ROLE OF LYSINE ACETYLATION AND DEACETYLATION IN PLANT METABOLISM

A Thesis

Presented to the Faculty of the Graduate School

of Cornell University

in Partial Fulfillment of the Requirements for the Degree of

Master of Professional Studies in Agriculture and Life Sciences

Field of Biotechnology Applications

by

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December 2020

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ABSTRACT

The sessile nature of plants has led to the development of a sophisticated array of signaling pathways that allow for dynamic regulation in gene expression, metabolic and cellular activities. At the molecular level, histone acetylation and deacetylation, which are epigenetic modifications, play pivotal roles in making plants adaptive to different environments and influencing the production of primary metabolites. However, recent mass-spectrometry-based analysis of plant cellular extracts discovered wide-spread lysine acetylation in mitochondrial and chloroplast proteins. This brief review provides an overview of recent developments in understanding lysine acetylation and its relationship with plant metabolism.

BIOGRAPHICAL SKETCH

Malika Sharma hails from Mumbai, India. She obtained her bachelor's degree in biotechnology from DY Patil University, Navi Mumbai in 2018. She started her Master of Professional Studies Program at Cornell University in August 2019 where she specialized in biotechnology application of plants. Her aim is to study medicinal plants and work towards scaling up their production using fermentation techniques. She is currently working as a research assistant at Boyce Thompson Institute. In her free time, she likes to paint and dance.

ACKNOWLEDGMENTS

I would like to thank my project mentor Prof. Klaas van Wijk without whose help this project would not have been possible. I would also like to express my gratitude towards the Plant Biology department at SIPS for providing me the resources to hone my skills in the field of plant biology. I am also thankful to all my friends for extending help within their capacities.

Last, but not the least, I thank my unbelievably supportive family, they have demonstrated rare and amazing patience throughout my lengthy working sessions.

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INTRODUCTION

Nucleosomes are conserved structures consisting of two copies of core histone proteins H2A, H2B, H3, and H4, assembled into an octamer by protein-protein interactions and a single linker protein, H1. Almost two turns of DNA (approximately 146–147 bp) are wrapped around the histone octamer. The H1 histone acts as a linking structure and stabilizes the octameric core. Nucleosomes condense to form the chromatin fibers. For years, the role of chromatin was only linked to packaging the DNA but its function for regulating DNA accessibility by modifying its structure has gained a lot of interest [1].Two mechanisms involved in these modifications have surfaced. The first involves remodeling of nucleosome patterning along the chromatin fiber by using energy of ATP hydrolysis in conjunction with Swi2 superfamily of proteins. The second mechanism involves covalent changes in the histone N-terminus by chromatin-dependent factors [2]. Reversible chromatin modification such as acetylation, methylation, glycosylation, and ubiquitination are essential for regulating plant development and plasticity. Histone acetylation particularly has been shown to play crucial role in plant stress response and influence plant metabolism, such as photosynthesis and respiration ([3], [4]). Histone acetylation is associated with an active chromatin state of genes, whereas histone deacetylation represses gene expression. Histone acetylation consists of addition of acetyl group, which is provided by acetyl-CoA, to the ε-amine group of N-terminal lysine residues and is catalyzed by histone acetyltransferases (HATs) and histone deacetylation is catalyzed by histone deacetylases (HDACs).

In addition to histones, non-histone protein acetylation(N-termini and lysines) and deacetylation (lysines) have also been found in many cellular compartments [3]. Lysine acetylation and deacetylation play a role in cellular responses and may be of great importance in regulating

metabolic pathways with the help of lysine acetyltransferases (KATs) and lysine deacetylases (KDACs), which are synonymous to HATs and HDACs. KATs catalyze the transfer of an acetyl moiety to the amine side chain of lysine. Lysine deacetylases catalyzes the reverse reaction and works through two mechanisms: 1) remove the acetyl group from lysine and release acetate and 2) utilize NAD+ as a coenzyme and give O-acetyl-ADP-ribose and nicotinamide (Fig1a) [5]. In plants and most bacteria, lysine acetylation was also observed to have occurred non-enzymatical [6]. This was observed at a pH>8, a level that can be reached during active respiration in the mitochondrial matrix, as well as in the chloroplast stroma (stromal pH is between 7 (in dark) and 8 (in light) during photosynthesis [7]. Lysine acetylation and deacetylation is a widespread PTM, which occurs in large number of proteins of diverse biological function (Fig. 1b). This review discusses its role in primary metabolism in plants.

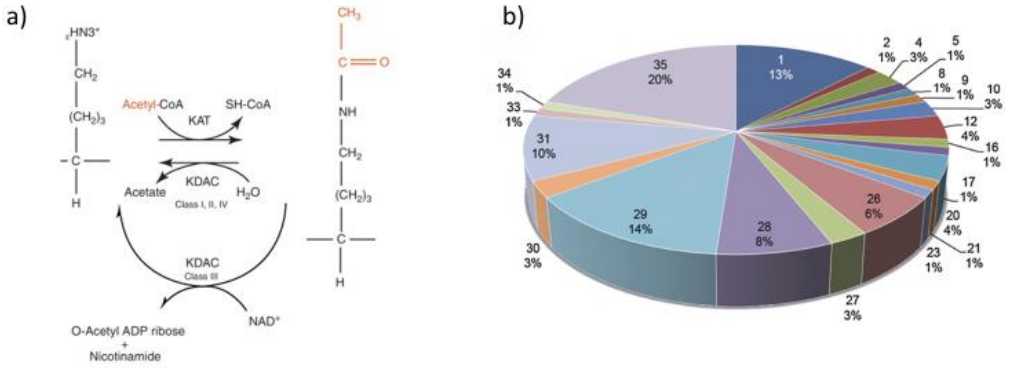

Figure 1 Mechanism of KATS and KDAC and lysine acetylated proteins a) Schematic representation of KAT and KDAC catalytic mechanism from [5], b) Pie chart representing Lys-acetylated proteins in *Arabidopsis thaliana.* The numbers represent the following categories: 1, photosynthesis; 2, major carbohydrate metabolism; 4, glycolysis; 5, fermentation; 8, TCA cycle; 9, mitochondrial respiratory chain; 10, cell wall; 12, nitrogen metabolism; 16, secondary metabolism; 17, hormone metabolism; 20, stress; 21, redox regulation; 23, nucleotide metabolism; 26, miscellaneous; 27, RNA regulation of transcription; 28, DNA synthesis; 29, protein synthesis, degradation, targeting, and posttranslational modification; 30, signaling; 31, cell organization and cell cycle; 33, development; 34, transport; 35, not assigned unknown proteins - from [3, 8].

LYSINE ACETYLATION/DEACETYLATION ENZYMES

KATs and KDACs have specific roles and are spread across the cell at different locations. In plants, the KATs are classified as General control non-depressible 5 (GCN5)-related Acetyl Transferase (GNAT), MOZ-YBF2/SAS3-SAS2/TIP60 (MYST), cAMP-responsive element Binding Protein (CBP) and TATA-binding protein Associated Factor 1 (TAF1)[9]. On the other hand, KDACs are grouped into three families: Reduced Potassium Dependency 3 (RDP3)/Histone DeAcetylase 1 (HDA1), Silent Information Regulator 2 or sirtuins (SIR2), and the plant specific HD-tuins (HD) [7]. Both KATs and KDACs are localized in the cytoplasm, nucleus, mitochondria, and chloroplast (Fig 2). Their role is to control cell fate and influence cell cycle progression, plant responses to environmental conditions, and gene interactions.

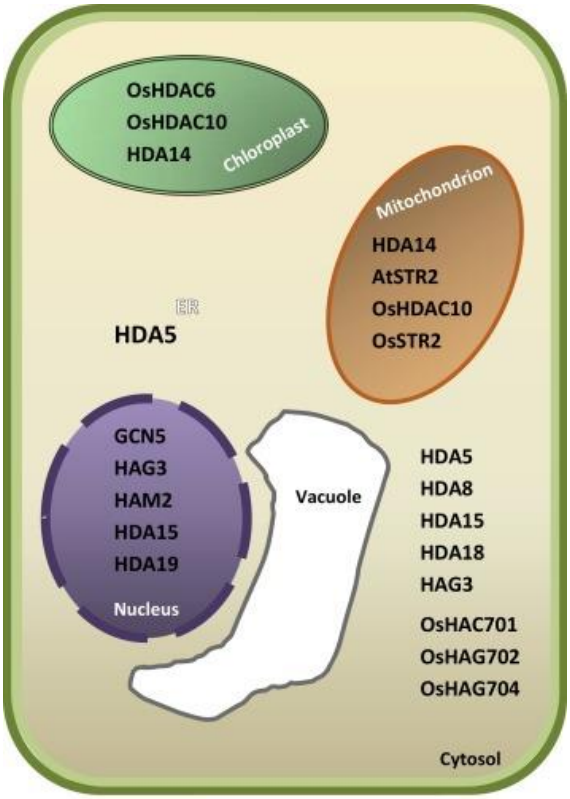

Figure 2. Subcellular Localization of Lysine Acetyltransferases (KATs) and Deacetylases (KDACs) in different plant species. Proteins shown include Arabidopsis KATs (GCN5, HAG3, and HAM2) and KDACs (HDA5, HDA8, HDA14, HDA15, HDA18, HDA19 and AtSRT2) and rice HATs (OsHAC701, OsHAG702, and OsHAG704) and HDACs (OsHDAC6, OsHDAC10, OsHDAC14 and OsSRT2). Abbreviation: ER, endoplasmic reticulum. from [9]

ACETYL-COENZYME A AND ACETYLATION

Acetylation was believed to be only involved in controlling gene expression and DNA-related processes through histone modifications, until the identification of several hundreds of acetylated proteins from mouse liver mitochondria and human HeLa cells and other human and bacterial cells [5]. The acetylation of several enzymes of cellular processes made it clear that there was a direct link between the PTM and metabolic pathways. Acetyl-CoenzymeA (Co-A) is a central metabolite involved in numerous anabolic and catabolic pathways and serves as a substrate for lysine acetylation (Fig 3). For this reason, acetylation is also known to influence the regulation of carbon flux (Fig 3). Acetyl-CoenzymeA synthetase (ACS) is an enzyme that catalyzes the synthesis of acetyl-CoA from pyruvate. Acetylation/deacetylation of ACS has potential to generate an autoregulatory loop that could enhance or dampen such controlling mechanisms [10].

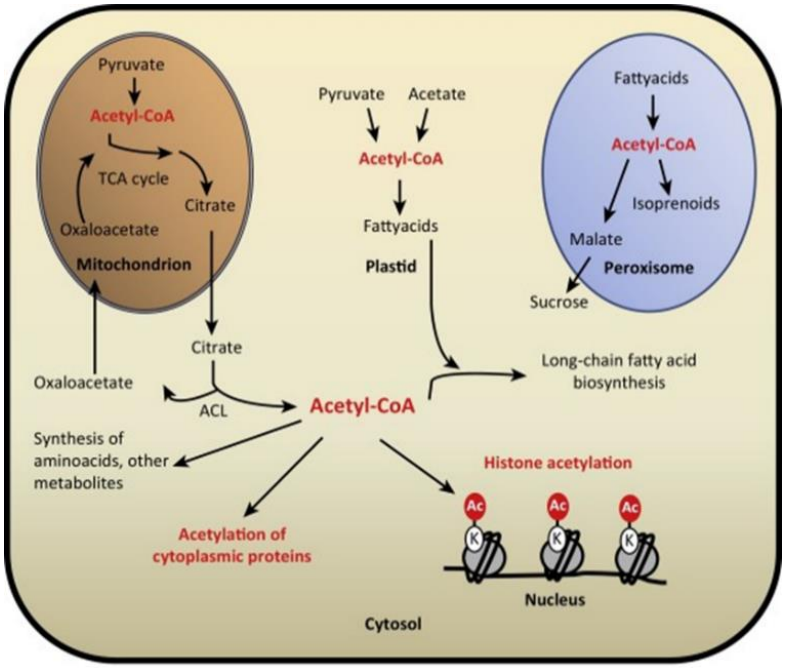

Figure 3. Acetyl-CoA is at the Nexus of Metabolism, Histone and Nonhistone Protein Acetylation and Gene Expression. In plant cells, acetyl-CoA is produced in different compartments, including mitochondria, plastids, peroxisomes, and cytosol. The cytosolic pool of acetyl-CoA produced by ATP citrate lyase (ACL) is used for histone and nonhistone protein acetylation in the nucleus and cytoplasm - from [9].

LYSINE ACETYLATION/DEACETYLATION IN PLASTIDS AND MITOCHONDRIA

Plastid proteins involved in electron transport chain, such as subunit H of the PSI reaction center, chlorophyll a/b-binding protein, and the b-subunit of the chloroplast ATP synthase, and the Calvin-Benson cycle (Rubisco, both large and small), Rubisco activase, aldolase, glyceraldehyde 3-phosphate dehydrogenase (GAPDH), and phosphoglycerate kinase were observed to be lysine acetylated [3] (Fig 4). Partial deacetylation of Rubisco Large subunit was shown to increase catalytic activity [7].

Association of Light harvesting complex (LHC) with PSII is also regulated by lysine acetylation. A variation in levels in lysine acetylation is observed in the pool of LHC tightly bound to PSII and loosely bound LHCII pool which show a lower level of Lys acetylation [11]. This suggests that lysine acetylation may also be involved in maintaining structure of interdependent proteins. Lysine acetylation also regulates mitochondrial functions since key enzymes of oxidative phosphorylation and photorespiration reside in the mitochondria (Fig 5).

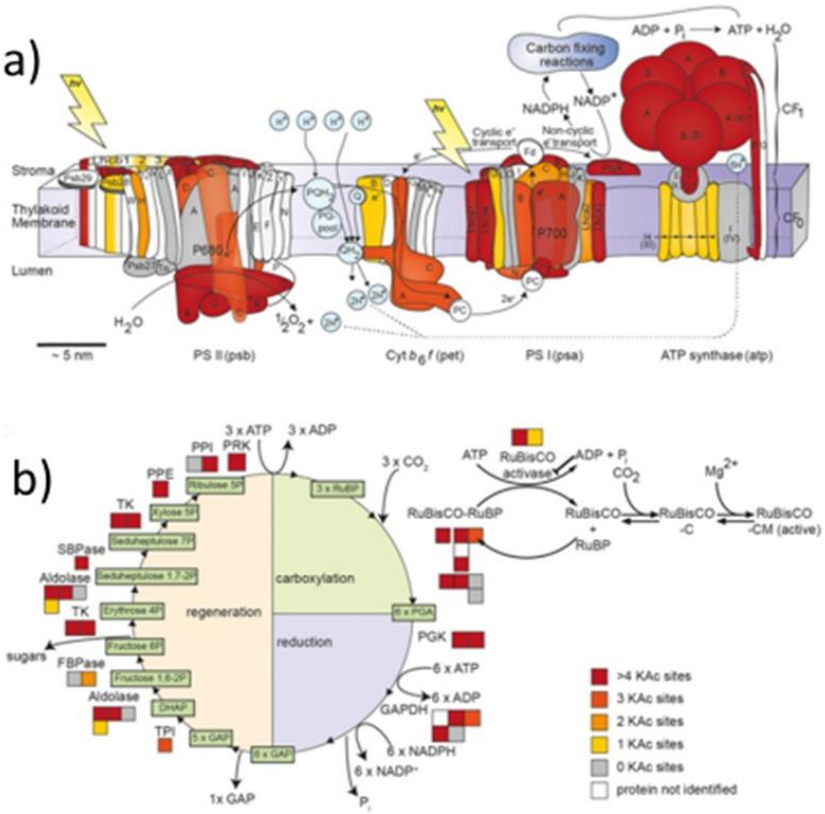

Figure 4 a,b. Distribution of proteins and number of lysine acetylated sites involved in photosynthesis. Color code: proteins not identified in the LC-MS/MS analyses (white), proteins without identified lysine-acetylated sites (gray), and proteins with one (yellow), two (orange), three (dark orange), or four or more acetylation sites (red). For the Calvin–Benson cycle, each box indicates a separate Arabidopsis AGI identifier. Cytb6f, cytochrome b6f; FBPase, fructose-1,6-bisphosphatase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; PGK, phosphoglycerate kinase; PPE, phosphopentose epimerase; PPI, phosphopentose isomerase; PRK, phosphoribulokinase; PSII, photosystem II; PSI, photosystem I; RuBisCO, ribulose-1,5-bisphosphate-carboxylase/oxygenase; SBPAse, seduheptulose-1,7-bisphosphatase; TPI, triose phosphate isomerase; TK, transketolase [7].

Non enzymatic lysine acetylation is more prevalent in the mitochondria than the cytosol because of its higher pH and elevated levels of acetyl-CoA. Lower number of acetylated proteins are found in the mitochondria and a reason for that could be the occurrence of fatty acid breakdown in glyoxysomes and not mitochondria. Information related to mitochondrial KATs is not available for plants but plant KDACs belonging to the sirtuin family have been successfully

characterized [6]. Lysine acetylation of enzymes involved in the TCA cycle and glycolysis were observed to be upregulated during seed germination as a source of energy in wheat. Similar observations were made during de-etiolation in *Zea mays*. A reason for this may be lack of mineral uptake system and photosynthetic apparatus, suggesting the importance of biochemically cycles that take place in the mitochondria.

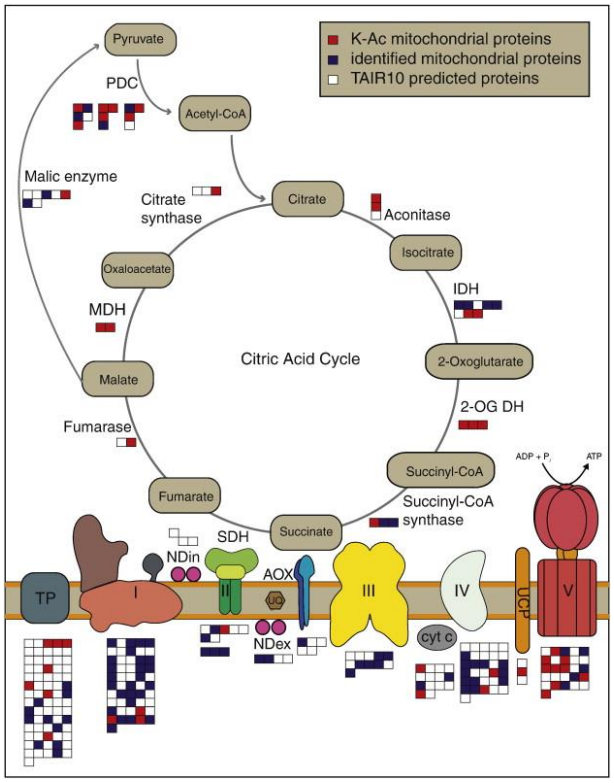

Figure 5. Overview of identified Arabidopsis lysine-acetylated proteins from mitochondrial energy metabolism. Color code: identified non-acetylated proteins before enrichment(blue), identified lysine-acetylated proteins(red) and TAIR10 proteins not identified (white) in the LC-MS/MS analyses. AOX, alternative oxidase; TCA, tricarboxylic acid; TP, transporter; I, complex I; II, complex II; III, complex III; IV, complex IV from [12].

CONCLUSION

Lysine acetylation/deacetylation was thought to occur in histones and regulate gene expression until it was observed in non-nuclear proteins. It was observed that most of the proteins and enzymes responsible in regulating plant central metabolism undergo lysine acetylation/deacetylation. Lysine acetylome profiling played a crucial role in identifying and classifying different acetyltransferases and deacetylases in cellular and subcellular organelles. This has helped in finding direct links to primary metabolism and identifying their functions in different processes such as seed germination and regeneration of new tissues and how PTMs have played an important role during de-etiolation [13-15].

By studying the regulatory potential of lysine acetylation, modifications in chloroplast and mitochondria metabolism could be made which can help in improving plant growth and may also be used to accumulate metabolic intermediates to eventually increase the nutritional values of certain crops. Lysine acetylated proteins have also been implicated in controlling plant biotic and abiotic stress responses. A characterization of cross signaling pathways and its link with abiotic and biotic stress responses, physiological effects of PTM during stress responses, especially lysine acetylation can also be the focus of future research.

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