

## Gene Silencing in Arabidopsis

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Chromatin is well known to play an important role in silencing and activation of gene expression. Histone modifications play many roles in this process, including modulating chromatin condensation, providing binding sites for specific factors, and targeting of DNA methylation. Purification of histones from *Arabidopsis* and analysis by nHPLC- $\mu$ ESI- LTQ-FT mass spectrometry has allowed us to obtain a relative quantitation of most of the acetylation and methylation sites on the histone H3 N-terminus. We find distinct differences between the abundance of active and silencing modifications on the two histone H3 variants and a very low abundance of both types of modifications on the same histone. As methylation of lysines can involve one, two or three methyl groups, we have also determined the relative abundance of each of these variations present in the two variants. In conjunction with these studies, we have also analyzed histone modifications in a mutant line lacking a functional KRYPTONITE gene (SU(VAR)3-9 homolog). Using western analysis, ChIP and mass spec, we find that KRYPTONITE is the primary enzyme responsible for dimethylation of histone H3K9. As this mutant was isolated as a suppressor of an epigenetic mutation in the SUPERMAN gene and resulted in a decrease in CNG methylation, we can conclude the dimethylation of H3K9 is the critical mark for both directing CNG DNA methylation and gene silencing<sup>1</sup>.

### **1. Dimethylation of histone H3 lysine 9 is a critical mark for DNA methylation and gene silencing in *Arabidopsis thaliana***

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