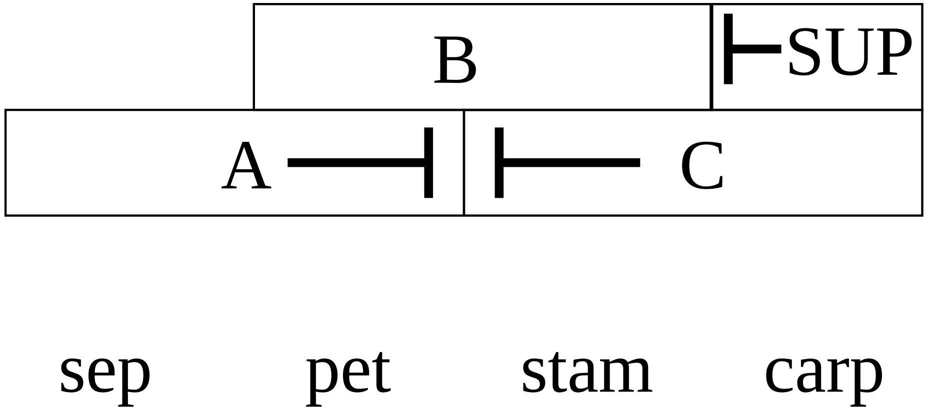
- How functions are combined within the ABC model

- How are flowers initially formed and ABC function gene expression induced ?

Two proteins (AP3 and PI) are required for B function
How do they act together?

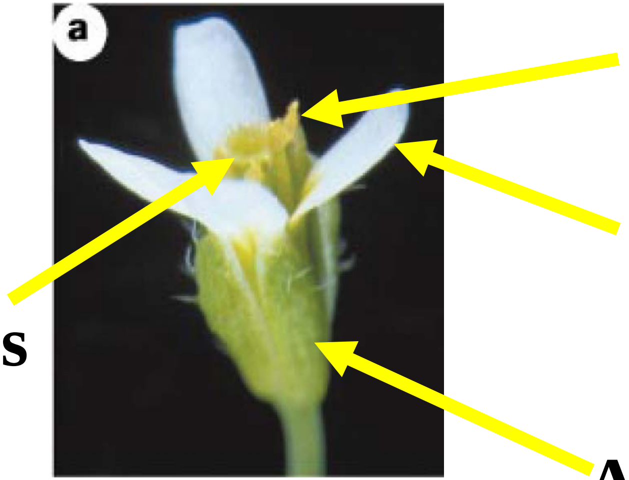
Wild type B=AP3 and PI



B ⊢SUP

A ⊣ ⊢ C

sep pet stam carp



C: Stamens

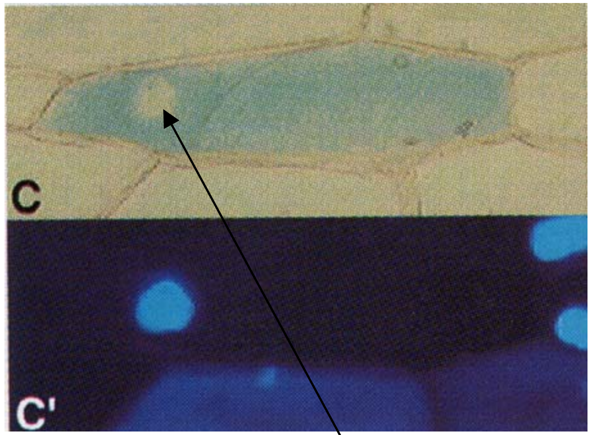
B: Petals

C: Carpels

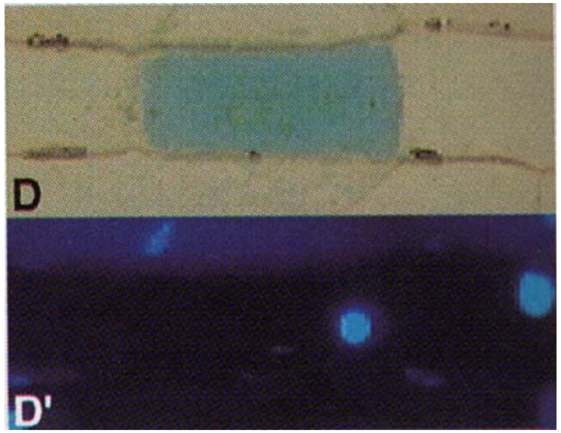
A: Sepals

Co-expression of AP3 and PI is required for nuclear localisation Shown using translational fusions to the GUS marker protein

AP3-GUS

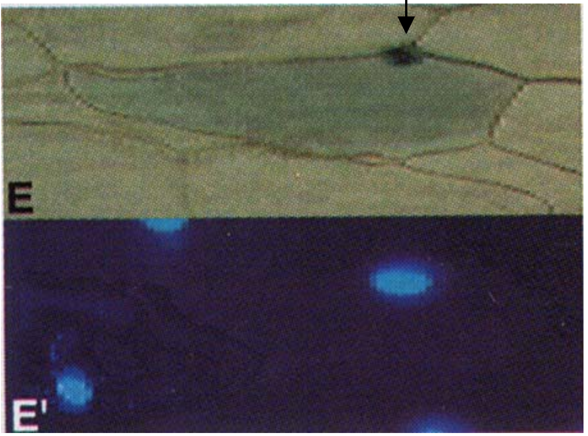


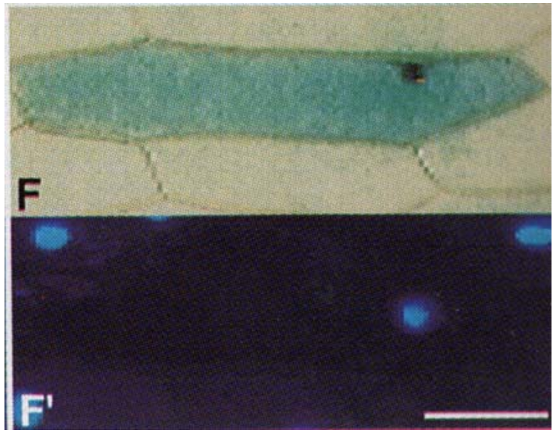
PI-GUS



nucleus

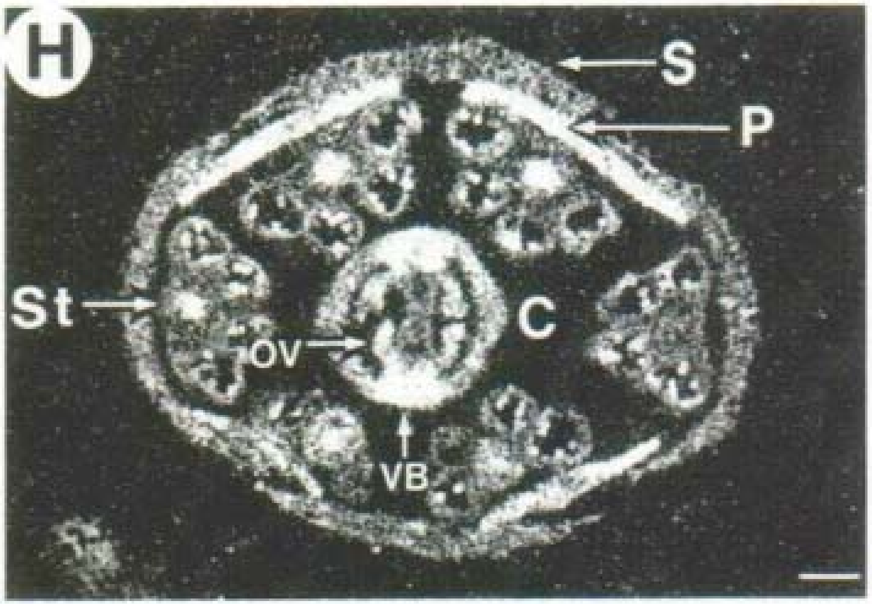
AP3-GUS
+
PI



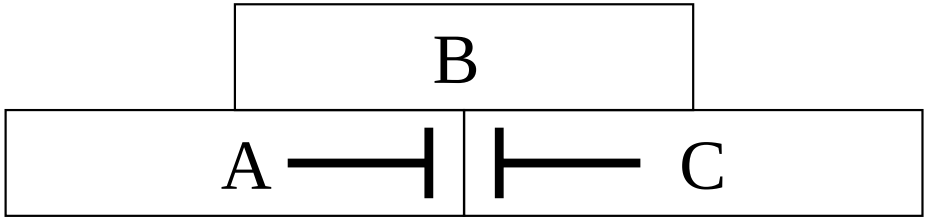


PI-GUS
+
AP3

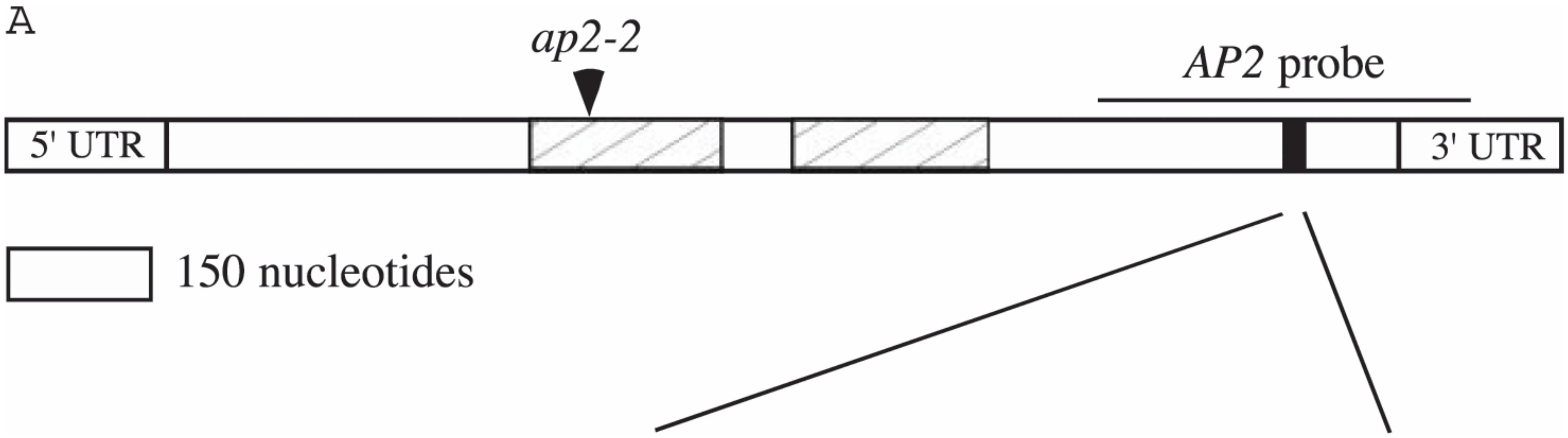
APETALA2 is a class A gene required for whorl 1 and 2, but is expressed in all whorls.
How is AP2 activity restricted to whorls 1 and 2 ?



Wild type

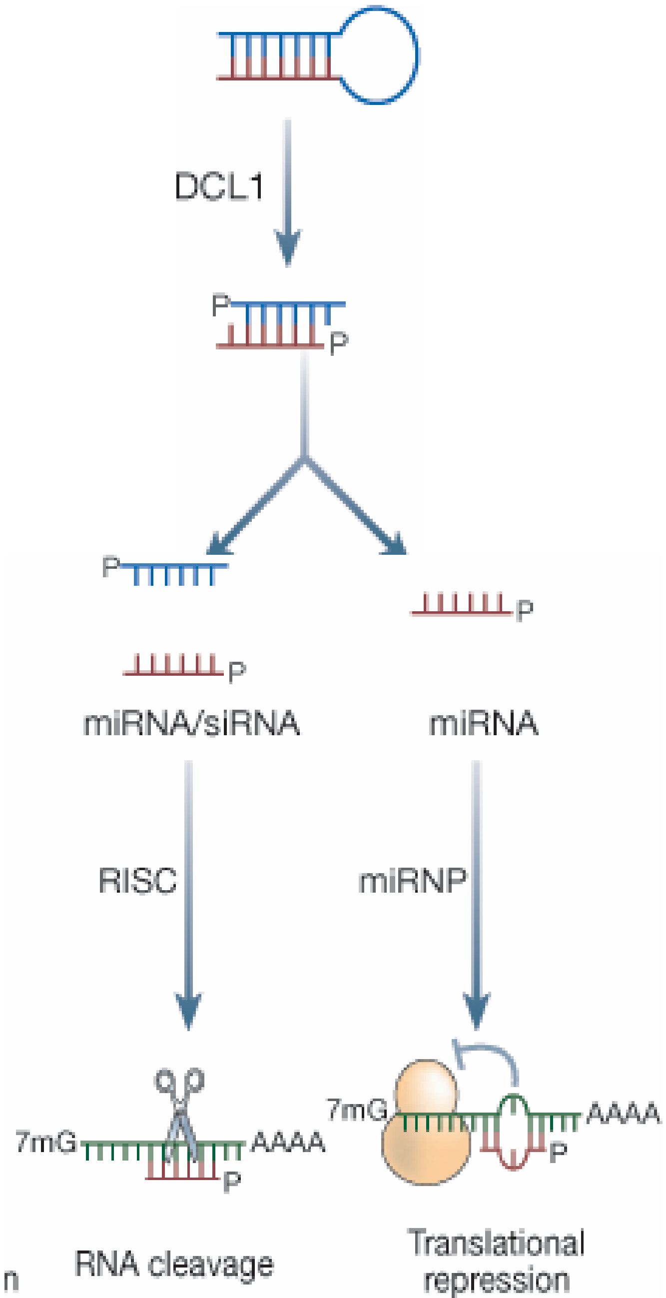


Arabidopsis microRNA172 has homology to the AP2 gene

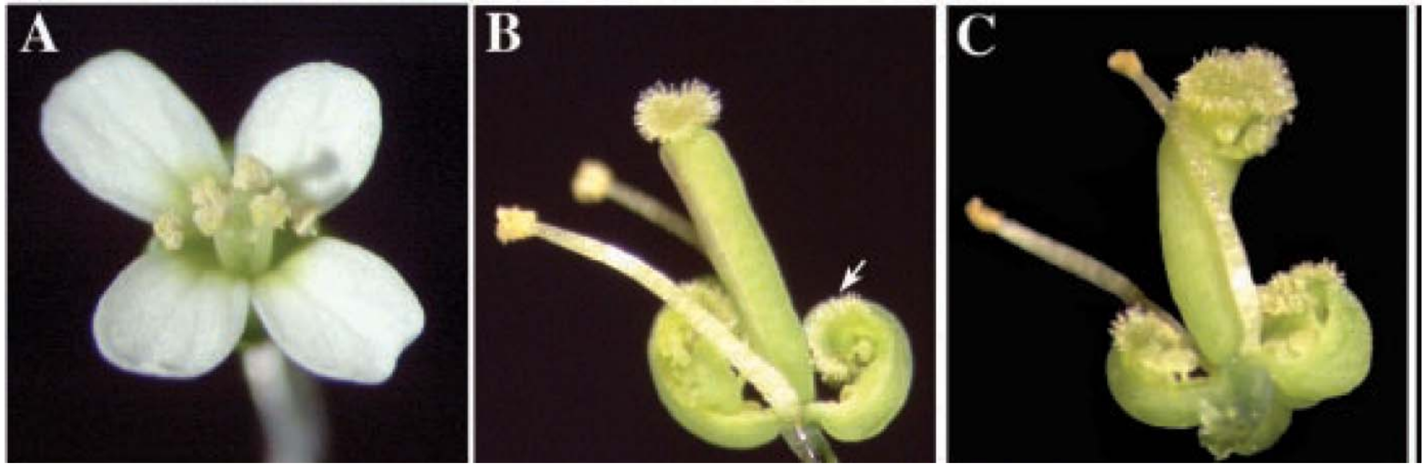


wild-type *AP2* RNA	5' gcu gca gca uca uca gga uuc ucu 3'
miRNA172a-1,-2	3' **ua cgu cgu agu agu ucu aag a** 5'
miRNA172b-1,-2	3' **ga cgu cgu agu agu ucu aag a** 5'
miRNA172c	3' **ua cgu cgu agu agu ucu aag g** 5'

microRNAs repress gene activity by interacting with the mRNAs of target genes either repressing translation or causing degradation of the mRNA

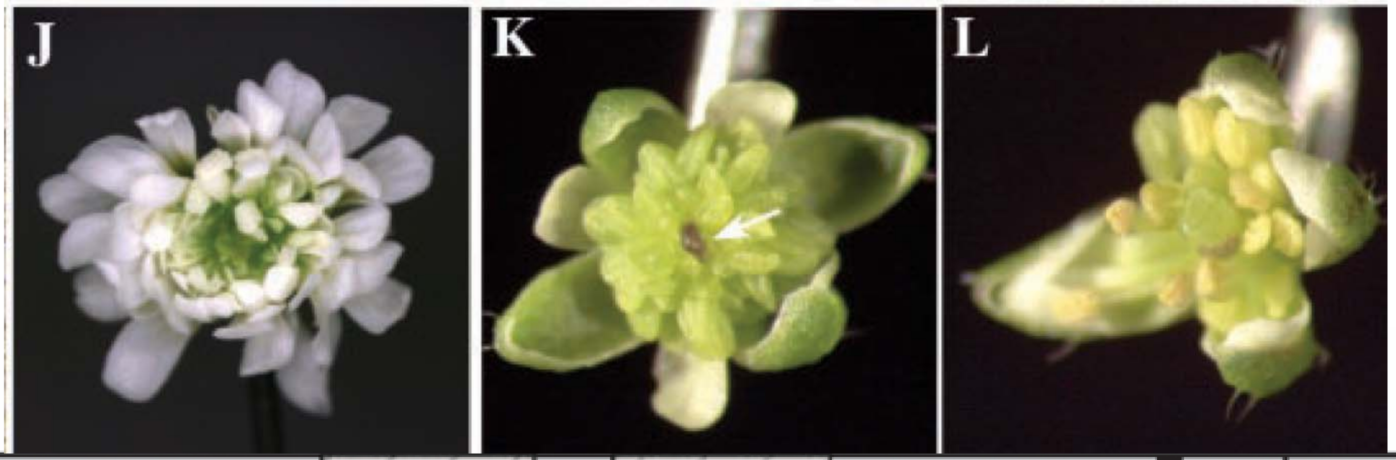


Expression of mutant forms of MIR172 from a viral promoter causes an ap2 mutant phenotype

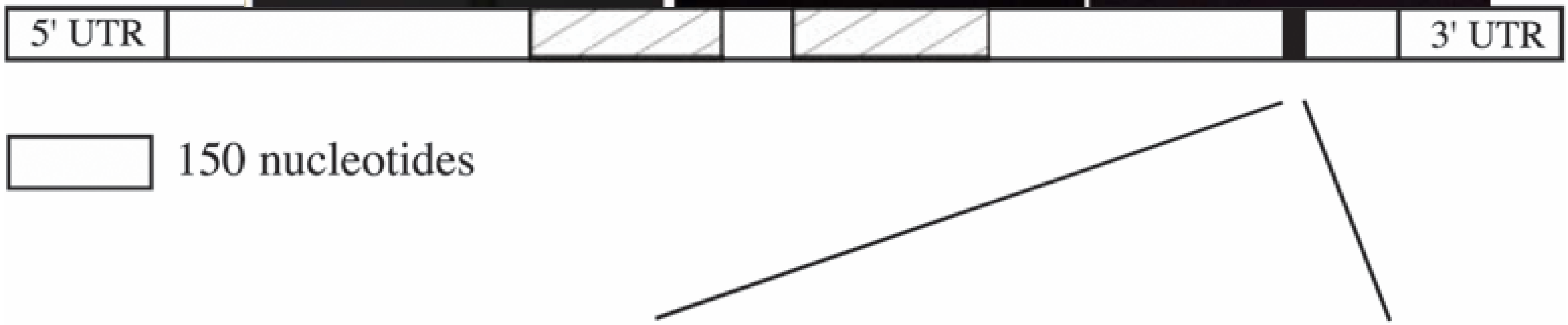


Expression of MIR172 at high levels in all cells prevents AP2 function

Expression of a mutant form of AP2 mRNA that has reduced homology to MIR172 causes increased petal number and more floral whorls



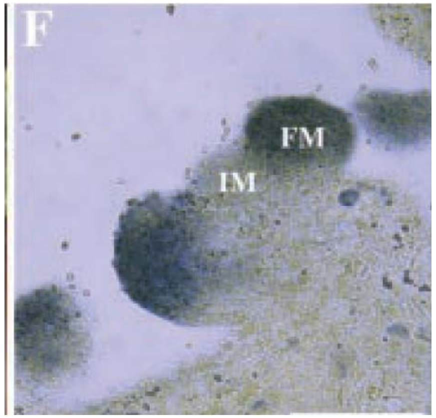
A



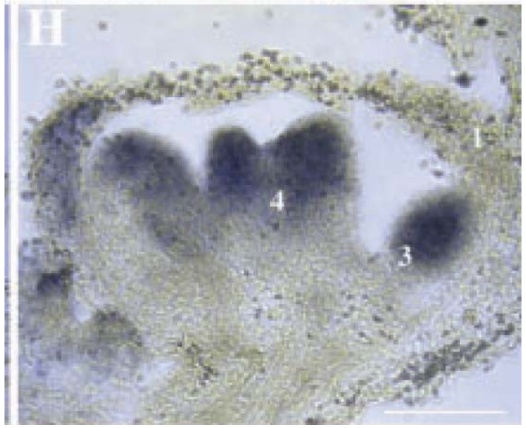
AP2 protein		A	A	A	S	S	G	F	S	
AP2m3 RNA	5'	gca	gcu	gcu	ucc	ucu	ggu	uuc	ucu	3'
AP2m1 RNA	5'	gca	gcu	gcu	ucc	uca	ggu	uua	ucu	3'
wild-type *AP2* RNA	5'	gcu	gca	gca	uca	uca	gga	uuc	ucu	3'
miRNA172a-1,-2	3'	**ua**	**cgu**	**cgu**	**agu**	**agu**	**ucu**	**aag**	**a**	5'
miRNA172b-1,-2	3'	**ga**	**cgu**	**cgu**	**agu**	**agu**	**ucu**	**aag**	**a**	5'
miRNA172c	3'	**ua**	**cgu**	**cgu**	**agu**	**agu**	**ucu**	**aag**	**g**	5'

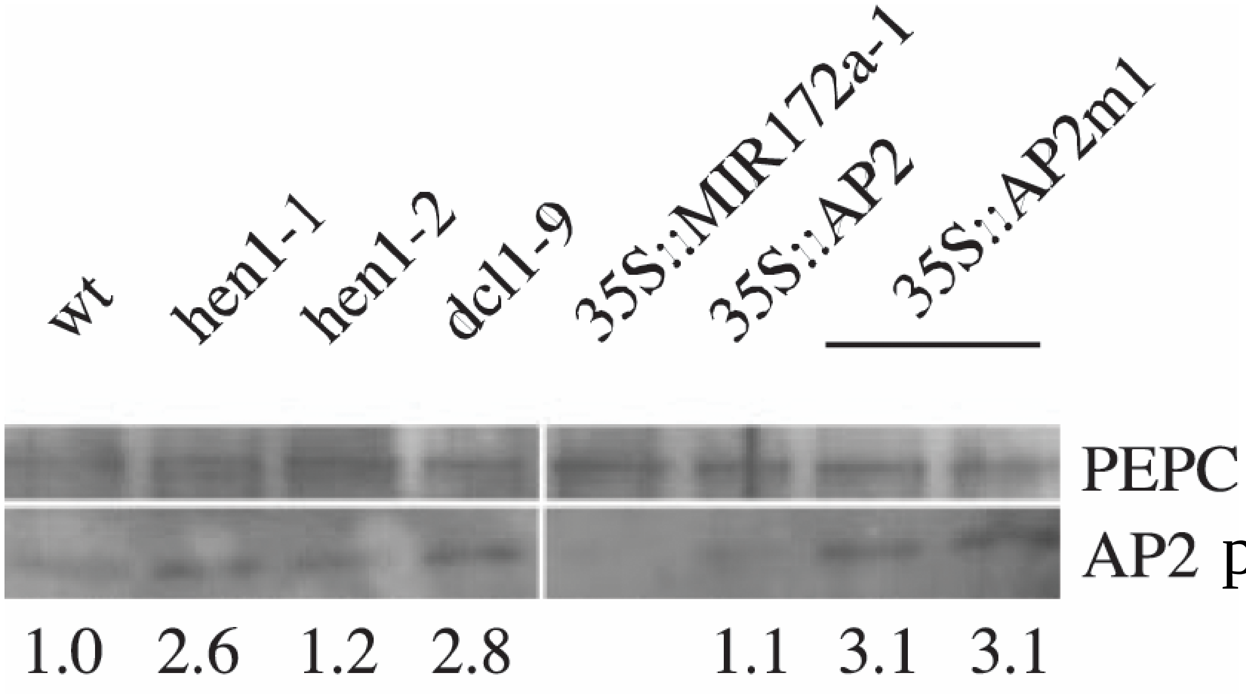
MIR172 is expressed only in the inner whorls in older floral primordia and reduces AP2 protein levels

Floral meristems



Older, Stage 7 flower



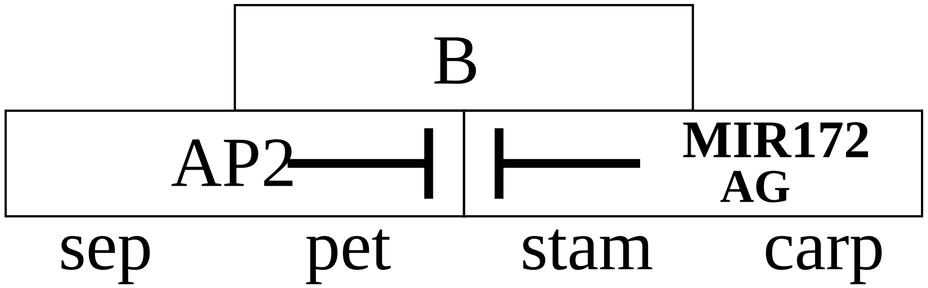


Control protein

protein

MIR172 restricts AP2 activity to the 1 and 2 whorl so that AG expression is prevented in these whorls but can occur in whorls 3 and 4

Wild type


B

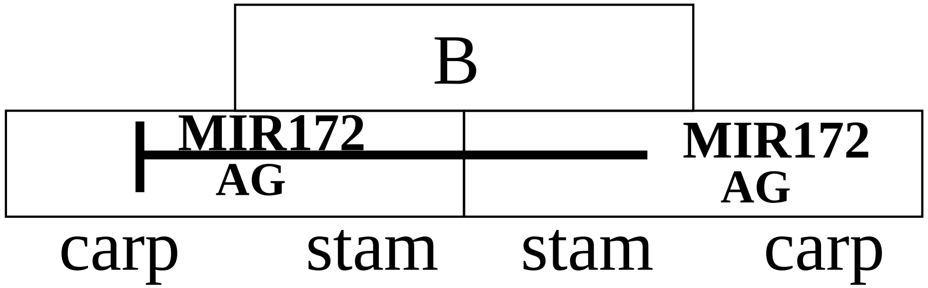
AP2

MIR172

AG

sep pet stam carp

35S::MIR172


B

MIR172

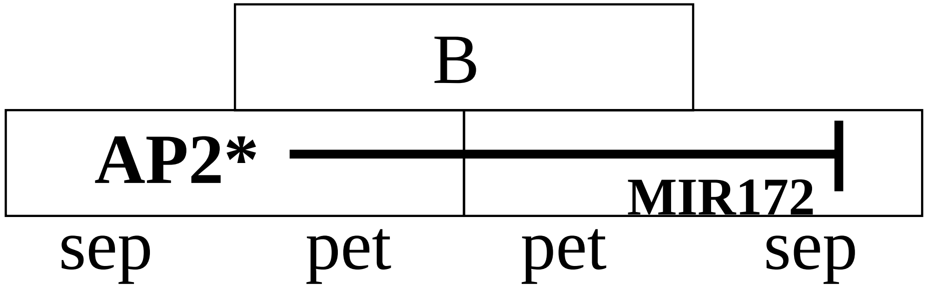
AG

MIR172

AG

carp stam stam carp

35S::AP2* not recognised by MIR172


B

AP2*

MIR172

sep pet pet sep

Misexpression of B or A and B in leaves is not sufficient to convert leaves to petals

Wild-type

B function

35S::AP3 35S::PI

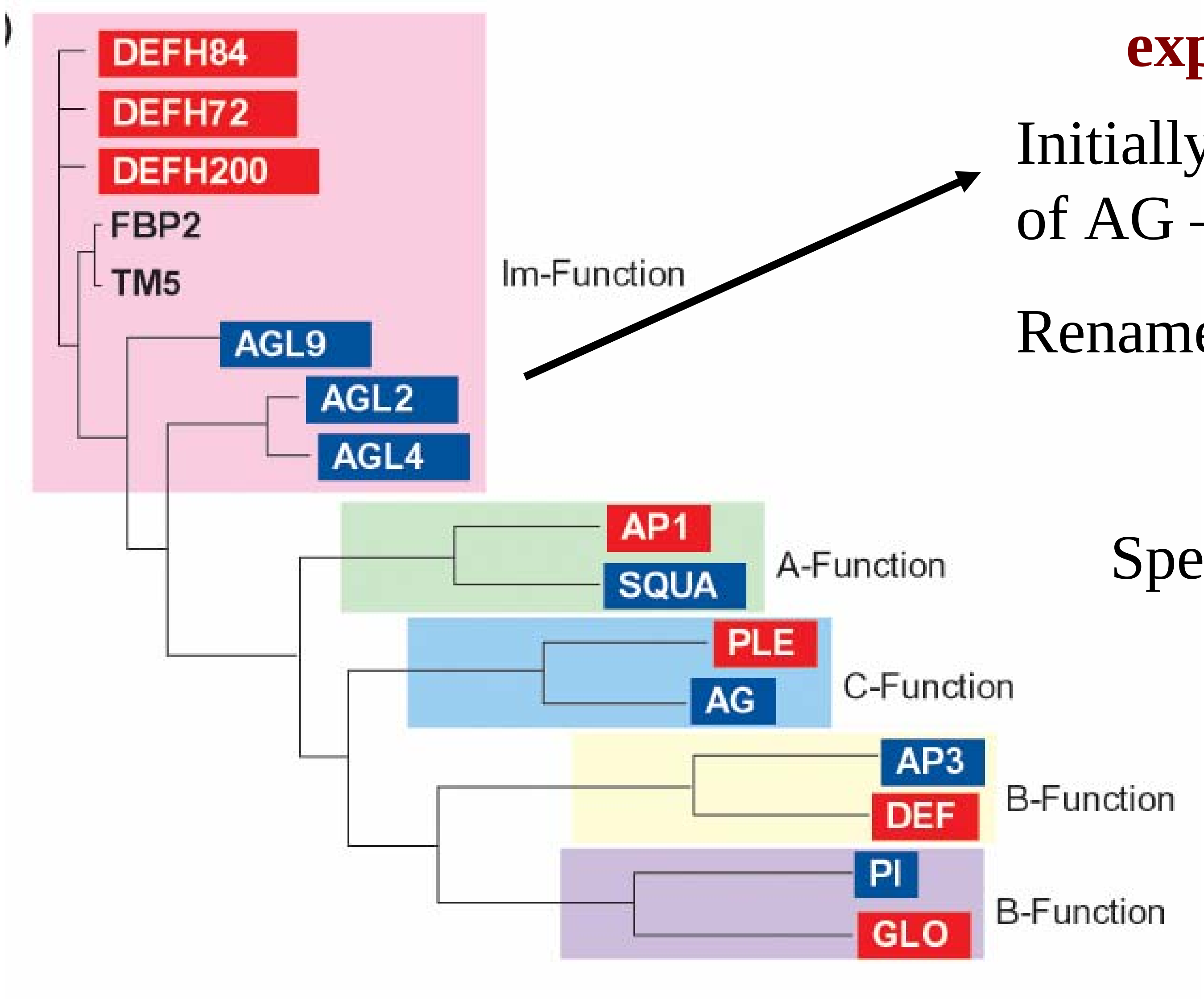
A function

35S::AP1

Suggests another floral-specific factor is absent in the leaves.

This was not identified by initial genetic screens

More MADS box genes expressed in the flower



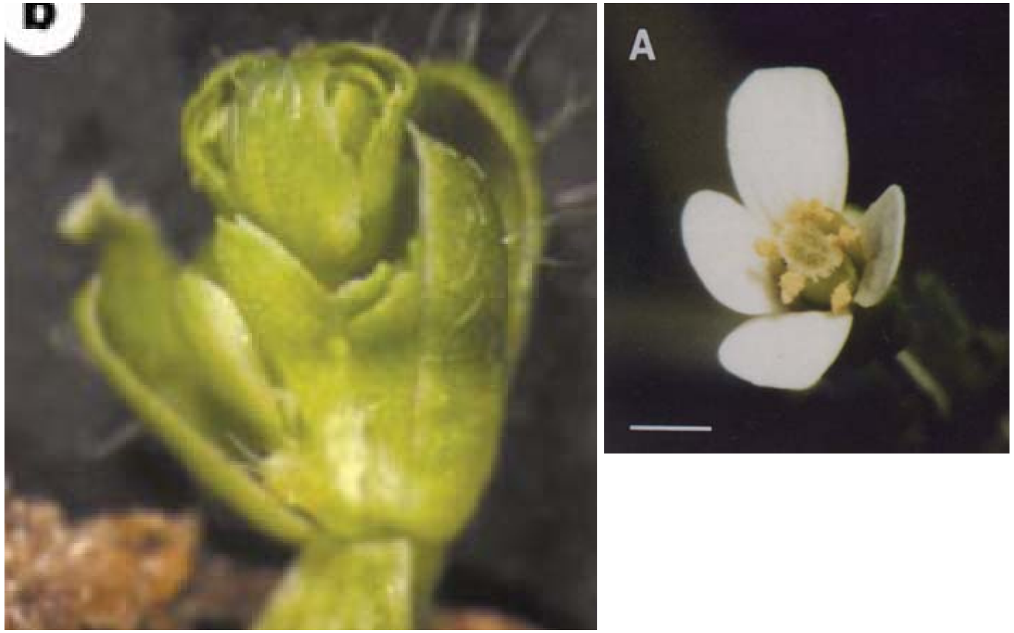
Initially identified as homologues of AG – AGL2, AGL4, AGL9

Renamed sepallata 1,2,3

Specific expression patterns – in whorls 2,3,4, although AGL2 and AGL9 are also expressed in whorl 1 of younger flowers.

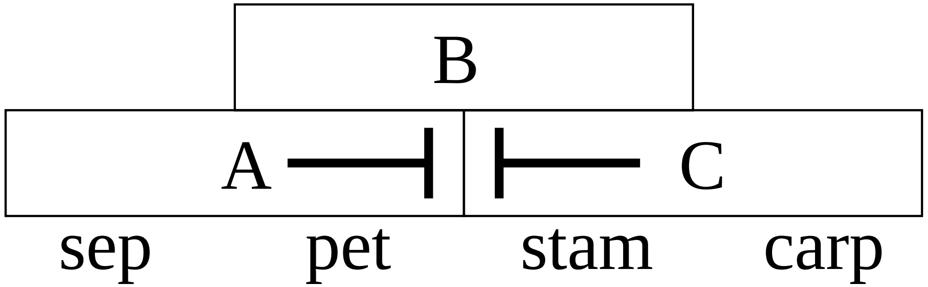
Genes in red from Antirrhinum
Genes in blue from Arabidopsis

Inactivation of SEP1 SEP2 and SEP3 in triple mutants



Were not identified in
Original mutant screens
Because of redundancy
Between proteins.

Wild type

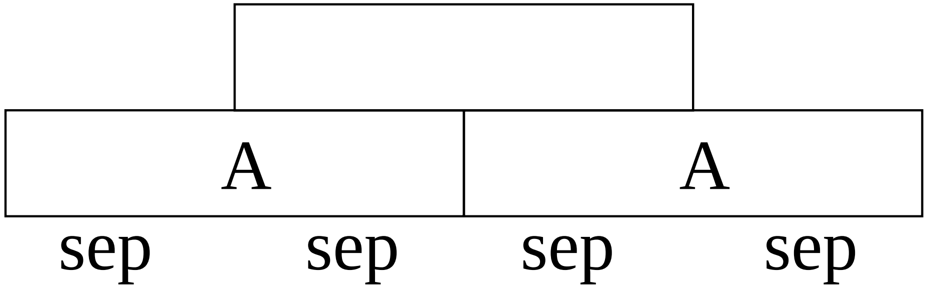


B

A ⊣ ⊢ C

sep pet stam carp

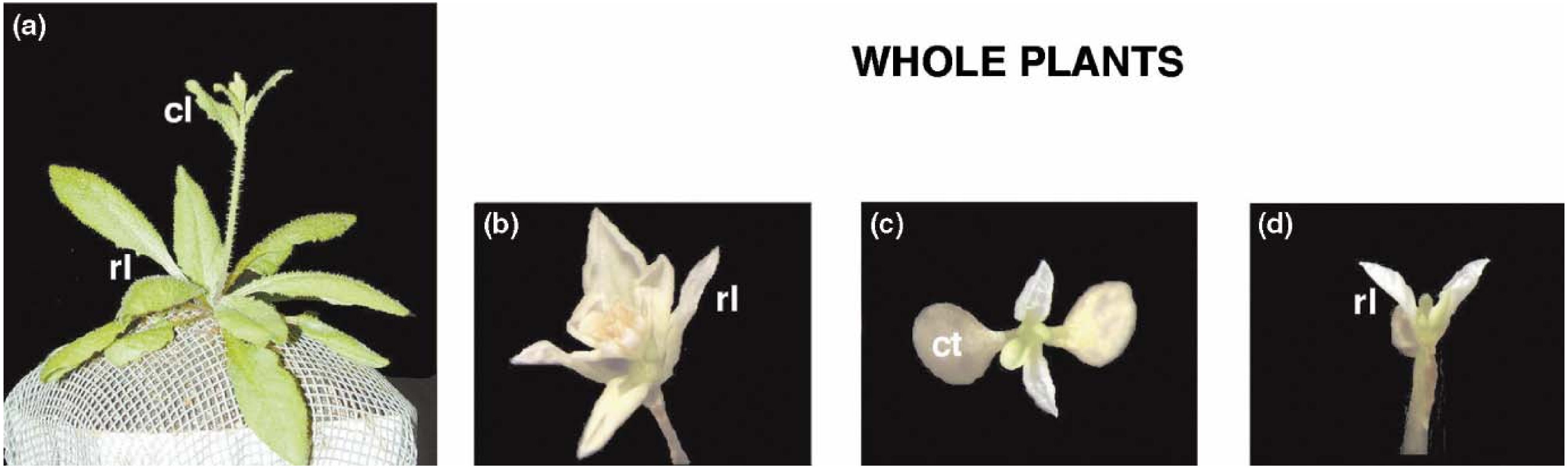
sep1 sep2 sep3



A A

sep sep sep sep

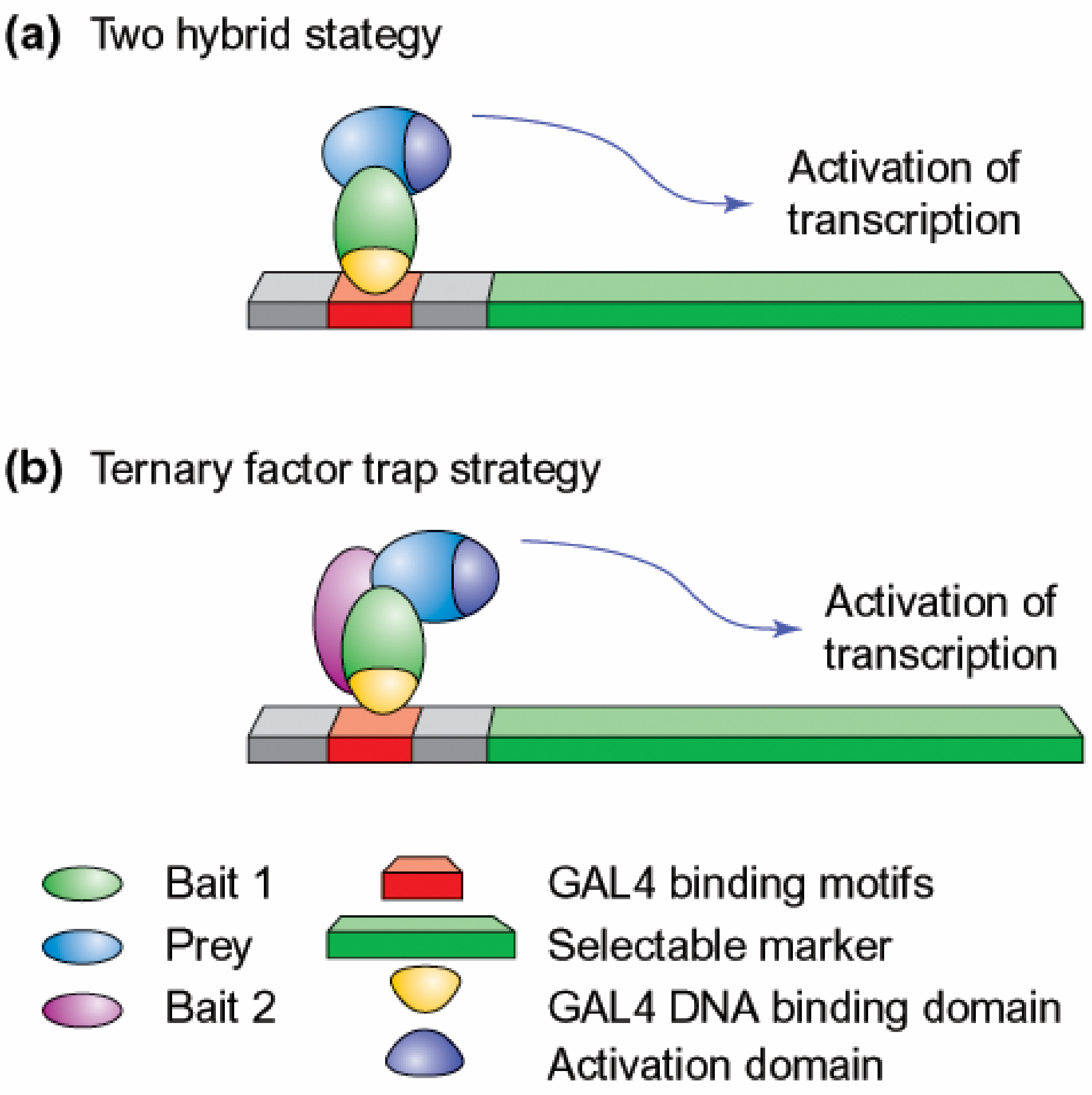
Misexpression of SEP1 AP1 PI and AP3 in leaves creates petals



Plants carrying combination of
35S::AP1 35S::SEP2 35S::PI 35S::AP3

**Therefore a combination of AP1 PI AP3 SEP is sufficient
To confer B function.**

Test for protein-protein Interactions in yeast.


(a) Two hybrid stategy

(b) Ternary factor trap strategy

Do the MADS box proteins form higher order protein complexes?

SEP proteins mediate multimeric complexes between PI/AP3 and AG or between PI/AP3 and AP1

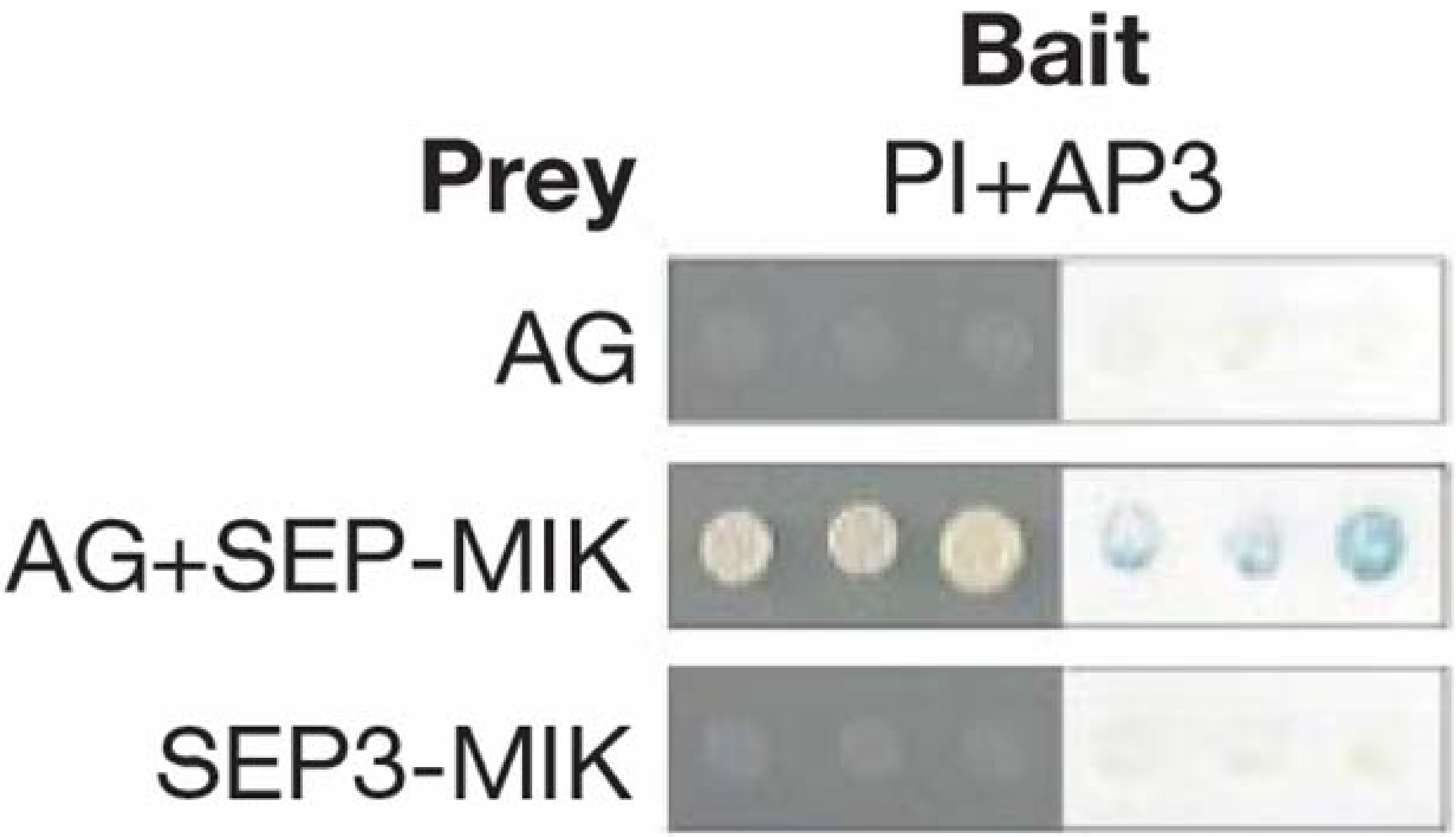
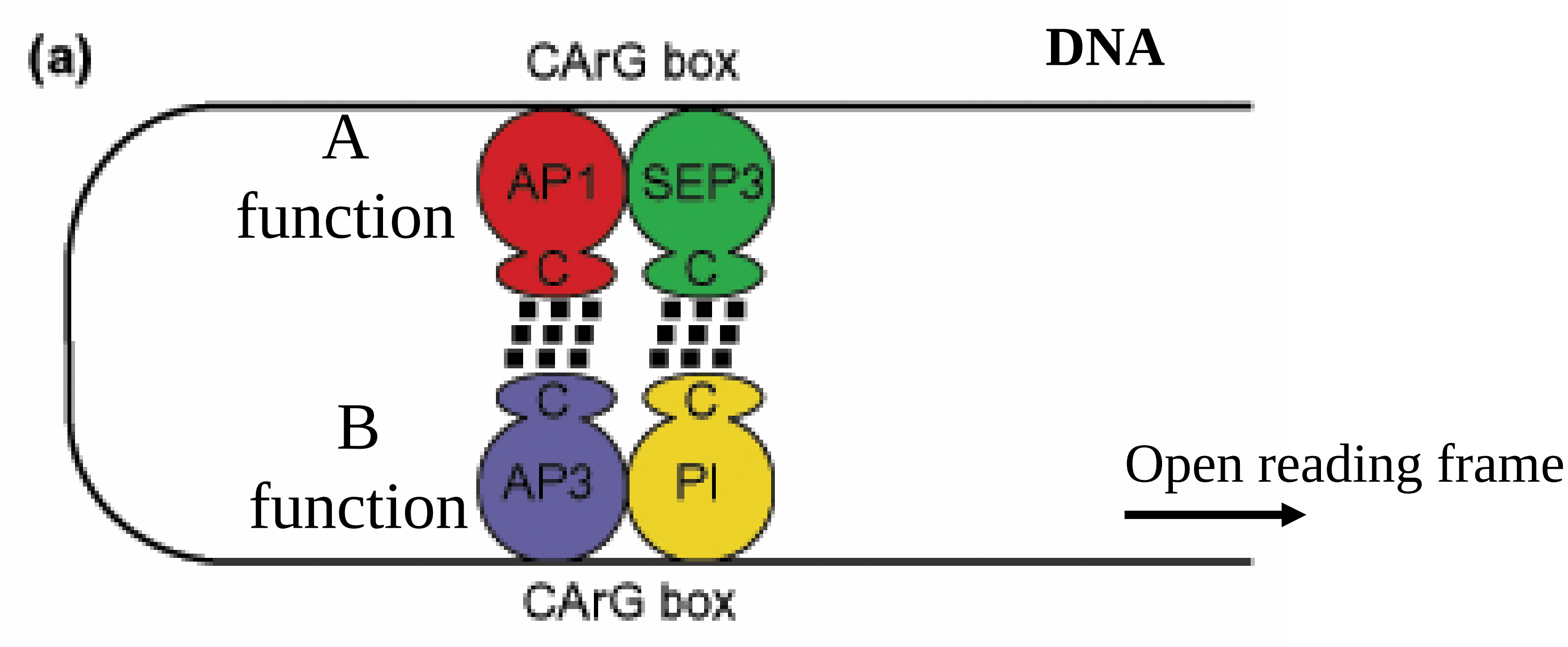


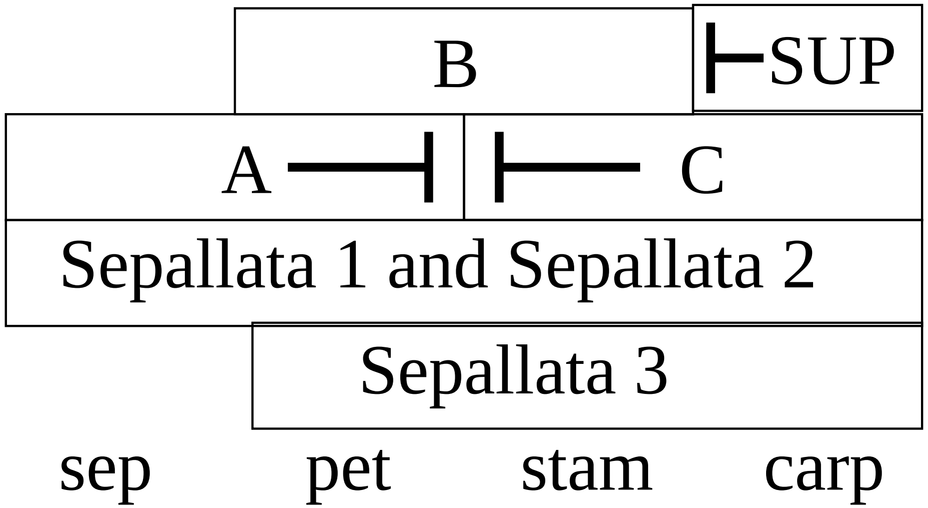
Plate with
No HIS

LacZ expression

SEP genes also provide activation domains



Model explains how A function (AP1) and B function (AP3/PI) Combine to specify the second whorl – petals.


B ⊢SUP

A ⊣ ⊢ C

Sepallata 1 and Sepallata 2

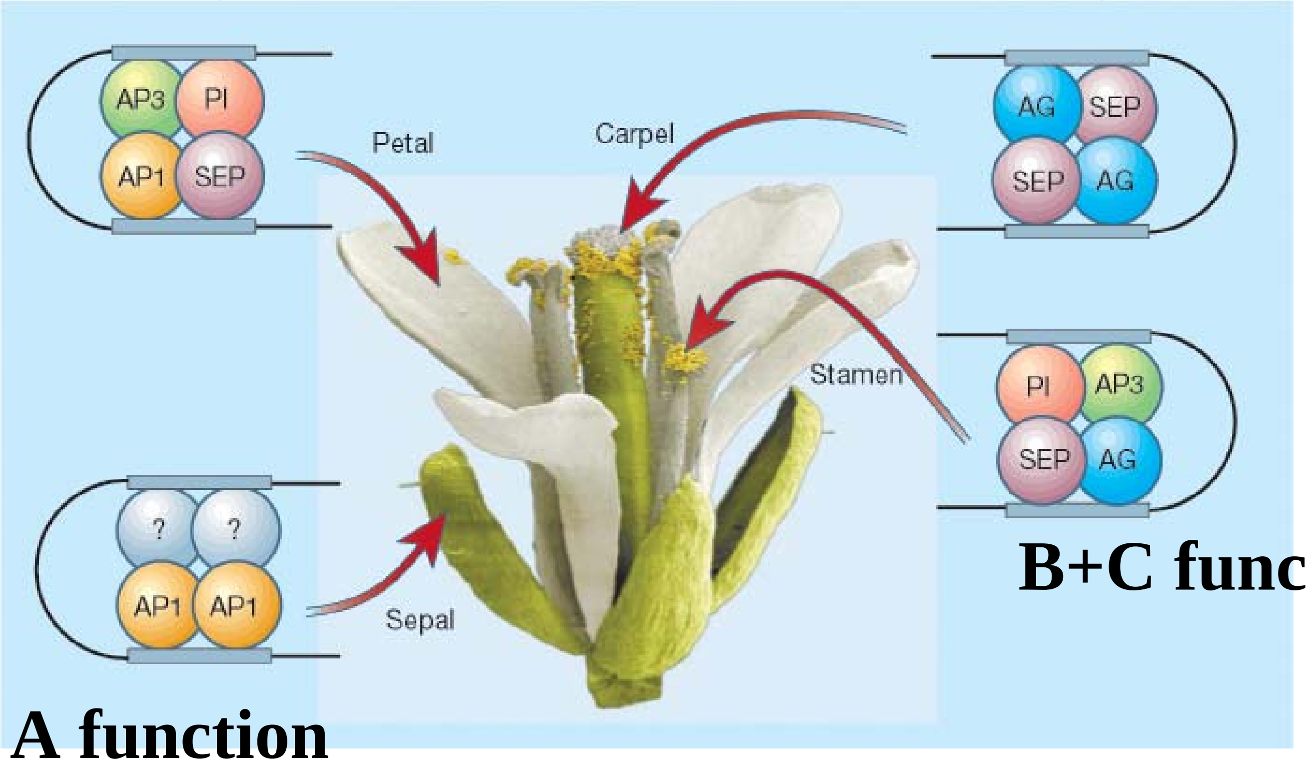
Sepallata 3

sep pet stam carp

Similar multimeric complexes of MADS box proteins are proposed to specifiy the other whorls

A+B function

C function

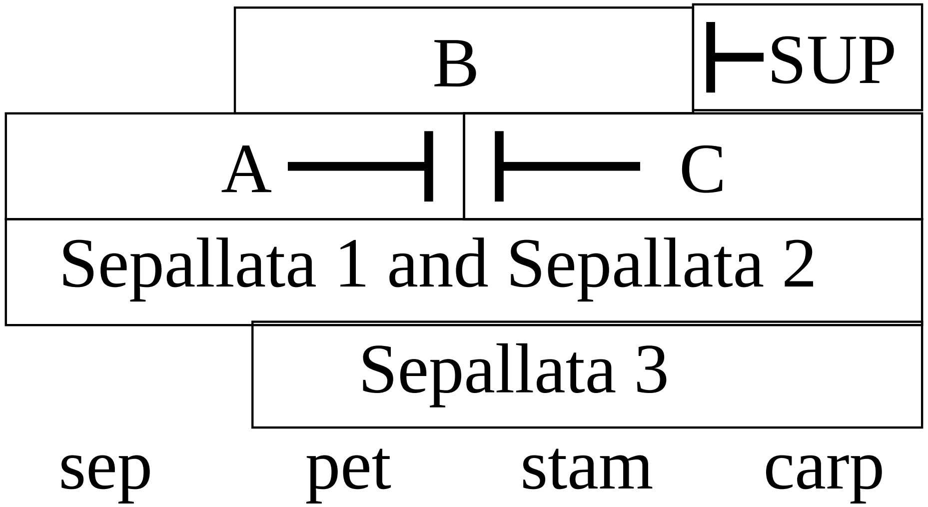


B+C function

A function

SEP proteins mediate multimeric complexes between PI/AP3 and AG or between PI/AP3 and AP1

Wild type



B

⊢SUP

A ⊣ ⊢ C

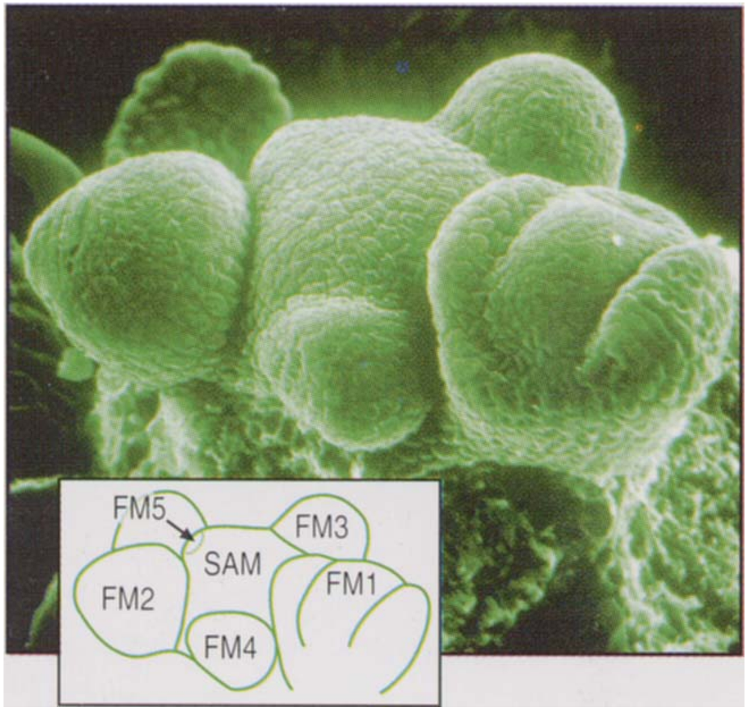
Sepallata 1 and Sepallata 2

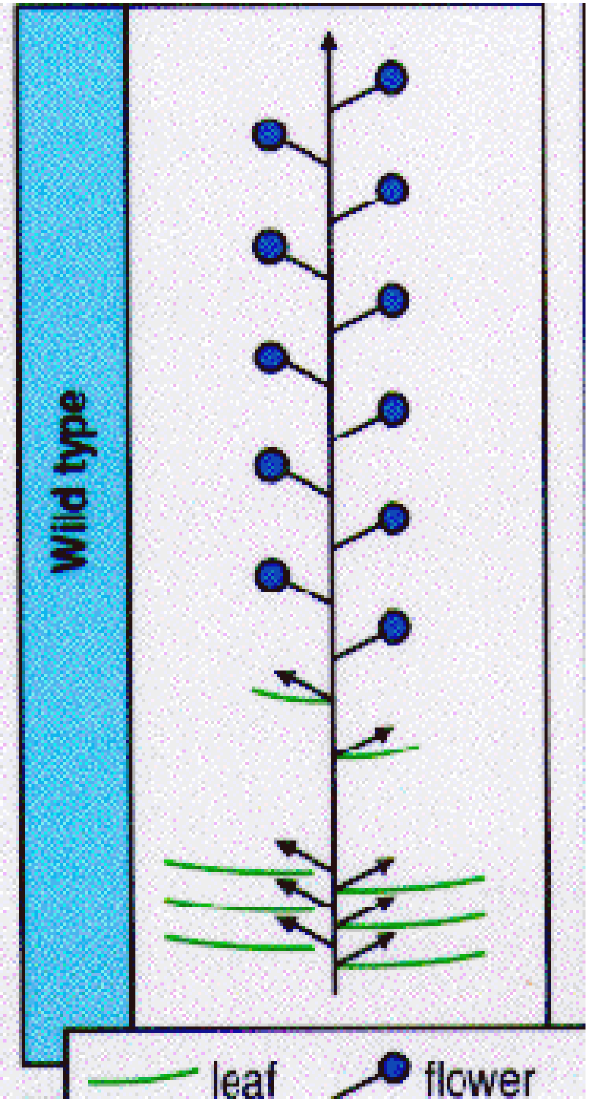
Sepallata 3

sep pet stam carp

Formation of multimeric complexes suggests mechanism for combining A/B and B/C functions within the ABC model.

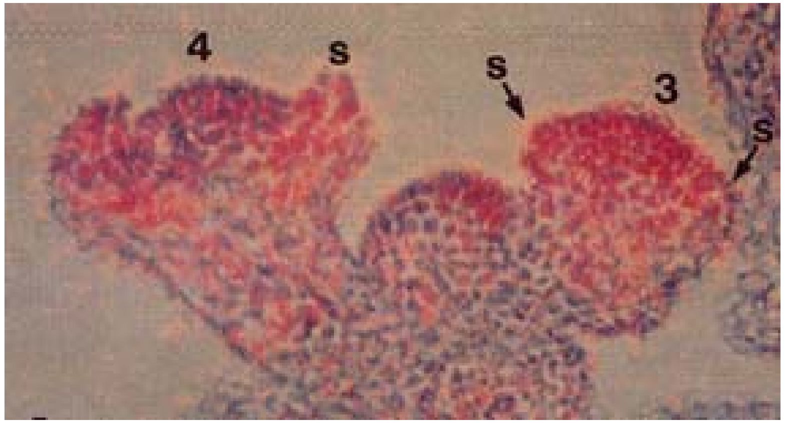
Flowers develop from stem cells called the shoot apical meristem

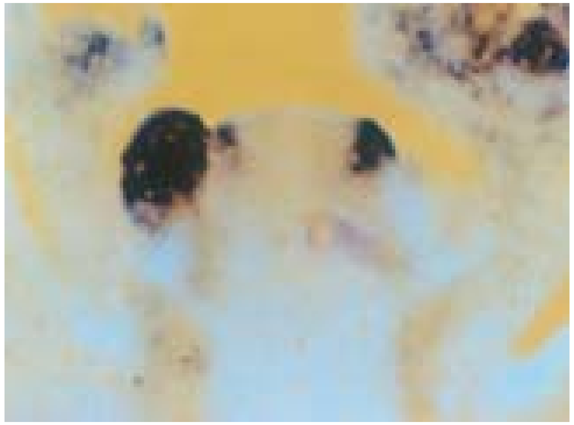




The FLORICAULA/LEAFY genes confer floral identity on primordia

flo mutant

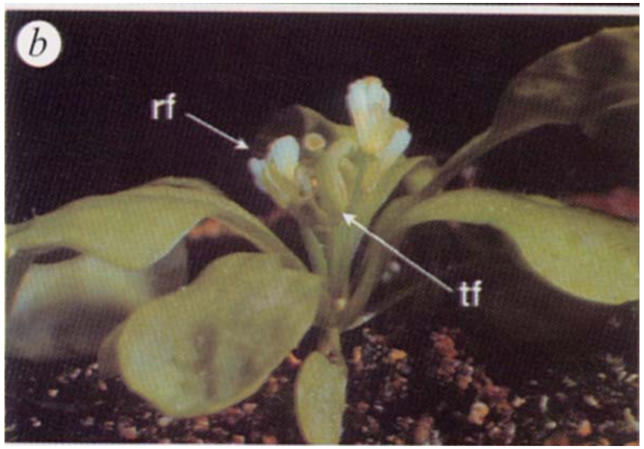


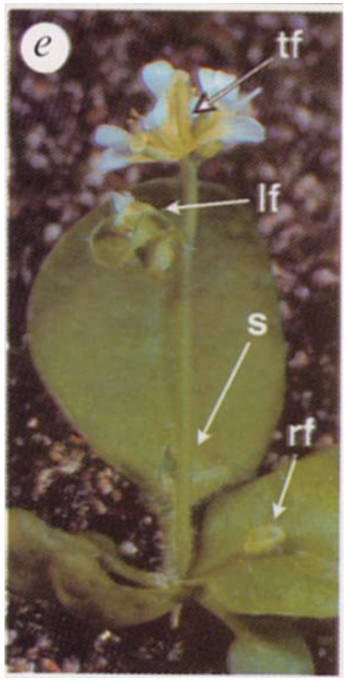


LFY expression

LEAFY is sufficient to confer floral identity on developing primordia when expressed from a viral promoter

35S::LFY plants





Wild-type

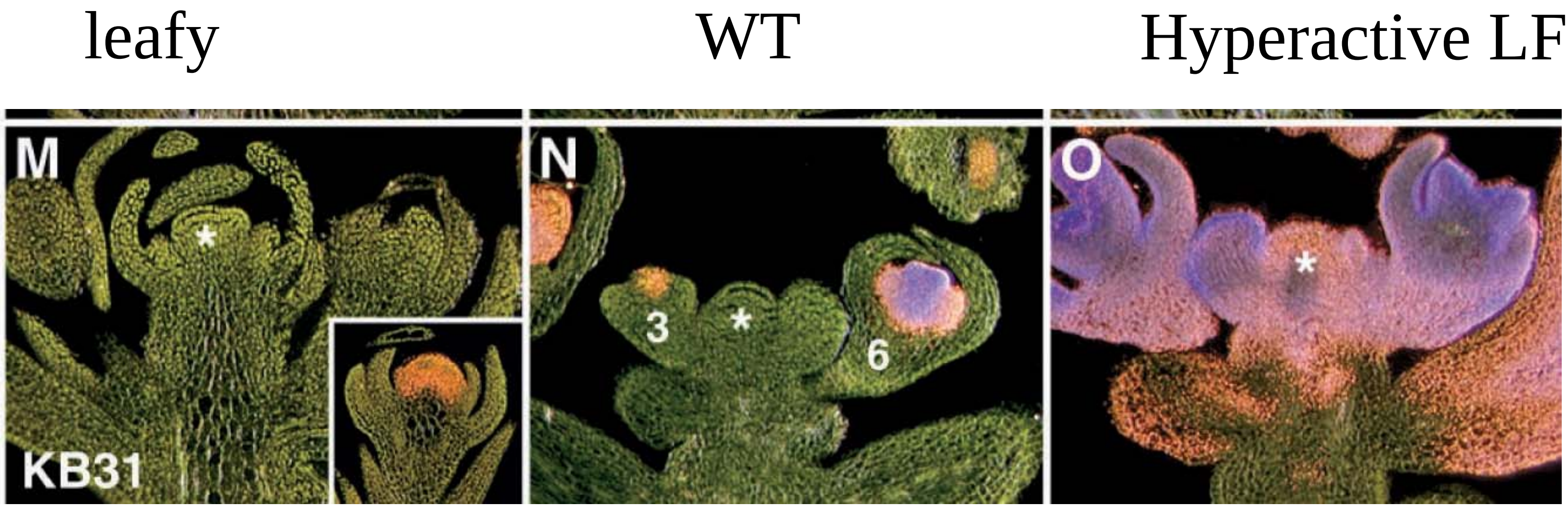
LEAFY is a plant-specific gene

No homologues in animal cells

A single copy gene in Arabidopsis

No protein domains suggesting a biochemical function for the protein.

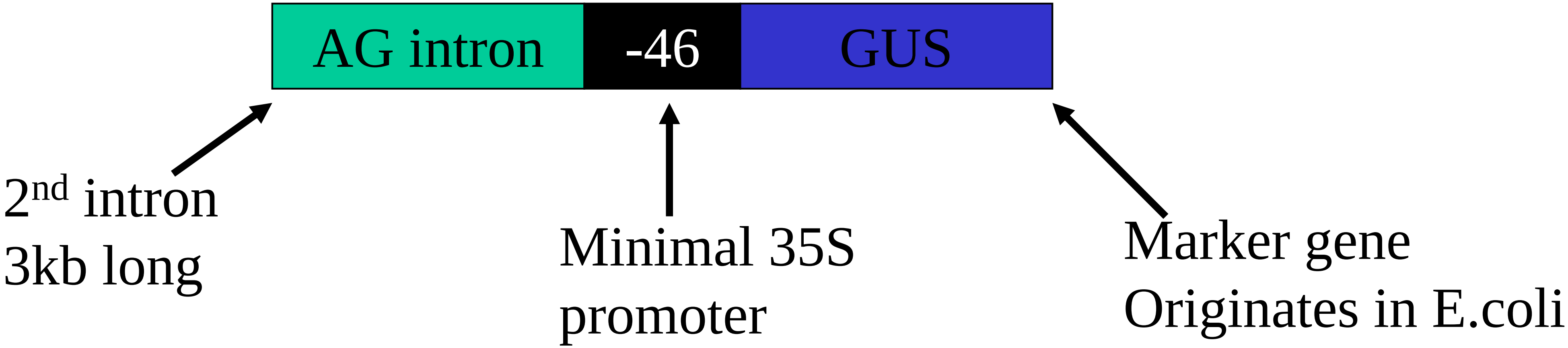
LEAFY activates AGAMOUS through a short enhancer in an intron



leafy

WT

Hyperactive LFY



AG intron

-46

GUS

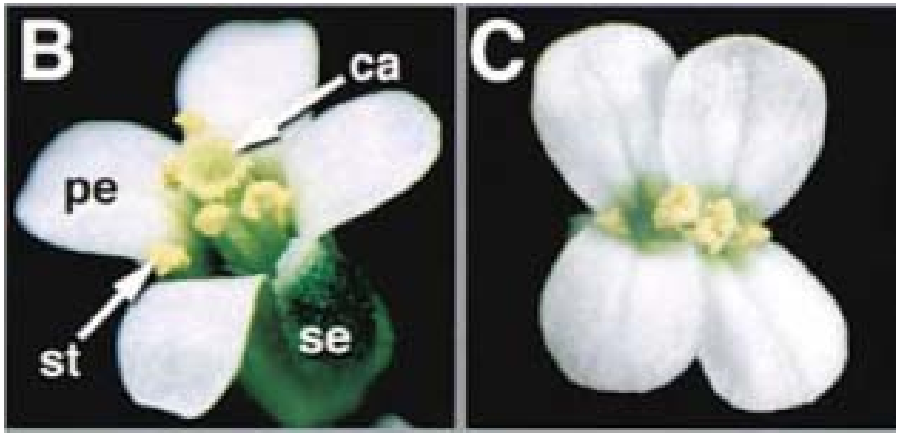
2^{nd} intron
3kb long

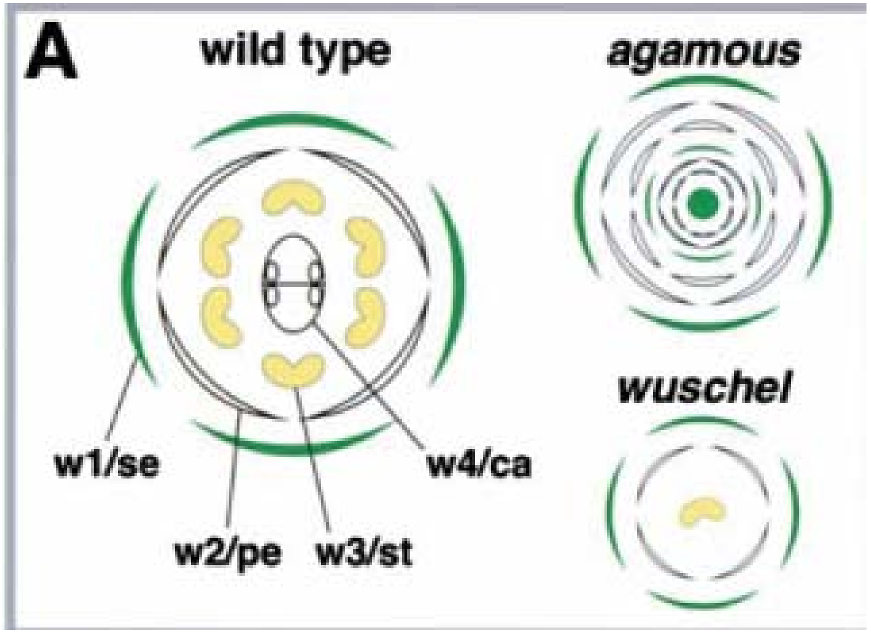
Minimal 35S
promoter

Marker gene
Originates in E.coli

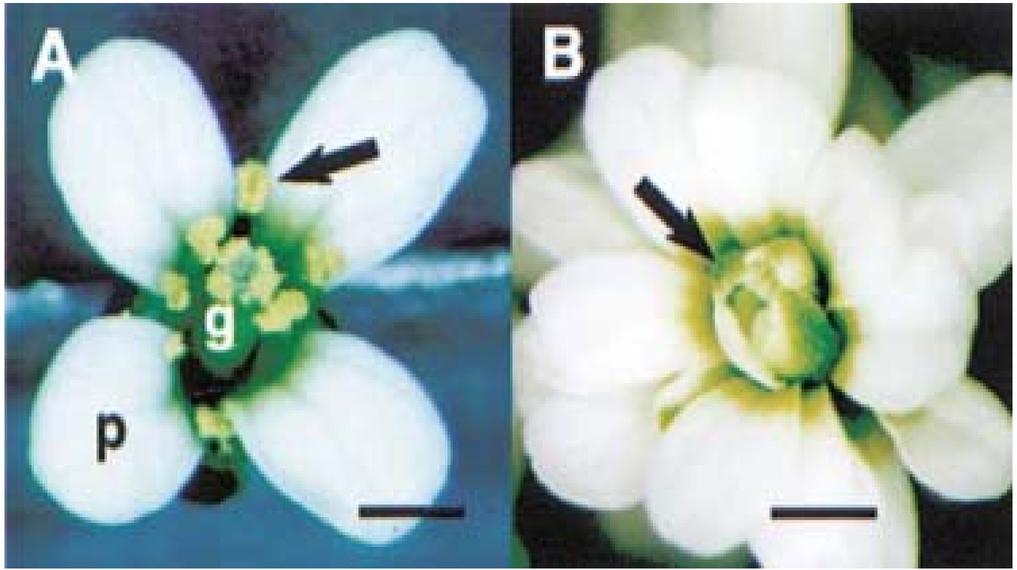
wuschel mutants have fewer 3rd whorl organs and no 4th whorl organs; they show the opposite phenotype to agamous mutants

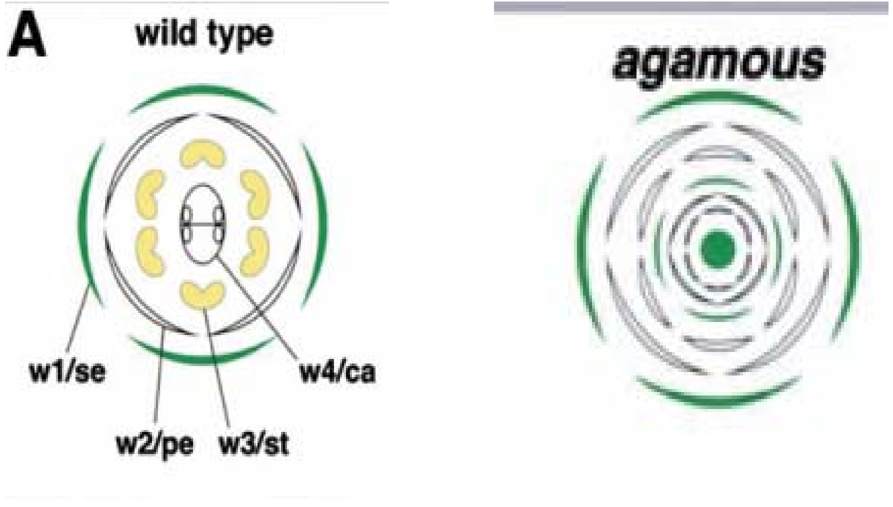
WT *wuschel*



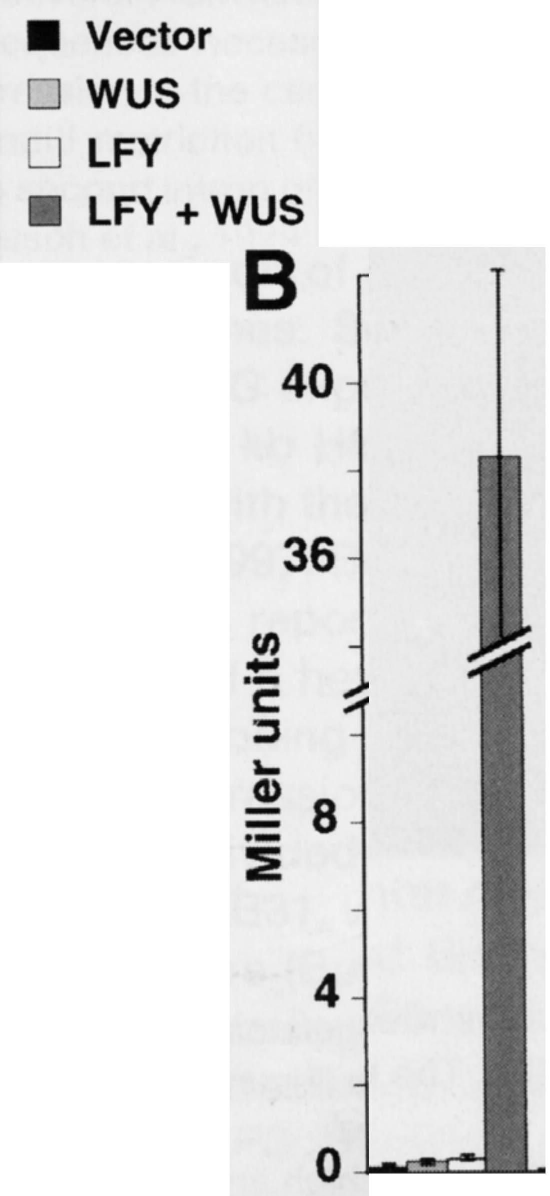


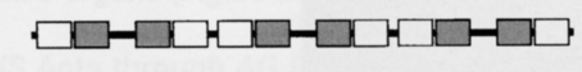
WT ***agamous***





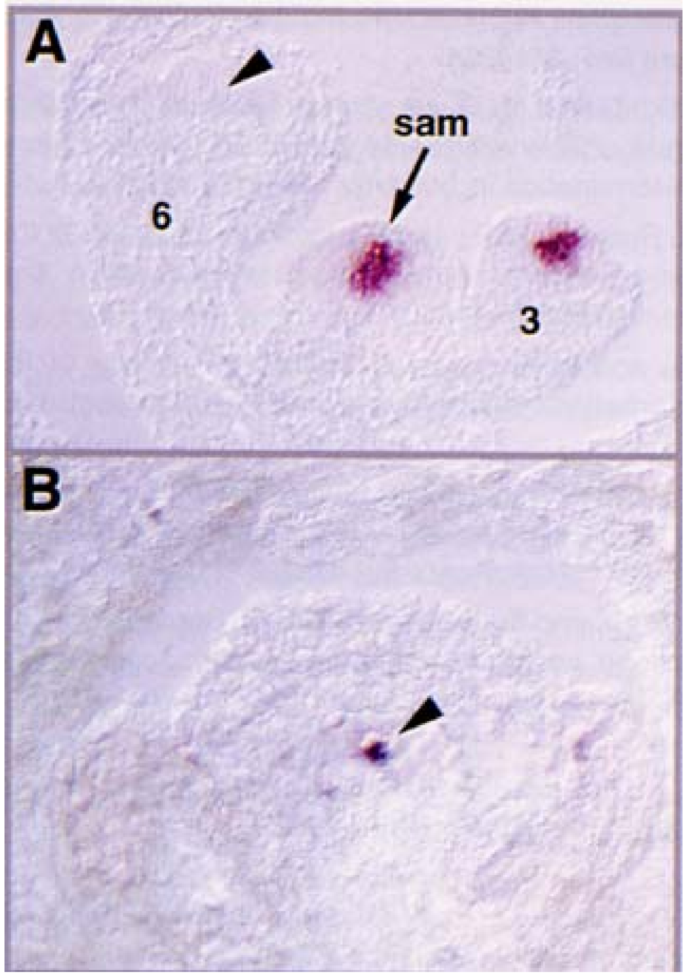
Both LEAFY and WUSCHEL bind to the AG promoter, and they activate transcription co-operatively in yeast cells





Co-activation
from AG sequences
in yeast

In WT plants *WUSCHEL* expression is only detected in young floral primordia, but persists for longer in *agamous* mutants

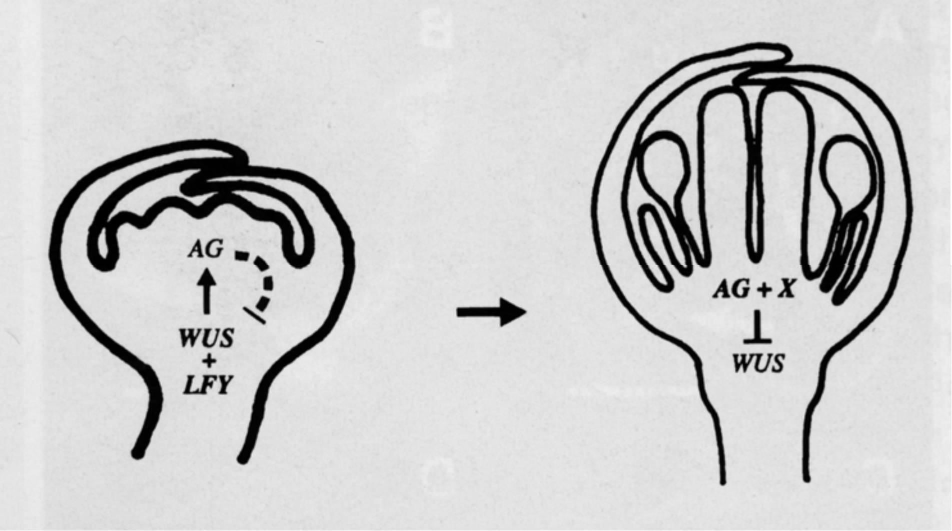


Wild-type flower
WUS not detected in older flower (stage 6)

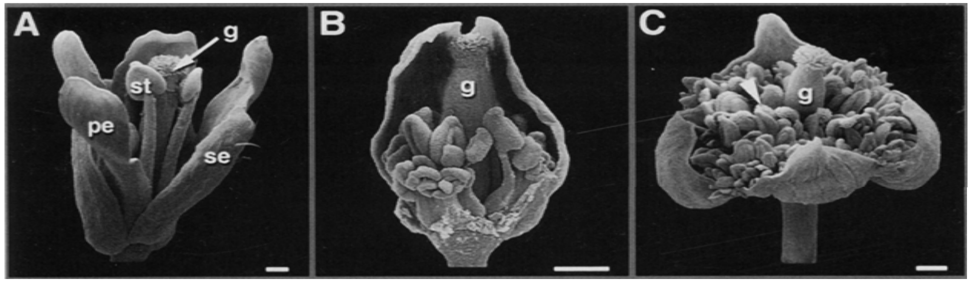
agamous flower
WUS still detected in stage 10 flower

AGAMOUS is required to repress WUSCHEL

A negative feed-back loop regulates WUSCHEL expression in the flower meristem



Therefore in an agamous mutant wuschel activity would be increased and would persist for longer.
This may explain why agamous mutant flowers do not stop after 4 whorls.
In wuschel mutants no AG expression and no WUS expression, so no 4th whorl development.



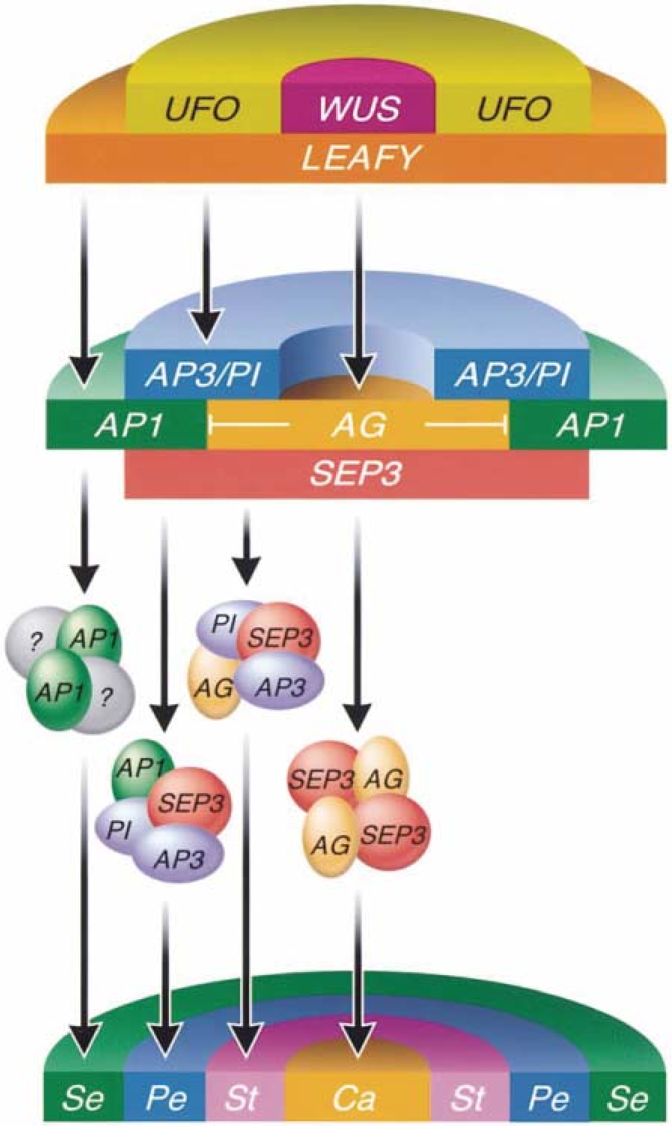
Wild-type

AP3::WUS weak

AP3::WUS strong

In support of this, expression of WUSCHEL from the AP3 promoter causes extra whorls to develop.

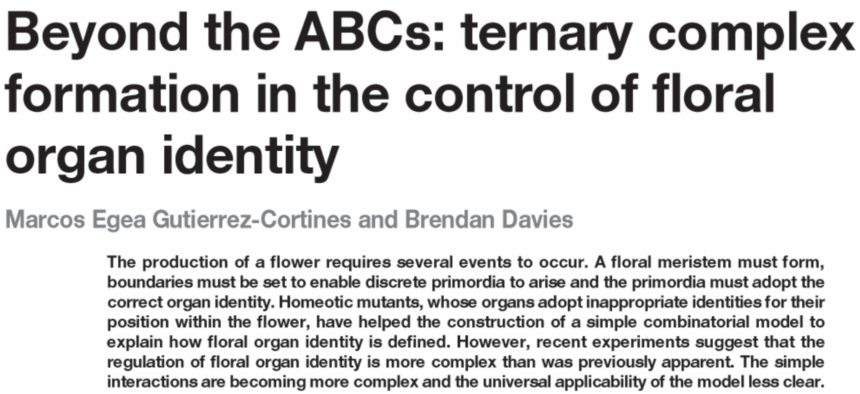
Diagram of floral development from the initial patterning of the primordium to development of floral organs



On the web site

- This presentation

-

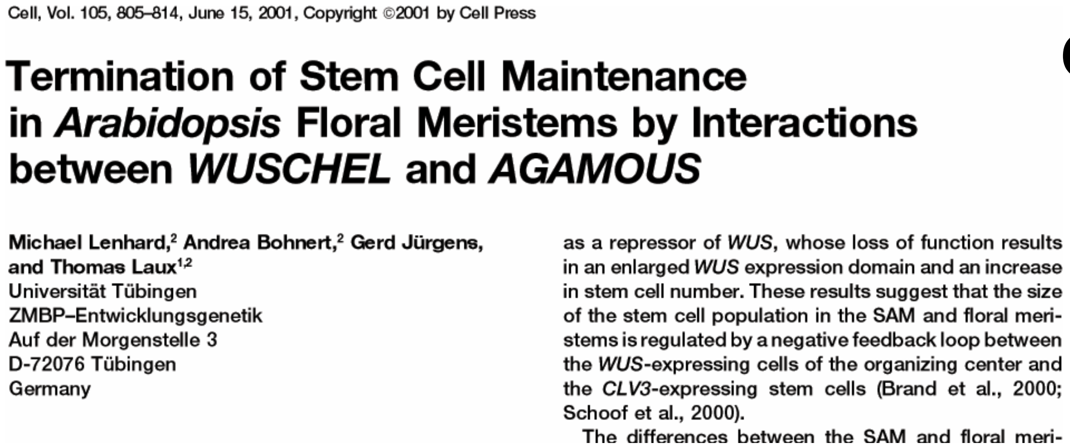


Beyond the ABCs: ternary complex formation in the control of floral organ identity

Marcos Egea Gutierrez-Cortines and Brendan Davies

The production of a flower requires several events to occur. A floral meristem must form, boundaries must be set to enable discrete primordia to arise and the primordia must adopt the correct organ identity. Homeotic mutants, whose organs adopt inappropriate identities for their position within the flower, have helped the construction of a simple combinatorial model to explain how floral organ identity is defined. However, recent experiments suggest that the regulation of floral organ identity is more complex than was previously apparent. The simple interactions are becoming more complex and the universal applicability of the model less clear.

-



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Termination of Stem Cell Maintenance in *Arabidopsis* Floral Meristems by Interactions between *WUSCHEL* and *AGAMOUS*

Michael Lenhard,[2] Andrea Bohnert,[2] Gerd Jürgens, and Thomas Laux[1,2]
Universität Tübingen
ZMBP–Entwicklungsgenetik
Auf der Morgenstelle 3
D-72076 Tübingen
Germany

as a repressor of *WUS*, whose loss of function results in an enlarged *WUS* expression domain and an increase in stem cell number. These results suggest that the size of the stem cell population in the SAM and floral meristems is regulated by a negative feedback loop between the *WUS*-expressing cells of the organizing center and the *CLV3*-expressing stem cells (Brand et al., 2000; Schoof et al., 2000).

The differences between the SAM and floral meri-

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Forschung

Abt. Entwicklungsbiologie de Pflanzen

George Coupland