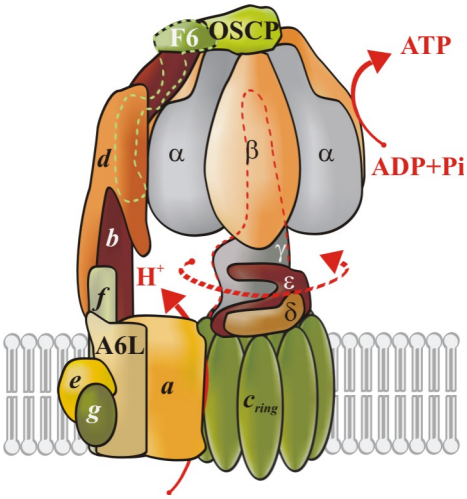

Figure 1: Schematic model of a monomer of mammalian ATP synthase. Thick arrows indicate proton translocation through the F_O and ATP synthesis within the $\alpha_3\beta_3$ catalytic unit. Dashed arrow shows the direction of γ subunit rotation.

1.2. ATP Synthase Gene Expression

Genes for *E. coli* enzyme are part of a polycistronic *unc* operon and encode subunits in the order of *a*,*c*,*b* of F_O and δ, α, γ, β and ε of F_1 [10]. Expression of the individual genes is regulated post-transcriptionally by the control of efficiency of translational initiation [11] and by mRNA degradation [12]. Contrary to the prokaryotic ATP synthase, the mitochondrial enzyme is encoded in two separate genomes [13]. Some of the F_O subunits are expressed from the mitochondrial genome (mtDNA) but majority of the ATP synthase proteins are products of nuclear genes (Table I.). Genes for subunits *a* (6) and A6L (8) are mostly part of the mtDNA whereas *Chlamydomonas reinhardtii* possesses nuclearly-encoded subunit *a* [14]. Genes for subunit *c* (9) are in the nucleus of Metazoa [15] and in mtDNA of some protists [16], yeast [17] and plant species [18-21]**.**

A subset of mammalian genes encoding ATP synthase subunits is expressed in tissue-specific manner like genes encoding subunits *c* [22-24] and γ [25-27]. Bioinformatic approach identified possible isoforms of subunit *g* in some metazoan species [28].

1.3. ATP synthase Assembly

After the proper import into the mitochondria, proteins that are due to become part of the ATP synthase complex are assembled in a step-by-step process which, at least in eukaryotic organisms, is aided by a substrate (subunit)-specific chaperones.

1.3.1. Consecutive Assembly of the Enzyme Subunits

Studies with bacterial ATP synthase imply that independent formation of the F_1 and F_O sectors is possible but assembly of the fully functional proton channel requires the expression of F_1 genes [29]. F_1 assembled in mitochondria isolated from the yeast cytoplasmic *petite* mutant [30] or from mammalian Rho^0 cells [31]. On the other hand, the assembly of a functional proton channel of the F_O portion was not observed in mitochondria lacking the β subunit [32]. Ordered assembly of the yeast F_O sector was demonstrated from the investigation of mutants for the mitochodrially-encoded subunits [33]. A principal role of subunit 9 (*c*) was suggested as in its absence subunits 6 and 8 (A6L) were not associated with the enzyme complex. Sequential addition of mitochondrially-synthesised subunits was initiated with the subunit 9 and followed by the assembly of subunit 8. Subunit 6 appeared to be added as the last mitochondrially-synthesised protein. Besides, subunits 6 could not be detected in cells devoid of any of the stator-stalk subunits *b* [34], subunit *d* [35], OSCP [36], subunit *h* [37], subunit *i/j* [38] and subunit *f* [39]. The peripheral stalk subunits *b*, *d* and OSCP have been proposed to assemble in the order of *b*, OSCP and subunit *d* [40].

Electrophoretic assays of subcomplexes of the human ATP synthase strengthened the concept of successive assembly of the enzyme together with the belated incorporation of mitochondrially-encoded subunits. Such conclusions have been inferred mainly from studies on three model situations: cells with mutated subunits *a* [41-43]; cells incapable of mitochondrial translation due to its arrest by doxycycline [44] or absence of mtDNA (Rho^0 cells) [43,45]; and cells with diminished synthesis of subunits *a* due to altered processing of the *ATP6-COX3* mRNA[46]. Nijtmans and collaborators hypothesized that the newly formed F_1 is attached to the ring of *c* subunits, which is most probably followed by the addition of the outer stalk components (such as subunits *b*, OSCP and F_6) among other nuclearly-encoded subunits. The completion of the membraneous part is achieved after the incorporation of mitochondrially-encoded subunits *a* and A6L [44].

1.3.2. Molecular Chaperones Specific for the F_1F_O Assembly

Work with respiratory deficient strains of *Saccharomyces cerevisiae* has provided evidence that assembly of the mitochondrial ATP synthase is dependent on proteins that serve substrate-specific chaperone-type functions: Atp10p, Atp11p, Atp12p, Atp22p, Atp23p and Fmc1p. Atp11p and Atp12p mediate formation of the F_1 moiety via interaction with the β and α subunit, respectively [47]. The role of Fmc1p is unclear, it seems to be required for assembly/stability of the yeast ATP synthase at heat stress conditions [48]. Atp10p, Atp22p and Atp23p are essential for formation of the F_O part [49-52], during which Atp10p assists in the incorporation of the subunit *a*. Atp23p exerts dual function during assembly of the yeast enzyme. It mediates the maturation of a newly synthesised subunit 6 precursor and, independent of its proteolytic activity, promotes incorporation of the mature subunit 6 into the F_O channel.

Proteins homologous to Atp11p [53,54] and Atp12p [54] suggest that both proteins can be involved in assembly of ATP synthases from variety of organisms. Approvingly, results from complementation analyses and/or two-hybrid screens showed that products of *D. yakuba* gene 2A5 [53] and *H. sapiens* genes *ATPAF1* and *ATPAF2* [54] are functional counterparts of yeast factors. Based on a sequence similarity, we further showed that both proteins are present in majority of eukaryotic lineages, which corresponds to the conservation of catalytic $\alpha_3\beta_3$ hexamer [55].

1.4. Supramolecular Organisation of ATP Synthase

Interaction of ATP synthase with mitochondrial translocases favours tight coordination between the final steps in oxidative phosphorylation [56,57]. Individual ATP synthases are also in physical contacts with each other in the mitochondrial inner membrane. ATP synthase dimers or even higher oligomers were recovered from mitochondria by a mild detergent lysis with subsequent analysis by native polyacrylamide gel electrophoresis [58-61].

There is mounting evidence that the supramolecular structures of ATP synthase are involved in maintaining the integrity of the mitochondrial cristae, which traverse the interior of the organelle. Consistent with this idea, yeast cells devoid of subunits *e*, *g* and *b* displayed aberrant mitochondrial morphologies characterised by numerous digitations and onion-like structures [60]. Remarkably, not only cells deficient in F_O subunits but also mutants deficient in F_1-α or F_1-β subunits or proteins that mediate F_1 assembly (*i.e.* Atp11p, Atp12p, or Fmc1p) show loss of cristae [62].

A model of supramolecular organisation of the ATP synthase has been presented [63]: a dimerisation interface includes subunits 6 (*a*), 8 (A6L), *b*, *i*, *f* and subunit *h*. Subunits *e* and *g* play a stabilisation role at the periphery of the dimerisation contact site and form the second interface facilitating the oligomeric arrangement.

1.5. Regulation of Mammalian ATP Synthase Biogenesis

The mitochondrial ATP production responses to various energy demands during ontogenetic development, hormonal stimuli, oxygen availability, workload etc. The biogenesis of ATP synthase is regulated at different levels of gene expression, which can be seen even for genes coding for subunits of the same complex.

Expression of *ATP5B* gene encoding F_1 β subunit seems to be governed at the level of transcription during the proliferation of liver mitochondria [64]. In contrast, during the differentiation process, expression of ATP synthase genes (*ATP5B*, *ATP6* and *ATP8*), is regulated at post-transcriptional level [65,66] by the control of mRNA stability [64] and translational efficiency [67,68] as previously demonstrated for the β subunit transcript.

Expression of *P1* and *P2* genes is differentially regulated in mammalian tissues [23,24]. While *P1* expression reflects the need for ATP synthase capacity arising from miscellaneous physiological stimuli (cold aclimation, cell differentiation and hormonal treatment), expression of the *P2* gene remains constitutive. Thus, key role of the *P1* gene in the biogenesis of mammalian ATP synthase was suggested [22,23].

Long term electrical stimulation of rabbit muscle upregulates ATP synthase β subunit [69]. Similarly, ATP synthase expression increases during skeletal and heart muscle contraction and is regulated via calcium signalling [70,71].

1.6. Genetic Defects of ATP synthase

Deficiency of ATP synthase as well as of other OXPHOS enzymes may originate from a mutation either in mtDNA or in ncDNA. Isolated defects of ATP synthase are associated with severe and often fatal clinical phenotypes with early onset during neonatal age or infancy. Brain and heart muscle are among the most affected tissues. Despite differencies in phenotypic manifestation, decreased ATP production and increased mitochondrial membrane potential and oxidative stress are common to both types of ATP synthase defects [72].

1.6.1. ATP synthase Defects due to Mutations in mtDNA

So far, maternally transmitted mutations leading to isolated deficiency of ATP synthase have been located within the *ATP6* gene. Most patients harbour T8993G or T8993C transversion ([73,74]). Clinical symptoms are proportional to the degree of heteroplasmy. At levels bellow 90-95 % it manifests as neurogenic muscle weakness, ataxia and retinitis pigmentosa (NARP). At high mutation loads close to homoplasmy (above 90-95 %) there develops degenerative encephalopathy - a maternally inherited Leigh syndrome (MILS). The NARP/MILS or familial bilateral striated necrosis (FBSN) have also been identified with two other mutations in *ATP6* gene, T9176G and T9176C [75]. A single case of FBSN was also caused by a nearly homoplasmic T8851C (i.e. conserved Trp→Arg) mutation.

While ATP hydrolysis is normal, ATP synthase activity is impaired in the cells with T8993G mtDNA mutation and correlates with the level of heteroplasmy; a 60–75% mutant mtDNA load is required for obvious clinical expression of central-nervous-system symptoms [76]. Despite functional inhibition, the amount of ATP synthase is unchanged [41] and so is the capability of oligomerisation [77].

Another point mutation, T9101C transition, manifests as Leber's hereditary optic neuropathy (LHON [78]), a disease characterised by loss of vision in young adults, more frequently males, caused by degeneration of the optic nerve but associated mainly with mtDNA mutations in genes encoding complex I subunits.

An ATP synthase deficiency associated with a two-base microdeletion at position 9205-6 (9205ΔTA) has only been detected in two cases that differ in biochemical and clinical symptoms. 9205ΔTA disturbs the termination codon of *ATP6* gene and sets the adjacent *COX3* immediately in frame.

1.6.2. ATP synthase Defects due to Mutations in ncDNA

Until know, 14 patients with isolated deficiency of ATP synthase have been documented [79]. All of them show 70-90% decrease in the content of ATP synthase while the amount of respiratory chain enzymes is normal or even increased. The patients´ phenotypes have surprisingly common features with early neonatal onset, severe and often fatal hyperlactacidemia and 3-methylglutaconic aciduria and hypertrophic cardiomyopathy. Most of the newborns had low birth weight. Half of the patients died and only two of the surviving patients are older than 10 years. Nevertheless, the length of survival has no correlation with the severity of the enzyme defect. No mutation in ATP synthase structural genes could be detected in the patients tested. Mutations in F_1-assembly genes were also

excluded and the level of their expression remains unaffected. The only exception is a patient harbouring a missence mutation in *ATPAF2* gene encoding Atp12 assembly protein, the only known pathogenic mutation of nuclear origin resulting in the selective reduction in ATP synthase [80]. The phenotype contrasts with prominent heart involvement in the previous cases and is marked by dysmorphic features, progressive encephalopathy, lactic acidosis and 3-methylglutaconic aciduria. One of the longer surviving patients developed severe peripheral neuropathy [79].

In analogy with mtDNA-based defects, the cells from patients with ATP synthase defects of nuclear origin display increased mitochondrial membrane potential [81,82] and increased generation of reactive oxygen species [82].

2. AIMS OF THE THESIS

The biogenesis of mitochondrial ATP synthase was studied with respect to its assembly from individual subunits under the assistance of specific proteins with chaperone-type function and with respect to mitochondrial disorders with selective dysfunction of the enzyme. The first part of the work focused on the distribution and structural features of potential assembly factors in various genomes. The second part was devoted to the expression pattern of specific mammalian chaperones. The particular aims were as follows:

1) To characterise the occurence of orthologues of the yeast ATP synthase assembly factors

- To find orthologues of the known yeast ATP synthase assembly factors based on sequence similarity across genomes
- To analyse structural features of the orthologues (and compare them with the results of mutagenesis studies on yeast counterparts)

2) To analyse expression of the mammalian ATP synthase assembly factors

- To evaluate the expression of assembly factors in mouse tissues
- To study the expression of assembly factors in five patients with isolated deficiency of ATP synthase of nuclear origin

3. SUMMARY OF THE RESULTS

The thesis consists of four publications. Two of them concentrate on the genome distribution of genes conding for factors specific for assembly of the ATP synthase and differential expression of mammalian assembly genes.

The other studies compare phenotypic features of selective ATP synthase deficiencies with respect to the genetic cause of the diseases. They also stress that the inability of mitochondria to supply sufficient ATP is not the only consequence of OXPHOS failure.

Differential expression of ATPAF1 and ATPAF2 genes encoding F(1)-ATPase assembly proteins in mouse tissues. Pickova A, Paul J, Petruzzella V, Houstek J.; *FEBS Lett.* 2003; 551(1-3):42-6.

This is the first article showing tissue-differential expression of ATP synthase assembly factors. Only two genes (*ATPAF1* and *ATPAF2*) have been found in mammalian cells to code for Atp11 and Atp12 chaperones specific to the F_1 assembly. Quantitative reverse-transcription polymerase chain reaction was used in order to assess the transcript content in several tissues (brown adipose tissue, kidney, liver, heart, brain and skeletal muscle) from adult (2-month-old) and young (5-day-old) mice. The nearly constant *ATPAF1* mRNA levels contrasts with highly variable expression profile of *ATPAF2*, which resembles expression profiles of F_1 α and β genes. The most striking feature is a strongly upregulated *ATPAF2* in brown adipocytes (Figure 2).

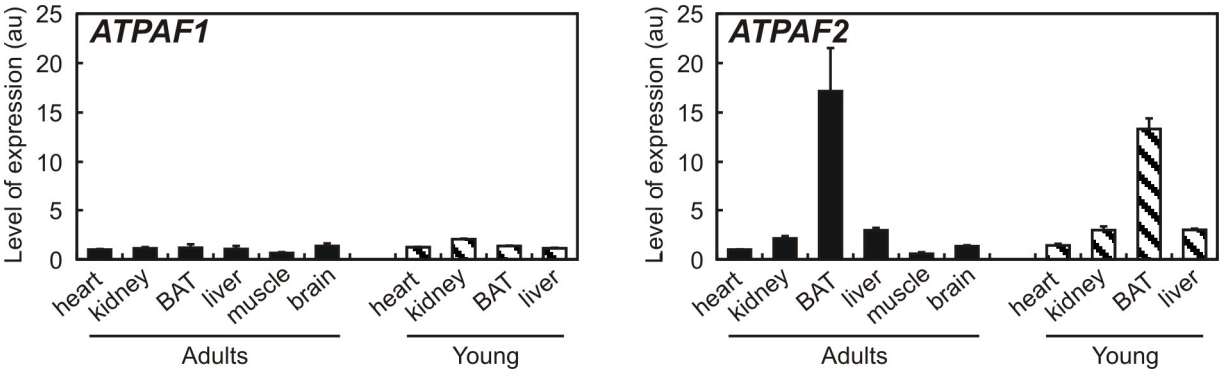

Figure 2: Tissue expression profile of ATPAF1 and ATPAF2 mRNAs in adult and 5-day-old mice analysed by quantitative RT-PCR. Transcript levels are normalised to GAPDH mRNA and are expressed relative to the mean value from adult's heart, which was set to 1. Data are means ± SD. au – arbitrary units, BAT – brown adipose tissue.

Further search for *cis*-acting elements in putative promoter region of human, mouse and rat genes revealed regulatory sites, commonly modulating the biogenesis of

mitochondria-related genes including ATP synthase genes, within *ATPAF2* but not *ATPAF1* gene. Thus *ATPAF2* seems to be highly regulated gene, while *ATPAF1* may provide with constant level of chaperone transcipt.

Assembly factors of F_1F_O-ATP synthase across genomes. Pickova A, Potocky M, Houstek J.; *Proteins.* 2005; 59(3):393-402.

The studies about assembly of the yeast ATP synthase had promted us to search available genomic data for orthologues of the known yeast chaperones. Atp11 and Atp12 appear to be ubuquitous proteins and are the only proteins found in metazoan species (Table II). Nevertheless, only Atp12 was identified within bacterial genomes, namely in the α-proteobacterial species. Atp10 was detected in a limited number of species in contrast to the distribution of its interaction partner, the subunit *a*. Surprisingly, the primary sequence of subunit *a* differed according to the presence or absence of *ATP10* gene (Figure 3). In organisms possessing Atp10p orthologue, the C-terminus of subunit *a* contains sonserved proline residue and the two last amino acids are hydrophobic, represented mainly by leucine or histidine. Fmc1 distribution is quite sporadic and is confined to fungal species. Atp22 was detected only in selected members of Saccharomycetales. Atp10p, Atp11p and Atp12p orthologues were further characterised with respect to primary and secondary structural features, which may serve as a basis for in-depth analysis and functional studies.

To conclude, the distribution of assembly genes strenghtened the structural and functional differences between the F_1 and F_O domains in eukaryotes. The F_1-assembly mechanism seems to be preserved among species. In contrast, F_O structure and biogenesis shows higher variability.

TABLE II. Distribution of ATP synthase assembly factors and their interacting subunits.

		Atp11	Atp12	Fmc1	Atp10	F_1-α	F_1-β	F_O-*a*
Metazoa	Chordata	●	●			●	●	●
	Arthropoda	●	●			●	●	●
	Nematoda		●			●	●	●
Fungi	Ascomycota	●	●	●	●	●	●	●
	Basidiomycota	●	●	●	●	●	●	●
	Encephalitozoon cuniculi							
Mycetozoa	*Dictyostelium discoideum*	●	●		●	●	●	●
Viridiplantae	*Chlamydomonas reinhardtii*	●	●			●	●	●
	Magnoliophyta	●	●		●	●	●	●
Rhodophyta	*Cyanidioschyzon merolae*	●	●		●	●	●	●
stramenopiles	*Thalassiosira pseudonana*	●	●		●	●	●	●
	Phytophthora sojae	●	●		●	●	●	●
Alveolata	Apicomplexa	●	●			●	●	
	Ciliophora (ciliates)	●	●			●	●	
Euglenozoa	Kinetoplastida	●	●			●	●	●
Entamoebidae	*Entamoeba histolytica*							
Diplomonadida	*Giardia intestinalis*							
Parabasalidea	*Trichomonas vaginalis*							
Proteobacteria	α-proteobacteria		●			●	●	●

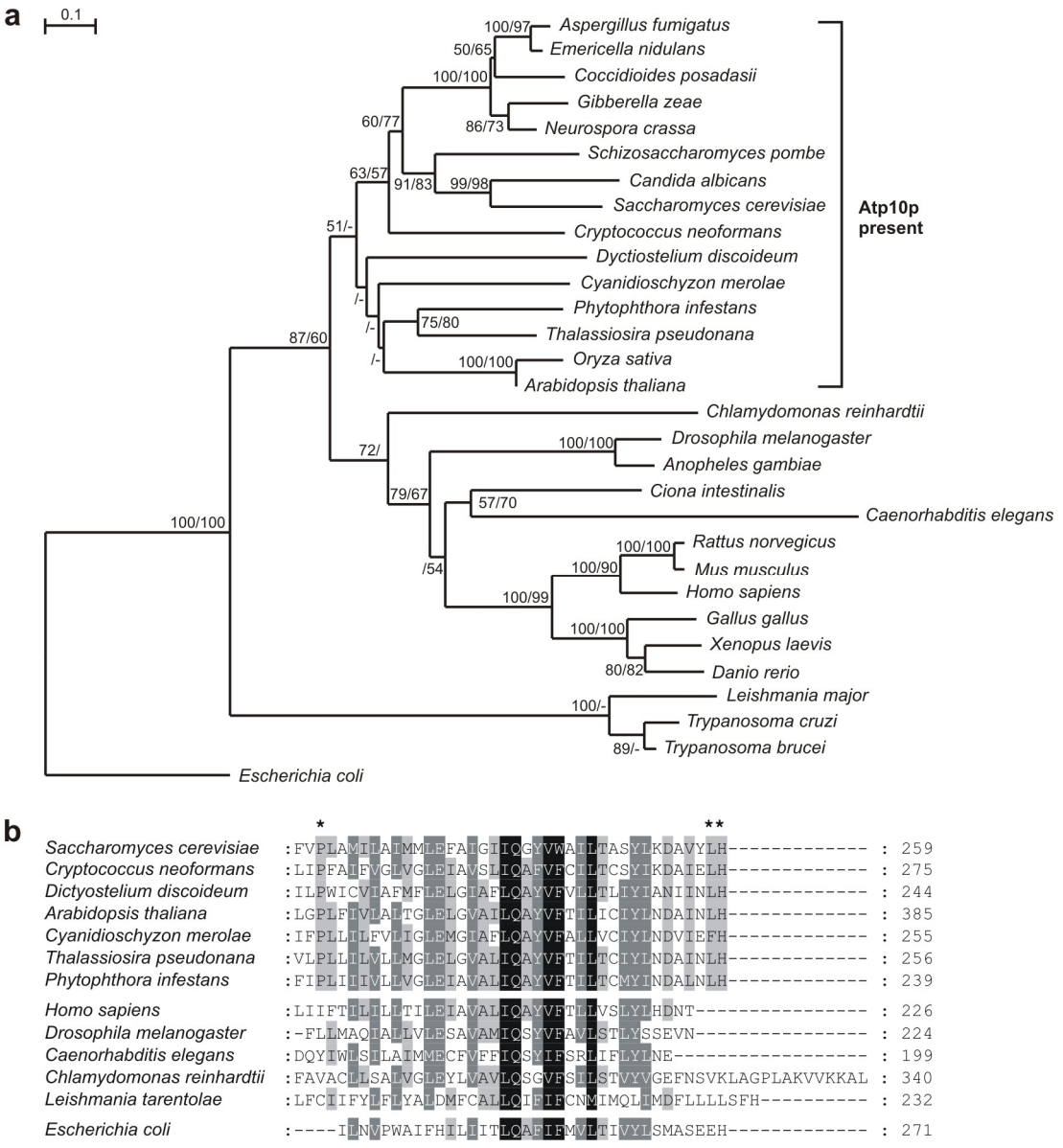

Figure 3: Phylogeny of ATP synthase subunit *a*. (a) Tree of the *a* subunit homologues constructed by the neighbour-joining (NJ) and maximum-likelihood (ML) methods on the basis of T-COFFEE multiple alignments. *Escherichia coli* homologue was used as an outgroup. Numbers next to the nodes are percentages of bootstrap confidence levels calculated from 500 NJ/ML replicates; only values over 50 % are shown, - means different branch topology between ML and NJ tree. The scale represents 0.10 fixed mutations per site. **(b)** Multiple alignment of the *a* subunit C-terminus from selected species based on the T-COFFEE algorithm. Asterisks mark residues specifically conserved in organisms possessing the *ATP10* gene.

Deficiency of mitochondrial ATP synthase of nuclear genetic origin. Sperl W, Jesina P, Zeman J, Mayr JA, Demeirleir L, Vancoster R, Pickova A, Hansikova H, Houst'kova H, Krejcik Z, Koch J, Smet J, Muss W, Holme E, Houstek J.; *Neuromuscul. Disord.* 2006; 16(12):821-9.

Here we compared and analysed clinical and biochemical data on 14 patients with isolated ATP synthase deficiency of nuclear origin characterised by a markedly decreased content of an otherwise fully assembled and active enzyme. Half of the patients is reported for the first time. The exact genetic cause of the defects is unknown. No mutated structural genes have been found in the cases tested. There is only one case in which mutation was localised to the *ATPAF2* gene, encoding F_1-α chaperone. Most of the patients present with remarkably uniform phenotype with onset in early neonatal age, hypertrophic cardiomyopathy, pronounce often fatal metabolic acidosis and high lactate levels. Some patients had elevated 3-methylglutaconic acid in urine. All surviving patiens had some degree of psychomotor retardation. Lack of striatal brain involvement and decreased amount of structurally normal ATP synthase distinguish these defects from those caused by mutations in *ATP6* gene. In conclusion, the ncDNA-based defects represent a spefic type of mitochondrial diseases that can be recognised at both clinical and biochemical level.

Mitochondrial diseases and genetic defects of ATP synthase. Houstek J, Pickova A, Vojtiskova A, Mracek T, Pecina P, Jesina P.; *Biochim. Biophys. Acta.* 2006; 1757(9-10):1400-5.

Defective biogenesis of ATP synthase complex leads to severe mitochondrial diseases. Here we review current knowledge on the ATP synthase defects and show that there are two types of isolated deficiency of ATP synthase with respect to the structural and functional features of ATP synthase as well as the consequences of the enzyme failure. The defects of mtDNA-origin are qualitative, i.e. the enzyme structure is altered and its function impaired. Known defects due tu mutations in nuclear genome are quantitative where the amount of correctly assembled enzyme is significantly lowered. Most of the current patients from the second group do not bear currently known mutations, however, expression of F_1-assembly genes is normal and no mutation has been located within their coding region. Common to both types of defects is the energy deprivation and increased oxidative stress.

4. CONCLUSIONS

The subsequent conclusions stem from our bioinformatic studies:

- We have performed a comprehensive survey of orthologues to Atp11p, Atp12p, Fmc1p, Atp10p and Atp22p from all available genomes.
- The distribution of the orthologues mirrors the structural and functional difference between the F_1 and F_O moieties: assembly of the catalytic domain depends on action of at least two specific factors, Atp11 and Atp12, with the exception of Nematoda whose genome bears homology with *ATP12* only.
- The presence of Atp12p orthologue in α-proteobacteria points to eubacterial origin of *ATP12*
- The membranous F_O part seems to be more versatile in terms of both structure and biogenesis: the presence of Atp10p orthologue matches a specific structure of the C-terminal part of subunit *a*.
- The finite occurence of Fmc1 and Atp22 may reflect specific requirements of some lineages.
- Sequence analysis of Atp11p, Atp12p and Atp10p orthologues provides basis for detailed molecular studies of these chaperones.

The expression studies have resulted in the following outcome:

- *ATPAF1* and *ATPAF2* are genes with low or moderate level of expression.
- The tissue distribution of *ATPAF2* correlates with the expression profiles of the α and β subunits and peaks in brown adipose tissue, which is marked by a diminished content of ATP synthase.
- *ATPAF1* manifested a constant expression in adult and 5-day-old mouse tissues.
- Nowadays, the primary cause of the 13 from the 14 published nuclear genetic defects remains enigmatic. The clinical and biochemical data, however, do not exclude mutation in different nuclear genes, which is also indicated by our results.
- Concerning the F_1-assembly genes, we have not found abberant transcript levels in five patients with selective ATP synthase deficiency. Similarly, the amount of Atp11 did not vary significantly among controls and patiens with ATP synthase defect. We also have not detected any mutation in the enzyme assembly or structural genes (collaboration with a research group of Prof. V. Pačes, unpublished data). Nevertheless, altered biogenesis of another currently unknown ancillary protein may lead to this type of defect.

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