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Histone Acetyltransferases in Plant Development and Plasticity

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Abstract: In eukaryotes, transcriptional regulation is determined by dynamic and reversible chromatin modifications, such as acetylation, methylation, phosphorylation, ubiquitination, glycosylation, that are essential for the processes of DNA replication, DNA-repair, recombination and gene transcription. The reversible and rapid changes in histone acetylation induce genome-wide and specific alterations in gene expression and play a key role in chromatin modification. Because of their sessile lifestyle, plants cannot escape environmental stress, and hence have evolved a number of adaptations to survive in stress surroundings. Chromatin modifications play a major role in regulating plant gene expression following abiotic and biotic stress. Plants are also able to respond to signals that affect the maintaince of genome integrity. All these factors are associated with changes in gene expression levels through modification of histone acetylation. This review focuses on the major types of genes encoding for histone acetyltransferases, their structure, function, interaction with other genes, and participation in plant responses to environmental stimuli, as well as their role in cell cycle progression. We also bring together the most recent findings on the study of the histone acetyltransferase HAC1 in the model legumes *Medicago truncatula* and *Lotus japonicus.*

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1. INTRODUCTION

In eukaryotes, the genomic DNA is packed with histone proteins to form structures known as nucleosomes, which in turn form higher-order structures of chromatin. The nucleosomes are evolutionarily well conserved among eukaryotic species [1]. Each nucleosome consists of two copies of the core histone proteins H_2A, H_2B, H_3, and H_4, assembled into an octamer by protein-protein interactions and a single linker protein, H_1. Almost two turns of DNA (approximately 146–147 bp) are wrapped around the histone octamer [2-4]. The H_1 histone as a linking structure stabilizes the octameric core [3].

The nucleosome is not only a mean of packaging large amounts of DNA into the nucleus. Nucleosome positioning on the chromatin strand plays a critical role in modulation of gene expression because it regulates accessibility of DNA to transcription factors and chromatin modifying enzymes. It can also repress transcription *in vivo* and *in vitro* [5]. Nucleosomal DNA represents a mechanical barrier to multiple protein factors (general transcription factors, sequence-specific DNA-binding activators and non-DNA-binding transcription co-activators (or adaptors) that must be bound to DNA for transcriptional activation. Two different mechanisms contribute to the relief of nucleosomal repression: the use of chromatin remodeling factors and the accomplishment of post-translational modifications of chromatin components, in particular histone acetylation. The amino termini of histones extend from the nucleosomal core and are modified by acetyltransferases and deacetylases during the cell cycle. These acetylation patterns may direct histone assembly and help regulate the unfolding and activity of genes. Gene activity is associated with histone acetylation that plays a major role in maintaining chromatin structure and function [6]. The highly acetylated histones are preferentially associated with the active genes, whereas the hypoacetylated histones are responsible for the inactive genes [7-9].

The amino-terminal tails of histones extending from the nucleosomal structure have positively charged lysine (lys^+) and arginine (arg^+) amino acids [10], as the high binding affinity between histones and DNA is based on their opposite charge. The histone tails represent approximately 25-30% of the mass of isolated histones [11, 12], thus ensuring sufficiently large surface for interactions with regulating factors. The acetyl group (CH_3COO^-) is negatively charged and therefore neutralizes the positive charge of histones. Consequently, it decreases the total positive charge of histones, reducing their affinity for negatively charged DNA, which facilitates the access of transcription factors to genomic sequence. The "histone code" hypothesis suggests that the existence of different chemical modifications of N-terminal histone tails provides binding sites for transcription factors that alter chromatin structure actively or promote transcription [13, 14].

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What are the effects of histone acetylation on chromatin structure? Lusser *et al.* [15] suggest three hypotheses concerning the acetylation-dependent modulation of chromatin structure. First, acetylation introduces negative charge in positive histones by acetyl groups (CH_3COO^-), neutralizing them. This process destabilizes the nucleosomal core, allowing transcriptional regulators to bind the regulatory promoter regions of DNA. Second, acetylation affects the packing of the chromatin and therefore could alter the accessibility of regulatory proteins to DNA regulatory regions. Third, acetylation is a specific signal that influences histone-protein interactions [15].

The basic mechanisms of histone acetylation and histone deacetylation are elucidated by detailed studies on the specialized enzymes-histone acetyltransferases (**HATs**) and histone deacetylases (**HDs**) [16-18]. Histone acetyltransferases (HATs) transfer acetyl (CH_3COO^-) groups to the NH_3^+ groups of lysine residues, while the opposing reactions of histone deacetylases (HDs) remove the acetyl groups.

2. STRUCTURE AND FUNCTION OF HISTONE ACETYLTRANSFERASES (HATs)

Based on their subcellular distribution, HATs have been divided into two classes: **HAT-A** and **HAT-B** [17, 18]. Type A HATs are located in cell nucleus and acetylate nucleosomal core histones. They function as transcriptional co-activators and therefore play an important role in the regulation of gene expression. Type A HATs are divided into five families that include the GCN5-related N-terminal acetyltransferases (GNATs), the MYST (MOZ, Ybf2/Sas3, Sas2 and Tip60)-related HATs, p300/CREB-binding protein (CBP) HATs and transcription initiation factor TAFII-250 [for TATA-binding protein (TBP)-associated factor].

Type B HATs are located in the cytoplasm and catalyze acetylation of free histones (not DNA associated), selectively acetylating lysine 5 and lysine 12 of histone H_4 [19, 20]. They are found in maize [21] and show high specificity to the acetylation of histone H_4 [22]. They function as a heterodimeric complex [21] and are related to the chromatin synthesis and assembly of nascent histones into chromosomes. A sequence analysis prognosticates a type B HAT homolog in *Arabidopsis thaliana* [23]. Newly identified genes by Pandey *et al.* [23] specify the homology group to which the gene belongs: HAG from GNAT and MYST families, HAC from CBP family, and HAF from TAFII250 family. These members have homologs in many eukaryotes including plants, particularly in *A. thaliana* [23-25].

In higher eukaryotes, the **GNAT** family is divided into three subfamilies: **GCN5** (General Control Nonderepressible protein 5), **ELP3** (Elongator complex Protein 3) and **HAT1**. In *Arabidopsis*, there are homologs corresponding to each of the subfamilies: **AtGCN5/HAG1**, **HAG3** and **HAG2** [23] and there are two **MYST** genes, named ***HAG4* (HAM1)** [26] and ***HAG5* (HAM2)** [26]. The *Arabidopsis* genome contains **five p300/CBP** (p300/CREB-binding protein) genes: ***AtHAC1*** (or *PCAT2*), ***AtHAC2*** (or *PCAT1*), ***AtHAC4*** (or *PCAT3*), ***AtHAC5*** (or *PCAT4*) and ***AtHAC12***, and two **TAFII250** genes: ***HAF1*** and ***HAF2*** [29].

Structural and functional studies on HAT proteins (Gcn5/PCAF, Esa1 and Hat1) have revealed a fundamental mechanism of histone binding and histone acetylation [30]. Sequence analysis of these proteins demonstrates that they contain a structurally conserved core domain and are part of different families, showing high sequence similarity within the families [31]. The Gcn5/PCAF family contains an N-terminal HAT-domain and a C-terminal bromodomain that is considered to be a targeting motif [32-34]. These proteins function as transcriptional co-activators or adaptors that assist the connection between components of the transcriptional machinery.

Identification of the four components of the Elongator complex, named ELO1, ELO2, ELO3, and DRL1/ELO4 shows the influence of histone acetylation on plant growth and development. The ELO3 is a histone acetyltransferase from the GCN5 class. The Arabidopsis *elo* mutants have decreased leaf and primary root growth due to reduced cell proliferation [35-37].

The CBP/p300 family also includes HAT- and bromodomains, as well as three cystine-histidine-rich domains (cys/his-1, cys/his-2 and cys/his-3) that mediate protein-protein interaction. While CBP/p300 proteins are transcriptional regulators, the TAFII250 proteins are believed to contain TBP (TATA-binding proteins), supporting transcriptional process [38]. This family is represented by one HAT-domain, two kinase domains and two bromodomains that have affinity to highly acetylated H_4 histone [33]. In the structure of MYST proteins, besides a HAT-domain, there is a small zinc-domain, located within the HAT-domain and a chromodomain that can bind to RNA [(Fig. **1**), 39].

The genes *AtHAC1*, *AtHAC2*, *AtHAC4*, *AtHAC5* and *AtHAC12* have ZZ-type zinc finger domains, TAZ-type zinc finger domains, Cys-rich HAT domain, KIX, and bromodomains [40]. The zinc finger domains (ZZ and TAZ) participate in the protein-protein interactions with transcription factors [41], whereas Cys-rich HAT domain at the C-terminal determines the HAT activity *in vitro* [27]. The HAT-proteins with bromodomains are distinguished with respect to the numbers of bromodomains and their presence [23].

Histone acetyltransferases play a crucial role in the control of cell fate and influence cell cycle progression, plant responses to environmental conditions, and gene interactions.

The nature of the mutations (T-DNA insertion and point mutation) identified in the *Arabidopsis* HAT gene *AtGCN5/HAG1* suggests that this gene participates in plant development pathways (meristem function, cell differentiation, leaf and floral organogenesis) and the responses to environmental stimuli, like light and cold [42]. The *atgcn5* mutants show pleiotropic defects as dwarfism, loss of apical dominance, smaller, upward, curled leaves, serrated leaves, abnormal root, aberrant meristem function, short petals and stamens, floral organ identity and reduced expression of light- and cold-inducible genes [43-46].

The p300/CBP family is involved in early development [47], probably due to the participation in apoptosis, prolif-

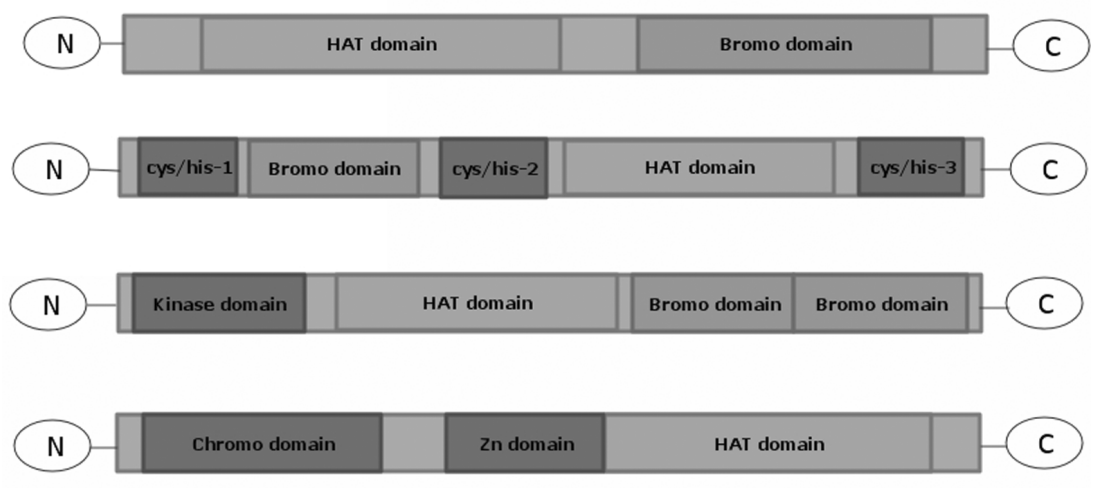

Fig. (1). Diagrams of the HAT protein domains: HAT domain-histone acetyltransferase domain; Zn domain-zinc binding domain; cys/his 1,2,3-cystine/histidine-rich domains.

eration and differentiation. Little is known about the effect of AtHAC1 on *Arabidopsis* development. The p300/CBP plays a crucial role in gene expression and activation, in particular as this family can acetylate histones and non-histone proteins [25, 48]. Furthermore, p300/CBP is not only HAT but also acts as a factor acetyltransferase, affecting multiple cellular processes [25]. p300/CBP can interact with DNA-binding transcription factors and can be part of activation complexes [49-51]. Deng *et al.* [52] show that the *AtHAC1* gene affects the vegetative and reproductive development of *Arabidopsis*. Mutations in *AtHAC1* lead to a late-flowering phenotype and pleiotropic developmental defects, such as short primary roots and partially reduced fertility. Likewise, mutations in the *Arabidopsis HAF2* gene could affect the photoperiod pathway and leaf greening [29].

In *Arabidopsis*, acetylation of N-terminal lysine residues (K9, K14, K18, K23 and K27) of H_3 histone (H3K9, H3K14, H3K18, H3K23 and H3K27) and lysine residues (K5, K8, K12, K16 and K20) of H_4 histone (H4K5, H4K8, H4K12, H4K16 and H4K20) has been related to the regulation of plant cell cycle and epigenetic processes [53]. Particularly, H3K9 acetylation is highly correlated with actively transcribed genes [54-56]. Numerous reports suggest that histone hyperacetylation is equivalent to actively transcribed genes, whereas hypoacetylated histones correspond to inert state of genes [8, 25, 57]. Using mass spectrometry analysis, Earley *et al*. [26] show that mono- (K16Ac), di- (K12Ac; K16Ac), tri- (K8Ac; K12Ac; K16Ac), and tetra- (K5Ac; K8Ac; K12Ac; K16Ac) acetylated isoforms of H_4 histone are related to H_4 hyperacetylation that occurs in the following order K16→K12→ K8→K5.

To avoid confusion and inaccuracy with the characterized members of the families of lysine demethylases, acetyltransferases, and lysine methyltransferases, Allis *et al.* [58] proposed a new nomenclature, where HATs (histone acetyltransferases) have been renamed KATs (lysine acetyltransferases). The authors aim to rationalize the nomenclature and facilitate the next generations scientists working in this research field.

3. SIGNIFICANCE OF HISTONE DEACETYLASES (HDS) FOR MODULATION OF EXPRESSION LEVELS OF HISTONE ACETYLTRANSFERASES

The major histone deacetylase groups include RPD3, HDA1, SIR2 (Silent Information Regulator 2), and HD2 families [59]. In *Arabidopsis*, the RPD3 family is represented by AtHD1 (AtHDA19), AtHDA6, AtHDA7, and AtHDA9 [60]. Phylogenetic analyses show that the RPD3/HDA1 family is found in all genomes of higher eukaryotes [23]. Similarly, homologous proteins have also been found in organisms that lack histones (bacteria and Archaea) [61]. The SIR2 family has no structural similarity to histone deacetylases of the other families, but is presented in all kingdoms, including bacteria [62]. In *Arabidopsis*, two SIR2 homologs, whose functions are not very clear, are identified; this family requires NAD as a co-factor [63] because SIR2 proteins are NAD-dependent histone deacetylases. The HD2 family is first discovered in maize [64] and considered to play a unique role in plants, as the HD2 class is found only in plants [64, 65]. In *Arabidopsis* are identified four HD2 homologs: AtHD2A, AtHD2B, AtHD2C and AtHD2D [65, 66]. The first three are highly expressed in shoot apical meristem (SAM), primary leaves and embryos, whereas the fourth homolog is expressed in flowers, young siliques and stems [67].

Disruption of AtHD1 by a T-DNA insertion, resulting in a null allele mutant (*athd1-t1*) induces a number of developmental abnormalities, site-specific and reversible acetylation changes in H_3 histone lysine 9 (lys9/H_3), H_4 histone lysine 12 (lys12/H_4), and H_4 histone tetra- lysines 5, 8, 12 and 16. Microarray analysis of *Arabidopsis* shows that the transcriptome is up or downregulated in *athd1* mutants, manifesting positive and negative control of transcriptional regulation [68].

Thus, histone acetylation and deacetylation play a unique and distinct role in transcriptional regulation tied to the developmental programs, where gene interactions are essential.

4. HATs TARGET GENES WITH A ROLE IN PLANT DEVELOPMENT

One of the fundamental developmental pathways in plants is the floral organogenesis. Flowering is controlled by multiple regulatory mechanisms, including histone modifications. In *Arabidopsis*, the flowering time is regulated by several fundamental pathways: the autonomous, vernalization, photoperiod and gibberellins (GA) [69, 70]. During vernalization, plants acquire competence to flower, whereas the long-day conditions promote flowering.

Flowering Locus C (FLC) is a key regulator of flowering time that is involved in all four pathways. When the MADS-box transcription factor (FLC) is highly expressed, flowering is delayed, whereas its low expression induces flowering [71]. The combination of phenotypic, genetic, molecular and biochemical analyses indicate that the expression level of FLC is regulated by modifications of histone proteins [55, 68]. The two members of the autonomous pathway, Flowering Locus D (FLD) and Flowering Locus VE, are involved in the inhibition of the expression levels of FLC by chromatin deacetylation. Other key players that are highly related to FLC are five MADS-box genes: *MAF1*, *MAF2*, *MAF3*, *MAF4*, and *MAF5* [72, 73]. MADS AFFECTING FLOWERING (MAFs) genes are basic regulators of floral transition in *Arabidopsis* [74, 75]. A number of genes involved in the autonomous pathway (*FCA*, *FPA*, *FLK*, *LD*, *FY* and *REF6*) have been shown to promote flowering by repressing FLC [52]. In addition to the repressed FLC expression, vernalization is another pathway sharing the same function [76]. The gene responsible for this inactivation is *Vernalization Insensitive 3* (*VIN3*) gene that participates in histone H_3 deacetylation [76].

Consistent with the studies by Servet *et al.* [42], AtGCN5 is related to the regulatory function of key genes, controlling flower meristem activity. Knock-out mutants (*Atgcn5*) mentioned above, show terminal flowers that are characterized by homeotic transformations of petals into stamens, sepals into filamentous structures, formation of ectopic carpels [44], over-proliferation of young buds, and development of abnormal structures around the inflorescence meristem [77]. Up-regulation of the meristem regulatory genes, *WUSCHEL* (*WUS*), *AGAMOUS* (*AG*), *LEAFY*, and *UFO* (*UNUSUAL FLORAL ORGANS*), is detected in the *gcn5* mutant phenotype, which suggests that AtGCN5 is involved in the regulation of the floral meristem activity through the WUS/AG pathway [44].

In *Arabidopsis*, the plant shoot derives from a small group of undifferentiated, actively dividing cells, which are defined by WUS. The studies by Gallois *et al.* [78] show that WUS expression induces shoot stem cell identity, leaf development, floral development and embryogenesis. Developmental functions of AtGCN5 involve direct repression of WUS transcription (by the association of the repressor in the *WUS* promoter region) and the up-regulation of *PLETHORA* (*PLT1* and *PLT*2). The *WUS* gene controls shoot and floral meristem stem cells, whereas *PLT1/PLT2* is required for the root meristem development [79].

In plants, the growing tips of the shoot axis and axillary buds represent a population of undifferentiated, actively dividing cells, which form SAM [80]. This group of dividing stem cells consists of two zones: the central meristem zone and the peripheral meristem zone. The first zone provides new cells, needed for the peripheral zone, whereas new organs originate from the second zone [81]. The *WUS* expression is an important determinant for the cell maintenance in the central zone [82]. The expression level of *WUS* is regulated by *CLAVATA3* (*CLV3*), as its antagonistic function controls the size of the stem cell population [83, 84]. *WUS* activates *CLV3* in the shoot apex [85].

Another key gene responsible for the regulation of floral development is *LEAFY* (*LFY*) [86]. Lohmann *et al.* [87] demonstrate that the floral organ identity gene *AGAMOUS* (*AG*) is activated by *WUS* in combination with *LFY*.

The knotted-like homeobox (KNOX) gene family is important for the differentiation of centrally located, slowly dividing stem cells and peripheral founder cells. These genes are expressed in SAM. During leaf initiation, the KNOX genes are downregulated in the peripheral cells. In *Arabidopsis*, the expression of KNOX genes is repressed by *ASYMMETRIC LEAVES1* (*AS1*) gene, showing cell differentiation in leaves [88, 89]. The histone modifications and histone acetylation and deacetylation in particular, regulate the expression level of *AS1* [90] and are involved in leaf-axis specification [89], the transition between juvenile and mature leaves [90], and leaf morphogenesis. In *Arabidopsis*, a bromodomain protein called GENERAL TRANSCRIPTION FACTOR GROUP E6 (GTE6) is similar to AS1 and its positive regulator, because it is associated with the promoter region of *AS1*. Therefore, GTE6 activates AS1 by mediating acetylation of histones [90, 91].

As it was already mentioned, the Elongater core enzyme, ELO3, is a conserved histone acetyltransferase from the GCN5 class. Nelissen *et al.* [92] confirmed acetylation of H3K14 in coding and $3'$ -untranslated regions of specific genes [the auxin repressor *SHORT HYPOCOTYL 2* (*SHY2*), and the auxin influx carrier *LIKE AUXIN RESISTANT 2* (*LAX2*)], that are involved in the distribution of auxin, a plant hormone, which plays a crucial role in *Arabidopsis* growth and development.

5. ROLE OF HISTONE ACETYLTRANSFERASES (HATs) IN PLANT RESPONSES TO INTERNAL AND EXTERNAL STIMULI

A number of biological processes, such as cell differentiation, growth and development, are affected by internal and external signals, like light [60], temperature, osmotic and oxidative stresses [92-94]. An important mechanism in mediating the changes of gene expression under these stimuli is chromatin remodeling. Histone acetylation and deacetylation can activate or repress gene expression. Misexpression of HATs and HDs leads to multiple defects in plant growth and development, which confirm their basic role in modifications related to changes in environmental conditions.

In contrast to animals, plants are sessile organisms that must adapt to their changing surroundings. Part of their adaptation responses are mediated by the modulation of histone acetylation. The light as an environmental stimulus regulates histone acetylation and participates in growth and development of plants through their entire life cycle [95, 96].

Light and nitrogen availability are two factors that control the expression of the C4-specific gene encoding phosphoenolpyruvate carboxylase (C_4-PEPC). The general role of C_4-PEPC is the primary fixation of carbon dioxide during the process of photosynthesis. This gene provokes hyperacetylation of the H_3 and H_4 histones in maize (*Zea mays*), as the availability of illumination is one of the necessary requirements [97]. The same authors suggest that the illumination represses histone deacetylation, resulting in the hyperacetylation of histones. Another gene involved in the hyperacetylation process of the H_3 and H_4 histones, and controlled by illumination is the *pea plastocyanin* (*PetE*) gene [98]. Basic components of the light pathway are the phytochromes (phy) and the cryptochromes (CRY) that are related to regulation of plant response to light. These photoreceptors activate the HY5 transcription factor (ELONGATED HYPOCOTYL 5), resulting in gene expression accordingly [99]. *HY5* is defined as a light-responsive gene that represents a fundamental target of AtGCN5 [100] and an important participant in the regulation of transcription, controlling photomorphogenesis [101]. AtGCN5 and HY5 are elements of the same light-regulatory pathway in the sense that they share genomic targets, such as light-responsive genes [100]. Molecular and genetic analyses demonstrate that AtGCN5 has an epistatic attitude towards HY5 [54]. As a result of light induction, AtGCN5 interacts with TAF1/HAF2, leading to increased levels of acetylation of H3K9Ac, H3K27Ac and H4K12Ac. This shows that AtGCN5 and TAF1 are important factors in light responses as they affect histone acetylation and gene expression [29, 54]. TAF1/HAF2 participates in the phytochrome pathway and interacts with HY5 activating light-induced gene expression [29].

As mentioned above, AtGCN5 participates in the light-regulatory pathway but is also involved in the cold response pathway [102]. Analyses of the processes of cold acclimation and vernalization have shown that AtGCN5 interplays together with C-repeat/DRE binding factors (CBF1) to regulate cold-regulated (*COR*) gene expression [43]. Plants must adapt their growth to low temperature (cold acclimation) and prevent early flowering. Different histone modifications contribute to this specific adaptation [103]. Histone acetylation acts antagonistically to histone deacetylation and controls the "on" or "off" switching of the cold-responsive gene expression.

Exposure to low temperatures affects two genes: *FLC* (*Flowering Locus C*) and *VIN3* (*Vernalization Insensitive 3*) that induce changes in histone modifications. If MADS-box floral repressor (FLC) is exposed to low temperatures, the *FLC* expression level is reduced contributing to the early flowering [104]. The cold acclimation provokes *VIN3* gene activity, which causes *FLC* silencing through histone deacetylation [105]. A hypothesis exists that the VRN2 dimethylation of the H_3K_9 and H_3K_{27} histones (H_3K_9me2 and H_3K_{27}me2) is a result of histone deacetylation, which leads to *FLC* repression [(Fig. **2**), 106, 107]. The interaction between the key regulator of the vernalization pathway (*FLC*) and the PHD-finger protein encoding gene (*VIN3*) correlates with the period of cold treatment. Therefore, the increasing period of cultivation leads to low *FLC* expression levels and high *VIN3* levels [105].

6. ROLE OF HISTONE ACETYLTRANSFERASES (HATs) IN CELL CYCLE PROGRESSION

During the interphase, the chromosomes are despiralized, whereas during mitosis they become highly compacted. The precise delivery of genetic information during cell division depends on the high condensation of chromosomes, occurring during cell cycle transition from interphase to mitosis. The highly dynamic balance of the histone acetylation and deacetylation contributes to the modulation of chromatin conformation and compactization [5, 108]. Acetylated histones are observed both in the transcriptionally active euchromatin and transcriptionally inactive heterochromatin. In gene-rich regions, during early S phase and mid-S phase, an increased histone acetylation is observed, whereas in late S phase, M phase, and G1 phase, the acetylation is reduced to a much lower level [109, 110]. Furthermore, the late S phase and G2 phase are associated with highly acetylated histones that deacetylate in the heterohromatic regions during the late G2 phase. In *A. thaliana*, Cools *et al.* [111] develop a synchronized root tip system under hydroxyurea treatment to study the genes involved in cell cycle progression. The addition of this genotoxic drug has dramatic impact on the transcription of various histone genes. The level of histone transcripts peaks at different times under hydroxyurea treatment. During this synchronization experiment appearance of various histones confirms results of Morales *et al.* [112], regarding the incorporation of histone proteins into nucleosomes. First, H_3 and H_4 associate to form tetramer followed by two H_2A-H_2B dimers, thus completing the nucleosome assembly. Finally, the H_1 linker protects the nucleosome structure. In *Arabidopsis*, the acetylation of K5, K8 and K12 of the H_4 histone is always immutable during G1, S and G2 phases, as H_4K_{16} leads to a cell-dependent modulation. Interestingly, the histone H_3K_{18} has a similar function as H_4K_{16} in the acetylation process [113].

It has been shown that ElO3 is required for shoot meristem cell cycle progression in *A. thaliana* seedlings. The main process during seed germination is DNA replication when new histone molecules are synthesized. The expression level of histone genes is a marker for DNA synthesis. In the *elo3* mutants almost no signal of H_4 are detected, suggesting that DNA replication is not observed in the mutants background [114].

In the centromeric regions of the chromosomes which are gene-poor, the H_4K_5, H_4K_8 and H_4K_{12} histones are highly acetylated during the interphase and rapidly deacetylate during mitosis, while the gene-rich regions are

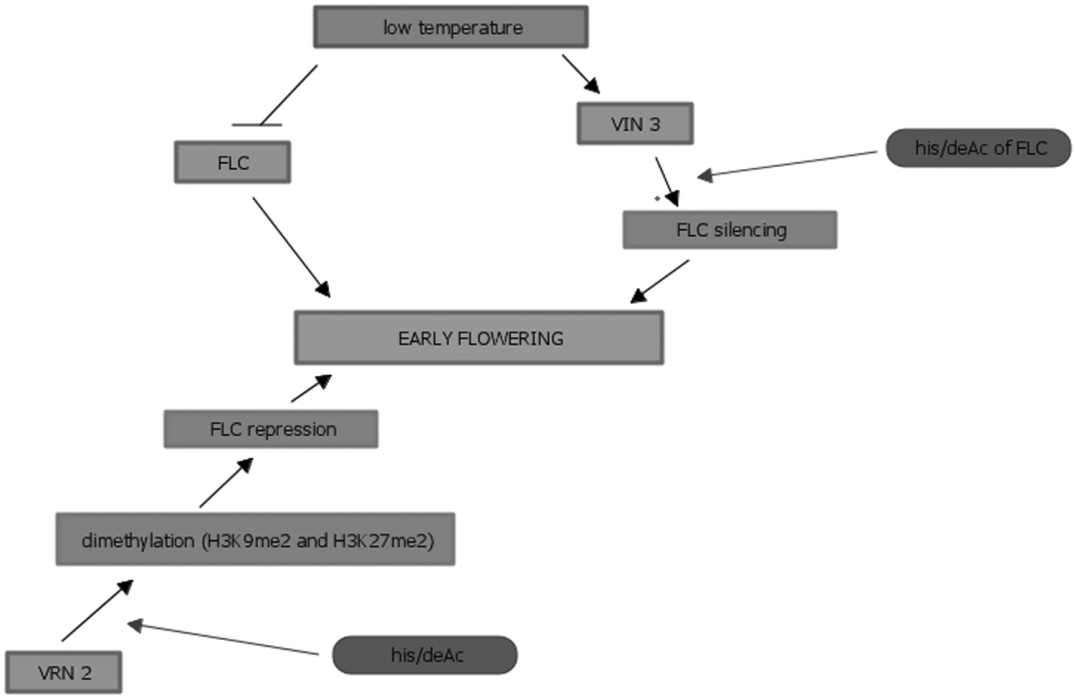

Fig. (2). Schematic representation of the major genes, promoting early flowering. *FLC*- Flowering Locus C; *VIN 3*- Vernalization Insensitive 3; *VRN 2*- Vernalization 2; his/deAc- histone deacetylation.

represented by highly acetylated levels of H_4K_8 and H_4K_{16}. The selectively acetylated lysine 5 and lysine 12 of the type B HATs H_4 histones (H_4K_5 and H_4K_{12}) quickly deacetylate during the telophase and interphase [113-115]. In plants, treatment with the DNA synthesis inhibitor trichostatin A (TSA) causes acetylation of lysine 5, lysine 12 and lysine 16 of the H_4 histone [116]. Germination of wheat seeds in the presence of TSA changes significantly the architecture of the interphase chromosome arms, suggesting that specific chromosome segments are remodeled by this treatment, but there is a tethering of both centromeres and telomeres to the nuclear envelope [117]. In lines carrying multiple transgene integrations that are usually colocalized during interphase, the TSA treatment leads to their dispersion and an increase in transgene activity. This suggests that the colocalisation/dispersion of the transgenes may be a function of specific interphase chromosome organization.

Histone acetylation and transcriptional activation are associated with changes in chromatin organization and chromatin remodeling, as the active genes in higher eukaryotes are positioned in potentionally mobile regions [118]. Also, the active genes that are undermethylated and with an increased acetylation, have a remarkable relation to the regulation of gene expression [119].

Jasencakova *et al.* [120] study the extent of H_4 histone and H_3 histone acetylation within euchromatin and heterochromatin during the cell cycle. They use a new approach, based on specific immunolabeling with antibodies for acetylated isoforms of H_4 histone [121]. DNA content into G1-, S- and G2-fractions of the histone isoforms manifests chromatin domains of isolated chromosomes. The identification of these specific chromatin domains is achieved through an *in situ* fluorescence hybridization or DAPI (4`,6-diamidino-2-phenylindole) staining. The results show that the extent of H_4 histone acetylation is linked to the replication process rather than to the transcription activity. Experiments conducted with field bean demonstrate high H_4 acetylation levels during or shortly after replication [120].

Maintaining genome integrity is critical for the survival of a species. UV radiation, genotoxic chemicals, ionizing radiation and the products of cell metabolism, such as reactive oxygen species and lipid peroxidation, constantly damage cells and are potential mutagens. The cellular DNA damage response system continuously surveys the genome, ready to propagate a number of signals based on the damage detected and its potential to repair. Many of the proteins involved in macromolecular complexes in the diverse cellular processes are acetylated: DNA replication, DNA damage and repair, chromatin remodeling, cell cycle, splicing, nuclear transport, and cytoskeleton reorganization [122].

7. RECENT PROGRESS IN THE INVESTIGATION OF HAC1 IN THE MODEL PLANTS *MEDICAGO TRUNCATULA, LOTUS JAPONICUS* AND *ARABIDOPSIS THALIANA*

In the project 'IFCOSMO': Integrated functional and comparative genomics studies on the model legumes *Medicago truncatula* and *Lotus japonicus* (supported by a grant from the Ministry of Education and Science, Bulgaria) are used forward and reverse genetic approaches in order to study the functions of unknown legume genes [123, 124]. One of the genes of particular interest is *M. truncatula* HAC1 (histone acetyltransferase, MT0G02960, PLAZA

2.5), which is an ortholog of the *Arabidopsis HAC1* (At1g79000, PLAZA 2.5), belonging to p300/CBP (p300/CREB-binding protein). *Agrobacterium tumefaciens*-mediated transformation approach was applied to obtain stable HAC1 transformants of the model species *M. truncatula*, *L. japonicus* and *A. thaliana*. Positive plants with *HAC1* overexpression and RNAi knockdown, and plants carrying transcriptional reporter (endogenous promoter of *HAC1* fused to GUS and GFP marker genes) are currently the subject of intensive investigation and phenotyping, focusing on the processes of lateral root formation, nodulation and somatic embryogenesis.

M. truncatula and *L. japonicus* plants with overexpression of *HAC1* had a better developed root system, in comparison with wild-type plants. The *M. truncatula* root system consisted of roots forming a rosette around base of the stem and many secondary branches that were much longer than the primary roots. The plants were larger and more vigorous with shoots, bearing more side branches than the wild-type plants. In *L. japonicus* overexpressing *HAC1*, the root system consisted of a primary root and secondary branches that were shorter and thicker than these in the wild-type plants. RNAi-mediated knockdown of *HAC1* in *L. japonicus* led to polyploid giant cells, which are associated with enlarged cell volume. Microscopic examination of *A. thaliana* with *HAC1* knockdown showed numerous giant leaf cells. Overexpression of *HAC1-GFP* in *A. thaliana* and *L. japonicus* revealed its distinct localization in the nuclei. Taken together, these data confirmed involvement of the studied *HAC1* gene in the cell cycle progression and development of both model legume plants.

FUTURE PERSPECTIVE

Histone acetylation/deacetylation is a conserved mechanism for epigenetic regulation of chromatin structure during plant development and stress conditions, modulating the cell cycle and maintaining genome stability. Although histone modifications are thoroughly researched in yeast and mammals, there have been very few studies in plants. Until now, most of the reports are limited to *A. thaliana* and tomato, and reveal histone modifications at sites that are unique to plant species. It has been shown that histone variants and modifications in the legume soybean are different from *A. thaliana.*

Plants are ideal model systems to study the effect of diverse exogenous and endogenous cues on acetylation patterns, in order to understand chromatin-mediated control of gene expression, and decipher the biological significance of the changes in histone modifications. These rapid modifications may underlie the higher developmental plasticity of plants, allowing them to respond rapidly to environmental stress. More comprehensive studies on the legume genomes, like *M. truncatula* and *L. japonicus*, will provide further insights into modifications of histone proteins and their functional significance in higher plants. The combined genome-wide analysis of histone acetylation patterns with the phenotypic data from the model legumes will allow identification of candidate genes involved in genetic traits of specific interest. Future efforts will focus on transferring the obtained knowledge for histone acetylation from model species to commercially viable legume crops.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflicts of interest.

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ABBREVIATIONS

HACs	=	Histone Acetyltransferases
HATs	=	Histone Acetyltransferases
HDs	=	Histone Deacetylases
GNATs	=	GCN5-related N-terminal Acetyltransferases
CBP	=	p300/CREB-Binding Protein
TBP	=	TATA-Binding Protein
GCN5	=	General Control Nonderepressible protein 5 MAFs = MADS Affecting Flowering
SAM	=	Shoot Apical Meristem

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