Review

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# The protein acetylome and the regulation of metabolism

**Shufan Xing and Yves Poirier**

Department of Plant Molecular Biology, Biophore Building, University of Lausanne, CH-1015 Lausanne, Switzerland

**Acetyl-coenzyme A (CoA) is a central metabolite involved in numerous anabolic and catabolic pathways, as well as in protein acetylation. Beyond histones, a large number of metabolic enzymes are acetylated in both animal and bacteria, and the protein acetylome is now emerging in plants. Protein acetylation is influenced by the cellular level of both acetyl-CoA and $NAD^+$, and regulates the activity of several enzymes. Acetyl-CoA is thus ideally placed to act as a key molecule linking the energy balance of the cell to the regulation of gene expression and metabolic pathways via the control of protein acetylation. Better knowledge over how to influence acetyl-CoA levels and the acetylation process promises to be an invaluable tool to control metabolic pathways.**

## Acetyl-coenzyme A and acetylation

Acetyl-coenzyme A (CoA) is a universal metabolite found in all organisms. An energy-rich thioester bond connects the acetyl group to CoA, facilitating the transfer of the acetyl moiety to a variety of molecules. In plant cells, acetyl-CoA contributes to the synthesis of numerous molecules including fatty acids, amino acids, isoprenoids, flavonoids, phenolics and alkaloids [1]. Acetyl-CoA also contributes to the acetylation of lignin and of glycans on proteins and the cell wall [2,3]. Numerous molecules derived from acetyl-CoA have high industrial value, either in the pharmaceutical or chemical industry, making the control of the carbon flux through acetyl-CoA an important topic for many biotechnological applications.

Beyond its implication in both primary and secondary metabolism, acetyl-CoA also participates in the direct acetylation of proteins, both at the N-terminal of protein and at internal amino acids, in particular the $N^{\varepsilon}$-position of lysine. While $N^{\varepsilon}$-acetylation of proteins, such as histones and transcription factors, have been known to be important for the control of gene expression, recent advances in analytical techniques have considerably expanded the number of proteins modified by $N^{\varepsilon}$-acetylation, making this post-translational modification (PTM) as prevalent and important as phosphorylation [4]. Of particular interest is the recent discovery that a large fraction of metabolic enzymes in animal, bacteria and plants are acetylated and that such modification can control enzyme activity and carbon flux through a pathway [5–8]. Being at the interface of both anabolic and catabolic pathways, acetyl-CoA is ideally placed to act as a regulatory molecule linking the energy balance of the cell to the regulation of gene expression and metabolic pathways via the control of protein acetylation and deacetylation.

Following a brief introduction on the metabolic pathways implicating acetyl-CoA in various organelles, the review will focus on the acetylation of amino acids in proteins and how it impacts numerous aspects of the cell functions, and in particular its emerging role on the control of metabolic pathways.

## Contribution of acetyl-CoA in metabolic pathways

Major metabolic pathways contributing to acetyl-CoA generation and utilization in plant cells have been recently reviewed [1] and only salient features of its role in anabolism and catabolism will thus be described here. Acetyl-CoA occurs both in the cytosol and in organelles, such as mitochondria, plastids and peroxisomes (Figure 1). Furthermore, a pool of acetyl-CoA must also exist in the nucleus to mediate histone acetylation. Acetyl-CoA is impermeable to membranes and thus must be synthesized in each subcellular organelle, with the exception of the nuclear pool, which probably at least partially derives from the diffusion of acetyl-CoA through the nuclear pores. In the plastid, a major flux towards acetyl-CoA synthesis occurs to mediate fatty acid biosynthesis via pyruvate and the pyruvate dehydrogenase complex (PDC) while lower level of acetyl-CoA can also be generated from acetate via a plastidial acetyl-CoA synthetase [1]. Plastidial acetyl-CoA is also involved in the synthesis of leucine and arginine via 2-isopropylmalate synthase and N-acetylglutamate synthase, respectively [9]. Acetyl-CoA is also involved in the synthesis of cysteine via the intermediate O-acetylserine, and the enzyme serine acetyltransferase has been localized to both cytosol, plastid and mitochondria [10].

In mitochondria, acetyl-CoA is synthesized from the PDC and is essentially converted to citrate, where it can either enter the tricarboxylic acid (TCA) cycle or be exported to the cytosol to contribute to acetyl-CoA synthesis via the ATP citrate lyase [11]. In both cytosol and plastids of all plants, and mitochondria of some grasses, acetyl-CoA can be converted to malonyl-CoA via the acetyl-CoA carboxylase [12]. Malonyl-CoA participates in the synthesis of fatty acids in plastids and their elongation in the cytosol, lipoic acid in mitochondria, and the formation of flavonoids, isoflavonoids, stilenoids and other manonylated compounds in the cytosol. A major contribution of cytosolic acetyl-CoA is the synthesis of isopentenyl pyrophosphate, the precursor of isoprenoids, via the mevalonate (MVA) pathway.

*Corresponding author:* Poirier, Y. (yves.poirier@unil.ch).

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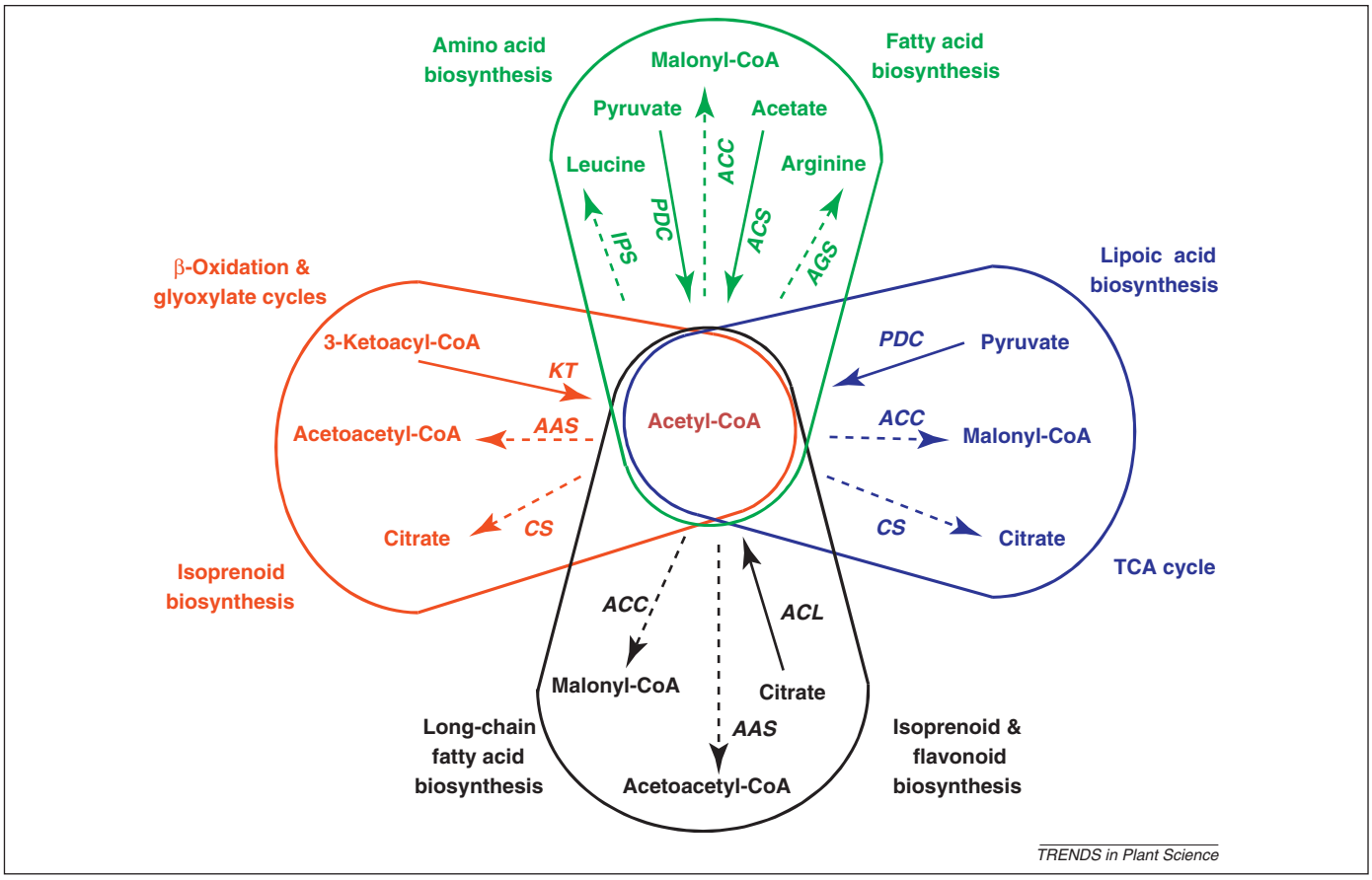



**Figure 1**. Major pathways involving acetyl-CoA in plants. The various subcellular compartments are shown in different colors: plastid, green; mitochondrion, blue; peroxisome, red; cytosol, black. Major enzymes generating acetyl-CoA (full arrows) are: ACL, ATP citrate lyase; ACS, acetyl-CoA synthetase; KT, 3-ketothiolase; and PDC, pyruvate dehydrogenase complex. Major enzymes utilizing acetyl-CoA (dashed arrows) are: AAS, acetoacetyl-CoA synthase; ACC, acetyl-CoA carboxylase; AGS, N-acetylglutamate synthase; CS, citrate synthase; and IPS, 2-isopropylmalate synthase. Not shown in the diagram is the utilization of acetyl-CoA for the synthesis of the cysteine intermediate O-acetylserine in the cytosol, plastid and mitochondrion. Amino acid degradation also implicates the generation of acetyl-CoA either in the peroxisome or mitochondrion.

In the peroxisome acetyl-CoA is generated by the degradation of fatty acids via the ß-oxidation cycle and then enters the glyoxylate cycle via the citrate synthase. These two catabolic and anabolic pathways participate in the conversion of fatty acids to sugars. Recent data indicate that at least some enzymes of the MVA isoprenoid pathway are localized to the peroxisomes, raising questions as to how carbon flux is partitioned and controlled between these two subcellular compartments [13]. Finally, degradation of amino acids, such as isoleucine, leucine and tryptophan generates acetyl-CoA and involves both peroxisomes and mitochondria [14].

## Acetyl-CoA and the acetylation of proteins

Acetyl-CoA is involved in three types of protein acetylation that together form the protein acetylome (Figure 2). O-acetylation occurs on the hydroxyl group of internal serine or threonine residues and is in competition with phosphorylation of the same residues. $N^{\alpha}$-acetylation refers to the addition of an acetyl group to the N-terminal amino acid of proteins. $N^{\alpha}$-acetylation is irreversible and is one of the most common co-translational protein modifications found in eukaryoates. By contrast, $N^{\varepsilon}$-acetylation occurs at the amino group of the side chain of internal lysine residues and is a reversible process, with a flux between acetylated and deacetylated states.

### *O-acetylation*

O-acetylation (Figure 2a) was initially discovered through the study of *Yersinia* Outer Protein J (YopJ), a virulence factor of *Yersinia pestis*, the causative agent of the plague. YopJ was shown to block the mitogen-activated protein kinase (MAPK) signaling pathway through the acetylation of serine and threonine residues that are required to be phosphorylated for kinase activation [15,16]. Thus O-acetylation precludes phosphorylation and blocks all signaling and activity functions associated with such phosphorylation. Although the YopJ/MAPK pathway remains the only described example of protein O-acetylation, and no orthologs of YopJ have been described in eukaryotes, its simple and yet powerful mode of action as a modulator of protein activity via the control of phosphorylation, itself a major PTM, makes it likely that O-acetylation should be found in other proteins. A major bottleneck is the difficulty in the detection of O-acetylation, because most methods based on mass spectrometry (MS) cannot distinguish between O-acetylation with other acetylation events and no specific antibodies to O-acetyl-serine or O-acetyl-threonine are available [17]. However, recent advances in analytical tools, developed in part for the detection of $N^{\varepsilon}$-acetylation, should enable a more systematic search of O-acetylation in eukaryotes, including plants.

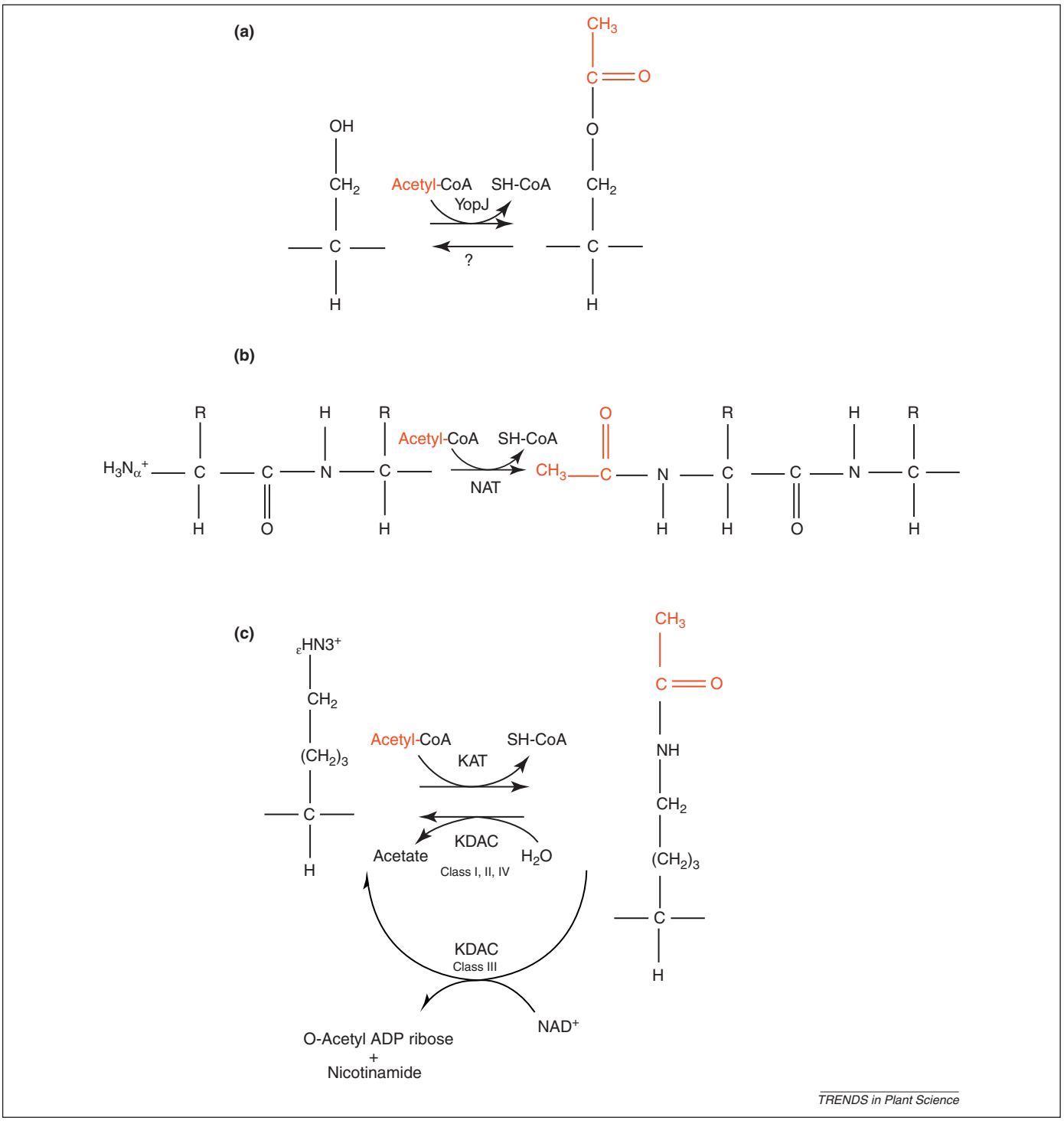



**Figure 2**. Various forms of protein acetylation. **(a)** In O-acetylation, the hydroxyl group of serine or threonine is acetylated by the enzyme YopJ of *Y. pestis*. It is unknown which enzyme, if any, can mediate the deacetylation reaction. **(b)** $N^{\alpha}$–acetylation involves the acetylation of the N-terminal amino group of proteins via the action of N-terminal acetyl transferases (NAT). This reaction is thought to be irreversible. **(c)** $N^{\varepsilon}$-acetylation involves the transfer of an acetyl moiety to side amino group of lysine via lysine acetyl transferases (KAT). Deacetylation occurs through two different mechanisms. Class I, II and IV deacetylases (KDAC) remove the acetyl group from lysine and release acetate, whereas class III KDAC, also called sirtuins, utilizes $NAD^+$ as a coenzyme and give O-acetyl-ADP-ribose and nicotinamide.

### *$N^{\alpha}$-acetylation*

$N^{\alpha}$-acetylation (Figure 2b) is estimated to occur in nearly 50% of proteins in *Saccharomyces cerevisiae* and over 80% of those in humans, but it is rarely present in prokaryotes [18]. Although the level of protein $N^{\alpha}$-acetylation in plants is not known, the high degree of functional conservation of the proteins involved in $N^{\alpha}$-acetylation across eukaryotes, including plants, would suggest that $N^{\alpha}$-acetylation is also very common in plants. Three major N-terminal Acetyl Transferases (NAT) protein complexes containing a catalytic and one or several auxiliary subunits have been well described in human and yeast, each capable of recognizing different N-terminal sequences for $N^{\alpha}$-acetylation [19]. The N-Acetyl Transferase A complex (NatA) contains the

N-Acetyl Transferase 1 (Nat1) and Arrest Defective 1 (Ard1) subunits, the N-Acetyl Transferase B complex (NatB) contains the subunits N-Acetyl Transferase 3 (Nat3) and Mitochondrial Distribution and Morphology 20 (Mdm20), while the N-Acetyl Transferase C complex (NatC) contains the Maintenance of Killer proteins Mak3, Mak10 and Mak31. Homologs to all these subunits are present in plants, and complementation of yeast *natc* mutants with the homologous *Arabidopsis* (*Arabidopsis thaliana*) genes has been reported [20]. NatA mainly recognizes N-terminal single amino acids, such as Ser, Ala, Thr or Gly. NatB specifically recognizes the N-terminal di-amino sequence Met–Glu, Met–Asp and Met–Asn, while NatC catalyzes the $N^{\alpha}$-acetylation of Met–Ile, Met–Leu, Met–Trp and Met–Phe [19].

$N^{\alpha}$-acetylation in yeast and mammals has been associated with several functions, including modification of protein activity, strength of protein–protein interaction, thermal stability, and protein targeting to organelles [21]. For example acetylation is required for tropomyosin binding to actin, it modifies the kinetic properties of the rat glycine N-methyltransferase, causes the loss of specific peptidase activities of 20S proteosome, modifies the interaction strength between the E2 enzyme Ubiquitin Conjugating 12 (Ubc12) and the E3 enzyme Defective in Cullin Neddylation 1 (Dcn1), directs some yeast proteins to the Golgi or inner nuclear membrane while it prevents post-translational translocation through the endoplasmic reticulum membrane of others [21,22]. Although the claim that $N^{\alpha}$-acetylation could be involved in protein stability, at least partially via the inhibition of ubiquitination, remained unsubstantiated for many years, recent research in yeast found that acetylated proteins are actually recognized by the ubiquitin ligase Degradation of Alpha 10 (Doa10) and degraded via the 26S proteasome [23]. An exciting recent study aimed at understanding the antiapoptotic activity of B Cell Lymphoma-xL (Bcl-xL) in human cells revealed that overexpression of Bcl-xL led to a reduction in levels of acetyl-CoA and of $N^{\alpha}$-acetylated proteins, and that restoring acetyl-CoA level by the addition of acetate or citrate restored $N^{\alpha}$-acetylation and conferred sensitivity to apoptotic stimuli [24]. These results are significant, because they show for the first time that protein $N^{\alpha}$-acetylation is regulated by acetyl-CoA availability and that acetyl-CoA can serve as a signal coupling apoptosis to metabolism via the regulation of protein $N^{\alpha}$-acetylation.

A single mutant has been described in *Arabidopsis* affecting a member of the NAT family, namely Mak3, the catalytic component of the NatC complex [20]. Initially identified as a mutant with reduced effective quantum yield, the *mak3* mutant is smaller than wildtype plants, has reduced CHLa/ CHLb value and carotenoid content as well as reduced level of proteins constituting photosystem II and most of the thylakoid multiprotein complexes. Although these phenotypes were suggested to be linked to the requirement of acetylation for the stability and/or import competence of organellar precursor proteins, such mechanisms have not yet been demonstrated. Interestingly, analysis of the plastid proteome identified 47 nuclear-encoded proteins that were $N^{\alpha}$-acetylated following processing, but no $N^{\alpha}$-acetylated protein was found in mitochondria except for a glutamate dehydrogenase with an intact targeting pre-sequence [25,26]. Blocking of the import of plastid proteins in mutants of the Translocation Outer membrane protein 159 (Toc159) led to the accumulation of unprocessed N-acetylated precursor proteins in the cytosol, indicating that N-acetylation may also be a common feature of plastid proteins before import [27]. Although $N^{\alpha}$-acetylation of the large subunit of Rubisco is a feature conserved across vascular and non-vascular plants, including algae, no function has yet been ascribed to this modification [28]. While none of the homologous proteins belonging to the Nat family have been localized to the plastid, it is perhaps more likely that protein homologous to the bacterial Rim involved in the $N^{\alpha}$-acetylation of some ribosomal protein in *Escherichia coli* may be responsible for the acetylation of plastid proteins, although none have yet been demonstrated to be localized to this organelle [29].

Another effect of protein $N^{\alpha}$-acetylation found in plants has been a 20-fold enhancement of the activity of an acetylated form of a 18-amino acid peptide derived from the bacterial elongation factor Tu in triggering media alkalinization (an indirect measure for the induction of defense response) [30]. Clearly, considering the power of the current tools of proteomic and genomics, many basic discoveries of the role of $N^{\alpha}$-acetylation in plants are just awaiting concerted efforts by researchers in this field.

### $N^{\varepsilon}$-acetylation of histones

$N^{\varepsilon}$-acetylation of protein was first described more than 50 years ago for histones. Because of the important function of histones on chromatin structure and transcriptional regulation, histone acetylation has been at the core of protein acetylation research for four decades [31–33]. Acetylation is just one kind of modification found on histones, which also includes methylation, phosphorylation, adjunction of ADP-ribose groups and peptides, such as SUMO and ubiquitin. The acetylation of histones typically occurs at the tails, which are rich in lysine. $N^{\varepsilon}$-acetylation neutralizes positive charges of the histone tails, resulting in a decrease of their affinity for negatively charged DNA and promoting the binding of transcription factors to DNA. Furthermore, acetylated histone lysine residues can be recognized and used as docking site for transcriptional co-regulators and chromatin remodeling factors that contain a module called the 'bromodomain' [34]. Acetylation of histones is associated with the relaxation of chromatin structure and gene activation, and typically occurs in the promoter and 5'-end of genes in *Arabidopsis* [35].

The level of histone acetylation is determined by the activity of both histone acetyl transferase (HATs) and histone deacetylase (HDACs) (Figure 2c). Because the same enzymes have been found to be regulating the acetylation of numerous non-histone proteins, they are now referred to as lysine acetyl transferase (KAT) and lysine deacetylase (KDAC). The KAT in *Arabidopsis* are encoded by 12 genes and can be grouped into four classes, which are named as GCN5-related N-Acetyl Transferase (GNAT), MOZ-YBF2/SAS3-SAS2/TIP60 (MYST), cAMP-responsive element-Binding Protein (CBP) and TATA-binding protein Associated Factor 1 (TAF1), based on comparative analysis with the KAT of yeast and animal [36–38]. Similar to KAT,

the KADC in *Arabidopsis* are encoded by 18 genes and can also be grouped into four types, including Reduced Potassium Dependency 3 (RDP3), Histone DeAcetylase 1 (HDA1), Silent Information Regulator 2 (SIR2) and the plant-specific Histone Deacetylase 2 (HD2) [36–38]. The SIR2 family of KDAC, also called class III KDAC or sirtuins, are distinct from other groups of KDAC in catalyzing deacetylation via a reaction depending on $NAD^+$ (Figure 2C). Some KAT contain bromodomains, enabling them to recognize and bind acetylated histones, indicating that acetylation of histones by one KAT may help to recruit other KAT on the same nucleosome [37]. It is important to note that both the recent discovery of the large abundance of non-histone proteins that are acetylated (see below), including in chloroplasts and mitochondria, as well as the identification of transcription factors in animal that auto-acetylate [39], makes it very likely that additional enzymes with KAT and KDAC activities will be identified in the future. Nevertheless, in contrast to the control of protein phosphorylation, that involves hundreds of kinases and phosphatases, the control of protein acetylation rests on a more restricted number of effectors, likely reflecting their involvement in the control of more global genetic and metabolic switches.

Analysis of mutants affected in either KAT or KDAC revealed that $N^{\varepsilon}$-acetylation plays key roles in a number of processes, including cell cycle, flowering time, response to environmental conditions such as light or pathogen attack, root and shoot development, hormone signaling and epigenetic processes [36,38]. Histone acetylation is dynamic and responds to environmental and developmental signals [40,41]. Dynamic changes in histone acetylation are mediated through the recruitment of KAT and KDAC via their interaction with transcription activators [e.g. Transcription Adaptor Protein 2 (ADA2) and CRE Binding Factor 1 (CBF1)], transcriptional repressors [e.g. Altered Cold-responsive Gene 1 (ACG1) and Apetala 2/ Ethylene Responsive Element Binding Protein (AP2/EREBP)], and/or molecules involved in signal transduction [e.g. Coronatine Insensitive 1 (COI1) for JA or Ethylene Responsive element binding Factor 7 (ERF7) for abscisic acid] [36].

General Control Non-repressible 5 (GCN5) is one of the best characterized of the known GANT-type KAT in plant. The *Arabidopsis gcn5* mutant shows various pleiotropic defects, including dwarfism, loss of apical dominance, aberrant meristem function, root and leaf development, short petals and stamens, floral organ identity, and reduced expression of light- and cold-inducible genes [38]. Mutations of other *KAT* genes also affect different aspects of plant growth and development. For example, the *Arabidopsis taf1* mutant was affected in light regulation and the greening of seedlings [42,43], the *CBP* genes affect flowering time [44,45], and the *MYST* genes are involved in gametogenesis [46].

Similarly to KAT, analysis of mutants in various KDAC also revealed their importance in a number of developmental processes or responses to the environment (reviewed in [47]). For example, HD2A and HD2B are involved in the establishment the adaxial–abaxial leaf polarity, RPD3A influences the expression of jasmonic acid and ethylene regulated pathogenesis-related genes, while *HDA18* contributes to the patterning of root epidermal cells [47].

## $N^{\varepsilon}$-acetylation of non-histone proteins, including metabolic enzymes

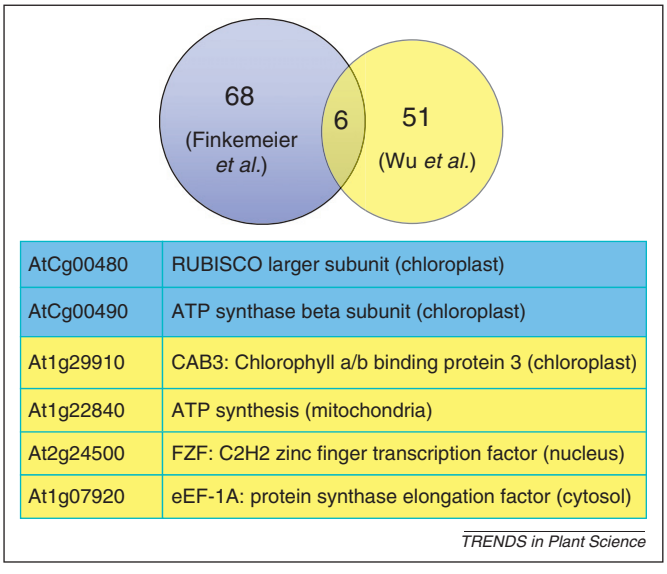
Until the late 1990s, only few proteins other than histones were known to be acetylated, including alpha-tubulin and the DNA-associated High Mobility Group proteins [48]. However, the discovery in 1997 that acetylation of the animal transcription factor p53 regulates its activity marked the beginning of a new search for acetylated proteins that was based on a candidate-based approach [49]. More than 100 proteins were then shown in bacteria, yeast and animal to be acetylated, including a large number of transcription factors and DNA-associated proteins as well as nuclear receptors [48]. While clearly the majority of acetylated proteins were involved in the control of gene expression and DNA-related processes, including recombination and DNA repair, there were notable exceptions, such as the acetylation of acetyl-CoA synthetase [50–52]. The year 2005 marked a turning point with the development of unbiased approaches made possible by the combination of the use of specific anti-$N^{\varepsilon}$-acetylated lysine antibodies with powerful and sensitive new MS-based analytical tools. Starting with the identification of several hundreds of acetylated proteins from mouse liver mitochondria and human HeLa cells [53], recent key studies revealed the presence of thousands of acetylated proteins in human cells and several hundred in bacteria [6,8,54–57]. These acetylated proteins are involved in a broad spectrum of cellular processes, including proteolysis, endocytosis and vesicular trafficking, mRNA processing, cell cycle, stress response, cytoskeleton dynamics, autophagy, as well as protein contributing to signaling cascades, including kinases and phosphatases. Interestingly, two recent studies focusing on the protein acetylome in mitochondrial and cytosolic fractions of human liver on one hand, and the acetylome of the bacteria *Salmonella enterica* on the other, revealed that a large number of metabolic enzymes are acetylated [6,8]. For example, the majority of enzymes involved in glycolysis, gluconeogenesis, the TCA cycle, as well as fatty acid synthesis and degradation were acetylated [6,8,58]. In both animal and bacteria, the level of protein acetylation was affected by the nature of the carbon source used for growth. Most interesting was the discovery that for a number of key enzymes, the acetylation status affected enzyme activity and controlled the direction of carbon flux in a pathway. For example, in human liver cells, fatty acids led to an increased acetylation of the ß-oxidation multifunctional enzyme enoyl-CoA hydratase/ 3-hydroxyacyl-CoA dehydrogenase, which itself led to increased activity of the enzyme [8]. In *S. enterica*, the level of acetylation of the enzyme glyceraldehyde 3-phosphate dehydrogenase (GAPDH), catalyzing the reversible conversion of glyceraldehyde-3-phosphate and 1,3-bisphosphoglycerate, was higher in cells grown on glucose versus acetate, and increased GAPDH acetylation led to increase activity towards glycolysis and decreased activity towards gluconeogenesis, thus revealing that acetylation of GAPDH controls the carbon flux in favor of glycolysis in cells grown on glucose [6]. Other examples of the control of metabolism via acetylation of enzymes include the inactivation of the mitochondrial acetyl-CoA synthetase via acetylation of the active site [50–52] and the downregulation of the urea cycle via the

acetylation of the carbamoyl phosphate synthase 1 [59] and ornithine carbamoyl transferase in response to nutrient signals [60].

Apart for limited studies on tubulin acetylation in some plants [61,62], our knowledge of protein $N^{\varepsilon}$-acetylation in plants was almost exclusively focused on histones [63,64]. However, two recent studies have aimed at initiating a wider description of the $N^{\varepsilon}$ acetylome in plants (Figure 3) [5,7]. The combination of these two studies resulted in the identification of 125 $N^{\varepsilon}$-acetylated proteins in *Arabidopsis* involved in a wide range of cellular processes, including photosynthesis (e.g. small and large subunit of Rubisco, and light-harvesting chlorophyll *a*/*b*-binding protein), protein metabolism (e.g. ubiquitin conjugating enzyme and ribosomal protein), gene expression (e.g. transcription factors), RNA metabolism (e.g. Argonaute 1), stress response (e.g. glutathione synthase and DNAJ heat shock protein), cell signaling (e.g. ethylene receptor ETR2), metabolite transport (e.g. ABC transporters), cytoskeletal organization (e.g. actin depolymerization factor ADF2) and cell wall synthesis (e.g. UDP-xylose synthase). Furthermore several enzymes involved in primary and secondary metabolism were also identified including a terpene synthase-like protein, a 3-ketoacyl-CoA synthase involved in very-long-chain fatty acid biosynthesis, a fructose-bisphosphate aldolase, a pyruvate decarboxylase, a cinnamyl-alcohol dehydrogenase, a cytochrome P450, several isoforms of glutamine synthase, a malate dehydrogenase, a phosphoglycerate kinase and a GAPDH [5,7]. Treatment with a human recombinant KDAC leading to partial deacetylation of either Rubisco, phosphoglycerate kinase or GAPDH resulted in an increase in their activities, while deacetylation of malate dehydrogenase led to a decrease in activity [5]. Notably, the link between malate dehydrogenase acetylation and increase activity has also been reported for the human enzyme [8]. For Rubisco, several of the acetylated Lys residues were previously found to be important either in catalysis or for interaction between domains, giving important clues as to effects of acetylation on Rubisco activity [5].

The control of the acetylation status of a broad range of non-nuclear proteins implies that KAT and KDAC are not exclusively localized to the nucleus. The mitochondrial localization of the human Sirtuin 3 (SIRT3) was one of the first examples of a non-nuclear KDAC [65]. While a systematic analysis of the subcellular localization of plant KAT and KDAC remains to be done, some rice (*Oryza sativa*) KDAC have been localized to the chloroplast and mitochondria [66]. Acetylation of the large subunit of Rubisco and ATP synthase ß subunit, two proteins that are encoded by the plastome and synthesized directly in the plastid, further supports the presence of some KAT (and likely also KDAC) in the plastid.

While the number of identified acetylated proteins, including metabolic enzymes, in *Arabidopsis* is relatively small compared to humans, the fact that only six proteins (including four located in the plastid) were found to be common in both studies (Figure 3) indicates that the depth of coverage is likely very low. Interestingly, at least 21 of the identified acetylated lysine residues identified in [5] were also found in their respective human homologs, implying the conservation of the function of acetylation in a broad range of organisms.




| | |
|---|---|
| AtCg00480 | RUBISCO larger subunit (chloroplast) |
| AtCg00490 | ATP synthase beta subunit (chloroplast) |
| At1g29910 | CAB3: Chlorophyll a/b binding protein 3 (chloroplast) |
| At1g22840 | ATP synthesis (mitochondria) |
| At2g24500 | FZF: C2H2 zinc finger transcription factor (nucleus) |
| At1g07920 | eEF-1A: protein synthase elongation factor (cytosol) |

**Figure 3**. The *Arabidopsis* acetylome. The Venn diagram shows the number of distinct acetylated proteins identified in the studies of Finkemeier *et al.* [5] and Wu *et al.* [7]. The six proteins found in common between these two studies are described in the table, with the genes in blue and yellow being encoded by the plastid and nuclear genomes, respectively.

## Could acetyl-CoA act to control plant metabolism via acetylation?

Considering that acetyl-CoA is a central intermediate in numerous plant anabolic and catabolic pathways as well as an essential substrate for acetylation, it is tempting to speculate whether acetyl-CoA could play a pivotal role in regulating the carbon flux through metabolic pathways via its effects on protein acetylation. Study of $N^{\varepsilon}$–acetylation in animals provides good evidence that such a system indeed exists. The Peroxisome proliferator-activated receptor Gamma Coactivator 1-alpha (PGC-1α) is the master regulator of mitochondria biogenesis in animal and acts through its interaction with a diversity of transcriptional factors [67]. The activity of PGC-1α, which is controlled by its acetylation status, coordinates a transcriptional response which increase mitochondrial activity under conditions of energy needs and attenuates it when energy needs are low [68,69]. PGC-1α is activated through deacetylation by Silent Information Regulation 2 homolog 1 (SIRT1) and inhibited through acetylation by GCN5 [70,71]. The activity of both GCN5 and SIRT1 is modulated by the energy status of the cell. SIRT1 belongs to class III KDAC that are dependent on $NAD^+$ (Figure 2C). Thus conditions that favor a high $NAD^+$/NADH ratio, such as fasting, favor SIRT1 activity and result in PGC-1α deacetylation and activation. By contrast, PGC-1α deactivation by acetylation is controlled via the production of acetyl-CoA in the nucleus by the ATP citrate lyase [72]. Downregulation of a nuclear ATP citrate lyase was shown to reduce histone acetylation, at least in part, via reduced acetylation activity of GCN5 [72]. Furthermore, synthesis of acetyl-CoA via acetyl-CoA synthetase was found to be inactivated by acetylation of the enzyme and reactivated through a SIRT-mediated deacetylation [73]. Thus, not only are

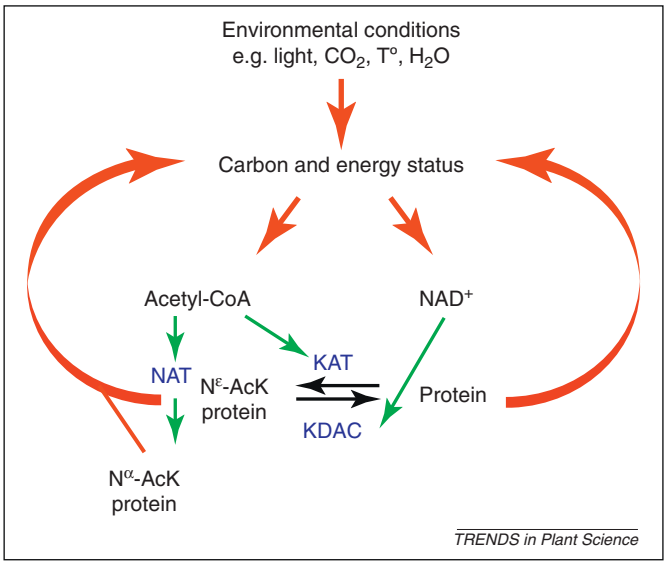



**Figure 4**. Model for the implication of acetyl-CoA and the acetylome on plant metabolism. The influence of environmental conditions on the cell energy status affects the level of acetyl-CoA and $NAD^+$ in various cell compartments. High acetyl-CoA will favor either $N^{\alpha}$– or $N^{\varepsilon}$-acetylation via increase in NAT or KAT activity, respectively, while high $NAD^+$ will activate deacetylation via increase activity of the class III KDAC (sirtuins) (green arrows). Protein acetylation or deacetylation can have either stimulatory or inhibitory effects on certain enzymes and metabolic pathways, which in turn can lead to either increase or decrease of the energy status, acetyl-CoA or $NAD^+$ levels (red arrows).

KAT dependent on acetyl-CoA, but they are themselves potential regulators of acetyl-CoA pools, along with KDAC.

The recent discovery that overexpression of the anti-apoptotic Bcl-xL protein in human cells led to a reduction in levels of acetyl-CoA and of $N^{\alpha}$-acetylated proteins, and that raising acetyl-CoA led to increases of both $N^{\alpha}$-acetylation and sensitivity to apoptotic signals, provide clear evidence that acetyl-CoA can serve as a signal regulating metabolism via protein $N^{\alpha}$-acetylation [24]. A working model of how both $N^{\alpha}$- and $N^{\varepsilon}$-acetylation could be modulated and could in turn influence plant metabolism is shown in Figure 4.

## Concluding remarks and future perspective

Our understanding of the role of acetyl-CoA and protein acetylation on the control of plant metabolism is clearly in its infancy. However, their importance in the control of metabolism in bacteria, yeast and animal, and the conservation of the enzymes involved in these pathways in plants, strongly suggest that acetyl-CoA and protein acetylation are most likely also key players in the control of plant metabolic pathways. Plants are also likely to have unique mechanisms involved in controlling metabolic flux via acetyl-CoA and protein acetylation, because a large part of the energy status in plants occurs through the chloroplast via photosynthesis.

To make rapid progress in understanding the role of acetyl-CoA and protein acetylation in plant metabolism, it will be essential to acquire an in-depth description of the acetylome in plants. In addition, it will be essential to demonstrate to what extent acetylation of metabolic enzymes is important in the control of plant metabolic pathways. Several key approaches will need to be combined to accomplish this, including: (i) determining how environmental conditions as well as the carbon and energy balance of plants cells can influence the level of protein acetylation; (ii) determining the effects of acetylation on enzymatic activity; and (iii) identifying KAT and KDAC involved in controlling the acetylation status of proteins in various organelles and determining how they are themselves regulated. Being only one of many PTM, protein acetylation competes with other modifications, such as phosphorylation, methylation, ubiquitylation and sumoylation, for the same lysine residues. Furthermore, examples of cooperation between acetylation at one site and phosphorylation or ubiquitination at other sites have been described, in some cases involving the interaction of KDAC with phosphatases or proteins involved in ubiquitination [74]. It is thus expected that a complex interplay and crosstalk exist between protein acetylation and other PTM that involves both antagonistic and cooperative interactions and that ultimately modulate protein activity and metabolic flux.

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