Allotopic Expression of a Gene Encoding FLAG Tagged-subunit 8 of Yeast Mitochondrial ATP Synthase

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Subunit 8 of yeast mitochondrial ATP synthase is a polypeptide of 48 amino acids encoded by the mitochondrial ATP8 gene. A nuclear version of subunit 8 gene has been designed to encode FLAG tagged-subunit 8 fused with a mitochondrial signal peptide. The gene has been cloned into a yeast expression vector and then expressed in a yeast strain lacking endogenous subunit 8. Results showed that the gene was successfully expressed and the synthesized FLAG tagged-subunit 8 protein was imported into mitochondria. Following import, the FLAG tagged-subunit 8 protein assembled into functional mitochondrial ATP synthase complex. Furthermore, the subunit 8 protein could be detected using anti-FLAG tag monoclonal antibody.

Key words: allotropic expression, ATP synthase, mitochondria, yeast

The adenosine triphosphate synthase (ATP synthase) also known as F\textsubscript{1}F\textsubscript{0}-ATPase is primarily responsible for production of energy used to drive cellular processes. The enzyme catalyses formation of adenosine triphosphate (ATP) from adenosine diphosphate (ADP) and inorganic phosphate (Pi) at the expense of the transmembrane electrochemical proton gradient generated by respiration chain. ATP synthase can also hydrolyse ATP and form a transmembrane proton gradient which can be used to facilitate ion transport and substrate uptake (Attardi & Schatz 1988).

The ATP synthase is a multisubunit complex. In yeast, the enzyme is composed of at least 17 subunits grouped into two components, F\textsubscript{1} and F\textsubscript{0} components. The F\textsubscript{1} component is comprised of subunits α, β, γ, δ, and ε, with stoichiometry of 3:3:1:1:1 (Cox et al. 1992). The F\textsubscript{0} component is composed of subunits b, oligomycin-sensitivity conferring protein (OSCP), d, e, f, g, h, i/j, k which are encoded by nuclear genes, and subunits 6, 8, and 9, which are encoded by mitochondrial genes (Stephens et al. 2000). The catalytic sites lay in the F\textsubscript{1} component while proton conductance takes place in the F\textsubscript{0} component. Based on studies carried out in Escherichia coli, it has been established that the catalytic mechanism of ATP synthase involves rotation of both subunits γ and ε (Noji et al. 1997). The enzyme therefore works as a molecular rotary motor (Senior & Weber 2004). It has recently been proven that the direction of subunit γ rotation during ATP synthesis is opposite to that of ATP hydrolysis (Diez et al. 2004).

The subunit 8 of yeast mitochondrial ATP synthase is a polypeptide of 48 amino acids with predicted molecular weight of 5.8 kDa (Macreadie et al. 1983). The protein is encoded by the mitochondrial ATP8 gene. As a mitochondrially encoded protein, subunit 8 is transcribed and translated within the organelle. Subunit 8 of the yeast mitochondrial ATP synthase is considered to be the homologue of the mammalian subunit 8. Interestingly, however, no homologue of subunit 8 is found in Escherichia coli (Cox et al. 1992). It has been considered that subunit 8 has an essential structural role in the mitochondrial ATP synthase, being required for the assembly of a functional F\textsubscript{0} component (Marzuki et al. 1989).

Detail analysis of subunit 8 structure and function is still lacking. Although subunit 8 has been considered to participate in proton translocation, the exact role of subunit 8 in this function remains unclear.

A major problem in applying mutagenesis techniques to study structure and function of mitochondrially encoded protein such as subunit 8 is the lack of a reliable method for delivering mutagenised genes back into the mitochondria. To circumvent this problem, allotropic expression system (diagrammatically illustrated in Figure 1) has been developed for subunit 8 (Gearing & Nagley 1986). Allotopic expression is deliberate relocation of organellar genes to the nucleus and delivery of the gene products from the cytoplasm to the corresponding organelle (Nagley & Devenish 1989). For allotopic expression of subunit 8 gene, Gearing et al. (1985) designed a completely novel subunit 8 encoding DNA sequence to be expressed in the nucleocytoplasmic system. In this nuclear version of subunit 8 gene, as many as 31 codons out of 48 natural mitochondrial codons were altered. One codon (for Thr22) had to be changed to comply with the nuclear codon dictionary, otherwise the mitochondrial CTA codon for threonine would be translated to leucine on the cytosolic ribosomes. The other 30 codon changes were made essentially to optimize the expression of subunit 8 gene. To ensure that the cytoplasmically synthesized subunit 8 was imported into mitochondria, a DNA sequence encoding a mitochondrial signal peptide was fused to the N-terminus of the subunit 8 gene.
Upon four days of incubation (Figure 3) indicated that the medium. Growth of transformants on ethanol medium observed M31, transformant colonies were transferred onto solid ethanol subunit 8 rescued the ethanol negative phenotype of the strain M31. The strain M31 is lack of endogenous subunit expression. The resulted recombinant plasmid was then used encoding the FLAG tagged-subunit 8 fused with mitochondrial subunit 8 topology and its interaction with other subunits. Detecting subunit 8 protein. This will provide tool for probing provide means for the use of immunochemistry methods for the present study, the subunit 8 is FLAG tagged in order to in vivo, the gene ATP8 gene, Su8 = subunit 8, SP = mitochondrial signal peptide, AS = ATP synthase subunit 8 is an essential subunit of the mitochondrial ATP synthase complex, the strain M31 is therefore unable to form functional mitochondrial ATP synthase. The M31 strain expressing FLAG-tagged subunit 8 protein successfully assembled into functional enzyme complexes. The M31 strain expressing FLAG-tagged-subunit 8 was then denoted strain FTC2.

The purpose of epitope tagging subunit 8 is to enable detection of subunit 8 protein using a specific antibody with high affinity to the tag. The ability to detect subunit 8 protein is important in analyzing subunit 8 topology and probing subunit 8 interaction with other subunits. The subunit 8 gene employed in the present study has been modified so as to incorporate coding sequences for a hexapeptide FLAG epitope tag (DYKDDD) at the C-terminus (see Figure 2). A commercially available anti-FLAG antibody (M2) is used to detect the tagged-artificial (nuclear version) ATP8 gene. The amino acid sequence of subunit 8 of yeast mitochondrial ATP synthase is shown in standard three letter amino acid code. Numbers refer to amino acid residues. The codon for natural mitochondrial ATP8 gene and the artificial (nuclear version) ATP8 gene are shown aligned. Different codons used in artificial gene are indicated by underline. Residues of the FLAG tag are indicated in bold. The subunit 8 and the FLAG tag are bridged by two serine residues. The nucleotide sequence of the mitochondrial signal peptide fused to the N-terminus of A-ATP8 gene is not shown. N-ATP8: natural mitochondrial ATP8 gene, A-ATP8: artificial (synthetic) ATP8 gene, *: stop codon.
The proteins were then transferred to PVDF membrane. The membrane was cut into two portions. One portion of the membrane containing subunit 8 protein was probed with anti-FLAG M2 antibody as a primary antibody. The second portion of the membrane containing subunit γ protein was probed with anti-subunit γ antibody. Detection of subunit γ was intended as a positive control.

Results (Figure 4) showed that the FLAG tagged-subunit 8 protein could be detected using anti-FLAG M2 monoclonal antibody. Subunit γ was also detectable in each sample. As expected, the non-tagged-subunit 8 protein isolated from the YM2 strain was not detectable.

The main goal of the present study is to examine as to whether the allotopically expressed FLAG tagged-subunit 8 protein is able to assemble into functional mitochondrial ATP synthase complexes as well as to ensure that the expressed FLAG tagged-subunit 8 can be detected using anti-FLAG tag monoclonal antibody. The present study has demonstrated that the allotopically expressed FLAG-tagged subunit 8 protein assembles into functional mitochondrial ATP synthase complexes. This indicates that the addition of FLAG tag at the C-terminus of subunit 8 protein does not abolish its ability to assemble and function in the enzyme complex. In addition, the FLAG tagged-subunit 8 protein can be detected using the anti-FLAG monoclonal antibody. The present study has therefore provided a further system for facilitating elucidation of subunit 8 structure and for probing subunit 8 interaction with other proteins in the mitochondrial membrane.

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Figure 3. Functional assessment of allotopically expressed FLAG tagged-subunit 8. Host cell (M31) and transformants (FTC2) were grown on solid ethanol medium. The growth of FTC2 on ethanol medium indicated that the FLAG tagged-subunit 8 is successfully imported into mitochondria upon its translation in the nucleocytosolic system, and is assembled into functional mitochondrial ATP synthase complex.

Figure 4. Detection of the FLAG tagged-subunit 8 protein. FLAG tagged-subunit 8 protein contained in mitochondrial lysate was detected in a Western blot analysis using the anti-FLAG M2 monoclonal antibody. Mitochondrial lysates were prepared from strains YM2 (strain M31 expressing subunit 8 without FLAG tag) and FTC2. The presence of subunit γ was also probed using anti-subunit γ antibody. γ = subunit γ of yeast mitochondrial ATP synthase, Su8 = subunit 8 of yeast mitochondrial ATP synthase.