ultrastructure. The findings presented in this work may be of great interest as they suggest that a defect in ATP synthase oligomerization has strong mitochondrial repercussions. Although it has to be formally investigated, we may speculate that ATP synthase oligomerization defect could be at the origin (or concomitant to) mitochondrial pathologies.

Reference


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S1.P19
Assembly of mitochondrial ATP synthase subunit c rings in mammalian HEK293 cells

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The biogenesis of eukaryotic mitochondrial ATP synthase (ATPase) proceeds as modular enzyme assembly from pre-assembled domains. Discoveries of accessory subunits DAPIt and MLQ, or assembly factors such as TMEM70 present uniquely in vertebrates suggest that ATPase biogenesis may show significant differences between higher and lower eukaryotes. Our previous studies in human HEK293 cells showed that shRNA downregulation of central stalk subunits γ, δ, or ε led to decreased content of ATPase, which is functionally and structurally normal. Importantly we did not detect any incomplete ATPase complexes with proton conductance uncoupled from ATP synthesis, which occur when these subunits are deleted in yeast. The only identified subcomplex in HEK293 with γ, δ, or ε knockdown was an assembly intermediate or aggregate of subunit c without apparent association of other ATPase subunits. In the present study we further explored the composition of the c subcomplex. We overexpressed subunit c isoform 1 with C-terminal FLAG tag for simplified detection and isolation in control HEK293, as well as in cells with shRNA knockdowns of subunits γ, δ, and ε. Native and SDS/PAGE experiments showed that the overexpressed c subunit can be incorporated into ATPase holoenzyme. The tagged variant was also detected in the accumulated subcomplex in knock-down cell lines. The distribution of tagged subunit c between the holoenzyme and the subcomplex reflected the pattern of the endogenous subunit. Interestingly, a small portion of the subunit c subcomplex of identical size was also found in control cells, suggesting that it might represent genuine assembly intermediate, and not only an aberrant complex formed in γ, δ, or ε downregulated cells. Anti-FLAG antibody immunoprecipitation led to successful pull-out of both the subunit c subcomplex, and the holoenzyme as evidenced by co-immunoprecipitation of other ATPase subunits, including the endogenous c subunit. These results indicate the formation of hybrid c-rings composed of both subunit variants. Mass spectrometric analysis of the precipitated fraction failed to identify any candidate subunit c chaperones/assembly factors associated with the accumulated subcomplexes. Our results suggest that in mammalian cells, subunit c rings may be formed by self-assembly, as previously shown in lower organisms. This study was supported by Grant Agency of the Czech Republic (P303/12/1363, 14-368046), and Grant Agency of Charles University (1160214).

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S1.P20
The novel TB drug Bedaquiline targets the mycobacterial F1Fo synthase rotor ring

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The increasing number of tuberculosis (TB) infections with multi-drug resistant (MDR) Mycobacterium tuberculosis strains causes a severe health problem, necessitating the development of new antibiotics (Koul et al., 2011). A novel antibiotic drug, Bedaquiline (Sirturo™, formerly TMC207), was found to be highly effective in the treatment of MDR-TB (Andries et al., 2005). It was reported that it targets the rotor ring of the energy converting enzyme F1Fo-ATP synthase (Haagsma et al., 2009; Segala et al., 2012), which is essential for the survival of mycobacteria. However, structural and biochemical information about the precise binding site of Bedaquiline and its mechanism of action are not known at the level of the isolated ATP synthase. We performed ATP synthesis inhibition experiments using two different antibiotics, Bedaquiline and Mefloquine, which efficiently inhibited the synthesis and hydrolytic activity of a mycobacterial ATP synthase. Furthermore, drug competition assays with the ATP synthase inhibitor dicyclohexylcarbodiimide (DCCD) using the isolated, mycobacterial c-ring showed that Bedaquiline competes with DCCD at the proton binding site. We present direct biochemical proof that the c-ring's ion binding site is the actual target of the drug; the binding is highly specific and has a high affinity with a Kd in the nanomolar range. To further explore the molecular basis of the interaction we performed isothermal titration calorimetry (ITC) using WT and mutant c-rings, which providing a list of residues involved in the interaction. Finally we also aim to solve the structure of the c-ring in complex with Bedaquiline using X-ray crystallography. 3D crystals of the c-ring/Bedaquiline complex and their X-ray diffraction power are presented. References: Andries K et al., (2005) A diarylquinoline drug active on the ATP synthase of Mycobacterium tuberculosis. Science 307: 223–227 Haagsma AC et al., (2009) Selectivity of TMC207 towards mycobacterial ATP synthase compared with that towards the eukaryotic homologue. Antimicrobial agents and chemotherapy 53: 1290–1292 Koul A et al., (2011) The challenge of new drug discovery for tuberculosis. Nature 469: 483–490 Segala E et al., (2012) New mutations in the mycobacterial ATP synthase: new insights into the binding of the diarylquinoline TMC207 to the ATP synthase C-ring structure. Antimicrobial agents and chemotherapy 56: 2326–2334.

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S1.P21
Single molecule rotation assay of FoF1 on arrayed lipid bilayer chamber system (ALBiCs)

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F-type ATP synthase (FoF1) is the rotary motor protein that mediates the energy conversion between phosphate transfer potential of ATP and proton motive force (pmf) across biomembranes via mechanical rotation of the inner rotor complex. To understand the