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Effect of Light Quality and Vernalization on Late-Flowering Mutants of *Arabidopsis thaliana*[1]

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ABSTRACT

We have analyzed the response to vernalization and light quality of six classes of late-flowering mutants (*fb, fca, fe, fg, ft,* and *fy*) previously isolated following mutagenesis of the early Landsberg race of *Arabidopsis thaliana* (L.) Heynh. When grown in continuous fluorescent illumination, four mutants (*fca, fe, ft,* and *fy*) and the Landsberg wild type exhibited a reduction in both flowering time and leaf number following 6 weeks of vernalization. A significant decrease in flowering time was also observed for all the mutants and the wild type when constant fluorescent illumination was supplemented with irradiation enriched in the red and far red regions of the spectrum. In the most extreme case, the late-flowering phenotype of the *fca* mutant was completely suppressed by vernalization, suggesting that this mutation has a direct effect on flowering. The *fe* and *fy* mutants also showed a more pronounced response than wild type to both vernalization and incandescent supplementation. The *ft* mutant showed a similar response to that of the wild type. The *fb* and *fg* mutants were substantially less sensitive to these treatments. These results are interpreted in the context of a multifactorial pathway for induction of flowering, in which the various mutations affect different steps of the pathway.

Light quality, photoperiod, and low temperature are among the most important environmental factors which affect the onset of flowering in plants (2, 5, 9). Substantial effort has been devoted to characterizing the ways in which different plant species sense and respond to these environmental variables (1, 2, 6, 15, 26), but the molecular mechanisms involved in floral induction remain unknown. Genetic analysis of species which are polymorphic for flowering time has been useful in revealing the action of some of the genes involved in the control of flower initiation (1, 13, 18). However, the interpretation of the effects of these genes has been complicated by the lack of isogenic lines and the lack of a method for identifying the genes or the gene products affected by the mutations. In these respects, *Arabidopsis* is an attractive model species for a genetic analysis of flowering because the rapid development of the molecular genetics of *Arabidopsis* (4, 8) may allow the cloning of genes which are otherwise evident only on the basis of a mutant phenotype.

Arabidopsis thaliana is a quantitative long day plant. Long photoperiods promote flowering in all *Arabidopsis* ecotypes. However, this is not an absolute requirement and plants of all ecotypes will flower in short days. Similarly, exposure to cold temperatures accelerates flowering of many geographical races of *Arabidopsis*. This requirement is also not absolute, and all ecotypes will eventually flower without exposure to cold temperatures (reviewed in 19–21). There have been several reports of the isolation of induced mutations in early races of *Arabidopsis* that resulted in delayed onset of flowering under certain environmental conditions (11, 12, 14, 16, 22, 25). Most of these mutations were recessive or semidominant. The most thoroughly characterized mutations of this class were isolated and studied by Hussein (11, 12) and others in the Van der Veen laboratory (14).

As an approach to understanding the molecular mechanisms which regulate flowering in *Arabidopsis*, we have extended the analysis of six loci previously implicated in this process through mutant analyses (14). A major goal of our analysis was to explore criteria by which we might determine whether these mutations affect flowering directly or indirectly. This is of particular concern since we have observed (unpublished) that mutants of *Arabidopsis* deficient in starch biosynthesis have a late-flowering phenotype under some circumstances. It has previously been noted that with certain mutants, vernalization appeared to have a positive effect in reducing flowering time (7, 11, 12, 16, 25). Therefore, we have examined the effect of light quality and vernalization on the phenotype of the late-flowering mutants. The results of this analysis provide evidence that at least one of the mutations has a direct effect on floral induction.

MATERIALS AND METHODS

Plant Material

All mutant lines of *Arabidopsis thaliana* (L.) Heynh. described here were originally isolated in the laboratory of Dr. Van der Veen following ethyl methane sulfonate-mutagenesis of the race Landsberg *erecta*, and were kindly provided by M. Koornneef. The late-flowering phenotype of each of the lines is due to the presence of a mutation which has been mapped (14) to one of the following loci: *fb, fca, fe, fg, ft,* and *fy*. All of the mutations are recessive, except for *fg* which is semidominant. Mutations *fb* and *fg* are now known to be allelic to

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mutations *gi* and *co*, respectively, described by Redei (22) (M Koornneef, personal communication). The specific lines used here have been given the following designations by M. Koornneef: W20 (Landsberg *erecta*), W51 (*fb*), W52 (*fca*), W54 (*fe*), W55 (*fg*), W56 (*ft*), W57 (*ft*). The seed used for the experiments reported here was harvested 22 to 24 months before use and, therefore, did not require cold treatment to overcome possible variation in dormancy between the various lines.

Growth Conditions

Plants were grown at a density of approximately 1 plant per 12 cm^2 in 12 or 20 cm pots containing a perlite:vermiculite:sphagnum (1:1:1) mixture irrigated with mineral nutrient solution at 2 week intervals (10). Plants were grown at 18°C and 95% RH under continuous illumination (90 ± 5 μmol m^{-2} s^{-1} photosynthetic photon flux) to avoid any interaction effects between day length sensitivity and cold requirements. In some experiments light was provided by cool-white fluorescent lamps with the irradiance spectrum shown in Figure 1. In other experiments, the fluorescent lamps were supplemented with incandescent lamps, which produced an additional peak of red light at 660 nm and an increase in the amount of far red irradiance (Fig. 1).

A. thaliana can be vernalized at the seed stage (19). Therefore, vernalization treatments were performed on seeds sown in pots and incubated at 4 ± 1°C in low light (about 2 μmol $m^{-2}s^{-1}$) for different times prior to transfer into the growth chamber. Since seeds germinated during the vernalization treatment, the result was that vernalized plants were at a slightly more advanced stage of germination than nonvernalized plants when moved into the growth chamber. This difference in developmental stage at the onset of the FT[2] and LN measurements was responsible for a small increase in FT of the nonvernalized plants with respect to the vernalized ones. However, it does not account for the increased LN of the non-vernalized plants with respect to the vernalized ones.

Experimental Scheme

Responses of mutants and WT to the different light quality regimes and vernalization treatments were measured by scoring two variables, FT and LN. FT was scored as the number of days from the time at which plants were put into the growth chamber to the time of opening of the first flower. LN was scored as the number of leaves on the rosette (excluding cotyledons) and the main flowering stalk at the time of opening of the first flower. FT and LN are correlated variables since plants delayed in flowering remain for a longer time as vegetative rosettes and accumulate a greater number of leaves. For each treatment, FT and LN were measured as the means of FT and LN scored for the plants growing in the same pot. Plants of the same genotype, growing in different pots at different locations in the same growth cabinet were considered replicate treatments. Mean comparisons between replicate treatments were performed using *t*-tests. Since no significant differences were found between replicates, data were pooled and means recalculated.

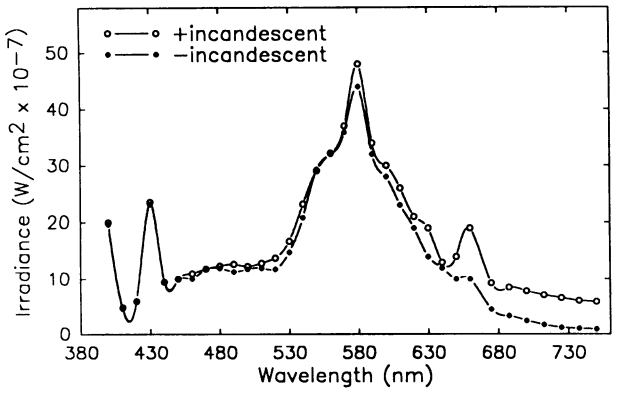

Figure 1. Irradiance spectrum of the two different light regimes used. Total irradiance in the 400 to 700 nm range was 90 ± 5 μmol $m^{-2}s^{-1}$.

[2] Abbreviations: FT, flowering time; LN, leaf number; WT, wild type.

RESULTS

Effect of Vernalization on Flowering Time and Leaf Number

Measurements of FT and LN for the six late-flowering mutants and the WT grown under constant fluorescent lights with a range of vernalization treatments from 0 to 41 d are shown in Figures 2 and 3, respectively. When not vernalized, all mutants showed substantially higher values for FT and LN than the WT, corroborating their late-flowering phenotype. After 41 d of vernalization there was a readily apparent decrease in both FT and LN for the *fca, fe, ft,* and *fy* mutants and the WT. When the response was expressed as percentage of reduction in FT or LN (Table I) three mutants (*fca, fe, fy*) showed a greater reduction than the WT for both variables, while the other three mutants showed similar (*ft*) or smaller reductions (*fb, fg*). In general, longer vernalization treatments resulted in lower FT and LN, except for mutants *fb* and *fg* which showed little reduction in either variable.

Effect of Light Quality on Flowering Time and Leaf Number

In preliminary experiments we observed considerable differences in flowering time between plants that had been grown with fluorescent lamps and plants grown with an incandescent supplement. Therefore, we measured the effect of these two light regimes (Fig. 1) on FT of the different mutants (Table II). The FT of all lines was reduced by the incandescent supplement, and mean differences in FT due to the treatment were statistically significant in all cases. The same three mutants (*fca, fe,* and *fy*), which were most affected by vernalization also showed the greatest reduction in FT by treatment with incandescent light. The *ft* and *fg* mutants showed a comparable reduction to the WT, while the *fb* mutant was less responsive than the WT (Table II). Incandescent light supplement produced a significantly smaller reduction in FT than 41 d of vernalization in WT and mutants *fca* and *fy*. For the other mutants, 41 d of vernalization or growth with incandescent supplement produced similar reductions in FT (Table I).

The effect of the incandescent light supplement on leaf

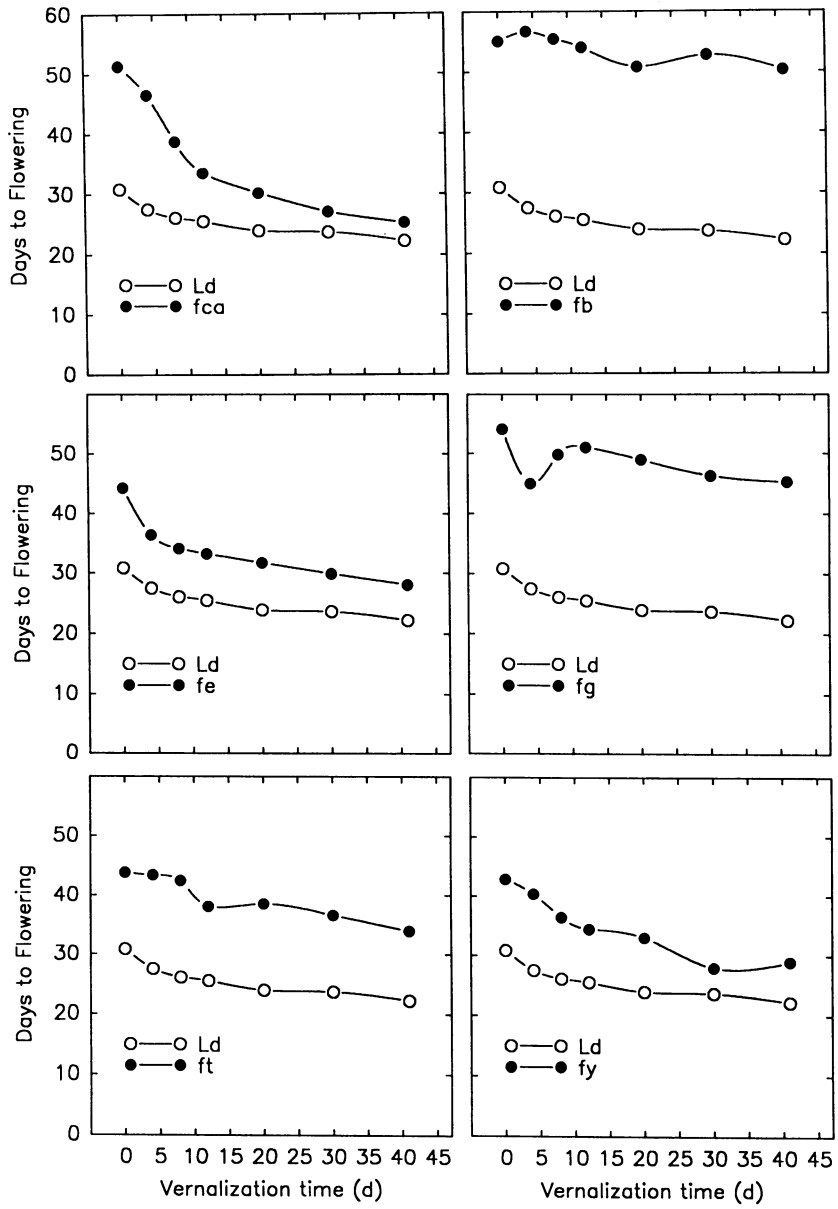

Figure 2. Effect of vernalization on FT of late-flowering mutants (*fca, fb, fe, fg, ft, fy*) and Landsberg WT (Ld). Each point represents the mean of measurements made on 15 to 20 plants pooled from two replicates. Standard errors are obscured by the symbols.

number was analyzed for the *fca* mutant and WT (Fig. 4; Table II). The reduction in LN promoted by incandescent illumination was significantly greater for the *fca* mutant than for the WT. As with FT, incandescent light supplement produced a substantially smaller reduction in LN of the *fca* mutant than 41 d of vernalization. However, reductions of WT LN promoted by incandescent supplement or 41 d of vernalization were indistinguishable.

Combined Effect of Incandescent Light and Vernalization

In order to determine if the effect of incandescent light was independent of the duration of the vernalization treatment, we scored FT and LN of WT and the *fca* mutant vernalized for different times and grown with or without incandescent supplement (Fig. 4). When the WT was not vernalized, incandescent supplementation caused a reduction in both FT and LN. However, after the shortest period of vernalization examined (5 d), there was no difference in FT or LN of WT plants grown with or without incandescent supplementation. There was a similar but more pronounced effect of incandescent supplementation on FT and LN of the *fca* mutant without vernalization. However, by contrast with the WT, periods of vernalization of up to 45 d did not completely eliminate the reduction of FT and LN by incandescent supplementation.

DISCUSSION

WT Response

Interpretation of the effects of the 'late-flowering mutations' is only possible when placed in the context of the WT response. In this respect, an important question pertains to why the Landsberg WT does not normally respond strongly to

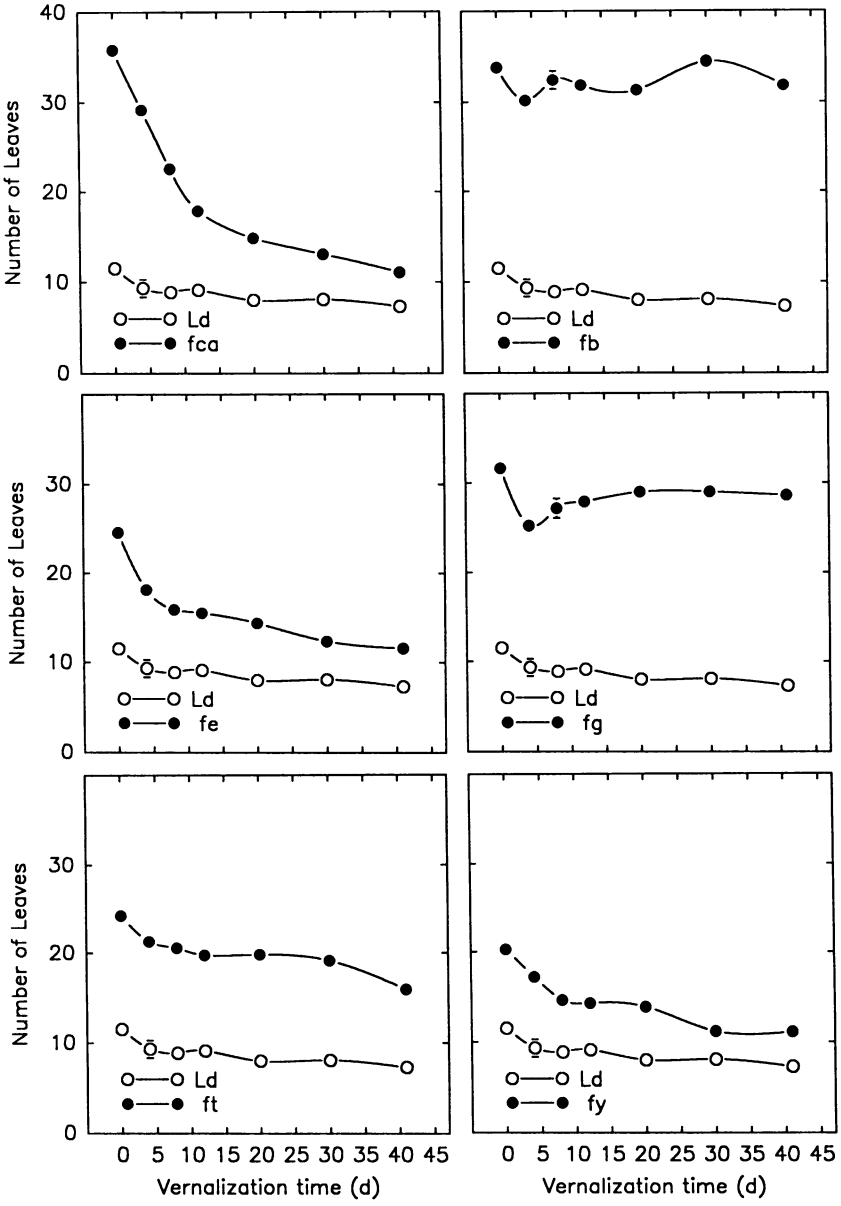

Figure 3. Effect of vernalization on LN of late-flowering mutants (*fca, fb, fe, fg, ft, fy*) and WT (Ld). Each point represents the mean of measurements made on 15 to 20 plants pooled from two replicates. Standard errors, indicated by bars, are generally obscured by the symbols.

Table I. *Effect of Vernalization on Flowering Time and Leaf Number*

The value "% Reduction" was calculated from the difference in FT or LN with or without 41 d of vernalization (Figs. 2 and 3).

Mutant	Reduction	
	LN	FT
	%	
WT	36	28
fb	6	9
fca	69	51
fe	53	37
fg	10	16
ft	34	23
fy	45	32

vernalization whereas other (winter) races of *Arabidopsis* do. Our results indicate that the Landsberg race responds to a vernalization treatment with a reduction in both LN and FT when grown under continuous fluorescent lights (Table I; Figs. 2, 3). Although it has not previously been described that the Landsberg race shows such a response, Napp-Zinn (19) reported some response of other early races to vernalization when they were grown under short photoperiods or low light intensity (400 lux $\simeq$ 8 μmol $m^{-2}s^{-1}$). Since both vernalization and incandescent lights have a similar effect on FT and LN of WT plants (Tables I, II), the vernalization response can only be observed when plants are grown under fluorescent lights and would not be found in plants grown under incandescent lamps or in natural light. Thus, we propose that a cold-inducible pathway is present in both early and late races but not normally manifest in the early ones. According to this

Table II. *Effect of Incandescent Supplementation on Flowering Time and Leaf Number under Constant Illumination.The numbers in parentheses are sample sizes. Reduction in FT and LN by the incandescent supplement was statistically significant with $P < 0.001$ in all cases.*

Mutant	Light Quality		Reduction[a]
	No incandescent	Incandescent	
	FT		%
WT	30.9 ± 2.0 (20)	25.0 ± 1.4 (46)	19
fb	55.1 ± 1.6 (12)	49.4 ± 1.7 (5)	10
fca	51.4 ± 2.7 (20)	36.3 ± 4.4 (26)	29
fe	44.3 ± 3.3 (15)	27.8 ± 2.3 (8)	37
fg	54.1 ± 2.9 (20)	44.4 ± 4.6 (9)	18
ft	43.9 ± 3.6 (21)	34.1 ± 1.0 (9)	22
fy	42.9 ± 2.4 (20)	30.5 ± 2.3 (8)	29
	LN		
WT	11.5 ± 1.4 (20)	7.2 ± 1.1 (46)	37
fca	35.9 ± 3.7 (20)	16.0 ± 2.5 (26)	55

[a] The value '% Reduction' was calculated from the difference in LN or FT between plants grown only in fluorescent illumination or in fluorescent illumination with incandescent supplement.

scheme, winter races arose as a consequence of mutations in the main induction pathway which resulted in the plant becoming dependent on the cold-inducible pathway.

WT plants also responded to an incandescent light supplement with a reduction in FT and LN (Table I). Using different *Arabidopsis* races Meijer (17) and Brown and Klein (3) showed that blue light, an important component of fluorescent lamps, is enough to induce flowering in this species. Both studies reported a positive effect of far red irradiation (730 nm) on flowering time and a lack of response to red light (660 nm). Based on these results, the effect of the incandescent supplement in our experiments is probably due to their higher far red component (Fig. 1).

Mutant Responses

At least four of the late-flowering mutants (*fca, fe, fy, ft*) responded to both vernalization and incandescent light by decreasing FT and LN. However, since the WT also responded to these stimuli, the question is whether the mutant responses were significantly different with respect to the WT. Comparison of the effects of 41 d of vernalization *versus* no treatment indicated that three mutants (*fca, fe, fy*) showed a greater reduction in FT and LN than WT (Table I). In the *fca* and *fe* mutants both 41 d of vernalization or growth with incandescent supplement can largely overcome the delay in flowering time produced by the mutation. When plants were grown under incandescent illumination after 60 d of vernalization, FT of the *fca* mutant and WT were 19.1 ± 0.1 and 19.2 ± 0.1 d, respectively. These means were not statistically different, indicating that, at least for the *fca* mutant, longer vernalization treatments can completely overcome the delay in FT caused by the mutation. Therefore, we conclude that the *fca* mutation, and possibly the *fe* mutation, specifically affects the activity of a gene involved in flowering. These results do not permit a distinction between an effect on the production of a flowering stimulus or the ability of the apex to respond. The

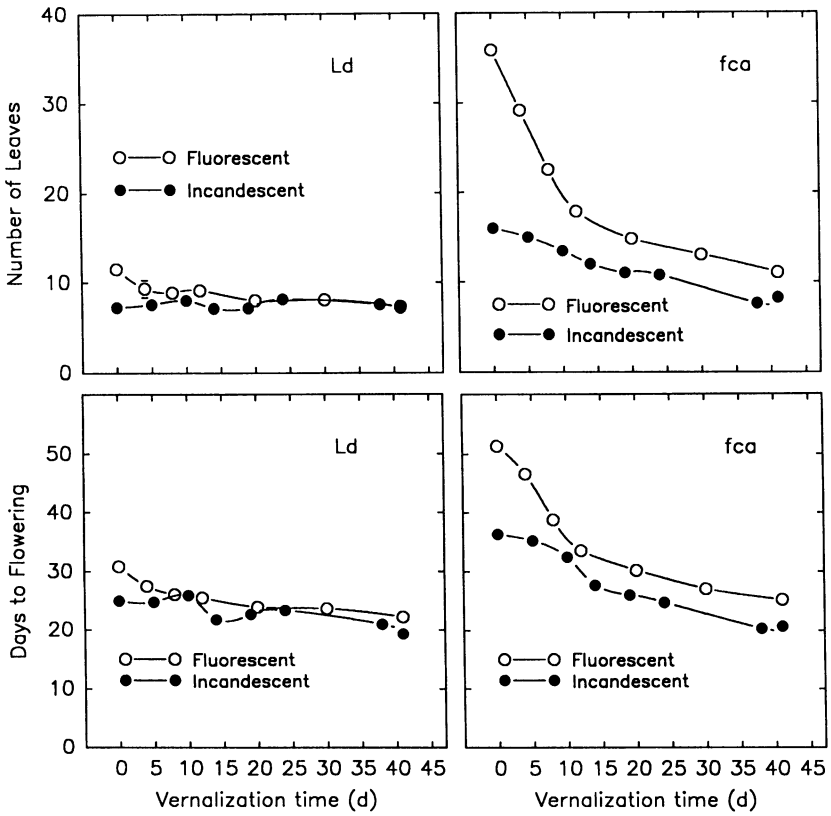

Figure 4. Effect of light quality on the response to vernalization of FT and LN of WT (Ld) and the *fca* mutant. Each point represents the mean of measurements made on 20 to 40 plants pooled from two replicates. Standard errors, indicated by bars, are generally obscured by the symbols.

observation that these mutants responded to both vernalization and incandescent supplementation indicates that the mechanisms by which cold temperatures and far red light induce flowering are not affected by these mutations. Since the combined effect of vernalization and incandescent supplement produced a greater reduction in FT and LN of the *fca* mutant than either treatment applied separately, it appeared that these treatments had an additive effect up to the point that a minimum FT and LN was reached.

The *fb, fg,* and *ft* mutants showed a similar or lower response to both vernalization and incandescent supplement than the WT. By contrast with the *fca, fe,* and *fy* mutants and the WT, vernalization produced in *fb* and *fg* a greater decrease in FT than in LN. There was a negative correlation between vernalization time and FT as found for the other mutants, but there was no correlation between vernalization time and LN. This indicates that most or all of the FT reduction observed is probably due to the fact that vernalized plants are in a more advanced germination state than nonvernalized plants, rather than to a direct effect of vernalization on FT. Since the response to both cold treatment and incandescent supplement seemed to be affected in these mutants, these mutations may alter the sensitivity to those floral inductive mechanisms. Alternatively, it seems possible that these mutations cause metabolic effects that only indirectly delay the onset of flowering.

Floral Induction Pathways in *Arabidopsis*

From the observed responses of Landsberg WT and late-flowering mutants of *Arabidopsis* to light quality and vernalization a tentative pathway of floral induction in this species can be proposed. Since the biochemical basis of floral induction is unknown, we refer to 'pathway' in a developmental rather than a biochemical context.

The observation that, in the early race Landsberg, flowering can be efficiently initiated without cold or far red induction indicates that this race possesses a floral induction mechanism (illustrated by A, A2, A3 in Fig. 5) which is independent of those environmental stimuli. Striking evidence for the constitutive nature of this mechanism is provided by the results of experiments in which *Arabidopsis* plants flower if grown throughout their life cycle in complete darkness on liquid medium supplemented with sucrose (23). Both blue light (3, 17) and availability of assimilates at the meristem (24) could be factors influencing floral induction through this pathway. This constitutive floral inductive mechanism may not be a unidirectional stepwise pathway since mutations which completely block floral induction have never been reported in *Arabidopsis*. Alternatively, it is formally possible that such mutations could be lethal. Whatever the mechanism, it is also present in late races of *Arabidopsis* since they ultimately flower even in the absence of cold induction (19–21). Analysis of the Landsberg WT responses also indicate that early races are responsive to far red light (pathway B) and cold temperatures (pathway C). We suggest that the existence of an active pathway A would render pathways B and C largely redundant in early races, except under those environmental conditions, such as short days or dim light (19), which depress the activity of pathway A. It should be noted that the primary effects of

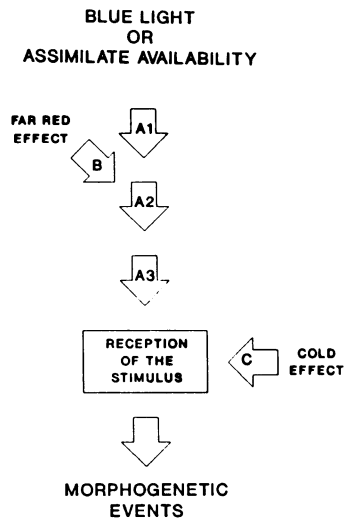

Figure 5. Hypothetical scheme of floral inductive pathways in *A. thaliana.*

the various pathways may be exerted in different tissues of the plant.

Late-flowering mutants described here can be classified into two main groups. The *fca, fe,* and *fy* mutants are more responsive than WT to both incandescent illumination and cold treatments and the delay in flowering caused by the mutation can be partially or completely offset through stimulation of pathways B or C. Thus, we propose that these mutations affect the activity of different steps in pathway A. According to this scheme, since the *fca* mutant is more responsive to vernalization than to incandescent illumination, the mutation affects segments A2 or A3. The possibility, suggested by several authors (2, 15), that vernalization increased the sensitivity to photoperiodic induction is also compatible with this scheme. Another conceivable explanation for this class of mutants would be that these mutations affect repressors of inductive pathways B and C. However, this hypothesis would imply that there are many loci responsible for these repressors and that these molecules are repressing both pathways simultaneously. Following the same scheme, the *fb, ft,* and *fg* mutants, which are less responsive to all inductive mechanisms, could be directly or indirectly affected in the response to whatever signals are produced along these inductive pathways.

In conclusion, the differences in the phenotypes of the various mutants suggests that there are several factors interacting in the flowering process, and that the effects of mutations affecting one of these factors can be offset in many cases by the action of the others. These results agree with the theory of a multifactorial control of flowering (1). The mutations analyzed here may represent only a subset of the possible number of mutations which can cause late-flowering. It is, therefore, essential to develop criteria by which the late-flowering phenotype associated with a particular mutation may be attributed to either direct or indirect effects of the mutation. In this respect, it seems likely that the isolation of the genes corresponding to one or more of the mutations

described here will provide an insight into the mechanisms which regulate flowering in higher plants.

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