Functional studies of A. thaliana MYST-family acetylases, HAG4 and HAG5, produced by cell-free translation

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Histone post-translational modifications (PTMs) play a crucial role in the regulation of gene expression in eukaryotes. Histone modifying enzymes, as master regulators, can add, identify and remove a wide array of modifications of core histones, namely histone H2A, H2B, H3 and H4. One of the most frequent PTM is acetylation, which is mainly associated with transcriptional activation. In *Arabidopsis thaliana* 12 histone acetyltransferases (HAT) can be found. We aimed to investigate two of the lesser-known MYST-family acetylases, HAG4/HAM1 (AT5G64610) and HAG5/HAM2 (AT5G09740). Previous *in vivo* studies confirmed their roles in plant fertility and significant phenotypic differences were observed in mutant plants. Despite the high similarity in their amino acid sequence, it was shown that HAG4 and HAG5 are a functionally redundant pair of genes. It is also known, that HAG4 and HAG5 primarily acetylate histone H4 (H4K5) and have a slight activity on H3. Previously, efficient *in vitro* expression and purification of HAG4 and HAG5 enzymes were not successful in *E. coli* expression systems. In the presented project, we show the succesful expression and purification of *Arabidopsis thaliana* HAG4 and HAG5, histone H3, H4 by cell-free wheat germ translation system. We aim to perform acetylation assays with HAG4 and HAG5 on histone H3, H4 and other putative substrates, which can be confirmed by mass spectrometry. Additionally, we aim to investigate the binding of acetyl-CoA to the enzymes, and the mechanism of the predicted autoacetylation of HAG4 and HAG5.

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