

- [1] Morales-Rios E., de la Rosa-Morales F., Mendoza-Hernandez G., Rodriguez-Zavala J.S., Celis H., Zarco-Zavala M., Garcia-Trejo J.J. (2010) *FASEB J.* **24**:599-608.
- [2] Minauro-Sanmiguel F., Bravo C., Garcia J.J., (2002) *J. Bioenerg. Biomembr.* **34** 433-443.

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## 1P52

### Studies of ATP synthase from Mycobacteria

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The ATP synthase catalyses the synthesis of ATP from ADP and Pi using a transmembrane proton motive force generated by the oxidative respiratory chain. The enzyme can also operate in reverse as an ATP-dependent proton pump. The ATP synthase from *Mycobacterium tuberculosis* was identified to be the molecular target of a new anti-tuberculosis drug, diarylquinoline TMC207 [1]. Detailed functional and structural studies of mycobacterial ATP synthase are required to understand how the enzyme could be exploited as a drug target. We are working on the ATP synthase from *Mycobacterium smegmatis*. It is a non-pathogenic, fast-growing mycobacterium and is an ideal model for *Mycobacterium tuberculosis*. Initial studies have been focused on the hydrophilic catalytic F<sub>1</sub> domain of the *M. smegmatis* ATP synthase lacking the delta subunit (MF<sub>1</sub>). The MF<sub>1</sub>-ATPase has been over-expressed in a mycobacterial expression system. The recombinant MF<sub>1</sub>-ATPase has been purified. Like F<sub>1</sub>-ATPases from some other eubacteria including *M. tuberculosis* it has a latent ATP hydrolysis activity, which is activated by treatment with trypsin or detergents. Crystals of the purified enzyme currently diffract X-rays to 3 Å resolution at a synchrotron source.

### Reference

- [1] K. Andries, P. Verhasselt, J. Guillemont, H.W. Gohlmann, J.M. Neefs, H. Winkler, J. Van Gestel, P. Timmerman, M. Zhu, E. Lee, P. Williams, D. de Chaffoy, E. Huitric, S. Hoffner, E. Cambau, C. Truffot-Pernot, N. Lounis, V. Jarlier, A diarylquinoline drug active on the ATP synthase of *Mycobacterium tuberculosis*, *Science* **307** (2005) 223-227.

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## 1P53

### Heterologous expression of the assembled and functional F<sub>1</sub>F<sub>0</sub> ATP synthase from *A. aeolicus* in *E. Coli*

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The F<sub>1</sub>F<sub>0</sub> ATP synthase catalyzes the synthesis of ATP from ADP and inorganic phosphate driven by ion (H<sup>+</sup> or Na<sup>+</sup>) motive forces across the membrane. The hyperthermophilic bacterium *A. aeolicus* possesses a nine-subunit F<sub>1</sub>F<sub>0</sub> ATP synthase (AAF<sub>1</sub>F<sub>0</sub>). Its genes are split into four clusters in the genome. AAF<sub>1</sub>F<sub>0</sub> differs from the F<sub>1</sub>F<sub>0</sub> ATP synthases of non-photosynthetic organisms, because it possesses a hetero-, not homodimeric peripheral stalk, composed of the b-subunits. Together with its high thermal stability, such unique properties make AAF<sub>1</sub>F<sub>0</sub> an interesting target for studying the mechanisms of energy conservation at the molecular level. Therefore, to complement the work on the native AAF<sub>1</sub>F<sub>0</sub>, we set out to produce it heterologously in *E. coli* (EAF<sub>1</sub>F<sub>0</sub>).

We could successfully express the subcomplexes F<sub>0</sub>-b<sub>1</sub>b<sub>2</sub>, F<sub>1</sub>-α<sub>3</sub>β<sub>3</sub>γ, F<sub>1</sub>-α<sub>3</sub>β<sub>3</sub>γε and the entire complex F<sub>1</sub>F<sub>0</sub>-α<sub>3</sub>β<sub>3</sub>γεab<sub>1</sub>b<sub>2</sub>c in *E. coli* by using constructs containing native and artificial operons. After purification by affinity chromatography, we characterized the complexes by blue native PAGE, Western-blotting using antibodies specifically developed against AAF<sub>1</sub>F<sub>0</sub> subunits, size exclusion chromatography, and mass spectrometry, showing that they correctly contain all respective subunits. Moreover, we could detect the assembled EAF<sub>1</sub>F<sub>0</sub> complexes by single-particle electron microscopy. Finally, we could show that the EAF<sub>1</sub>F<sub>0</sub> purified from *E. coli* possesses a comparable ATP-hydrolysis activity to AAF<sub>1</sub>F<sub>0</sub>, using in-gel activity and phosphate determination assays.

In conclusion, our system allows the generation of AAF<sub>1</sub>F<sub>0</sub> in *E. coli*, thus offering advantages over the use of costly and non-manipulatable *A. aeolicus* cells. The recombinant AAF<sub>1</sub>F<sub>0</sub> is amenable to new experimental approaches, such as site-directed mutagenesis, *in vivo* complementation assays, and amino acid labeling. Therefore, our work provides a valuable genetic platform to study the structure and function of AAF<sub>1</sub>F<sub>0</sub>.

- [1] Peng G, Bostina M, Radermacher M, Rais I, Karas M, Michel H (2006) *FEBS Lett* **580**: 5934-5940.
- [2] Deckert G, Warren PV, Gaasterland T, Young WG, Lenox AL, Graham DE, Overbeek R, Snead MA, Keller M, Aujay M, Huber R, Feldman RA, Short JM, Olsen GJ, Swanson RV (1998) *Nature* **392**: 353-358.
- [3] LeBel D, Poirier GG, Beaudoin AR (1978) *Analytical biochemistry* **85**: 86-89.

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