Cytosine methylation, methyltransferases and flowering time in *Arabidopsis thaliana*

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Statement of Originality

This thesis contains no material which has been accepted for the award of any other degree or diploma in any University. To the best of my knowledge, this thesis contains no material previously published, or the result of any work by any other person, except where due reference is made in the text.

Ruth Kathleen Genger

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Abstract

Environmental signals such as photoperiod and temperature provide plants with seasonal information, allowing them to time flowering to occur in favourable conditions. Most ecotypes of the model plant *Arabidopsis thaliana* flower earlier in long photoperiods and after prolonged exposure to cold (vernalization). The vernalized state is stable through mitosis, but is not transmitted to progeny, suggesting that the vernalization signal may be transmitted via a modification of DNA such as cytosine methylation. The role of methylation in the vernalization response is investigated in this thesis.

Arabidopsis plants transformed with an antisense construct to the cytosine methyltransferase *METI* (AMT) showed significant decreases in methylation. AMT plants flowered significantly earlier than unvernalized wildtype plants, and the promotion of flowering correlated with the extent of demethylation. The flowering time of mutants with decreased DNA methylation (*ddm1*) was promoted only in growth conditions in which wildtype plants showed a vernalization response, suggesting that the early flowering response to demethylation operated specifically through the vernalization pathway.

The AMT construct was crossed into two late flowering mutants that differed in vernalization responsiveness. Demethylation promoted flowering of the vernalization responsive mutant *fca*, but not of the *fe* mutant, which has only a slight vernalization response. This supports the hypothesis that demethylation is a step in the vernalization pathway.

The role of gibberellic acid (GA) in the early flowering response to demethylation was investigated by observing the effect of the *gai* mutation, which disrupts the GA signal transduction pathway, on flowering time in plants with demethylated DNA. The presence of a single *gai* allele delayed flowering, suggesting that the early flowering response to demethylation requires a functional GA signal transduction pathway, and that demethylation increases GA levels or responses, directly or indirectly.

In most transgenic lines, AMT-mediated demethylation did not fully substitute for vernalization. This indicates that part of the response is not affected by *METI*-mediated methylation, and may involve a second methyltransferase or a factor other than methylation. A second Arabidopsis methyltransferase, *METIIa*, was characterized and compared to *METI*. The two genes are very similar throughout the coding region, and share the location of their eleven introns, indicating that they diverged relatively recently. Both are transcribed in all tissues and at all developmental stages assayed, but the level of expression of *METI* is significantly higher than that of *METIIa*. The possible functions of *METI*, *METIIa*, and other Arabidopsis cytosine methyltransferase genes recently identified are discussed.

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Dedicated to my dear mother

Kathleen Elizabeth Genger

1929 - 1994

and my loving father

Johannes Jacobus Genger