



SEARCH FOR COMPOUNDS ABLE TO MODULATE F_0F_1 ATP SYNTHASE IN SWITCHING FROM LIFE ENZYME TO CELL DEATH EXECUTOR

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Abstract — Mitochondria are the main site of energy power in eukaryotic cells. The enzyme F_0F_1 synthase is responsible for ATP production driven by the transmembrane proton gradient. The maintenance of a very low permeability of the inner mitochondrial membrane is crucial for this mechanism, since sudden opening of the permeability transition pore (PTP) leads to matrix swelling and outer membrane rupture, with release of pro-apoptotic factors. Recently, it has been suggested that dimers of ATP synthase in mammals could represent the main component of the mitochondrial PTP, a feature modulated by calcium and involving the matrix protein Cyclophilin D (CyPD). This study would help to develop new tools for the identification of plant secondary metabolites, in particular flavonoids, able to modulate PTP and therefore acting on the programmed cell death mediated by mitochondria. Therefore, this project would represent the first screening for plant molecules able to interfere with programmed cell death, as a preliminary study for the development of drugs active in PTP-related pathologies.

Index Terms — TRANS2CARE, Cyclophilin ATP synthase, permeability transition pore, programmed cell death, cyclophilin D

1 BACKGROUND

Mitochondria are double-membrane organelles, which represent the energy power of eukaryotic cells. In aerobic conditions, the production of ATP in mitochondria is exerted by F_0F_1 ATP synthase. Nevertheless, mitochondria are also involved in the early stages of programmed cell death (PCD) through the release of pro-apoptotic factors from the intermembrane space. Such a release can be due to a sudden opening of the inner mitochondrial membrane permeability transition pore (PTP) to small molecules. This implies a matrix swelling, leading to outer membrane rupture and release of pro-apoptotic factors. Recently, it has been proposed that ATP synthase, when present as a dimer, forms the mitochondrial PTP in the presence of high calcium concentration (Bernardi, 2013). Consistently, the modulator of permeability transition, the matrix protein Cyclophilin D (CyPD), has been identified also as ATP synthase interaction factor (Giorgio et al., 2009).

Evidence of PTP formation by ATP synthase dimers has been obtained in mammalian mitochondria (Giorgio et al., 2013), although the underlying mechanisms remain to be established. Furthermore, little information is available for PTP in other organisms, where mitochondria are involved in PCD, in particular in plants (Vianello et al., 2012).

2 OBJECTIVES

This research will contribute to clarify the enzymatic mechanisms that allow the switch of ATP synthase to PTP formation and the physiological effectors involved in this process. Potential compounds, such as plant secondary metabolites (e.g. flavonoids), which could interfere with PTP formation will be identified. Then, their ability to modulate PCD pathway, mediated by mitochondria, will be examined. This would represent a preliminary step for the development of drugs active in PTP-related pathologies (tumors, ischemia, neurodegenerative diseases).

3 APPROACH & METHODS

General approach

The structural and functional properties of ATP synthase from different origins will be compared to define the essential elements involved in the process. This will constitute the basis for the following step in which molecules, able to modulate the PTP/ATP synthase switch activity, will be identified.

Methods

The structural and functional characterization of the ATP synthase will be performed by proteomic and immunological approaches. The mammal model will be used for comparison with ATP synthase from plant mitochondria. PTP formation will be followed by spectrophotometric and fluorimetric assays. The role of essential components of ATP synthase for PTP formation will be tested by knock-out mutants of *Arabidopsis*. The potential modulators of ATP synthase switch to PTP in both mammalian and plant mitochondria will be tested by fluorimetric techniques.

4 RESULTS

Dimerization of ATP synthase has been characterized in mammalian mitochondria by biochemical approaches, such as native electrophoresis, immunoblotting and enzymatic activity detected *in situ* (Giorgio et al., 2009). Identification of ATP synthase interaction factors has been performed by immunoprecipitation and cross-linking agents. It has been demonstrated that ATP synthase dimers

interact through the lateral stalk (OSCP subunit) with the matrix protein Cyclophilin D (CyPD), which is a well-known inducer of PTP closure, and such interaction is weakened by PTP-blocker Cyclosporin A (CsA) (Fig. 1) (Giorgio et al., 2009; 2013). Moreover, when isolated ATP synthase dimers were inserted into planar lipid bilayer, electrophysiological measurements demonstrated that, in the presence of calcium, they are able to form a channel with properties identical to those described for PTP (Giorgio et al., 2013). A hypothetical model of ATP synthase transition to PTP has been then formulated (Bernardi, 2013) (Fig. 2).

Plant mitochondria possess a similar active ATP synthase complex, but it has not been associated to PTP formation yet. Nevertheless, some components have been identified, such as OSCP and CyPD, but it is still not clear if they could interact in PTP modulation.

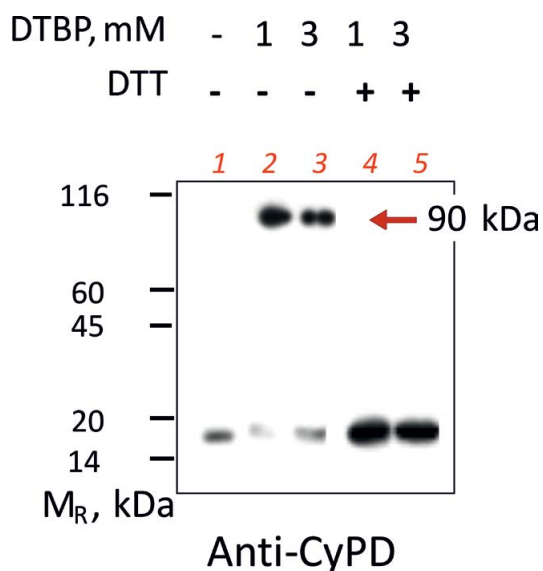


Figure 1: CyPD interacts with mitochondrial ATP synthase through the lateral stalk connecting F_0F_1 . ATP synthase from mammalian mitochondria was separated by SDS-polyacrylamide gel electrophoresis and immunodetected with Ab against CyPD, before (lane 1) and after treatment with the crosslinker DTBP in absence (lanes 2 and 3) or presence of DTT (lanes 4 and 5) able to revert the effect. The 90 kDa crosslinked proteins (arrow) reacted also with Ab against the lateral stalk subunits of ATP synthase (not shown) (modified from Giorgio et al., 2009).

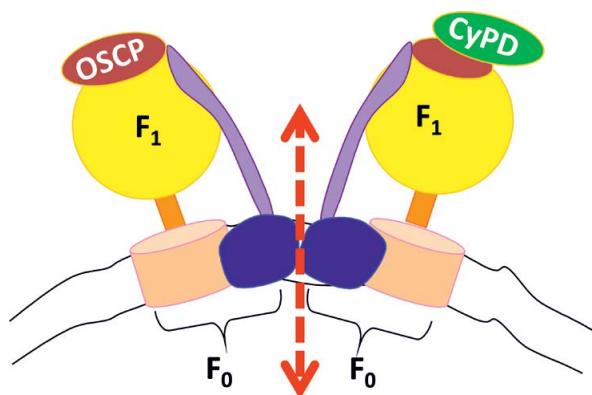


Figure 2: Hypothetical transition of F_0F_1 ATP synthase dimers to form the PTP. ATP synthase dimers can undergo PTP formation when Ca^{2+} rather than Mg^{2+} is bound, possibly at the catalytic sites, in a reversible process favored by binding of CyPD to OSCP. Adenine nucleotides counteract PTP formation in synergy with Mg^{2+} . Red arrow denotes the hypothetical pathway for solute diffusion between two FO subunits (adapted from Bernardi, 2013).

5 POTENTIAL NEW PRODUCTS & SERVICES

This study will allow the identification of plant secondary metabolites (i.e. flavonoids) able to modulate ATP synthase switch to PTP. This would represent the first screening toward the realization of potential natural pharmaceuticals/drugs able to interfere with programmed cell death. Furthermore, the analysis of the components involved in ATP synthase activity and regulation would lead to identify proteins correlated to PCD. Their quantification in different organisms/tissues by immunological/proteomic techniques would lead to design diagnostic tools for PTP-related pathologies.

6 CURRENT COLLABORATIONS

6.1 With other researchers

University of Padova, Department of Biology, Department of Biomedical Sciences; University of Udine, Department of Food Sciences; University of Trieste, Department of Life Sciences (LP, Trans2Care).

7 CONTACT OR COLLABORATIONS NEEDED

Future collaboration with clinical laboratories is needed.

8 COMMUNICATION TOOLS

- The high level of expertise in the field of biochemistry, as well as the high level of applicability, quality and performance of the presented research will be disseminated through high quality scientific publications.
- This research has been presented to the biomedical community at National and International congresses.

9 FUNDS NEEDED

9.1 For basic research (investigation of biological mechanisms): € 40,000

9.2 For applied research (solutions for real-world problems): € 100,000

9.3 For pilot & demonstrator activities (to develop a prototype): € 150,000

10 CONCLUSIONS

The mitochondrial permeability transition is an essential event in mediating the early stage of the PCD pathways. The recent evidence of ATP synthase involvement in PTP formation opened an innovative and still unexplored research field to find agents able to modulate the PTP. This investigation would open new opportunities to identify plant natural products able to interfere with the PTP phenomenon and therefore would represent the first step to design diagnostic tools for PTP-related pathologies, which comprise a wide range of human diseases.

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