

Soutenance

Soutenance de thèse

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A 14h - Salle des séminaires de l'IBS

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Probing the oligomeric organization of the mitochondrial ADP/ATP carrier in native membranes

Thèse de Doctorat de l'Université Joseph Fourier

The transport of small molecules through the inner mitochondrial membrane is essential in eukaryotic metabolism and is selectively controlled by a family of integral membrane proteins, the Mitochondrial Carrier Family (MCF). The ADP/ATP carrier (AAC), which is responsible for the import of ADP to the matrix of mitochondria and the export of newly synthesized ATP toward the cytosol, is the best-known and characterized MCF member. Although its structure sheds light on several aspects of the carrier activity, additional investigations are still required to decipher the whole transport mechanism, to understand the specificity and to characterize the controversial oligomeric state of the protein. For many years, based on studies mainly carried on detergent solubilized AAC the general consensus has been in favor of a dimeric organization of the carrier. The AAC three-dimensional structure, monomeric, broke this dogma.

In order to get a precise insight into the *in vivo* oligomeric organization of AAC we combined several approaches. Fluorescence resonance energy transfer (FRET) measurements were performed directly on mammalian and *E.coli* cells expressing AAC labeled with several types of FRET probes. In parallel, different functional assays were established to control the state of the mitochondria in these cells and the transport activity of these AAC fusions. Lastly, measurements of the respiration rate coupled to the titration of the inhibitory effect of carboxyatractyloside on isolated rat liver mitochondria were used to investigate the organization of AAC in native mitochondria within two regimes of oxidative phosphorylation.

Taken together the results described herein revealed that 1) AAC can function mechanistically as a monomer, 2) the organization of AAC in native membranes might be related to the state of the mitochondria and be involved in regulation