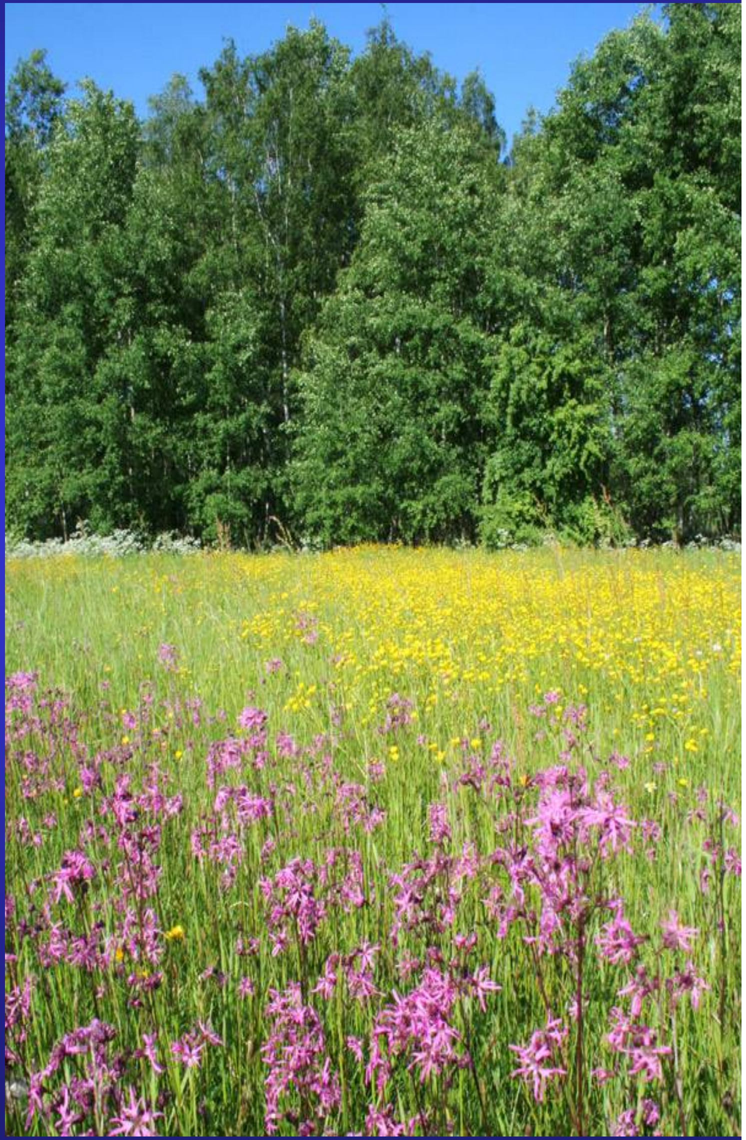
3) Plant reproduction

a) Flowering induction

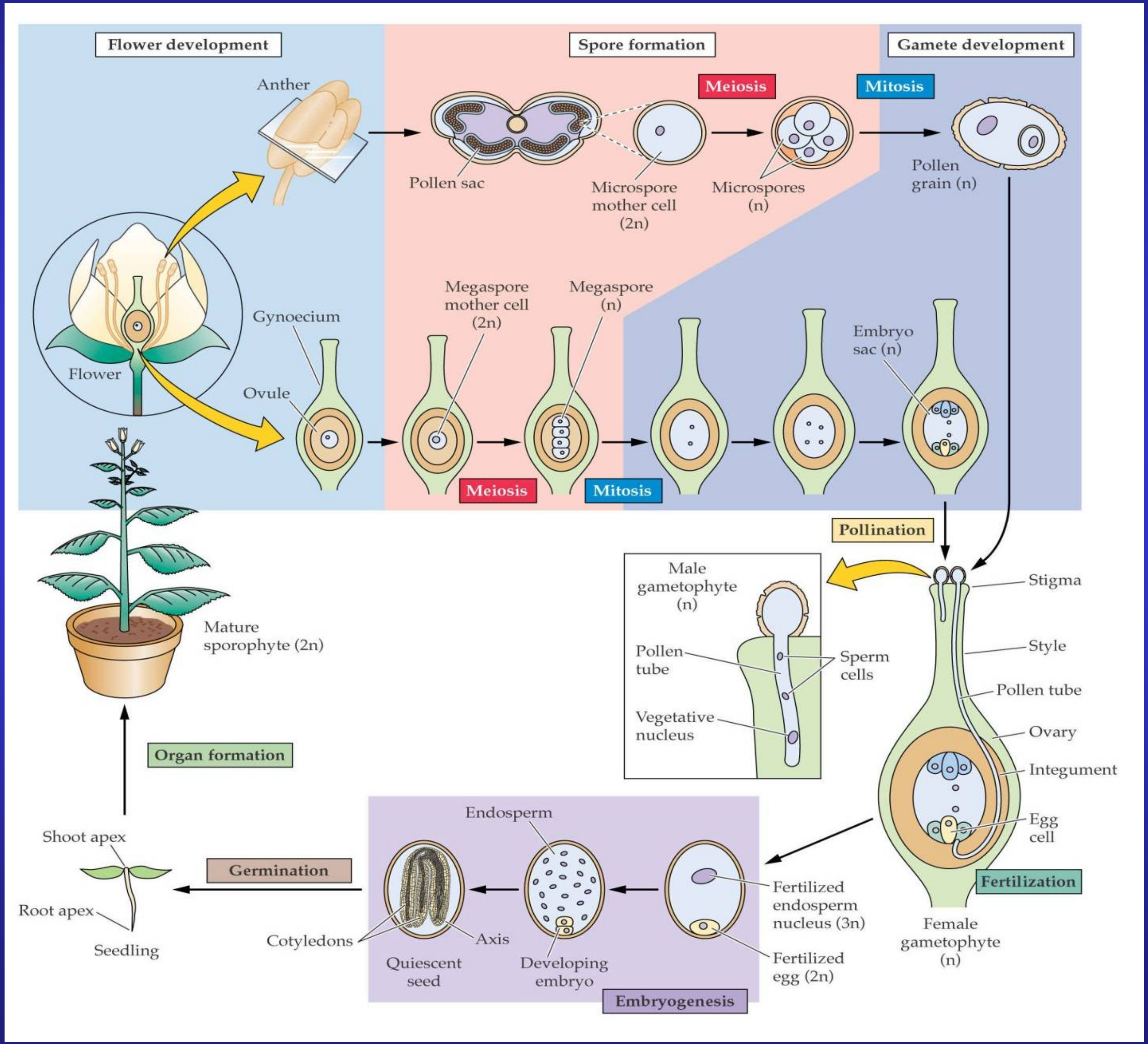
b) Flower development - brief morphology

c) Genetic and molecular analysis of flower development



PMP

Leaf cycle of plants



a) Flowering induction

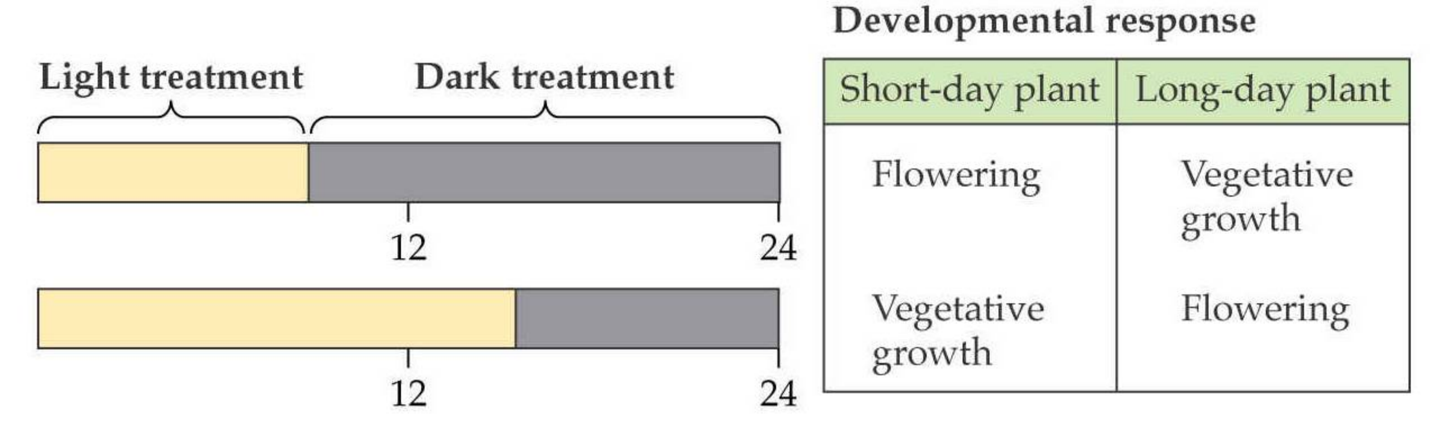
Flower induction – reprogramming of vegetative meristems:
site of secondary shoots, where flower organs are developing

Ability of plants to reprogram the vegetative development to the reproductive development shows important developmental difference between plants and animals.

By this reprograming the plant starts its own senescence and death.

The reprogramming must be exactly timed.

Flowering is induced by a photoperiod = ratio between length of day and night



Developmental response

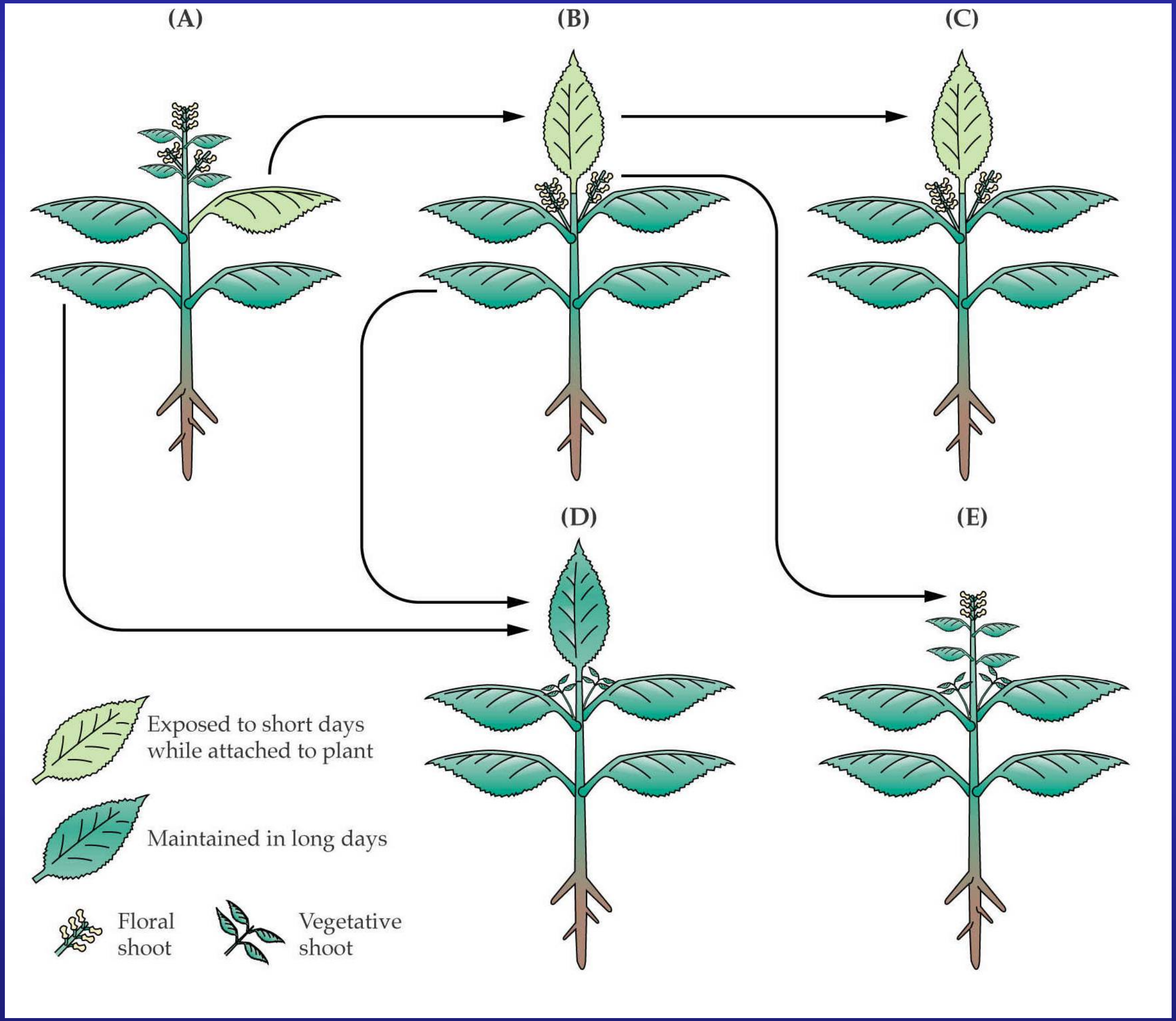
Short-day plant	Long-day plant
Flowering	Vegetative growth
Vegetative growth	Flowering

Short-day plant – flowering is induced by short day and long night (e.g. chrysanthemum, strawberry, spinach)

Long-day plants – flowering is induced by long day and short night (e.g. wheat, *Arabidopsis*)

Neutral plants – flowering is not induced by photoperiod (e.g. tomato)

Leaf – perceives photoperiod signal inducing transition of plants to flowering phase



Phytochromes = photosensitive pigments in the cytoplasm – they absorb red light and are involved in flowering induction, germination, and in sensing the photoperiod.

Phytochromes are encoded by 5 genes: *PHYA, PHYB, PHYC, PHYD, PHYE*

PHYA and *PHYB* regulate flowering time

Mutant phyB flowers early

Mutant phyA flowers late

Mutant phyAphyB flowers earlier than *phyB*

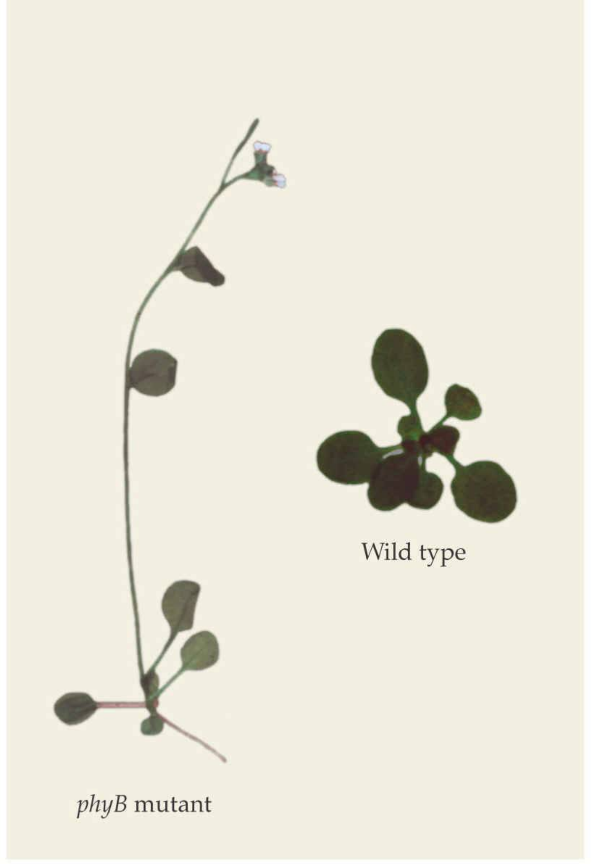
⇩

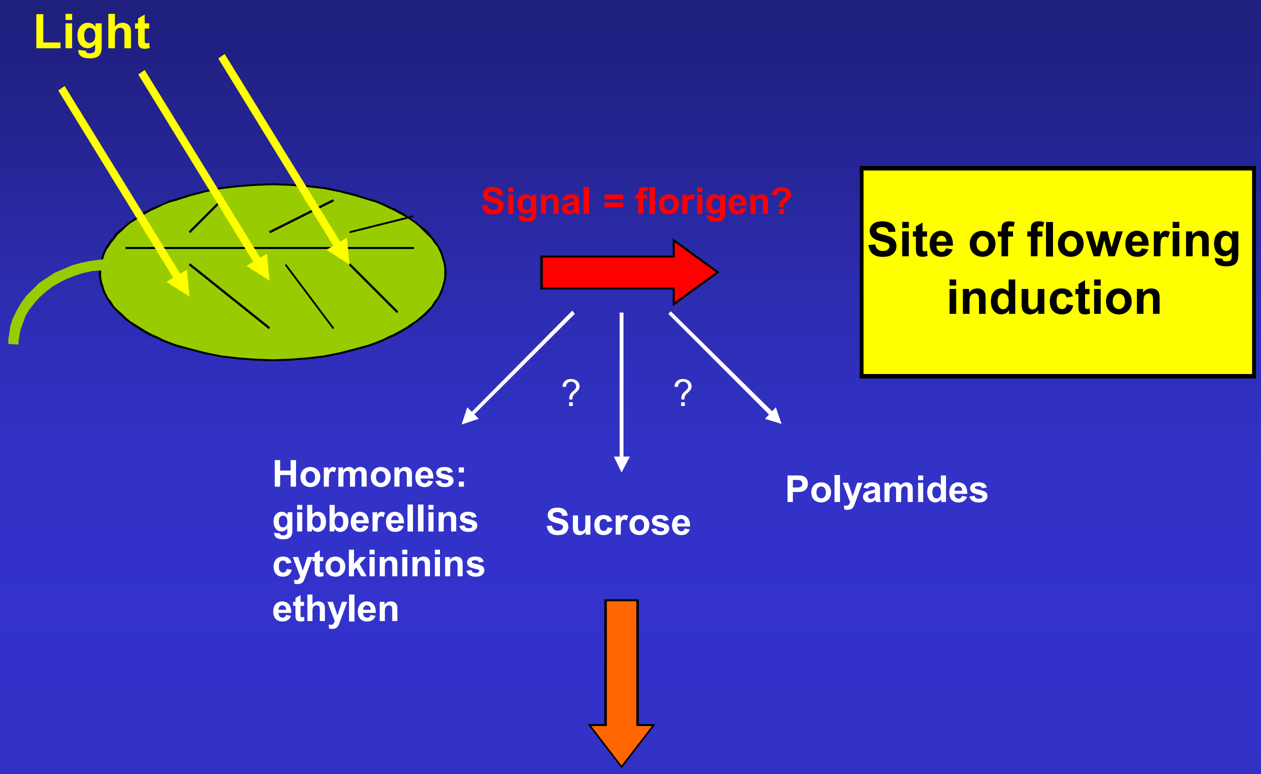
Flowering is complex process

+

Cryptochromes – sense blue light

Photosynthesis pigments





Light

Signal = florigen?

Site of flowering induction

? ?

Hormones:
gibberellins
cytokininins
ethylen

Sucrose

Polyamides

Hypothesis multifactorial triggering:
Functionality of one molecule influences
other functions

Term florigen – Mikhail Chailakhyan, Russia 1936 (experiments in 1932-1934)

Corbesier and Coupland (2005) – hypothesis: Florigen is RNA or protein, which is transported from leaves to meristem

Huang T *et al*. (2005)
Science 309: 1633-1772

Abe M *et al*. (2005)
Science 309: 1052-1056

Wigge PA *et al*. (2005)
Science 309: 1056-1059

Light (long day)

***CO* (*CONSTANS*)**

CO protein
(transcription factor)

***FT* (*FLOWERING LOCUS T*)**

Expression of *FT*

mRNA of *FT*

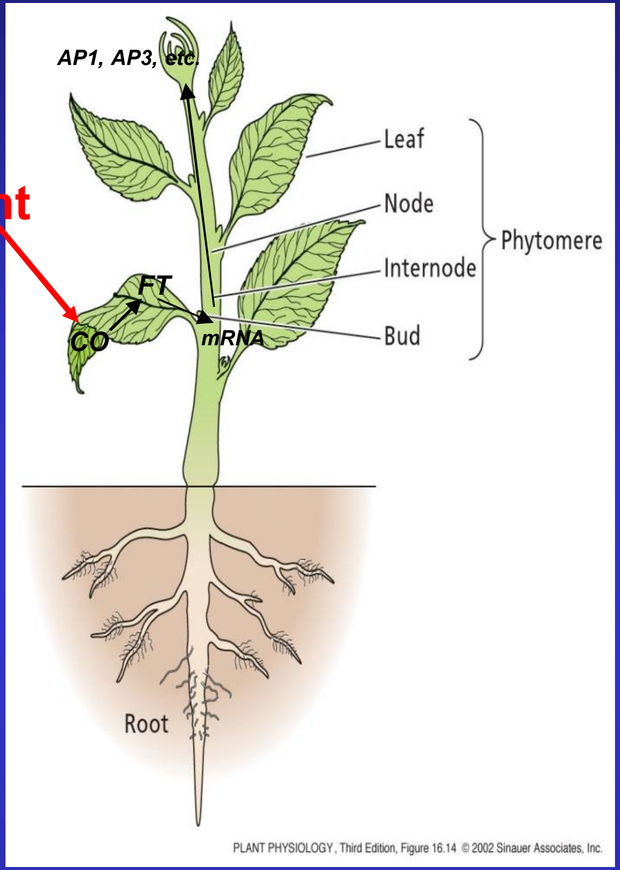
Shoot apex

FD + **FT**

FD (FLOWERING LOCUS D)

AP1
AP3
LFY
CAL

Flowering induction



PLANT PHYSIOLOGY, Third Edition, Figure 16.14 © 2002 Sinauer Associates, Inc.

! **UPDATE 2007**

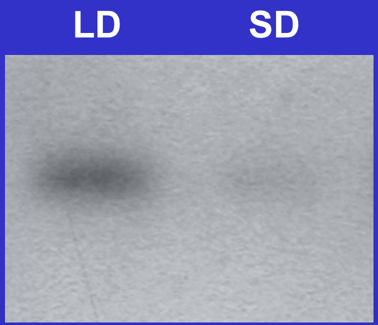
Jaeger KE, Wigge PA (2007) Cur Biol 17: 1-5

Mathieu J et al. (2007) Cur Biol 17: 1055-1060

Protein FT is transported from leaves is sufficient for flowering induction

***CONSTANS* (*CO*)** – identified by analysis of late flowering mutant; Functional *CO* codes for transcription factor and is up-regulated in long day (LD = long day); *CO* activates expression of flowering genes

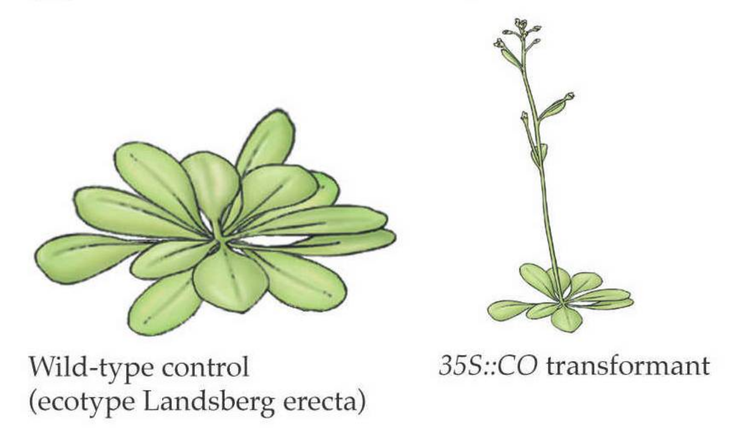
Northern blot = amount of RNA



Flowering induction

Construct: strong promoter *35S* + gen *CO*

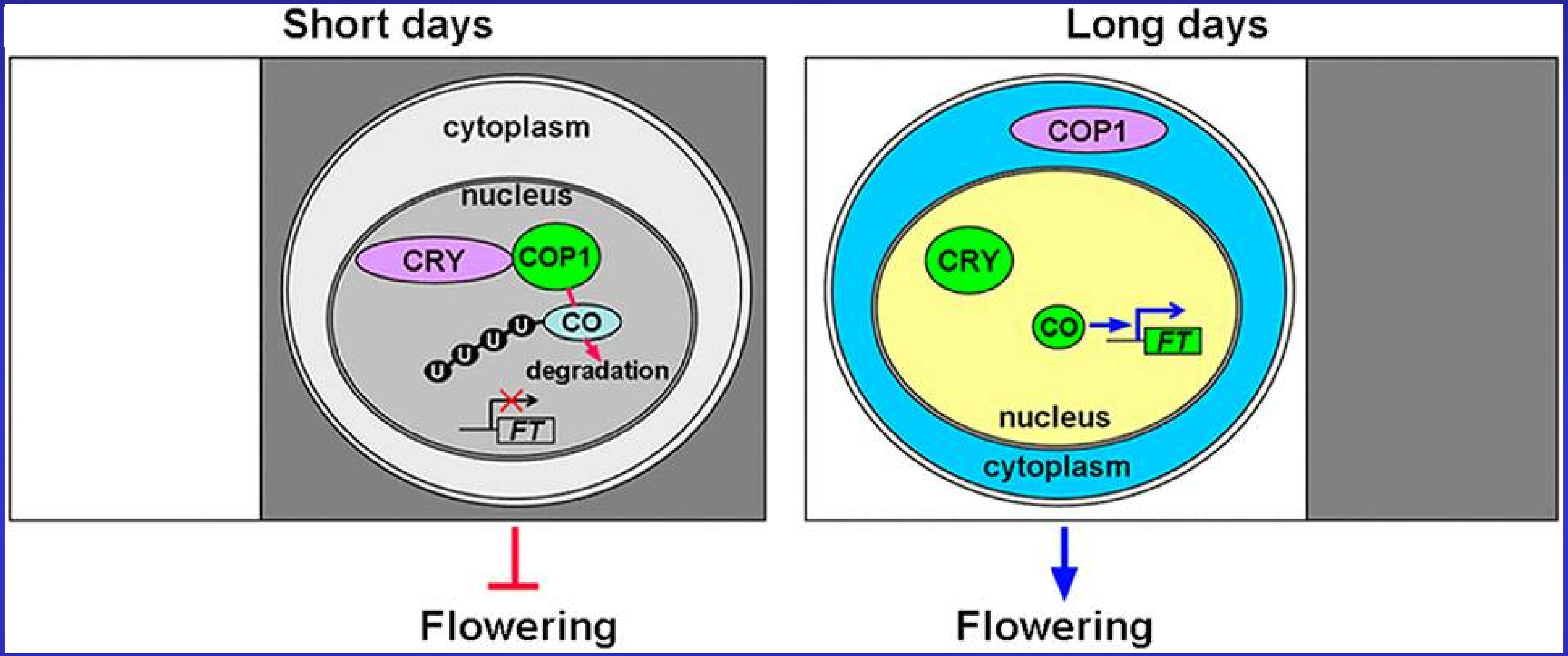
Flowering is induced even in short day



CO functions as a transcription factor and as co-activator as well

UPDATE 2008

Liu L-J et al. (2008) Plant Cell 20: 292-306



Short day (SD) CRY1 off => COP1 accumulation in the nucleus => ubiquitination of CO => FT is not expressed => inhibition of flowering

Long day (LD) CRY1 on => translocation of COP1 to cytoplasm => CO accumulation in the nucleus => FT is expressed – induction of flowering

Genes determining the identity of flower meristems

LEAFY (LFY)
TERMINAL FLOWER1 (TFL1)
APETALA1 (AP1)
CAULIFLOWER (CAL)

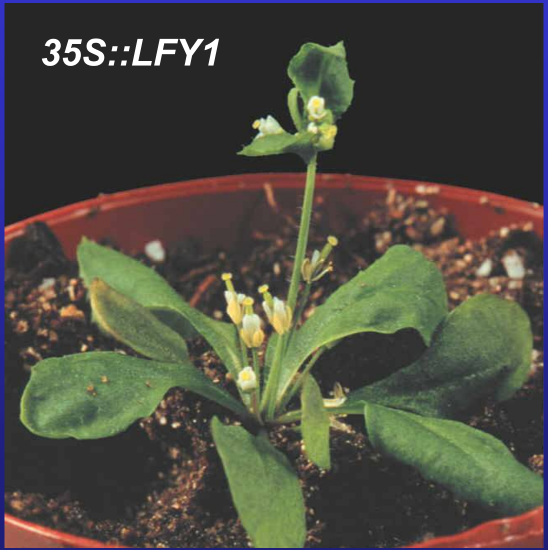
LEAFY (LFY) – mutant *lfy* produces more flower shoots than WT plants; flowers are green and have only organs similar to sepals and petals

Indeterminate growth

Ectopic (and constitutive) expression of *LFY1* => premature flowering; shoots are changing to flowers.

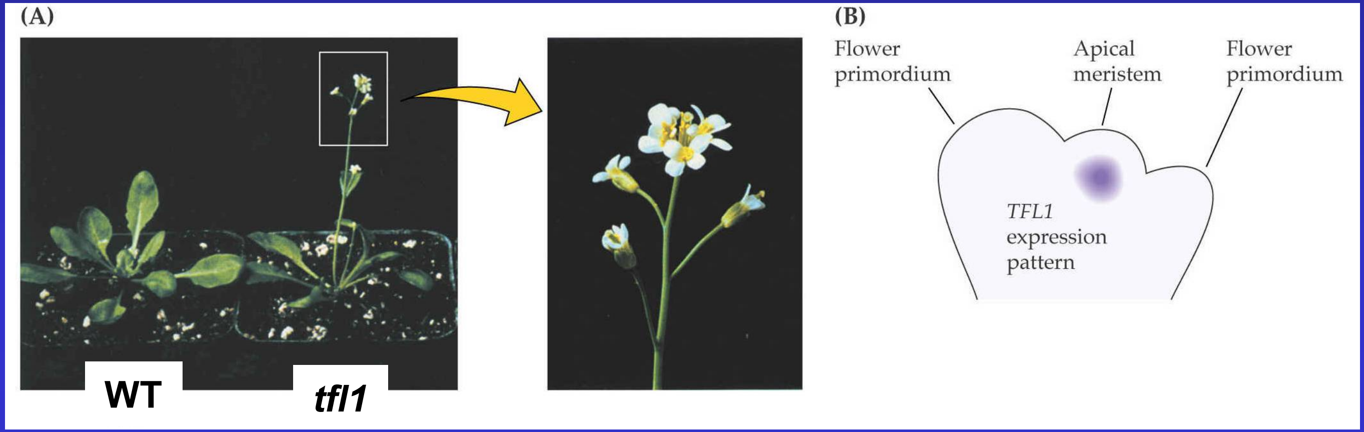
↓

Normal function of *LFY* = switching indeterminate growth to determinate growth



Determinate growth

TFL1 – mutant *tfl1* flowers early; it forms primary flower shoot, it does not form lateral shoots = phenotype opposite to *lfy*; *TFL1* is expressed in the apical meristem, instead in the flower primordia



Normal function of *TFL1:* in maintains **In**determinate growth

TFL1 → **bumb** ← **LFY**

TFL1 and **LFY** products act against each to other

In early phases of flower development, the function of *LFY* is similar to the functions of the genes *AP1, AP2 and CAL* and their mutually amplify each other.

Flower induction – 5 developmental pathways

1. Photoperiod (phytochromes, cryptochromes, *CO*, *FT*, mRNA *FT*, FT/FD protein, *SOC1*)

SOC1 = Suppressor Of Constans1;
Transcription factor containing MADS box

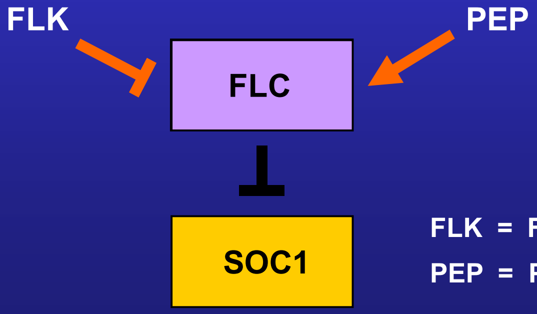
2. Autonomous and vernalization

Autonomous

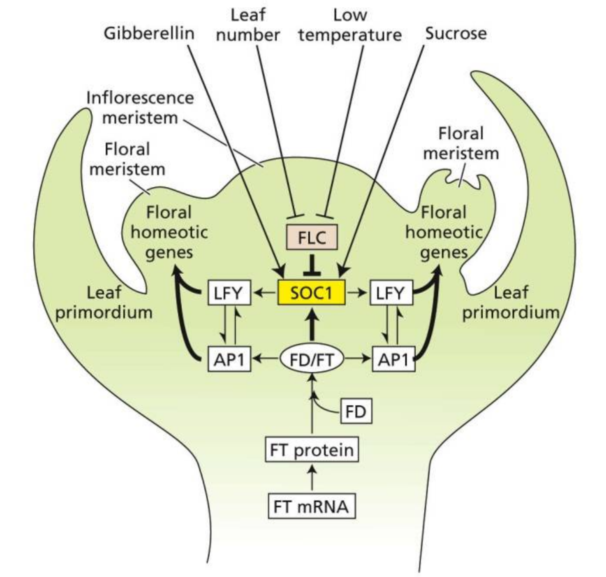
Internal signals = number of leaves – reduces expression of *FLOWERING LOCUS C* (*FLC*) => stimulation of *SOC1*

UPDATE 2009

Ripoll JJ et al. (2009) Developmental Biology 333: 251-262


FLK
PEP
FLC
SOC1

FLK = FLOWERING LOCUS K
PEP = PEPPER



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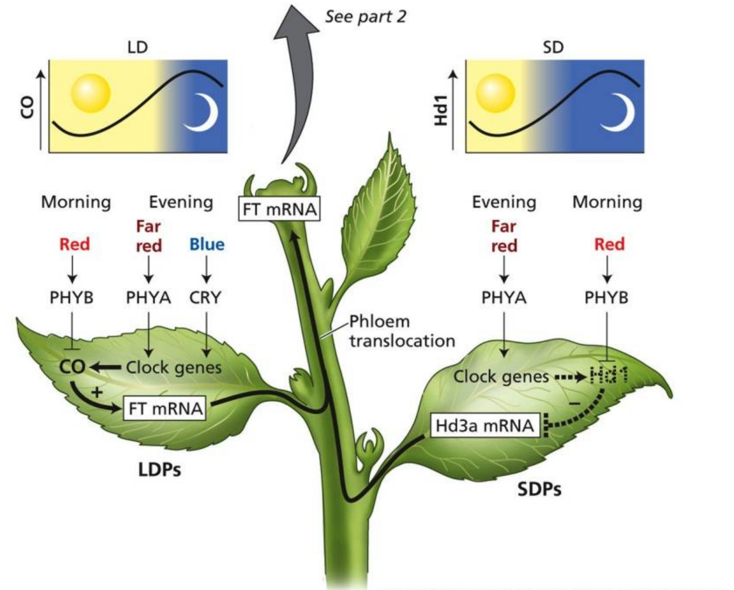


PLANT PHYSIOLOGY, Fourth Edition, Figure 25.33 (Part 1) © 2006 Sinauer Associates, Inc.

Vernalization: low temperature

- reduction of repressor *FLOWERING LOCUS C* (*FLC*) => stimulation *SOC1*

3. Carbohydrate

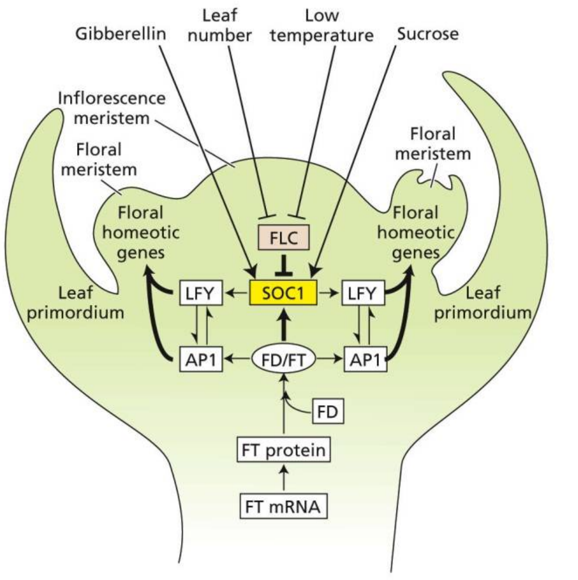
- reflects metabolic status of plants = sucrose stimulates flowering induction SOC1 => expression

4. Gibberellin – required for premature flowering and for short-day-induced flowering

- gibberellins induce transcription factors of GAMYB family => stimulation of *LFY*

- gibberellins interact directly with SOC1 => activation of *LFY*

- GA_4 is active gibberellin



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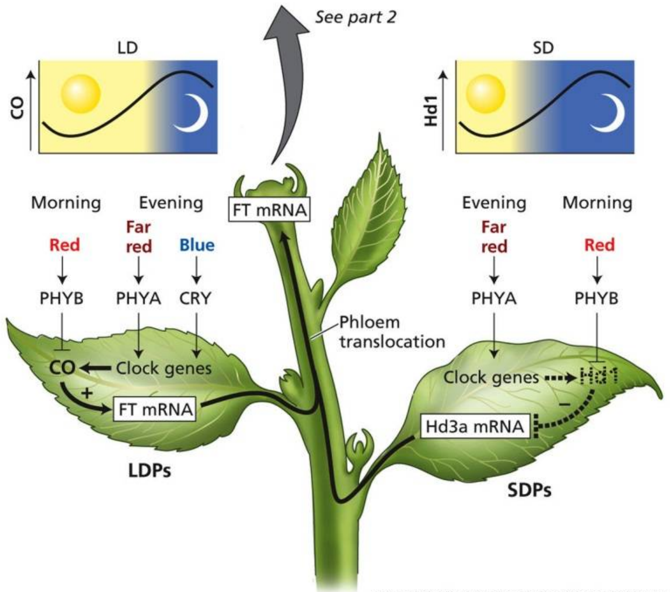


PLANT PHYSIOLOGY, Fourth Edition, Figure 25.33 (Part 1) © 2006 Sinauer Associates, Inc.

UPDATE 2010

Fornava et al. (2010) Cell 14: 550-550e

5. Aging

- Reflects age of plant

Young plant => high level of **miR-156**

⊥

SPL

↙

LFY
SOC1 → Flowering induction

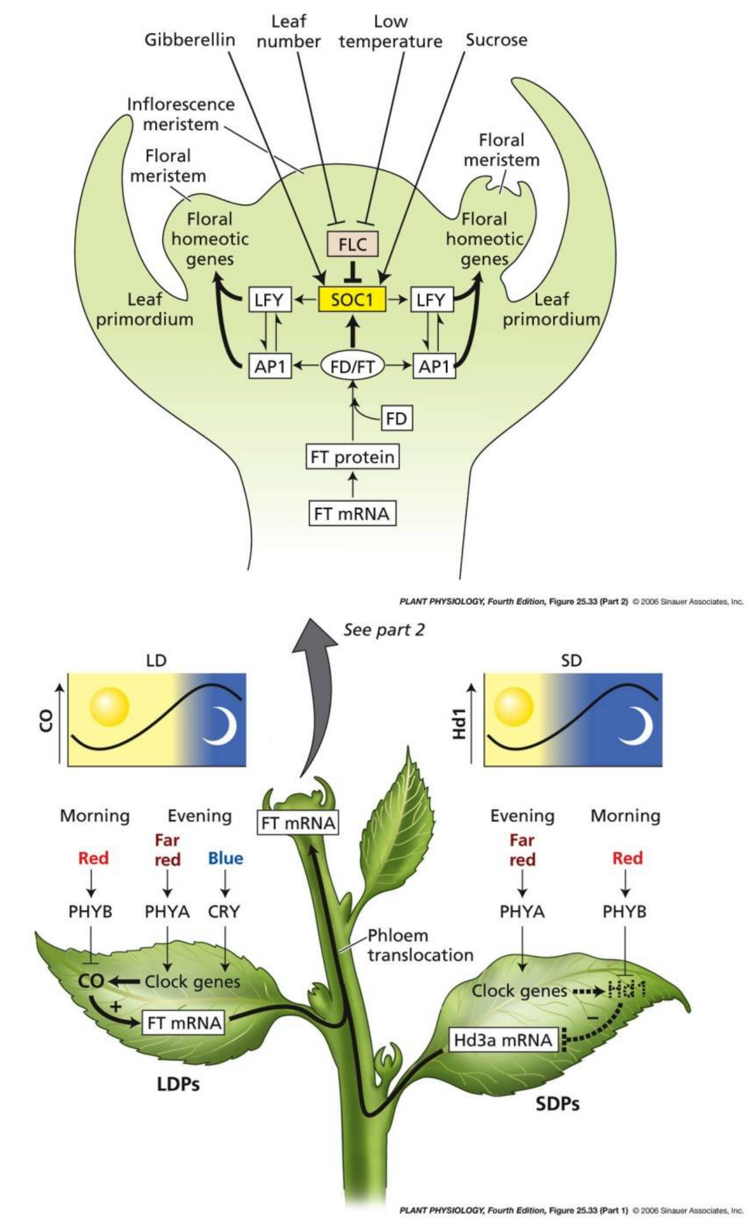
Pathways 1 to 5 converge in **SOC1**

↓

Induction of LFY and AP1

↓

Induction of flower homeotic genes **(model ABC)**



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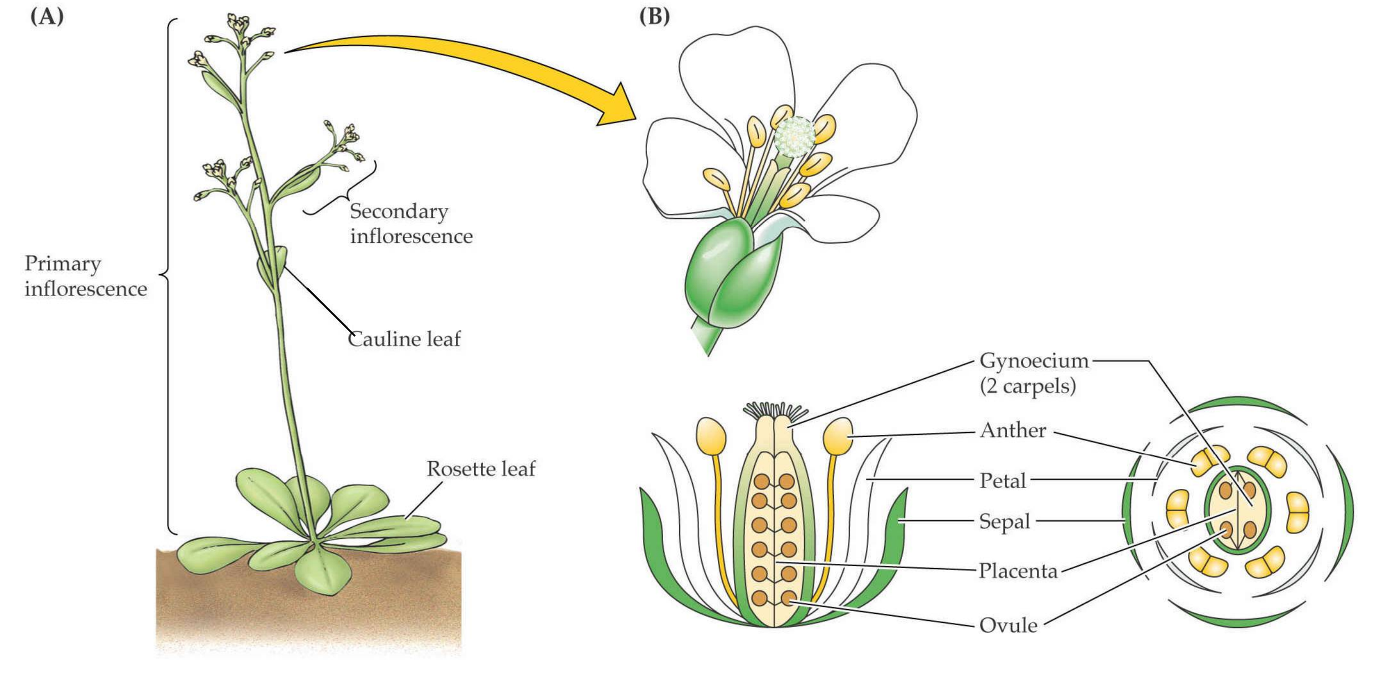
b) Flower development – brief morphology

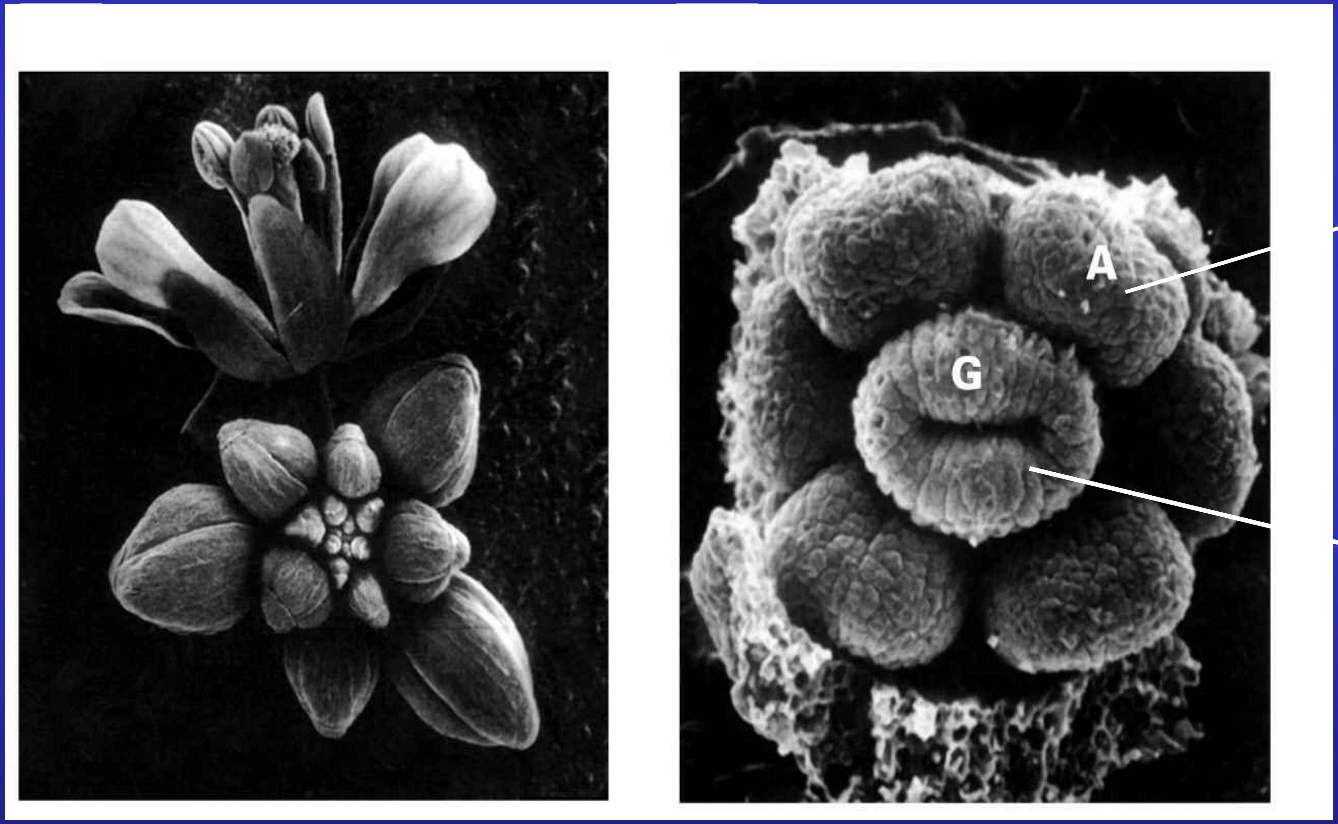
Model plant *Arabidopsis* => 1st lecture PMP

Structure of *Arabidopsis* flower and its development

Signal => flower induction => apical meristem produces flowers



Flower are formed in a curl around the central meristem



Anther

Gynoecium
(carpels)

c) Genetic and molecular analysis of flower development

Analysis of genetic mutations, which alter specification of flower development allow to define and distinguish basic processes on molecular level

ABC model

Thanks to mutations (T-DNA mutant library), which results in reduction or in changes of positions of flower organs, 4 genes were identified that play key role in development of flower organs: ***AP2, AP3, PI, AG***.

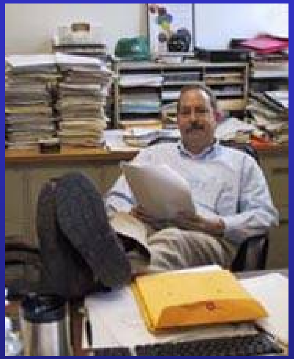
Isolation and characterization of mutants → Gene cloning and proposal of ABC model

Bowman JL et al. (1991) Development 112: 1-20

Prof. M. Koornneef (Wageningen, Holandsko)

Prof. John Bowman

http://www.dbs.ucdavis.edu/

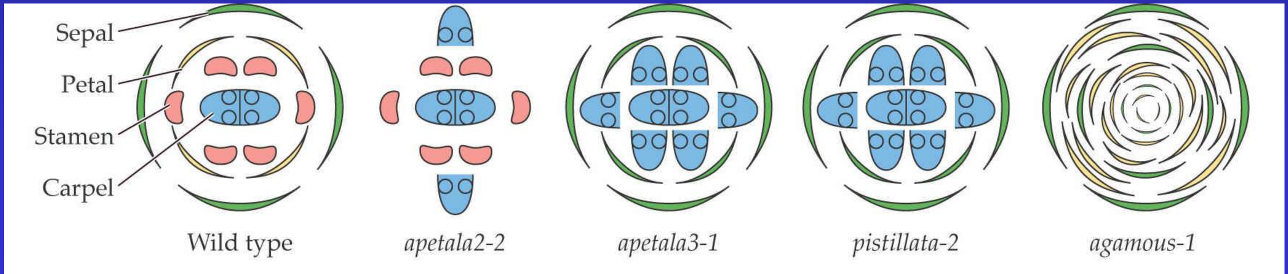


Prof. Elliot Meyerowitz

http://www.its.caltech.edu/~plantlab/

Genes playing key role in development of flower organs (flower homeotic genes):

Flower phenotypes of the mutants



Mutant *ap2* : sepals → carpels petals → stamens

Mutant *ap3* :

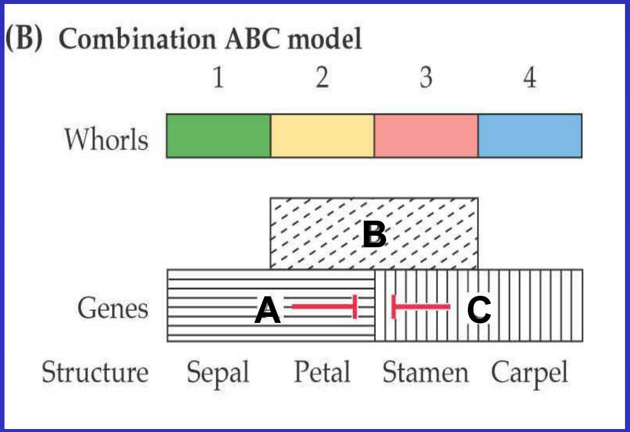
Mutant *pi* : petals → sepals stamens → carpels

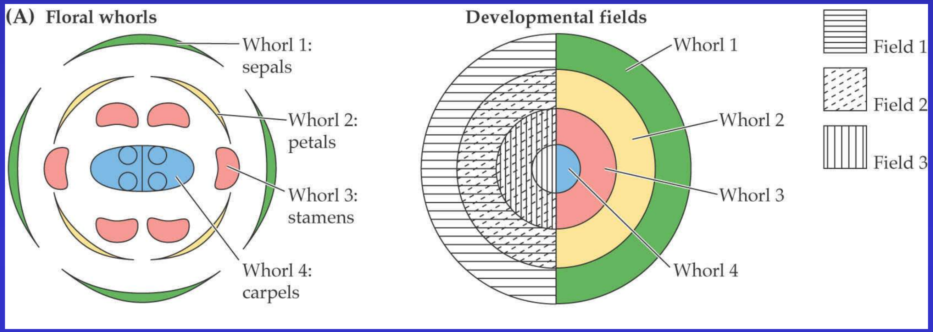
Mutant *ag* : stamens → petals carpels → 2. *ag* flower

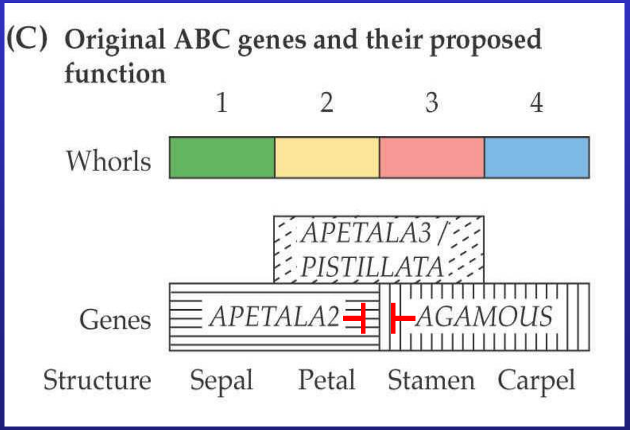
AP2, AP3, PI, AG were named homeotic genes, because they were revealed also in other plant species. Unlike classic homeotic genes, they do not code for proteins with homeodomain.

ABC model of flower organ development

Flower primordium consists of 3 concentric and overlapping fields (whorls) of gene activity: A, B, C





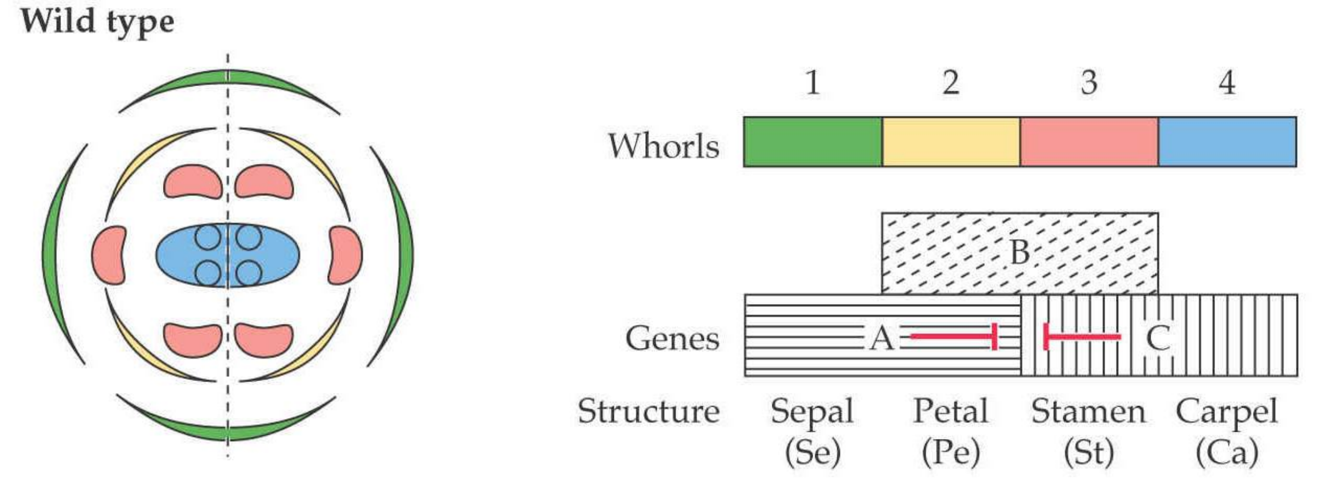


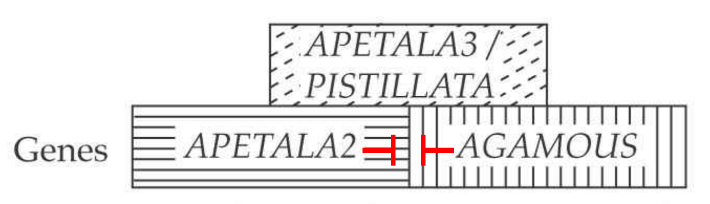
AP2 controls field **A** = **sepals** + **petals**

AP3 / PI controls field **B** = **petals** + **stamens**

AG controls field **C** = **stamens** + **carpels**

ABC model explains development of specific organs in WT flower

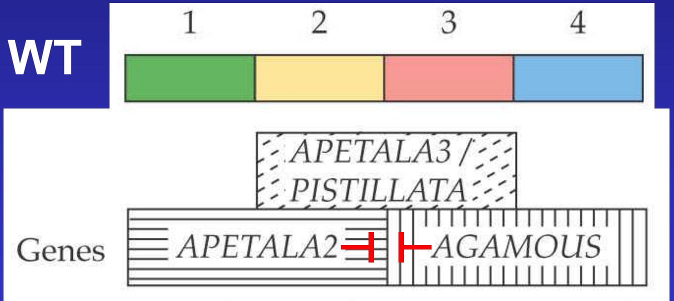




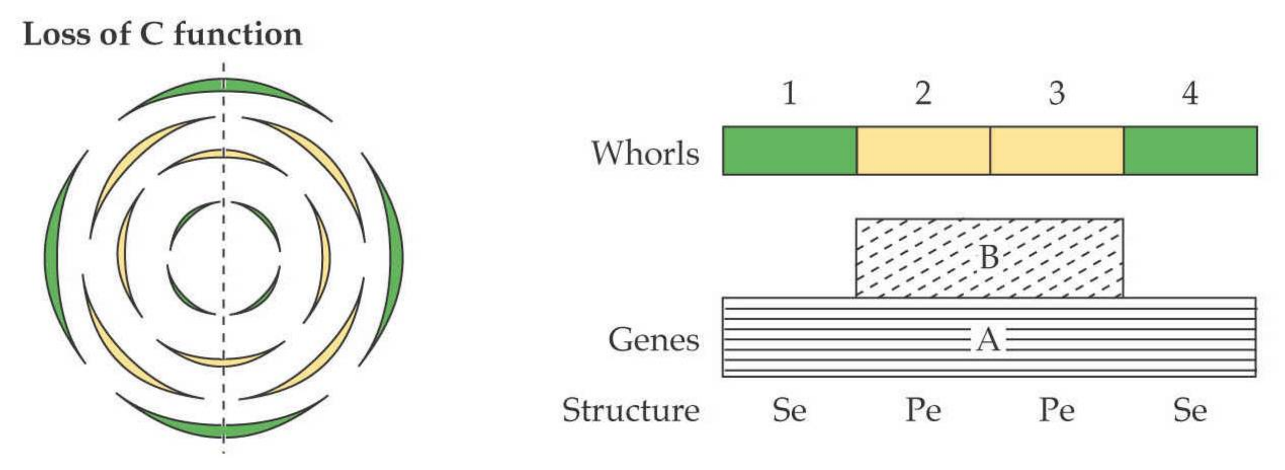
WT : all genes function normally (correctly)

ABC model explains development of specific organs in the mutants

WT



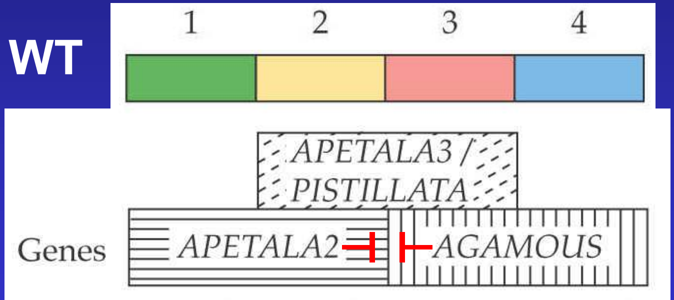
ag



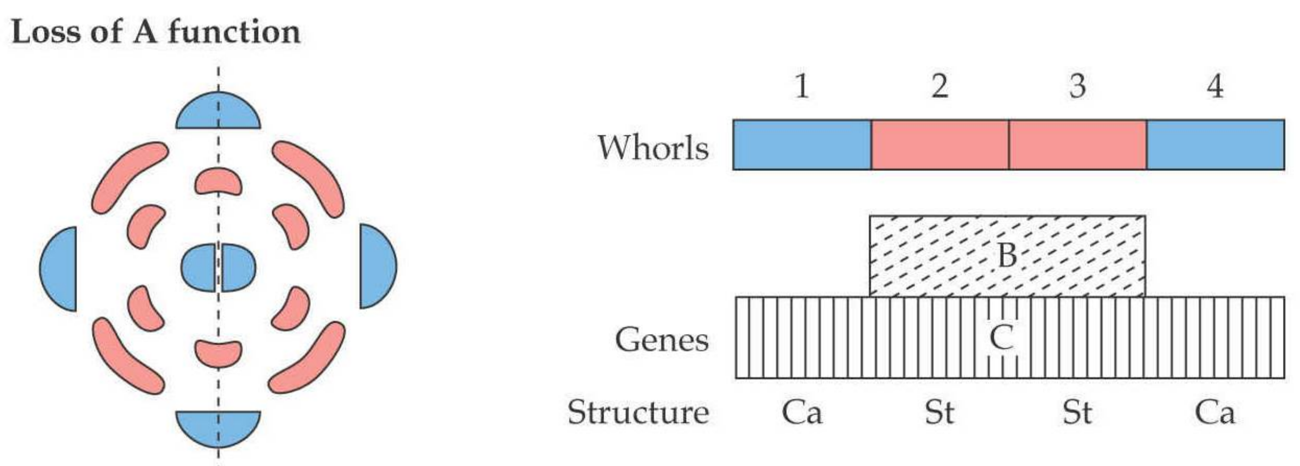
AG gene is off => *AG* does not operate against *AP2* => *AP2* expands into field 3 a 4 => **stamens** ⟶ **petals** **carpels** ⟶ **sepals**

ABC model explains development of specific organs in the mutants

WT

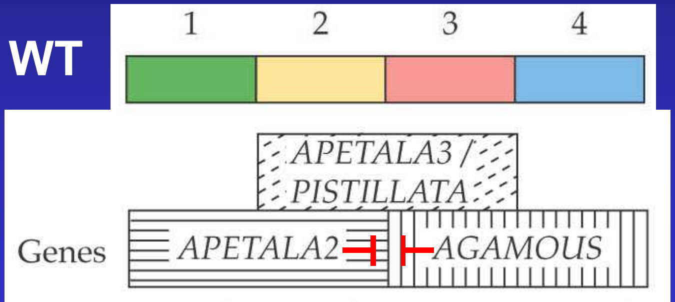


ap2

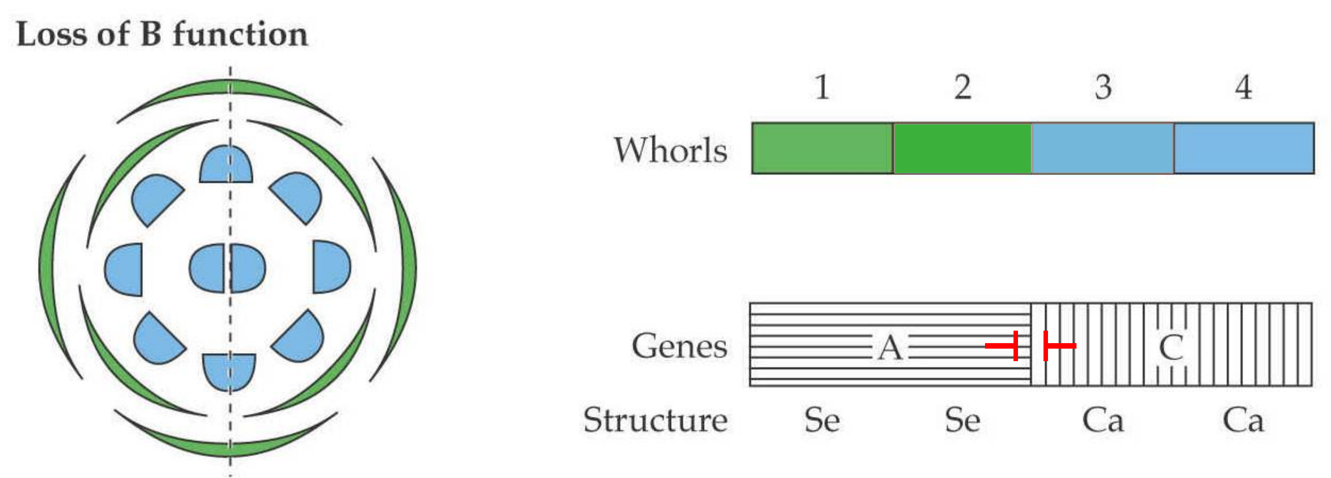


AP2 gene is off => *AP2* does not operate against *AG* => *AG* expands into field 1 a 2 => **sepals** → **carpels** **petals** → **stamens**

ABC model explains development of specific organs in the mutants

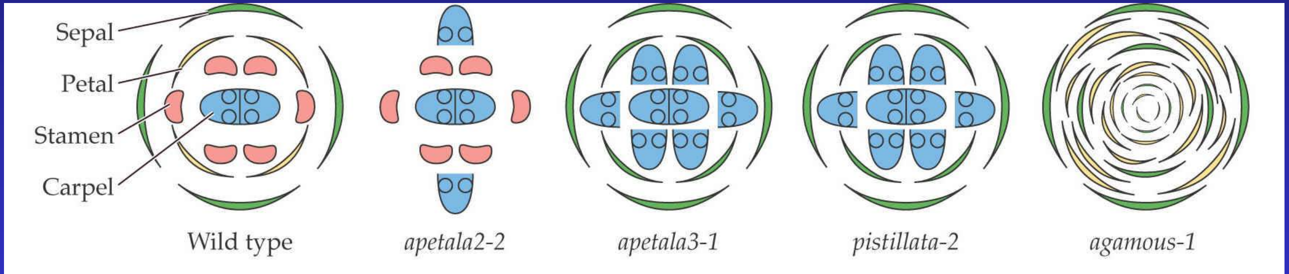


ap3 / pi

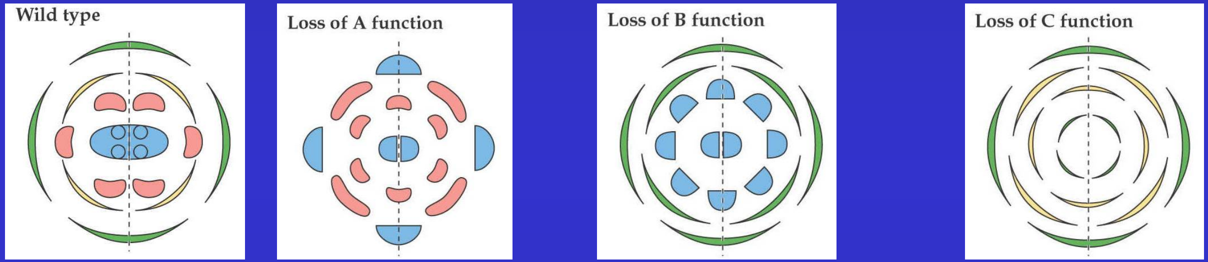


AP3 / PI genes are off => *AP3/PI* does not act in the combination with *AP2* in the field 2 neither in the field 3 => **petals** → **sepals** **stamens** → **carpels**

Observed phenotypes



Phenotypes predicted from the ABC model



Variance from observed phenotype

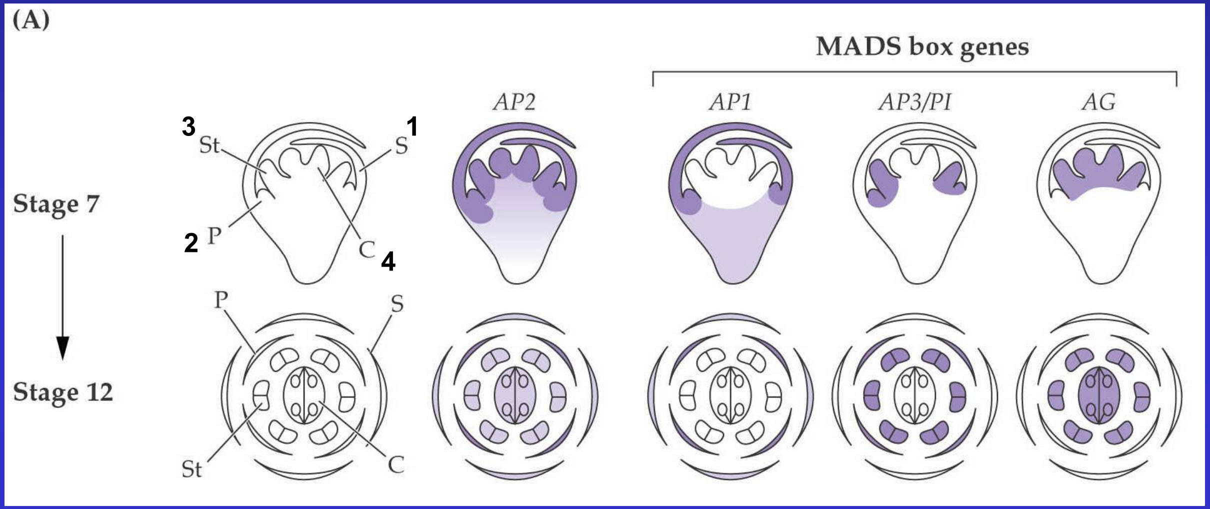
Variance from observed phenotype

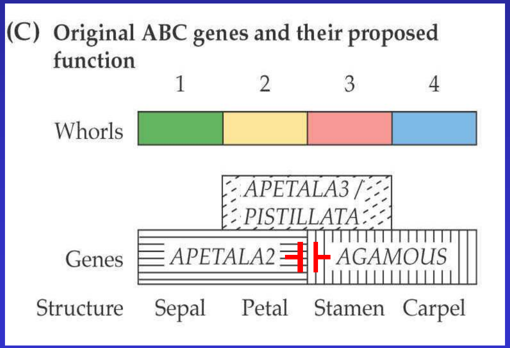
ABC model corresponds well with the observed phenotypes of the mutants, although not completely.

Model revision

ABC model was revised based on the study of gene expression using hybridization *in situ* during flower development.

Gene expression in the organs in WT plants



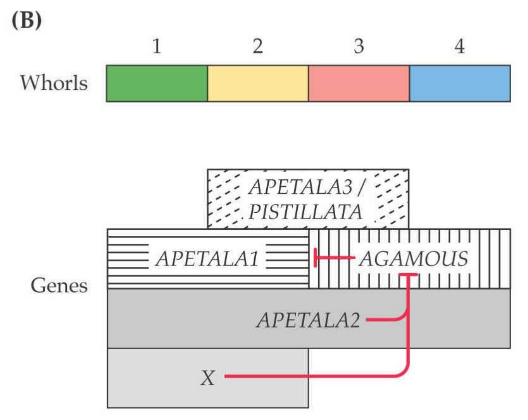


Expression of *AP2* in 1 and 2 **OK**. **But**, expression in 3 and 4 is unexpected as the original model proposes a suppression by *AG*.

But, when *AP2* is expressed in 3 and 4, then expression of AG in these fields should be suppressed – but it is not!

OK, *AP3* / *PI* is expressed in 2 and 3

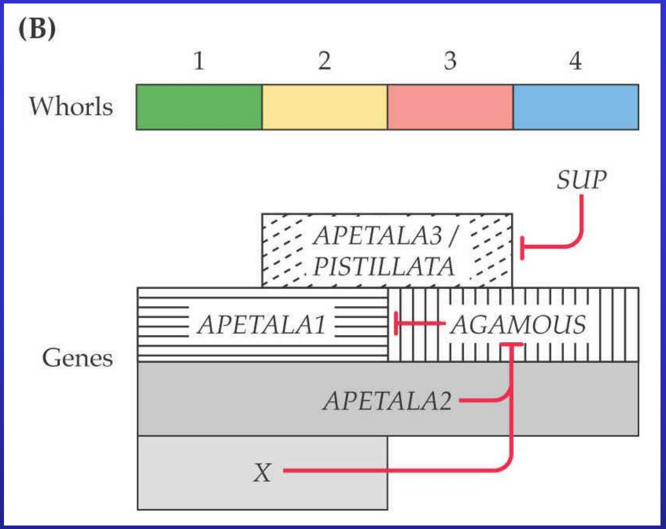
Model revision: unknown gene *X* + gene *AP1*



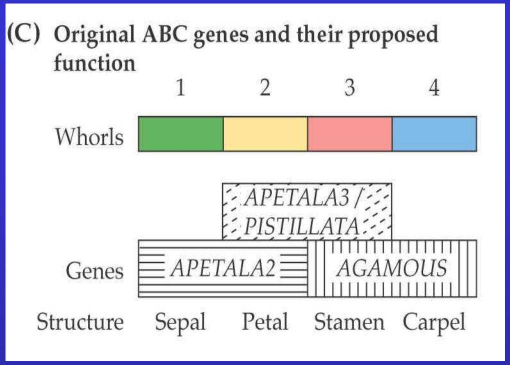
Another revision of the ABC model

Mutant *sup (superman)* was found – expression of gene *AP3 / PI* expands into field 4, where stamens started to be formed. It means that gene *SUP* negatively regulates *AP3 / PI*

The revised ABC model



The original ABC model

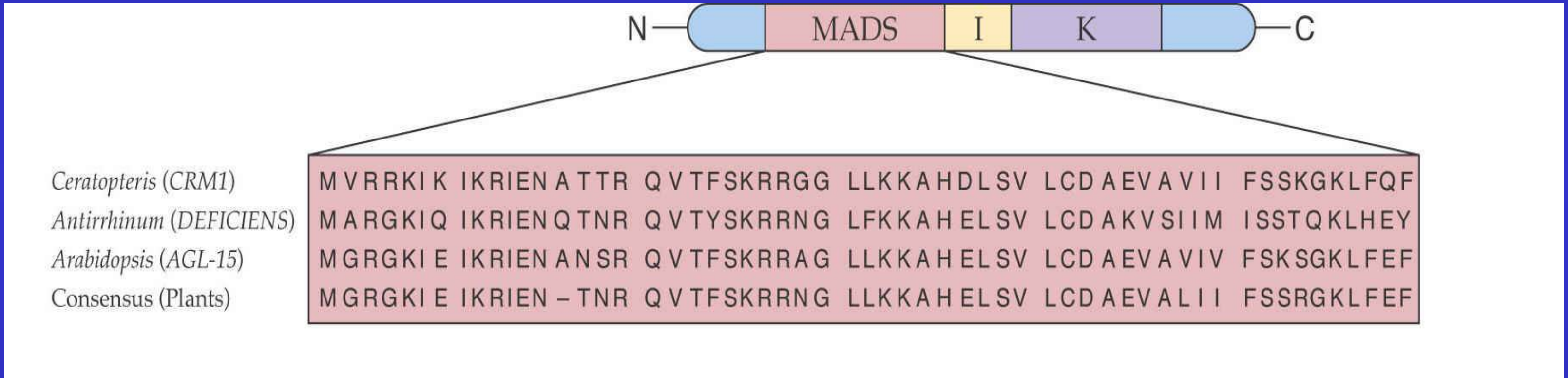


Sakai H et al. (2000) Plant Cell 12: 1607-1618

Nakagawa H et al. (2004) Plant Cell 16: 920-932

Products of homeotic genes = proteins

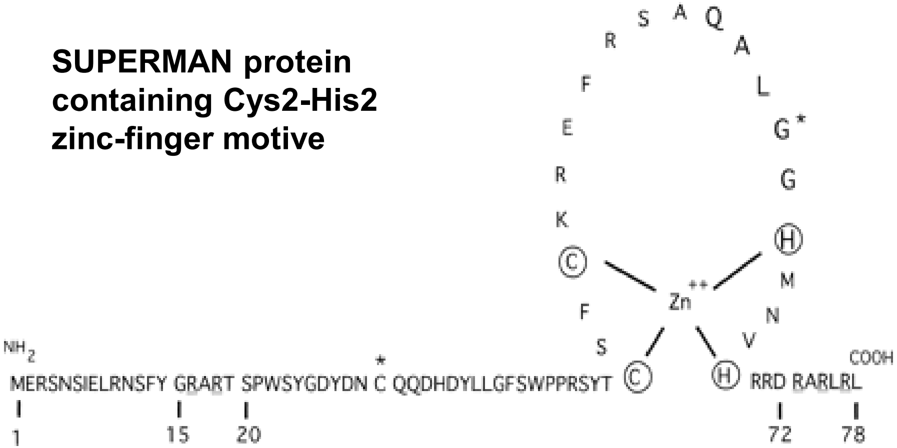
AG, PI, AP1, AP3 – a family of evolutionary conserved transcription factors. Each of them contains conserved DNA-binding domain: **MADS box**.



Ceratopteris (*CRM1*)	MVRRKIK	IKRIENATTR	QVTFSKRRGG	LLKKAHDLSV	LCDAEVAVII	FSSKGKLFQF
Antirrhinum (*DEFICIENS*)	MARGKIQ	IKRIENQTNR	QVTYSKRRNG	LFKKAHELSV	LCDAKVSIIM	ISSTQKLHEY
Arabidopsis (*AGL-15*)	MGRGKIE	IKRIENANSR	QVTFSKRRAG	LLKKAHELSV	LCDAEVAVIV	FSKSGKLFEF
Consensus (Plants)	MGRGKIE	IKRIEN–TNR	QVTFSKRRNG	LLKKAHELSV	LCDAEVALII	FSSRGKLFEF

These transcription factor control expression of other genes specific for flower organs.

SUP – transcription factor that binds with specifically using Cys2 – His2 zinc-finger motive, and in *Arabidopsis* it controls by this way formation of the interface stamen – carpel.



SUPERMAN protein containing Cys2-His2 zinc-finger motive

Dathan N et al. (2002) Nucleic Acids Research 15: 4945-4951

http://nar.oupjournals.org/cgi/content/full/30/22/4945

AP2 – is not similar to known proteins. It belongs to a new large family of proteins able to bind to DNA. It contains serine-rich acidic domain, which binds to DNA.

Okamuro JK et al. (1997) PNAS USA 94: 7076-7081

http://www.pubmedcentral.gov/picrender.fcgi?tool=pmcentrez&action=stream&blobtype=pdf&artid=21287

Basic principles of the control of flower development based on genetic and molecular analyses of homeotic genes:

1) Genes, coding for transcription factors control spread spectrum of genes that specify fate of a flower.

2) These genes function in mutual combinations.

3) Products of some from these genes (e.g. AP2 and AG; AP3/PI and AP1) control each other their activity.