# Chapter Five

## The role of gibberellic acid in the early flowering response of *METI* antisense plants

### 5.1 Introduction

Gibberellic acids (GAs) are a large class of tetracyclic diterpenoids endogenous to plants, of which the active forms are regulators of plant growth and development. GAs are involved in processes including breaking seed dormancy, endosperm mobilization in germinating cereal grains, stem elongation, initiation of flowering, and fruit development (Crozier, 1983; Pharis and King, 1985).

Application of GA promotes flowering in a range of plant species (reviewed in Lang, 1965; Zeevaart, 1983; Pharis and King, 1985). Many species that flower early in response to GA also flower early in response to long days or vernalization (Zeevaart, 1983; Pharis and King, 1985), raising the possibility that GA may be involved in these responses. This response to exogenous GA is paralleled by the effect of photoperiod and vernalization on GA metabolism. When the long day plant spinach is transferred from growth in short to long days, levels of $GA_{20}$ and $GA_{29}$, the last two products of the GA biosynthetic pathway in spinach (Figure 5.1), rise, while the level of $GA_{19}$, the immediate precursor to $GA_{20}$, falls (Metzger and Zeevaart, 1980, 1982). Examination of GA metabolism in cell-free extracts of spinach indicated that the activity of enzymes catalyzing two steps in the GA biosynthetic pathway, $GA_{53}$ to $GA_{44}$ and $GA_{19}$ to $GA_{20}$, was increased in long days (Gilmour *et al*., 1986). The gene encoding gibberellin 20-

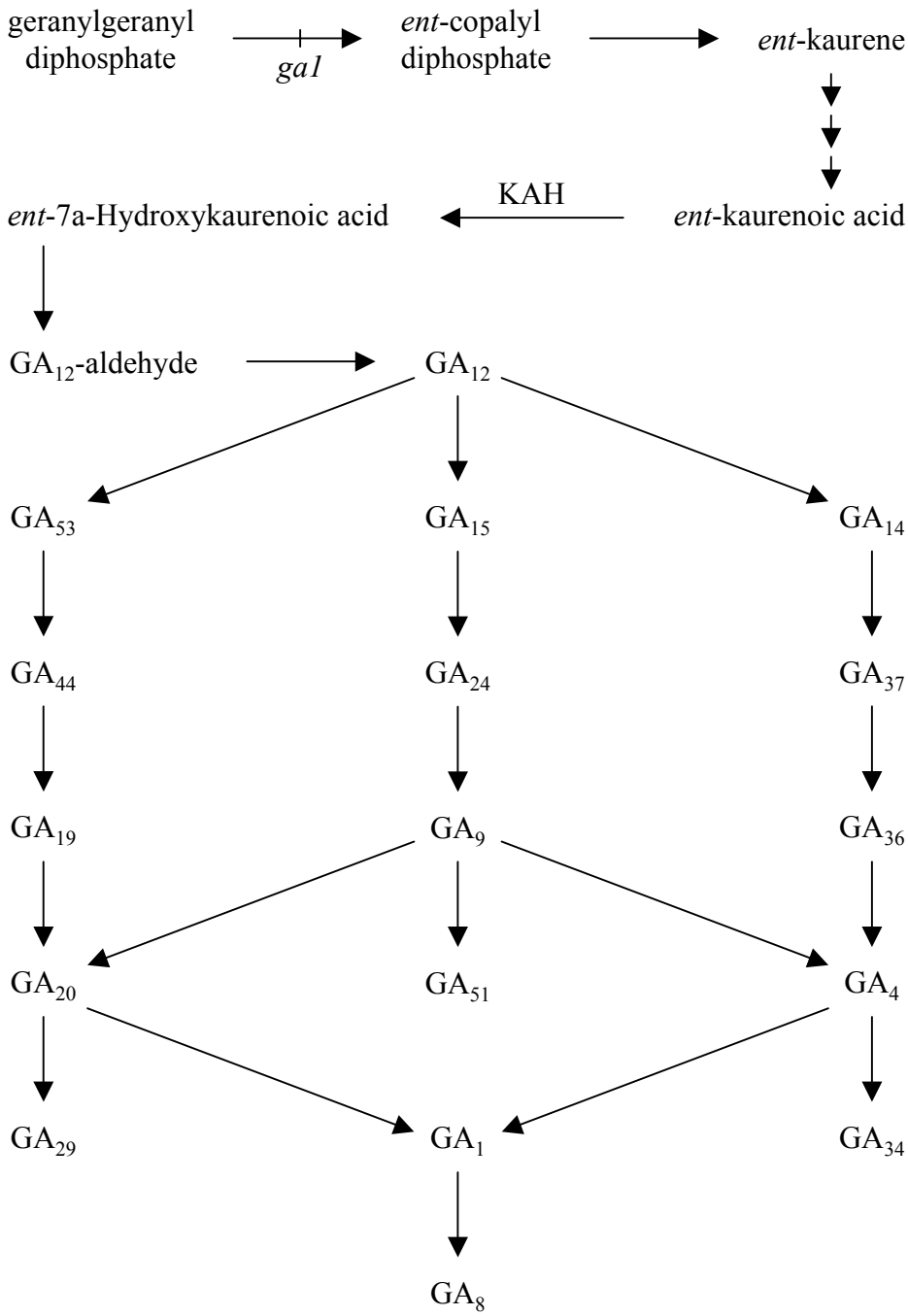



Figure 5.1: Gibberellin biosynthesis pathways, based on Ross *et al*. (1997). The position of the Arabidopsis *ga1* mutation is shown.

oxidase, the enzyme which catalyzes the conversion of $GA_{53}$ to $GA_{44}$ to $GA_{19}$ to $GA_{20}$, and $GA_{19}$ to $GA_{17}$, is more highly expressed in spinach plants grown in long days than in plants grown in short days (Wu *et al*., 1996).

Changes in GA metabolism also result from prolonged cold treatment. Both *Arabidopsis thaliana* and *Thlaspi arvense* are vernalization responsive and flower early when treated with exogenous GA (Bagnall, 1992; Wilson *et al*., 1992; Metzger, 1988b). GA metabolism in the shoot apex of *Thlaspi* changes after prolonged exposure to cold (Hazebroek and Metzger, 1990; Hazebroek *et al*., 1993). *Thlaspi* plants exposed to 6°C for 4 weeks showed a large increase in turnover of applied [$^{2}$H] and [$^{3}$H] kaurenoic acid (KA), but no changes in the metabolism of applied [$^{14}$C] $GA_{12}$ aldehyde (Hazebroek and Metzger, 1990). These changes were observed in the shoot apex; vernalization did not lead to changes in GA metabolism in leaves. This distinction is important, as vernalizing temperatures are perceived by dividing cells in the shoot apex (and elsewhere) but not by mitotically inactive cells such as those in fully expanded leaves (Wellensiek, 1964; Metzger, 1988a; Burn *et al*., 1993a). As only the metabolism of KA was affected, vernalization may regulate steps in GA biosynthesis between KA and $GA_{12}$ aldehyde (Figure 5.1). Further work on *Thlaspi* showed that, after vernalization, the activity of kaurenoic acid hydroxylase (KAH), which converts KA to 7β-hydroxy KA, increased rapidly in the shoot apex, and KA levels in the shoot apex declined rapidly (Hazebroek *et al*., 1993). Neither of these changes was seen in leaves.

The phenotype of dwarfed, GA-deficient mutants of Arabidopsis also points to the involvement of GAs in promoting flowering. One severe mutant, *ga1-3*, lacks a 5 kb portion of the 7 kb *GA1* locus (Sun *et al*., 1992; Sun and Kamiya, 1994) and is extremely GA deficient (Barendse *et al*, 1986). *GA1* encodes the enzyme ent-kaurene synthetase A, which catalyses the first committed step in the GA biosynthetic pathway (Sun and Kamiya, 1994; Figure 5.1). The *ga1-3* mutant flowers somewhat later than wildtype in continuous light, but does not flower when grown in short days unless treated with exogenous GA (Wilson *et al*., 1992). Vernalization does not promote flowering in *ga1-3* (Wilson *et al*., 1992); this is consistent with vernalization promoting flowering through increased GA levels, a path which would be blocked in *ga1-3*. As described in the previous chapter, the Class I and II late flowering Arabidopsis mutants show a correlation between response to GA and response to cold treatment (Chandler and Dean, 1994). Again, these findings indicate that vernalization may promote flowering through increases in the biosynthesis of GAs.

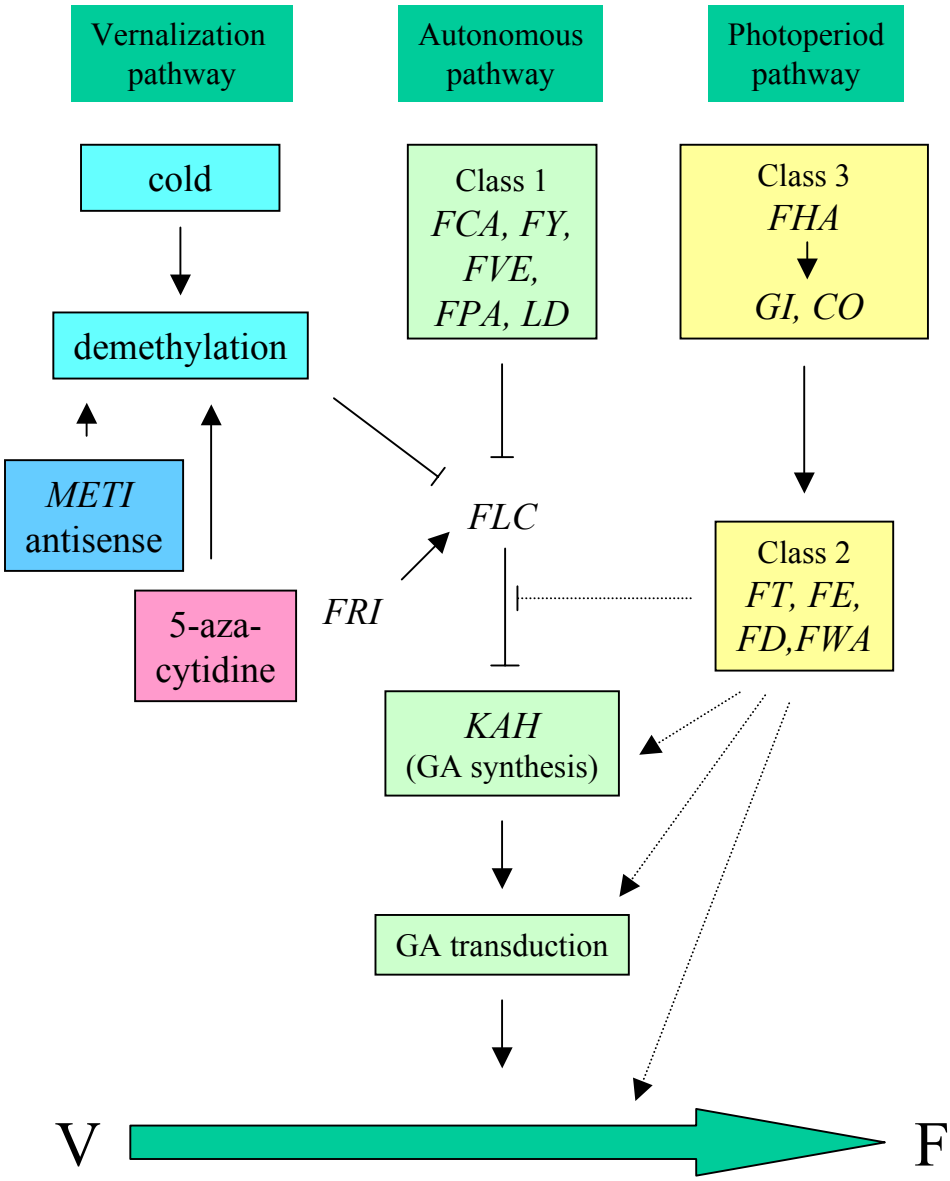
Burn *et al*. (1993a) formed the hypothesis that vernalization leads to demethylation of the promoter of a gene(s) important for the initiation of flowering, leading to increased expression of that gene and early flowering. Based on the changes observed in *Thlaspi* GA metabolism after vernalization (Hazebroek and Metzger, 1990; Hazebroek *et al*., 1993), Burn *et al*. (1993a) proposed that expression of the KAH gene was blocked by methylation of its promoter, or by a similar blockage of a gene regulating KAH, and that this block was released by vernalization and consequent demethylation of the promoter.

Burn *et al*. (1993a) hypothesized that resulting expression of the KAH gene in the apex would lead to an apex specific increase in GA levels and early flowering.

A complementary hypothesis has been suggested based on the characterization of the Arabidopsis late flowering *flf*-1 mutant (Sheldon *et al.*, 1999). The *flf*-1 mutant shows increased levels of *FLF* (= *FLC*) mRNA, indicating that FLC acts as a repressor of flowering. The level of *FLC* mRNA is reduced by vernalization in *flf*-1 and wildtype plants, and in *METI* antisense plants (Sheldon *et al*., 1999). This suggests that vernalization promotes flowering by down-regulating *FLC*, and suggests that this down-regulation may be due to demethylation. This may occur directly: demethylation of the *FLC* promoter may prevent binding of a transcription factor which only binds to methylated DNA, or allow a protein that represses *FLC* transcription to bind to the demethylated promoter. Alternatively, the down-regulation could occur indirectly, involving a repressor of *FLC* for which expression is up-regulated by demethylation (Sheldon *et al*., 1999). Figure 5.2 shows a model incorporating the hypothesis of Burn *et al*. (1993a) with the findings of Sheldon *et al*. (1999) regarding the role of *FLC*.

The *flf*-1 mutant flowers early in response to either vernalization or GA application; however, induction of this early flowering response requires an extended cold period, or repeated application of GA, compared to wildtype plants (Sheldon *et al.*, 1999). Though vernalization down-regulates *FLC*, the level of *FLC* mRNA is not altered in either *flf*-1 or wildtype plants treated with GA (Sheldon *et al.*, 1999). These results suggest that GA acts downstream of *FLC* in promoting flowering, and that FLC acts to antagonize GA activity

Figure 5.2: Vernalization dependent and independent pathways to flowering. This model postulates that the effect of demethylation, whether through low temperature, 5-aza-cytidine application or *METI* antisense expression, is to down-regulate *FLC*, releasing kaurenoic acid hydroxylase (KAH) from direct or indirect repression by *FLC*. Increased KAH activity results in increased levels of GA, promoting flowering.


Vernalization pathway
Autonomous pathway
Photoperiod pathway
cold
demethylation
Class 1
FCA, FY, FVE, FPA, LD
Class 3
FHA
GI, CO
METI antisense
5-aza-cytidine
FRI
FLC
Class 2
FT, FE, FD,FWA
KAH
(GA synthesis)
GA transduction
V
F

in promoting flowering. This could occur through reduced production or increased degradation of GA, or through repression of some element of the GA signal transduction pathway.

The molecular basis for the early flowering phenotype of *METI* antisense plants may be caused by down-regulation of *FLF* due to demethylation, resulting in increased GA levels, increased sensitivity to GA, or de-repression of the GA signal transduction pathway. If early flowering in *METI* antisense plants is due to a change in GA metabolism or signaling, then applied GA may not promote flowering of *METI* antisense plants to the same extent as in wildtype plants. To test the role of GA in the early flowering phenotype of *METI* antisense plants, the effect of exogenous GA on the flowering time of *METI* antisense plants and C24 plants was measured.

The Arabidopsis *gai* mutant (GA insensitive) has a phenotype superficially similar to that of the GA-deficient (*ga*) mutants, being dwarfed and late flowering (Wilson *et al*., 1992). However, while the *ga* mutant phenotype can be rescued by GA application, *gai* does not respond to exogenous GA (Wilson *et al*, 1992), and rather than being GA-deficient, *gai* accumulates GAs (Talon *et al*., 1990). GAI acts to repress GA responses, and is opposed by GA or a GA signalling intermediate (Peng *et al*., 1997). The GAI (wild-type) and gai (mutant) proteins differ by a 17 amino acid deletion, suggesting that the product of the mutant allele does not perceive GA (or the GA signalling intermediate), and therefore maintains repression of GA responses in the presence of GA (Peng *et al*., 1997). This is consistent with the semidominant nature of the *gai* mutation (Peng and Harberd, 1993;

Wilson and Somerville, 1995). Unlike the *ga1-3* mutant, the *gai* mutant responds to vernalization, although its vernalization response is reduced compared to that of the wildtype background, L*er* (Wilson *et al*., 1992). Cold treatment may decrease the insensitivity of *gai* to GA. If, as proposed by Peng *et al.* (1997), the mutant gai protein does not recognize GA (or a GA signalling intermediate) due to a conformational change, cold treatment may alter the conformation of the mutant protein, allowing recognition of GA or an intermediate.

If demethylation by the *METI* antisense promotes flowering solely through increased GA levels, expression of the antisense construct in the *gai* background should have no effect on flowering time. Plants homozygous for the antisense construct were crossed to *gai* homozygotes, and the flowering time of the F1 progeny was compared to the flowering time of F1 progeny of the *GAI* wildtype background, L*er*, and *METI* antisense. As *gai* is semidominant, any increase in GA levels due to expression of *METI* antisense may have some effect in the F1s, but the effect should still be less than on the wildtype controls.

## 5.2 Materials and Methods

### 5.2.1 Effect of $GA_3$ on flowering time of *METI* antisense plants

The flowering time of T3 plants from line #10.5, which is homozygous for the *METI* antisense, was measured with and without addition of $GA_3$ to the growth medium to a final concentration of $10^{-5}$ M, and with or without a cold treatment. The wildtype control

was C24. Growth conditions were as described in Section 2.1, and plants were grown under Sylvania F58W/133 fluorescent tubes in an eight hour photoperiod. Light intensity ranged from 110 μE at the edge of the cabinet to 180 μE in the centre. The racks of tubes were moved daily to ensure that all plants received equal illumination. The plants were observed daily, and the dates of germination and bolting, defined as first elongation of the primary inflorescence, were recorded, as was the rosette leaf number at the time of bolting.

### 5.2.2 Effect of *gai* mutation on flowering time of *METI* antisense plants

Three sets of crosses were made: between homozygous *gai* plants and homozygous *METI* antisense plants of T3 line #10.5 (*gai*-AMT; AMT = antisense methyltransferase); between homozygous *gai* plants and wildtype C24 (*gai*-C24); and between homozygous *METI* antisense plants of T3 line #10.5 and Landsberg *erecta*, the wildtype background of *gai* (L*er*-AMT). In each case, reciprocal crosses were made and F1 seed from both crosses pooled.

Fifty seeds for each of the *gai*-AMT and *gai*-C24 crosses, and twenty seeds for the L*er*-AMT cross were grown as described in Chapter 2. Tubes were placed at 4° C for two days to ensure even germination. All tubes were then transferred to a growth cabinet at 22° C. Plants were grown under Sylvania F58W/133 fluorescent tubes in an eight hour photoperiod. Light intensity varied from 100 μE at the sides of the cabinet to 190 μE in the centre, and the tubes were moved daily to ensure that all plants received equal

illumination. The plants were observed daily, and the dates of germination and bolting (elongation of the primary inflorescence) were recorded, as was the rosette leaf number at the time of bolting.

After the flowering time was recorded, crossing was verified by screening F1 plants for the presence of both L*er* and C24 chromosomes by a PCR assay. The primers used were the nga111 primers developed by Bell and Ecker (1994), one pair of a set of primers designed to flank simple sequence repeats in the Arabidopsis genome. These primers distinguish between C24 and L*er* on the basis of the size of the amplified product, and are codominant, allowing heterozygotes to be easily scored. PCR conditions and primer sequences are given in Section 2.3.3.

Plant material of confirmed F1 plants for each cross was bulked, and DNA was prepared using a CTAB-CsCl gradient method, described in Section 2.2.1. Methylation of cytosines in *Taq*I sites was measured by a thin layer chromatography method, described in Section 2.4. 5-methylcytosine levels of *gai*-AMT and L*er*-AMT were normalized to the *gai*-C24 control. The estimate of 5-methylcytosine level was repeated three times, and an average was taken of the three levels.

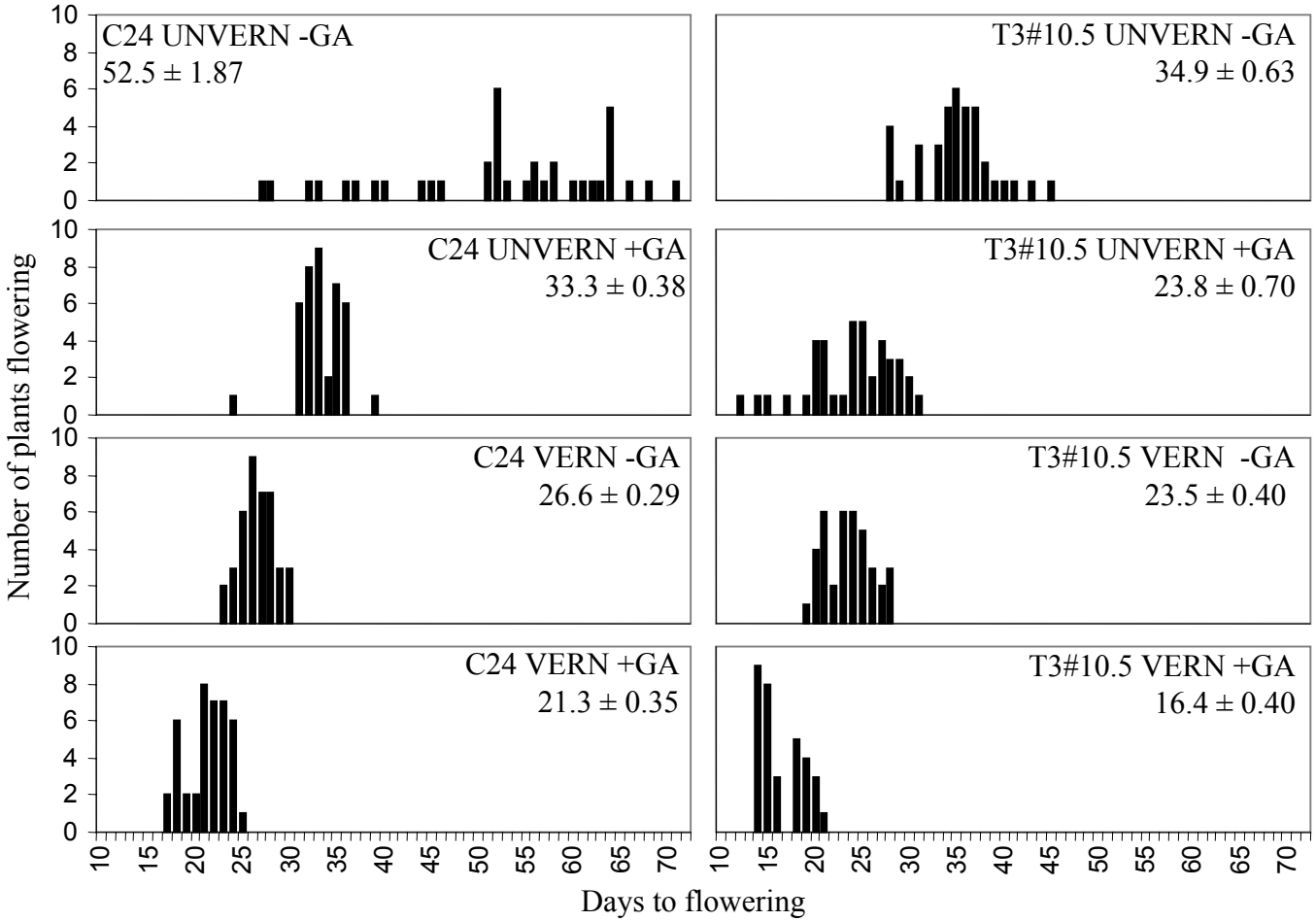
## 5.3 Results

### 5.3.1 Effect of GA on flowering time of *METI* antisense plants

The effect of exogenous GA on the flowering time of *METI* antisense plants was measured and compared to its effect on flowering time in wildtype C24 plants. As previously reported in Arabidopsis, incorporation of $GA_3$ into the growth medium had a large promotive effect on flowering (reviewed in Napp-Zinn, 1969; reviewed in Zeevaart, 1983). For the unvernalized wildtype C24 plants (Figure 5.3), time to bolting was reduced by approximately 19 days, so that $GA_3$-treated C24 plants flowered in 63% of the time taken by untreated C24 plants. Addition of $GA_3$ also reduced time to bolting for T3#10.5 unvernalized plants (Figure 5.3), by approximately 11 days, resulting in an average flowering time that was 68% of that for untreated transgenics.

Flowering of vernalized C24 and *METI* antisense plants was also promoted by $GA_3$ application, so that for both wildtype and transformed plants, the combination of vernalization and exogenous $GA_3$ resulted in earlier flowering than either treatment alone (Figure 5.3). Average flowering time for vernalized C24 plants treated with $GA_3$ was 64% of average flowering time for untreated vernalized C24 plants, while $GA_3$-treated *METI* antisense plants flowered in 69% of the time taken by untreated *METI* antisense transgenics. This indicates that vernalization did not saturate the early flowering response; it is also apparent that $GA_3$ treatment did not saturate the early flowering response, as $GA_3$-treated plants flowered earlier if vernalized. However, the promotion of

Figure 5.3: Effect of vernalization and/or $GA_3$ addition on flowering time of *METI* antisense and C24 plants. Average days to flowering ± standard error is shown for each treatment.


C24 UNVERN -GA
52.5 ± 1.87
T3#10.5 UNVERN -GA
34.9 ± 0.63
C24 UNVERN +GA
33.3 ± 0.38
T3#10.5 UNVERN +GA
23.8 ± 0.70
C24 VERN -GA
26.6 ± 0.29
T3#10.5 VERN -GA
23.5 ± 0.40
C24 VERN +GA
21.3 ± 0.35
T3#10.5 VERN +GA
16.4 ± 0.40
Number of plants flowering
Days to flowering

flowering seen for vernalized plants treated with $GA_3$ was less than the sum of the promotion of flowering recorded for vernalized plants and unvernalized $GA_3$-treated plants. This initially suggested that together, the two treatments saturated the flowering response. However, vernalized, $GA_3$-treated T3#10.5 plants flowered earlier than vernalized, $GA_3$-treated C24 plants, demonstrating that the combination of vernalization and $GA_3$ addition was not saturating.

Both C24 and *METI* antisense plants flowered earlier in response to vernalization, regardless of $GA_3$ addition. However, the vernalization response of C24 plants was substantially decreased by treatment with $GA_3$. In the absence of $GA_3$, vernalized C24 plants flowered in 50% of the time taken by unvernalized plants; this represents a decrease of approximately 26 days in the average flowering time. Addition of $GA_3$ reduced the C24 vernalization response: vernalized C24 plants flowered in 64% of the time taken by unvernalized C24 plants, a decrease of 12 days in average flowering time. This indicates that $GA_3$ addition is partially substituting for cold treatment. Addition of $GA_3$ had a negligible effect on the vernalization response of T3#10.5 plants. Vernalized T3#10.5 plants flowered in 67% of the time taken by unvernalized plants (approximately 11 days earlier on average). For T3#10.5 plants treated with $GA_3$, vernalized plants flowered in 69% of the time taken by unvernalized plants (approximately 7 days earlier on average).

These results suggest that addition of $GA_3$ may have a slightly smaller promotive effect on flowering of *METI* antisense plants than on flowering of wildtype plants, and, more

significantly, show that $GA_3$ reduces the vernalization responsiveness of C24 but not the *METI* antisense line T3#10.5. These observations are consistent with the hypothesis that reduced methylation partially substitutes for the promotive effect of $GA_3$ on flowering. This substitution could act through increased levels of GA, either in the whole plant or the shoot apex, through increased sensitivity to GA, or though up-regulation of the GA signal transduction pathway.

### 5.3.2 Effect of reduced methylation on flowering time of *gai* mutants

The effect of demethylation on the delayed flowering phenotype of the *gai* mutant was examined by measuring the flowering time of F1 progeny of crosses between *gai* and T3#10.5 (AMT) plants, and comparing it to that of F1 progeny of crosses between *gai* and C24 (the wildtype control for T3#10.5) and F1 progeny of crosses between T3#10.5 and L*er* (the wildtype control for *gai*). Confirmation that the plants were F1 progeny of the parental lines was obtained by screening with the SSLP PCR primers nga111 (Bell and Ecker, 1994); 45 *gai*-AMT F1 plants, 43 *gai*-C24 F1 plants, and 19 L*er*-AMT plants were identified. The flowering time results for these plants are summarized in Table 5.1 and Figure 5.4. The experiment was terminated at 82 days, by which time the medium was dehydrated and plants were beginning to senesce. All L*er*-AMT plants had flowered within this time, with an average flowering time of 42.3 ± 4.4 days, and a range of 20 to 74 days. Of the 45 *gai*-AMT plants, 29 (64.4%) flowered, with the earliest flowering at 21 days and the latest at 82 days. None of the *gai*-C24 plants flowered within the 82 days of the experiment.

| | *gai*-AMT | *gai*-C24 | L*er*-AMT |
|---|---|---|---|
| No. plants (total) | 45 | 43 | 19 |
| No. plants (bolted) | 29 | 0 | 19 |
| Percentage bolted | 64.4 | 0 | 100 |
| Average ± s.e. | n.a. | n.a. | 42.26 ± 4.39 |

Table 5.1: Analysis of flowering time of F1 plants from crosses between the *gai* mutant and either the T3 *METI* antisense line 10.5 (AMT) or C24, and between L*er* and AMT. As not all plants flowered, the average days to flower is shown only for the L*er*-AMT F1 plants.

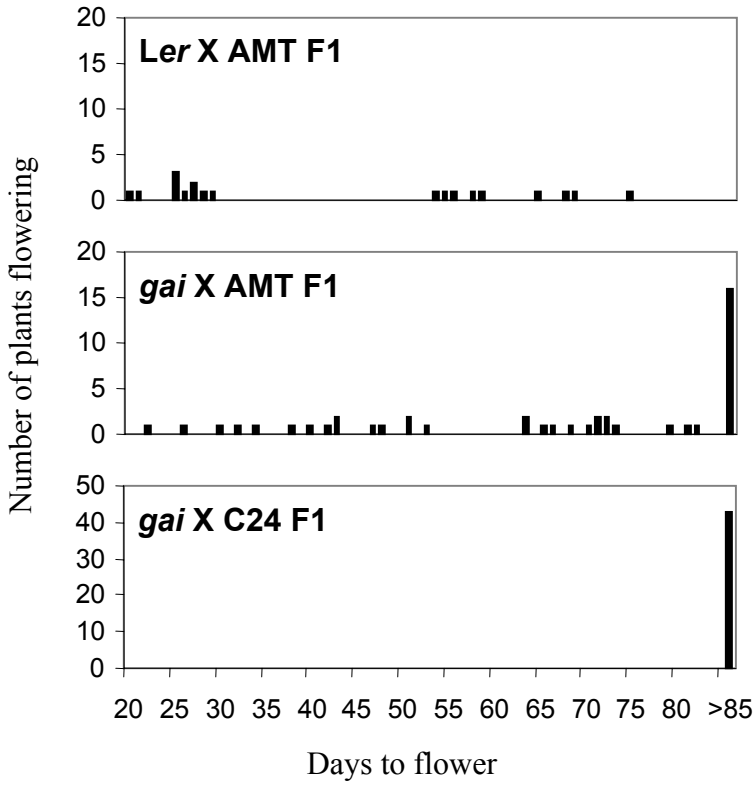



Figure 5.4: Days from germination to elongation of primary inflorescence for F1 plants from crosses between L*er* (wildtype control for *gai*) and *METI* antisense (AMT), *gai* and AMT, and *gai* and C24 (wildtype control for AMT). Plants in the "days to flower" category ">85" failed to flower within the period of the experiment.

The methylation levels of bulked plant material from the *Ler*-AMT and *gai*-AMT F1 plants was measured in comparison with the methylation level of the *gai*-C24 F1 plants. Cytosine methylation in the L*er*-AMT and *gai*-AMT F1 plants was reduced relative to the *gai*-C24 F1 plants. The L*er*-AMT F1s had an average methylation level of 46.4 ± 6.3%, while the average level for *gai*-AMT F1s was 34.3 ± 1.6%. These methylation levels are not significantly different.

## 5.4 Discussion

If vernalization, GA and DNA demethylation all promote flowering via the same pathway (Figure 5.2), the effects of these factors in combination should, at most, be additive. This hypothesis was tested by observing the effect of exogenous $GA_3$ on the flowering time of unvernalized and vernalized Arabidopsis plants transformed with *METI* antisense (Finnegan and Dennis, 1993; Finnegan *et al*., 1996), in conditions where neither the cold treatment nor the application of GA saturated the early flowering response. If the early flowering phenotype of *METI* antisense plants is due to the action of increased endogenous GA, exogenous $GA_3$ would be expected to have no effect on the flowering time of these plants, or at least a lesser effect than on wildtype C24.

Flowering of plants from the *METI* antisense line T3#10.5 was promoted by the addition of $GA_3$, but not to the same extent as for C24. Also, the vernalization response of C24 was reduced by the addition of GA, while the vernalization response of T3#10.5 was

unchanged. These results are consistent with the early flowering phenotype of the *METI* antisense plants being due either to increased levels of GA, or to increased responsiveness to GA. It is also possible that reduced methylation affects an intermediate in the GA signal transduction pathway, allowing an increased flow through this pathway.

The effects of the *METI* antisense, of vernalization and of GA application were additive. The early flowering response was not saturated by the three week cold treatment, as vernalized plants flowered earlier after treatment with GA. Nor was the effect of *METI* antisense in line T3#10.5 saturating, as another *METI* antisense line had been shown to flower earlier (Chapter 3), and as T3#10.5 plants were able to flower early in response to vernalization. It is therefore possible that GA application promoted flowering via the same pathway as both vernalization and *METI* antisense, but these results are not conclusive. It is equally possible that the additive effect of the treatments was due to the non-saturating action of each in separate pathways.

The *gai* mutant, which is late flowering compared to wildtype, is impaired in recognition of GA (Peng *et al*., 1997). If the early flowering phenotype of *METI* antisense plants is due to a response to GA, expression of *METI* antisense in the *gai* mutant should not cause early flowering.

F1 plants resulting from crosses between a *METI* antisense line, T3#10.5 (AMT), and *gai* or L*er* had significantly reduced methylation levels. As the *gai* mutation is semidominant, some promotion of flowering would be expected if GA levels were

increased in *gai*/*GAI* plants. The presence of the *METI* antisense construct promoted flowering of *gai*/*GAI* plants; however, L*er*-AMT plants flowered earlier still. That is, when perception of GA is impaired in *METI* antisense plants by the presence of a single *gai* allele, flowering is delayed. This suggests that the promotive effect of *METI* antisense depends, at least in part, on activation of the GA signal transduction pathway, possibly through increased GA levels. If the promotive effect of *METI* antisense was independent of GA, the presence of the *gai* allele should not have affected flowering time, so that no differences in flowering time would be seen between *gai*-AMT and L*er*-AMT F1 plants. However, these results do not imply that *METI* antisense has a direct effect on GA levels or responses, merely that an active GA signal transduction pathway is necessary for phenotypic expression of the promotive effects of demethylation.

Results reported in previous chapters are consistent with the hypothesis that demethylation promotes flowering by the vernalization dependent pathway. It is possible that demethylation acts in more than one pathway to promote flowering. Alternatively, demethylation and increases in GA may be steps in the vernalization dependent pathway.

Expression of the *FLC* gene, which delays flowering (Michaels and Amasino, 1999; Sheldon *et al*., 1999), is reduced in plants with DNA demethylated due to expression of *METI* antisense (Sheldon *et al*., 1999). Results reported in Chapter 4 indicate that vernalization and demethylation both promote flowering by down-regulating *FLC*. Landsberg *erecta*, the background ecotype of the *gai* mutant, has very low levels of *FLC*. The F1 plants used for the flowering time experiments reported in this chapter carry a

C24-derived *FLC* allele and a C24-derived *FRI* allele, and would therefore be expected to have higher *FLC* levels than L*er* or *gai* (Sheldon *et al*., 1999; Michaels and Amasino, 1999), and correspondingly to have an increased response to vernalization or demethylation. This increased *FLC* level would be counteracted, in the *gai*-AMT plants, by the demethylation caused by the AMT transgene, leading to promotion of the flowering of *gai*-AMT F1 plants relative to that of *gai*-C24 plants.

Plants over-expressing *FLC* flower earlier in response to exogenous GA, but require a much higher level of applied GA than wildtype plants. The level of *FLC* mRNA is not reduced by GA treatment (Sheldon *et al*., 1999). These results suggest that *FLC* acts upstream of GA in the pathway to flowering, and that *FLC* acts to block the promotion of flowering by GA (Figure 5.4; Sheldon *et al*., 1999). Potentially, *FLC* could repress GA biosynthesis, increase degradation of "florigenic" GAs, or repress some component of the GA signal transduction pathway. As the activity of the KAH enzyme has been shown to be increased after vernalization (Hazebroek and Metzger, 1990; Hazebroek *et al*., 1993), the *FLC* gene product could repress this enzyme, either at the transcriptional, post-transcriptional, or protein level (Figure 5.2).

Taken together, the above observations suggest that demethylation allows an increased response to GA by reducing the level of FLC. *FLC* expression is also reduced in vernalized plants, which show transient DNA demethylation (Sheldon *et al*., 1999; Finnegan *et al*., 1998b), and plants overexpressing *FLC* require longer vernalization periods than wildtype plants to induce early flowering (Sheldon *et al*., 1999). This

implies that reduced methylation mediates the vernalization response by down-regulating *FLC* and allowing greater responsiveness to GA.

It is unlikely that GA levels are increased in the *METI* antisense plants, as they do not show the phenotype which would be expected if this was the case. Wildtype plants treated with GA have elongated hypocotyls and stems, and paler leaves than untreated plants. They also flower early compared to untreated plants, and exhibit parthenocarpy and reduced male fertility (Jacobsen and Olszewski, 1993). While *METI* antisense plants flower early, have, in some cases, pale leaves, and show floral abnormalities which sometimes cause male sterility (Finnegan *et al*., 1996), they do not show the elongation of stems and hypocotyls which is characteristic of plants treated with GA. It is possible, however, that *METI* antisense plants have increased GA levels only in the shoot apex, if some other factor necessary for increased GA production occurs only in the apex. Sheldon *et al*. (1999) showed that *FLC* mRNA was decreased in whole seedlings after vernalization, and in the leaves of *METI* antisense plants; therefore *FLC* is not a candidate for an apex-specific factor limiting GA production. Further research on the interaction of the *FLC* gene product with GA activity, especially in the apex, may elucidate how GA metabolism and/or signaling is affected in vernalized plants and in *METI* antisense plants.