Exploring Proteasome Subunit RPN11 Acetylation in *Saccharomyces cerevisiae*

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Introduction. Protein N-terminal acetylation is a post-translational modification found in up to 90% of human proteins, yet its function remains poorly understood. In yeast, where orthologous enzymes facilitate acetylation of proteins, the proteasome subunit Rpn11 is acetylated by the NatB complex. A deletion of NatB reduces Rpn11's stability and function.

Methods. To determine how critical NatB-dependent acetylation is for Rpn11, we engineered yeast cell lines using CRISPR-Cas9 where Rpn11 became a substrate for different N-terminal acetylases (Nat) or no acetylase at all. Next, we deleted the different Nat complexes in these backgrounds and analyzed strains for the presence and stability of Rpn11 and proteasomes (by immunoblotting and activity assays), as well as their cellular fitness (by various growth and stress assays).

Results. Western blot analysis revealed no change in proteasome subunit presence under optimal growth conditions when Rpn11 was mutated. However, the NatC knockout was detrimental when Rpn11 was altered to become a NatC-compatible substrate in protein folding stress conditions (canavanine). Furthermore, growth assays, western blots, and proteasome activity assays indicated that Rpn11 lacking any form of acetylation was less stable under heat stress (37°C for yeast).

Conclusions. N-terminal acetylation of Rpn11 is critical for maintaining protein stability under specific stress conditions. These findings suggest that the cellular role of N-terminal acetylation, though widespread, may be context-dependent and especially important during proteotoxic/protein-folding stress. Understanding how acetylation modulates proteasome function could shed light on the broader physiological significance of this conserved post-translational modification.

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