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Initial description of the developing soybean seed protein Lys-N$^{\epsilon}$-acetylome

Colin L. Smith-Hammond[a,b], Kirby N. Swatek[a,b], Mark L. Johnston[c], Jay J. Thelen[a,b], Ján A. Miernyk[a,b,c,*]

[a]Division of Biochemistry, University of Missouri, Columbia, MO, USA
[b]Interdisciplinary Plant Group, University of Missouri, Columbia, MO, USA
[c]Plant Genetics Research Unit, USDA, Agricultural Research Service, University of Missouri, Columbia, MO, USA

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ABSTRACT

Characterization of the myriad protein posttranslational modifications (PTM) is a key aspect of proteome profiling. While there have been previous studies of the developing soybean seed phospho-proteome, herein we present the first analysis of non-histone lysine-N$^{\epsilon}$-acetylation in this system. In recent years there have been reports that lysine acetylation is widespread, affecting thousands of proteins in diverse species from bacteria to mammals. Recently preliminary descriptions of the protein lysine acetylome from the plants *Arabidopsis thaliana* and *Vitis vinifera* have been reported. Using a combination of immunoenrichment and mass spectrometry-based techniques, we have identified over 400 sites of lysine acetylation in 245 proteins from developing soybean (*Glycine max* (L.) Merr., cv. Jack) seeds, which substantially increases the number of known plant N$^{\epsilon}$-lysine-acetylation sites. Results of functional annotation indicate acetyl-proteins are involved with a host of cellular activities. In addition to histones, and other proteins involved in RNA synthesis and processing, acetyl-proteins participate in signaling, protein folding, and a plethora of metabolic processes. Results from *in silico* localization indicate that lysine-acetylated proteins are present in all major subcellular compartments. *In toto*, our results establish developing soybean seeds as a physiologically distinct addendum to *Arabidopsis thaliana* seedlings for functional analysis of protein Lys-N$^{\epsilon}$-acetylation.

Biological significance
Several modes of peptide fragmentation and database search algorithms are incorporated to identify, for the first time, sites of lysine acetylation on a plethora of proteins from developing soybean seeds. The contributions of distinct techniques to achieve increased coverage of the lysine acetylome are compared, providing insight to their respective benefits. Acetyl-proteins and specific acetylation sites are characterized, revealing intriguing similarities as well as differences with those previously identified in other plant and non-plant species.

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* *Corresponding author at:* USDA, ARS, PGRU, 102 Curtis Hall, University of Missouri, Columbia, MO 65211, USA. Tel.: +1 573 882 8167; fax: +1 573 884 7850.
E-mail addresses: colin.smithhammond@mail.missouri.edu (C.L. Smith-Hammond), knsty4@mail.missouri.edu (K.N. Swatek), mark.johnston@ars.usda.gov (M.L. Johnston), thelenj@missouri.edu (J.J. Thelen), jan.miernyk@ars.usda.gov (J.A. Miernyk).

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1. Introduction

For decades now protein O-phosphorylation has been the most-studied and best-understood posttranslational modification (PTM) [1–3]. However, within the past five years the total number of identified Lys-N$^{\varepsilon}$-acetylated proteins has increased by several orders of magnitude [4–7]. It is not just for histones [8] or tubulin [9] anymore.

Protein lysine acetylation (KAc) (herein, unless explicitly stated otherwise, "lysine acetylation (KAc)" means protein Lys-N$^{\varepsilon}$-acetylation) is an evolutionarily conserved reversible PTM, and steady-state lysine acetylation is the sum of the protein Lys-acetyltransferase (KAT) and protein Lys-deacetylase (KDAC)-catalyzed reactions [10,11]. Acetylation neutralizes the positive charge that lysine has under physiological pH conditions, and in some instances directly modulates protein interactions [12,13]. The reversibility of KAc is important for its regulatory role, and the dynamic nature of global KAc can be easily seen when specific inhibitors of KAT or KDAC are employed [14].

Based upon several decades of observations, it is well established that reversible KAc is important in regulating gene expression, [8,15] autophagy [16–18], and function of the cytoskeleton [19]. However, prior to publication of the results of more recent large-scale proteomics screens of PTM [4,6,7,20], it was not anticipated that KAc would have a major role in regulation of intermediary metabolism. The results from high-throughput screening have consistently identified KAc as a modification to a plethora of cytoplasmic and mitochondrial enzymes [6,7,17]. Most extant reports broadly describe "signaling networks," but in a few instances changes in catalytic activity have been attributed to KAc [21,22].

In many instances, regulation of the biochemical activity of a protein by PTM can be hierarchical and quite complex. Examples include phosphorylation:ubiquitination/sumoylation [23], and even phosphorylation:O-glycosylation of the same serine residue [24]. There are an increasing number of examples of this type of PTM cross-talk with both histone and non-histone instances of KAc [25–28]. The phosphorylation-acetylation switch is particularly intriguing in terms of regulatory networks [29].

While the lysine acetylation of plant tubulin [30] and histone proteins [31] has been known for decades, only recently has the general protein acetylome been described for the reference plant *Arabidopsis thaliana* [32,33], as well as mesocarp and exocarp from *Vitis vinifera* [34]. Herein we present the first description of the general protein acetylome from developing soybean seeds, and compare results obtained with collision-induced dissociation (CID) versus electron transfer dissociation (ETD) tandem mass spectrometry (MS/MS).

2. Materials and methods

2.1. Reagents

Unless otherwise noted, all reagents were from the Sigma Chemical Company.

2.2. Plants

Soybean (*Glycine max* (L.) Merrill, cv. Jack) plants were glasshouse-grown with supplemental lighting (16 h light/8 h dark, 26 °C day/21 °C night). Plants were not nodulated, and were treated weekly with an all-purpose fertilizer (Osmocote 14-14-14, ScottsMiracle-Gro). Seeds were harvested, sorted, and stored frozen at −80 °C until used.

Our system of staging seed development is a modification of that described by Meinke et al. [35] and is based upon whole seed fresh weight and color as follows: S(tage) 1 < 15 mg; S2, 27–42 mg, storage cells have large central vacuoles; S3, 70–90 mg, storage protein accumulation has begun, and subdivision of the vacuole is occurring; S4, 115–150 mg; S5, 200–250 mg, filling of the storage vacuoles; S6, 300–350 mg, green maturing seeds; S7, 300–350 mg, mature yellow seeds; S8, 200–250 mg, fully mature, yellow, dehydrating seeds; S9, quiescent, yellow/tan-colored, and fully dehydrated seeds. Please see Fig. S1 of [36] for a schematic representation of the system. The results presented herein are from analysis of S4 seeds, a stage of rapid accumulation of storage oil and proteins.

2.3. Tissue printing

A detailed description of the tissue printing [37] will be presented elsewhere. Briefly, S4 seeds were frozen at −80 °C encased in Tissue-Tek freezing medium (Sakura Finetek), and 25 micron sections were cut at −20 °C. Backed nitrocellulose (Invitrogen) was pre-wetted with TBS (50 mM tris base, pH 7.4, 150 mM NaCl) containing 20% (v/v) methanol, pressed against the seed for three seconds, then gently removed.

Prints were stained for total protein using 0.1% Ponceau S (w/v) in 1% acetic acid (v/v). After being photographed, protein-stained prints were destained with deionized H_2O until the background was no longer pink. Monoclonal antibodies to acetyl-Lys (#9681, Cell Signaling Technology) were conjugated with the fluorescent probe Cy5 using an AbD Serotec LYNX rapid antibody conjugation kit. Antibody buffer (50 mM tris base, pH 7.4, 120 mM KCl, 5 mM EDTA, 0.25% w/v nonfat dry milk plus 0.05% v/v Nonidet P-40) was used to dilute the modified antibodies to 1:500. The antibody solution was incubated with the print overnight at 4 °C after which the prints were washed thrice for 10 min each in PBS (3.2 mM Na_2HPO_4, 0.5 mM KH_2PO_4, 1.3 mM KCl, 135 mM NaCl, pH 7.4) containing 0.1% Tween 20, 0.05% Nonidet P-40, and 0.05% Brij 35. Images were viewed using a Lecia MZFLII stereomicroscope with a Texas Red (Cy5) filter set, and captured using the Optronics MagnaFire camera. Quantification of the fluorescent image was accomplished by using the 3-D Surface Plot function of the Fiji distribution of ImageJ [38]. Images were assembled using ACDSee Canvas software.

2.4. Protein preparation

An albumin fraction was prepared from S4 seeds (fresh mass 115–150 mg), then depleted of seed storage proteins (SSP) with minor modifications to a previously described method [39]. Seeds were pulverized in liquid N_2 using a mortar and pestle, and resultant tissue powder suspended in three volumes