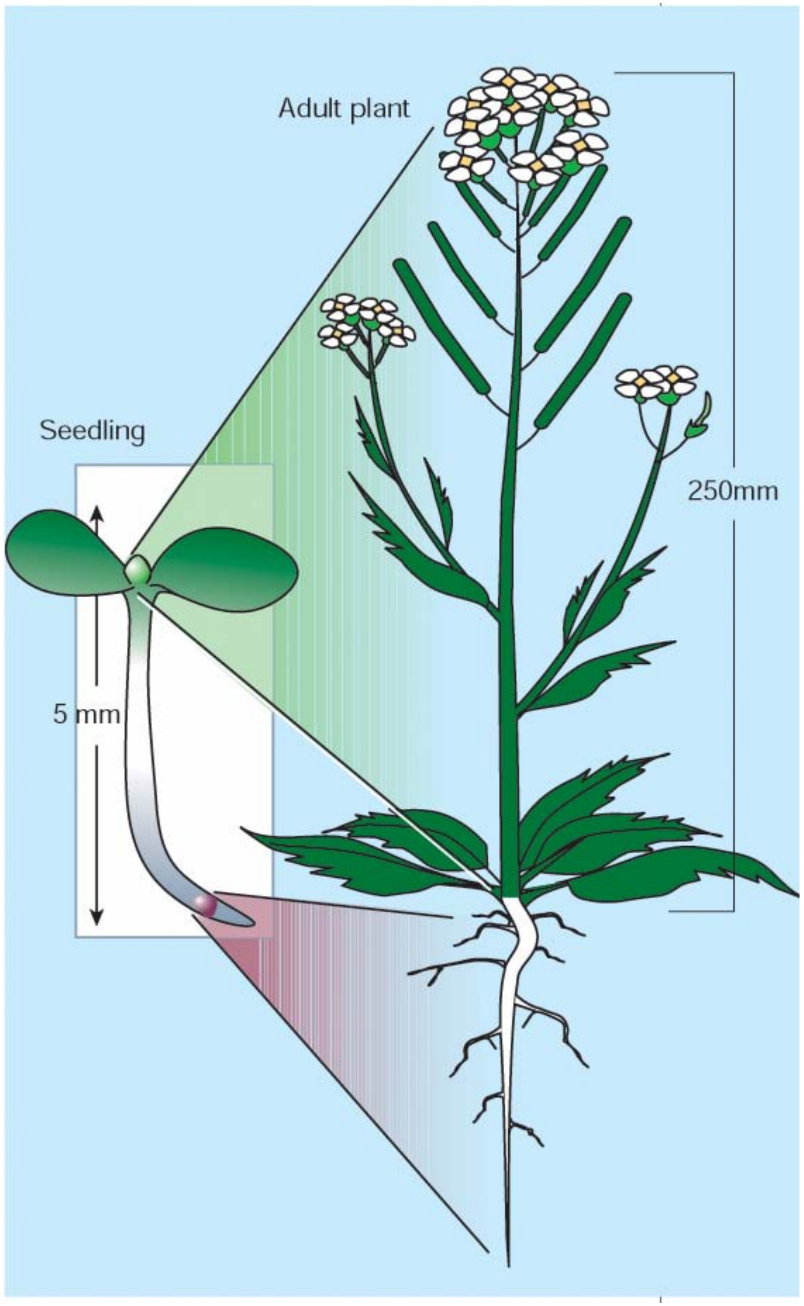
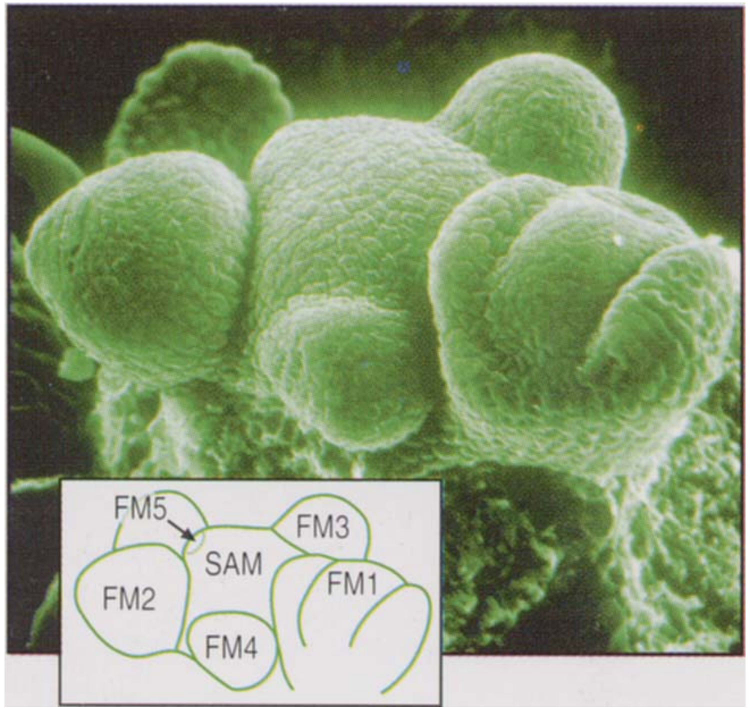
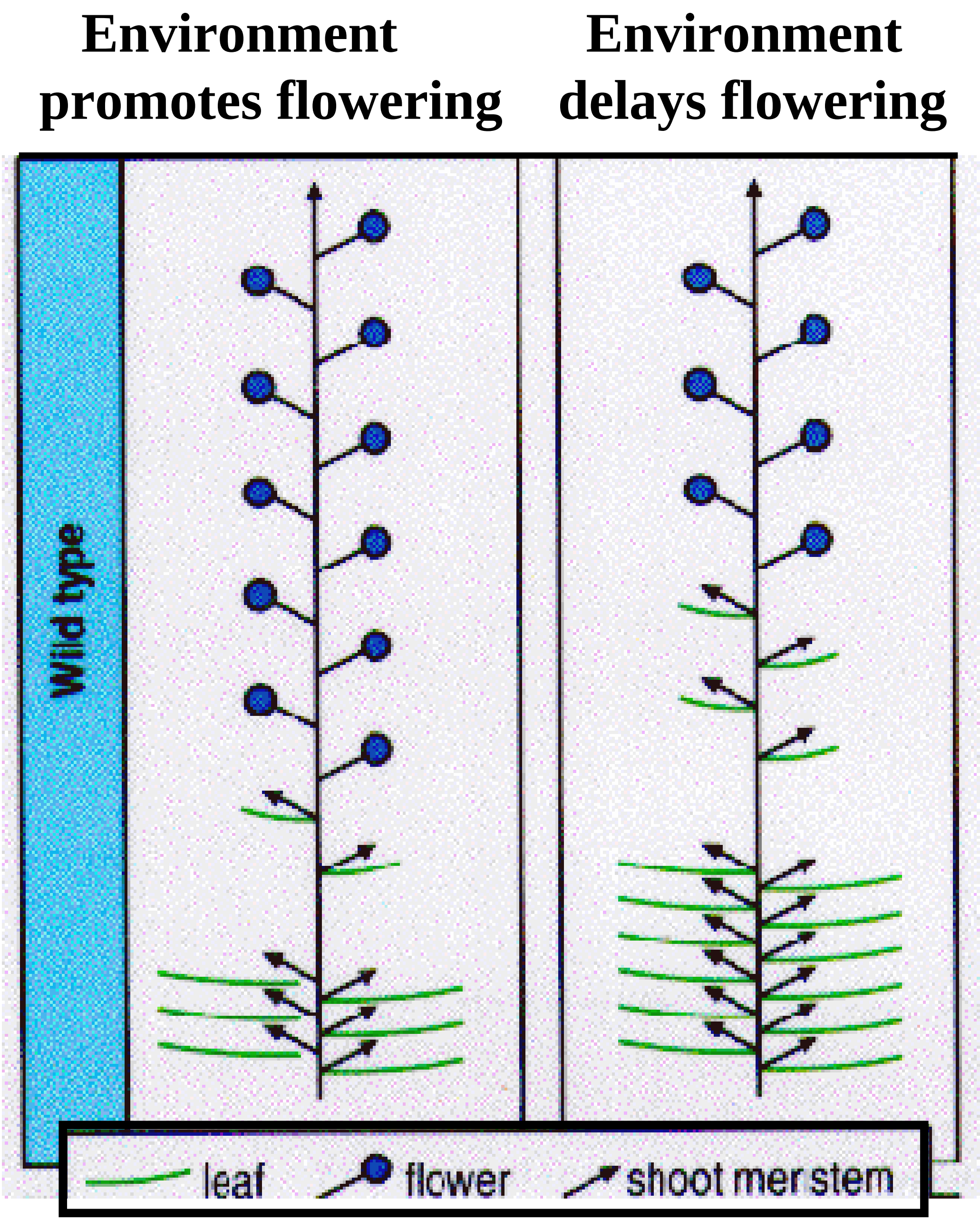




How is the transition from vegetative growth to flowering controlled ?

- How is it regulated by environmental conditions?

Environmental signals can influence the identity of the lateral organs formed at each node

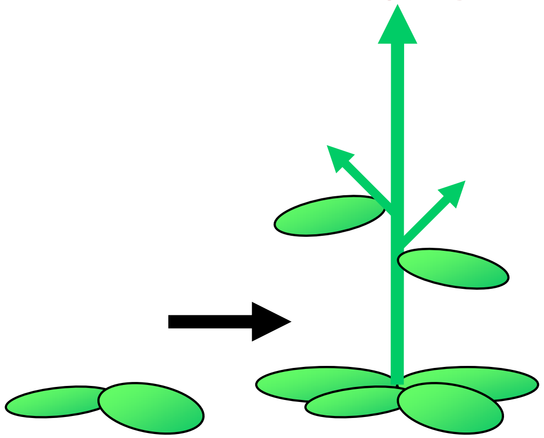


Environments that Promote flowering Of Arabidopsis are

-long daylengths, photoperiod

-Long exposure to low Temperature, vernalization

Long-day plants flower when daylength exceeds a critical daylength



Examples:
Arabidopsis, wheat, barley, sugar beet

Long days — Flowering

Short days — No Flowering

Two classes of mutation reduce the response to daylength

Long days

Wild-type *constans*

Late-flowering in long days
No effect in short days
constans, gigantea, ft, cry2

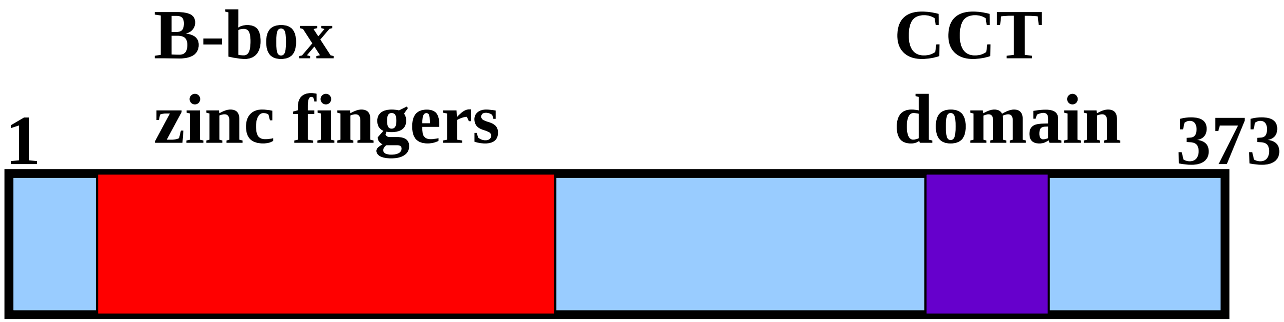
Short days

lhy-14

Wild-type

Early-flowering in short days
No effect in long days
lhy, toc1

CONSTANS has two motifs that are required for its function

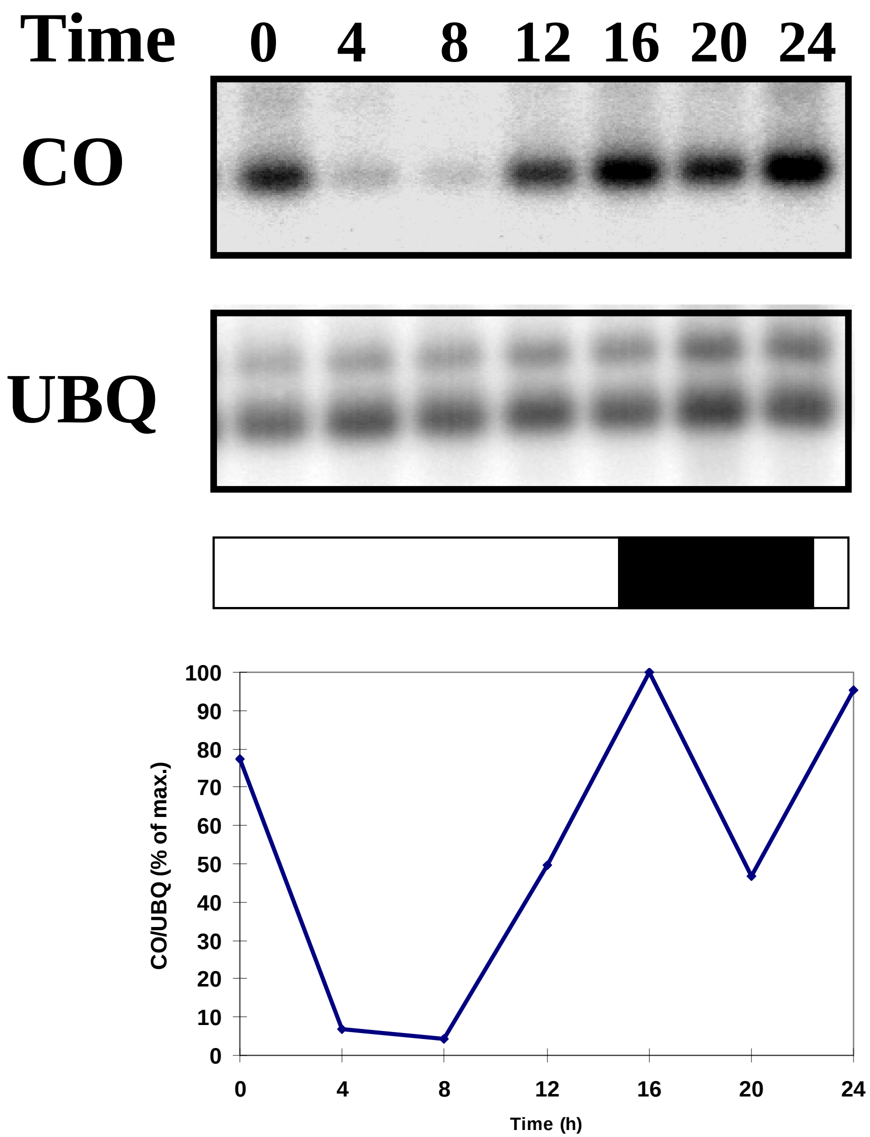


B-box proteins act in protein complexes that regulate transcription in animal cells

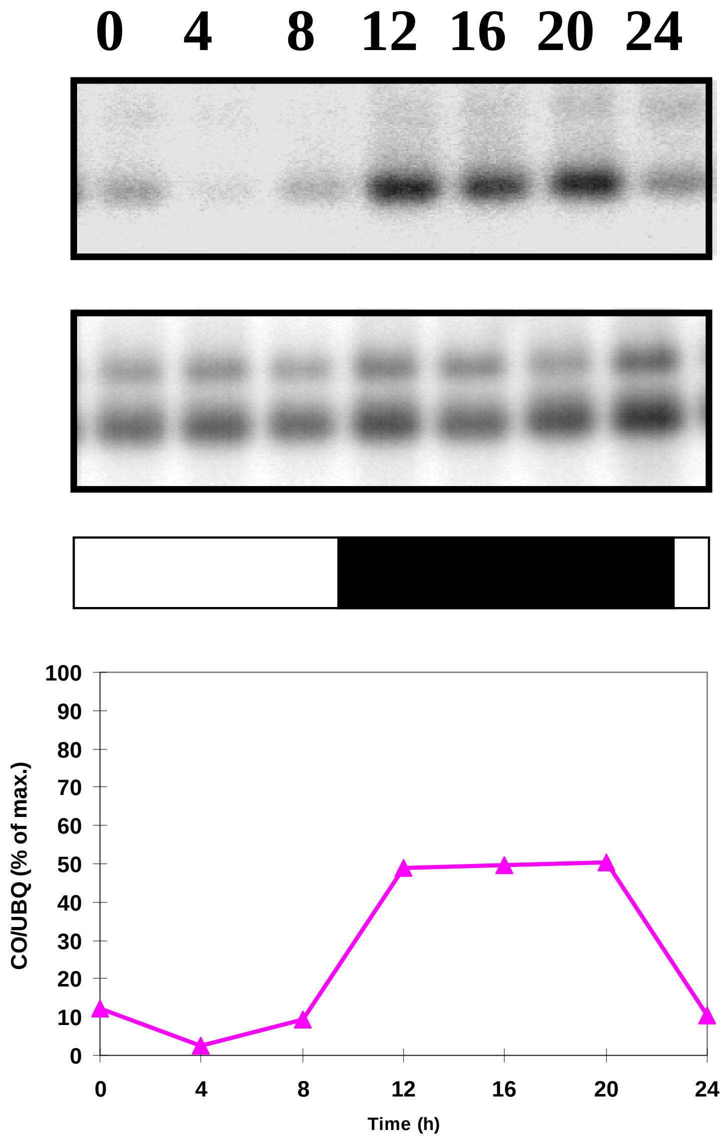
CCT is plant specific named after CONSTANS, CONSTANS-like and TOC1.

CO Expression Under Long and Short Days

LONG DAYS



SHORT DAYS

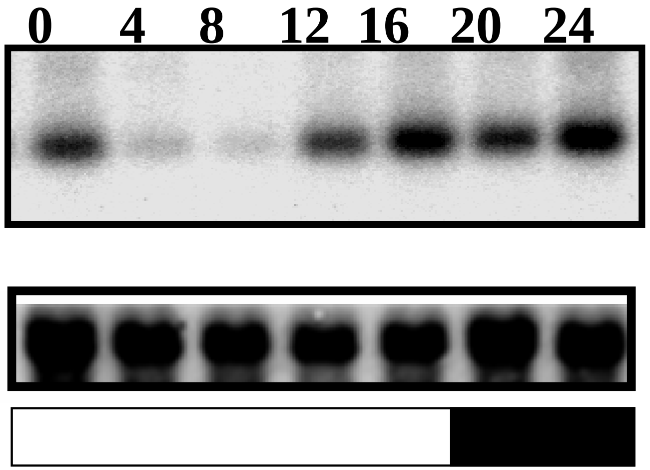


CO mRNA is expressed throughout the day and at higher levels in *35S::CO* plants, and this causes early flowering in all daylengths

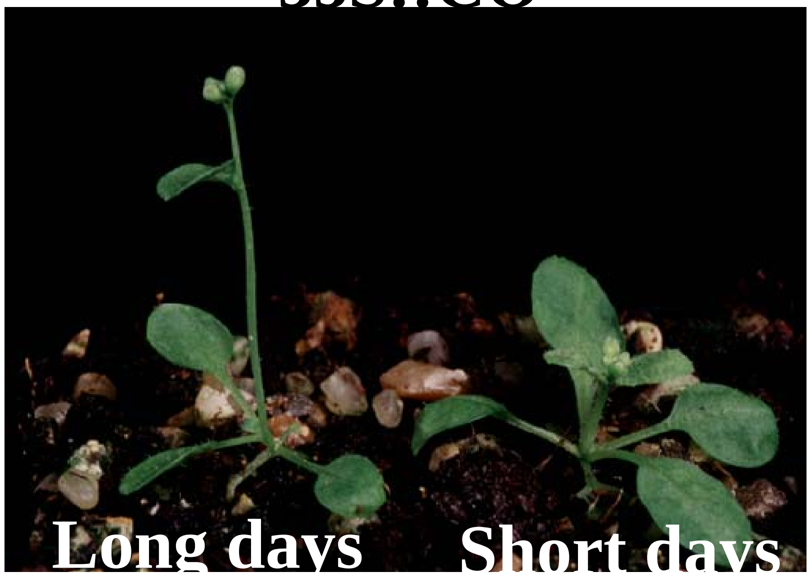
hours

WT CO

35S::CO CO mRNA

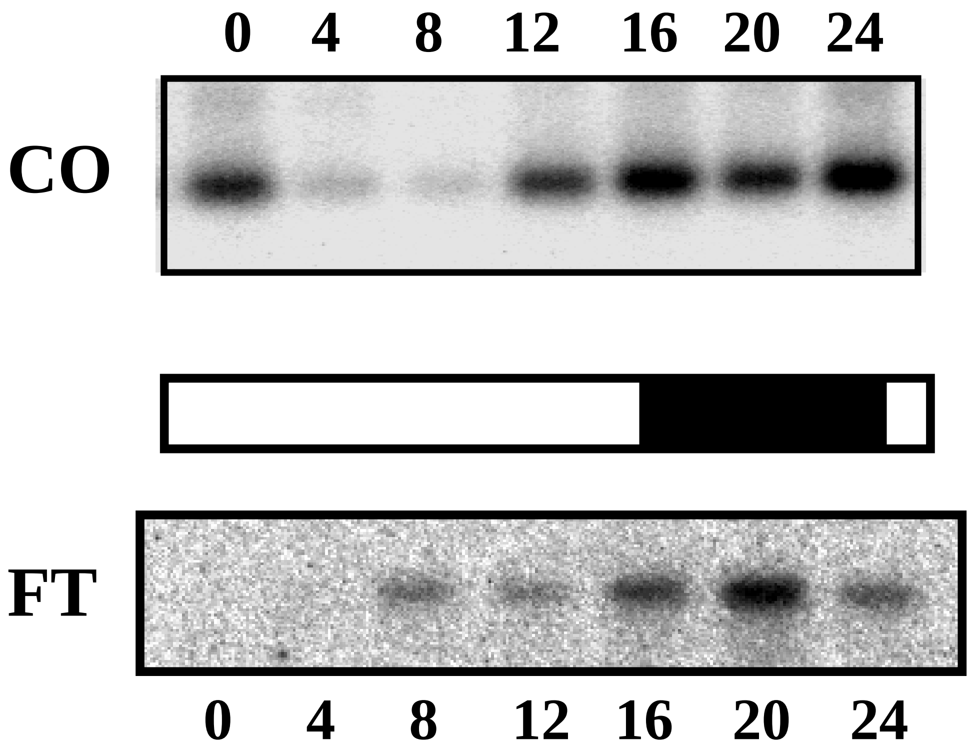


35S::CO



	Leaf Number
35S::CO	
Long Days	5.0
Short Days	4.8
Wild-type	
Long Days	8.6
Short Days	27.0

Comparison of diurnal rhythms in CO mRNA and mRNA of FT, a target gene of CO

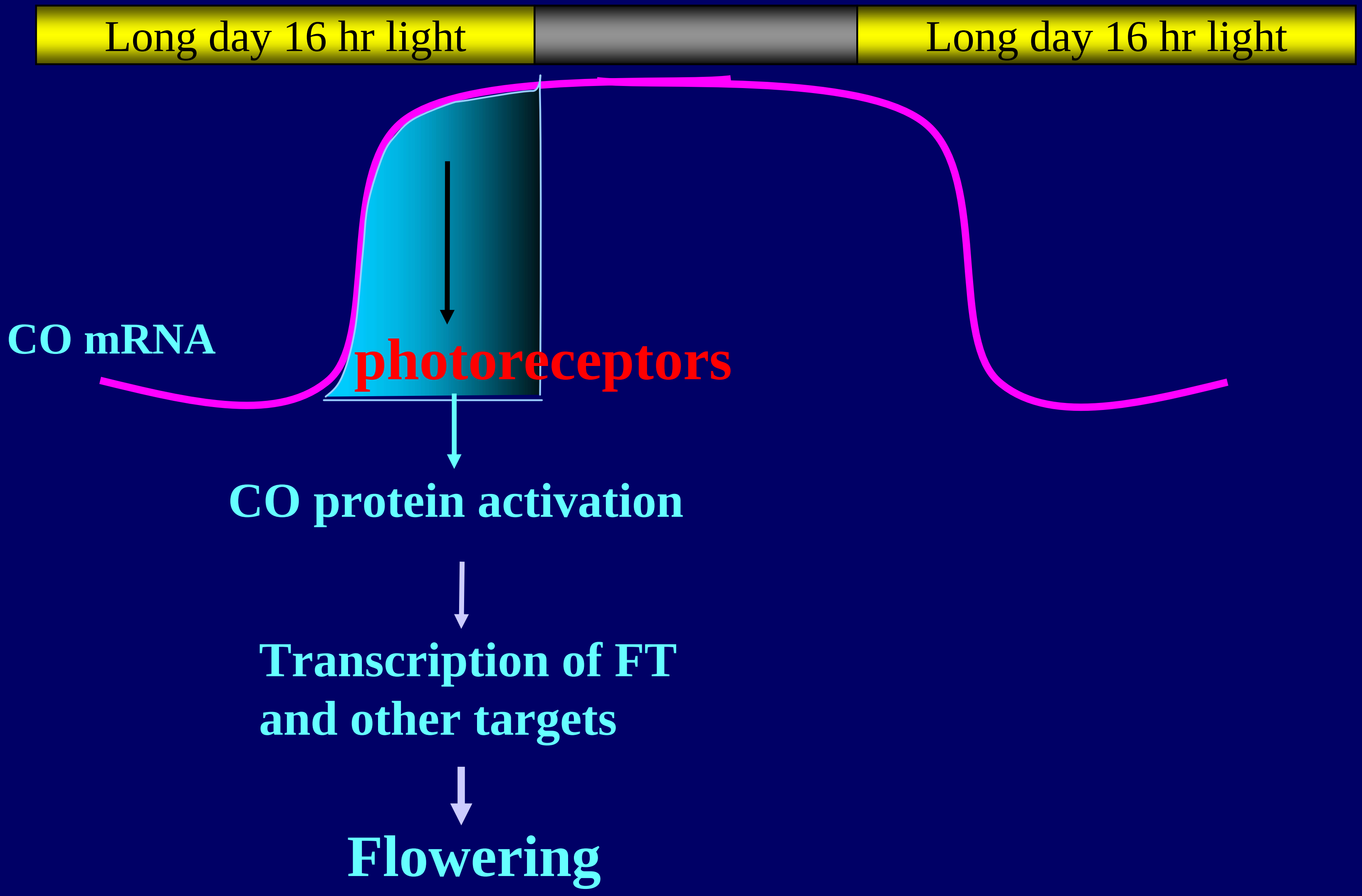


FT expression –

Reduced in co mutants
Reduced in short days
Overexpressed in 35S::CO

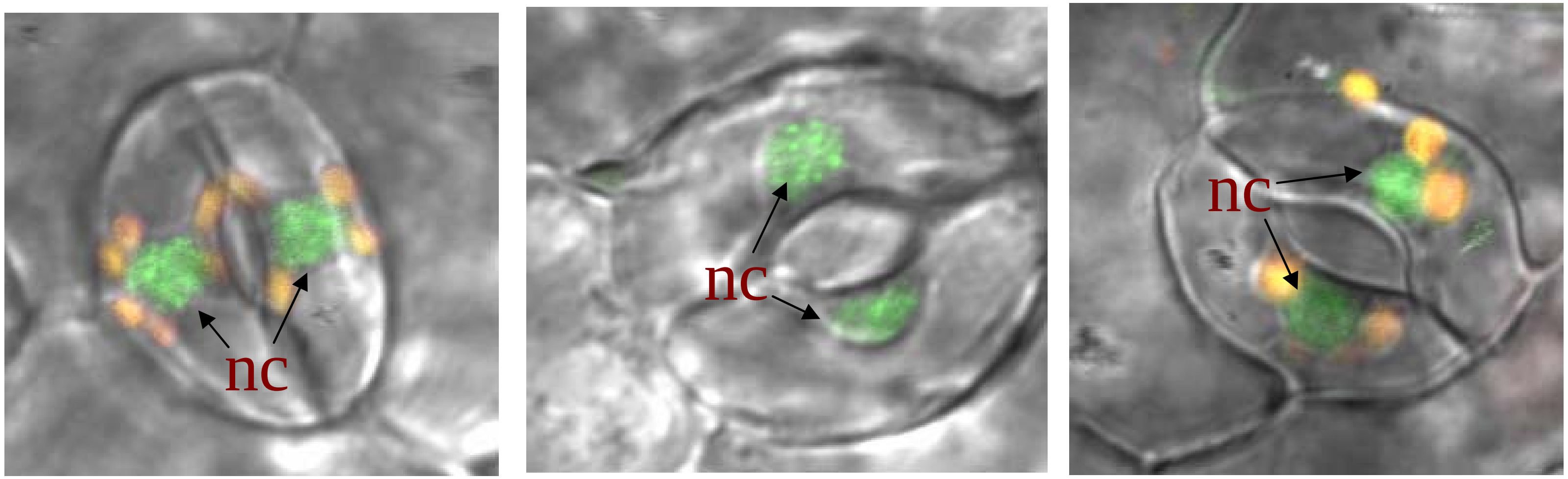
ft mutants similar phenotype to co mutants

A model for the response of Arabidopsis to daylength



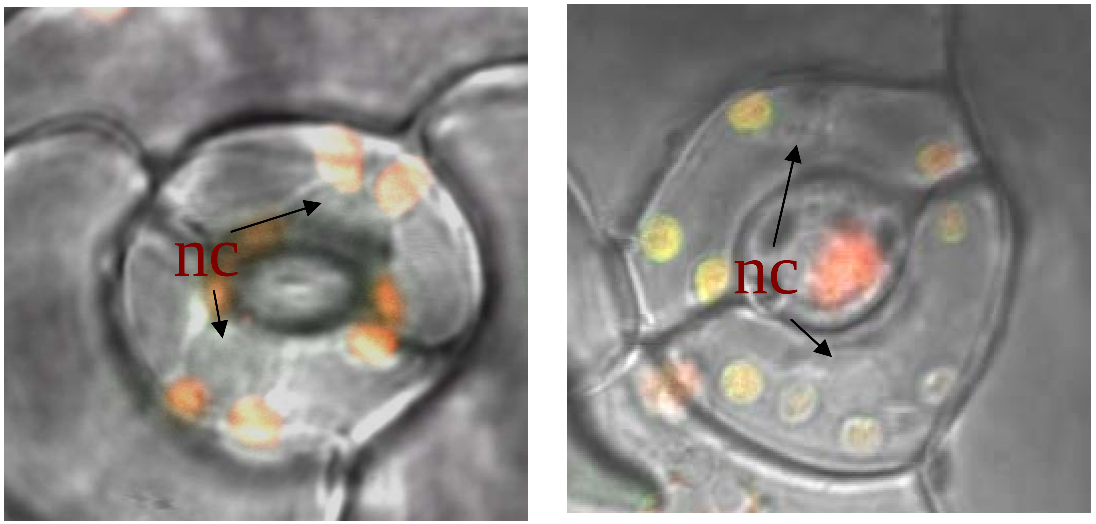
Exposure to light stabilises CO protein in the nucleus
35S::GFP:CO plants exposed to different light qualities

Light conditions in which CO is active



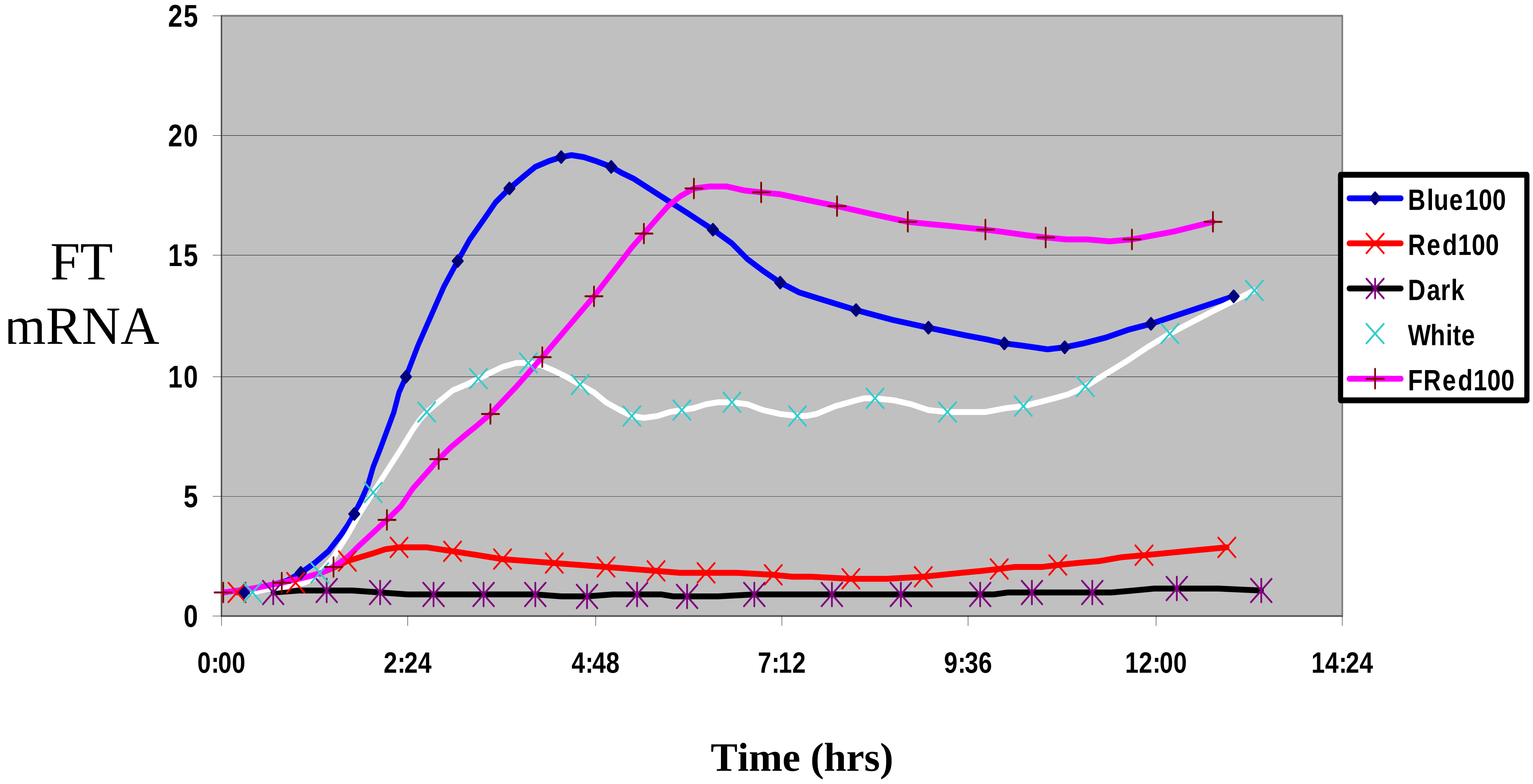
White **Blue** **Far-red**

Light conditions in which CO is inactive

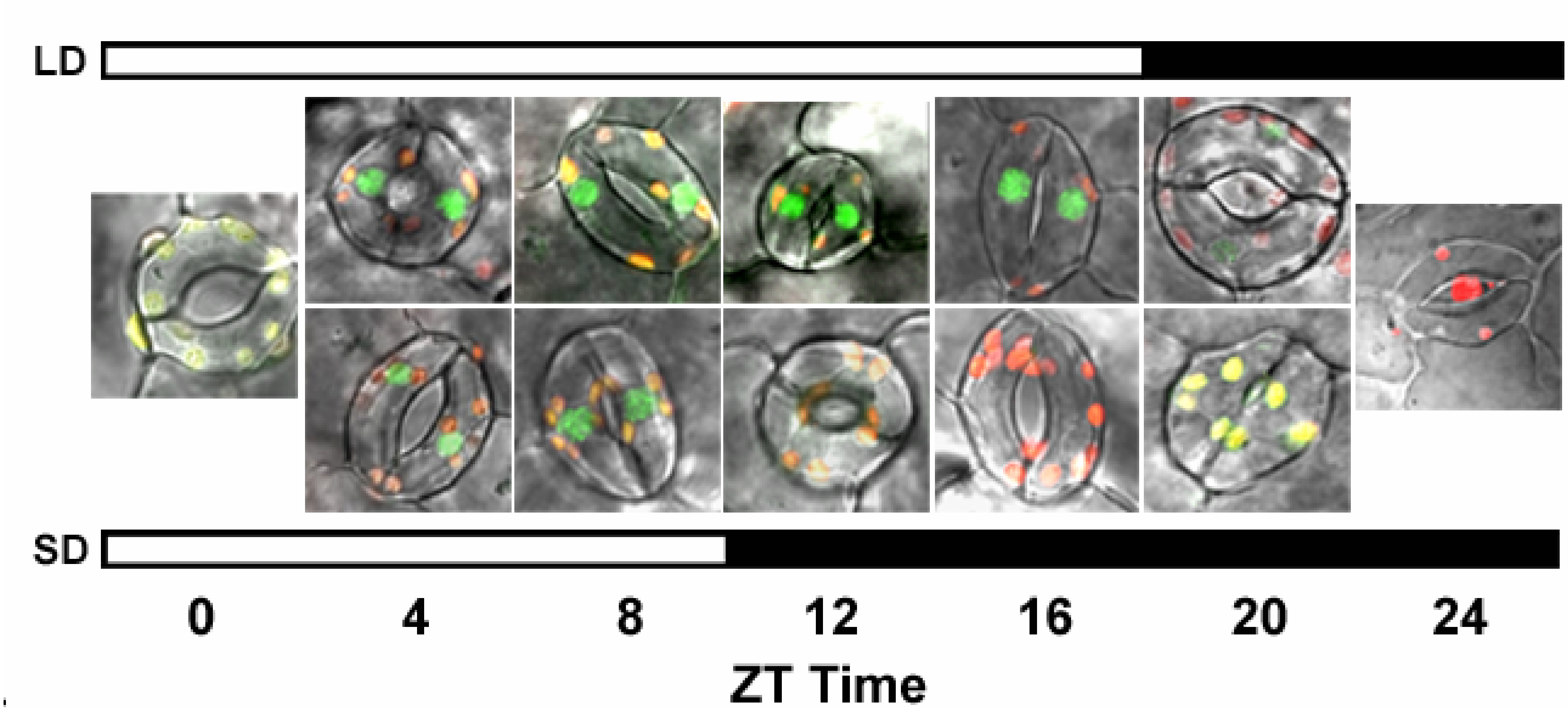


Dark **Red**

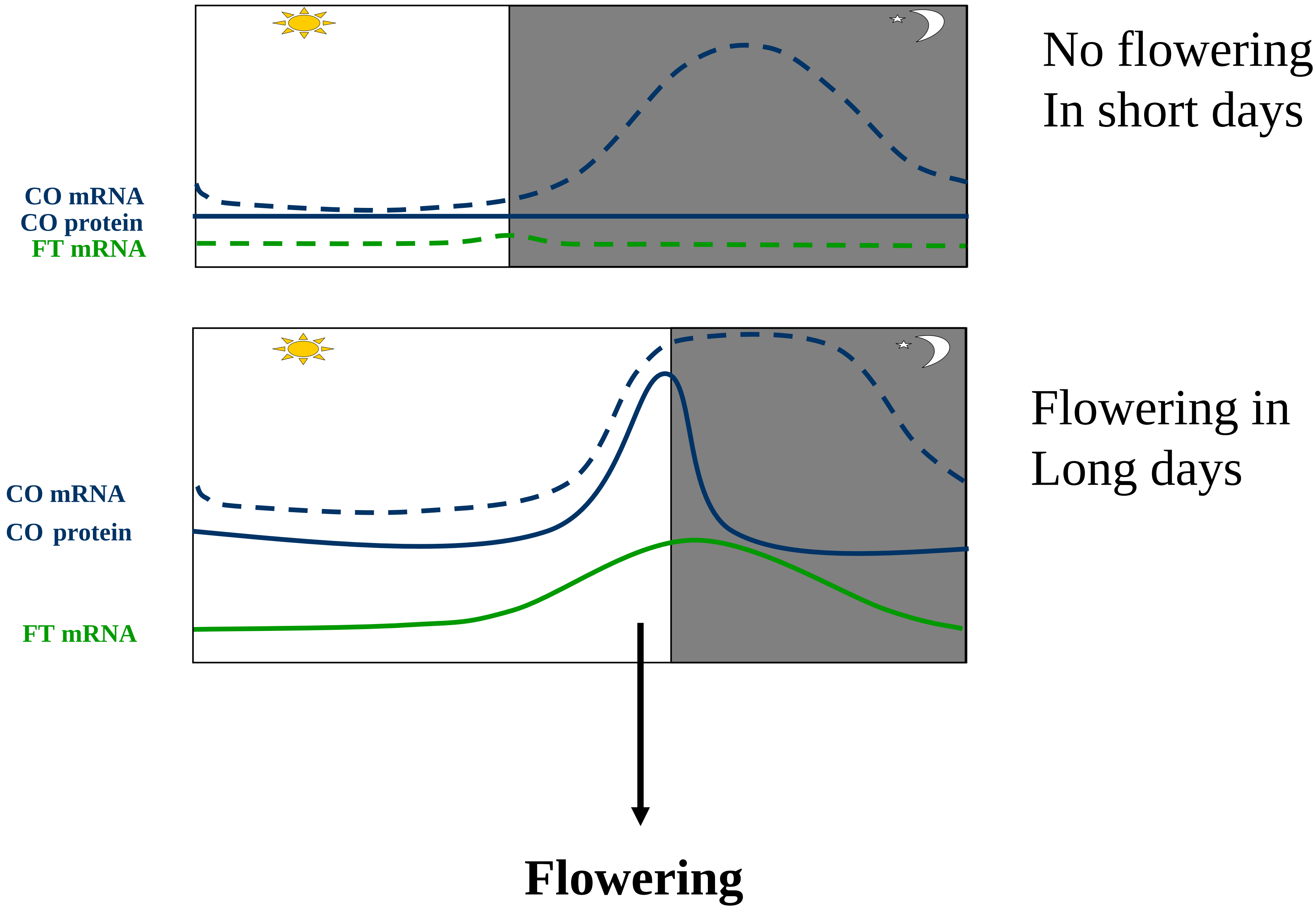
Activation of FT by 35S::CO occurs in blue and far-red light, but not red light



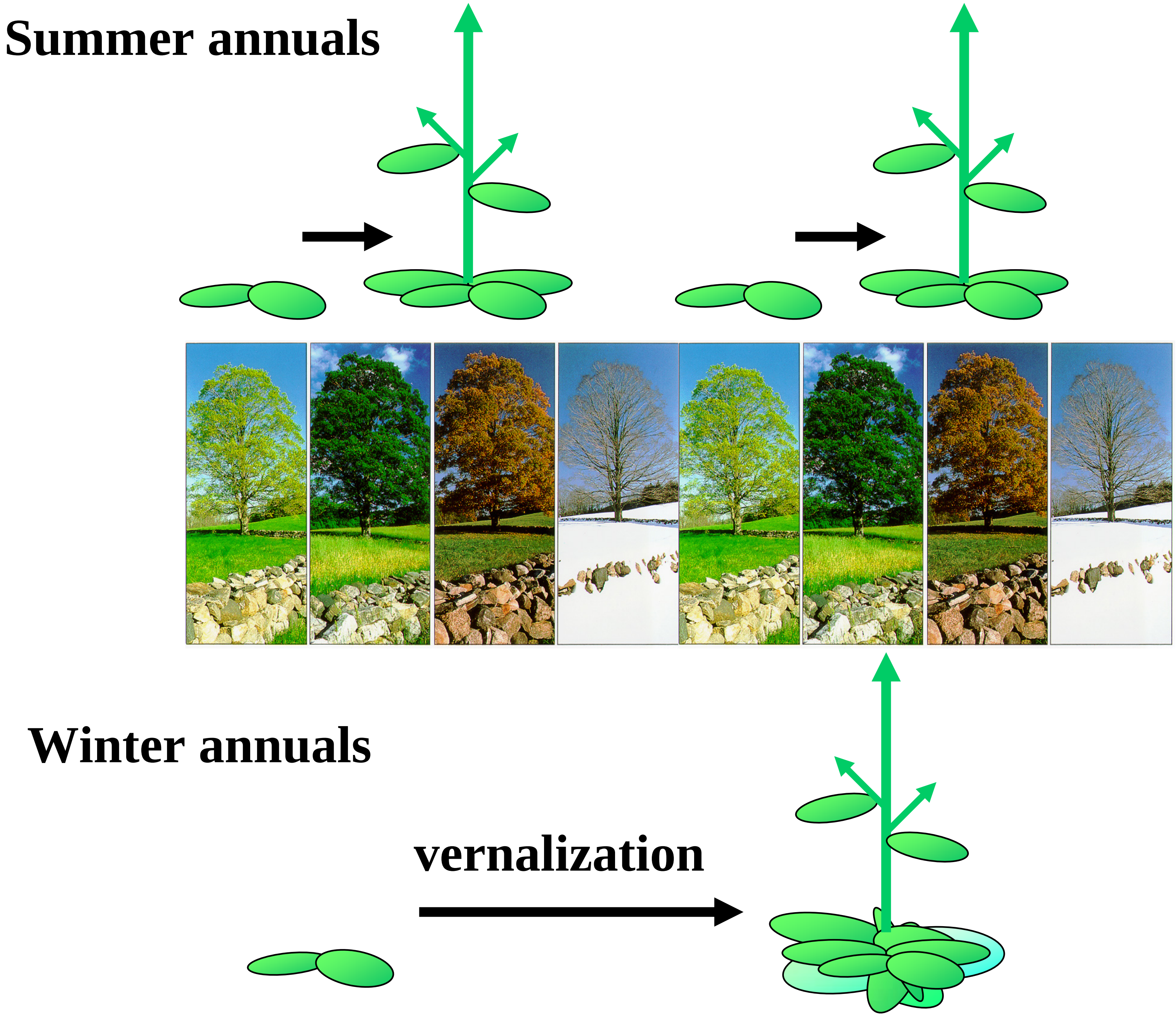
CO:GFP accumulates during the photoperiod in *35S::CO:GFP* plants



Coincidence of light and CO mRNA leads to stabilisation of CO protein and activation of FT under long days



Arabidopsis varieties differ in their reproductive strategies



Features of vernalization

1. **Occurs at shoot meristem**

2. **Requires several weeks of exposure to low temperatures. This treatment called vernalization.**

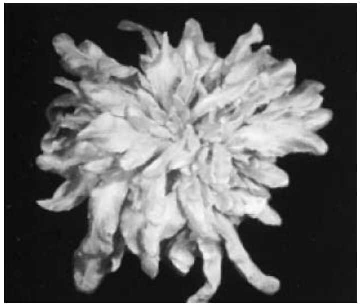
3. **Effects of vernalization maintained through many mitotic divisions.**
   If a plant is vernalized as a young seedling this effect can be „remembered“ for several months.

4. **Effects of vernalization reset at meiosis.**
   The progeny of a vernalized plant behave do not inherit the effect of vernalization, and behave as if they have not been vernalized.

Analysis of the genetic basis of vernalization response

No vernalization | **8 weeks vernalization**

Santa Fe (winter annual)



Columbia (Summer annual)

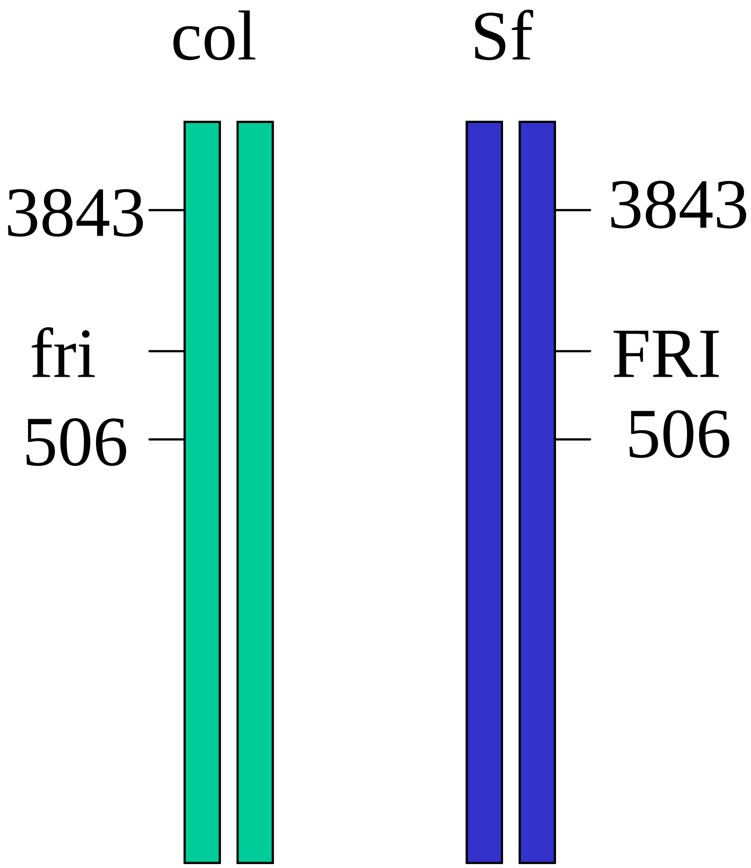
To identify genes that confer a vernalization response
- intercross Sante Fe and Columbia

Genetics of crossing Stockholm with Li-5

	Late flowering	Early flowering
Columbia	0	55
Santa Fe	40	0
Sf x Col	135	46

Indicates 3:1 segregation with late flowering dominant.

Gene that confers late flowering, FRIGIDA, also Occurs in other vernalization responsive varieties

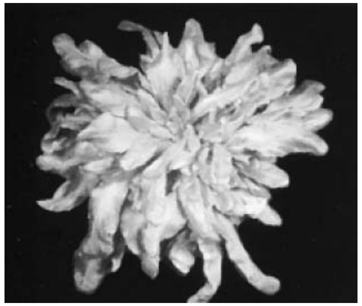


Chromosome 4

In crosses between other Examples of vernalization responsive and non-responsive strains alleles of FRIGIDA shown to underlie the difference. Including European strains Stockholm and Limburg-5.

FRI encodes a 609 amino acid protein of unknown function containing two coil-coil domains implicated in protein protein interaction.

How does FRIGIDA delay flowering?



FRI plant – very late flowering

↓

Mutagenise with radiation

↓

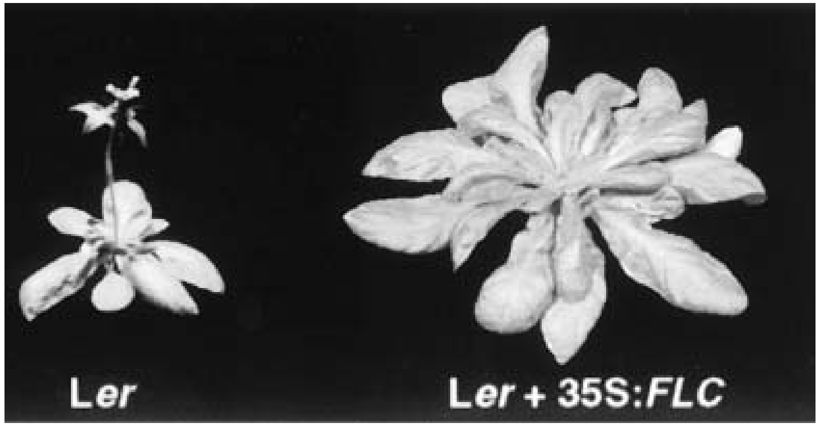
Screen for early flowering plants

flc *FLC*

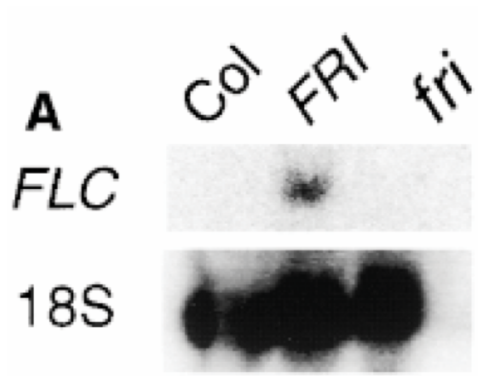
3 loss of function Alleles of FRI

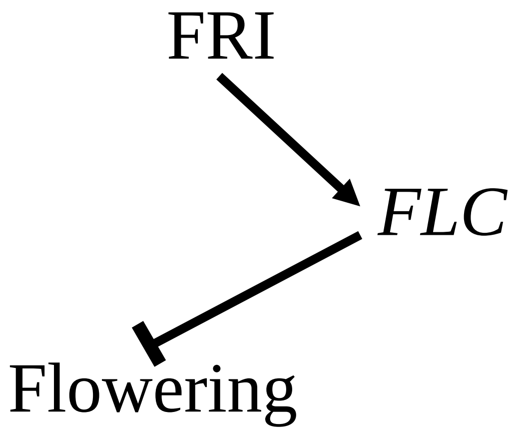
4 loss of function Alleles of FLC

FLC encodes a MADS box transcription factor that represses flowering



Overexpression of FLC delays flowering





FRIGIDA promotes expression of the floral repressor FLC

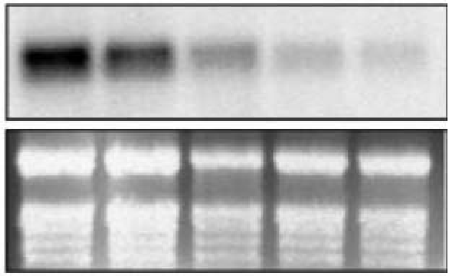
In winter annuals vernalization reduces abundance of the floral inhibitor FLC and accelerates flowering

FRI FLC **plants**

Time at 4C

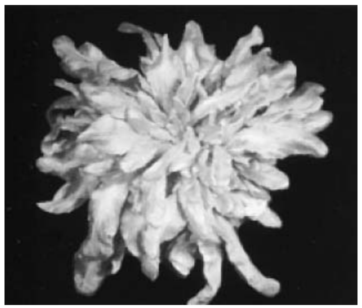
(days) 1 7 14 21 28

FLC



No vernalization **8 weeks vernalization**

Santa Fe



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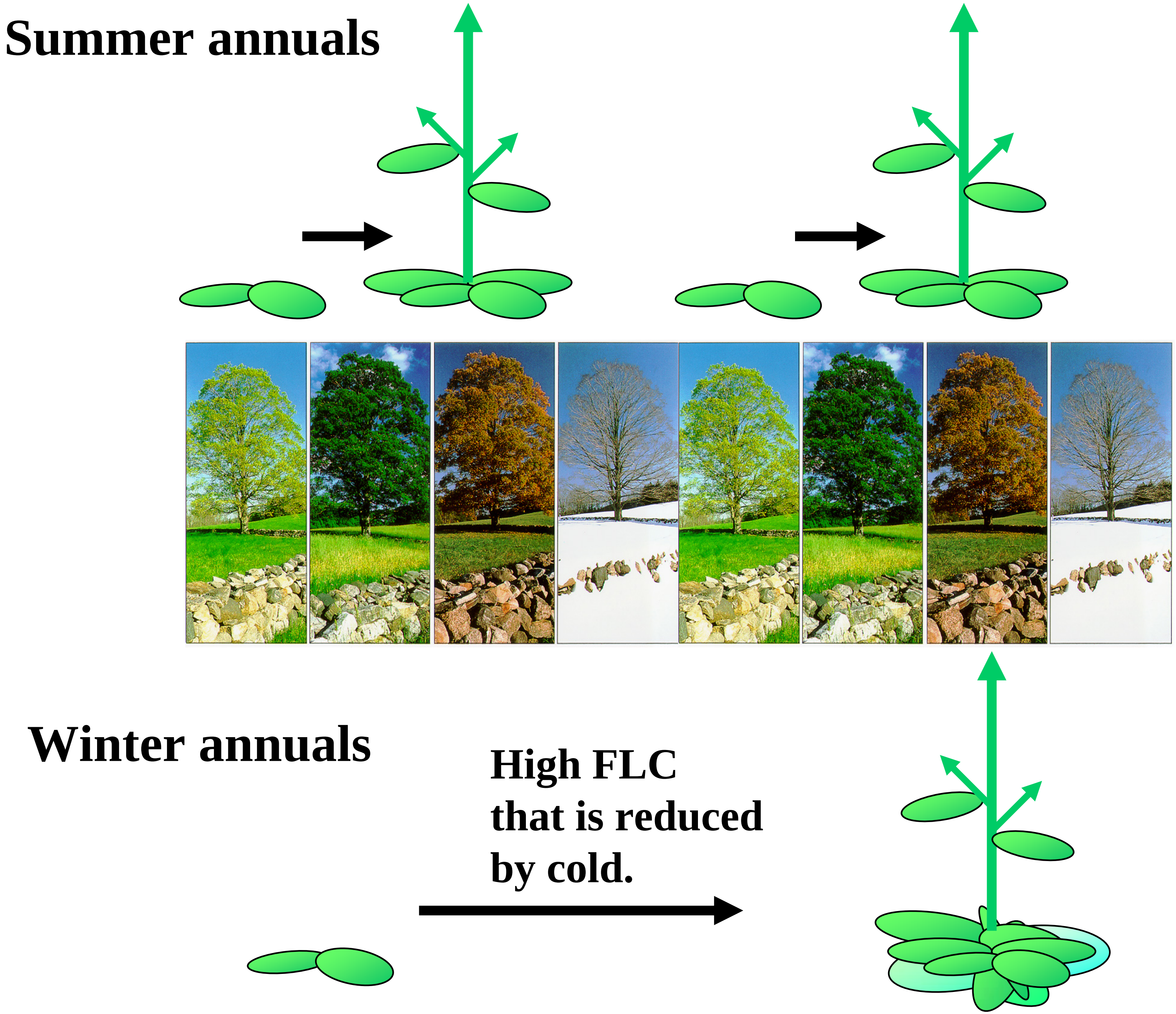


Identification of genes required for vernalization response - how is FLC expression controlled by low temperatures?

Late flowering, high FLC

↓

Treat with mutagen - EMS

↓

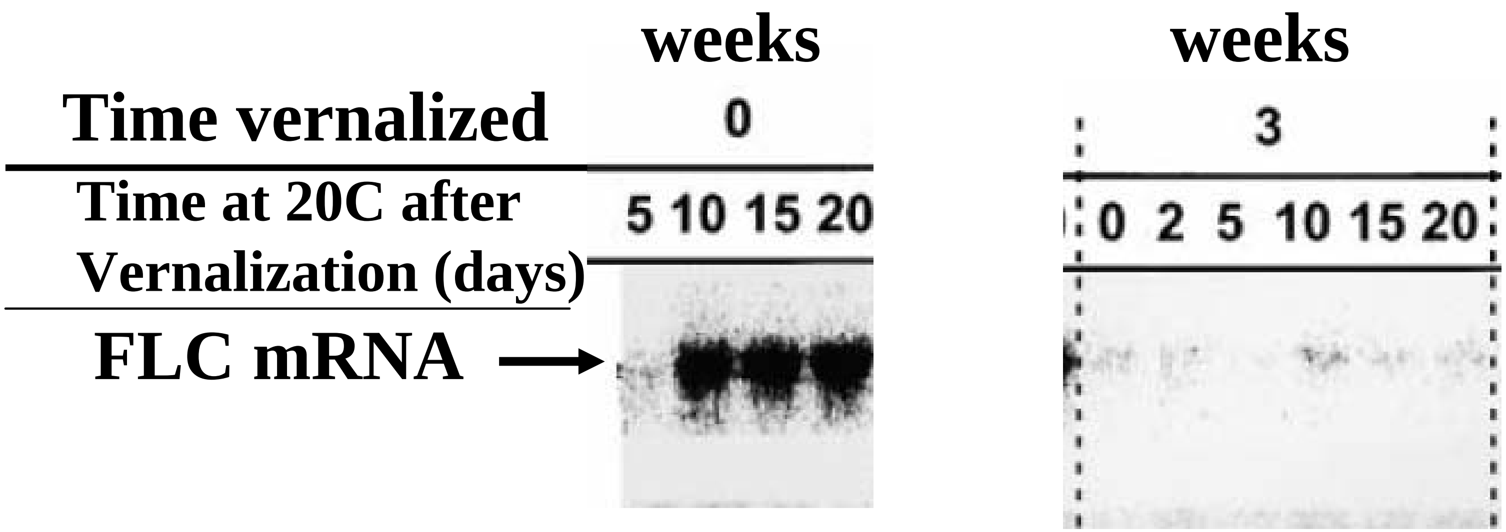
Give vernalization treatment to all plants

↙ ↘

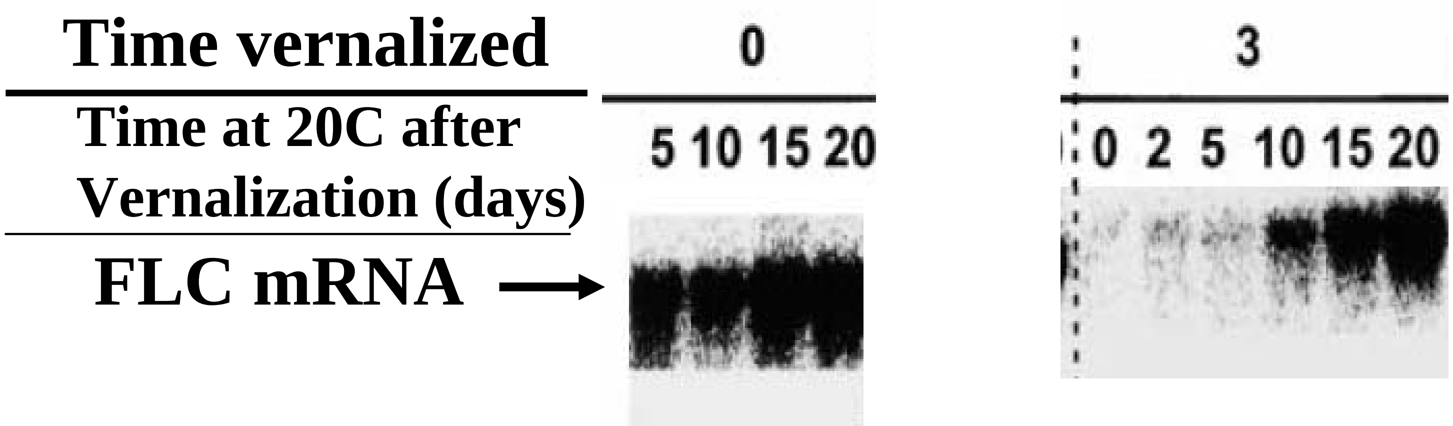
Most plants flower early.

Some plants flower late. These are vernalization and vernalization insensitive mutants.

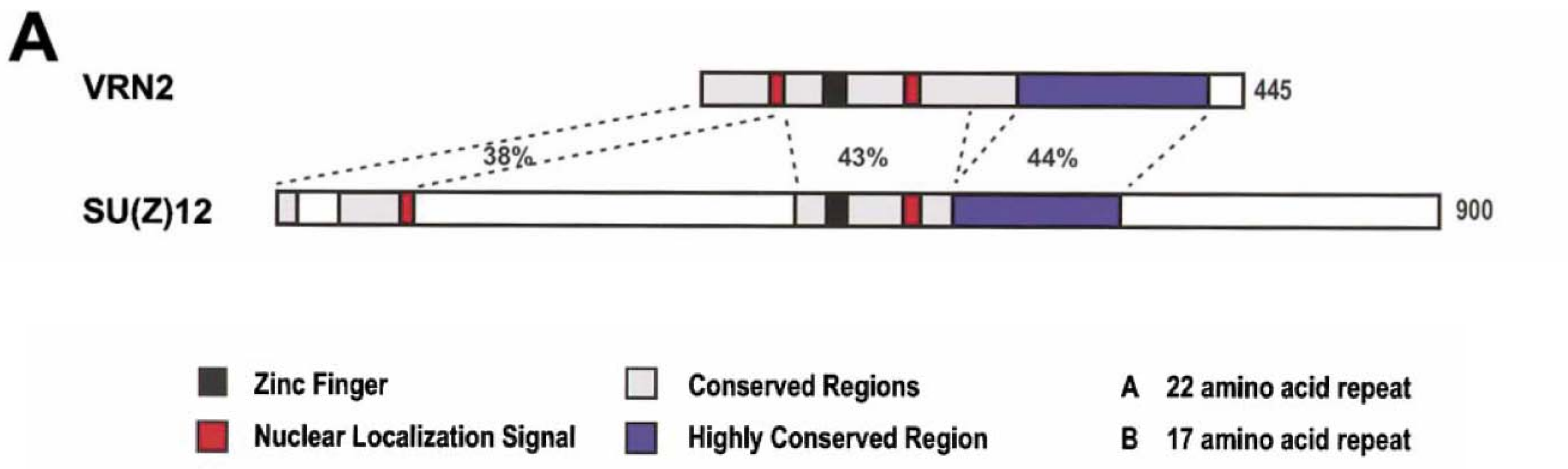
The role of VERNALIZATION2 is to maintain the repression of FLC expression



vrn2 mutant

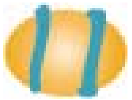


VERNALIZATION2 encodes a gene related to Drosophila Polycomb-group (PcG) genes



In Drosophila, PcG proteins act in large protein complexes. They maintain the repression of transcription of homeotic genes, once the pattern of expression of these genes has been established during early embry development.

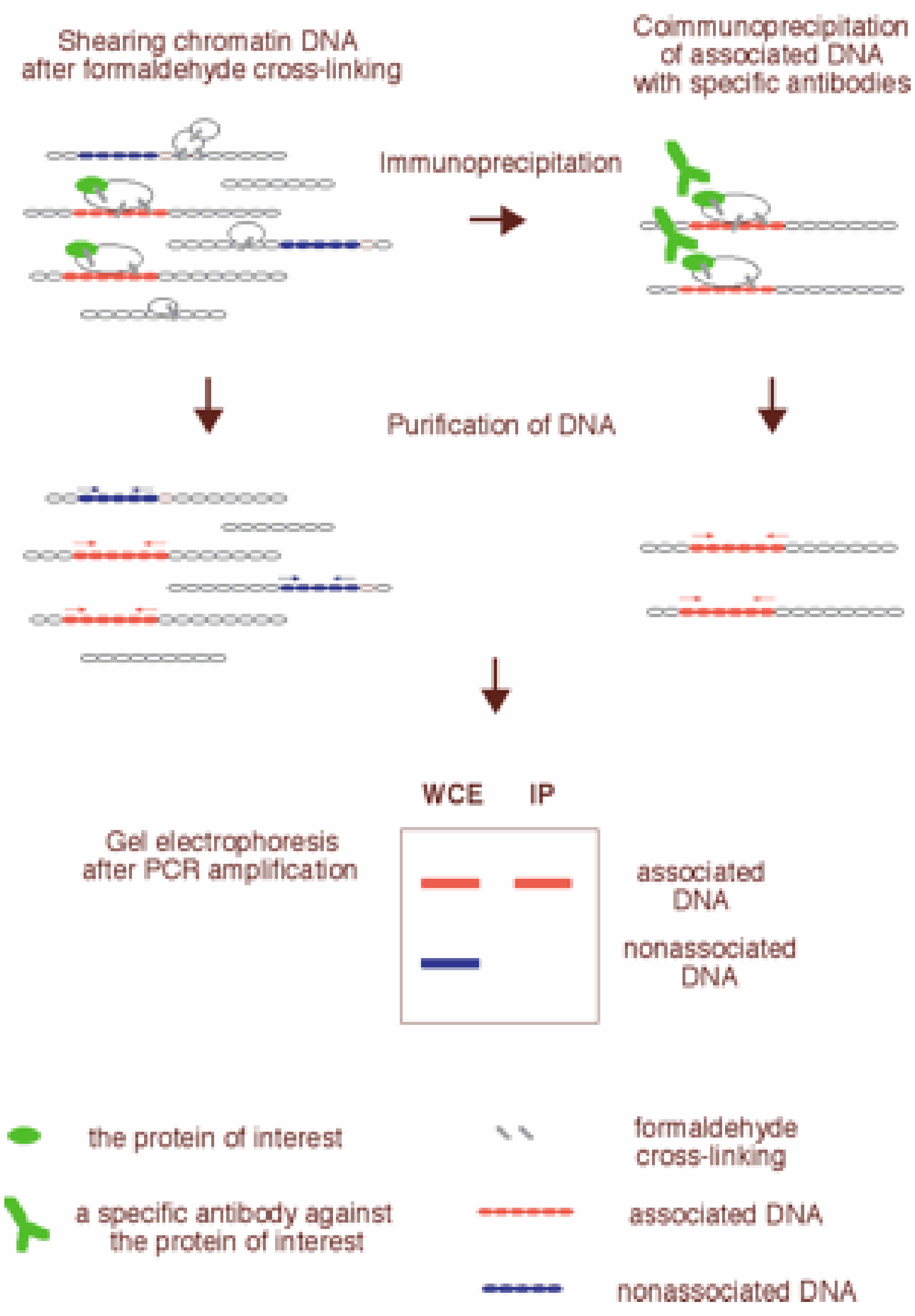
Polycomb-group complexes in Drosophila repress gene expression by modifying histones.



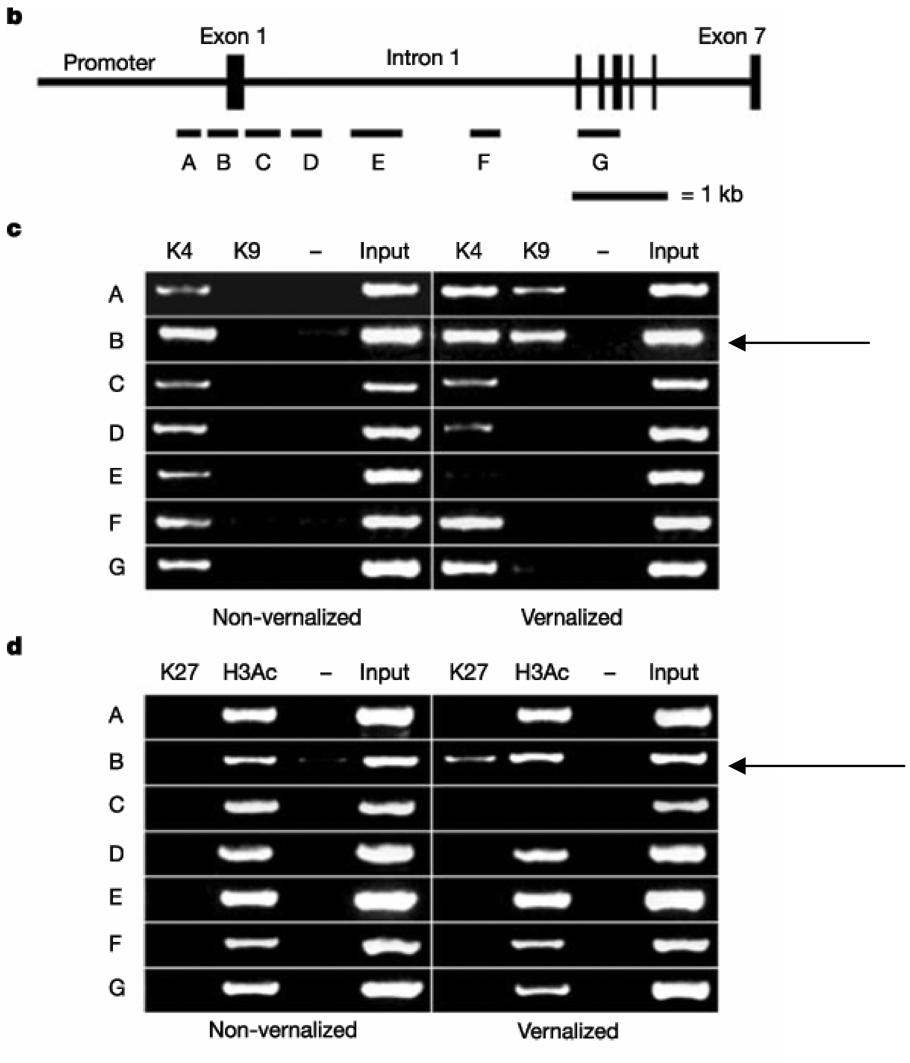
A nucleosome contains two copies of H2A, H2B, H3 and H4 wrapped around 147 bp of DNA

Modification of histones can alter gene expression.
Histone 3 is a target for modifications – those above activate gene expression, those below repress it.
Polycomb-group proteins promote the methylation of K9 and K27

Chromatin immunoprecipitation (ChIP) to identify DNA bound by specific proteins

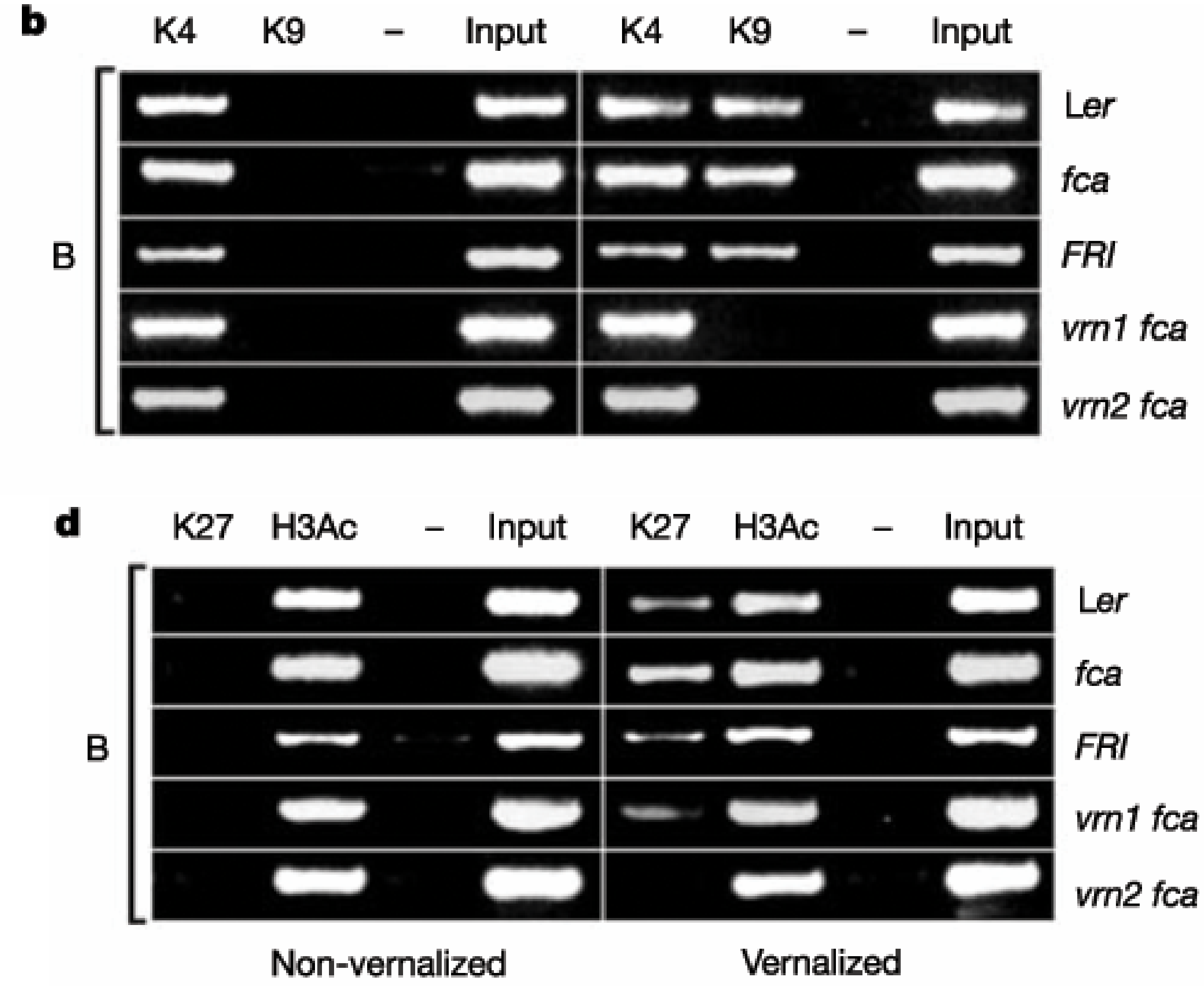


Methylated histones appear on the FLC gene after vernalization



Methylation of H3
K9 and K27 appears only
After vernalization when
The FLC gene is
repressed

ChIP of B fragment at 5‘end of FLC gene using
Specific antibodies against modified H3 histones

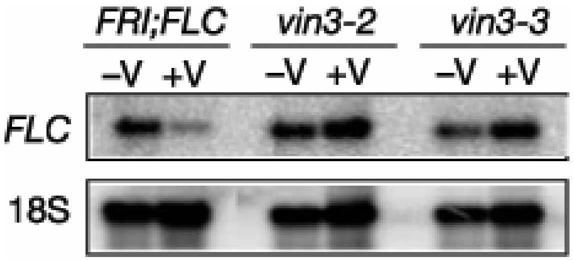


Methylation of K9
And K27 after
Vernalization
Requires VRN2

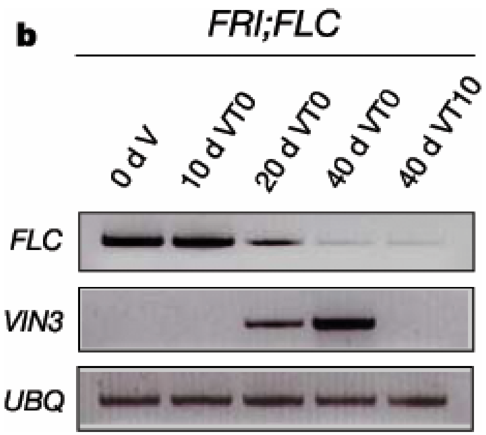
FLC expressed **FLC repressed**

F
L
C

VERNALIZATION INSENSITIVE 3 gene is required to reduce FLC



In the vin3 mutant FLC expression
Is not reduced by cold

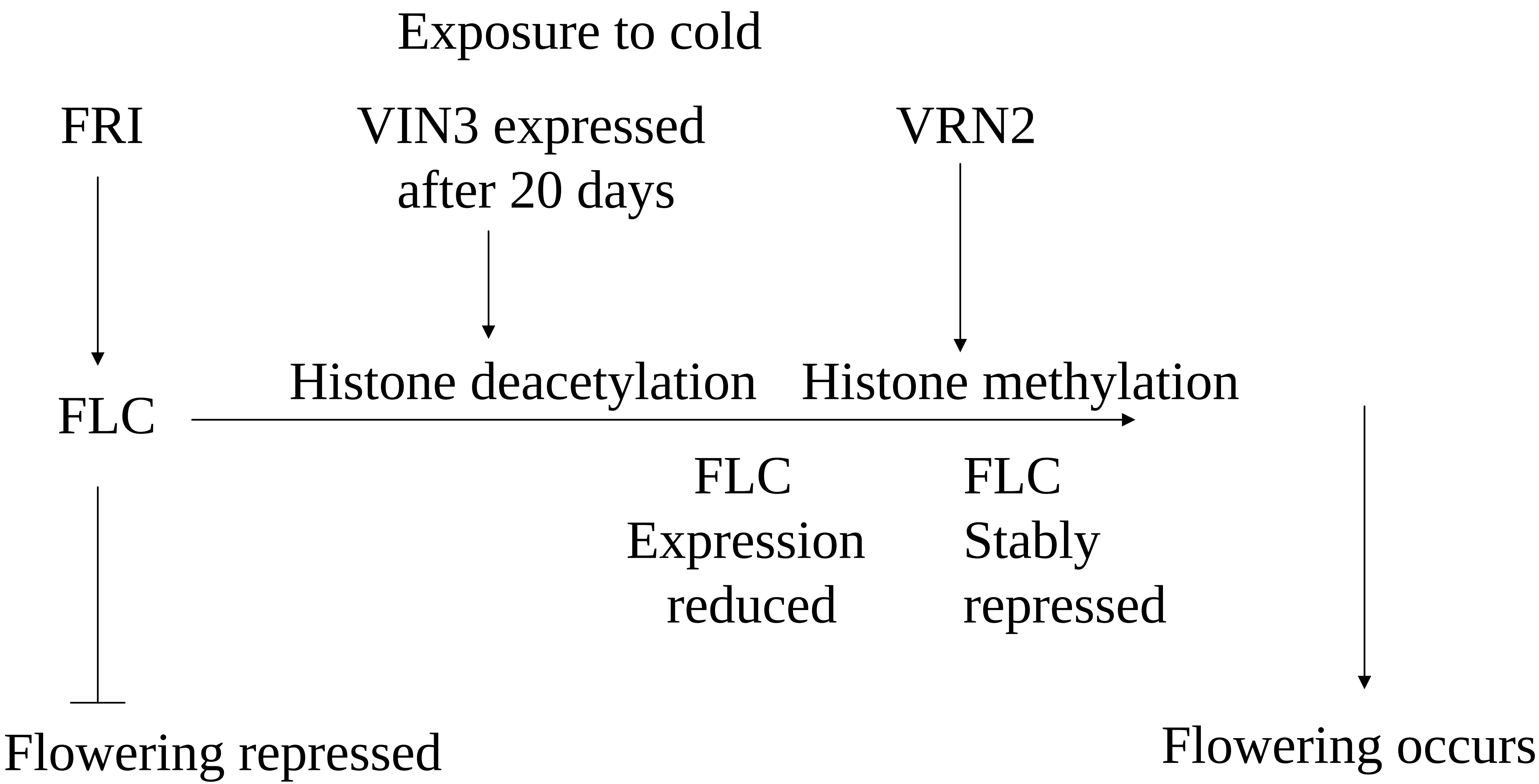


VIN3 expression rises during vernalization, suggesting has an early role in vernalization response

V
I
N
3
e
x
p
r

In the vin3 mutant deacetylation and methylation of histones on the FLC gene are blocked

Effect of vernalization on FLC expression and flowering



The photoperiod and vernalization responses converge on the same target genes: SOC1 and FT