

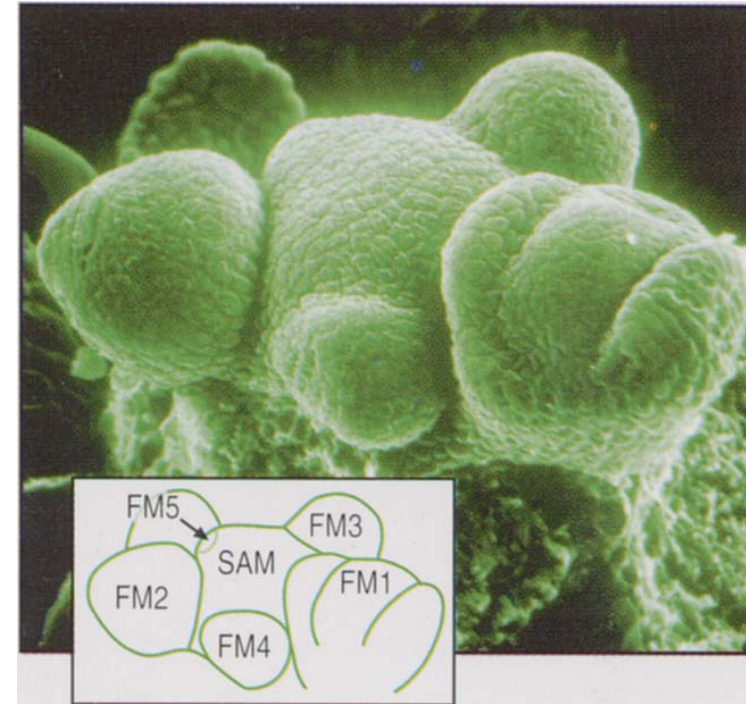
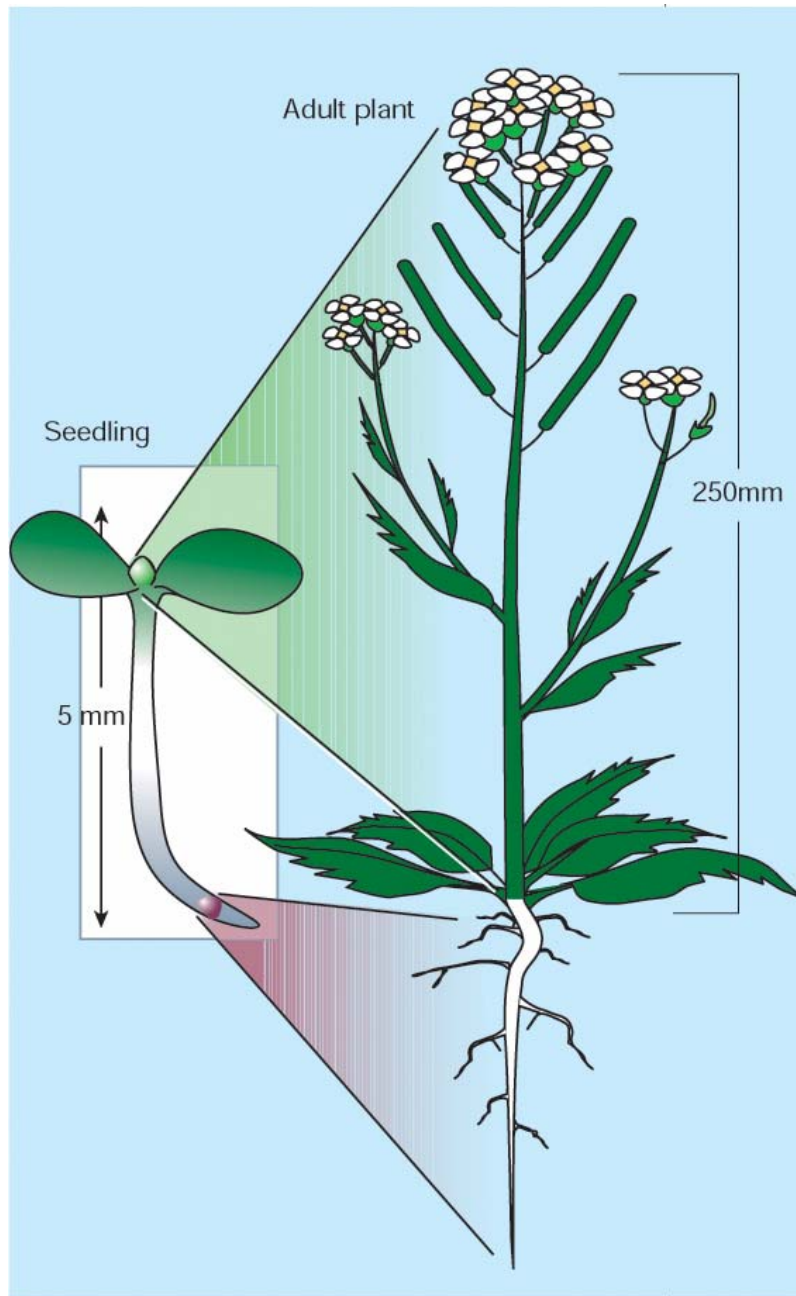
WEB ADDRESS:

[www.mpiz-koeln.mpg.de](http://www.mpiz-koeln.mpg.de)

Forschung

Abt. Entwicklungsbiologie de Pflanzen

George Coupland



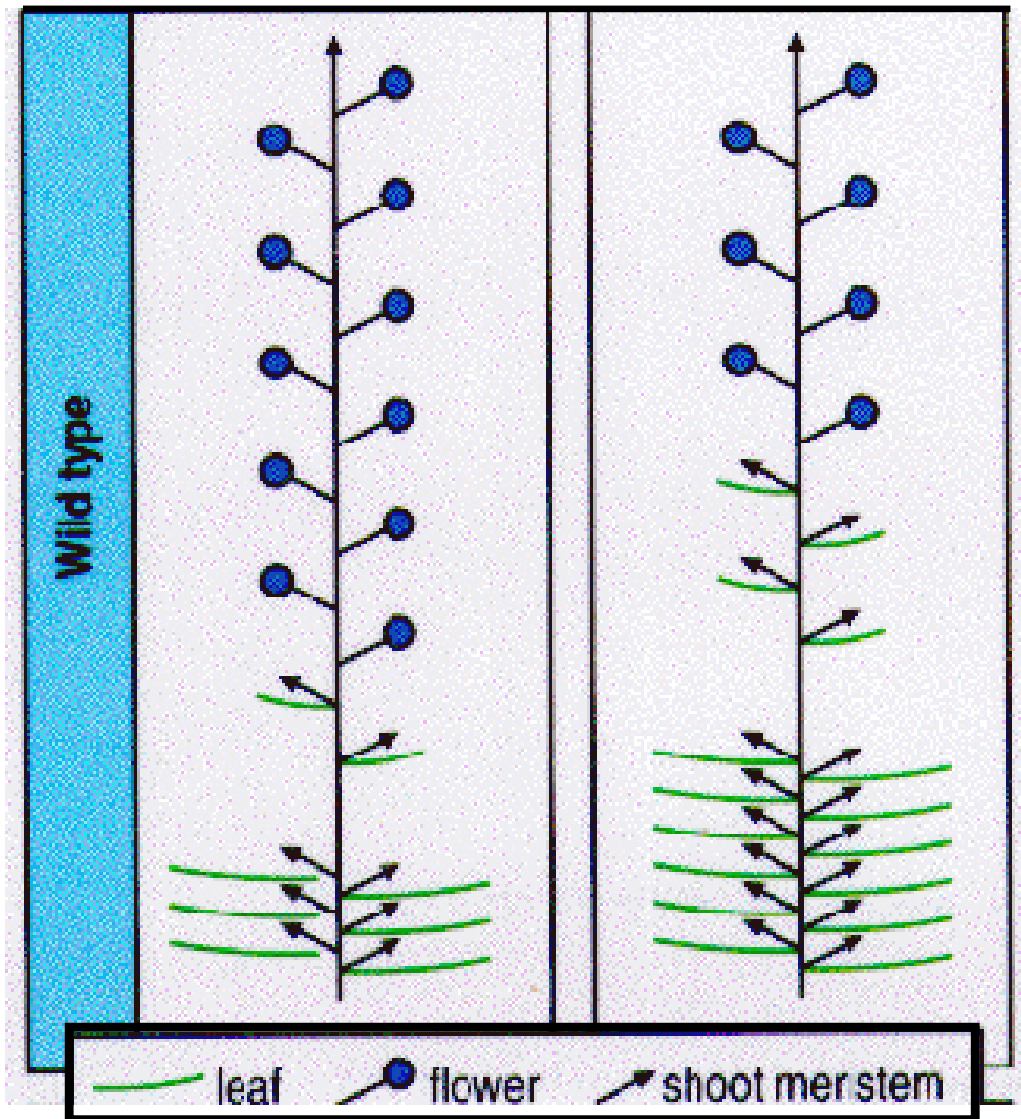
**How is the transition from vegetative growth to flowering controlled ?**

**- How is it regulated by environmental conditions?**

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

Environment  
promotes flowering

Environment  
delays flowering

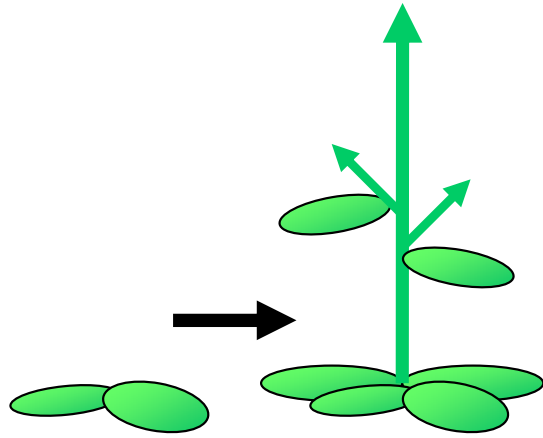


Environments that  
Promote flowering  
Of Arabidopsis are

-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**

# Many mutations delay flowering of *Arabidopsis*



**Wild-type**

*constans*



**Late flowering mutants can be placed in groups  
based on their responses to environmental conditions**

**Later flowering in long days, same as WT in short days**

**No response to vernalization** *constans, gigantea, ft, cry2*

**Later flowering in long days and short days**

**Flower faster after vernalization** *fca, fpa, fy, ld*

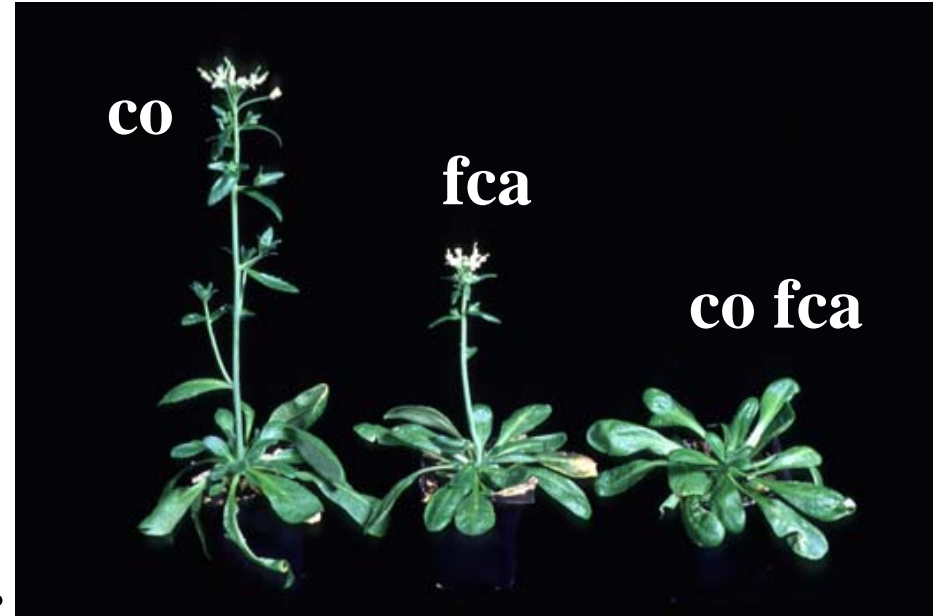
**Impair Gibberellic acid (GA) biosynthesis or response**

**Mainly later flowering in short days** *gal,*  
*gibberellic acid insensitive*

# These three groups proposed to define genetic pathways

## 1. Double mutant analysis

Combining mutants within groups causes no enhancement of phenotype. However, combining mutants between groups causes strong enhancement.



## 2. Clear groupings based on physiology.

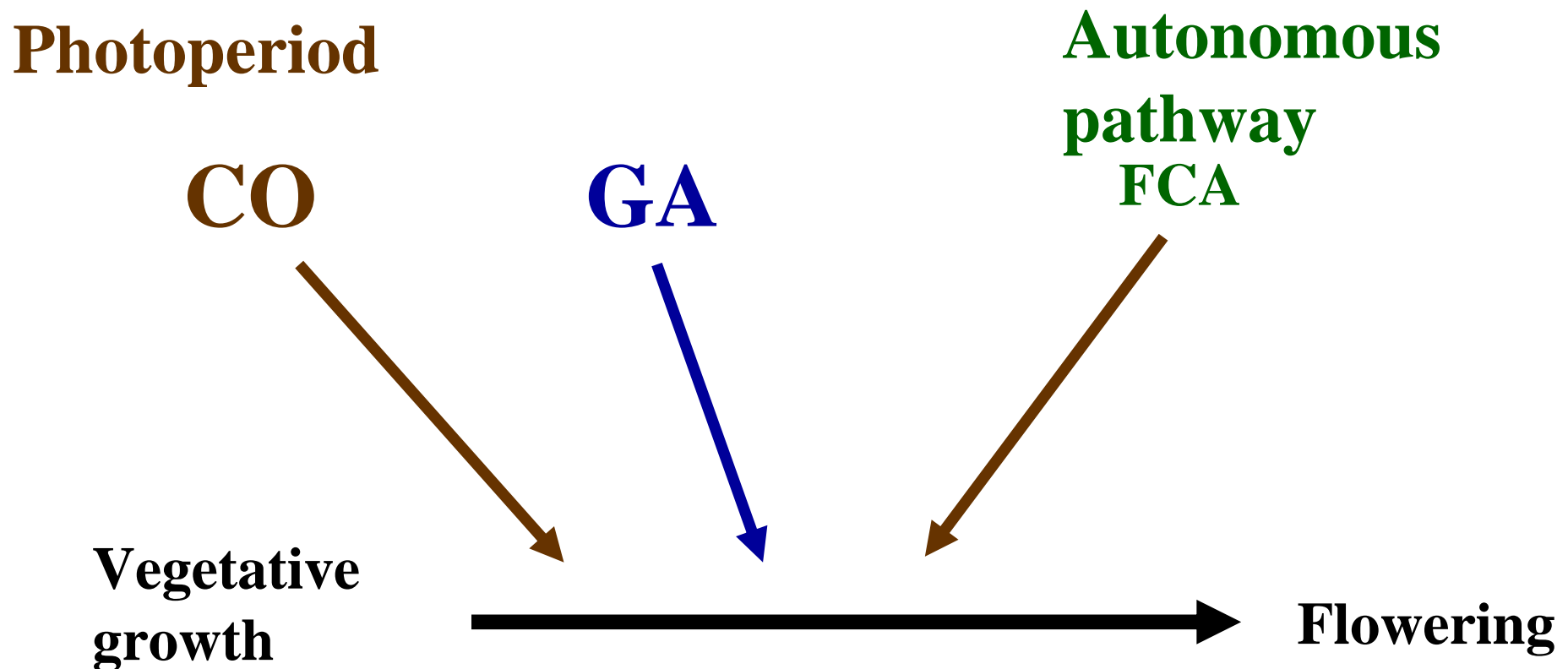
# Inactivating all three pathways prevents flowering



|            | LN in long days          |
|------------|--------------------------|
| WT         | 9.0                      |
| co         | 20.0                     |
| fca        | 31.0                     |
| ga1        | 16.0                     |
| co fca ga1 | over 90 (never flowered) |



# Flowering time regulation in Arabidopsis involves three genetic pathways



# 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



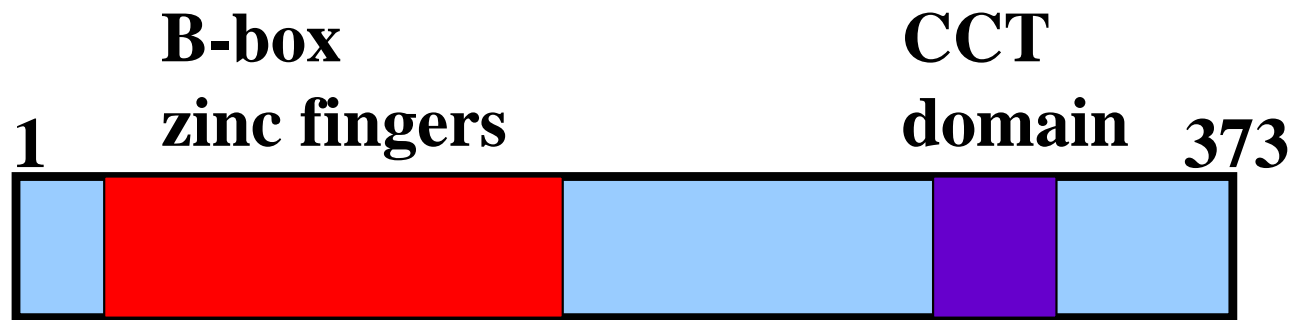
*lhy-14*

Early-flowering in short days

No effect in long days

*lhy, toc1*

# **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

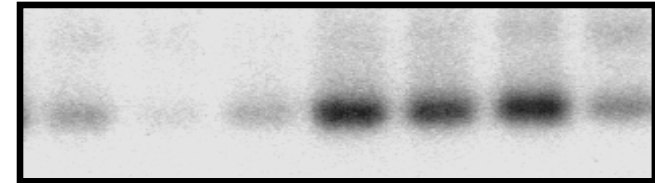
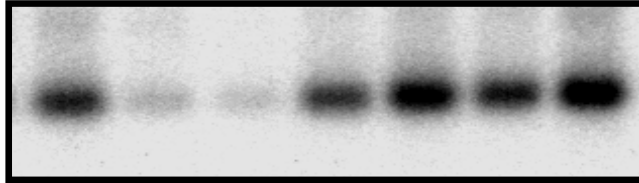
## LONG DAYS

## SHORT DAYS

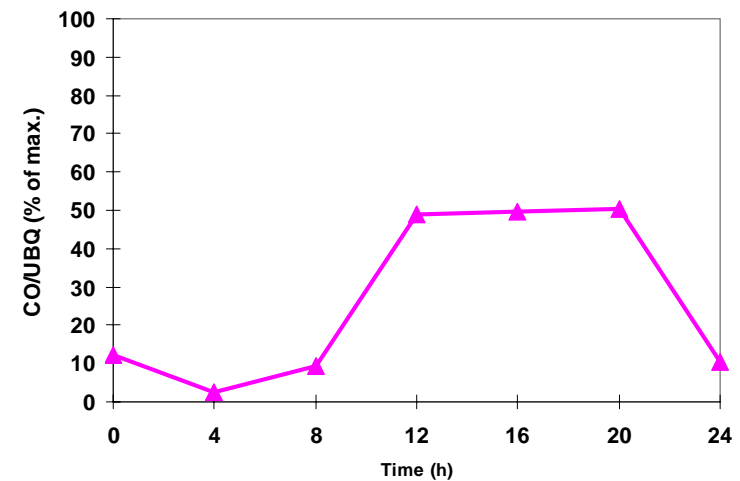
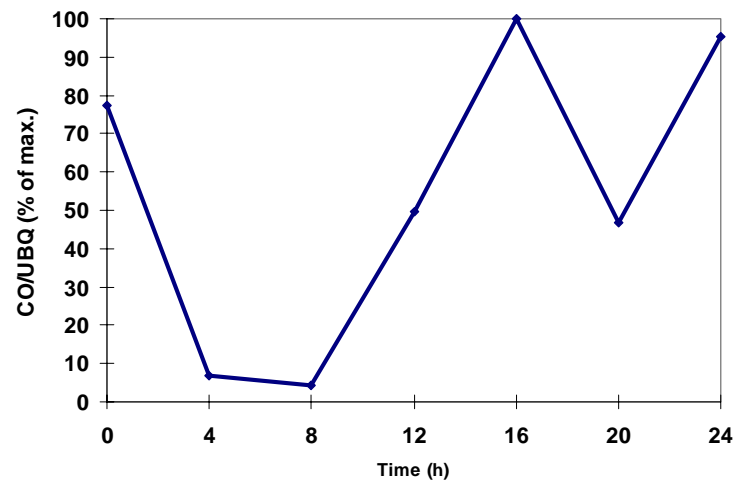
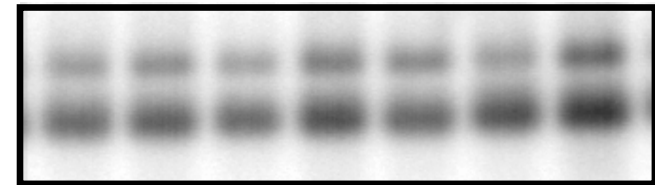
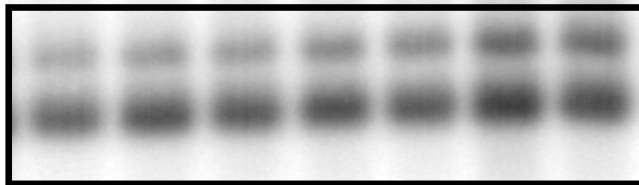
Time 0 4 8 12 16 20 24

0 4 8 12 16 20 24

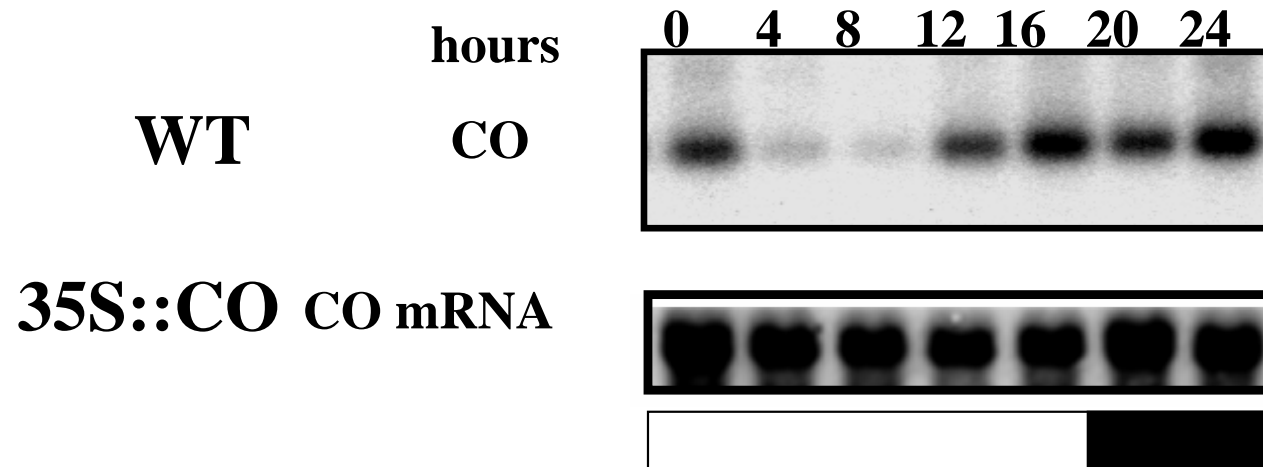
CO



UBQ

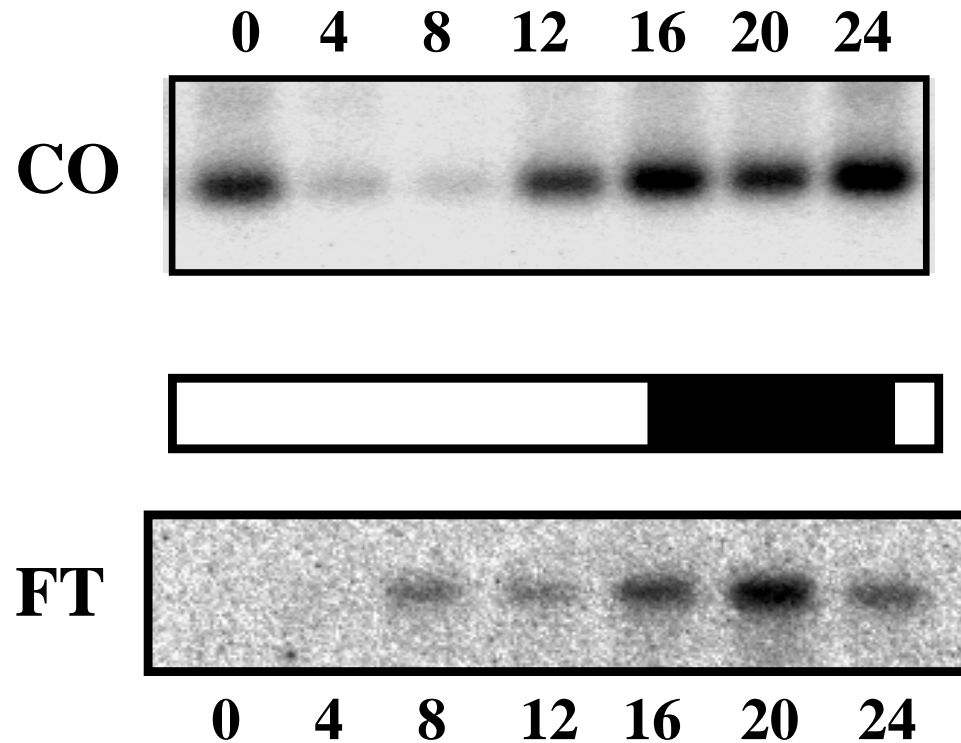


**CO mRNA is expressed throughout the day  
and at higher levels in 35S::CO plants,  
and this causes early flowering in all daylengths**



|                  |  | Leaf Number |
|------------------|--|-------------|
| <b>35S::CO</b>   |  |             |
| Long Days        |  | 5.0         |
| Short Days       |  | 4.8         |
| <b>Wild-type</b> |  |             |
| 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

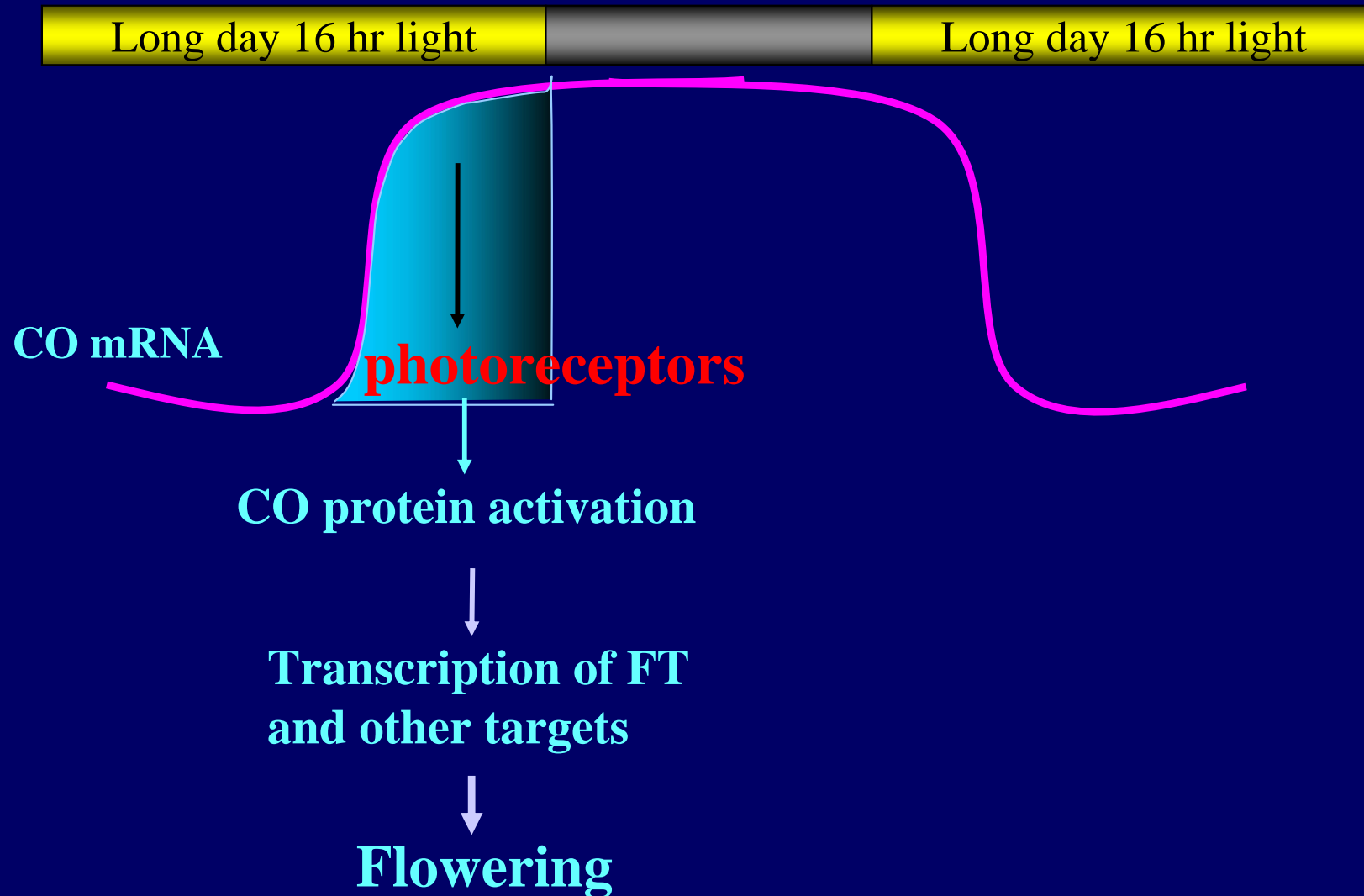
Overexpressed in 35S::CO

ft mutants similar

phenotype to co mutants



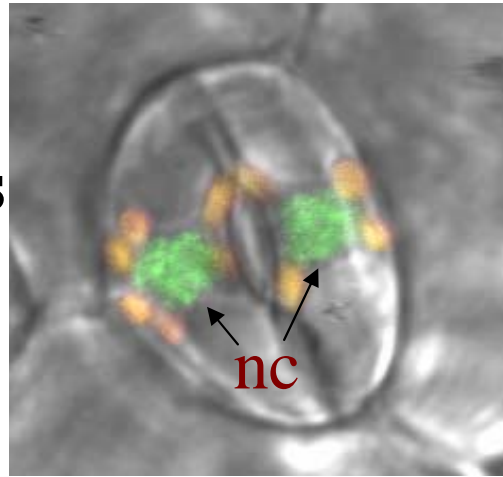
# A model for the response of Arabidopsis to daylength



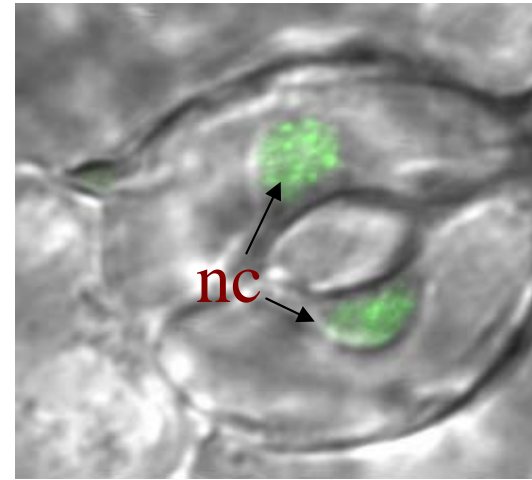
# Exposure to light stabilises CO protein in the nucleus

## *35S::GFP:CO* plants exposed to different light qualities

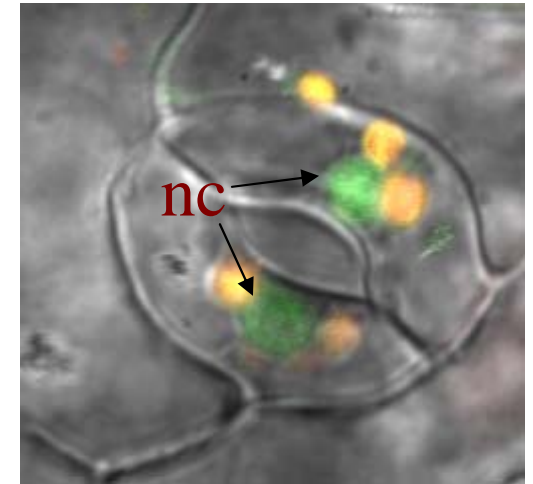
Light conditions  
in which CO is  
active



White

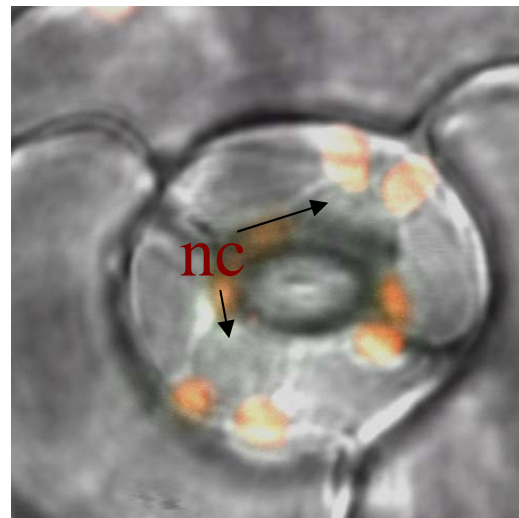


Blue

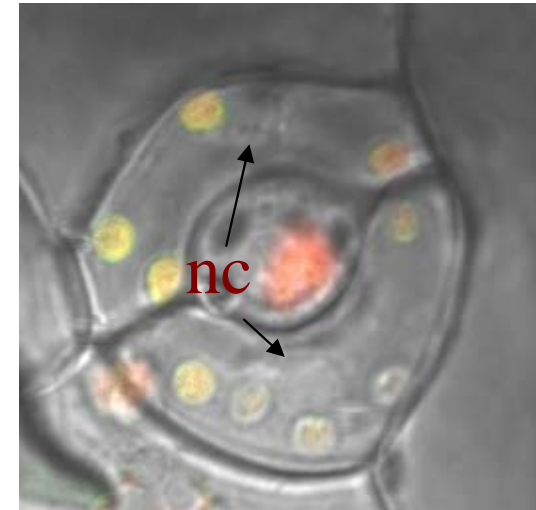


Far-red

Light conditions  
in which CO is  
inactive

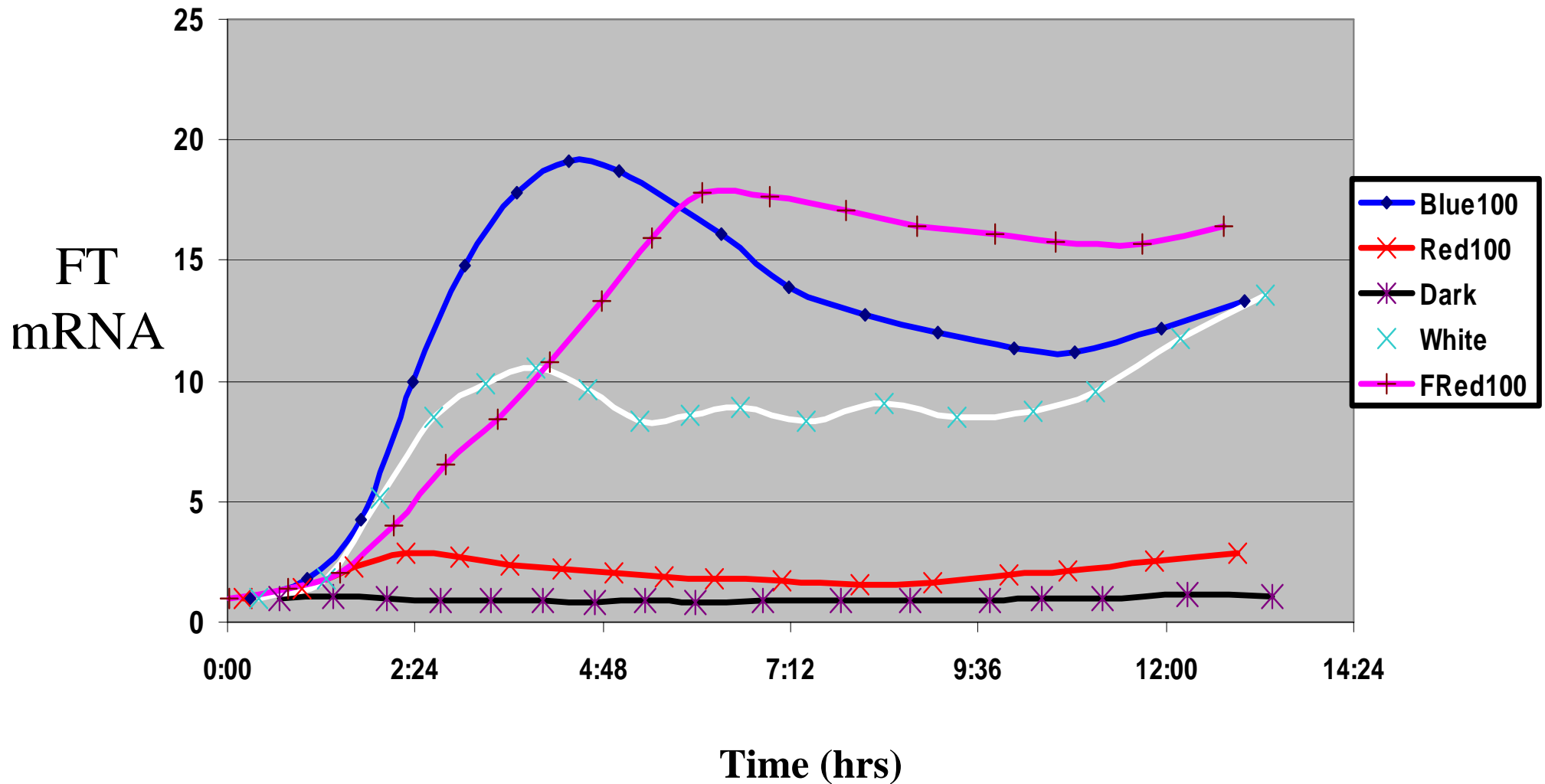


Dark

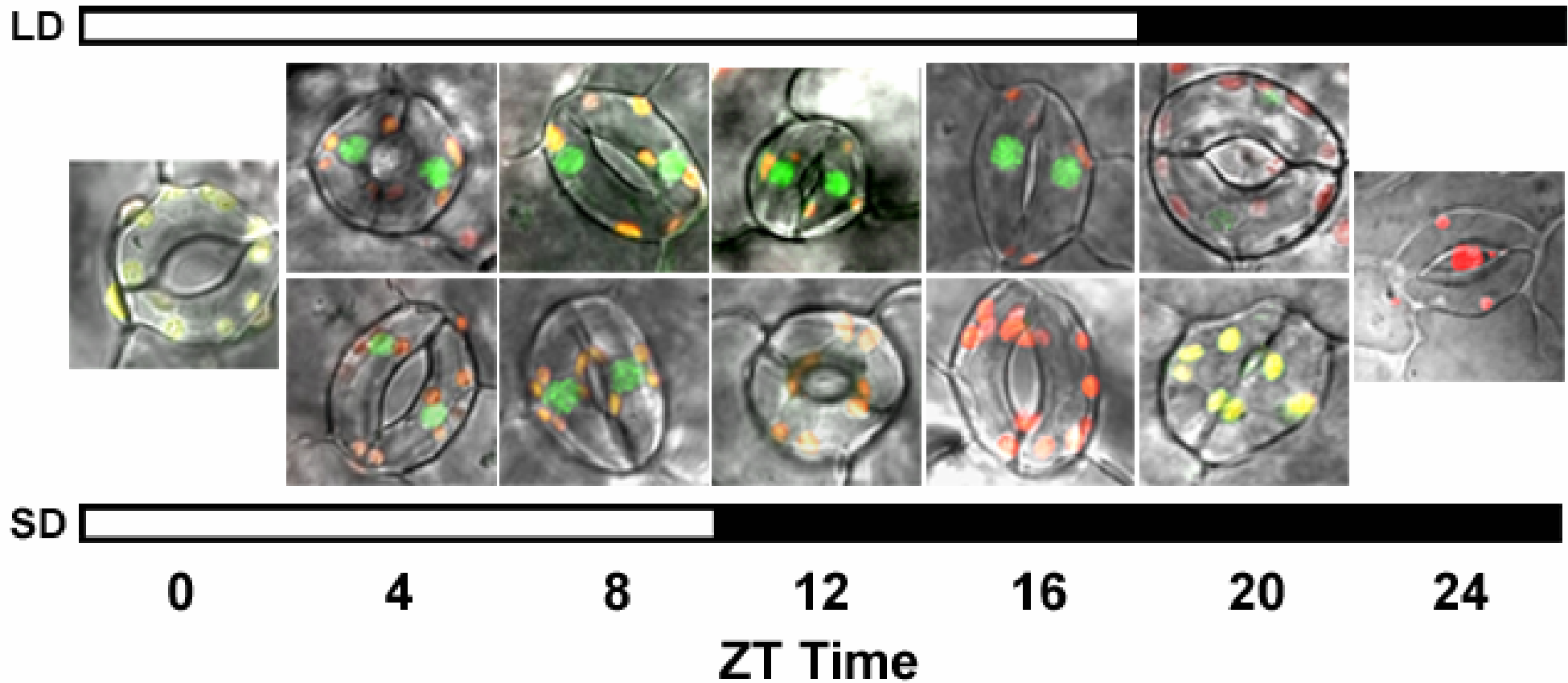


Red

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

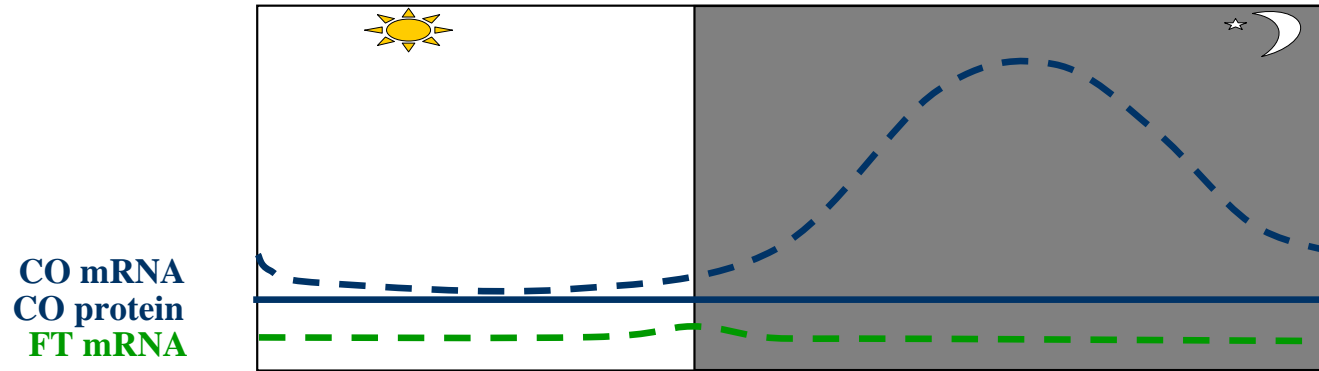


# CO:GFP accumulates during the photoperiod in *35S::CO:GFP* plants

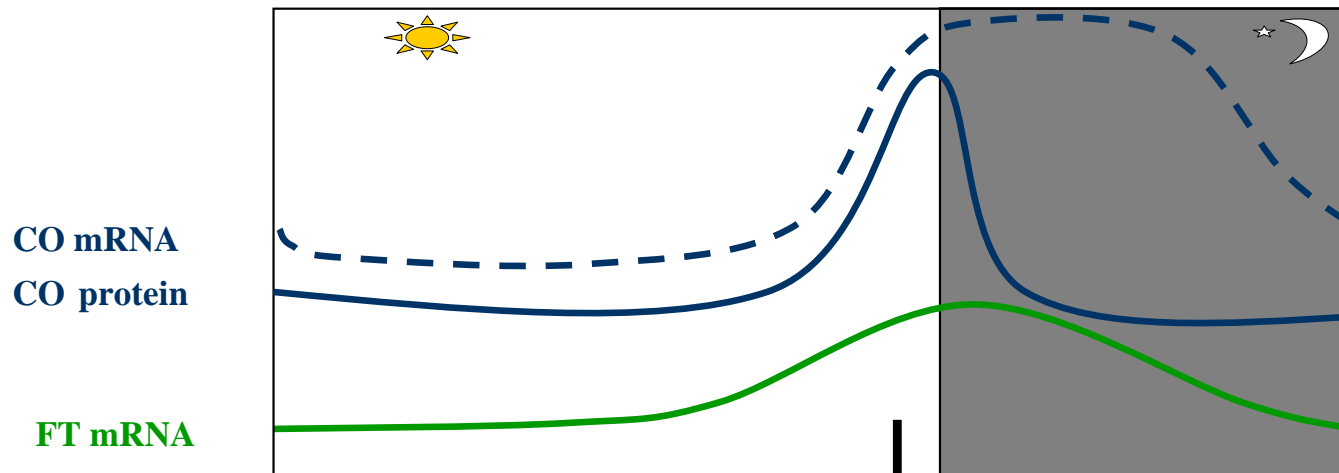


# Coincidence of light and CO mRNA leads to stabilisation of CO protein and activation of FT under long days

No flowering  
In short days

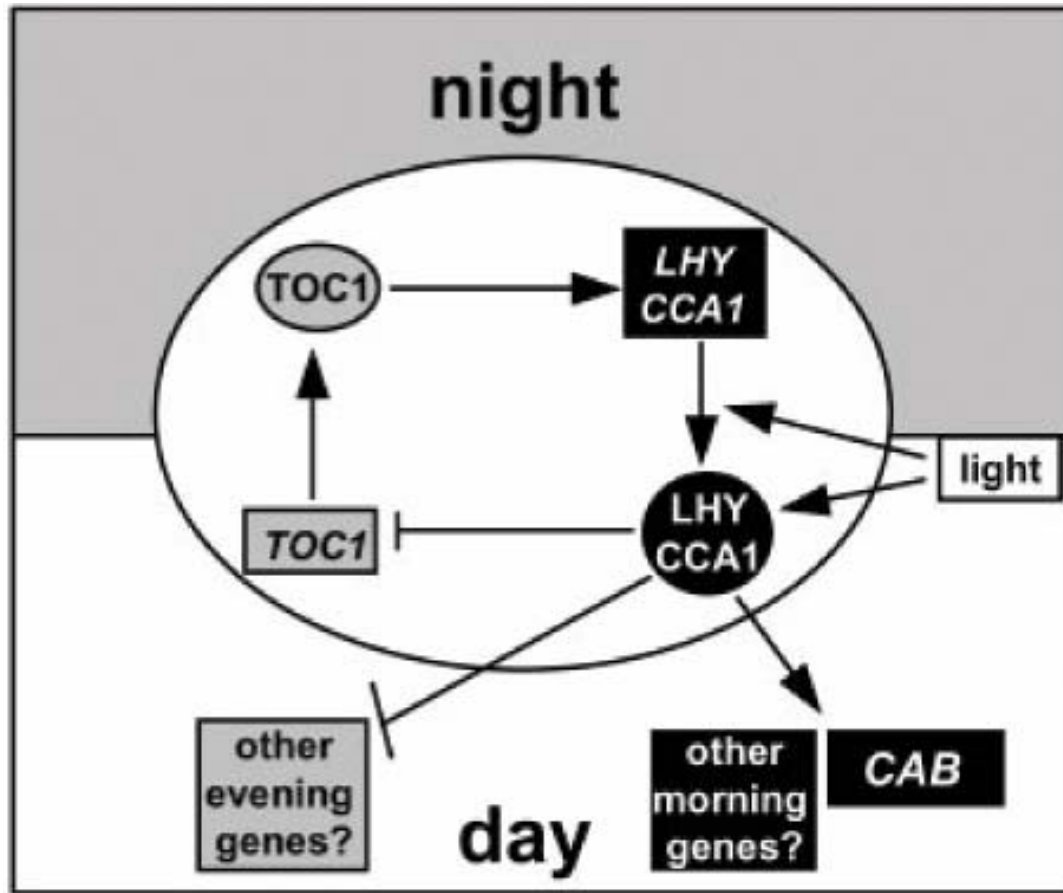


Flowering in  
Long days



Flowering

# The circadian clock of Arabidopsis is a negative feed-back loop regulating transcription



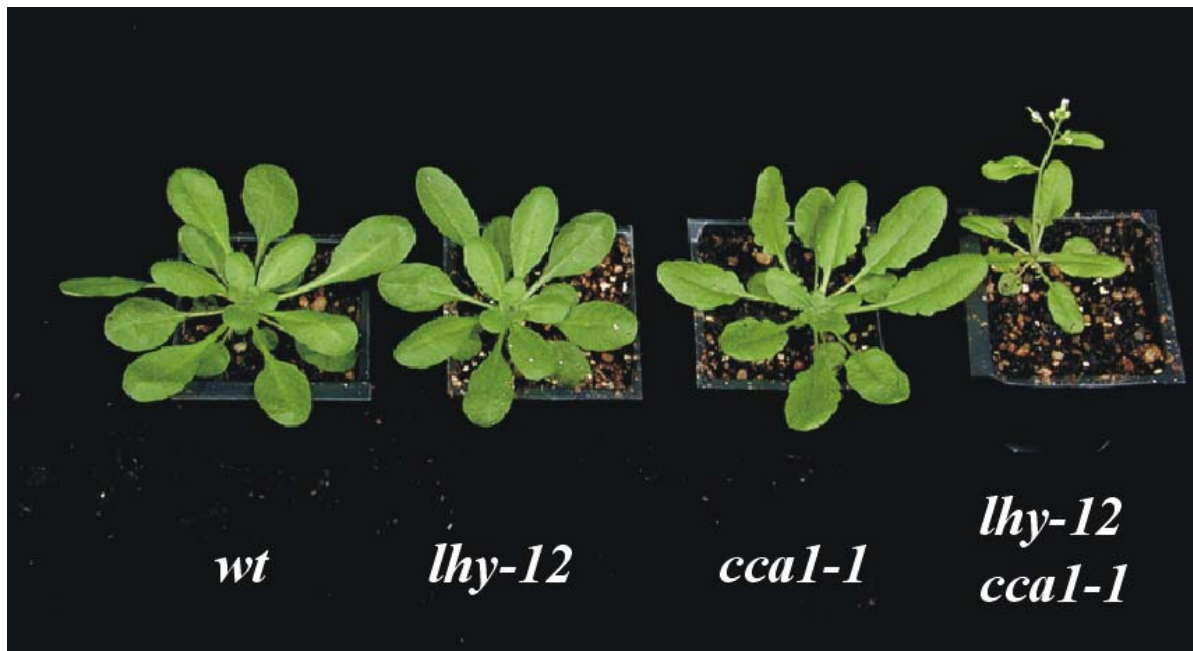
LHY/CCA1 – MYB  
Transcription factors  
Expressed in morning

TOC1 – similar to  
Two component regulators  
from bacteria. Expressed  
in the evening

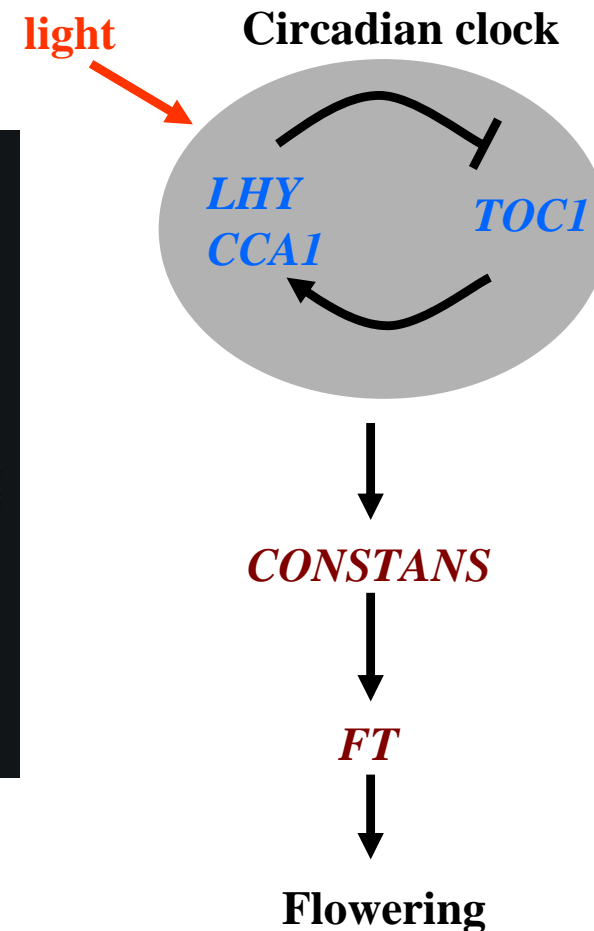
The circadian clock regulates 6% of Arabidopsis genes  
including CONSTANS



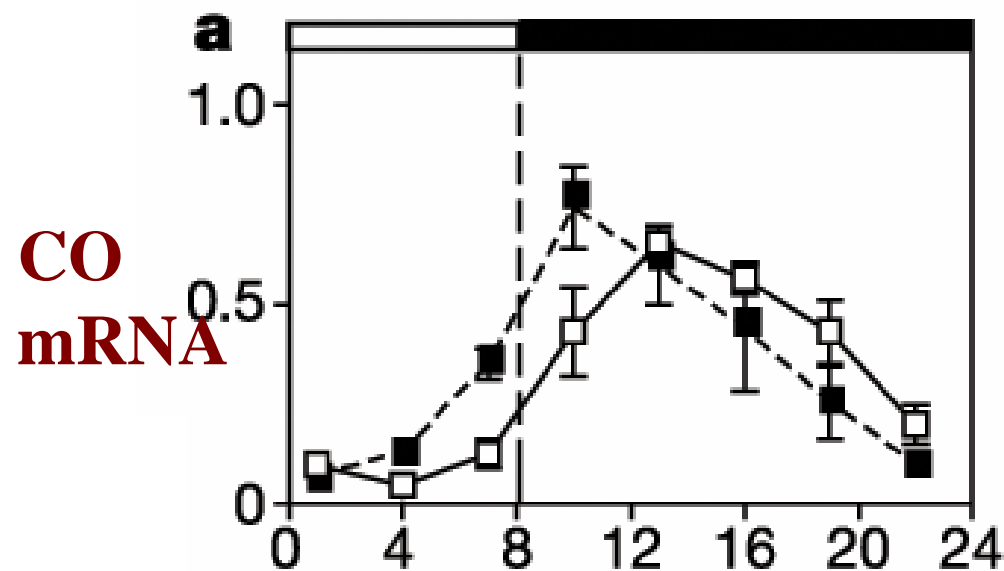
Mutations in circadian-clock components cause early flowering and this requires the CO and FT genes



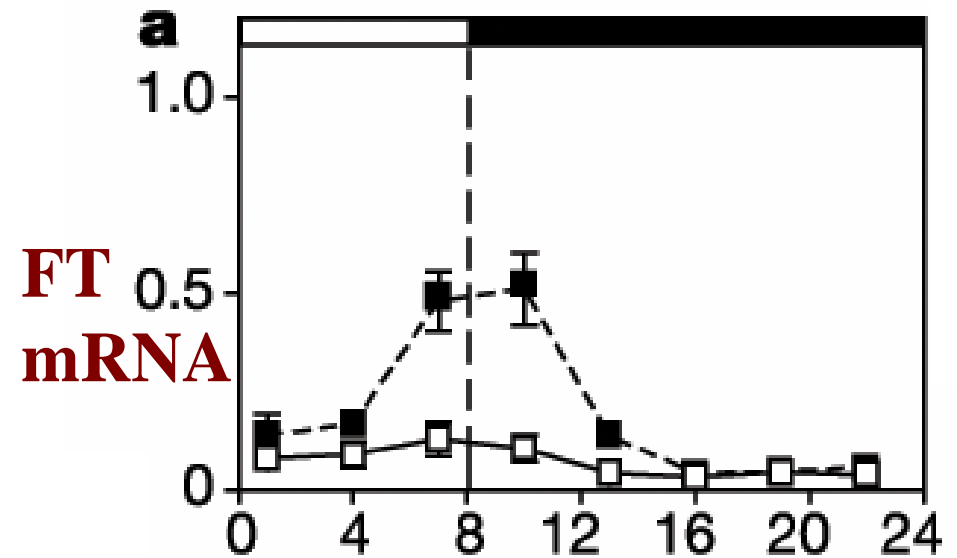
Mutations in *lhy*, *cca1* or *toc1* cause  
Early flowering under short days



# Mutations in TOC1 change the timing in CONSTANS expression causing FT to be expressed under short days

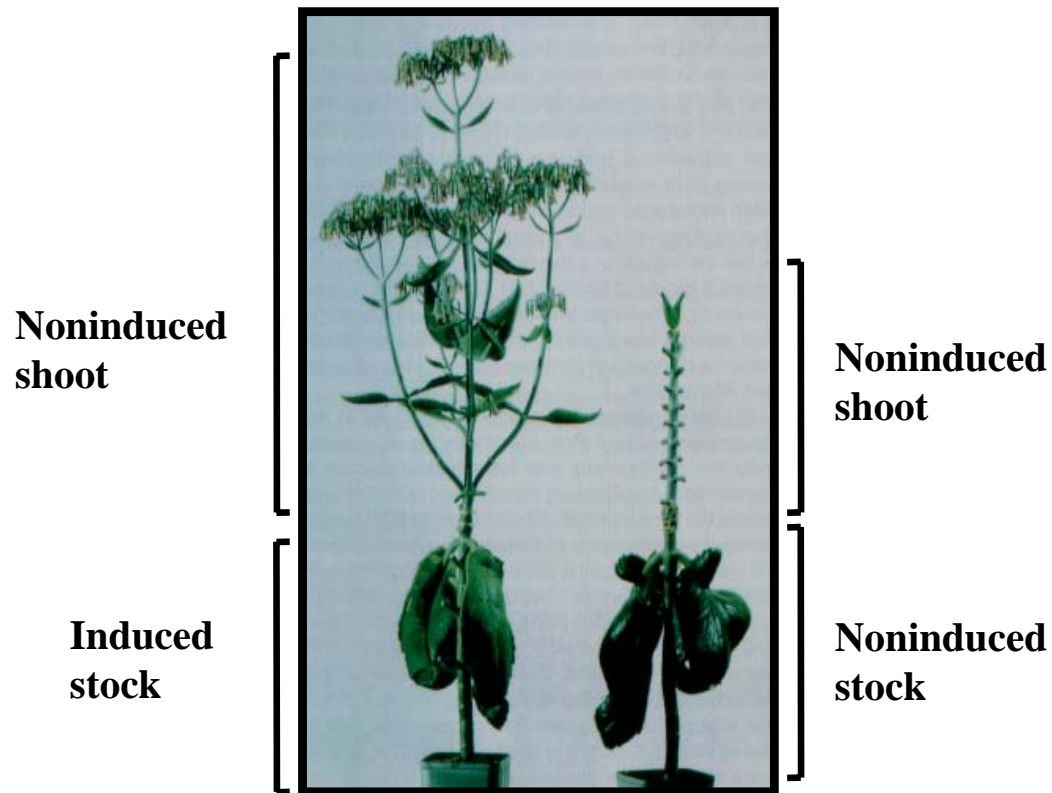


■ **toc1 mutant**  
□ **WT plant**



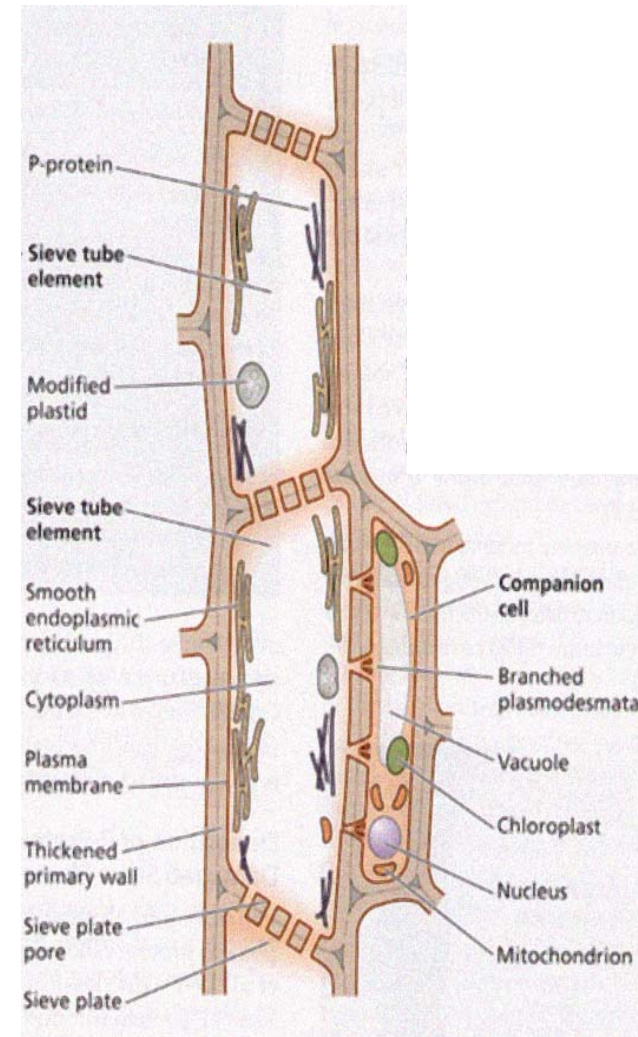
# A floral stimulus crosses a graft junction and induces floral development at the apex of the plant

## Grafting approaches



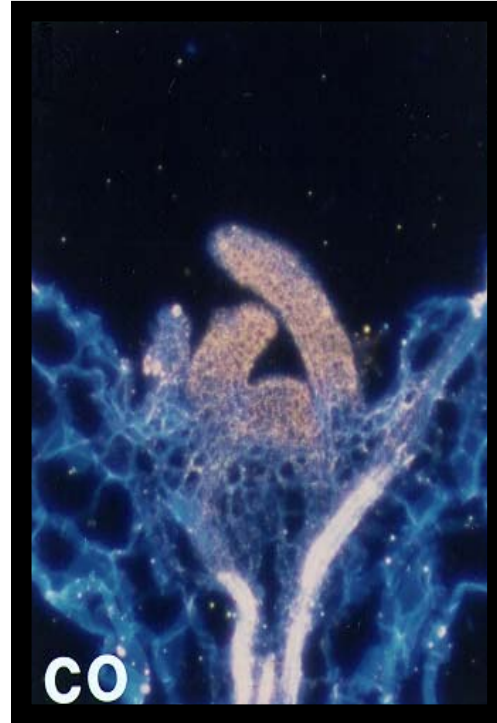
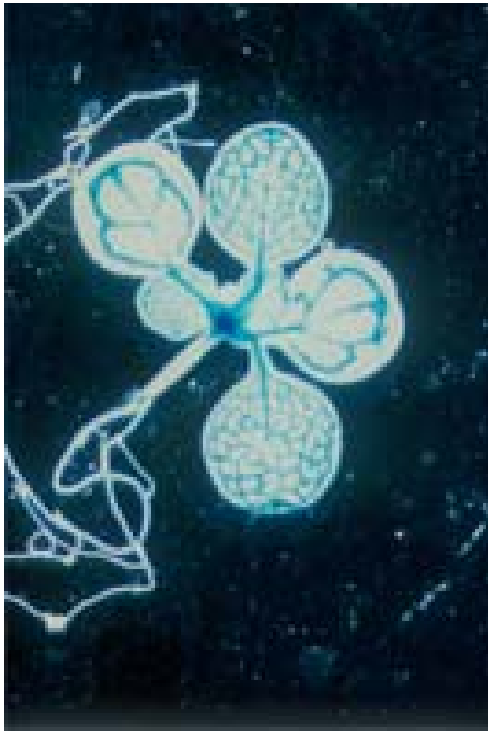
*Bryophyllum diagremontianum*

J. Zeevaart, MSU

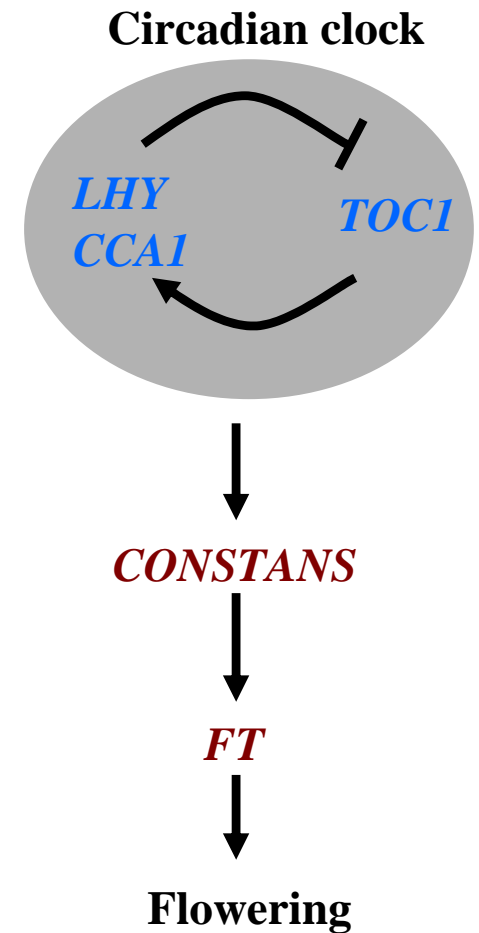
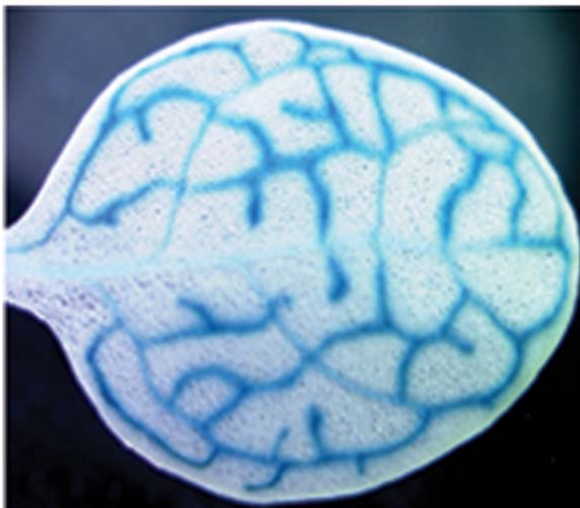


Phloem sieve elements transport photosynthate as well as some proteins and RNAs from the leaf to the apex of the plant

# *CONSTANS* shows a wide expression pattern in the leaf vascular tissue and the meristem

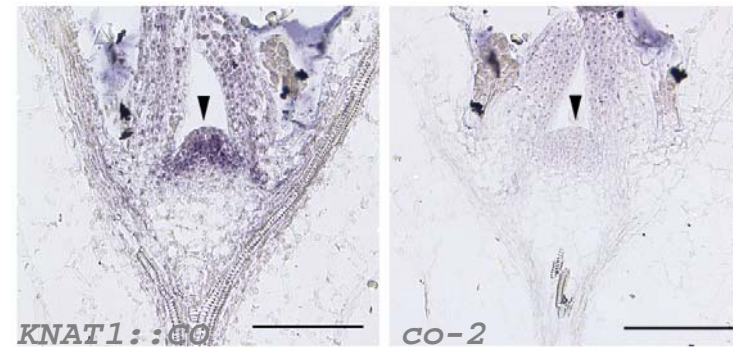
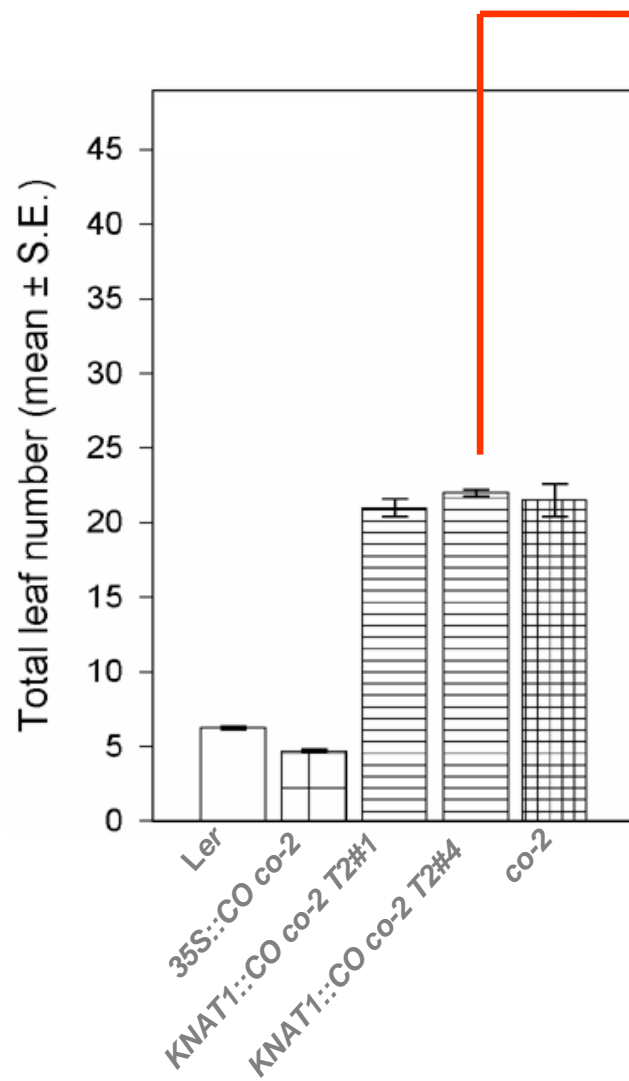


*CO* mRNA *in situ* hybridisation on an 8-day-old

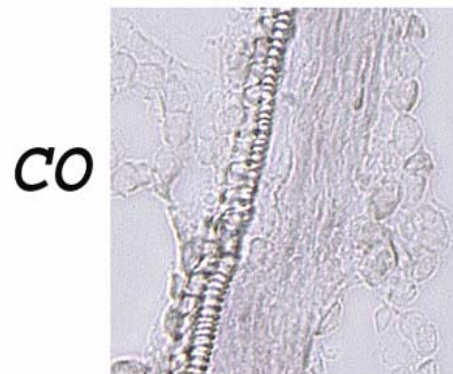
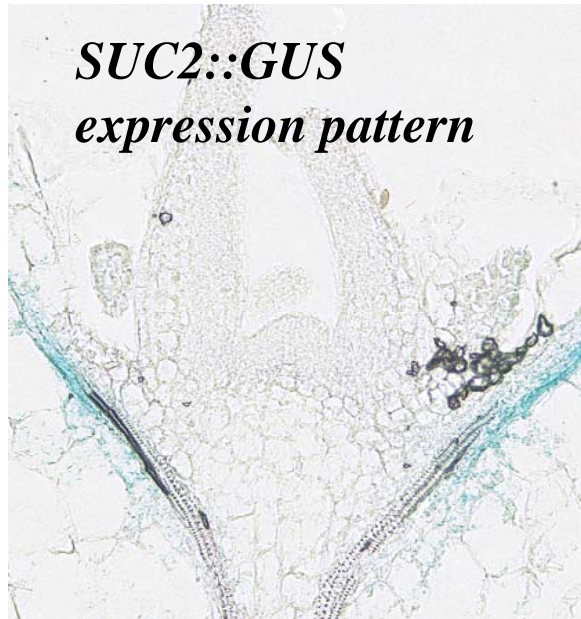




# *CO* does not promote flowering when expressed in the SAM



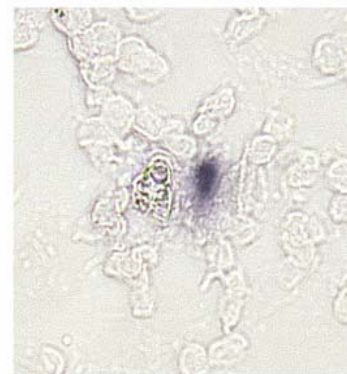
# *CO* acts in the phloem to trigger flowering



*Ler*

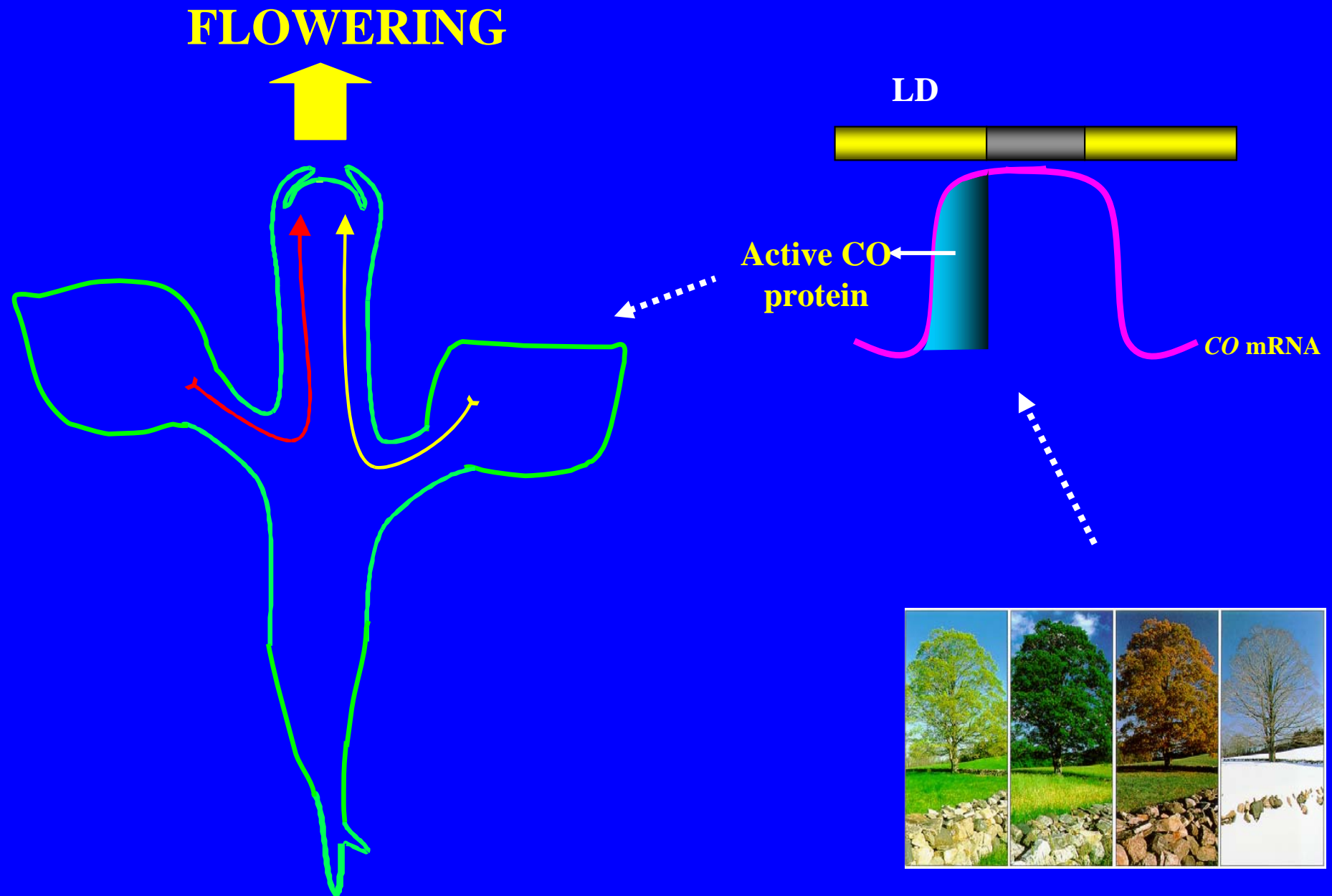


*SUC2::CO*

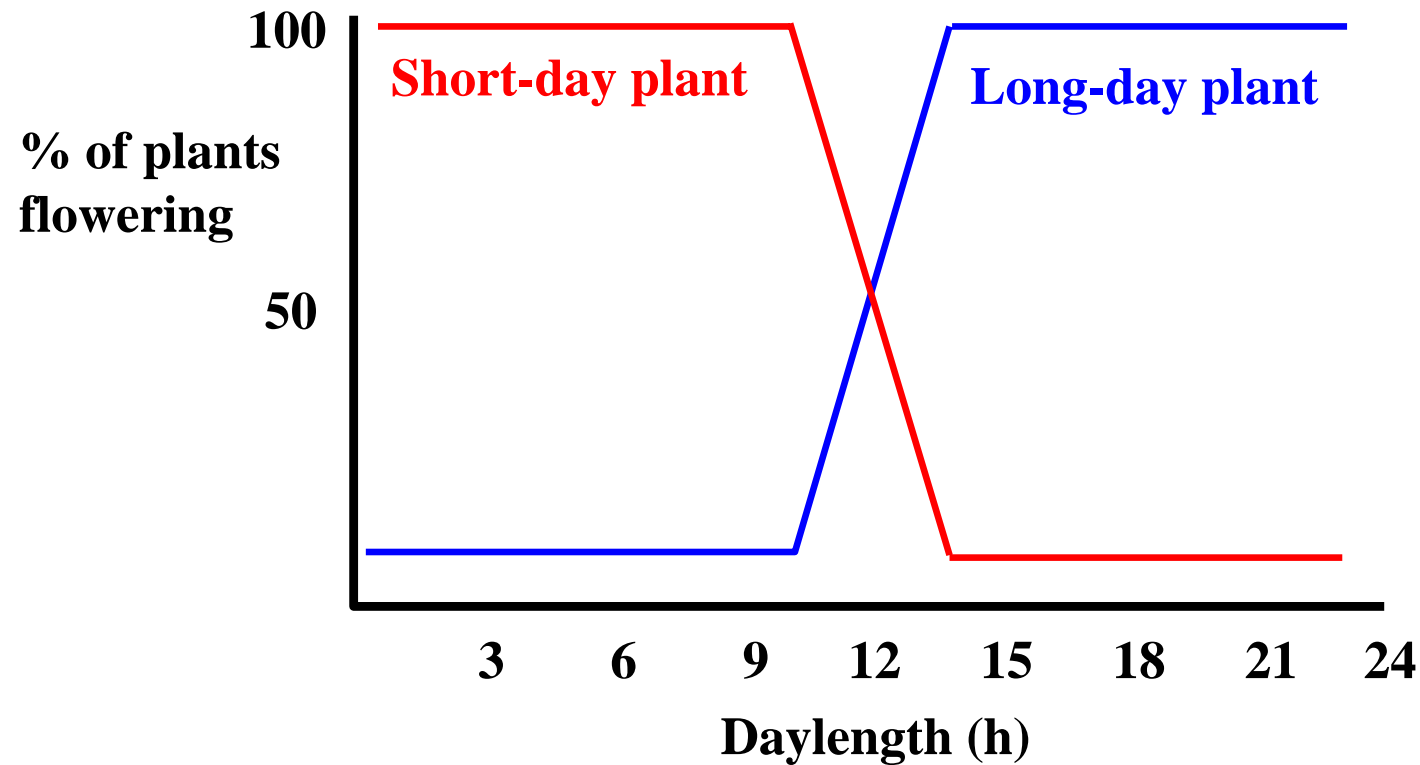




# Control of flowering in response to photoperiod in *Arabidopsis thaliana*



# Distinct response types: long and short-day plants



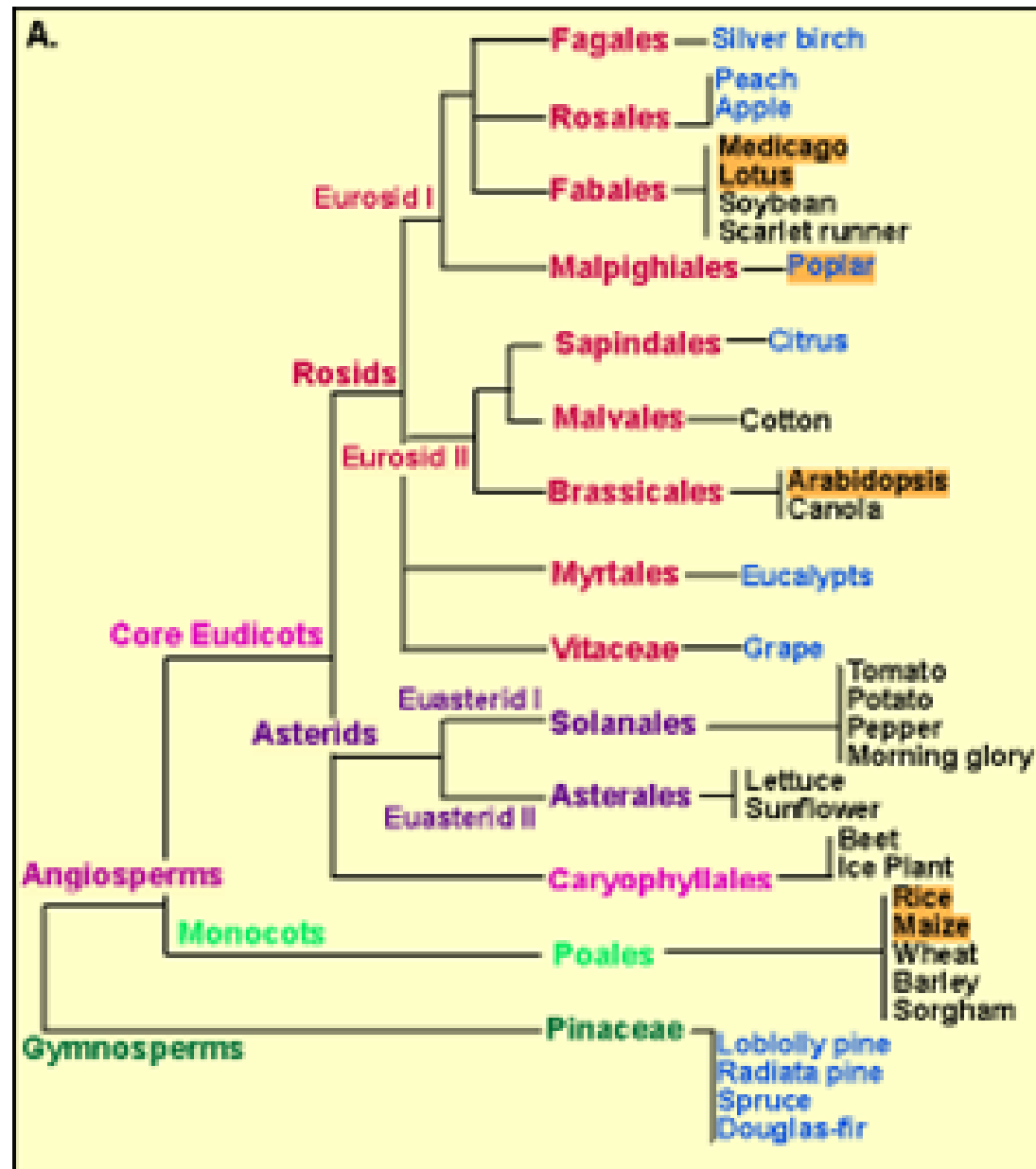
*Arabidopsis*



*Pharbitis nil*



# Long-day and short-day responses have evolved independently in different families of flowering plants



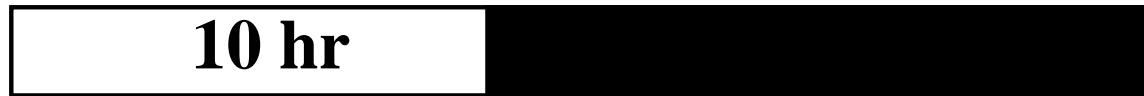
# Contrasting long and short-day responses

**Arabidopsis  
and other**

**Long day plants?**



**CO → FT → Flowering**



**CO**

**Short day  
plants**



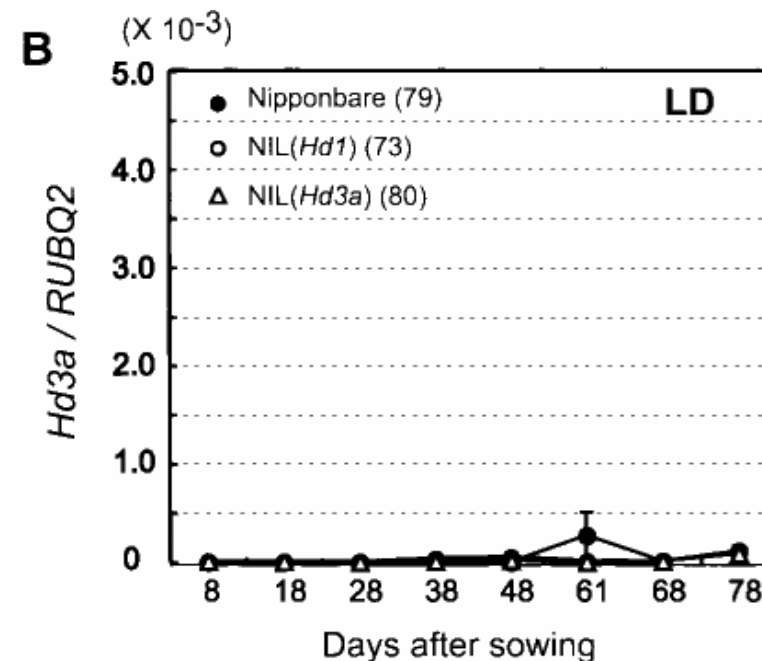
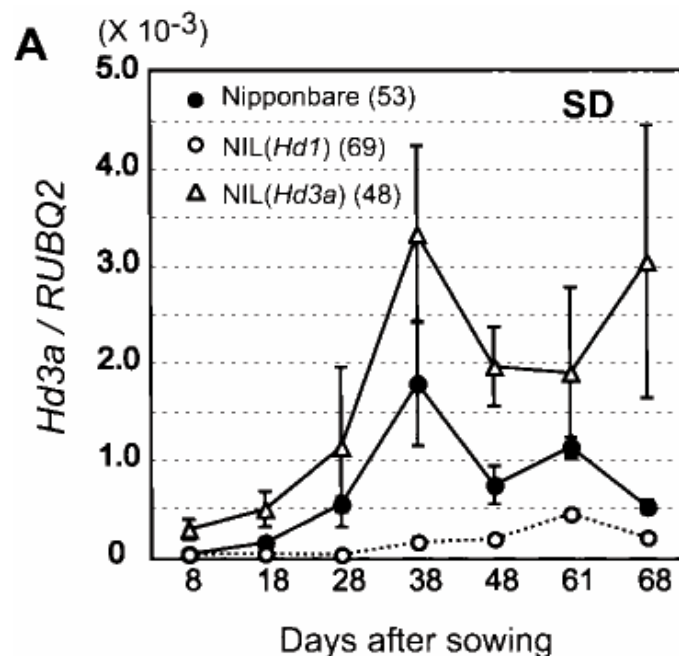
**→ Flowering**

# Conservation of sequence and expression pattern of *Arabidopsis* flowering genes in rice

CO rice orthologue – HEADING DATE 1

FT rice orthologue – HEADING DATE 3

HD3 – expressed in short days, but not long days.  
Reversed compared to *Arabidopsis*



# A conserved flowering-time pathway in rice and Arabidopsis confers the opposite effect to day length

*Arabidopsis*  
Long days



*GI*



*CO*



*FT*

Promotion of  
flowering



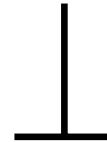
*Rice*  
Long days



*OsGI*



(*OsCO*) *Hd1*



(*OsFT*) *Hd3*

Inhibition of  
flowering



Rice data from:  
Hayama et al. (2003)  
Nature 422, 719



# In rice flowering occurs under short days because CO represses FT under long days

