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# Heterochromatin proteins and the control of heterochromatic gene silencing in *Arabidopsis*

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## Summary

The SU(VAR)3–9 protein family was first identified in animals as heterochromatin-associated proteins and found to control establishment of heterochromatic chromatin domains by histone H3 lysine 9 methylation. In *Arabidopsis* ten *SU(VAR)3–9* homologous *SUVH* genes are found where *SUVH1*, *SUVH2* and *SUVH4* represent different subgroups of genes. Also the SUVH1, SUVH2 and SUVH4 proteins represent heterochromatin-associated proteins and display differential effects on control of heterochromatic histone methylation marks. In *Arabidopsis* the heterochromatin specific histone methylation marks are mono- and dimethyl H3K9, mono- and dimethyl H3K27 and monomethyl H4K20. In contrast to animal systems trimethyl H3K9, trimethyl H3K27 and di- and trimethyl H4K20 do not index chromocenter heterochromatin in *Arabidopsis*. SUVH2 shows a central role in control of heterochromatin formation and heterochromatic gene silencing in *Arabidopsis*. Loss-of-function of SUVH2 results in significant reduction of all heterochromatin-specific histone methylation marks and causes DNA hypomethylation at chromocenter heterochromatin. SUVH2 overexpression leads to ectopic heterochromatisation accompanied with significant growth defects. SUVH2 shows strong dosage-dependent effects on transcriptional gene silencing. In *Arabidopsis* different experimental systems connected with transcriptional gene silencing have been used for genetic dissection of molecular mechanisms controlling epigenetic processes. Molecular analysis of the genes identified by the isolated modifier mutants suggests that transcriptional gene silencing in plants is caused by heterochromatisation. A new efficient experimental system for the analysis of transcriptional gene silencing has been established with the help of *LUCIFERASE* transgene repeats. The different lines established show either complete or partial silencing of the luciferase

*Abbreviations:* *E(var)*, enhancer mutation of position-effect variegation; E(Z), enhancer of Zeste protein; HMTase, histone methyltransferase; RIGS, repeat-induced gene silencing; *Su(var)*, suppressor mutation of position-effect variegation; *Su(var)3–9*, suppressor gene encoding a histone H3K9 methyltransferase; SUV4-20, histone H4 lysine 20 methyltransferase; SUVH, SU(VAR)3–9 homologous protein of plants; TGS, transcriptional gene silencing

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transgene repeats. These lines have been successfully used either for mutant isolation or for functional analysis of SUVH proteins in control of heterochromatic gene silencing.


## Introduction

Heterochromatin and euchromatin are structurally and functionally distinct chromosomal domains. Heterochromatin in contrast to euchromatin remains condensed and densely stained throughout the cell cycle. It is regularly found as blocks of constitutive heterochromatin around centromeres, at nucleolus organiser regions and at telomeres. Regions of constitutive heterochromatin are enriched in repetitive sequences and contain only few genes. Facultative heterochromatin is formed by heterochromatisation of euchromatic regions and represents a basic epigenetic mechanism for permanent gene inactivation during development. By heterochromatisation transposons, repeated coding sequences, transgene repeats and also a significant number of unique genes become silenced. The basic molecular mechanisms of heterochromatic gene silencing are conserved among organisms and were first resolved in animal model systems. Gene silencing by heterochromatisation was first detected as position-effect variegation in *Drosophila* (Muller, 1930). In position-effect variegation euchromatic genes show variegated expression due to gene silencing by heterochromatisation after their juxtaposition to heterochromatin by chromosomal rearrangements. Heterochromatic gene silencing in position-effect variegation reflects the repressive effects of heterochromatin on active genes and has successfully been used for genetic dissection of gene silencing processes by isolation of dominant suppressor mutation of position-effect variegation (*Su*(*var*)) and enhancer mutation of position-effect variegation (*E*(*var*)) mutations (Reuter and Wolff, 1981; Sinclair et al., 1983; Wustmann et al., 1989). With isolation of the suppressor gene encoding a histone H3K9 methyltransferase (*Su*(*var*)*3–9*) gene (Tschiersch et al., 1994) and demonstration of its function in histone H3 lysine 9 (H3K9) methylation (Rea et al., 2000; Schotta et al., 2002), a central function controlling heterochromatin formation and gene silencing was identified. The SU(VAR)3–9 protein contains the chromo and the SET domain, which are two conserved motifs of chromatin proteins (Jones and Gelbart, 1993; Tschiersch et al., 1994). Orthologs of SU(VAR)3–9 have been identified in *Schizosaccharomyces pombe* as Clr4p (Ekwall et al., 1996; Ivanova et al., 1998), in *Neurospora crassa* as DIM5 (Tamaru and Selker, 2001) and in mammals as SUV39H1 and SUV39H2 (Aagaard et al., 1999; O'Carroll et al., 2000). In all these organisms these proteins function as heterochromatin-specific histone methyltransferases (HMTase), which selectively methylate histone H3 at lysine 9.

Beside hypoacetylation of lysine residues, methylation of histone H3 at lysine 27 and H4 at lysine 20 are other characteristic histone modification marks in heterochromatin (Jenuwein and Allis, 2001). Lysine residues can be mono-, di- and trimethylated, which increase considerably the complexity of indexing processes in chromatin. Furthermore, in most organisms, DNA in heterochromatin is hypermethylated at cytosine residues. Histone-modifying enzymes (acetyltransferases, deacetylases and methyltransferases) as well as DNA methyltransferases are evolutionary conserved proteins.

## SU(VAR)3–9 homologues proteins in *Arabidopsis* are heterochromatin associated

In *Arabidopsis thaliana* we identified 37 genes encoding SET domain proteins (Baumbusch et al., 2001). The SET domain, a 130–160 amino acid motif, is found in four groups of proteins typified by *Drosophila* enhancer of Zeste protein (E(Z)), TRX, ASH1 and SU(VAR)3–9 (Jenuwein et al. 1998). In *Arabidopsis* 10 *SU(VAR)3–9* homologous (*SUVH*) and five *SU*(*VAR*)*3–9* related (*SUVR*) genes have been identified. Tree construction of SU(VAR)3–9 homologous protein of plant (SUVH) proteins from different plant species revealed four clearly distinct subgroups of *SUVH* genes in angiosperms (Naumann et al., 2005). Phylogenetic analysis of 21 SUVH protein sequences from *Arabidopsis*, rice, *Pinus taeda*, *Physcomitrella patens* and *Ceratopteris richardii* indicates an early phylogenetic split of *SUVH* genes during evolution of seed plants. It is furthermore suggested that the intron less genes of the *SUVH1* (*SUVH1*, *3*, *7*, *8* and *10*), *SUVH2* (*SUVH2* and *9*) and *SUVH5* (*SUVH5* and *6*) groups originated from an intron containing *SUVH4* like gene via retrotransposition (Baumbusch et al., 2001; Naumann et al., 2005).