Histone acetylation: a requirement for petunia floral scent

Konstantinos E. Vlachonasios1,2,*

1 Department of Botany, School of Biology, Aristotle University of Thessaloniki, Greece
2 Natural Products Research Centre of Excellence (NatPro-AUTh), Center of Interdisciplinary Research and Innovation of Aristotle University of Thessaloniki (CIRI-AUTh), Thessaloniki, Greece
*Correspondence: kvlachon@bio.auth.gr

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Biosynthesis of volatile organic compounds (VOCs) in plants depends on the biosynthetic pathways of the primary metabolite. These regulations require molecular coordination at different levels on both primary and secondary metabolic networks by specific transcription factors that temporarily activate transcription and scent emission in plant flowers. However, very little is known about the epigenetic regulation of VOC biosynthetic genes. Now, Patrick et al. (2021) report that H3K9 acetylation is required for proper gene expression of primary and secondary metabolite pathways involved in petunia floral scent. This work reveals that chromatin regulatory mechanisms are essential for activation of VOC biosynthesis in petunia flowers.

Plants synthesize a plethora of secondary metabolites that offer a wide range of functions, including plant growth, development, and defence against biotic and abiotic environments (Pichersky et al., 2006). One subset of those metabolites consists of VOCs classified in four major groups: terpenoids, phenylpropanoids/benzenoids, fatty acids, and carotenoid derivatives, as well as nitrogen- or sulfur-containing compounds (Schuurink et al., 2006). Plants use VOCs for communication and interaction with the surrounding environment, including attraction of pollinators and seed dispersers, defence against biotic factors and abiotic stresses, and for plant–plant signalling (Picazo-Aragonés et al., 2020).

The flowers of Petunia hybrida cv. Mitchell emit high levels of phenylalanine-derived phenylpropanoid/benzenoid acid volatiles in the evening. Therefore, petunia has emerged as a model system to study volatile benzenoid synthesis, emission, and regulation (Dudareva et al., 2013). Phe synthesis occur via the shikimate pathway that connects carbon metabolism to Phe (Maeda and Dudareva, 2012). The major volatile phenylpropanoid (C6-C3) and benzenoid (C6-C1) compounds are produced from catalysis of Phe to trans-cinnamic acid through l-phenylalanine ammonia-lyase (PAL) action. The formation of the volatile phenylpropanoid-related (C6-C2) compounds originates directly from Phe (Dudareva et al., 2013). VOC synthesis differs throughout plant development due to the differential gene expression patterns in distinct cell types (Picazo-Aragonés et al., 2020). Genes involved in the synthesis of VOCs exhibit transcriptional activation that coincides with the VOC emission (Colquhoun et al., 2010). Several R2R3-type MYB transcription factors control phenylpropanoid/benzenoid biosynthetic genes (Liu et al., 2015). Suppression of ODORANT I (ODOI) and EMISSION OF BENZENOIDS I and II (EOBI and EOBII) in petunia down-regulates the expression levels of many VOC biosynthetic genes and, as a result, a decrease in the emission of most volatiles was observed (Liu et al., 2015). However, knowledge on the mechanisms that regulate the transcriptional reprogramming of VOC biosynthesis is still limited.

Patrick et al. (2021) demonstrated that histone acetylation regulates volatile phenylpropanoid/benzenoid biosynthesis pathway genes. Using ChIP-seq analysis in second day post-anthesis petunia flowers, it was found that histone H3K9 acetylation levels were elevated on many genes that are involved in the shikimate, general phenylpropanoid, and volatile phenylpropanoid/benzenoid pathways. Moreover, several genes involved in monolignol biosynthesis and eugenol/isoeugenol synthesis also have higher histone H3 acetylation levels. The increased histone H3K9 acetylation levels were in turn associated with gene activation during anthesis. Therefore,
Box 1. Possible histone acetylation mechanisms in the VOC biosynthetic pathway in petunia

The regulation of VOCs and the release of petunia floral scent are mediated by histone modification. Furthermore, H3K9 acetylation is correlated with transcription activation of VOC biosynthetic genes (Patrick et al., 2021). Histone H3K9 acetylation is detected in genes involved in almost all steps of the shikimate pathway (blue), the primary steps of VOC biosynthesis. Moreover, genes (blue arrows) involved in the first steps of Phe-derived phenylpropanoid/benzenoid volatile compounds also received a histone acetylation mark. Therefore, in the first 2 d of open petunia flowers, several protein complexes that contain HATs should be recruited to the promoter and the loci of those genes. One of them could be the elongator complex that includes the ELP3/HAG3 acetyltransferase, since ELP3 is expressed during this developmental stage (Patrick et al., 2021). In Arabidopsis, ELP3 is involved in RNAPII transcription elongation through acetylation of H3K14 in the coding and 3'-untranslated regions of specific genes (Nelissen et al., 2010). Several other HAT-containing complexes, such as GCN5 or HAC, could also be recruited to the loci of VOC biosynthetic genes. In response to internal or external stimuli, specific HATs and the associated proteins are recruited to the promoter region of the responsive genes by particular transcription factors (Grasser et al., 2020). The HAT complex action will trigger histone acetylation (H3K9, H3K14, H3K23, H3K27, H4, and others), which is followed by transcription activation or repression. The question is what kind of signals recruit the HAT complex into promoters of the VOC biosynthesis genes.

One possibility is the regulation of the rhythmic release of VOCs by transcription factors encoded by clock genes (Dudareva et al., 2013). In petunia, the morning component LATE ELONGATED HYPOCOTYL (LHY) controls the daily expression of many VOC biosynthetic genes and specific transcription factors such as ODO1, by restricting their expression in the evening (Fenske et al., 2015). Acetylation and deacetylation of the H3 histone of the clock genes could change chromatin accessibility and subsequently affect the expression of the VOC biosynthetic genes and the emission of VOCs.

Another scenario is that specific transcription factors, such as ODO1, EOBI, and EOBII, recruit HAT complexes to the promoters of genes involved in the phenylpropanoid/benzenoid biosynthetic pathway independently of circadian rhythms. During flowering development in Arabidopsis, histone acetylation and several HATs play a significant role by controlling hormone responses and orchestrating gene expression profiles in several floral organs (Poulos and Vlachonasios, 2018; Grasser et al., 2020). Furthermore, ethylene regulates VOC emission after pollination in petunia by controlling several VOC genes (Underwood et al., 2005). Ethylene treatment increases H3 acetylation levels on ethylene-responsive genes in Arabidopsis seedlings (Wang and Qiao, 2019). The specificity of modulation of the histone acetylation response to produce different VOCs relies on the partner(s) of the HAT complex(es). Identification of the HAT-containing complex in primary and secondary metabolic networks will reveal details of the molecular mechanism that underlies VOC biosynthesis of flowers.
the genes involved in the shikimate and Phe biosynthetic pathway are a target of histone H3K9 acetylation, and the biosynthesis of VOCs is regulated at both the epigenetic and transcriptional level.

Histone acetylation neutralizes the positively charged Lys residues on histone tails. As a result, the interaction between DNA and histones weakens, making chromatin more accessible to transcription factors. The transcription factors will activate or repress gene expression (Roth et al., 2001). Histone acetylation is catalyzed by histone acetyltransferases (HATs). In eukaryotes, four types of nuclear-localized HATs have described. The GNAT (GCN5-related N-terminal acetyltransferase), MYST (MOZ, Ybf2/SAS3, SAS2, and Tip60-related), p300/CREB-binding protein (CBP), and TAF1 (TATA-binding associated factor) families. In Arabidopsis, three GNAT-type proteins—General Control Nonderepressible 5 (GCN5, also known as HAG1), Elongator complex protein 3 (ELP3 also known as HAG3), and HAG2; two MYST types—HAM1 and HAM2 (known also as, HAG4 and HAG5); five p300/CBP types—HAC1, HAC2, HAC4, HAC5, and HAC12; and two TAF1 types—HAF1 and HAF2 (Pandey et al., 2002)—are known. Multiple HATs are responsible for H3K9 acetylation, while several HATs such as GCN5 specifically acylate H3K14. Besides histones, GCN5 and other HATs could also acetylate numerous proteins (Sterner and Berger, 2000). The enzymatic specificity of the HATs reflects their specific role in the regulation of gene expression, as acetylated Lys of histones is recognized by bromodomain-containing proteins. These histone modification readers can cause diverse epigenetic consequences (Marmorstein and Zhou, 2014).

Histone acetylation modulates chromatin dynamics in plant responses to internal and external cues to regulate downstream gene expression as signalling outputs (Jiang et al., 2020). Furthermore, histone acetylation affects multiple processes that span the entire life cycle, including specific metabolic or developmental processes and adaptation to environmental cues (Grasser et al., 2020) (Box 1).

The histone acetylation and the transcriptional omics on the second day after anthesis of petunia flower points toward the molecular processes that contribute to VOC biosynthetic genes. This work could be used as a reference point to reveal the role of histone modifications in coordination of terpenoid biosynthesis genes or volatile, fatty acid derivative compounds in other plants. Epigenetic factors are relevant modulators of rapid responses to the environment, enabling plants to adapt to stress events more efficiently and preparing the offspring for future challenges. Moreover, volatile secondary metabolites are essential for plant biotechnological applications including food, health, and industrial products, and the perfume industry (Plasmeijer et al., 2020). The regulation of VOC biosynthesis at the chromatin level will have many metabolic engineering implications. For instance, the use of transgenes with the local chromatin environment that could be turned on or off by histone acetylation will facilitate the optimum regulatory circuits to produce high-level specialized metabolites.

**Keywords:** Benzenoid, chromatin, ELP3, floral scent, GCN5, histone acetylation, histone acetyltransferase, H3K9ac, H3K14ac, phenylpropanoid, VOC.

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