stalks. Recently it was demonstrated (i) that cyclophilin D binds to the FO OSCP subunit, resulting in partial enzyme inhibition; (ii) that CyPD binding requires high Pi, while the CyPD inhibitor CsA displaces CyPD from OSCP resulting in enzyme reactivation; and (iii) that ATP synthase dimers generate Ca^{2+}-dependent currents indistinguishable from those of the permeability transition pore (PTP), suggesting that the PTP forms from a Ca^{2+}-dependent conformational change of FOF1 dimers [1]. These findings imply that many modulators of the PTP may act on the ATP synthase. The most potent PTP inhibitors are H^+; indeed, the pore is blocked at acidic matrix pH that also promotes CyPD release from the inner membrane. Diethylpyrocarbonate (DPC) prevents PTP inhibition by H^+ through carbethoxylation of His residues, and also prevents the release of CyPD from the inner membrane induced by acidic pH [2]. We found that DPC also prevents the release of CyPD from ATP synthase induced by acidic pH. As is the case for the PTP, the effect of DPC was reversed by hydroxylamine, indicating that it can be traced to carbethoxylation of His residue(s). This in turn suggests that reversible protonation of the unique histidyl residue of OSCP (bovine His112) may play a critical role in modulation of the CyPD–ATP synthase interaction. Consistently, when OSCP subunit separated from mitochondria treated with DPC was digested with trypsin and analysed by ESI-MS, a mass shift of +72 Da of the OSCP 95-113 peptide was determined, which is consistent with carbethoxylation of the unique His112. In conclusion, DPC is proving very useful to address the role of OSCP His112 in modulation of CyPD binding to ATP synthase/PTP by matrix pH, which will be further addressed by mutagenesis.

References

[1] V. Giorgio, S. von Stockum, M. Antoniel, A. Fabbro, F. Fogolari, M. Forte, G.D. Glick, V. Petronilli, M. Zoratti, I. Szabó, G. Lippe, P. Bernardi, Dimers of mitochondrial ATP synthase form the permeability transition pore, PNAS, 110 (2013) 5887–92.
[2] A. Nicolli, E. Basso, V. Petronilli, R.M. Wenger, P. Bernardi, Interactions of cyclophilin with the mitochondrial inner membrane and regulation of the permeability transition pore, and cyclosporin A-sensitive channel, J. Biol. Chem., 271 (1996) 2185–92.

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S1.P2

The mechanism of binding of an intrinsically disordered mitochondrial inhibitor protein to F1-ATPase

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IF1 is an 84 amino acid length peptide that inhibits the ATP hydrolysis activity of F1Fo-ATPase. In solution, IF1 forms homodimers with two domains: a coiled-coil dimerization domain comprising residues 48–84, and a N-terminal inhibitory domain, from residues 1–45, that is intrinsically disordered. In the structure of bovine F1-ATPase inhibited with residues 1–60 of the bovine inhibitor protein IF1, one inhibitor protein (I1-60) interacts with five of the nine subunits of F1-ATPase, and I1-60 is bound tightly at the αDPβDP catalytic interface. Formation of the inhibited complex requires ATP hydrolysis. It has been proposed that the first interaction between the inhibitor and F1-ATPase is with the βE-subunit (the most open state), and that the inhibitor becomes entrapped progressively as two ATP molecules are hydrolysed. We have solved three novel F1–IF1 structures that support this proposal. Crystals of F1-ATPase were grown in the presence of a large molar excess of one of three inhibitors: I1-60His, I1-60His K39A, and I1-60His F22W Y33W. The resultant complexes all have multiple copies of the inhibitor bound to one F1-ATPase molecule. The structures reveal the binding cycle of the inhibitor to F1-ATPase showing how the intrinsically disordered inhibitory domain of IF1 becomes gradually more ordered as it interacts with F1-ATPase. The folding pathway of IF1 is shown with a greater level of structure possible for IF1 as its interactions with F1-ATPase become progressively more extensive.

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S1.P3

Regulatory conformational changes of the epsilon subunit in single FRET-labeled F_1 and F_oF_1-ATP synthase

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Subunit ε is an intrinsic regulator of the bacterial F_oF_1-ATP synthase. The C-terminal domain of ε can extend into the central cavity formed by the a and b subunits as revealed by the recent X-ray structure of the F_1 portion of the *Escherichia coli* enzyme [1]. This insertion blocks the rotation of the central g subunit and, thereby, prevents wasteful ATP hydrolysis. We developed an experimental system including a microfluidic single-molecule trap [2] to observe how epsilon inhibits the F_1 portion and the holoenzyme F_oF_1-ATP synthase. Labeling the C-terminal domain of the ε and g subunits specifically with two different fluorophores for single-molecule Förster resonance energy transfer (smFRET) allowed monitoring of the conformation of ε of the F_1 portion [3] or the reconstituted enzyme in real time [4].

References

[1] G. Cingolani, T. M. Duncan, Structure of the ATP synthase catalytic complex (F1) from *Escherichia coli* in an autoinhibited conformation, Nat. Struct. Mol. Biol. 18 (2011) 701–707.
[2] S. D. Bockenhauer, T. M. Duncan, W. E. Moerner, M. Börsch, The regulatory switch of F_1-ATPase studied by single-molecule FRET in the ABEL Trap, Proc. SPIE 8950 (2014) 89500H.
[3] M. Börsch, T. M. Duncan, Spotlighting motors and controls of single F_oF_1-ATP synthase, Biochem. Soc. Trans. 41 (2013) 1219–1226.
[4] T. M. Duncan, M. G. Düser, T. Heitkamp, D. G. G. McMillan, M. Börsch, Regulatory conformational changes of the e subunit in single FRET-labeled F_oF_1-ATP synthase, Proc. SPIE 8948 (2014) 89381J.

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S1.P4

Redox regulation of cyanobacterial chimera F1-ATPase comprises interplay between the γ-subunit "neck" region and the turn region of the βDELSEED-loop

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ATP synthase (FOF1) is an important enzyme for energy conversion in the cell and can be found in energy-transducing membranes of bacteria, mitochondria and chloroplasts. The enzyme

is prone to drop into ADP inhibition which is a common regulation mechanism to prevent wasteful ATP hydrolysis when an electrochemical ion gradient across the membrane is not available. The soluble portion F1 is capable of ATP hydrolysis, accomplished by the minimum catalytic core subunits $\alpha_3\beta_3\gamma$. A special feature of cyanobacterial and chloroplast F1 is an inserted amino acid sequence in the γ-subunit. In chloroplasts, the insertion is slightly extended and additionally contains two cysteines thus enabling thiol modulation. This higher plant-specific molecular switch was transferred to a chimera F1 by inserting the small cysteine-containing chloroplast sequence into a cyanobacterial γ-subunit [1]. Reduction of the disulfide bridge elevates latent ATPase and might represent a mechanism to prevent wasteful ATP hydrolysis in the dark. Although there are indications that suppression of ATP hydrolysis under oxidizing conditions involves ADP inhibition properties [1], the propagation of structural information between the site of thiol modulation in the γ-subunit and the catalytic sites in the β-subunits remains unclear. Here, a chimera F1 redox mutant study concluded a critical interplay between the γ-subunit "neck" region and the turn region of the βDELSEED-loop (i.e. βI397 and βL398, ATP synthase of *Thermosynechococcus elongatus* BP-1 numbering). Several mutants were designed with non-effective, weakened, enhanced and reversed thiol modulation. At selected positions of putative interactions, redox regulation of ATP hydrolysis appeared to be affected by substituent properties such as hydrophobicity and bulkiness. Removal of charged residues within the βDELSEED motif suggested that ionic interactions with the γ-subunit play a less important role in processing thiol modulation. For the first time, β-subunit residues were assigned to receive information produced by disulfide/dithiol formation in the γ-subunit. [1] Y. Kim, H. Konno, Y. Sugano, T. Hisabori, Redox regulation of rotation of the cyanobacterial F1-ATPase containing thiol regulation switch, J. Biol. Chem. 286 (2011) 9071–9078.

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S1.P5

Generation of a mitochondrial membrane potential in the absence of mtDNA: Can we obtain fresh insight by going back to the root?

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Increasing evidence places the root of the eukaryotic tree of life between the supergroup Excavata and all other eukaryotes, making these organisms important subjects to study fundamental aspects of mitochondrial biology and bioenergetics. The excavate parasite *Trypanosoma brucei* shows unusual and fascinating mitochondrial features, including a gigantic organellar DNA network (the kinetoplast or kDNA) and extensive posttranscriptional mRNA editing. As *T. brucei* switches between insect and mammalian hosts, mitochondrial function and energy metabolism undergo dramatic transformation. In insect stage parasites, oxidative phosphorylation requires FoF1-ATP synthase to generate ATP. In the bloodstream stage the enzyme operates as a proton pump to maintain the essential mitochondrial membrane potential, ΔΨm. In *T. brucei*, ATP synthase subunit 6 (a), an Fo subunit critical for proton transport, is kDNA-encoded, but trypanosomes can exist without kDNA due to specific mutations (e.g. L262P) in the nuclearly-encoded gamma subunit of F1 [1], a situation reminiscent of petite-negative yeast [2]. In yeast and mammalian cells, survival of mtDNA loss is proposed to use an alternative mode for generating ΔΨm that involves the ADP/ATP carrier and F1 [2], but the precise mechanism remains unclear. We use kDNA0 bloodstream form *T. brucei* to investigate: 1) The consequences of the gamma mutation on FoF1 structure, 2) the molecular mechanism of compensation for kDNA loss, and 3) the consequence of kDNA deletion on life cycle progression. High resolution clear native electrophoresis shows dramatic consequences to FoF1 structure in the presence of an L262P mutation. Cells show reduced or abolished expression of subunit 6 and oligomycin resistance. The apparent selection for cells that no longer express intact FoF1 suggests that L262P uncouples the enzyme, resulting in a lethal proton leak. Differentiation studies demonstrate that cells do not require kDNA for formation of transmissible intermediate stages, but that kDNA is indispensable for progression to the insect form. [1] S. Dean, M.K. Gould, C.E. Dewar, A.C. Schnaufer, Single point mutations in ATP synthase compensate for mitochondrial genome loss in trypanosomes, Proc. Natl. Acad. Sci. U.S.A., 110 (2013) 14741–14746. [2] X.J. Chen, G.D. Clark-Walker, Specific mutations in alpha- and gamma-subunits of F1-ATPase affect mitochondrial genome integrity in the petite-negative yeast Kluyveromyces lactis, EMBO J, 14 (1995) 3277–3286.

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S1.P6

Dimer and monomer of F1F0-ATP synthase in *Ustilago maydis*: Structural composition

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The F1F0-ATP synthase produces more than 90% of cellular ATP coupled to the proton electrochemical potential. This enzyme consists of two major sectors, the sector F0 which contains the c9–10, a and b subunits, and is powered by the proton electrochemical potential; and the soluble and catalytic sector F1, containing the alpha, beta, gamma, delta and epsilon subunits. In yeast and mammalian mitochondria, F0 contains an additional protein called OSCP [1–2]. In the last years, complex V has been described as a homo-dimer (V2), which plays a role in the architecture of the mitochondrial cristae; particularly, V2 is recognized as the main entity involved in the structure of the crista tips. Actually, the identity of the dimerizing proteins suggests that this is species-specific. Although the knowledge about V2 composition and stoichiometry and its role in the crista architecture has been studied, little is known about the kinetic behavior of this homo-oligomer. In this work, the dimer (V2) and the monomer (V1) of the mitochondrial F1F0-ATP synthase from the dimorphic basidiomycete *Ustilago maydis* were studied. The V1 and V2 were solubilized with the soft detergent digitonin and isolated using a sucrose gradient (1.5 to 0.5 M), and their subunit composition obtained by MS/MS. The V2 contained all V1 subunits, plus subunits e, g and h. Analysis of the sequences of the subunits shows that alpha subunit was shorter than beta subunit. However the alpha gene had similar length than their homologues; this suggested a post-translational modification of this subunit without affecting its ATP hydrolysis activity. Additionally, the prohibitins (PHB1 and PHB2) and ADP/ATP translocase were identified in both oligomeric forms of the F1F0-ATP synthase. [1] F. Minauro-Sanmiguel, S. Wilkens, J.J. García, Structure of dimeric mitochondrial ATP synthase: novel F0 bridging features and the structural basis of mitochondrial cristae biogenesis, PNAS. 102 (2005) 12356–12358. [2] S. Couoh-Cardel, S. Uribe-Carvajal, S. Wilkens, J.J. García-Trejo, Structure of dimeric F1F0-ATP synthase, The Journal of biological chemistry. 285 (2010) 36447–36455.

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