THE MITOCHONDRIAL F$_0$-F$_1$ ADENOSINE TRIPHOSPHATASE:

ISOLATION BY SEPHAROSE HEXYLAMMONIUM CHROMATOGRAPHY

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INTRODUCTION

Most ATP is synthesized by oxidative and photophosphorylation in mitochondria and chloroplasts respectively. Peter Mitchell proposed a theory which formulates that ATP is synthesized by the ATPase complex utilizing an electrochemical gradient generated by substrate oxidation or by light$^1$. The energy stored in this electrochemical potential is also utilized as a driving force in other processes such as active transport and motility.

After nearly two decades of controversy the general features of this hypothesis now have firm experimental support. It is now widely accepted that proton translocation and the role of $\Delta \mu \text{H}^+$ are crucial for ATP synthesis although arguments persist over the details of the coupling pathway and mechanisms$^2$. It has been shown that the coupling factor F$_0$-F$_1$ is responsible for the synthesis of ATP after it was reconstituted in liposomes in which this complex was the only protein component present$^3$-$^6$. The generation of the $\Delta \mu \text{H}^+$ to drive ATP synthesis can be achieved artificially by changing the external pH or generation of a K$^+$-diffusion potential with valinomycin.

The F$_0$-F$_1$ ATPase complex is composed of two sectors the proton translocating portion (F$_0$) which is an intrinsic membrane component and the soluble F$_1$ sector. They can be separated and their functions studied as autonomous entities. These $\text{H}^+$-ATPases are found in mitochondria, chloroplasts and many bacteria$^7$ and are remarkably similar in structure.
The sector of the complex catalyzing ATP synthesis which is the $F_1$ portion can be easily dissociated from the membrane and is soluble in aqueous buffers lacking detergents. The portion of the molecule remaining in the membrane after removal of $F_1$ is $F_0$, often termed proton channel.

Two models have been very helpful in the study of this complex. The $F_0$-$F_1$ ATPase from thermophilic bacterium$^3$ and the one from *Escherichia coli*$^8$ these two complexes have been thoroughly studied.

On the one hand the reconstitution studies which were performed in PS3 thermophilic bacterium were indeed very encouraging for further research on the same line with other $F_0$-$F_1$ systems. On the other, knowledge of the structure and function of the ATPase complex from *E. coli* derived from genetic studies developed very fast. The first mutant affecting the $F_1$ ATPase described by Butlin et al$^9$ was the beginning of this challenging field.

The $F_1$ portion of the complex is composed in most systems of five subunits termed $\alpha$, $\beta$, $\gamma$, $\delta$, $\varepsilon$ in order of decreasing molecular weight$^7$. In mammalian systems and endogenous low molecular weight polypeptide also known as the natural ATPase inhibitor$^{10}$ has been described. This protein is believed to regulate the catalytic activity of the enzyme$^{11-13}$.

The hydrophobic membrane sector of the ATPase complex in eukariotic systems is not well known. It catalyzes the conduction of protons from one side of the membrane to the other in a passive manner. Proton conducting abilities of proteolipids extracted with organic solvents have been demonstrated$^{14-15}$ although the resulting preparations show different sensitivities to proton channel inhibitors depending apparently on the organic solvent used for the extraction.

In bacteria it is composed of three subunits commonly known as $\alpha$, $\beta$ and $c$$^{16,17}$. Subunit $c$ binds Dicyclohexylcarbodiimide (DCCD) in a stoichiometric fashion resulting in a complete inhibition of proton conductance.

The link between $F_0$ and $F_1$ in mitochondria is believed to occur by means of three polypeptides these are the oligomycin sensitivity conferring protein (OSCP), $F_6$ and $F_B$ which have been well characterized$^{18-20}$. The approach now is to determine the interactions between these polypeptides and other subunits of the ATPase complex.

The study of the mitochondrial $F_0$-$F_1$ complex has been attempted by several groups$^{21-25}$ by solubilizing the complex with various detergents, the purification procedures involve mainly molecular sieve chromatography and differential centrifugation.