University of Cyprus
Department of Biological Sciences

Ph.D. Thesis Defense

Student Presentation

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Building CTF 01, Room 102, Panepistimioupoli Campus

This seminar is open to the public

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"FUNCTIONAL CHARACTERIZATION OF HISTONE N-TERMINAL ACETYLTRANSFERASE NAA40 REVEALS A NOVEL ROLE IN GENOME INTEGRITY"

N-terminal acetylation (Nt-Ac), catalyzed by N-terminal acetyltransferases (NATs), is one of the most abundant protein modifications in eukaryotes. Human N-alpha-acetyltransferase 40 (NAA40) is unique within the NAT family due to its specificity for the N-terminal tails of histones H4 (Nt-acH4) and H2A (Nt-acH2A). Although NAA40-mediated histone Nt-Ac has been linked to various cellular phenotypes, the molecular mechanisms underlying these effects remain poorly understood. This Ph.D. study aimed to functionally characterize NAA40 and its associated histone modification, leading to the identification of a new substrate, mapping of nuclear interaction partners, and uncovering a novel role in replication stress. In the first part, it is demonstrated that histone H2A.X is N-terminally acetylated (Nt-acH2A.X) in human cells and that NAA40 specifically acetylates this histone variant. Importantly, Nt-acH2A.X is responsive to UV-induced DNA damage, suggesting that this modification is dynamic and potentially involved in the DNA damage response. In the second part, to further define the nuclear localization and interaction network of NAA40, an affinity purification-mass spectrometry (AP-MS) approach is established, unveiling the nuclear interactome NAA40 and providing insights into its potential regulatory roles within nucleus. In the third part, a novel link between NAA40, its associated histone Nt-Ac and

replication stress is identified. Loss of NAA40 or its catalytic activity confers resistance to cancer cells *in vitro* and *in vivo* against replication stress-inducing agents. Molecularly, Nt-acH4 levels decrease during replication stress, while NAA40 loss facilitates replication recovery. Mechanistically, nucleosome affinity purifications demonstrate that Nt-acH4 selectively repels the replication stress-associated MMS22L-TONSL complex. In line with this, the presence of this complex is required for the replication stress resistance observed upon NAA40 depletion. Collectively, the findings within this doctoral thesis establish histone variant H2A.X as a novel *bona fide* substrate of NAA40, defines the nuclear interactome of this enzyme and uncovers a previously unrecognized role for NAA40 and histone Nt-Ac in the regulation of replication stress and maintenance of genome integrity.