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ASSAYING ATP SYNTHASE ROTOR ACTIVITY

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1. INTRODUCTION

Current focus during non-invasive investigations into disturbances of oxygen utilization is on either the level of NADH, using techniques such as near infra-red spectroscopy (NIRS), or on ATP/ADP,Pi ratios, by techniques such as NMR or MRI. These tools bracket the process of oxidative phosphorylation. In order to investigate molecular disturbances further, it is necessary to resort to destructive techniques, such as electron transport chain complex analysis by enzyme assay following tissue homogenisation, or mutation analysis following DNA extraction. Although these investigations are extremely informative in many instances, the enzyme assays are technically challenging even in competent hands. There is also always doubt about the integrity of the tissue before and during assay. Therefore those assays are routinely normalized against a mitochondrial 'housekeeping' enzyme, citrate synthase. Furthermore one of these enzymes, ATP synthase, can only be measured as its reverse reaction, as an ATP hydrolase (ATPase). So unreliable are the techniques available for this assay, however, that values for this enzyme are generally not reported in routine laboratory diagnosis. One published exception to that statement is the report by Baracca et al.[1] who studied ATP synthesis in isolated sub-mitochondrial particles prepared from platelets of two individuals with mitochondrial DNA T8993G mutations in the ATPase 6 gene encoding subunit a. By contrast, the mechanism of this enzyme complex and its role in the fundamental process by which mitochondria convert chemical energy in the form of food into the usable energy currency of the cell, namely ATP, is now known down to the molecular level[2-4]. Several reports have demonstrated the mechanism of ATPase function in vitro[2-4]. It is the purpose of the present work to assess whether it might be possible to harness this knowledge to design cellular probes to assay the activity of complex V in its synthetic mode in vivo.

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2. STRUCTURAL AND MECHANISTIC STUDIES ON ATP SYNTHASE

The structure of the complexes of the electron transport chain (ETC) in humans and other mammals has been largely predicted from comparative studies using bacterial or yeast models. The tools in those investigations include DNA and protein sequencing and comparative genomics and proteomics, coupled with secondary structure studies. Confirmation of those predictions has been made possible by X–ray diffraction and solution NMR[5]. From the results of such approaches, it has been possible to build representations of complex V such as that shown in Figure 1. The human complex V is believed to consist of at least eight different subunit components in total assembled into the two sectors identified by classical biochemical techniques and electron microscopy as F_1 and F_0. These sectors have subunit composition $\alpha_3\beta_3\gamma_1\delta_1\varepsilon_1$ and $a_1b_2c_{12}$ respectively. The mechanism of ATP generation in this model involves a rotor (of stoichiometric composition $c_{12}\gamma_1\varepsilon_1$) rotating clockwise when viewed from the mitochondrial matrix side in a stator that consists of $a_1b_2\alpha_3\beta_3\delta_1$ subunits. Gavin, Devenish and Prescott[6] have implicated two other subunits in anchoring the stator within the membrane, namely OSCP and subunit b. In recent years, several investigators [2,3], have turned their attention to the physical demonstration of the mechanism of the ATP synthase rotor.

Such approaches generally have used non-mammalian ATP synthase as the in-vitro study material and laser microscopy as the investigating tool. The techniques used in those studies have necessarily involved analysis of the reverse reaction, i.e. ATPase analysis. A typical such investigation involves the attachment of some component of the structure to a solid surface and the attachment of a signalling molecule to another part of the structure. Using such approaches, it has been convincingly demonstrated that ATPase activity is indeed accompanied by rotation at a rotary torque of 40 pN nm [2,7].

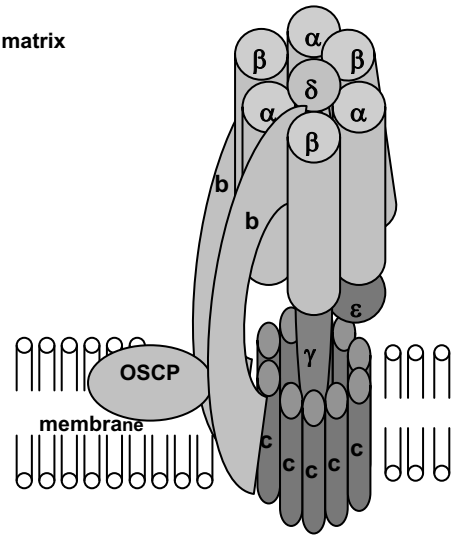

Figure 1. Model of ATP synthase (Complex V of oxidative phosphorylation). Rotation of the rotor ($c_{12}\gamma_1\varepsilon_1$) within the stator is driven by proton binding with c subunits through a channel in the stator ($a_1b_2\alpha_3\beta_3\delta_1$). An additional component to anchor the stator within the membrane (OSCP) is shown although precise positioning within the complex is speculative.