

# The Physiological Role of N-terminal Acetylations

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## ABSTRACT

Numerous different protein modifications occur in the cell and many of them are crucial for protein function. N-terminal acetylation is, along with N-terminal methionine cleavage, the most common protein modification among eukaryotic organisms. Despite the high abundance, surprisingly few examples of functionally important N-terminal acetylations have been reported and strains deleted in genes coding for the enzymes that catalyze the addition of the acetyl groups are viable. This thesis mainly concerns the N-terminal acetyl transferase NatB in *Saccharomyces cerevisiae*. NatB consists of the catalytic subunit Nat3p and the associated subunit Mdm20p. We demonstrate that a *nat3Δ* strain grows slowly, displays abnormal morphology and exhibits a protein expression profile resembling the protein expression of wild-type cells subjected to stress, while an *mdm20Δ* strain exhibits normal growth and morphology. The discrepancy in phenotype indicates that the two subunits have partially separated functions. We do, however, show that the NatB substrates Act1p, Rnr4p and Tfs1p are dependent on both NatB subunits to be acetylated. In total, we present four novel NatB substrates in this thesis. One of these substrates, Tfs1p, is studied in detail. Tfs1p is an inhibitor of both carboxypeptidase Y (CPY) and Ira2p, a GTPase-activating protein of Ras. We show that the N-terminal acetyl group of Tfs1p is required for CPY inhibition but not for Ira2p inhibition. Using a series of Tfs1p derivatives only able to inhibit either Ira2p or CPY we also demonstrate that Tfs1p mediated Ira2p inhibition has a more profound impact on growth and stress resistance than CPY inhibition. It is also shown that the inhibition of the two substrates have opposite effects on cell survival after heat-shock. Furthermore, we report that proteins that are predicted to be acetylated by NatB are unevenly distributed among functional categories. NatB substrates are, for example, dramatically overrepresented among proteins involved in cell cycle progression and DNA maintenance. It is demonstrated that strains deleted in *NAT3* or *MDM20* are highly sensitive to various DNA damaging agents, indicating that NatB substrates involved in DNA related processes contain functionally important N-terminal acetylations.