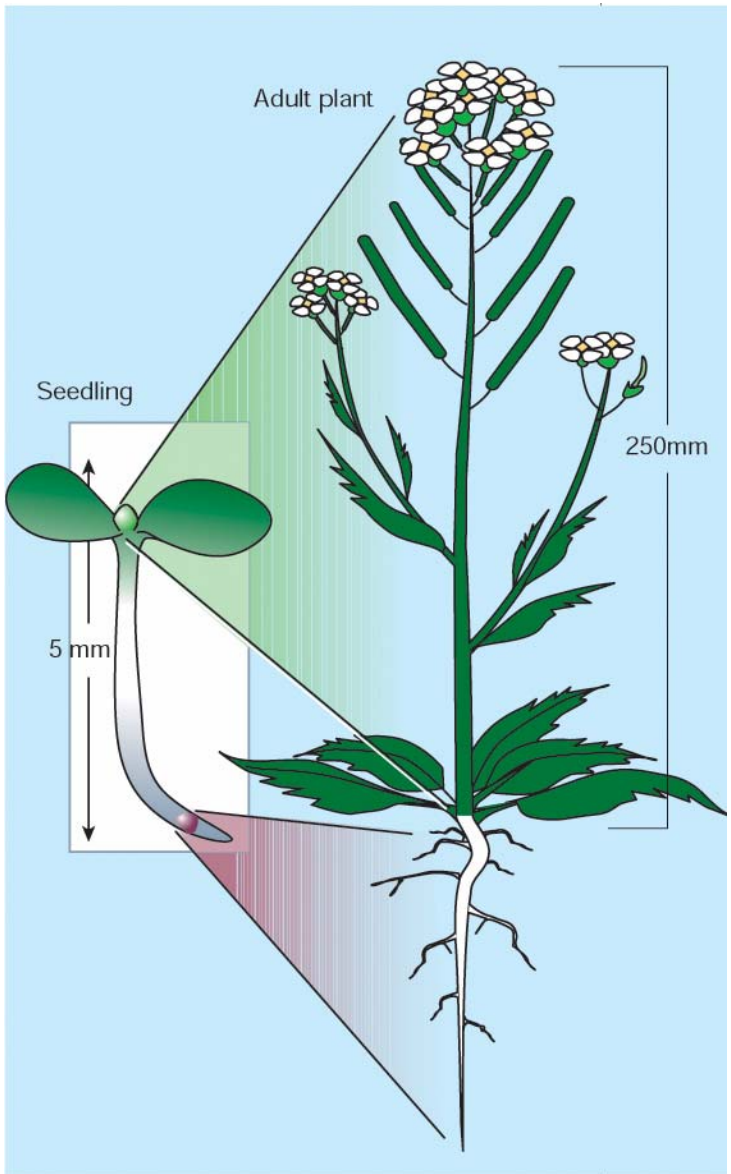
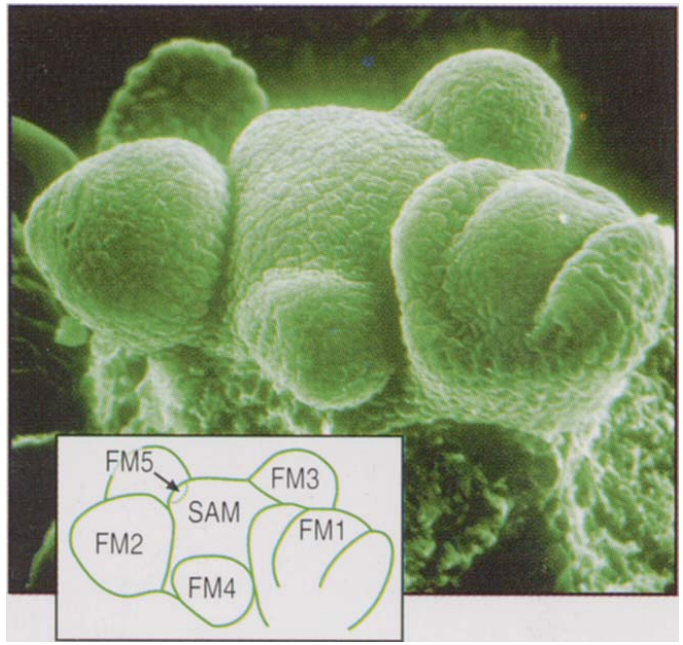
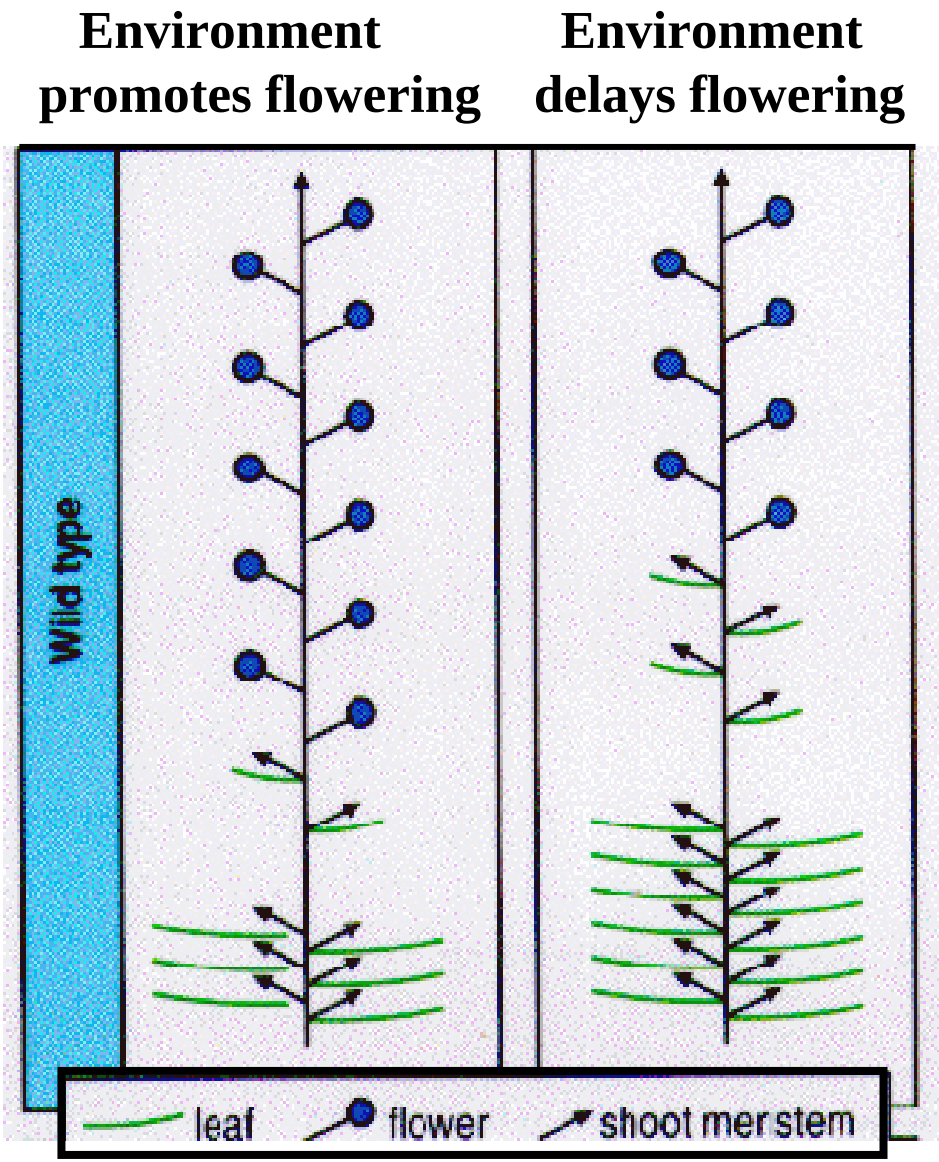




How is the transition from vegetative growth to flowering controlled ?

- How is it regulated by environmental conditions?

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

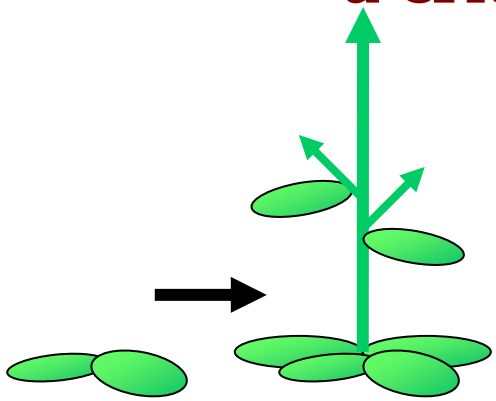


Environments that Promote flowering Of Arabidopsis are

-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*

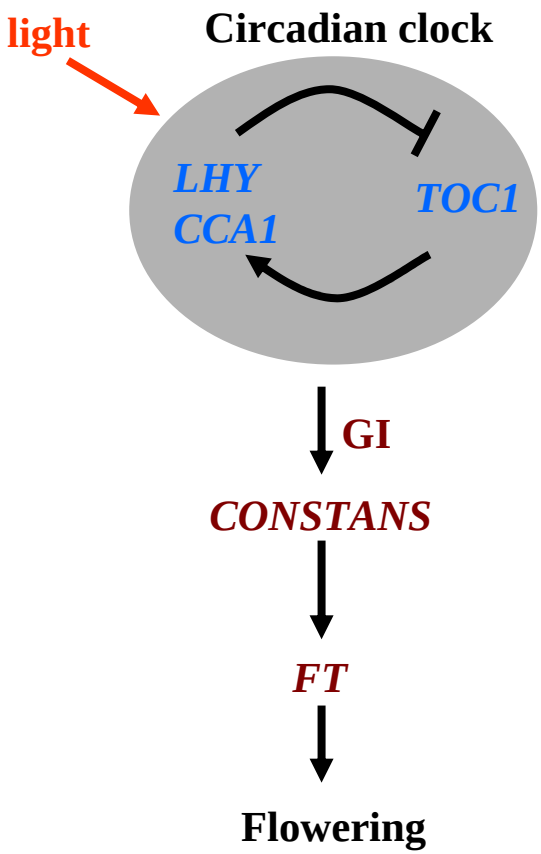
Late-flowering in long days
No effect in short days
constans, gigantea, ft, cry2

Short days

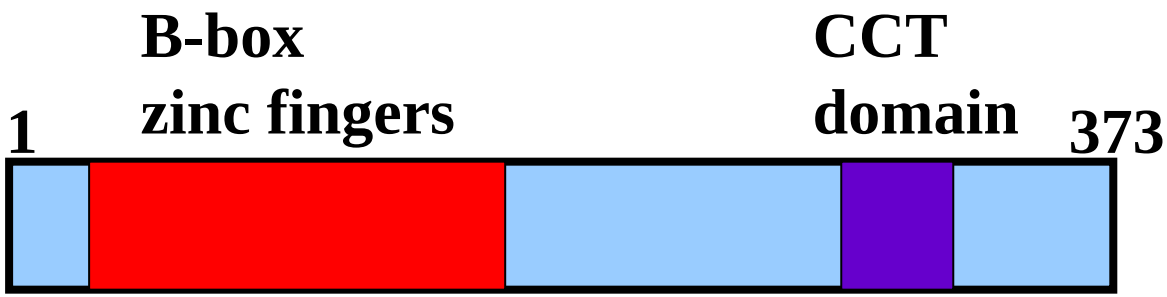
Wild-type *lhy-14*

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

Photoperiodic flowering pathway



CONSTANS has two motifs that are required for its function



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

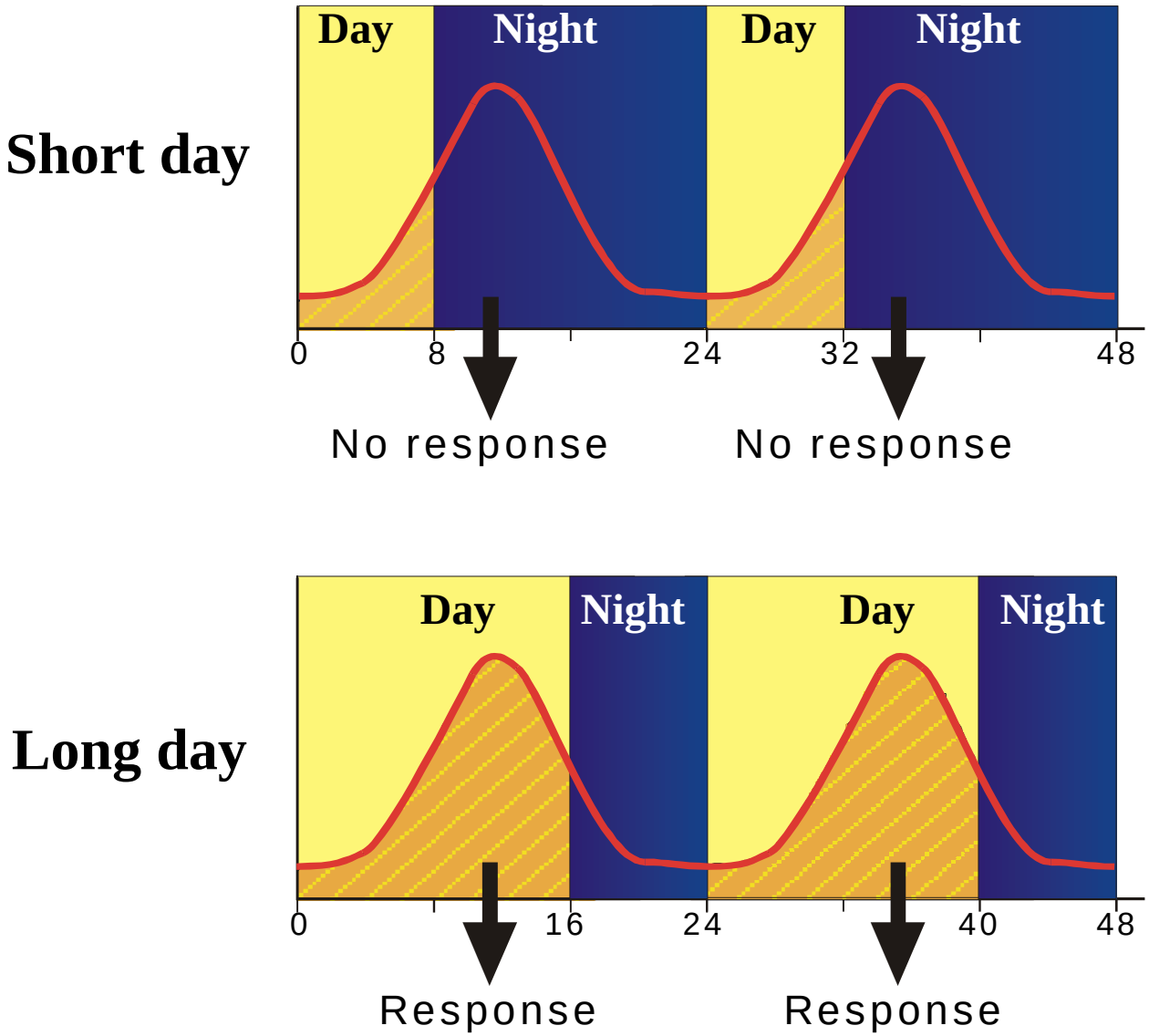
CCT is plant specific named after CONSTANS, CONSTANS-like and TOC1.

Circadian rhythms provide a mechanism for measuring time in photoperiodism

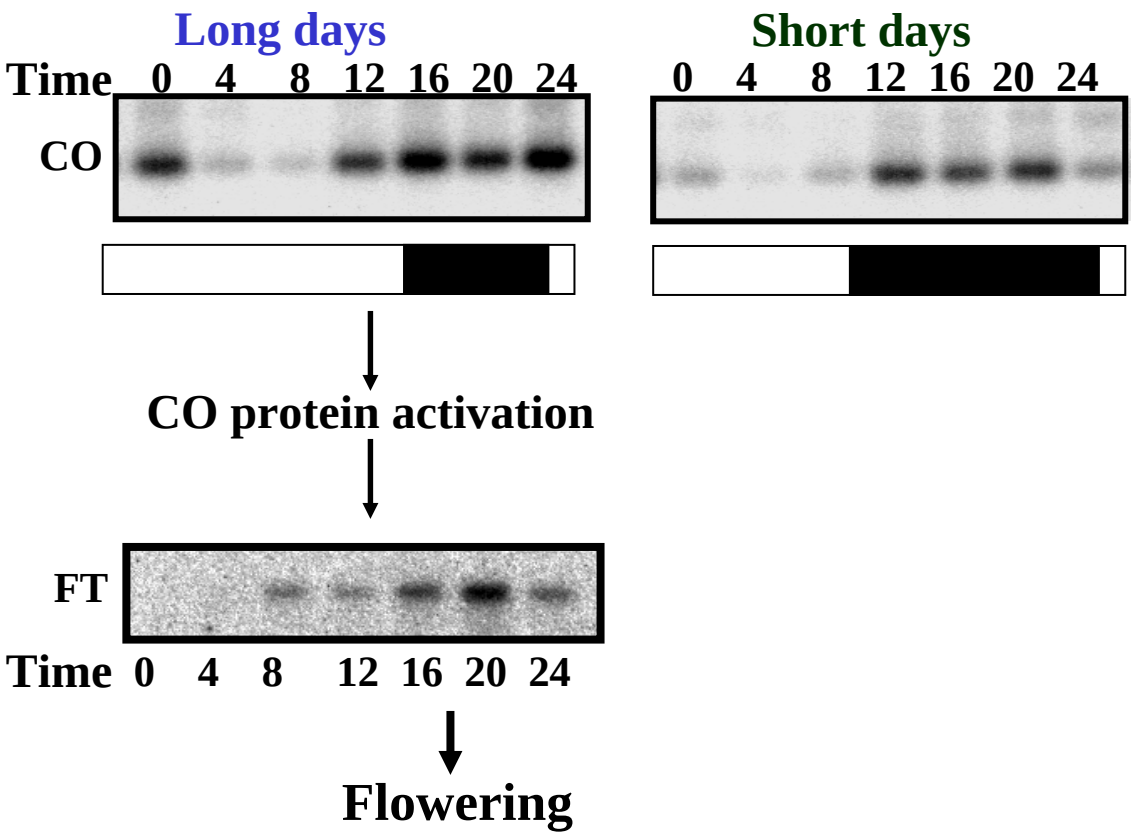
LHY::LUC *CCA1::LUC* *CAB2::LUC* *CCR2::LUC*

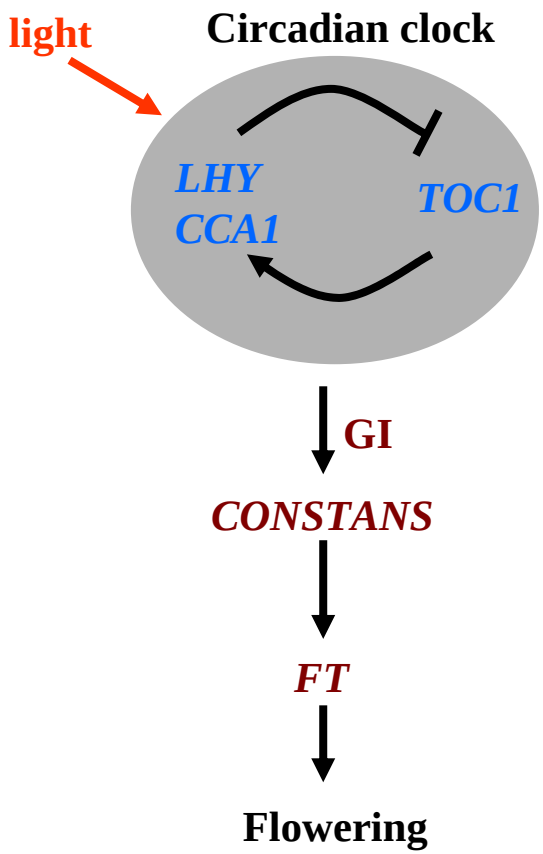
Early morning **mid- morning** **evening**

Circadian rhythms and light signaling combine to confer a photoperiodic response: external coincidence model of photoperiodism

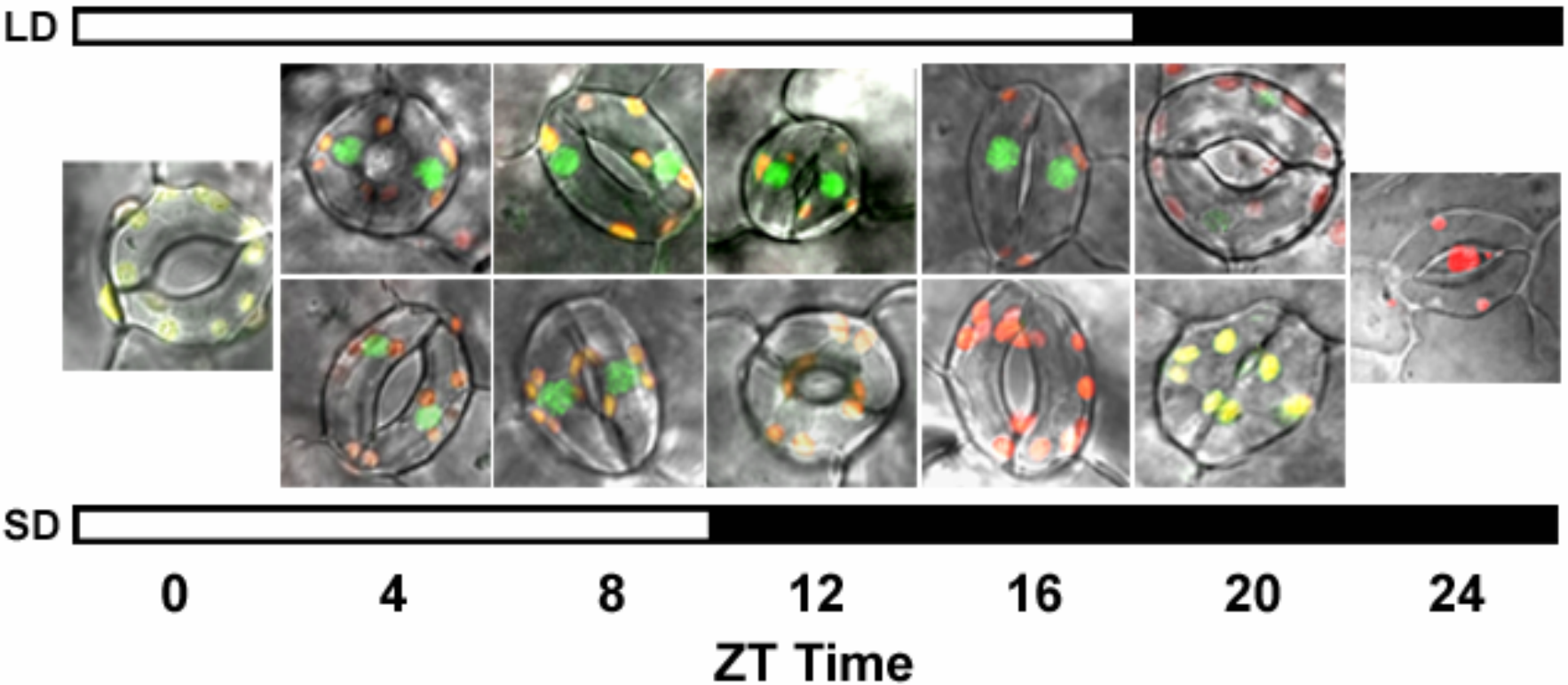


Transcriptional regulation of *CONSTANS* by the circadian clock enables discrimination between long and short days

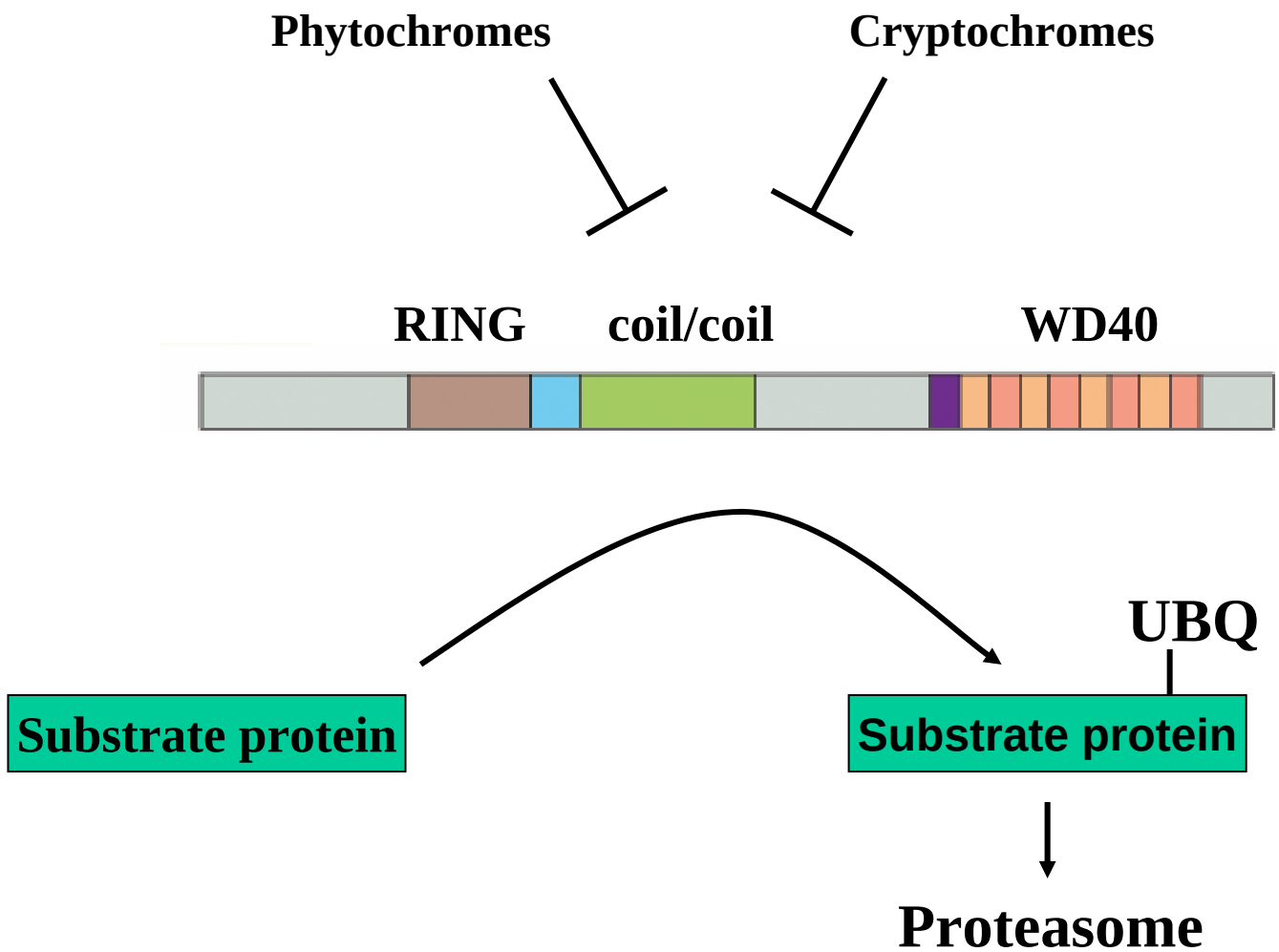




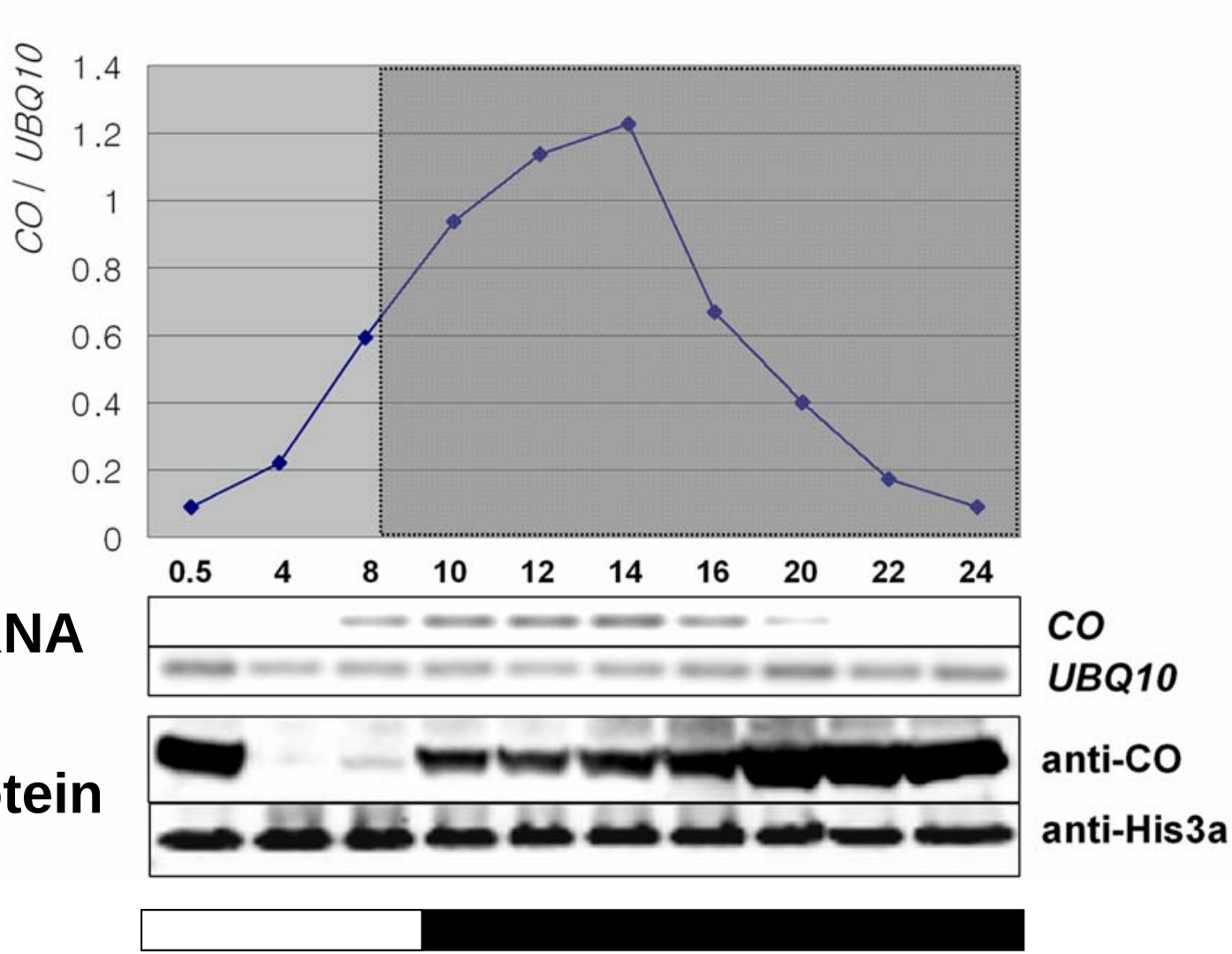
CO:GFP accumulates during the photoperiod in *35S::CO:GFP* plants



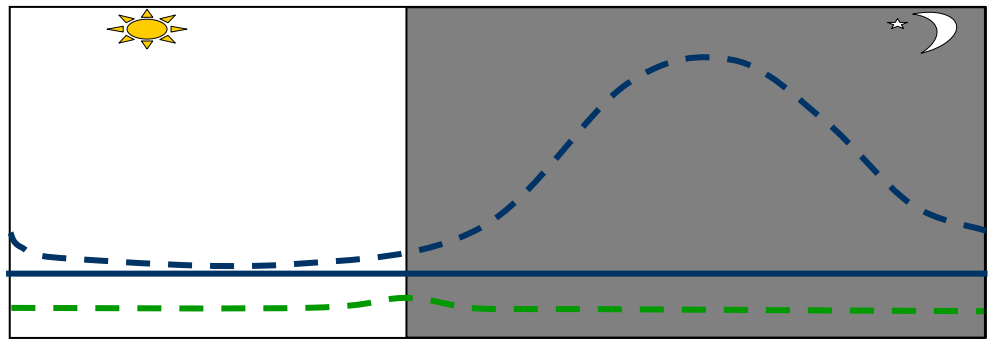
CONSTITUTIVE PHOTOMORPHOGENIC 1
is a component of a light-regulated ubiquitin ligase that is active in the dark



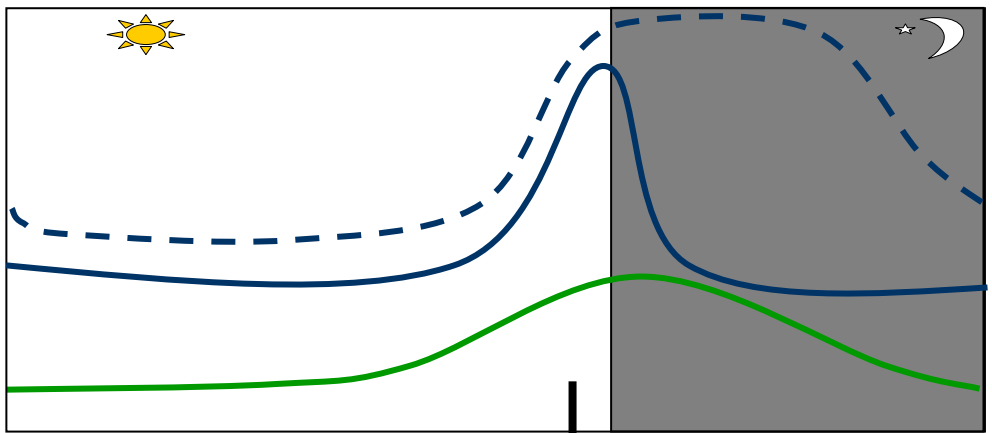
CO protein is relatively stable during the night in *cop1* mutants grown under short days



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



No flowering
In short days

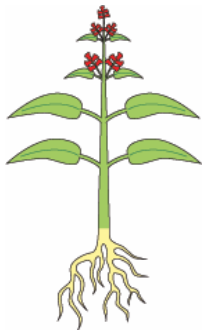


Flowering in
Long days

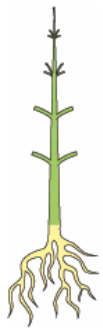
Flowering

Photoperiod is detected in the leaf and CO and FT are expressed in leaf vascular tissue

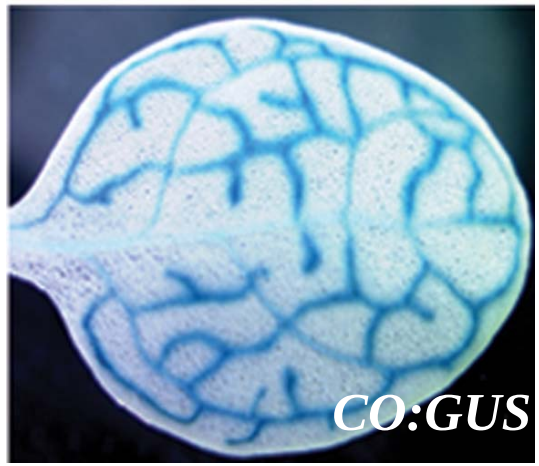
Grafting in *Perilla*



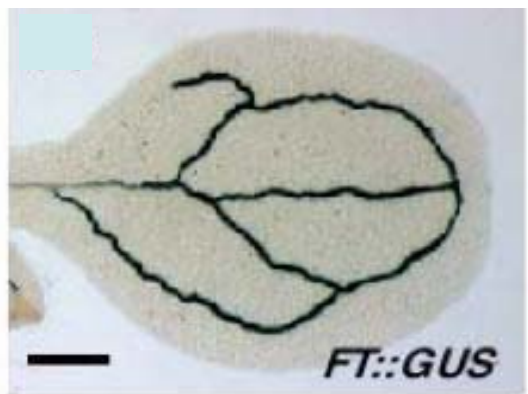
Induced leaf donor



Non-induced recipient shoot

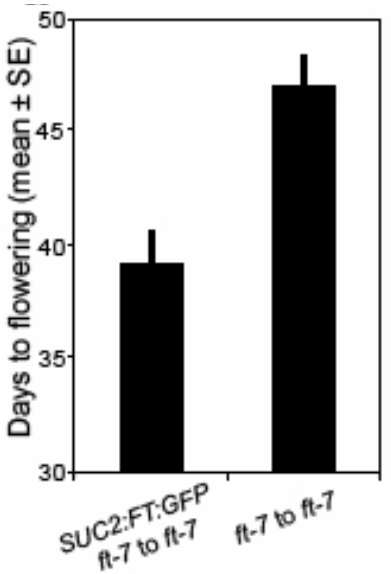


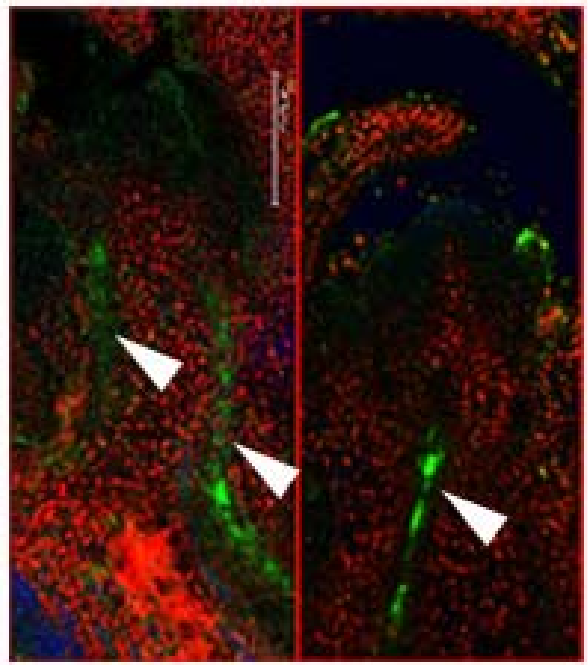
Flowering



Grafting *SUC2:FT:GFP* to *ft* mutants causes earlier flowering and FT:GFP protein is detected in the *ft* mutant

Flowering times of grafted plants





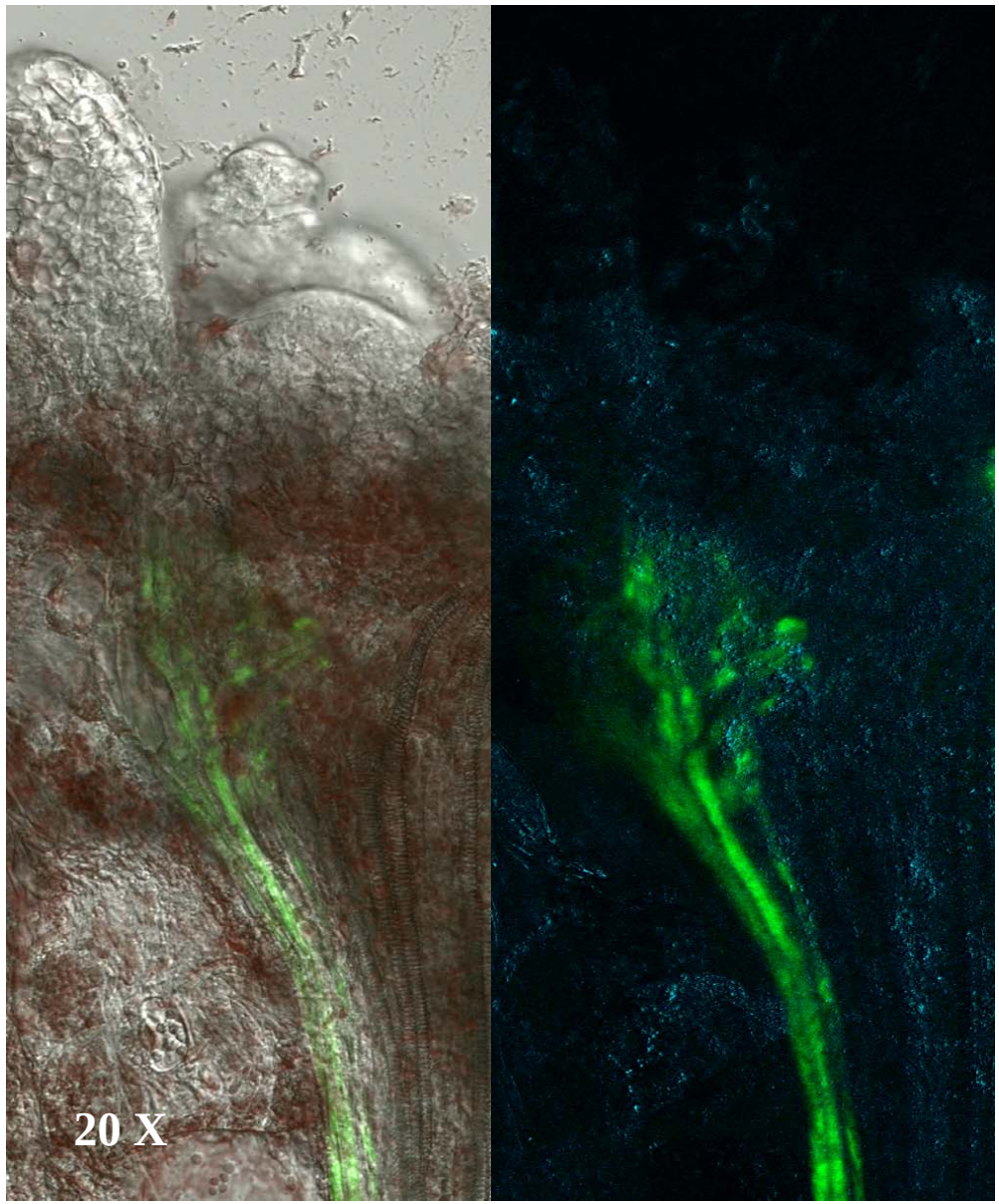
SUC2:FT:GFP ***ft-7* scion**

***ft-7* shoot grafted to *SUC2:FT:GFP* plant**

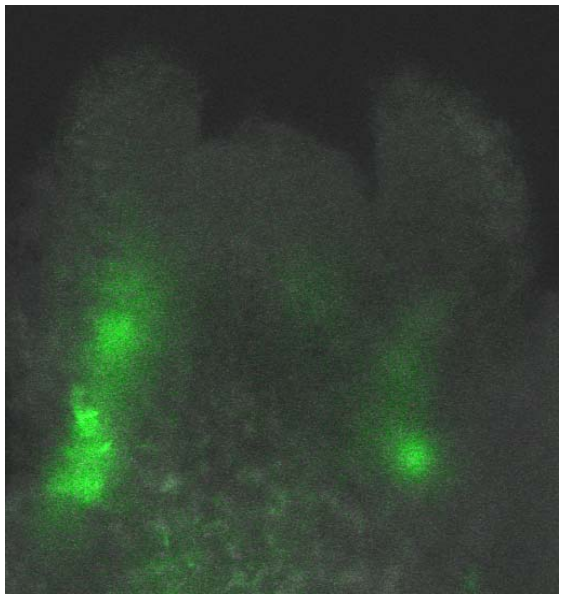
Colin Turnbull
Imperial College

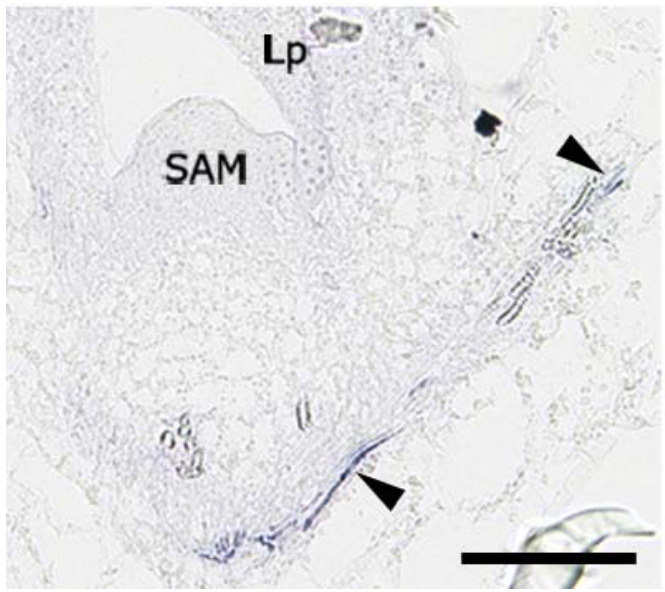
Pattern of detection of FT:GFP at the shoot apex of *SUC2::FT:GFP* plants

6-day old plants



Comparison of distribution of GFP mRNA and protein in SUC2:FT:GFP plants





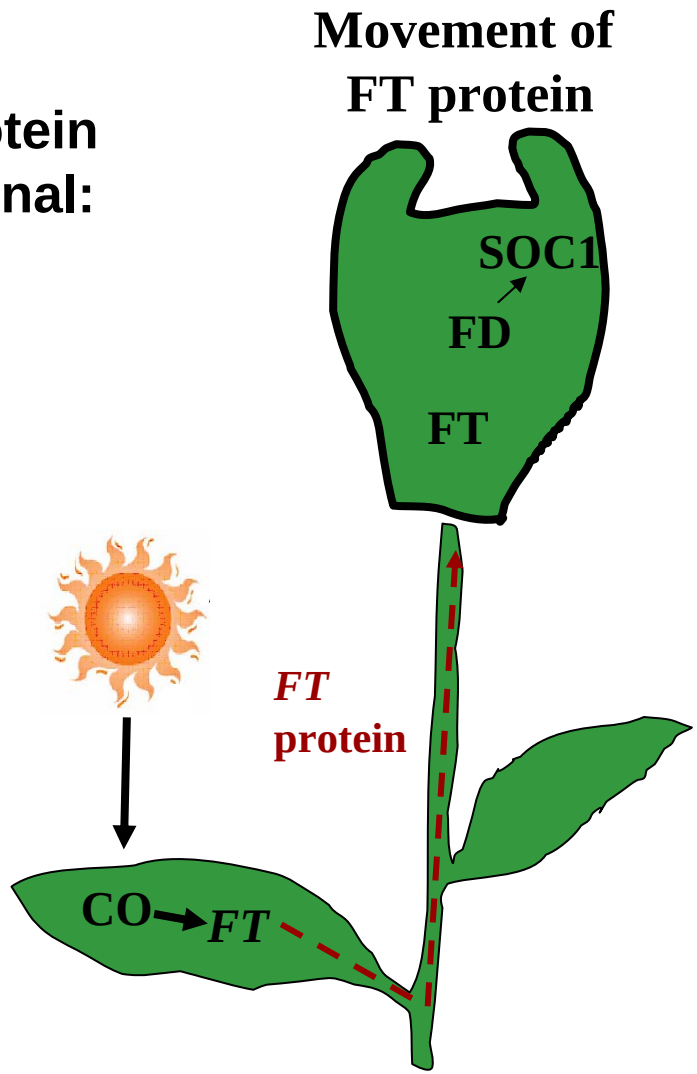
FT:GFP protein is found in cells of the meristem
but FT:GFP mRNA is only found in the vascular tissue

FT protein moves from the leaves to the meristem during floral induction

A series of papers proposing FT protein as a conserved mobile flowering signal:

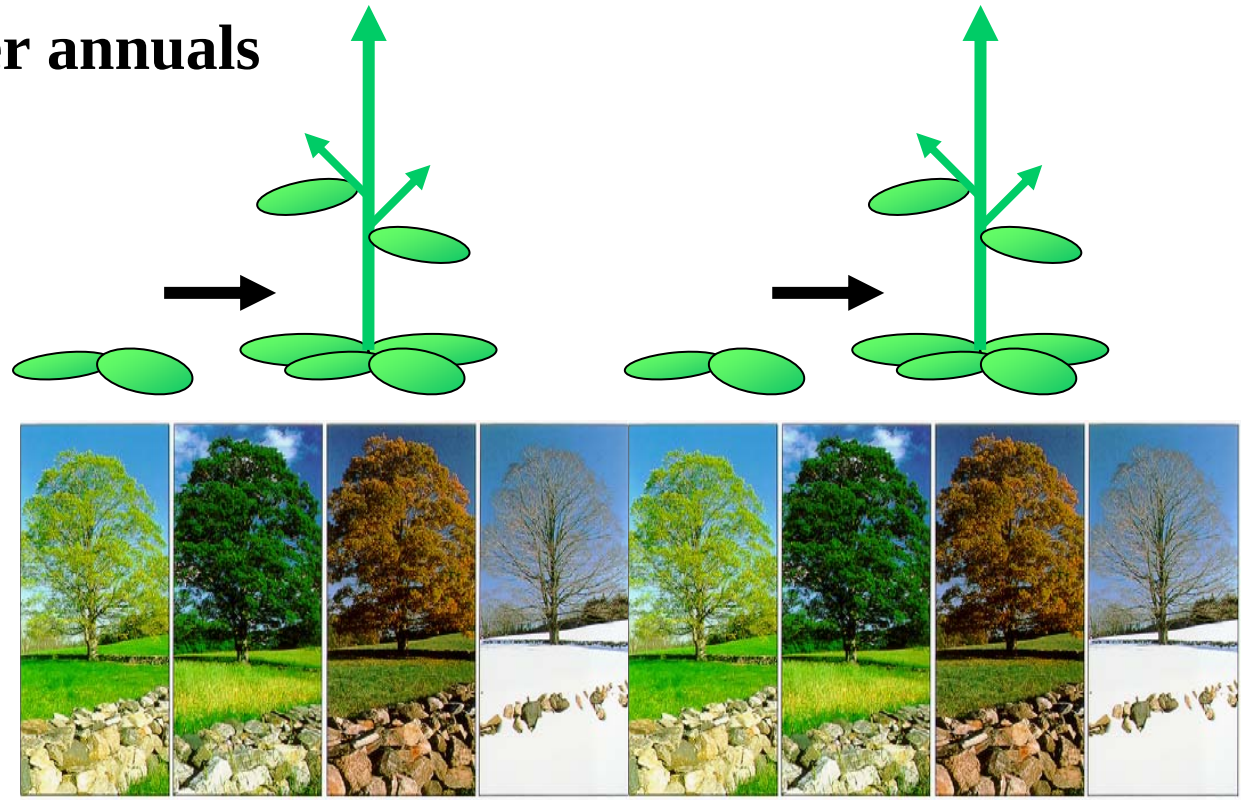
Corbesier et al (2007) Science
Tamaki et al (2007) Science

Jaeger, Wigge (2007) Current Biology
Mathieu et al (2007) Current Biology
Lin et al (2007) Plant Cell



Arabidopsis varieties differ in their reproductive strategies

Summer annuals



Winter annuals

vernalization

Features of vernalization

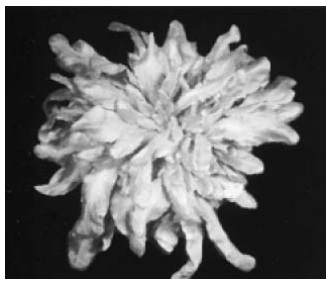
1. **Occurs at shoot meristem**

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)		

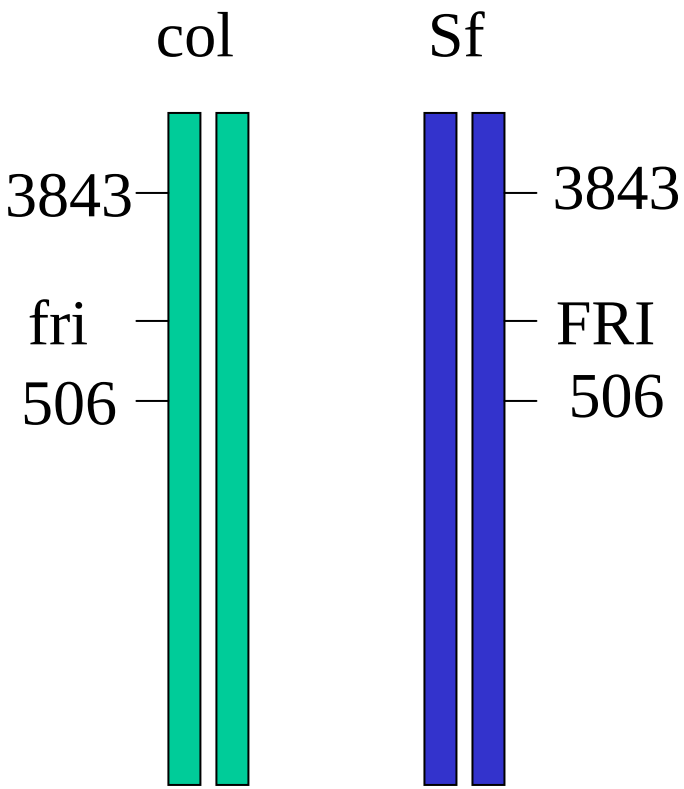
To identify genes that confer a vernalization response
- intercross Sante Fe and Columbia

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



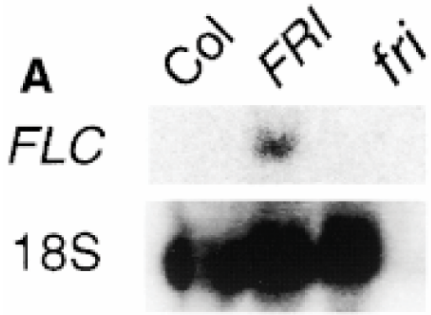
In crosses between other Examples of vernalization responsive and non-responsive strains alleles of FRIGIDA shown to underlie the difference. Including European strains Stockholm and Limburg-5.

Chromosome 4

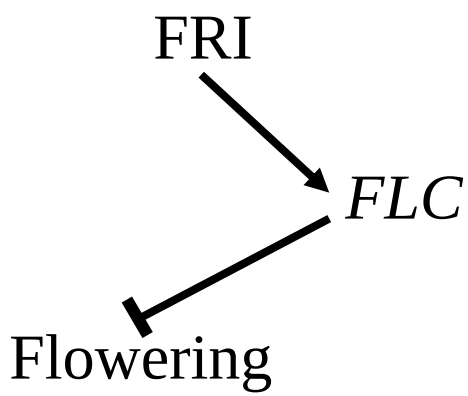
FRI encodes a 609 amino acid protein of unknown function containing two coil-coil domains implicated in protein protein interaction.

FRIGIDA delays flowering by activating transcription of FLC, which encodes a MADS box transcription factor that represses flowering

Overexpression of FLC delays flowering



FRIGIDA promotes expression of the floral repressor FLC

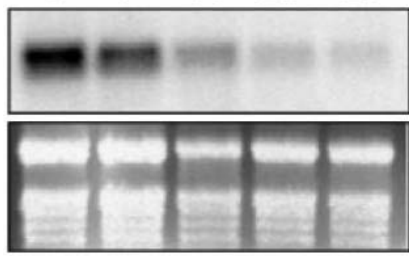


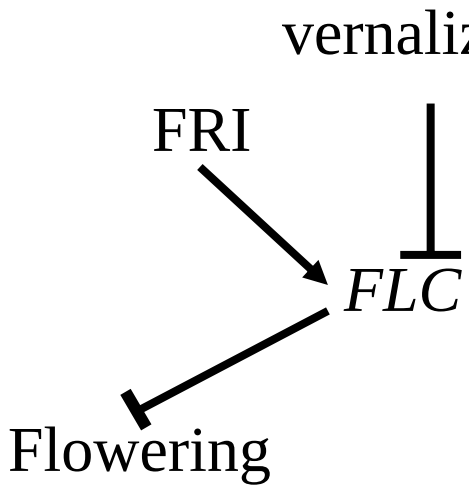
In winter annuals vernalization reduces abundance of the floral inhibitor FLC and accelerates flowering

***FRI FLC* plants**

Time at 4C (days) **1 7 14 21 28**

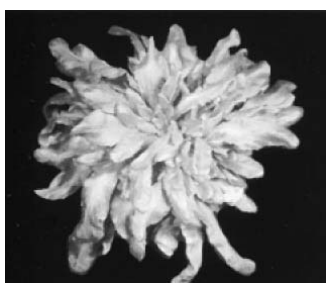
FLC





No vernalization **8 weeks vernalization**

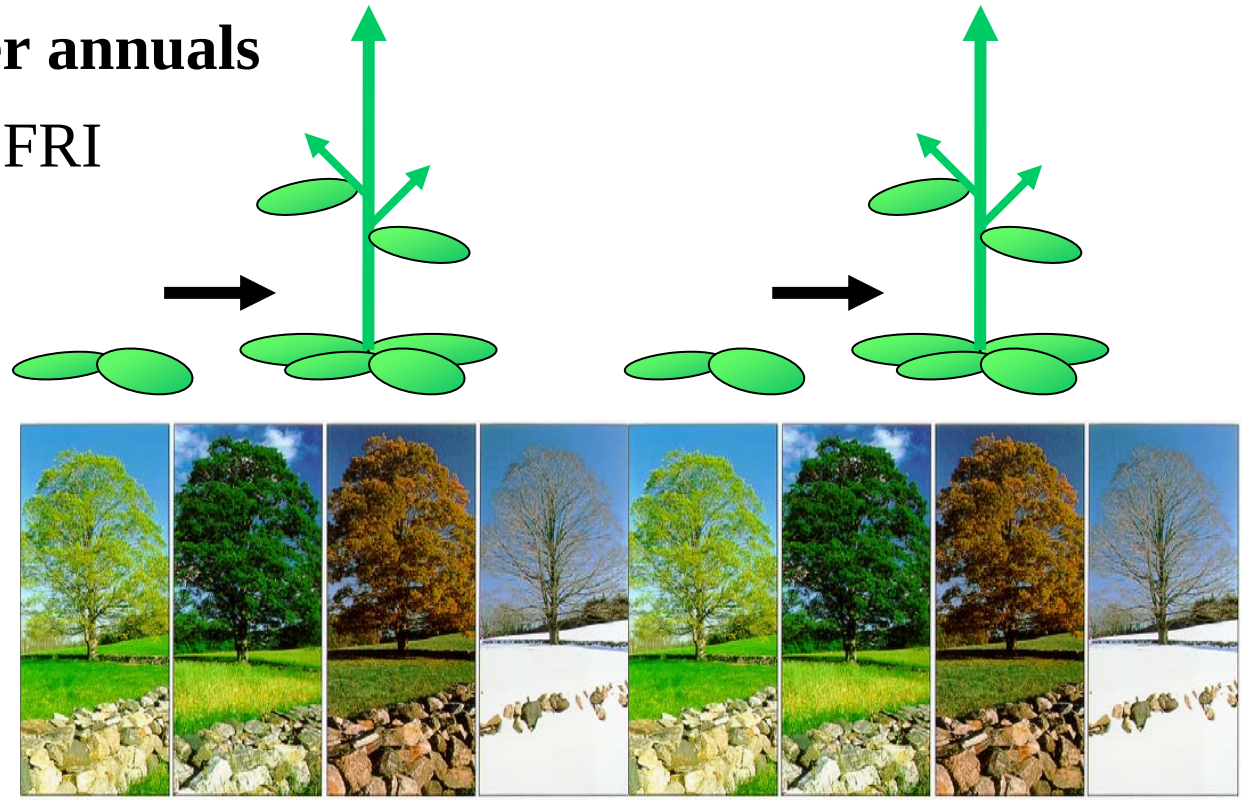
Santa Fe



Arabidopsis varieties differ in their reproductive strategies

Summer annuals

No FRI



Winter annuals

FRI

High FLC that is reduced by cold.

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

Late flowering, high FLC

↓

Treat with mutagen - EMS

↓

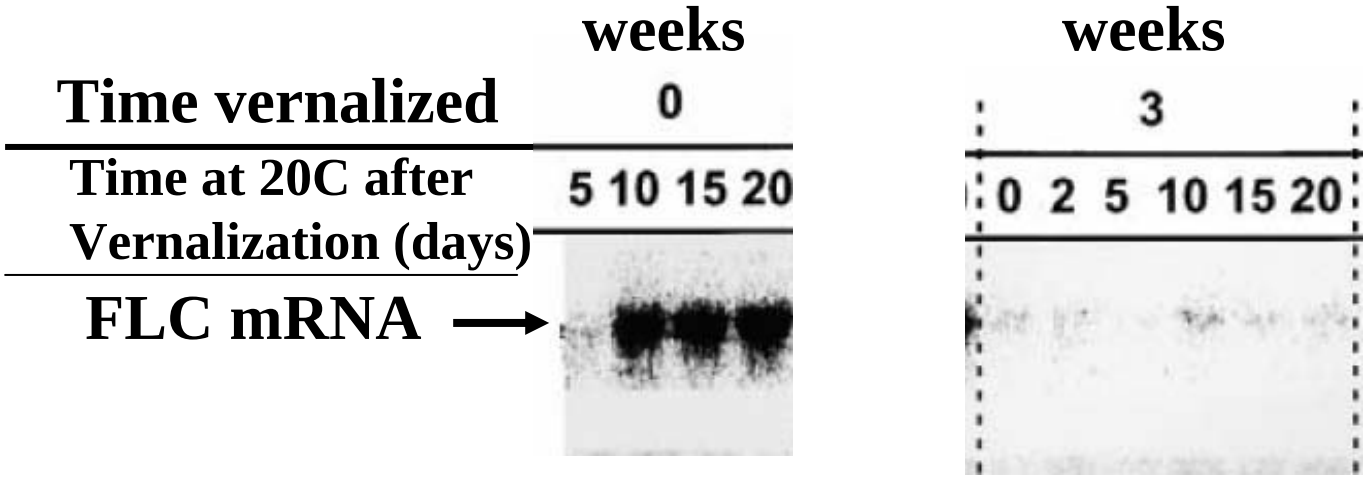
Give vernalization treatment to all plants

↙ ↘

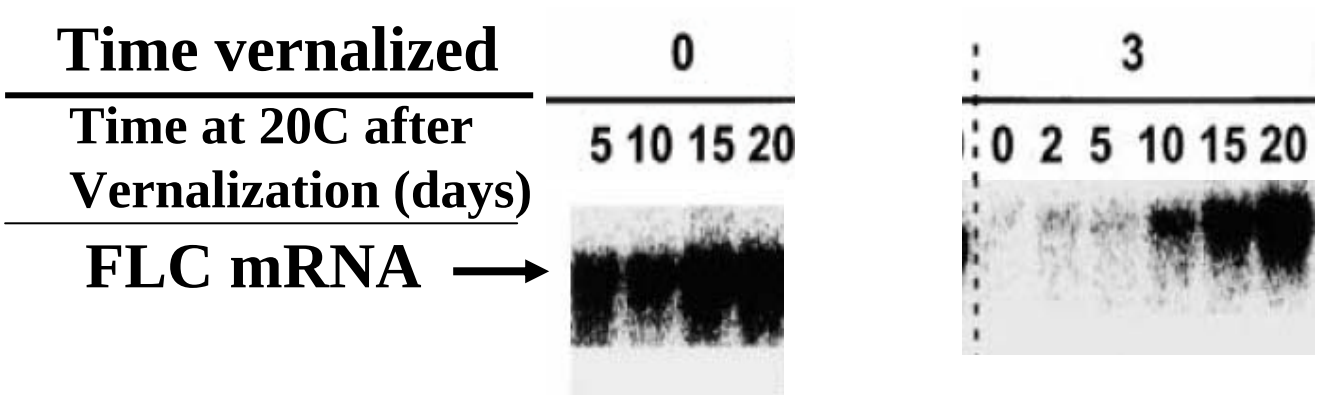
Most plants flower early.

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

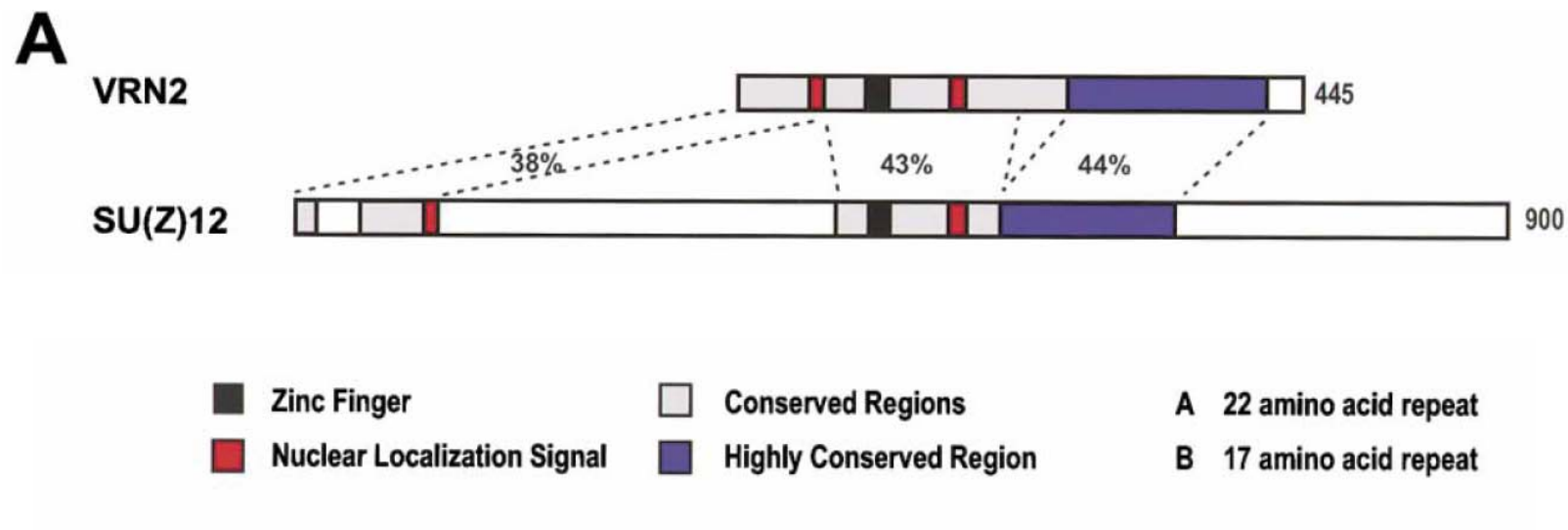
The role of VERNALIZATION2 is to maintain the repression of FLC expression after vernalization



vrn2 mutant



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



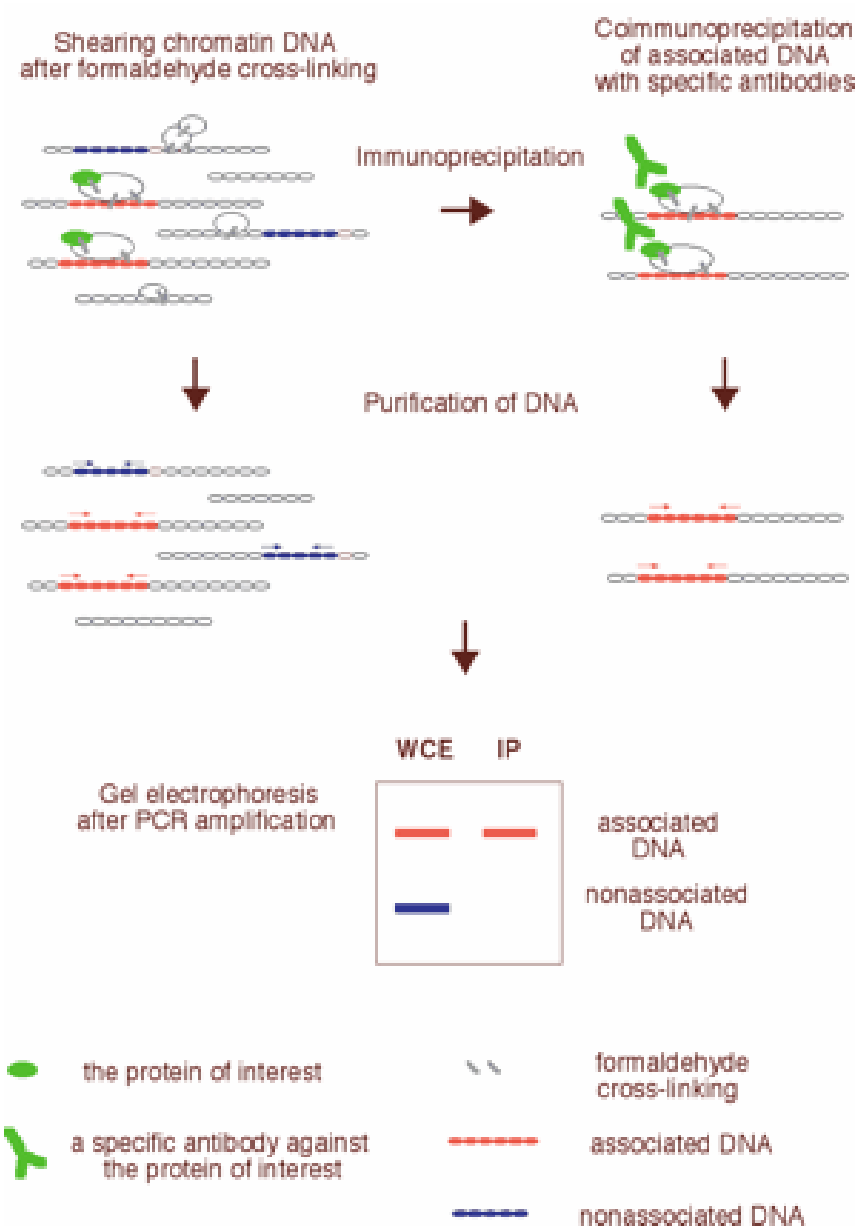
In Drosophila, PcG proteins act in large protein complexes. They maintain the repression of transcription of homeotic genes, once the pattern of expression of these genes has been established during early embry development.

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

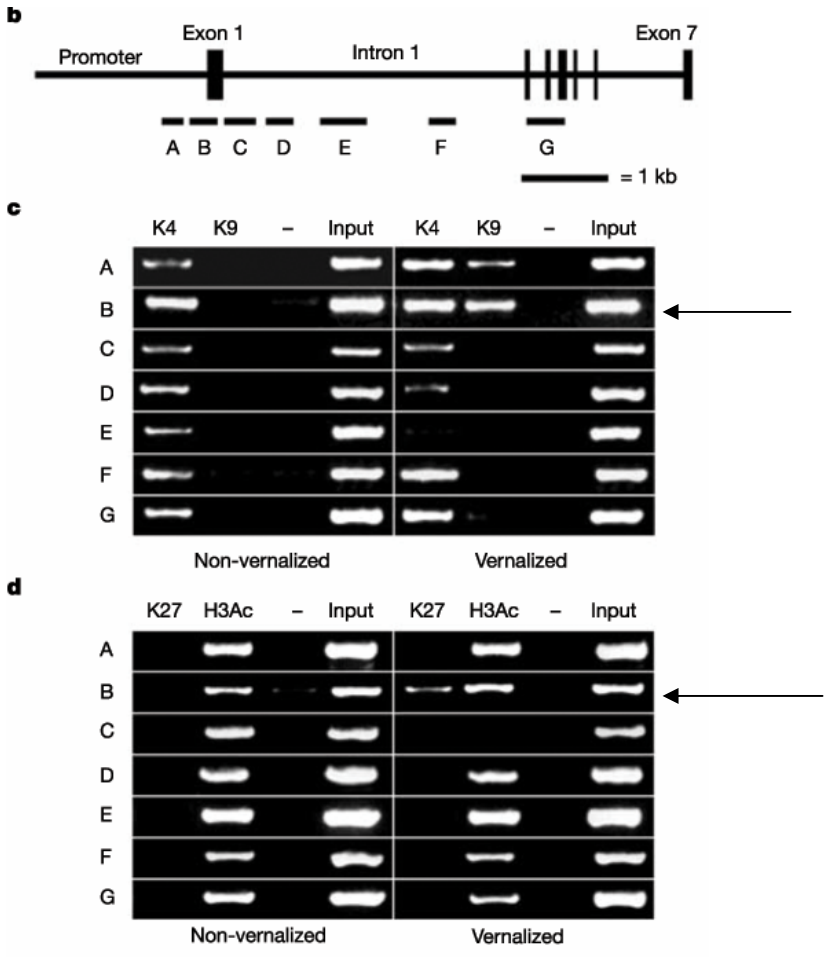
A nucleosome contains two copies of H2A, H2B, H3 and H4 wrapped around 147 bp of DNA

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

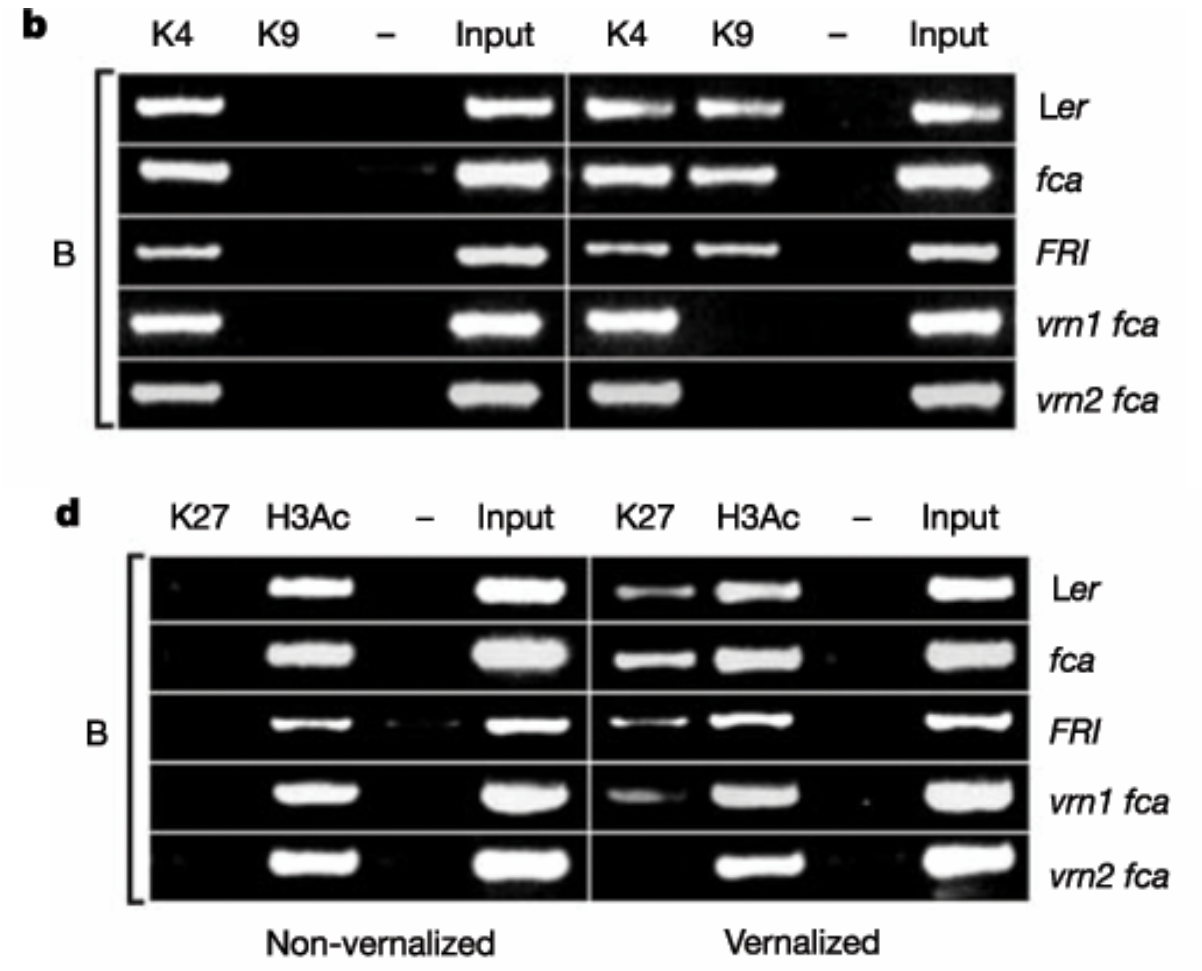


Methylated histones appear on the FLC gene after vernalization



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

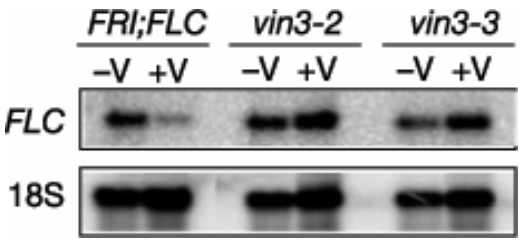
ChIP of B fragment at 5‘end of FLC gene using
Specific antibodies against modified H3 histones



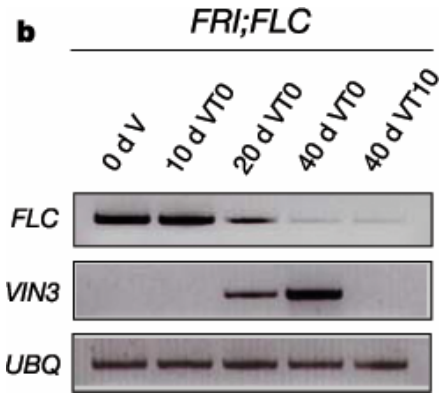
FLC expressed **FLC repressed**

Methylation of K9
And K27 after
Vernalization
Requires VRN2

VERNALIZATION INSENSITIVE 3 gene is required to reduce FLC



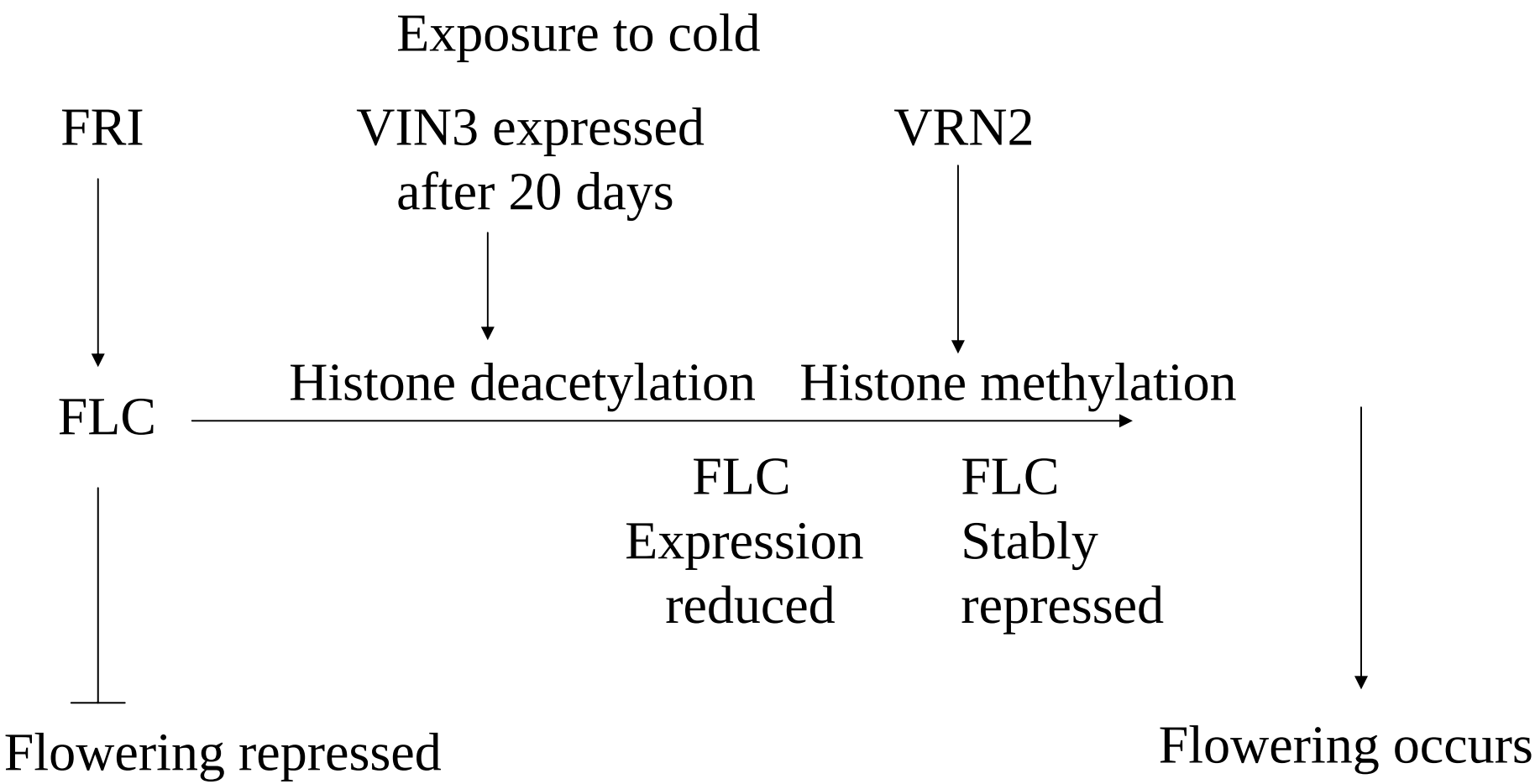
In the vin3 mutant FLC expression
Is not reduced by cold



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

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

Effect of vernalization on FLC expression and flowering



Convergence of vernalization and day length control on regulation of FT