Review

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Downstream effects of the *atp4Δ* mutation in *Saccharomyces cerevisiae*

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Abstract

Expression of *atp4* in yeast cells produces subunit 4, a structural component of ATP synthase in the mitochondria embedded in the membrane and making up the peripheral stalk. The gene is thought to have a role in the oligomerization of ATP synthase, which contributes to the curvature of the mitochondrial membrane, cristae formation, and membrane stability. Studies have shown that in the absence of this gene, yeast cells display abnormal mitochondrial structures and membrane instability, supporting this idea. Mutant strains had an overall smaller life span in comparison to the wildtype and displayed deficiencies in its growth and respiration, specifically its oxygen consumption rate and ATP levels. These results have suggested the role of subunit 4 in the assembly of the proper ATP synthase structure that drives these attributes. In addition to mitochondrial membrane stability and cell respiration, the protein may also have a role in mitochondrial genome maintenance. The gene's potential involvement in mitochondrial DNA (mtDNA) replication has been proposed due to rapid losses of mtDNA in the mutant strain. Future studies addressing the gap in the literature surrounding the mechanisms driving these observations have been encouraged.

Introduction

The brewer's yeast *Saccharomyces cerevisiae* is an extremely common model organism used to help study cellular processes in many organisms including humans. Countless studies have analyzed the many different types of yeast strains that are known, in order to better understand the components involved in biochemical pathways occuring in the cell (Wahlbom et al., 2003; Ohya et al., 2005; Bao et al., 2015; Tekarslan-Sahin et al., 2018). *Atp4* is a gene that encodes the protein subunit 4, a component of ATP synthase which is involved in one of the most important processes in the cell, generating ATP that used as energy to power all cellular activities and processes. Therefore, a consolidated review of the literature studying this gene is important to have done and gain a better understanding of what is known already and what is yet to be discovered. This review aims to focus on the *atp4* knockout mutant strain, first introducing what is currently known about the functional gene with regard to its structure and known roles, and then an overview of the knowledge around the mutant strain.

Protein Structure and Localization

The *atp4* gene in *Saccharomyces cerevisiae* expresses the protein ATP synthase subunit 4, also known as subunit b, which is a known structural

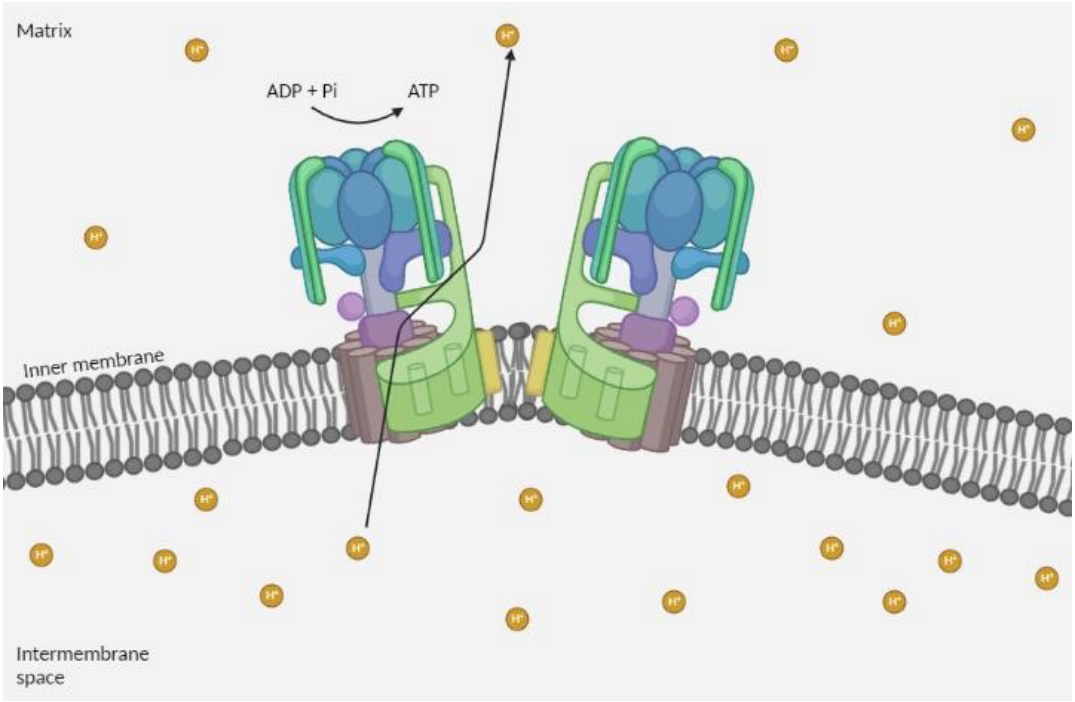

Figure 1: Illustration of the ATP4 gene product, subunit 4/b, as a structural component in an ATP synthase dimer, and its interactions with surrounding proteins. Diagram demonstrates ATP synthesis via the translocation of protons (dark orange) through ATP synthase into the mitochondrial matrix. The ATP synthase complex allows protons in the intermembrane space to move into the matrix down its electrochemical gradient, releasing energy that turns the turbine and produces ATP from ADP and phosphatases (Pi). The c-ring (brown) rotation is shown to be guided by subunit 4/b (green). Subunit 4/b is shown to interact with subunit c and subunit g (gold). The protein is shown to be embedded in the membrane making up a key component of the peripheral stalk (green + gold). The figure demonstrates its importance in maintaining inner membrane stability and curvature induced by dimerization. Dimerization of ATP synthase is shown with subunit 4/b as the interface, as suggested. Created with BioRender.com.

component of mitochondrial ATP synthase. Specifically, it is a component of the stator (peripheral) stalk that connects the F_1 and F_0 regions of the enzyme together (Figure 1) and prevents the F_1 region from spinning with the rotor (Lau et al., 2008; Srivastava et al., 2018). Both studies used cryo-electron microscopy to obtain a high-resolution structure of the complete, intact protein which allowed the researchers to isolate and characterize the different components. Lau et al. (2008) showed that at the F_0 region, subunit b in the peripheral stalk is partially adjacent to the c-ring subunits that make up the rotor, with subunit a between them at a certain depth in the membrane. This suggests that subunits b and a may interact with each other and interact together with the c-rings, perhaps guiding its rotation (Figure 1). Srivastava et al. (2018) similarly showed that the subunit was embedded to the membrane as a part of the F_0 domain and suggested that it may be involved in the movement of protons as well. Overall, findings have shown the protein to have many implications in the structure, maintenance and function of ATP synthase.

Role in Oligomerization and Cristae Structure

Some studies about *atp4* and its protein product have proposed that they have an important role in the oligomerization of ATP synthase (Weimann et al., 2008), a process that is critical for the formation and maintenance of the mitochondrial cristae in *Saccharomyces cerevisiae* (Habersetzer et al., 2013) and thought to have a critical involvement in effective energy transduction. Lau et al. (2008) proposed that subunit b could act as an interface between two monomers of the enzyme. Deletion of an intermembrane portion of the protein subunit showed deficiency in the oligomerization of the enzyme, as well as defects in the mitochondrial

morphology (Weimann et al., 2008). This implies that the gene has important involvement in the stability of ATP synthase in the mitochondrial membrane and cristae integrity (Figure 1). Similarly, Soubannier et al. (2002) found that altering subunit 4 ultimately led to a lack of oligomerization due to their instability and displayed an abnormal mitochondrial membrane phenotype. The authors suggested that this could be attributed to a substantial decrease in the amount of another ATP protein subunit, subunit g, which was previously known to be involved in ATP synthase dimerization.

Mutant yeast strains that had subunit g absent displayed similar results. Western blot analyses (Soubannier et al., 2002) and cross-linking studies (Soubannier et al., 1999) indicated that subunit 4 and g are near each other, implying that perhaps there is an interaction between them that contributes to both ATP synthase oligomerization and mitochondrial membrane stability (Figure 1).

Mitochondrial Membrane Abnormalities

As previously mentioned, it has been well demonstrated that the mutant yeast strain with the *atp4* gene knocked out or the protein products non-functional would have mitochondria that are abnormal in structure (Lau et al., 2008; Soubannier et al., 2002; Weimann et al., 2008). Researchers have made implications that decreased biogenesis of fully formed cristae in these mutants could be linked to the lack of ATP synthase dimers observed (Paumard et al., 2002).

Respiratory Growth and Deficiencies

The chronological life span (CLS) of the mutant strain, *atp4Δ*, was shown to be decreased in comparison to the wildtype by using a propidium iodide fluorescence staining method which binds to cell DNA and indicates cell viability (Kwon et al., 2015). The same researchers also showed that by measuring the maximal cell mass using optical density, the maximum for the mutant strain at the stationary phase of fungal growth was about half that of the wildtype. In addition to these attributes, the oxygen consumption rate and intracellular ATP levels were both decreased in the mutant strain compared to the wildtype and showed respiratory deficiencies such as being incapable of using non-fermentable carbon sources, such as ethanol and glycerol. This restraint was shown to come from the strain being unable to undergo a diauxic shift, a process in yeast cells that switches the source of their metabolism when the primary source glucose becomes limited (Galdieri et al., 2010).

Loss of Mitochondrial DNA Replication

Merz & Westermann (2009) demonstrated that in the *atp4Δ* mutation, cells interestingly showed a loss of mitochondrial DNA (mtDNA) compared to the wildtype. While the connection between the protein and this mechanism is unclear, these results align well with what has been previously proposed about the role of the *atp4* protein in the assembly of a few mitochondrial complexes by Paul et al. (1989). Their genome deletion mutant analysis revealed the loss in mtDNA to be instantaneous, similar to that of the mutant deletion of *mip1*, which is a key subunit in mitochondrial DNA polymerase that is essential for mitochondrial DNA replication (Genga et al., 1986). They, therefore, considered it a possibility that *atp4* has a role in regulating the amount of mtDNA present and that in the mutant strain, replication had been quelled.

Summary and Conclusion

Significant progress in the last few decades has been made in the understanding of ATP synthase subunits such as Atp4p and what they contribute, but there are still gaps present in the literature that future studies can address and investigate. While studies have been done to showcase the protein's involvement in the dimerization and oligomerization of ATP synthase (Lau et al., 2008; Soubannier et al., 2002; Weimann et al., 2008) it is still unclear how *atp4* is functionally contributing to this process. It also hasn't been properly investigated how it interacts with other protein subunits, such as subunit g. Perhaps using approaches to effectively identify protein-protein interfaces could be utilized to better clue in on the interactions between these two ATP synthase components (Zhou & Win, 2007; Soni & Madhusudhan, 2017). Paul et al. (1989) proposed the protein's potential role in the assembly of

mitochondrial complexes, and Merz & Westermann (2009) demonstrated the gene's potential role in mtDNA replication, but the molecular mechanism that explains their observations has yet to be known.

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