Regulation of Flowering Time by Arabidopsis Photoreceptors

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The shift in plants from vegetative growth to floral development is regulated by red–far red light receptors (phytochrome A and phytochrome B), blue–ultraviolet A light receptors (cryptochrome), and blue–ultraviolet B light receptors (Cry2). A mutation in the Arabidopsis thaliana Cry2 gene encodes a blue-light receptor apoprotein (CRY2) that is allelic to the late-flowering mutant, fha1. Flowering in CRY2 mutants is completely dependent on photoperiod. Cryptochrome 2 (cry2) is a positive regulator of the flowering-time gene CO, the expression of which is regulated by photoperiod. Analysis of flowering in cry2 and phyB mutants in response to different wavelengths of light indicated that flowering is regulated by the antagonistic actions of phyB and cry2.

The blue e–ultraviolet A (UV-A) light receptors, cryptochromes, and red–far-red light receptors, phytochromes, mediate light-regulated plant growth and development from seed germination to flower initiation. Phytochrome A (phyA), phytochrome B (phyB), and cryochrome 1 (cry1) function in both early photomorphogenesis (1–5) and floral induction (6–9). We report that in Arabidopsis thaliana, the
tivity by preventing proteasomal proteolysis of AA-NAT protein. A reasonable hypo-

thetical mechanism underlying the action of cAMP is inhibition of proteasomal tar-
getting by ubiquitination (13).

Cyclic AMP appears to regulate mam-

malian AA-NAT activity through comple-

mentary stimulation of transcription and inhibition of proteasomal proteolysis of

AA-NAT protein. Although transcription-

al control is not important in all vertebrates (14), inhibition of AA-NAT proteasomal proteolysis may be conserved (13, 15). β-Arrestin agonists may act in a similar man-

ner to control degradation of proteins in other tissues (13, 16).

These findings indicate that proteasomal proteolysis has a role in neural regulation in vertebrates, as in invertebrates (17). Our results indicate that receptor-regulated proteasomal proteolysis can function as a pre-

cise, selective, and very rapid neural switch. In the pineal gland, this mechanism

regulates the conversion of minute-to-minute changes in environmental input into pro-

found global changes in physiology (18). Such neurally regulated and selective

proteasomal proteolysis may play a similarly important role in other aspects of vertebrate physiology and behavior.

REFERENCES AND NOTES


9. Norepinepherine, isoproteronol, dibutyryl cAMP, and 8-bromocyclic AMP reproducibly increased AA-NAT activity (2) and AA-NAT (4) in parallel in pineal organ culture (2) and in isolated pinealocytes (20).


11. Cells (~106) were homogenized in 200 μl of 50 mM tris (pH 7.5), 500 mM NaCl, 1 mM ethylenediamine tetraacetic acid (Sigma), leupeptin (ICN), aprotinin (ICN), (metallo) ethylenediamine tetraacetic acid (Sigma), pepstatin (ICN), pepstatin (ICN), and protein A–Sepharose (1 hour, 4°C). AA-NAT activity was measured as indicated for experiments.

12. Rat pinealocytes or pineal glands were treated in

brain extract homogenate, and protein A–Sepharose (1 hour, 4°C). AA-NAT activity and AA-NAT are stable under these conditions. AA-

NAT was labeled by incubation (1 hour, 30°C, final vol-

tume = 20 μl) with [35S]ATP (adenosine triphosphate), 100 mM NaCl, 50 mM tris-HCl (pH 7.5), 1

mM DTT, 10 mM MgCl2, and 33 U of protein kinase A (PKA, Promega); radioactive bands were analyzed and quantitated as described (4). This technique is quantitative within the range of AA-NAT values used in these experiments.

13. Ubiquitination occurs via


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The blu e–ultraviolet A (UV-A) light recep-

tors, cryptochromes, and red–far-red light receptors, phytochromes, mediate light-regulated plant growth and development from seed germination to flower ini-

tiation. Phytochrome A (phyA), phytochrome B (phyB), and cryochrome 1 (cry1) function in both early photomorpho-
genesis (1–5) and floral induction (6–9). We report that in Arabidopsis thaliana, the
blue-light receptor cry2 (10) plays a major role in floral induction.

We isolated two Arabidopsis mutant alleles that accumulate no CRY2 protein (Fig. 1A) (11). The mutations, cry2-1 and cry2-2, resulted from partial and complete deletion, respectively, of the CRY2 gene (Fig. 1C). Young seedlings of the cry2 mutants are impaired in blue-light-dependent hypocotyl inhibition and cotyledon opening (10), and cry2 mutant plants flowered later than normal (Fig. 1, E and F). The late-flowering phenotype of cry2 mutants is recessive. Plants heterozygous for the cry2 locus (cry2/CRY2) flowered at about the same time as the wild-type plants, although lower levels of CRY2 protein was detected in these plants (Fig. 1F). Because of prolonged vegetative growth, the number of rosette leaves of cry2 mutant plants was roughly twice normal at flowering (Fig. 1F), as with the late-flowering mutants (12).

Arabidopsis is a facultative long-day plant for which flower initiation is accelerated in long-day (LD) but delayed in short-day (SD) photoperiods (13, 14). We examined the effect of cry2 on photoperiod-regulated floral induction (Fig. 2) (15). A similar amount of total irradiation was provided for plants grown under LD and SD to minimize the effect of different day lengths on photosynthesis. The difference between the cry2 mutant and the wild-type plants in the flowering time (and the number of rosette leaves) was the greatest for plants grown in LD; this difference diminished when the plants were treated with fewer LD periods (Fig. 2, A and B). Under uninterrupted SD photoperiods, cry2 mutant plants flowered slightly earlier than the wild-type in SD (Fig. 2, A and B). Therefore, the mutation in the CRY2 gene results in a partial loss of photoperiodic regulation of flowering time. The transgenic

![Image](https://example.com/figure1.png)

**Fig. 1.** Isolation of cry2 mutants and characterization of cry2/fha. (A) cry2-1 and cry2-2 mutants accumulate no CRY2 protein. Samples from hy4 (hy4-304, a cry1 mutant) cry2-1, cry2-2 mutant (11), and Columbia wild-type (wt) plants were prepared and analyzed, using immunoblot as described (10). The blot was probed with antibody to CRY2 (upper), and re-probed with anti-CRY1 (lower). (B) fha-1 and fha-2 mutants accumulate little CRY2 protein. Samples of cry2-1, its Columbia parent (Col), fha-1, fha-2, and their wild-type parent (Ler) were analyzed as in (A). (C) A Southern blot of the genomic DNA of the wild-type (Col) and the cry2-1 and cry2-2 mutant plants. Genomic DNA was isolated (25) using the cetyltrimethylammonium bromide method, digested with the restriction enzymes Eco RV and Eco RI, separated on a 1% agarose gel (10 μg per lane), transferred to a Nylon membrane, and hybridized with γ-32P-labeled CRY2 cDNA. (D) The diagram shows mutations in the CRY2 gene (GenBank accession: U43397) of different cry2/fha mutant alleles (not to the exact scales). The CRY2 gene is boxed, genomic sequences surrounding CRY2 are represented by solid lines (5') and thick dashed lines (3'), and the thin dashed lines represent deletions. The CRY2 sequences (29) at and flanking mutations found in fha-1 and fha-2 are shown in comparison with the corresponding CRY2 sequence of the wild type (Ler). (E) Thirty-four-day-old plants of the cry2 mutant (cry2-1) and wild-type Columbia (wt) grown under continuous white light. (F) An immunoblot showing the absence or presence of CRY2 protein in the homozygous (cry2/cry2), heterozygous (cry2/CRY2) lines, and wild-type plants (CRY2/CRY2). The flowering time and number of rosette leaves at the emerging of the first flowering buds for each were the averages of a population with more than 20 plants.

![Image](https://example.com/figure2.png)

**Fig. 2.** The photoperiodic response of cry2 mutant and CRY2-overexpressing plants. Seeds of cry2 and the corresponding wild type (Col) (A and B) or the CRY2-overexpressing line H2-9 (CRY2+) and the corresponding wild type (ws) (C and D) were sown on compound soil with a similar density (24 seeds per 3-inch by 5-inch pot), kept at 4°C for 4 days, and grown in LD [18 hours of light (~100 μmol m⁻² s⁻¹) 6 hours of darkness]. One pot of each line was transferred to SD [9 hours light (~200 μmol m⁻² s⁻¹) 15 hours darkness] at the time shown on the abscissa or was grown in continuous LD (Cont. LD). “Days to flower” are measured as the days between the date plants were placed under light to the date the first flower bud appeared (A and C). The number of rosette leaves were scored at the day the first flower bud appeared (B and D). The flowering time and the number of rosette leaves shown were the averages of total plants in each pot; the standard deviations are shown.
Gly254 is conserved in both photolyases and sense mutation would result in loss of CRY2 (Fig. 1D). It is unclear why such a mis-

RNA levels of CO in different samples were detected by reverse transcription–polymerase chain reaction using the primers specific for either CO or AP2 as described (20). We used 20 μg of total RNA, isolated from leaf tissues of different lines of plants grown under either LD or SD for 15 days, to synthesize the first strand of cDNA in a 100-μl reaction using a reverse transcription system according to the manufacturer’s instructions (Promega); the reaction was diluted 10-fold, and 1 μl was used in a 50-μl polymerase chain reaction (preheat at 94°C for 2 min, then 25 cycles, each of 55°C for 30 s, 68°C for 2 min, and 94°C for 30 s; 5 μl of each sample was fractionated in 1% agarose gel, blotted to a Nylon membrane, and the DNA was hybridized with the 32P-labeled CO or AP2 probe, accordingly. The relative intensity of CO bands was calculated by normalization of the intensities of the CO2 bands with the intensities of the corresponding AP2 bands; both were quantified from the digitized autoradiography using the NIH Image program (National Institutes of Health, Research Service Branch, National Institute of Mental Health, Bethesda, Maryland).

The flowering time was measured as described (Fig. 2) for the Columbia wild type (Col) and the cry2 (cry2-1) and phyB (phyB-9) mutant plants grown under continuous red (75 to 90 μmol s⁻¹ m⁻²), blue (75 to 85 μmol s⁻¹ m⁻²), or blue-plus-red light (60 to 80 μmol s⁻¹ m⁻²), with a ratio of red-light intensity to blue-light intensity of approximately 2 to 3 (19). Means of three independent experiments (individual samples contain more than 20 plants) with slightly different fluence rate from one experiment to another and the standard errors are shown.

were only slightly reduced (Fig. 3), which may explain why cry2 mutant plants flowered late only in LD (Fig. 2, A and B). Transgenic plants overexpressing CRY2 had CO mRNA levels significantly higher than the wild type in SD but not in LD (Fig. 3), correlating with the flowering time of the transgenic plants (Fig. 2, C and D). These results indicate that cry2 is a positive regulator of CO in response to photoperiod. cry2 is apparently not the only photoreceptor regulating CO expression; there was a twofold increase of CO mRNA in the hy1 mutant impaired in the biosynthesis of the phytochrome chromophore (22), and activity of CO is required for the early-flowering phenotype of hy1 and phyB mutants (20). Thus, cryptochrome 2 and phytochromes appear to function antagonistically in the regulation of CO gene expression.

Blue light (wavelength of ~400 to 500 nm) and red light (~600 to 700 nm) promote and inhibit flowering of Arabidopsis, respectively (24, 25), suggesting different functions of phytochromes and crytochromes in the flowering-time determination. Consistent with previous reports (24, 25), wild-type plants grown under continuous blue light flowered earlier (within 15 days after germination) than plants grown under a similar intensity of red light (more than 30 days after germination) (Fig. 4; red, blue). Considering that blue light promotes flowering and the cry2 mutant flowered late, it may be expected that cry2 mutants might flower later than the wild type if grown in continuous blue light. To our surprise, cry2 mutant plants grown under continuous blue (or red) light flowered at about the same time as the wild type (Fig. 4; red, blue). Because cry2 mutant plants flowered late under white light, we examined the flowering time of cry2 mutant plants grown under light containing both blue and red wavelengths. Under this condition, cry2 mutant plants flowered significantly later than wild-type plants (Fig. 4; red + blue). Thus, the delayed flowering of cry2 mutant plants under white light can be phenocopied by growing the mutant plants under blue-plus-red light.

Our results suggest that phytochromes mediate the red-light–dependent inhibition of flowering, whereas cry2 mediates the blue-light–dependent inhibition of phytochrome function. Phytochromes inhibit flowering in the absence of blue-light–dependent CRY2 activity such that red-light–grown wild-type plants flower late. In blue light, wild-type plants flower early, implying either the presence of a blue-light–dependent activator or the absence of a red-light–dependent inhibitor. Normal flowering of cry2 mutant plants in blue light indicates that the function of cry2 alone does not promote flowering under blue light. Thus, accelerated flowering of
wild-type plants in blue light can be at least partially explained by the absence of the activity of the red-light–dependent inhibitors, phytochromes. Under white light or blue-plus-red light, red-light–dependent phytochrome activity and blue-light–dependent cry2 activity function in an antagonistic manner. In these light conditions, cry2 mutant plants flower late because the red-light–dependent phytochrome activity inhibiting floral initiation remains unassailed as a result of the lack of the blue-light–dependent cry2 activity in the mutant plants.

We suggest that the function of both phytochromes and cry2 in flowering-time regulation is mediated by CO. The function of phytochromes proposed in our model is consistent with the observation that Arabidopsis hy1 and hy2 mutants, defective in the biosynthesis of phytochrome chromophore, flower earlier than the wild-type plants (6). It is not clear how many phytochrome species are involved in mediating red-light–dependent inhibition of flowering, although phyA is probably not associated with the flowering inhibition because the phyA mutant does not flower early (6). phyB mutant plants flower earlier than the wild-type plants grown under white light (6, 7), an effect mediated by CO (20). Thus, phyB could be one of the phytochromes that mediates red-light–dependent inhibition of flowering (4). Indeed, the early-flowering phenotype of phyB is dependent on red light (Fig. 4). In blue light, however, phyB mutant plants flowered at about the same time as the wild type (Fig. 4; blue). Consistent with our model, phyB mutation can suppress the late-flowering phenotype of cry2 under blue-plus-red light, whereas the cry2 mutation cannot suppress the early-flowering phenotype of phyB in red light (26).

Although our model explains the mode of action of cry2 and phyB in the regulation of flowering time of Arabidopsis, phyA and cry1 appear to function in different ways in the regulation of PHYB activity in the recombinant inbred map (http://cipl.hungen.upenn.edu/~atgc/ATGC_epi.html). FRA was mapped to about 4 centimorgans from the PHYA gene (12). For the complementation test, the flowering time of F0 progenies of cry2 knockout (Col background) 

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**Src Activation in the Induction of Long-Term Potentiation in CA1 Hippocampal Neurons**

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Long-term potentiation (LTP) is an activity-dependent strengthening of synaptic efficacy that is considered to be a model of learning and memory. Protein tyrosine phosphorylation is necessary to induce LTP. Here, induction of LTP in CA1 pyramidal cells of rats was prevented by blocking the tyrosine kinase Src and Src activity was increased by stimulating presynaptic LTP. Direct overexpression of Src in the postsynaptic neuron enhanced excitatory synaptic responses, occurring LTP. Src-induced enhancement of 3-hydroxy-5-methylisoxazolepropionic acid (AMPA) receptor–mediated synaptic responses required raised intracellular Ca2+ and N-methyl-d-aspartate (NMDA) receptors. Thus, Src activation is necessary and sufficient for inducing LTP and may function by up-regulating NMDA receptors.

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**REFERENCES AND NOTES**

12. The strategy used in the isolation of the cry2 mutant is based on the observation that overexpression of CRY2 resulted in a short hypocotyl under blue light, especially low intensity of blue light (10). Fast-neuron-mutagenized Columbia (Col-4) seeds were sown on compound soil, kept in a cold room (4°C) in especially low intensity of blue light (2 to 5 mol m–2 s–1) for 2 to 3 days. Seedlings that grew taller than average were selected. Individual isolates were analyzed for the CRY2 expression using the immunoblot as-say (10). Two cry2 alleles were identified from 636 isolates initially selected from ~130,000 M1 seeds. Lights and filters used are as described (10).
15. M. Ahmad and A. R. Cashmore, Phytochemistry 36, 8, 9, and the relative importance of individual photoreceptors in mediating photoperiodic signals may be different in other plant species (9). It will also be interesting to learn the relationship between cry2 in photoperiodism and the circadian clock associated with blue-light–entrained circadian rhythms in plants (27).