

Structural and Functional Analysis of FLAG Tagged-Subunit 8 of Yeast *Saccharomyces cerevisiae* Mitochondrial ATP Synthase

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Abstract

Yeast mitochondrial ATP synthase is a multisubunit complex composed of at least 17 different subunits. Subunit 8 of yeast mitochondrial ATP synthase is a hydrophobic protein of 48 amino acids encoded by the mitochondrial *ATP8* gene. Although ATP synthase from eukaryotes and prokaryotes shows a similar basic structure, no homologue of subunit 8 is found in prokaryotes such as *Escherichia coli*. Subunit 8 has three distinct domains; an N-terminal domain, a central hydrophobic domain and a C-terminal domain. In order to elucidate its structure and function, a set of nuclear genes encoding subunit 8 variants was designed to incorporate a FLAG tag at the C-terminus and a mitochondrial signal peptide at the N-terminus. Each gene was cloned into a yeast expression vector and then allotopically expressed in a yeast strain lacking endogenous subunit 8. Structural and functional analysis showed that the hydrophobic character of the central hydrophobic domain of subunit 8 is critical for the ATP synthase function. Subunit 8 is sensitive to charge manipulation at the C-terminus. The positively charged residues at the C-terminal domain are important for subunit 8 assembly and hence its function.