

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 β DELSEED-loop (i.e. β I397 and β L398, 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 β DELSEED 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, $\Delta\Psi_m$. 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 $\Delta\Psi_m$ 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. Schnauffer, 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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