BPY-605

PHOTOPERIODISM AND ITS ROLE IN FLOWER DEVELOPMENT

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Why do all of them flower at the same time, that too every year in the same month?

What is Photoperiodism?

- Photoperiodism is the sum total of plant's physiological responses to the duration of light received i.e. day length
- It is different from phototropism
- Phototropism is the differential growth of plant in response to light stimuli. For example shoots bend towards the light while roots bend away from it. Phototropism has no correlation with the duration of light
- In Photoperiodism flowering and other developmental processes are regulated in response to the photoperiod, or day length

It has significant role in-

- Most importantly in flowering
- It has significant role in
- bud dormancy
- Control of vegetative trait
- Tuberization in plants
- Bulb formation
- Simultaneous leaf fall in deciduous tree
- Dark carbon fixation in CAM plants

The Discovery

- The concept of Photoperiodism was given by W.W. Garner & H.A. Allard of U.S Department of Agriculture, studied flowering in Maryland mammoth variety of Tobacco plant in 1920.
- M.M. Variety was a single gene mutant tobacco that didn't flower in the spring or summer, like wild type.
- Under controlled experiments, in light-tight boxes where they could manipulate the amount of light and dark, they discovered that flowering only occurred if the day length (amount of light) was 14 hours or less.
- They called the Maryland Mammoth a **short-day plant** because it required a light period *shorter* than a **critical length** to flower.

Classification into SDP,LDP and DNP

- **Short-day plants** flower when daylight is less than a critical length. They flower in the late summer, fall, or early winter.

- **Long-day plants** flower when daylight is increasing. They flower in the spring and early summer.

- **Day-neutral plants** do not flower in response to daylight changes. They flower when they reach a particular stage of maturity or because of some other cue like temperature or water, etc. This is the most common kind of flowering pattern.

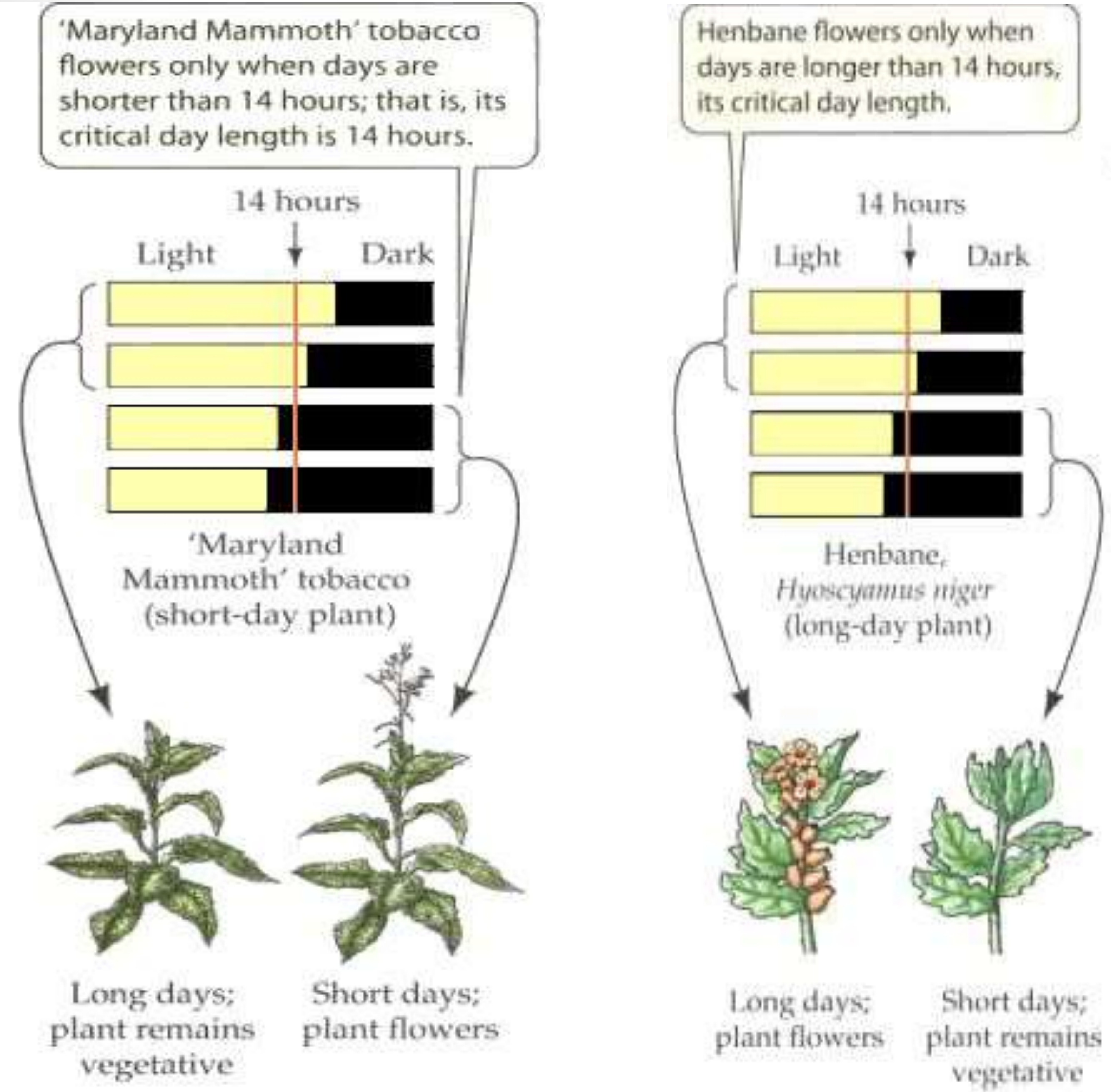

'Maryland Mammoth' tobacco flowers only when days are shorter than 14 hours; that is, its critical day length is 14 hours.
14 hours
Light
Dark
'Maryland Mammoth' tobacco (short-day plant)
Long days; plant remains vegetative
Short days; plant flowers
Henbane flowers only when days are longer than 14 hours, its critical day length.
14 hours
Light
Dark
Henbane, Hyoscyamus niger (long-day plant)
Long days; plant flowers
Short days; plant remains vegetative

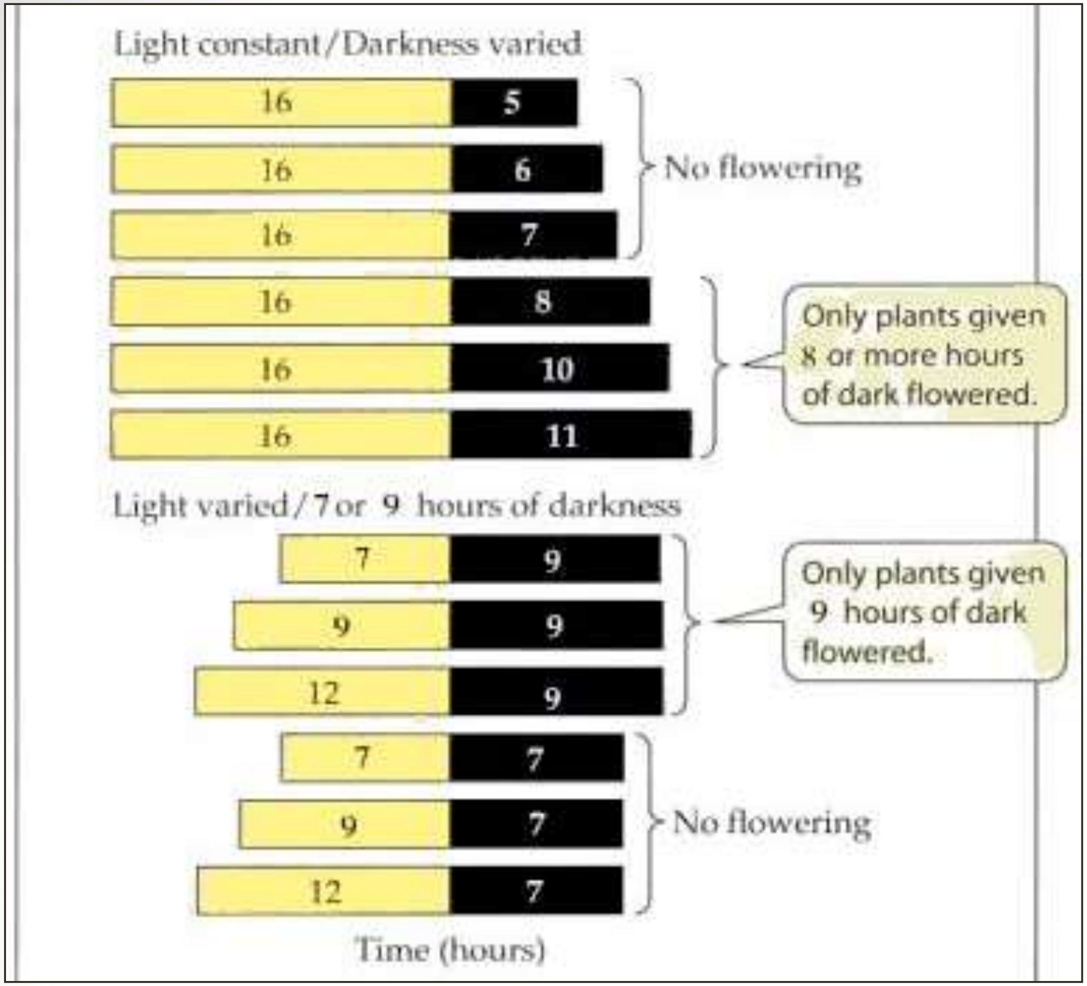
Table showing Photoperiodic responses of some common plants.

Short Day Plants	Long Day Plants	Day Neutral Plants
Chrysanthemums (*Chrysanthemum spp.*)	Barley (*Hordeum vulgare*)	Balsam (*Impatiens balsamina*)
Cocklebur (*Xanthium strumarium*)	Cabbage (*Brassica oleracea*)	Beans (*Phaseolus spp.*)
Cosmos (*Cosmos sulphureus*)	Carrot (*Daucus carota*)	Chillies (*Capsicum annuum*)
Dahlias (*Dahlia variabilis*)	Henbane (*Hyoscyamus niger*)	Cotton (*Gossypium hirsutum*)
Goosefoot (*Chenopodium rubrum*)	Larkspur (*Delphinium ajacis*)	Cucumber (*Cucumis sativus*)
Hemp (*Cannabis sativa*)	Lettuce (*Lactuca sativa*)	Dandelion (*Taraxacum spp.*)
Morning Glory (*Ipomoea purpurea*)	Onion (*Altium cepa*)	Jerusalem artichoke (*Helianthus tuberosus*)
Poinsettia (*Euphorbia pulcherrima*)	Petunia (*Petunia spp.*)	Maize (*Zea mays*)
Rice (*Oryza sativa*)	Poppy (*Papaver somniferum*)	Potato (*Solanum tuberosum*)
Soya beans (*Glycine max*)	Radish (*Raphanus sativus*)	Rhododendrons (*Rhododendron* spp.)
Tobacco (*Nicotiana tabaccum*)	Spinach (*Spinacea oleracea*)	Tobacco (*Nicotiana tabaccum*)
Violets (*Viola papilionacea*)	Wheat (*Triticum aestivum*)	Tomato (*Lycopersicum*)

What is the plant actually measuring?

- In 1940 it was found that **Photoperiodism** has nothing to do with day length—it is completely dependent on a **critical night length**.
- These findings initially reported from experiments on cocklebur are as-

1. The critical night length for the cocklebur is 8 hours: as long as the cocklebur plant has at least 8 hours of **continuous darkness**, it will flower
2. What was originally called a **short-day** plant is actually a **long-night** plant
3. If the night is punctuated by light for a few minutes, then it will not flower!



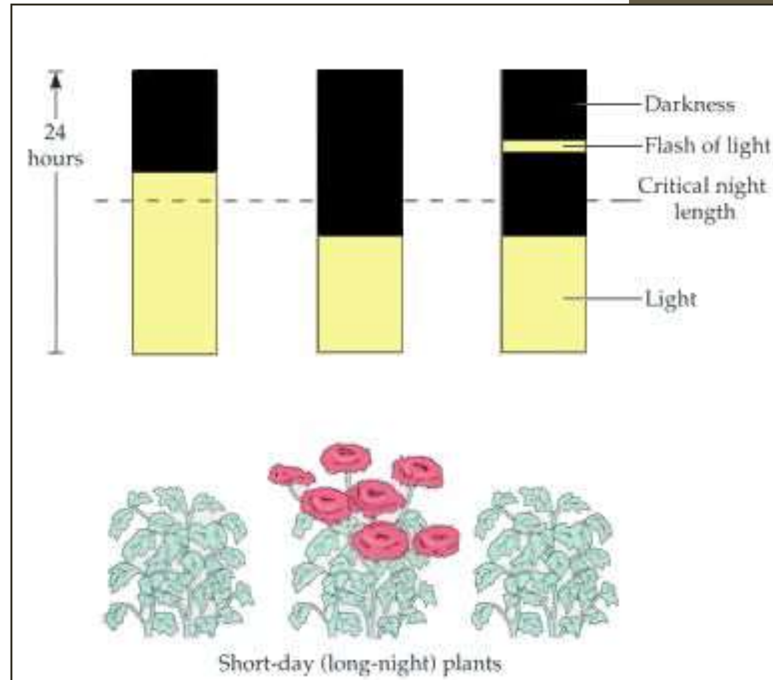
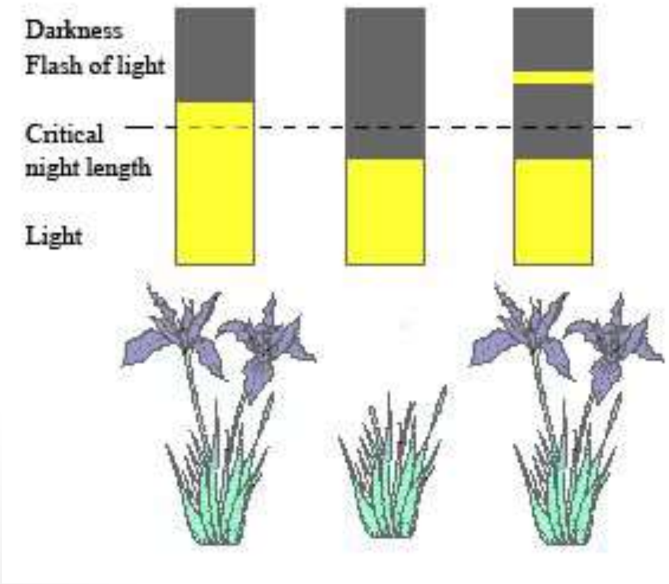


Fig- effect of alternate light and dark periods on flowering in cocklebur (from campbell's biology 5th edition)

What is ……..measuring? Contd..

- **Long-day Plants are Actually Short-night Plants!**
- Similarly, what were once thought to be **long-day** plants are actually **short-night** plants: they flower only when the night is shorter than a critical length.
- A few minutes of light during the night will shorten the night length, therefore causing flowering to occur!



Part of figure 39.16, page 766, Campbell's
Biology, 5th Edition

What must change to change a vegetative shoot into a flower

- What is a Flower?

- Following must change

1. Accumulation of a certain level of biomass so that plant can change from sexual immaturity into a sexually mature state (i.e. a transition towards flowering)
2. the transformation of the apical meristem's function from a vegetative meristem into a floral meristem or inflorescence
3. the growth of the flower's individual organs

Difference between vegetative and floral growth

- Vegetative Growth

1. Objective organ is a leaf/ shoot
2. Phyllotaxis may be alternate or opposite or spiral
3. It may be determined or not determined

- Floral Growth

1. objective organ is a flower or a group of flowers
2. Phyllotaxis is verticillate or whorled
3. Always determined

Molecular signals for floral transition

- The following four genes in *Arabidopsis_thaliana* possess both common and independent functions in floral transition

1. *FLOWERING LOCUS T* (*FT*)
2. *LEAFY* (*LFY*)
3. *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS1* (*SOC1*, also called *AGAMOUS-LIKE20).*
4. MADS-box-type genes, which integrates responses to photoperiod, vernalization and gibberellins.

Molecular framework for floral architecture

- The flower arises from the activity of three classes of genes, which regulate floral development

1. **Meristem identity genes**. Code for the transcription factors required to initiate the induction of the identity genes. They are positive regulators of organ identity during floral development.

2. **Organ identity genes**. Directly control organ identity and also code for transcription factors that control the expression of other genes, whose products are implicated in the formation or function of the distinct organs of the flower

3. **Cadastral genes**. Act as spatial regulators for the organ identity genes by defining boundaries for their expression. In this way they control the extent to which genes interact thereby regulating whether they act in the same place at the same time.

The ABC Model (Haughn & Somerville 1988)

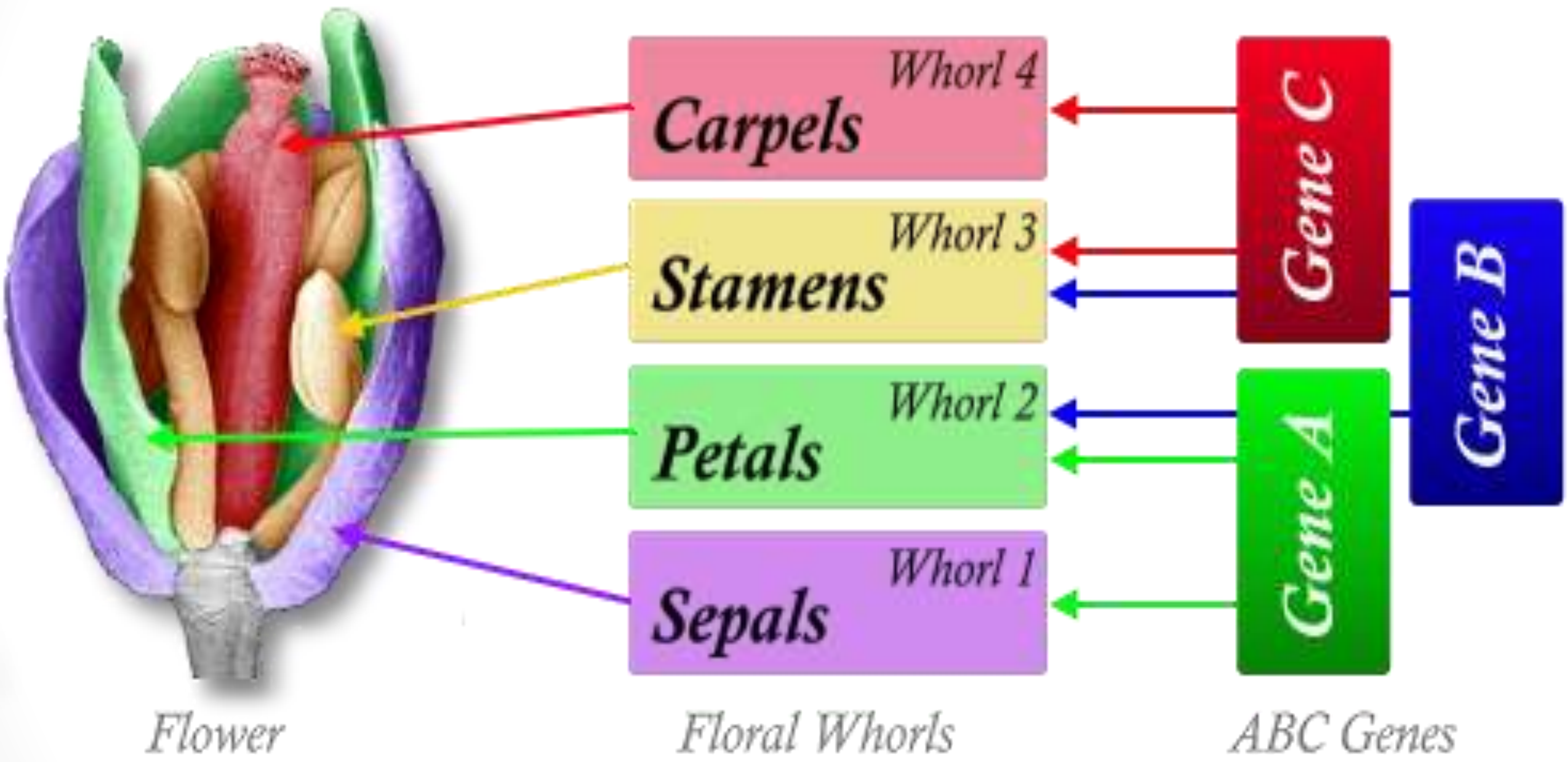


Fig- The single or additive expression of the homeotic genes in the right hand column have repercussions for the development of the organs in the central column and determine the nature of the whorl in the flower

Evidences for the model

- Support for the model came through following 2 developments

1. Firstly, the identification of the exact genes required for determining the identity of the floral meristem. In *A. thaliana* these include APETALA1 (*AP1*) and LEAFY (*LFY*).

2. Secondly, genetic analysis was carried out on the aberrant phenotypes for the relative characteristics of the flowers, which allows the characterization of the homeotic genes implicated in the process.

Genes exhibiting type A function

- In *A. thaliana*, function A is mainly represented by two genes *APETALA1* (*AP1)* and *APETALA2* (*AP2*). *AP1* is a MADS-box type gene, while *AP2* belongs to the family of transcription_factors that are only found in plants

- AP2 has also been shown to complex with the co-repressor TOPLESS (TPL) in developing floral buds to repress the C-class gene *AGAMOUS* (*AG*)

- In *Antirrhinum*, the orthologous_gene to *AP1* is *SQUAMOSA* (*SQUA*). The homologs for *AP2* are *LIPLESS1* (*LIP1*) and *LIPLESS2* (*LIP2*), which have a redundant function

- A total of three genes have been isolated from *Petunia hybrida* that are similar to *AP2*: *P. hybrida APETALA2A* (*PhAP2A*), *PhAP2B* and *PhAP2C*

Genes exhibiting type B function

- In *A. thaliana* the type-B function mainly arises from two genes, *APETALA3* (*AP3*) and *PISTILLATA* (*PI*), both of which are MADS-box genes

- The above genes have orthologs in *A. majus*, which are DEFICIENS (*DEF*) and *GLOBOSA* (*GLO*) respectively

- The *GLO*/*PI* lines that have been duplicated in *Petunia* contain *P. hybrida GLOBOSA1* (*PhGLO1*, also called *FBP1*) and also *PhGLO2* (also called *PMADS2* or *FBP3*)

Genes exhibiting type C function

- In *A. thaliana*, the C function is derived from one MADS-box type gene called *AGAMOUS* (*AG*), which intervenes both in the establishment of stamen and carpel identity as well as in the determination of the floral meristem

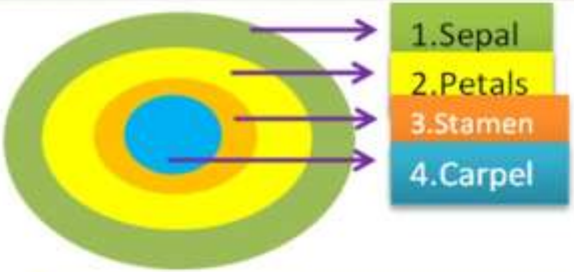
- The *PLENA* (*PLE*) gene is present in *A. majus*, in place of the *AG* gene, although it is not an ortholog. However, the *FARINELLI* (*FAR*) gene is an ortholog, which is specific to the development of the anthers and the maturation of pollen

- The genes that are closer homologs of *AG* in *Petunia* are *pMADS3* and *floral-binding protein 6* (*FBP6*)

ABC Model of Flower Development

Whorl 1: Sepal A alone	Whorl 2: Petal (A+B)	Whorl 3: Stamen(B+C)	Whorl 4: Carpel C alone

A C
B



Case 1: mutation in A gene

Whorl 1: Carpel C alone	Whorl 2: stamen (C+B)	Whorl 2: stamen (C+B)	Whorl 4: Carpel C alone

C C
B

ABC Model

apetala 2

Case 2: mutation in B gene

Whorl 1: Sepal A alone	Whorl 2: Sepal A alone	Whorl 3: Carpel C alone	Whorl 4: Carpel C alone

A C

apetala 3

Pistillata

Case 3: mutation in C gene

Whorl 1: Sepal A alone	Whorl 2: Petal (A+B)	Whorl 3: Petal (A+B)	Whorl 4: Sepal A alone

A A
B

agamous

The Quartet Model of Flower Development (Melzer and Thiessen 2010)

- There are D and E functions as well
- D function genes are are called *FLORAL BINDING PROTEIN7* (*FBP7*) and *FLORAL BINDING PROTEIN1L* (*FBP1l*). They have a role in development of ovule
- Four other MADS genes (*SEPALLATA*s) are involved in establishing the floral nature of flower organs in *Arabidopsis* often collectively called E function genes.
- SEP proteins likely act in multimeric combination with A, B and C function MADS proteins
- Reason for Less acceptance of this model- D and E are identified more popularly as meristem identity genes and not floral identity genes

MADS Box

- The **MADS box** is a conserved sequence motif. The genes which contain this motif are called the MADS-box gene family
- The length of the MADS-box reported by various researchers varies somewhat, but typical lengths are in the range of 168 to 180 base pairs (how many amino acids are there in MADS domain then?)
- The MADS box encodes the DNA-binding MADS domain.
- MADS-box gene family got its name later as an acronym referring to the four founding members

1. MCM1 from the budding yeast, *Saccharomyces cerevisiae*,

2. AGAMOUS from the thale cress *Arabidopsis thaliana*,

3. DEFICIENS from the snapdragon *Antirrhinum majus*,[10]

4. SRF from the human *Homo sapiens*

Functions of MADS-box genes

- The floral homeotic MADS-box genes (such as *AGAMOUS* and *DEFICIENS*) participate in the determination of floral organ identity according to the ABC model of flower development

- Another function of MADS-box genes is flowering time determination. In *Arabidopsis thaliana* the MADS box genes *SOC1* and *Flowering Locus C* (*FLC*) have been shown to have an important role in the integration of molecular flowering time pathways.

Various Models regarding Photoperiodism and flowering

- Following 3 models are significantly discussed

1. The Hourglass Model
2. Circadian Rhythm Model

2A. The External Co-incidence Model

2B. The Internal Co-incidence model

The Hourglass Model

- The hourglass model assumes the gradual accumulation of a chemical product in the organism
- A certain quantity of this chemical is necessary to trigger a physiological response .
- The threshold is reached if the product is not first degraded. It may be degraded by dark and only accumulates during the light phase or it may accumulate during dark and be degraded by light.
- If the light (or the dark) is long enough threshold is reached and a physiological response, such as maturation of the reproductive system, is initiated
- The Molecule involved was assumed to be Phytochrome based upon its similar behavior

Phytochrome

- Phytochrome is a homodimer: two identical protein molecules each conjugated to a light-absorbing molecule.
- Plants make 5 phytochromes: **PhyA**, **PhyB**, as well as C, D, and E.
- There is some redundancy in function of the different phytochromes, but there also seem to be functions that are unique to one or another. The phytochromes also differ in their absorption spectrum; that is, which wavelengths (e.g., red vs. far-red) they absorb best.
- Phytochromes exist in two interconvertible forms
  - $\mathbf{P_R}$ because it absorbs red (R; **660 nm**) light;
  - $\mathbf{P_{FR}}$ because it absorbs far-red (FR; **730 nm**) light.
- These are the relationships:
  - Absorption of red light by $\mathbf{P_R}$ converts it into $\mathbf{P_{FR}}$.
  - Absorption of far-red light by $\mathbf{P_{FR}}$ converts it into $\mathbf{P_R}$.
  - In the dark, $\mathbf{P_{FR}}$ spontaneously converts back to $\mathbf{P_R}$.

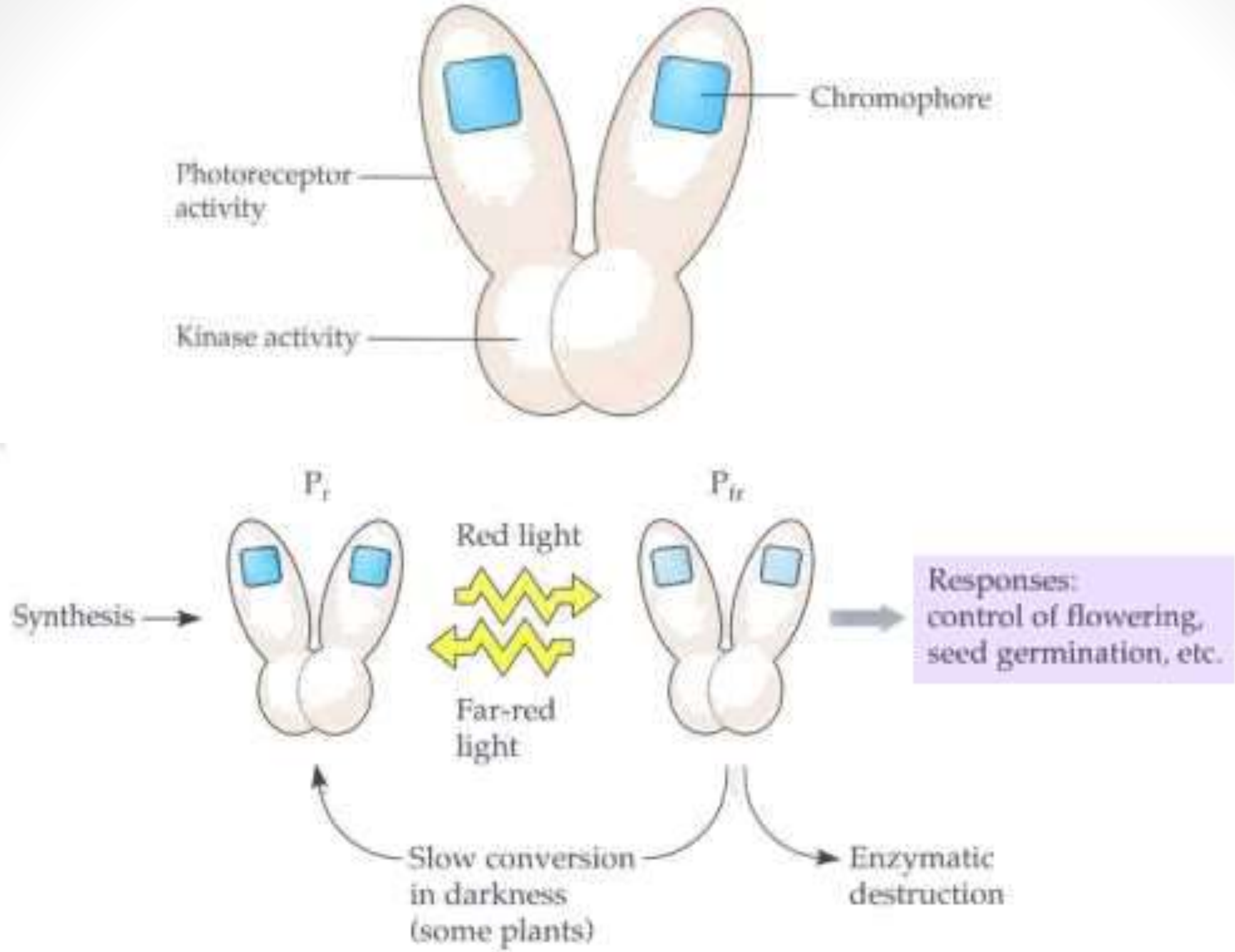


Fig- Structure and interconversion of phytochrome (Figures 39.19 and 39.20, page 769, Campbell's *Biology, 5th Edition)*

Experimental evidences

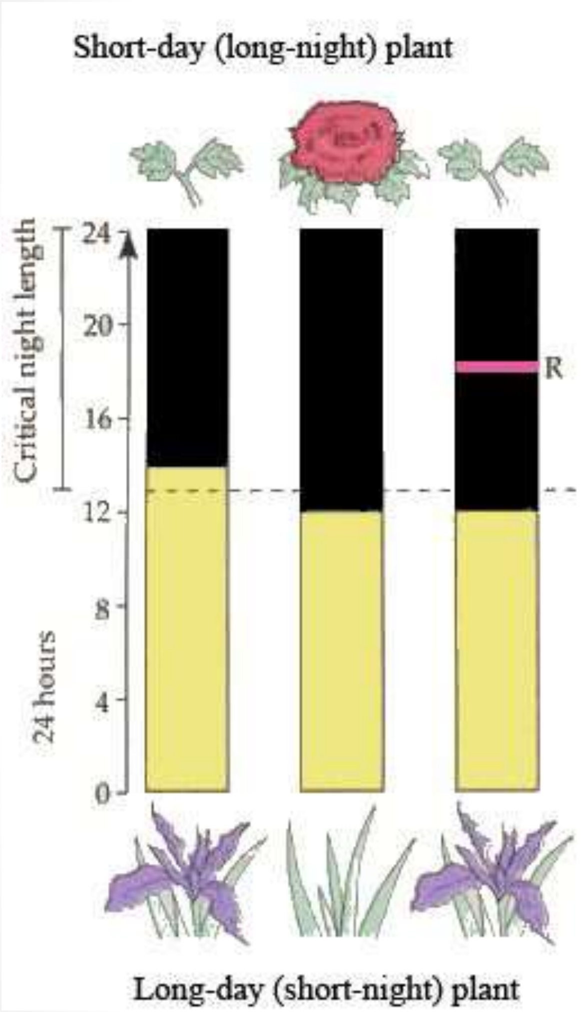
- Red light, of wavelength 660 nm, is the most effective in interrupting night length.
- Experimental results have confirmed this fact:

1. Short-day (long-night) plants experiencing a long night will *not* flower if exposed briefly to 660 nm light sometime during the night.

2. Long-day (short-night) plants exposed briefly to a 660 nm light *will* flower even if the total night length exceeds the critical number of hours.

- Shortening of night length by **red light (R)** can be negated by a flash of **far-red light (FR)** of 730 nm. When this occurs, the plant perceives no interruption in night length.
- No matter how many times red light is flashed, as long as it is followed by far-red light the effects of red light are canceled
- True for both LDPs and SDPs

Experimental evidences



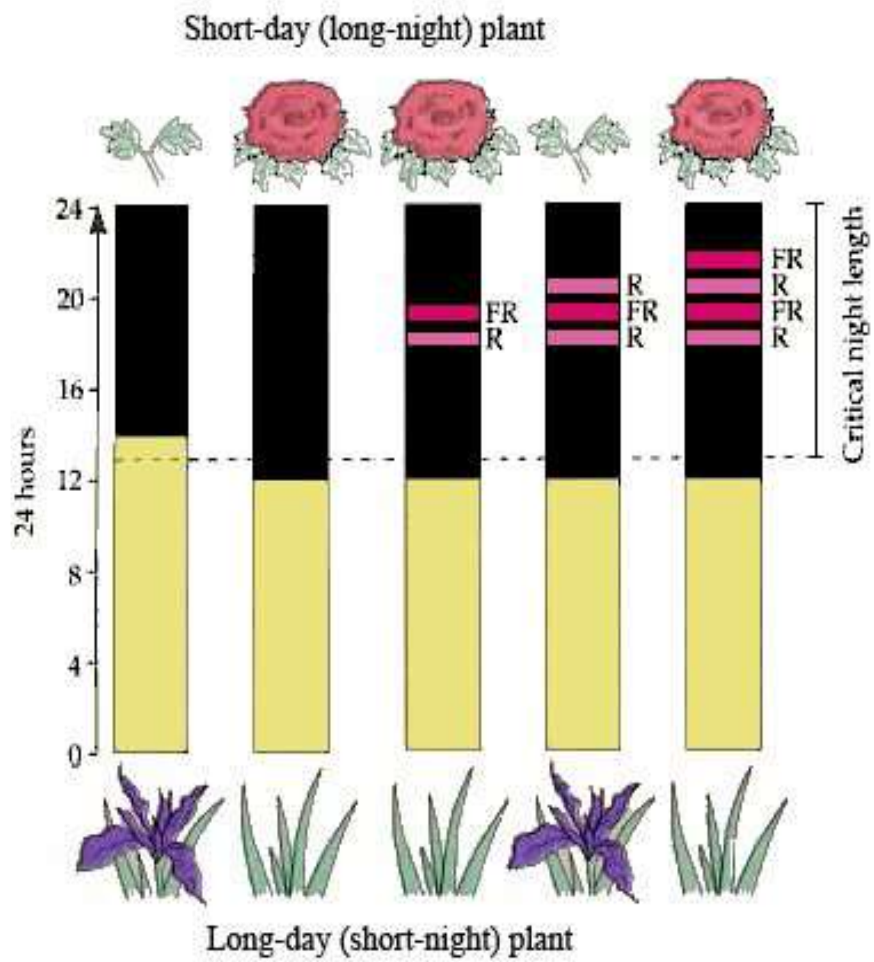


Fig- Effects of flashes of red and far red light on flowering in LDP and SDP (figure 39.18, page 768, Campbell's *Biology, 5th Edition)*

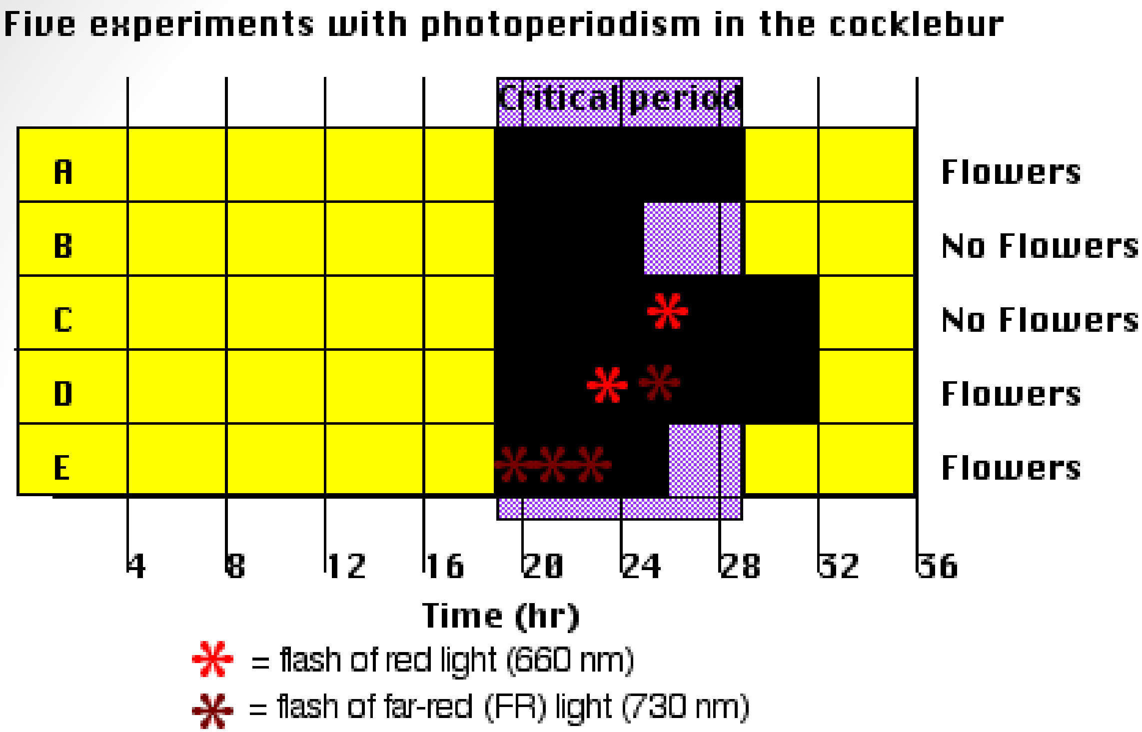


Fig- Sunlight is richer in red (660 nm) than far-red (730 nm) light, so at sundown all the phytochrome is P_{FR}.
During the night, the P_{FR} converts back to P_R.
The P_R form is needed for the release of the flowering signal.
Therefore, the cocklebur needs 8.5 hours of darkness in which to

convert all the P_{FR} present at sundown into P_R

carry out the supplementary reactions leading to the release of the flowering signal ("florigen").

If this process is interrupted by a flash of 660-nm light, the P_R is immediately reconverted to P_{FR} and the night's work is undone (C)
A subsequent exposure to far-red (730 nm) light converts the pigment back to P_R and the steps leading to the release of "florigen" can be completed (D)
Exposure to intense far-red light at the beginning of the night sets the clock ahead about 2 hours or so by eliminating the need for the spontaneous conversion of P_{FR} to P_R (E).

The Circadian Rhythms

- These are rhythms of biological activities that fluctuate over a period of approximately 24 hours even under **constant environmental conditions** (e.g. continuous darkness).
- Under constant conditions, the cycles may drift out of phase with the environment.
- However, when exposed to the environment (e.g., alternating day and night), the rhythms become **entrained**; that is, they now cycle in lockstep with the cycle of day and night with a period of exactly 24 hours.
- The entrainment of the rhythms requires that light is detected by the

1. phytochromes (absorb red light)
2. cryptochromes (absorb blue light)

External Coincidence Model(Bunning 1936)

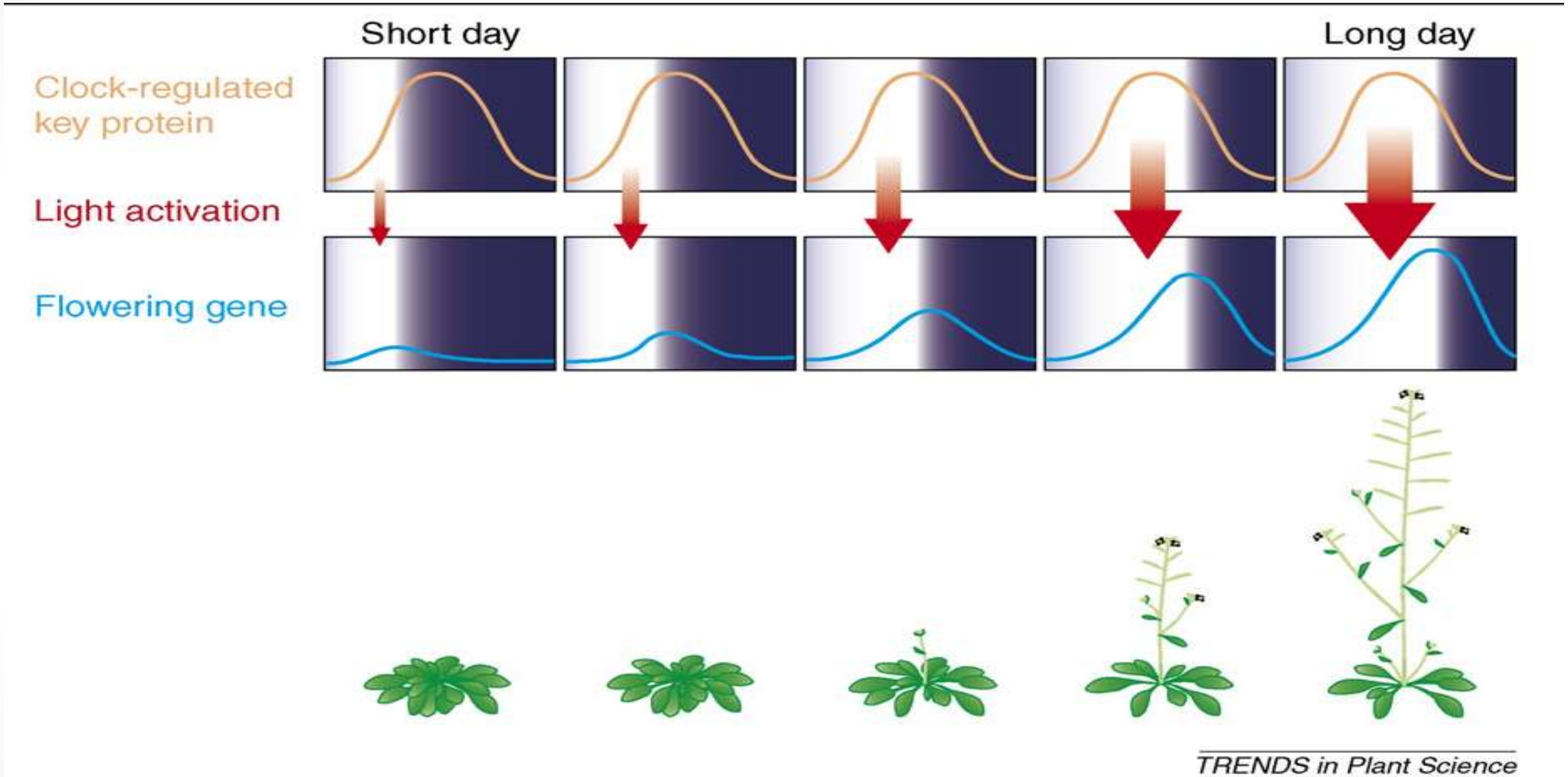


Fig- The external coincidence model: an example of the photoperiodic flowering response in long-day (LD) plants. The function of the clock-regulated key regulator, which induces the expression of the flowering gene, is regulated by light, therefore, flowering will be accelerated when the late-afternoon expression of the key regulator and the presence of daylight coincide.

External Coincidence contd..

- In this model, light plays two crucial roles

1. Entrains the rhythm of photosensitivity i.e. to reset the circadian clock
2. Acts a signal to regulate the activity of the key regulator and effect a response

- Hence the photoperiodic flowering pathway can be separated into

1. Functional domain 1- circadian clock
2. Functional domain 2- circadian regulated day length measurement mechanism

The Arabidopsis Circadian Clock

- Clock is reset by light signals that are perceived by phytochrome (phyA to phyE) and cryptochrome (cry1 and cry2)
- EARLY FLOWERING 3 (ELF3) protein functions as a cyclic repressor of light signaling- Gating response
- Core oscillator consists of negative feedback loop with

1. **Morning factors-** CIRCADIANCLOCK ASSOCIATED 1 (CCA1) and LATE ELONGATED HYPOCOTYL (LHY) proteins
2. **Evening factors**- TIMING OF CAB EXPRESSION 1 (TOC1), EARLY FLOWERING 4 (ELF4) and LUX ARRHYTHMO(LUX) proteins

- Four PSEUDO-RESPONSE REGULATOR (PRR3, PRR5,PRR7 and PRR9) proteins,which are homologs ofTOC1, are also associated with clock function because they comprise the interlocking loops in the CCA1–LHY circuit
- A clock-associated F-box protein ZEITLUPE (ZTL)

The Arabidopsis Day length measurement mechanism

- Day-length measurement mechanism consist of circadian regulation of CONSTANS (CO) gene expression and the light-regulation of CO protein stability and activity
- The CO gene encodes a Bbox-type zinc-finger transcriptional activator that induces the expression of the floral integrator *FLOWERING LOCUS T* (*FT*) and *SUPPRESSOR OF OVEREXPRESSION OF CO 1* (*SOC1;* also known as *AGL20*) genes in a light-dependent manner
- Products of FT and SOC1 when accumulated beyond a threshold induce flowering
- In long day photoperiods Co abundance is high at the beginning and end of the photoperiod
- CO deficient mutants- flower late in inductive photoperiod
- CO overexpressing mutants – early flowering independent of day length

How does Circadian clock regulate CO transcription?

- A number of transcription factors such as FLAVIN-BINDING, KELCH REPEAT, AND F-BOX 1 (FKF1), GIGANTEA (GI), ELF3, CYCLING DOF FACTOR 1 (CDF1), and RED AND FAR-RED INSENSITIVE 2 (RFI2) proteins are known to be involved in regulating CO transcription

- FKF1 and GI are activators of CO transcription, whereas ELF3, CDF1 and RFI2 are repressors

- The promoter regions of FKF1 and GI genes contain several evening elements, suggesting that the core clock components CCA1 and LHY directly regulate transcription of both evening genes

- Generating the LD-specific daytime expression of CO is the first important process for producing day-length dependent FT expression in the photoperiodic flowering pathway.

How is CO protein stability regulated

- CO protein stability is highest in the late afternoon in LD and is regulated by light signals perceived by phyA, phyB, cry1 and cry2 photoreceptors phyA and cry signals protect CO protein from degradation whereas phyB signals promote degradation

- Under SD conditions,COprotein is unstable throughout the day

- SUPRESSOR OF PHYA-105(SPA1), and its homologs, SPA3 and SPA4, are involved in regulating CO stability- these are negative regulators of phyA signalling.

- SPA1 also physically interacts with CONSTITUTIVE PHOTOMORPHOGENIC 1 (COP1) RING finger E3 ubiquitin ligase to regulate the stability of transcription factors affecting CO stability

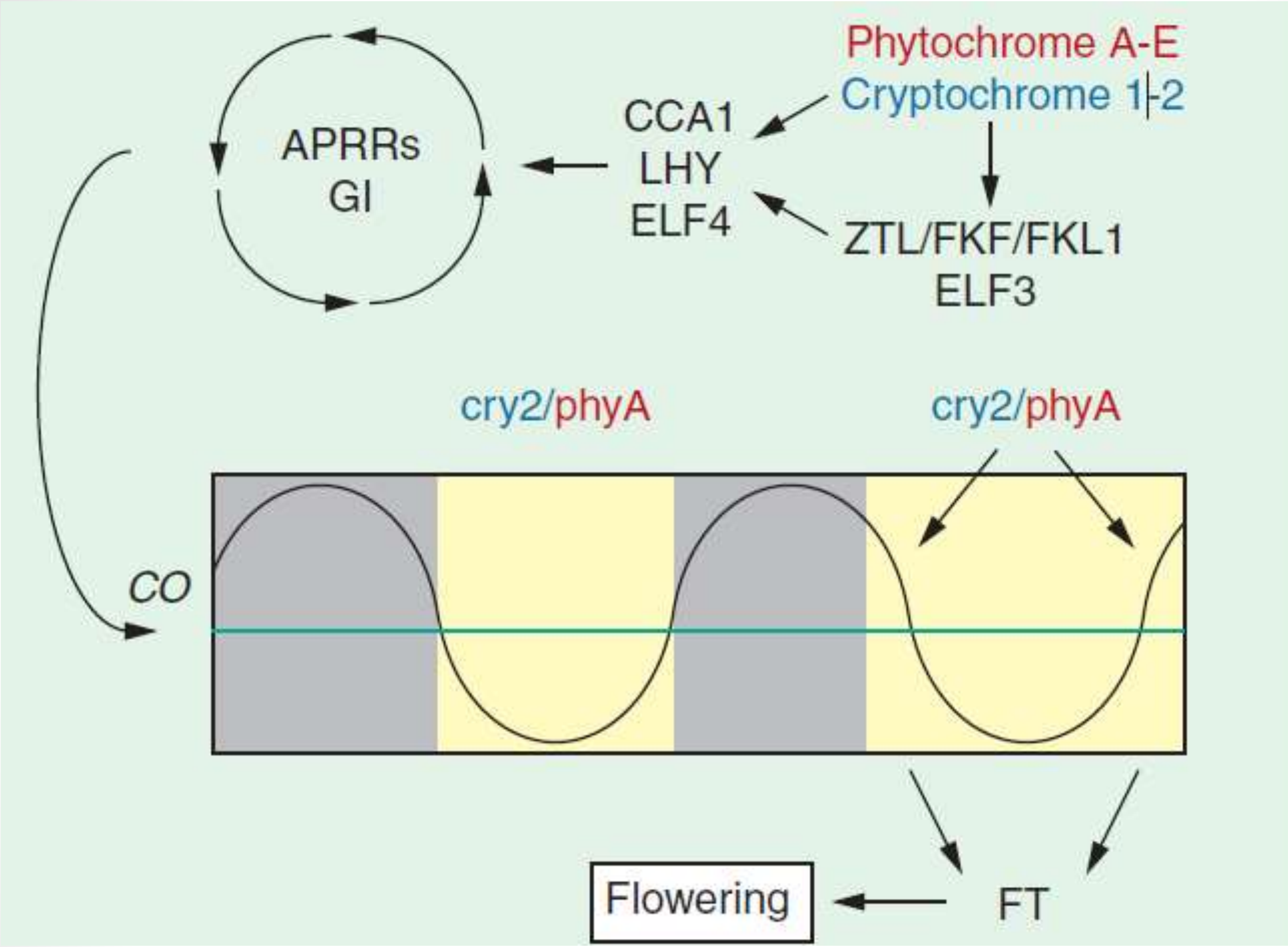


Fig- A molecular model of photoperiodic regulation of flowering time in Arabidopsis (Kay et. al. 2012)

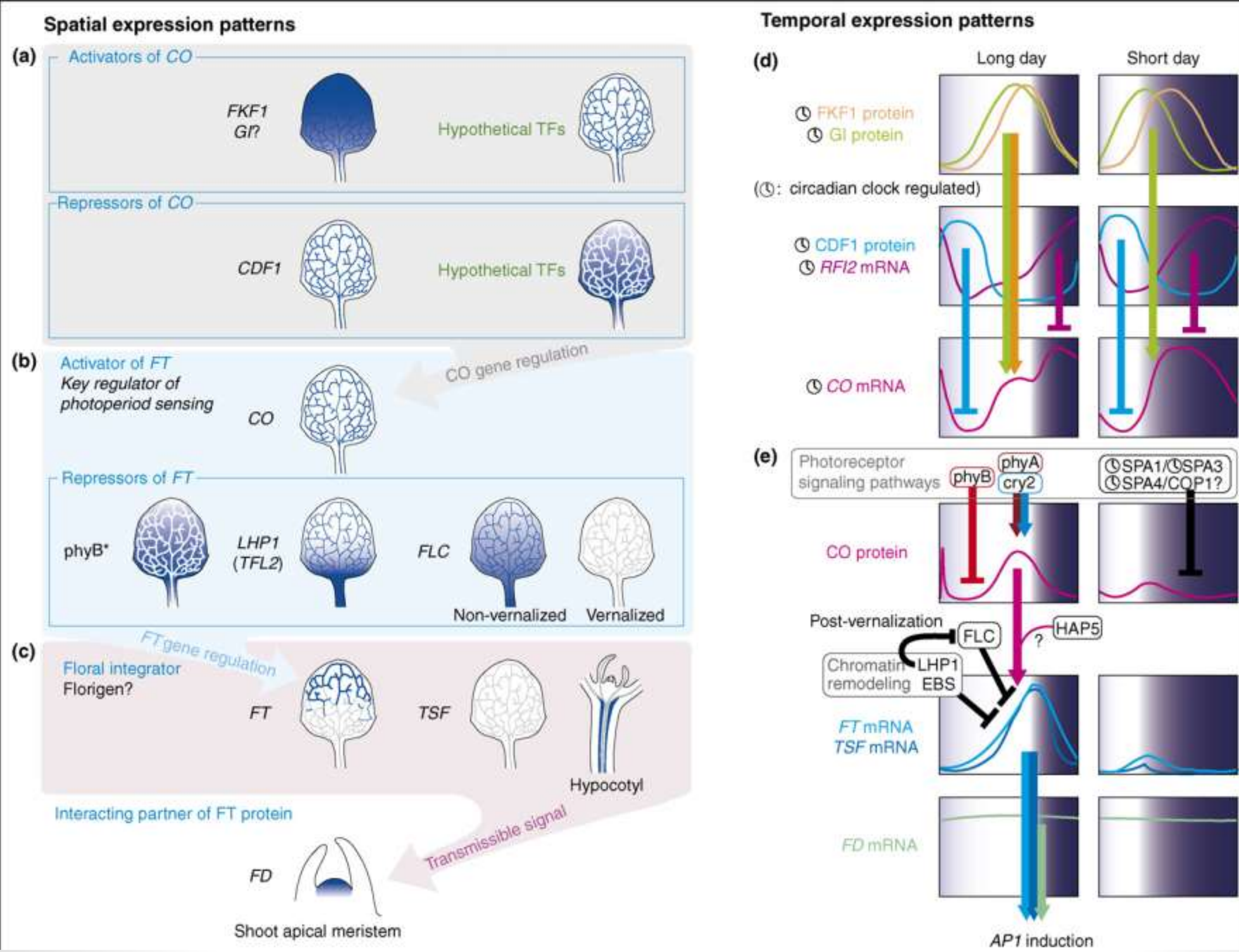


Fig-Spatial and temporal regulation of CO and FT expression under different photoperiods (Kay et.al. 2010)

Other findings

- The heading date is a complex trait controlled by multiple genes known as quantitative trait loci (QTLs). One major QTL, named *Hd1*, was recently identified as a *CO* homolog.
- The Hd1 has both conserved domains and also exhibits sequence similarity with CO and CO-like *Arabidopsis* proteins in regions between these domains.

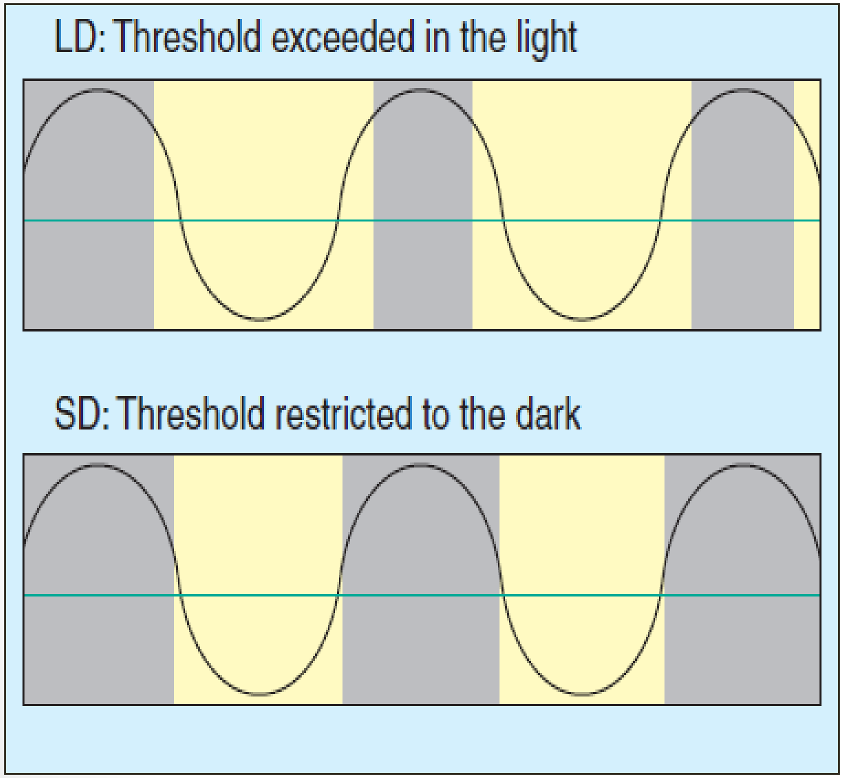
- In the obligatory short-day plant *Pharbitis*, a CO homolog (*PnCO*) was identified by differential analysis of induced versus non-induced tissues.
- When overexpressed in transgenic *Arabidopsis* plants, *PnCO* was capable of promoting flowering of *co* mutants

Internal Coincidence Model, (Colin Pittendrigh and Dorothea Minis 1964)

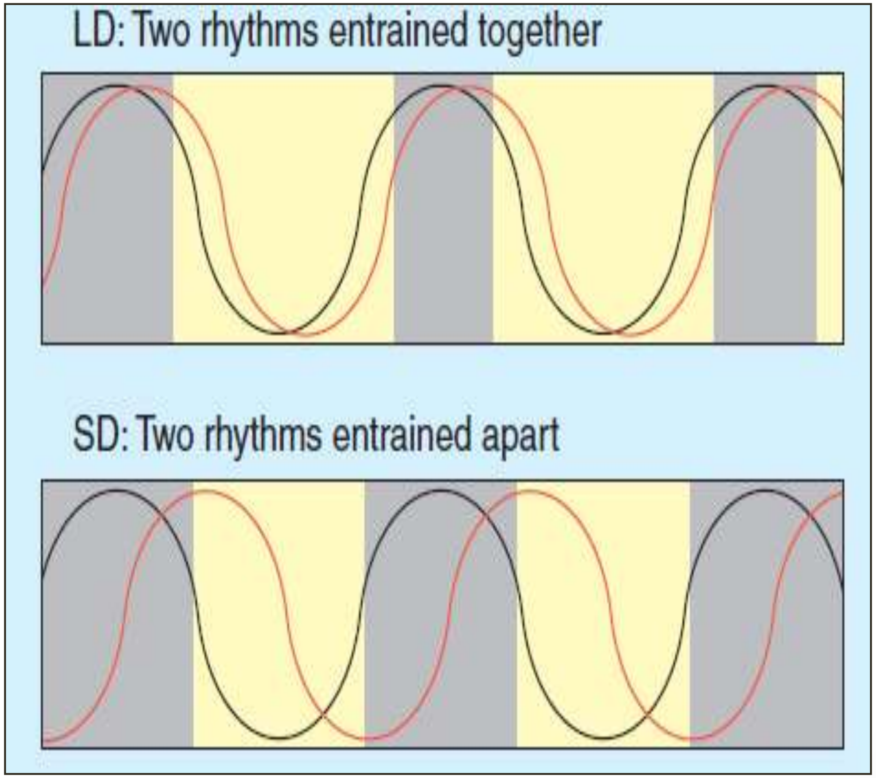
- Light has only one role - to entrain the circadian system
- It is based on the fact that photoperiodic flowering involves more than one circadian pacemaker
- Each of the oscillators will behave differently under the influence of the light-dark cycles, and assume different phase-relationships with the entraining cycle
- Changing photoperiods may alter the internal phase-relationships between two or more rhythms, bringing them into permissive or inhibitory modes

Comparing external and internal coincidence

External Coincidence



Internal Coincidence



Summary

- Physiological response of plants to day length perception are termed as Photoperiodism. (however the plant actually measures night length)
- These responses vary from SDP to LDP to DNP
- Photoperiod primarily affects flowering through a molecularly regulated framework of photoreceptors, transcription factors , Homeotic genes (ABC) and integrative genes (MADS-Box)
- Apart from the Phytochrome mediated hourglass mechanism photoperiodic flowering is more regulated by the circadian mechanism where CO is the key regulator
- Daylength effects CO expression which in turn changes FT or SOC1 expression to induce flowering
- It is also believed that other than CO there may be some more Key regulators that may be involved in triggering flowering through internal coincidence.

Telling Floral Time

Certain flowers open and close on schedule, taking their cues from circadian rhythms. A gardener could build a "flower clock" with blooms opening each hour.

KEY
■ Opens by ■ Closes by

SOURCE: THE SCIENCE MUSEUM OF VIRGINIA

8 A.M.
Scarlet Pimpernel
■ 8 a.m. ■ 2 p.m.

9 A.M.
Dandelion
■ 9 a.m. ■ 5 p.m.

10 A.M.
Morning Glory
■ 10 a.m. ■ evening

11 A.M.
Water Lily
■ 11 a.m. ■ 2 p.m.

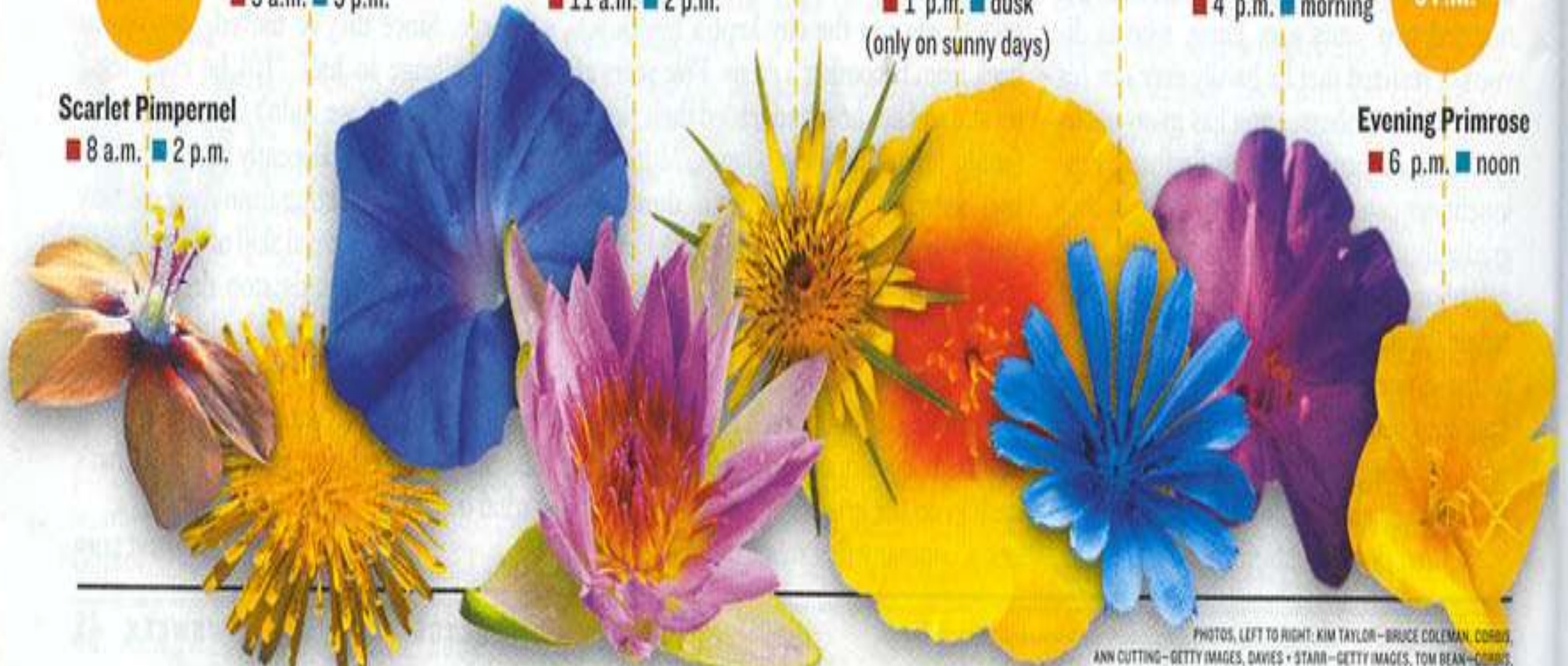
NOON
Goatsbeard
■ noon ■ 6 p.m.

1 P.M.
California Poppy
■ 1 p.m. ■ dusk
(only on sunny days)

2 P.M.
Chicory
■ 2 p.m. ■ 5 p.m.

4 P.M.
Four O'Clock
■ 4 p.m. ■ morning

6 P.M.
Evening Primrose
■ 6 p.m. ■ noon



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Thank you

Questions ?