Title: Post-Translational Modifications of ATP Synthase Modulate Mitochondrial Bioenergetics in Yeast

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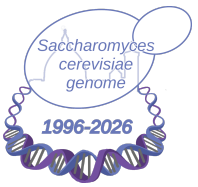
Abstract content:
Post-translational modifications (PTMs) are key regulatory mechanisms that modulate protein activity, stability, localization, and interactions. Phosphorylation is a well-characterized, reversible modification that controls signaling, metabolism, and stress responses. In contrast, AMPylation (adenylylation) has recently emerged as an important PTM, involving covalent attachment of AMP to residues that can also be phosphorylated. Evidence suggests that AMPylation and phosphorylation may act cooperatively or competitively, adding complexity to cellular regulation.
Using a large-scale approach to identify AMPylated mitochondrial proteins in wild-type cells and in strains lacking Fmp40—the only known AMPylase in *Saccharomyces cerevisiae* —we identified seven AMPylated subunits of ATP synthase, many modified at sites also known to undergo phosphorylation, underscoring the complex PTM-based regulation of this enzyme. We demonstrate that AMPylation/phosphorylation of Ser29 in the δ subunit (Atp16) modulates ATP synthase activity and oxidative phosphorylation (OXPHOS) coupling under both fermentative and respiratory conditions, resulting in increased respiratory control ratios and enhanced ATP production relative to oxygen consumption (P/O ratio). This regulatory mechanism is critical for maintaining mitochondrial membrane potential (ΔΨ).
In contrast, blocking AMPylation/phosphorylation of Atp4 ATP synthase subunit, leads to a more pronounced decrease in ΔΨ, whereas alterations in respiratory control ratios are opposite to those observed for the Atp16-S29 substitution. Our findings indicate that OXPHOS coupling is regulated in a complex manner by PTMs of ATP synthase subunits. Given that mutations in ATP synthase genes underlie numerous inherited disorders, elucidating the interplay between AMPylation and phosphorylation is essential for understanding the molecular mechanisms governing OXPHOS and its disease-associated dysregulation.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