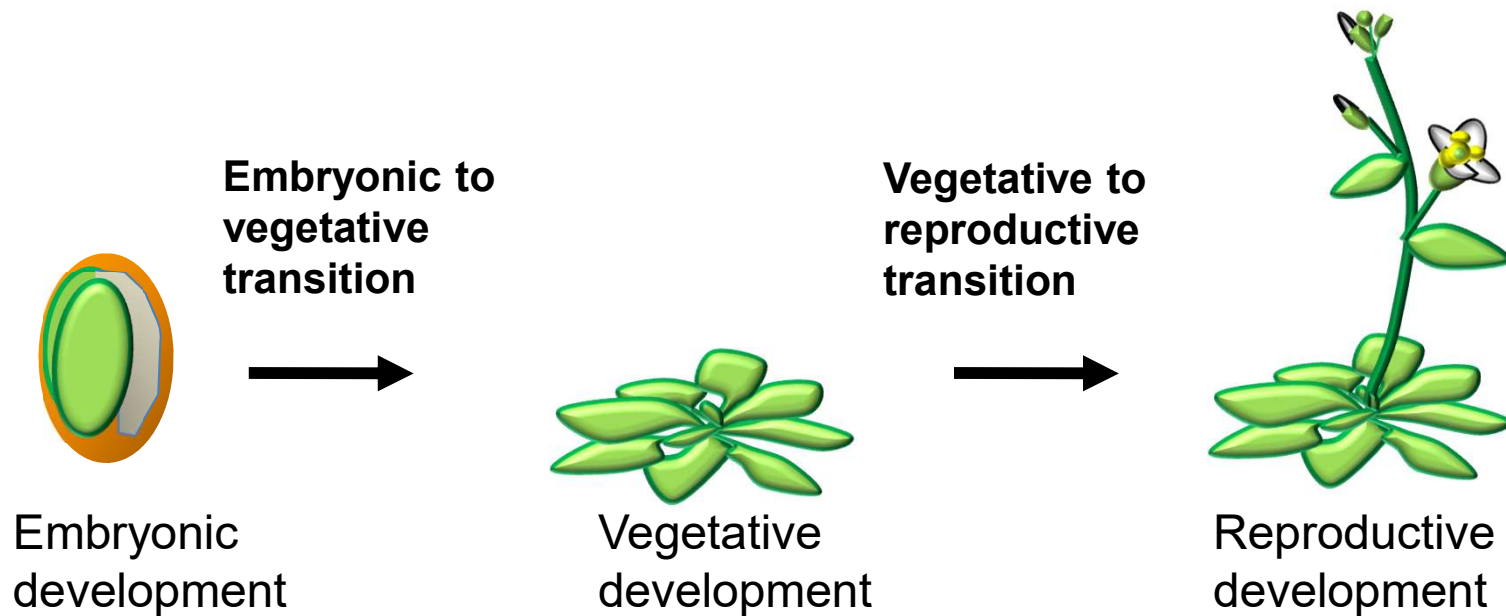
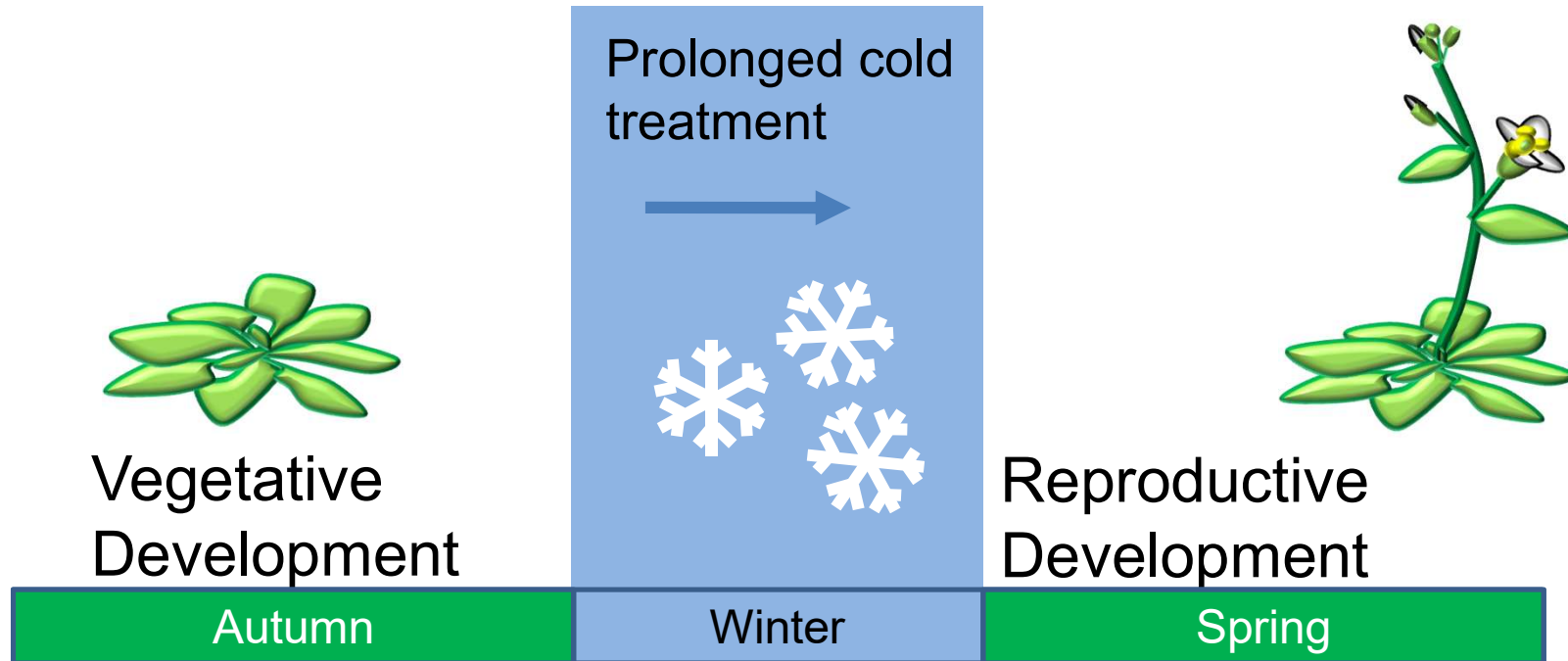


Epigenetic regulation of endogenous genes and developmental processes

Epigenetic programming in plants helps control developmental transitions

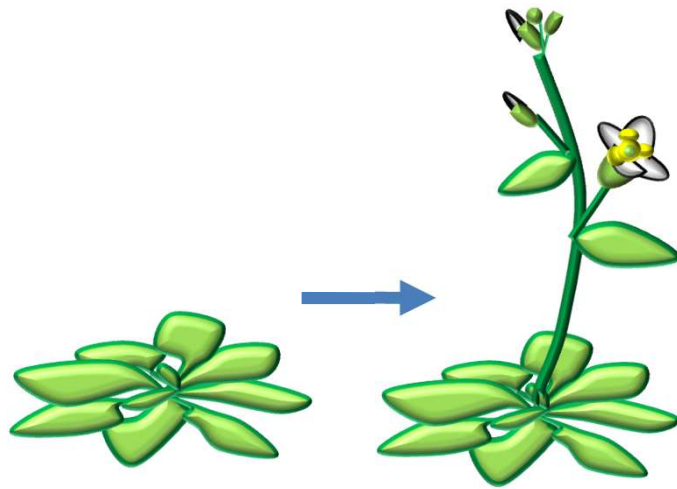


Epigenetic control of flowering time



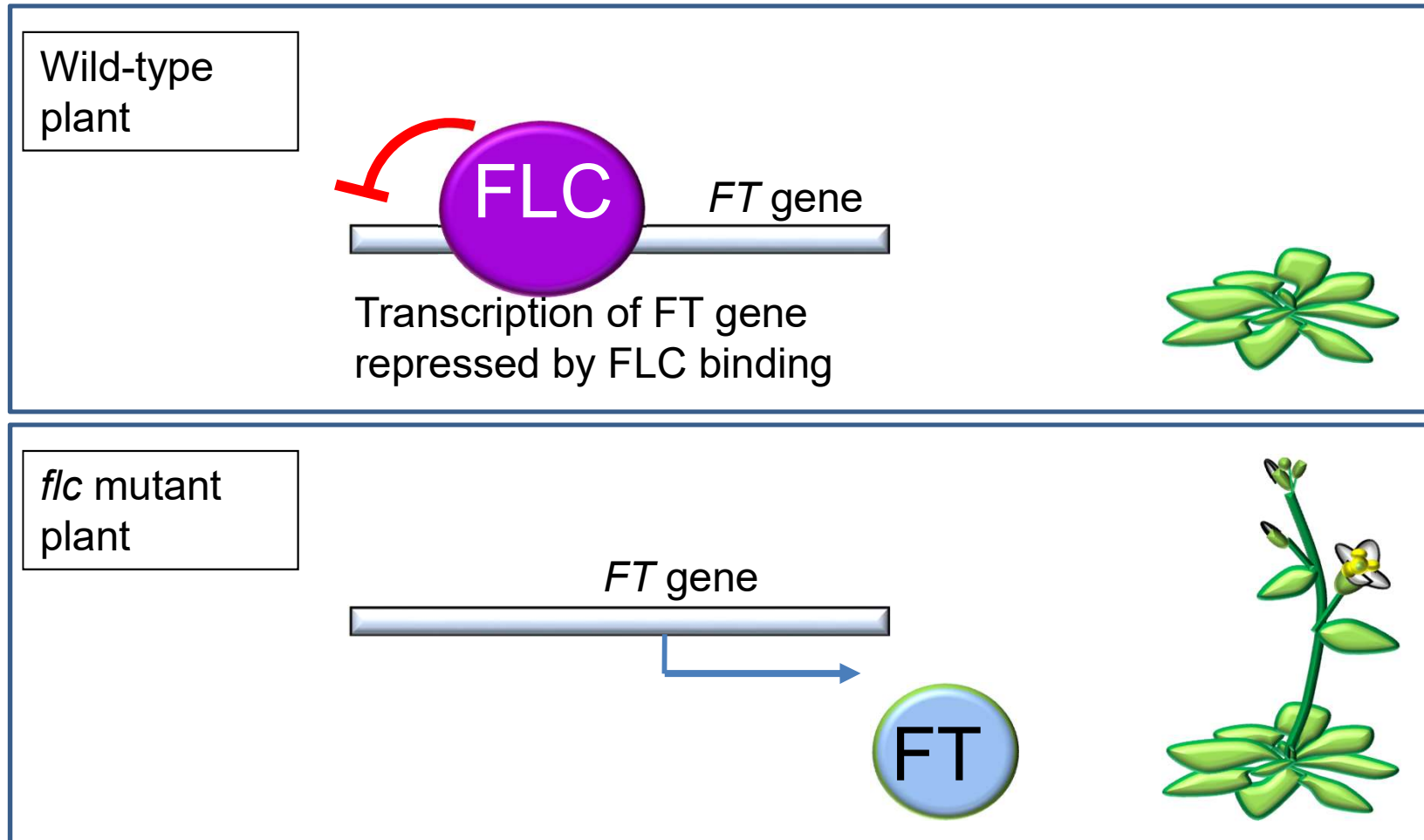
Some plants require a prolonged cold period (***vernalization***) - as experienced during winter, before they will flower.

FLOWERING LOCUS C (FLC) mutants flower early



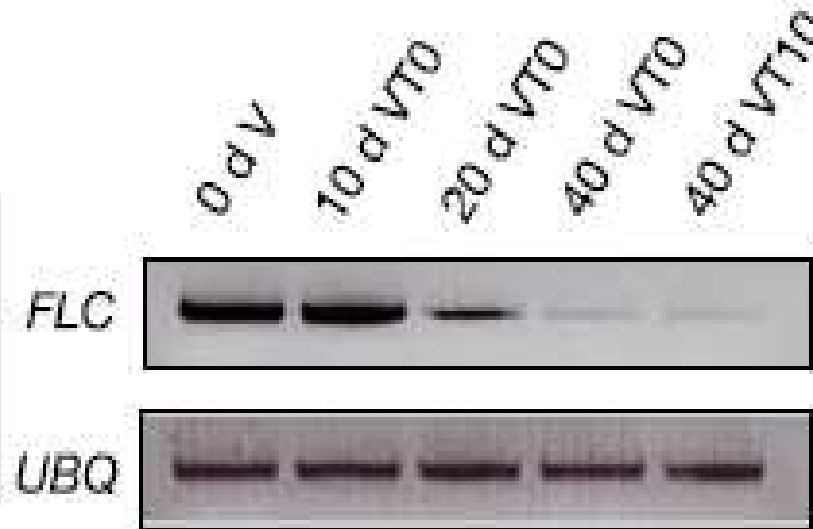
FLC is an inhibitor of flowering;
removing FLC removes the
vernalization requirement

FLC inhibits *FT*, an activator of flowering

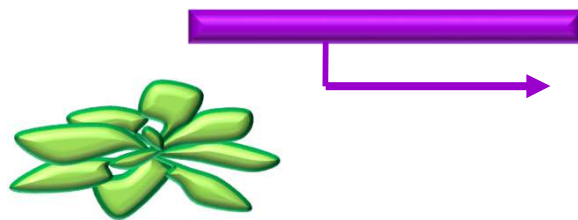


FLC is silenced by vernalization

After 40 days at 4°C, *FLC* is not expressed. Ten days after return to 22°C *FLC* expression is still off.



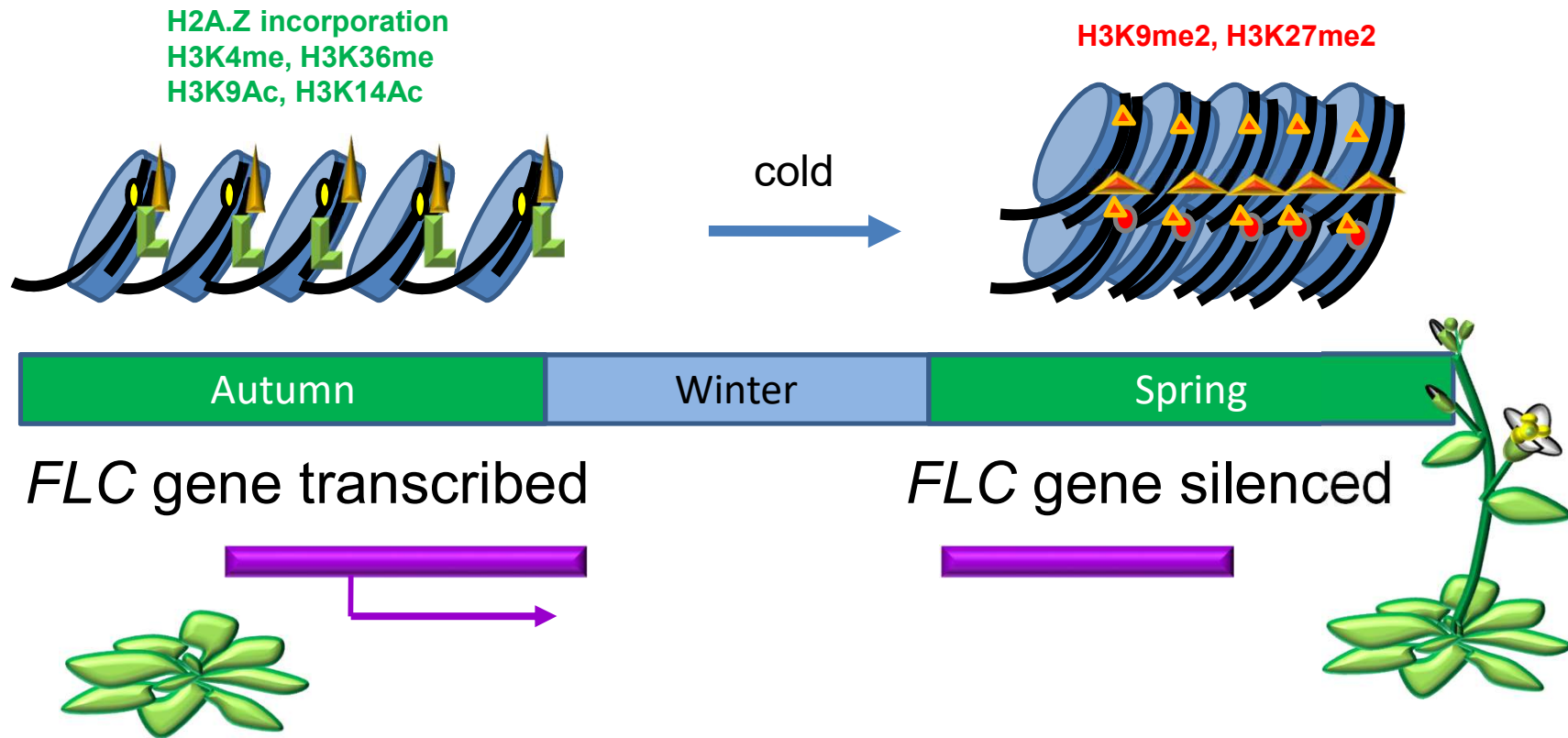
FLC gene transcribed



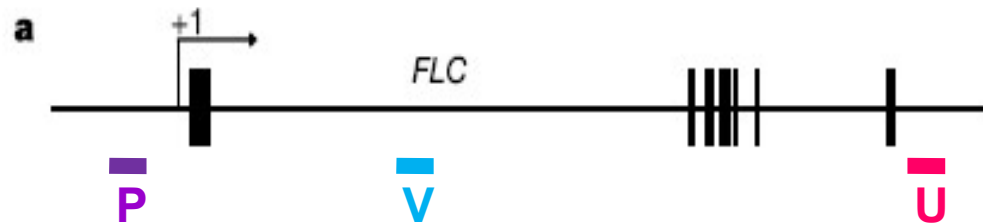
FLC gene silenced



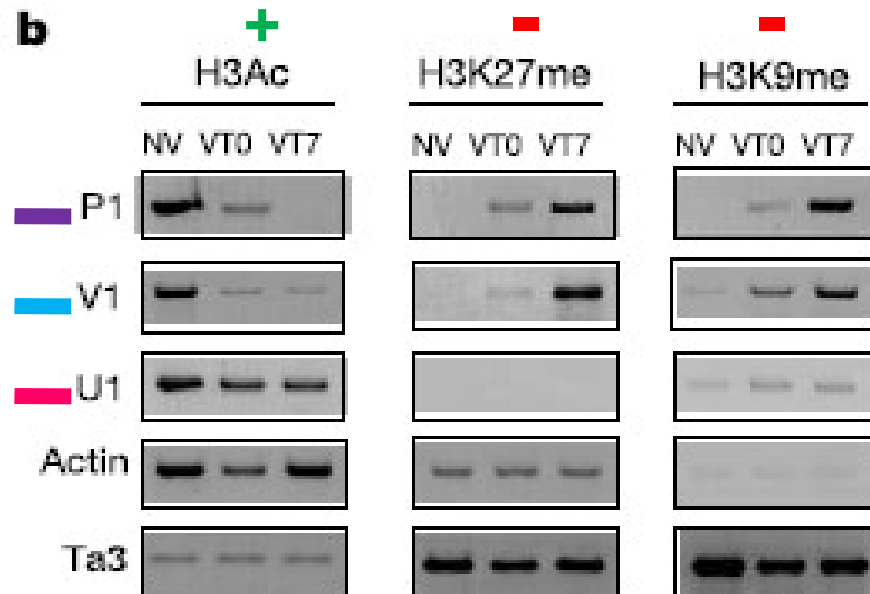
FLC is regulated by epigenetic modifications



The *FLC* gene is epigenetically modified during vernalization

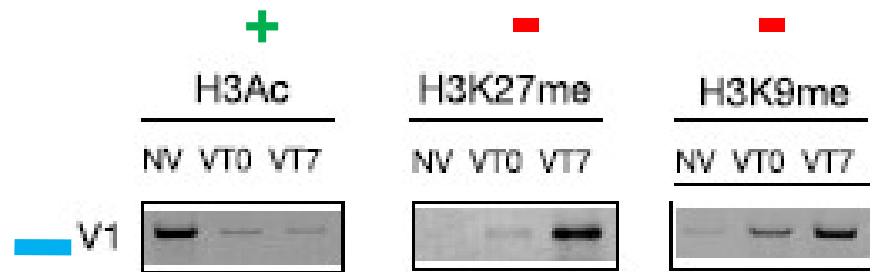
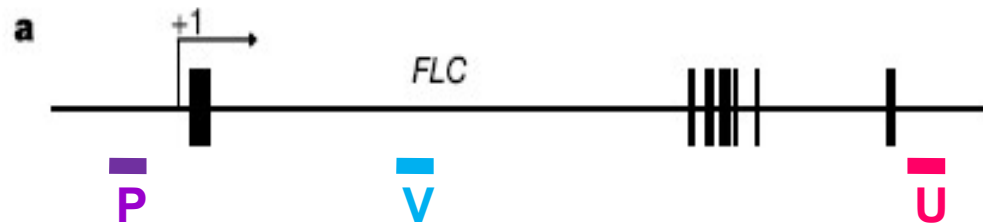


The *FLC* gene promoter (**P**), intron (**V**) and 3'UTR (**U**) were examined for histone modifications. Before vernalization, **P** and **V** showed activating modifications (**H3Ac**), and after vernalization they showed inhibitory modifications (**H3K27me**, **H3K9me**).

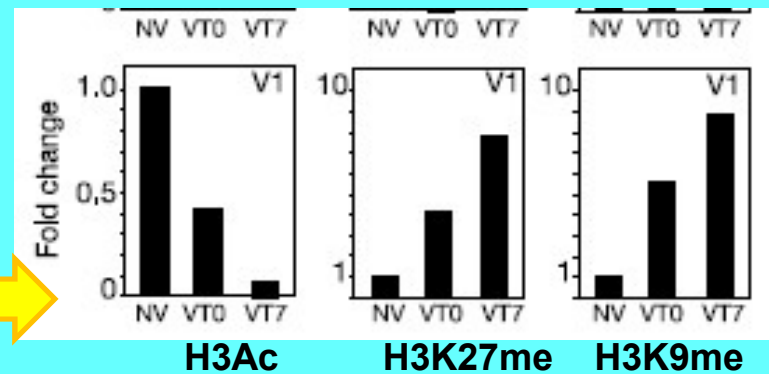


NV = no vernalization
VT0 = 40 days at 4°
VT7 = 40 days at 4° followed by 7 days at 22°

The *FLC* gene is epigenetically modified during vernalization

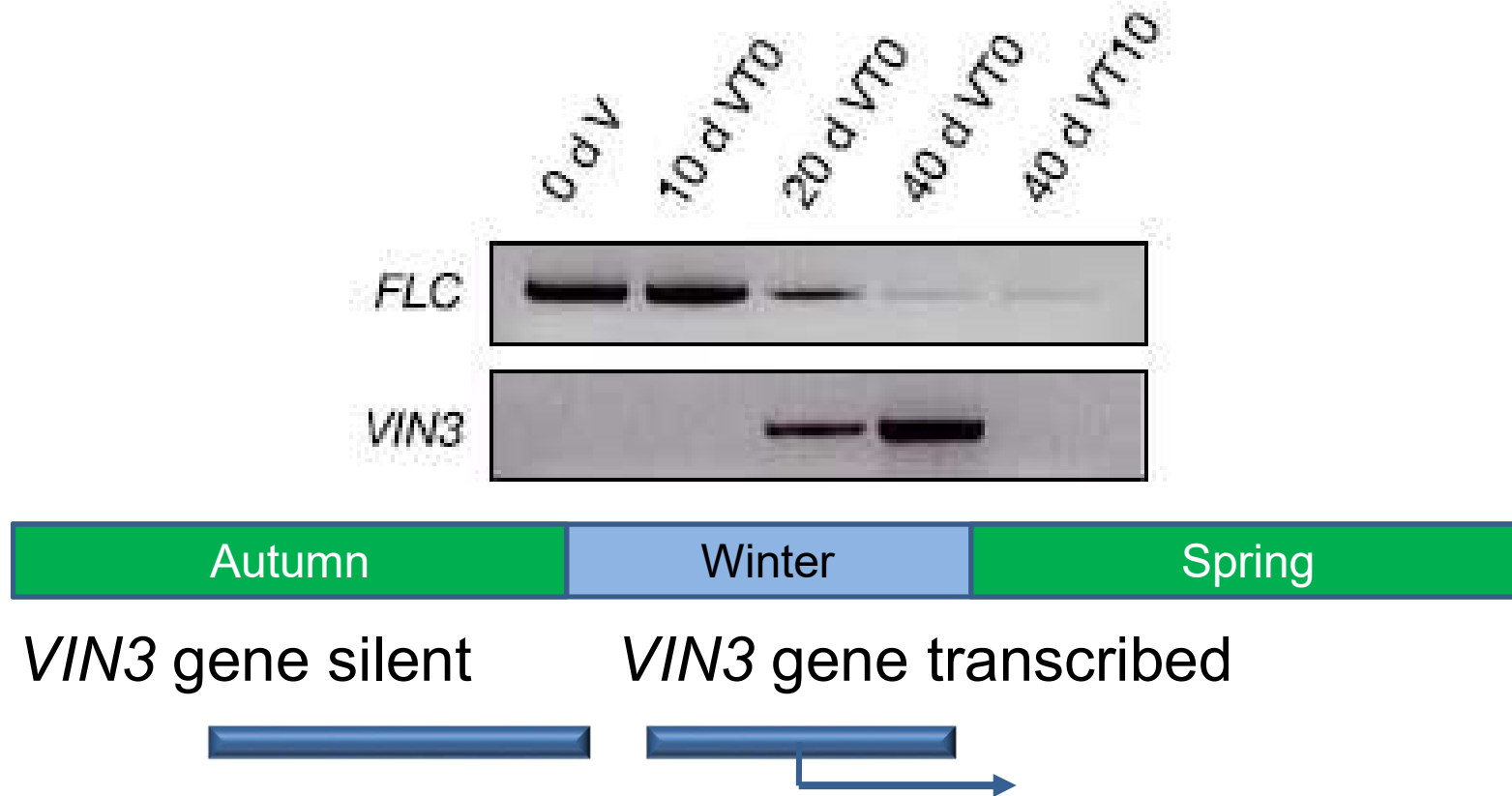


Quantification at intron

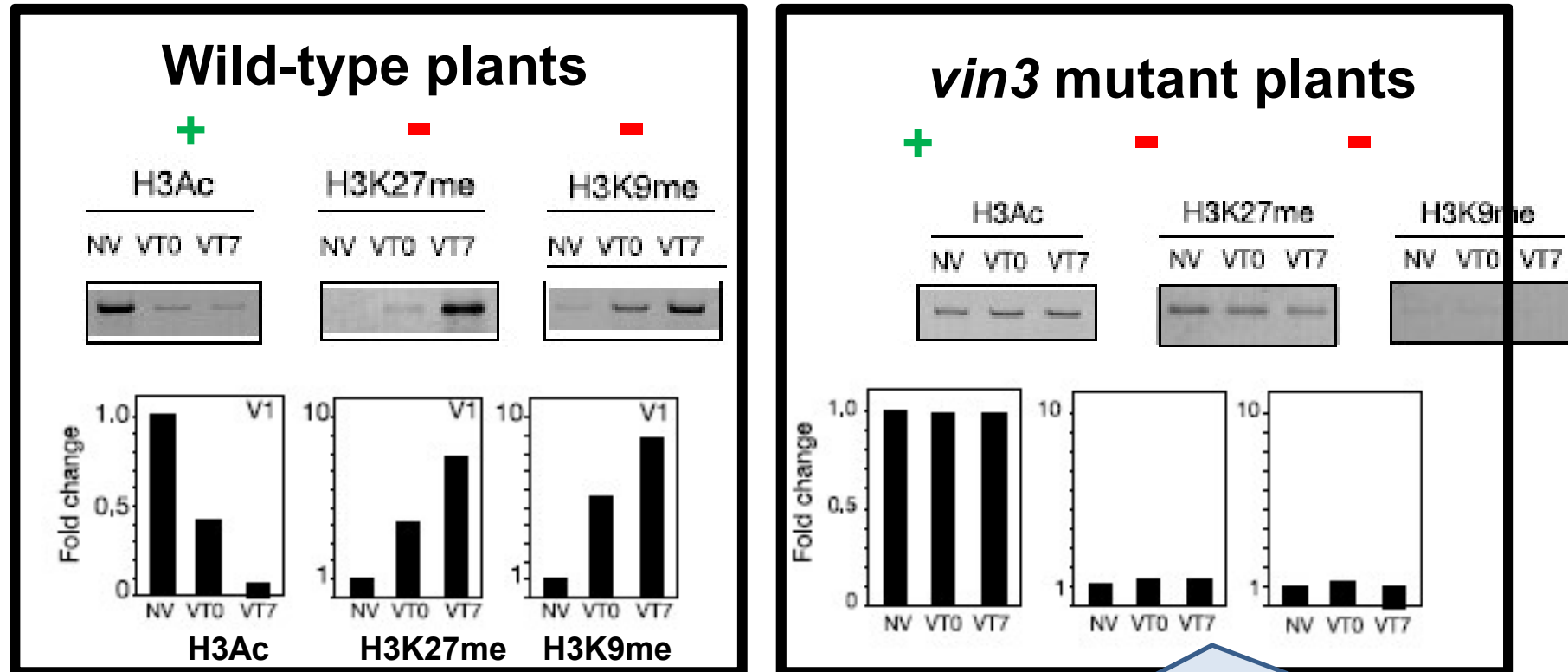


NV = no vernalization
VT0 = 40 days at 4°
VT7 = 40 days at 4°
 followed by 7 days at 22°

VIN3 is induced by vernalization

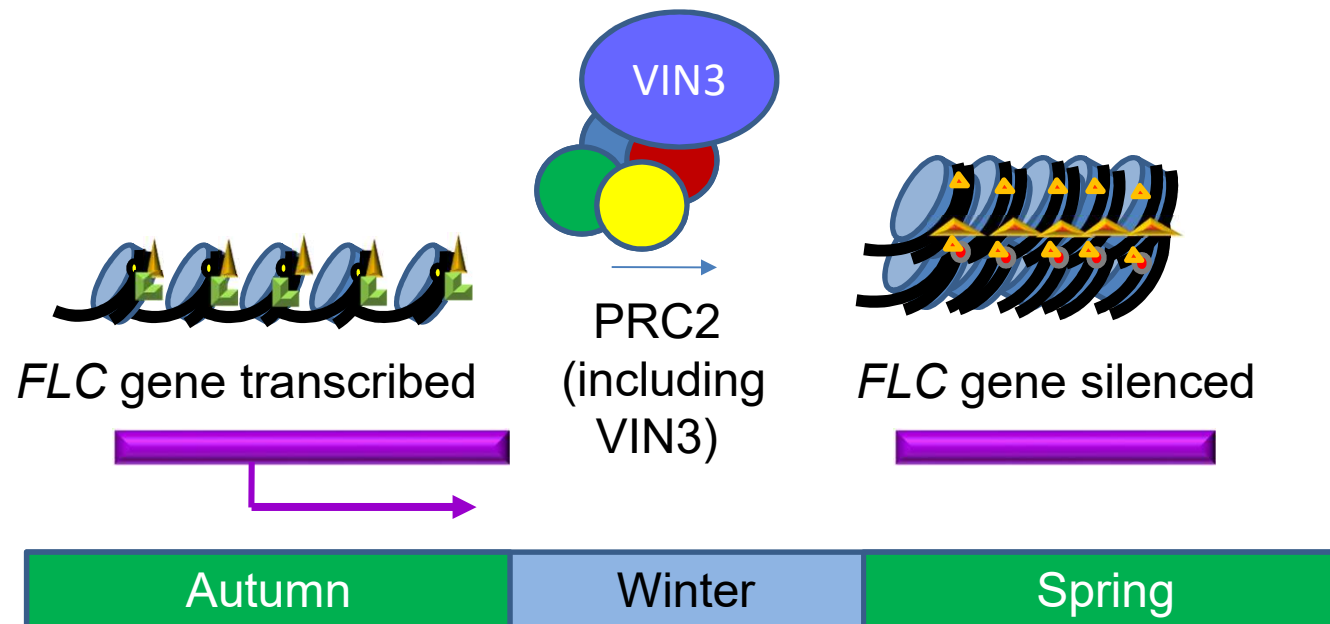


The *VIN3* gene is necessary for *FLC* modification during vernalization

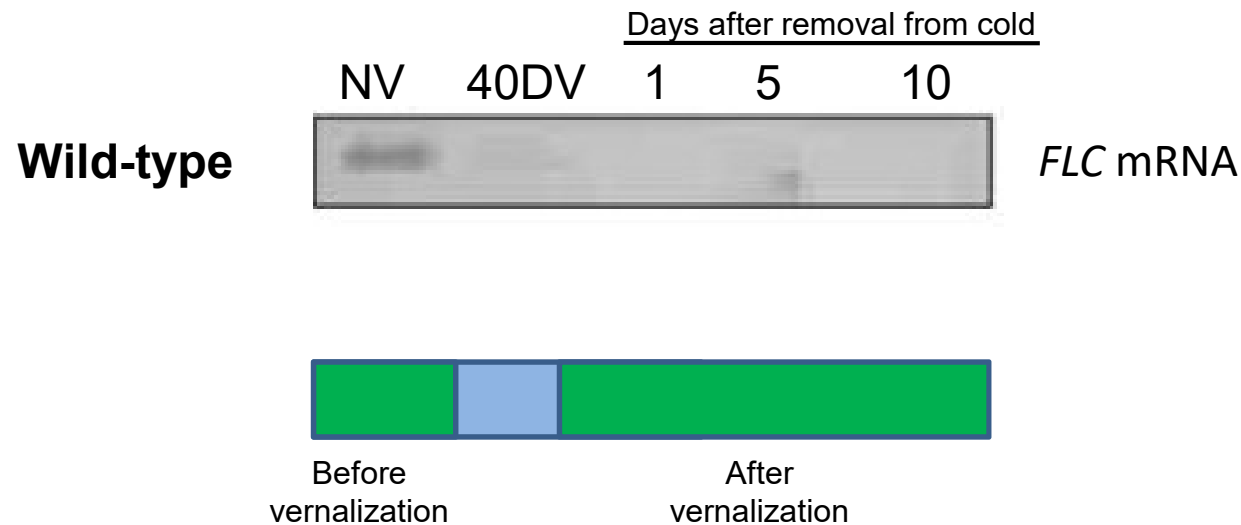


In the *vin3* mutant, the *FLC* gene remains epigenetically "ON", even when vernalized.

VIN3 and the PRC2 complex epigenetically silence *FLC*

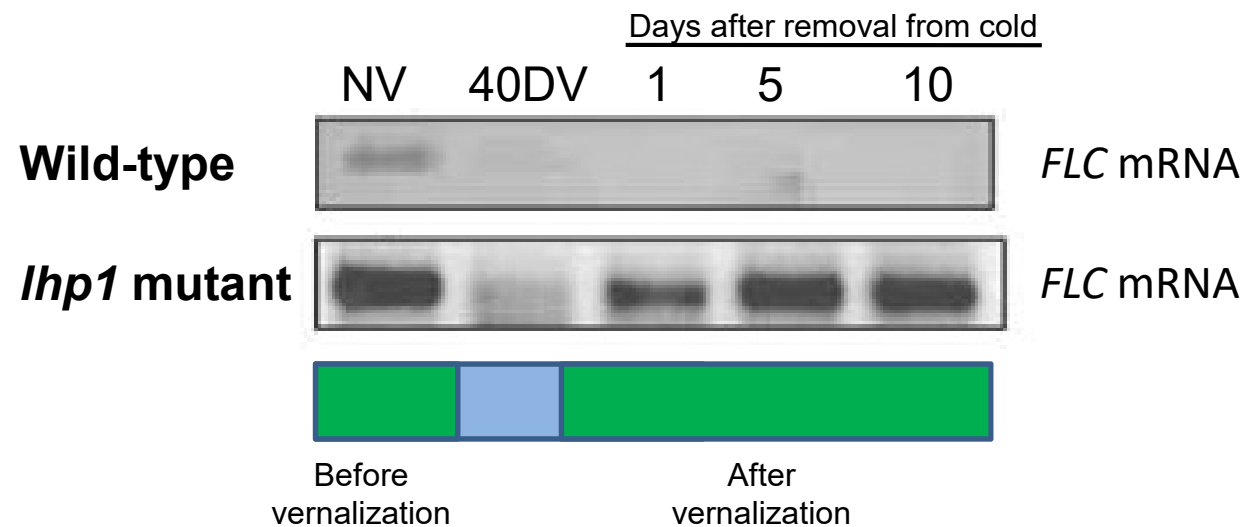


LHP1, a component of PRC1-like, is required to maintain *FLC* silencing



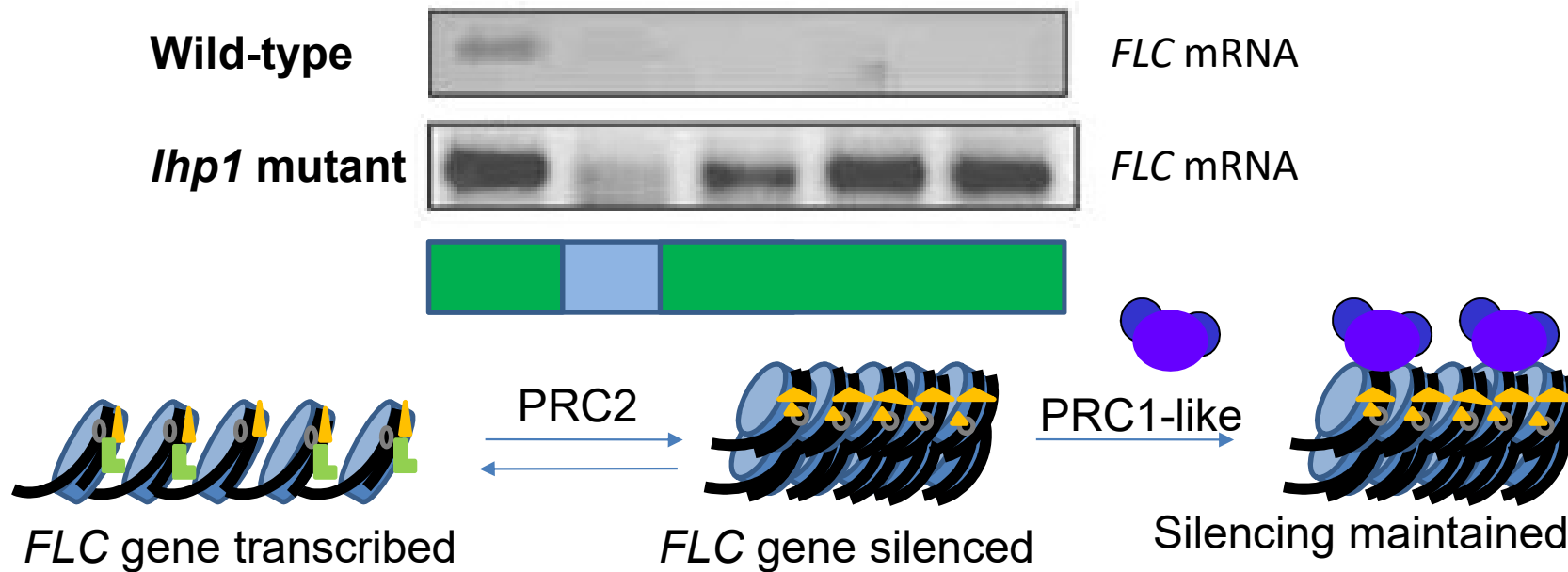
In wild-type plants, *FLC* levels remain low after returning plants to warm temperatures.

LHP1 is required to maintain *FLC* silencing



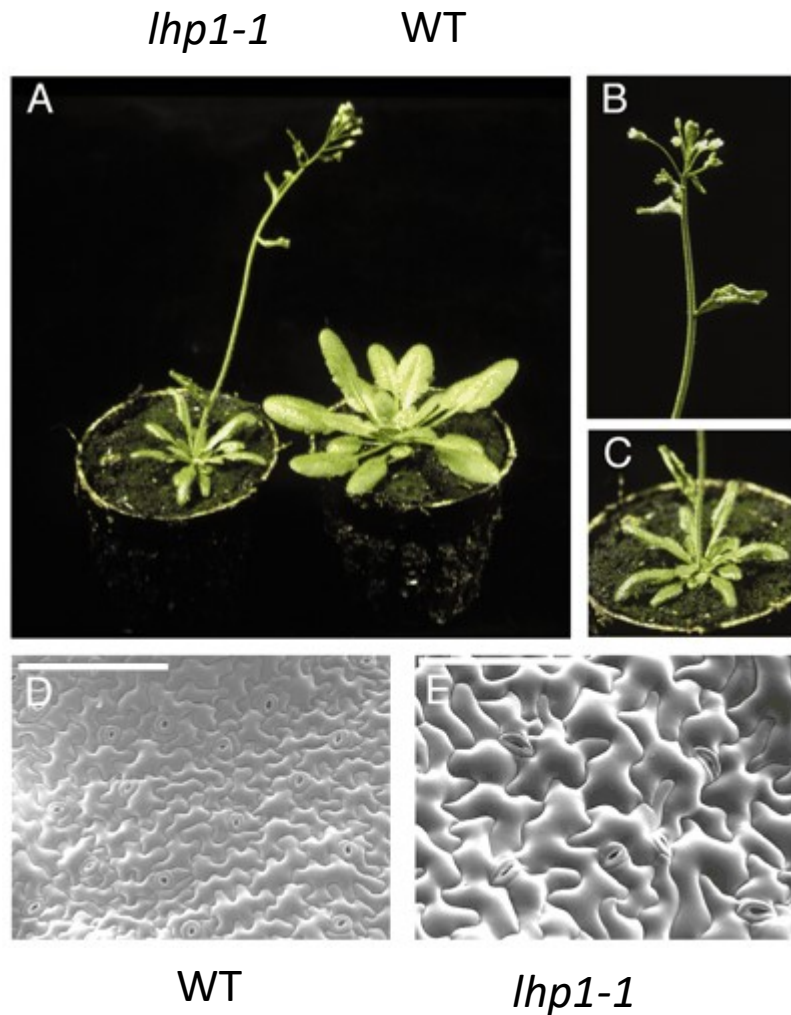
In *lhp1* mutants, *FLC* levels increase after returning plants to warm temperatures indicating that the silencing is not stable.

LHP1 is required to maintain *FLC* silencing

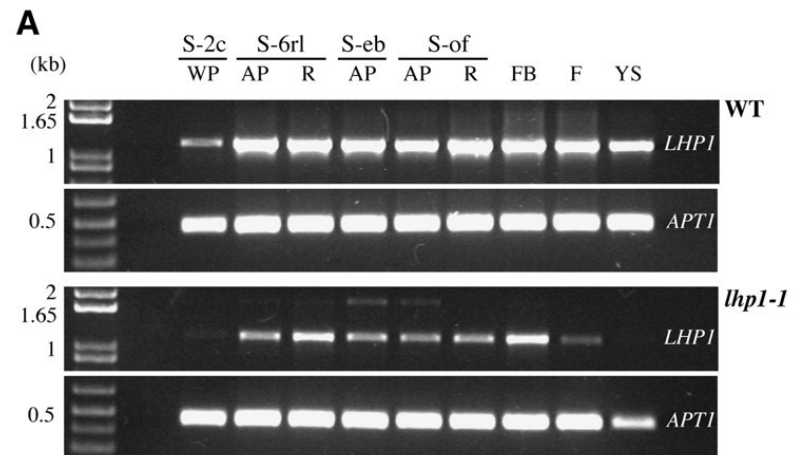


Reprinted by permission from Macmillan Publishers, Ltd: NATURE GENETICS. Sung, S., He, Y., Eshoo, T.W., Tamada, Y., Johnson, L., Nakahigashi, K., Goto, K., Jacobsen, S.E., and Amasino, R.M. (2006) Epigenetic maintenance of the vernalized state in *Arabidopsis thaliana* requires LIKE HETEROCHROMATIN PROTEIN 1. Nature Genetics 38: 706–710. Copyright 2006.

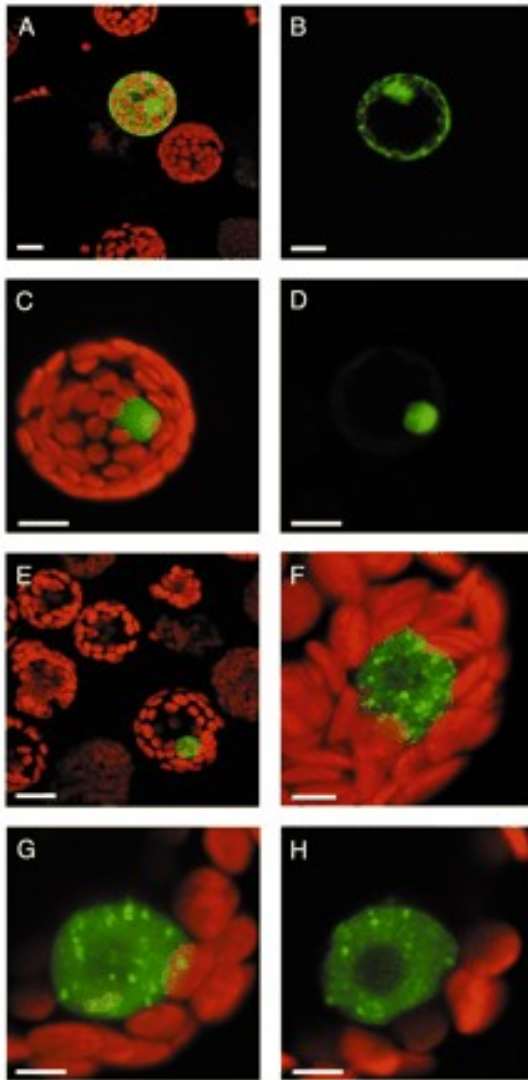
Mutations in *LIKE HETEROCHROMATIN PROTEIN 1* affect flowering time and plant architecture in *Arabidopsis*



Mutante per LHP1 mostra anticipo della fioritura, dimensioni ridotte e alterata morfologia fogliare



Mutations in *LIKE HETEROCHROMATIN PROTEIN 1* affect flowering time and plant architecture in *Arabidopsis*



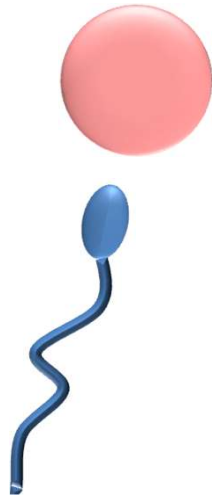
LHP1 è localizzato nel nucleo

LHP1 has a specific subnuclear localisation in tobacco mesophyll protoplasts, in transient assays. Chloroplasts appear red and GFP fluorescence is green; when the two fluorescences overlap, the yellow colour appears. (A,B) Protoplasts expressing GFP alone (pAVA121 plasmid). (A) Projection. (B) Section. (C,D) Protoplast expressing GFP-VirD2NLS. (C) Projection. (D) Section. The GFP fluorescence is uniformly distributed throughout the nucleus. (E-H) Protoplasts electroporated with the LHP1-GFP construct. A diffuse nucleoplasmic distribution and discrete particles are observed. (E) Projection of several protoplasts. One protoplast expresses the LHP1-GFP fusion in the nucleus, the others are not transformed. (F) Close-up view of the nucleus (projection). (G-H) Sections. Scale bar, (A-E) 10 μm ; (F-H) 2 μm .

Epigenetic control of flowering time

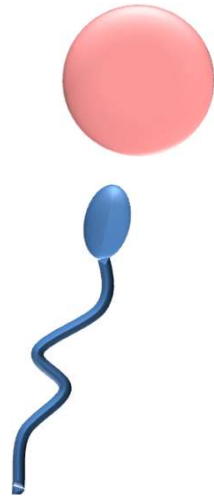
- During vernalization, silencing of *FLC* allows expression of *FT* and other flowering promoters
- VIN3 and PRC2 complex proteins epigenetically modify *FLC* to silence it.
- How cold induces *VIN3* expression is not currently known.
- Maintaining the silenced state requires PRC1-like complex which includes LHP1.

Genomic imprinting



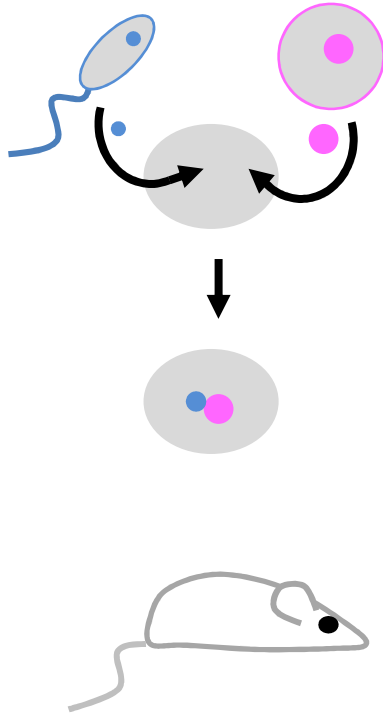
- The zygote receives two copies of each gene, one from the mother's genome and one from the father's.
- At most loci, both copies are active.

Genomic imprinting



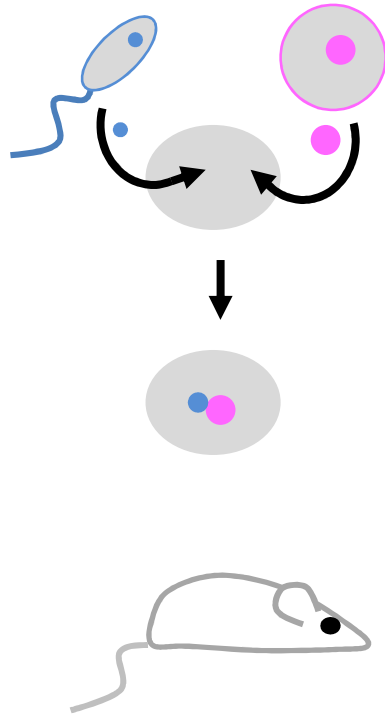
- The zygote receives two copies of each gene, one from the mother's genome and one from the father's.
- At most loci, both copies are active.
- Some loci, **imprinted** loci, show a "parent of origin effect".
- Expression of these loci is controlled by epigenetic factors.

Imprinting – nuclear transplant experiment

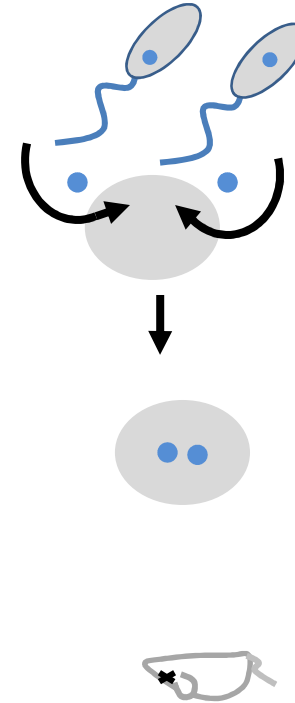
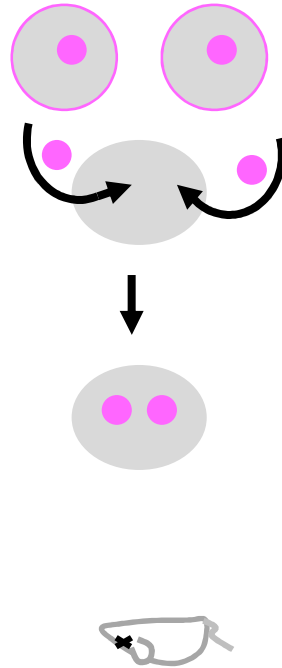


Placing a sperm and an egg nucleus into an enucleated fertilized cell leads to a normal embryo.

Imprinting – nuclear transplant experiment

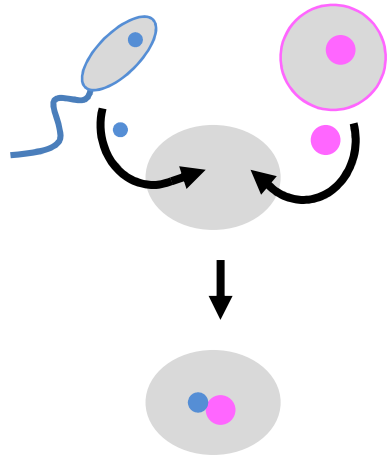


experiment



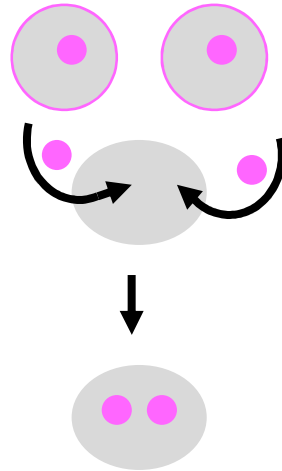
Zygotes that receive only maternal or only paternal nuclei do not survive.

Imprinting – nuclear transplant experiment

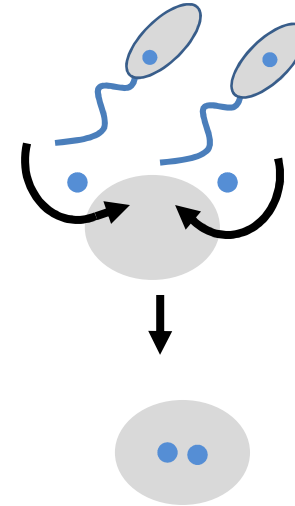


The two parental genomes are not equivalent

experiment



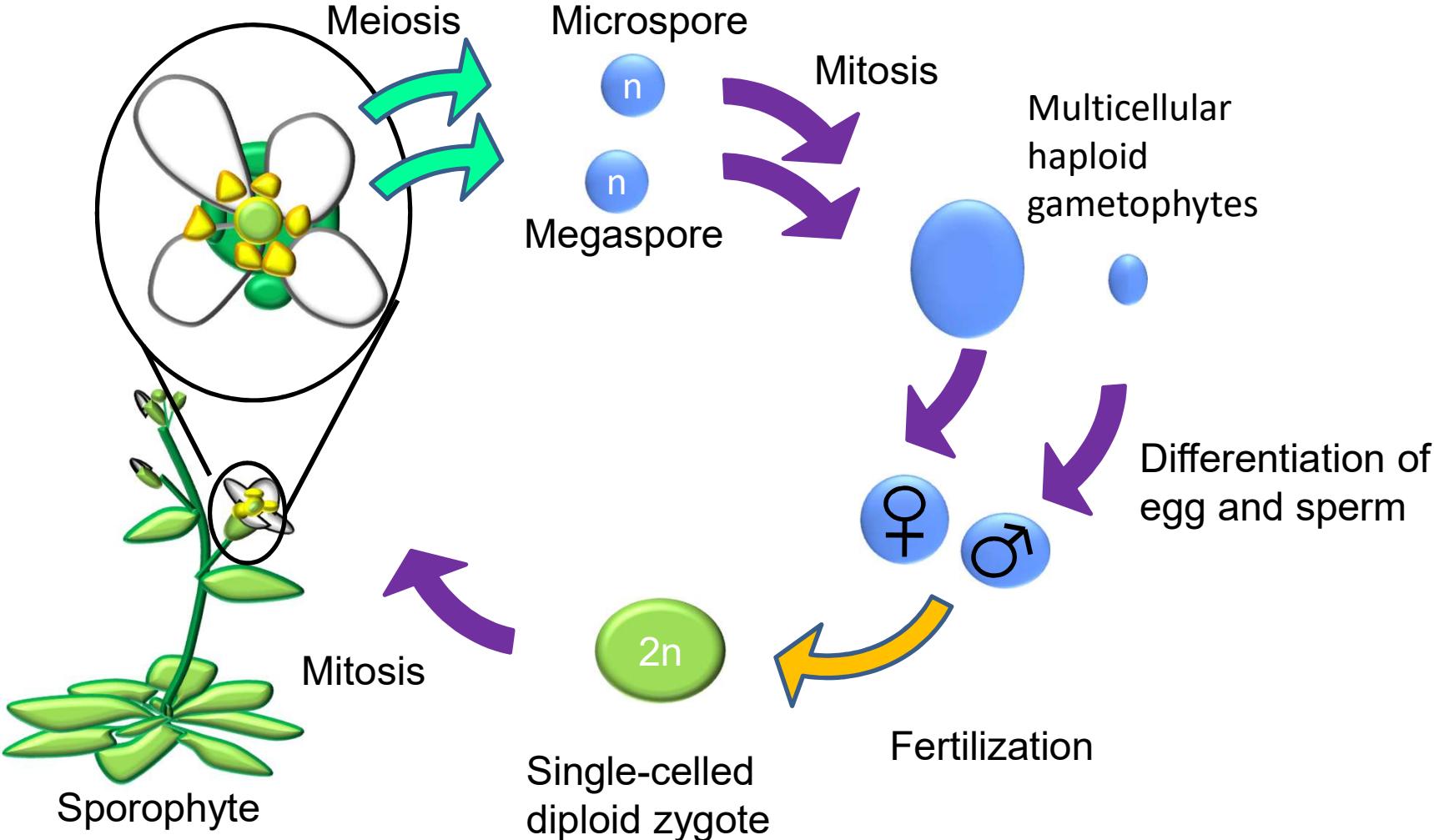
Zygotes that receive only maternal or only paternal nuclei do not survive.



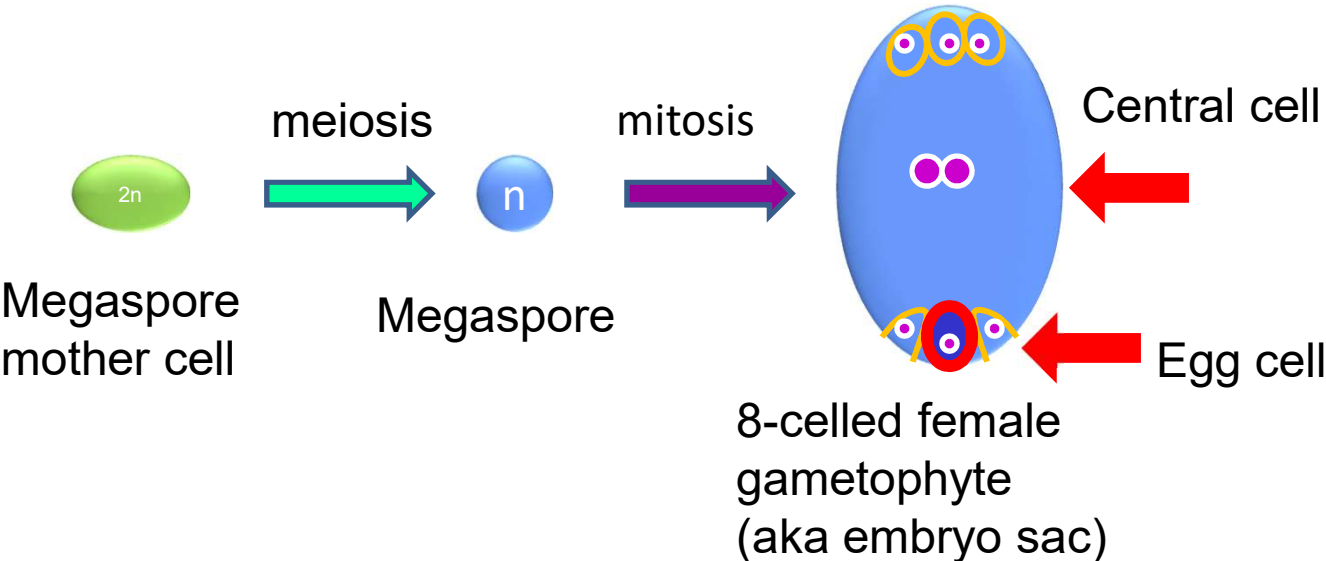
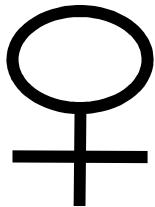
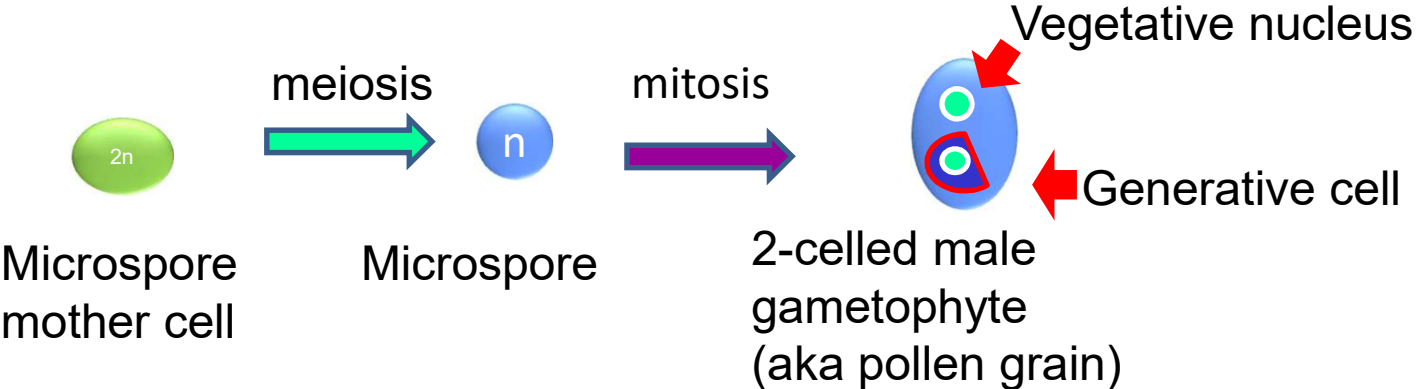
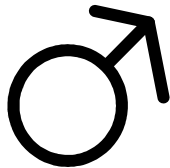
Imprinting in angiosperms

- Angiosperms show genomic imprinting too, but their reproduction is a bit more complicated than that of animals.....

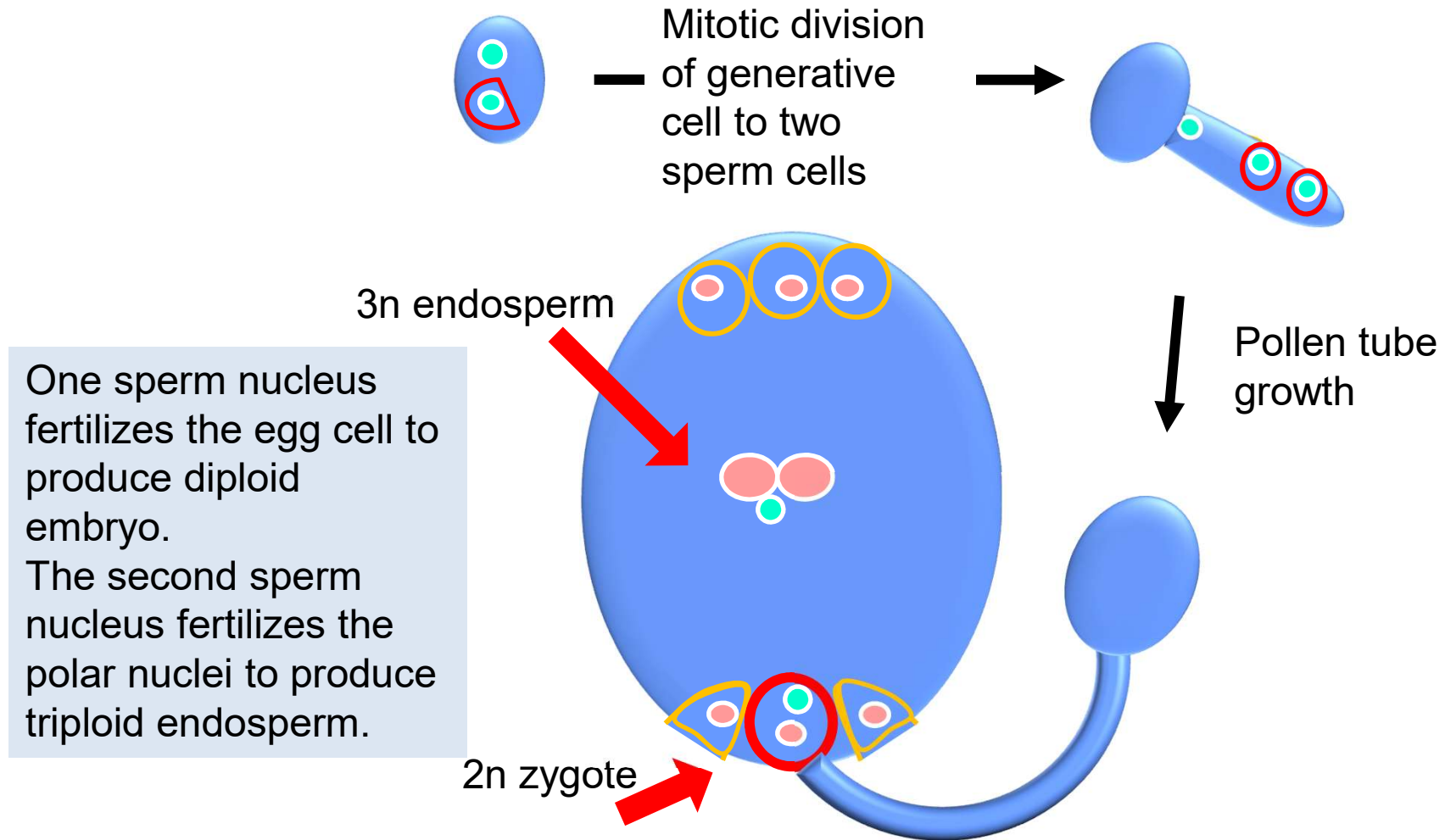
Angiosperm reproduction



Development of male and female gametophyte

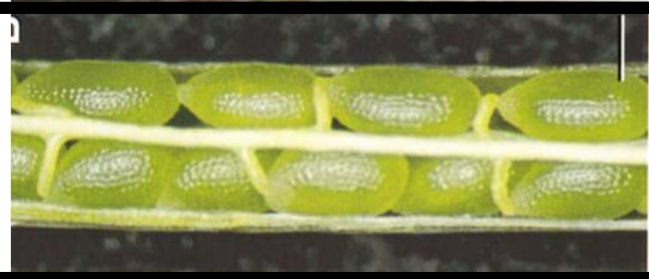


Double Fertilization



The *MEDEA* (*MEA*) gene is imprinted

♂ *MEA/mea* x ♀ *MEA/MEA*
All seeds viable

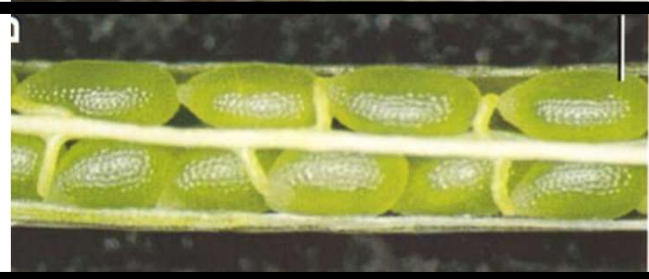


♂ *MEA/MEA* x ♀ *MEA/mea*
50% of seeds abort



The *MEDEA* (*MEA*) gene is imprinted

♂ *MEA/mea* x ♀ *MEA/MEA*
All seeds viable



♂ *MEA/MEA* x ♀ *MEA/mea*
50% of seeds abort



In the second cross, 50% of the seeds receive the mutant *mea* allele from their mother.

These seeds abort, even though they also have a wild-type *MEA* allele inherited from their father.

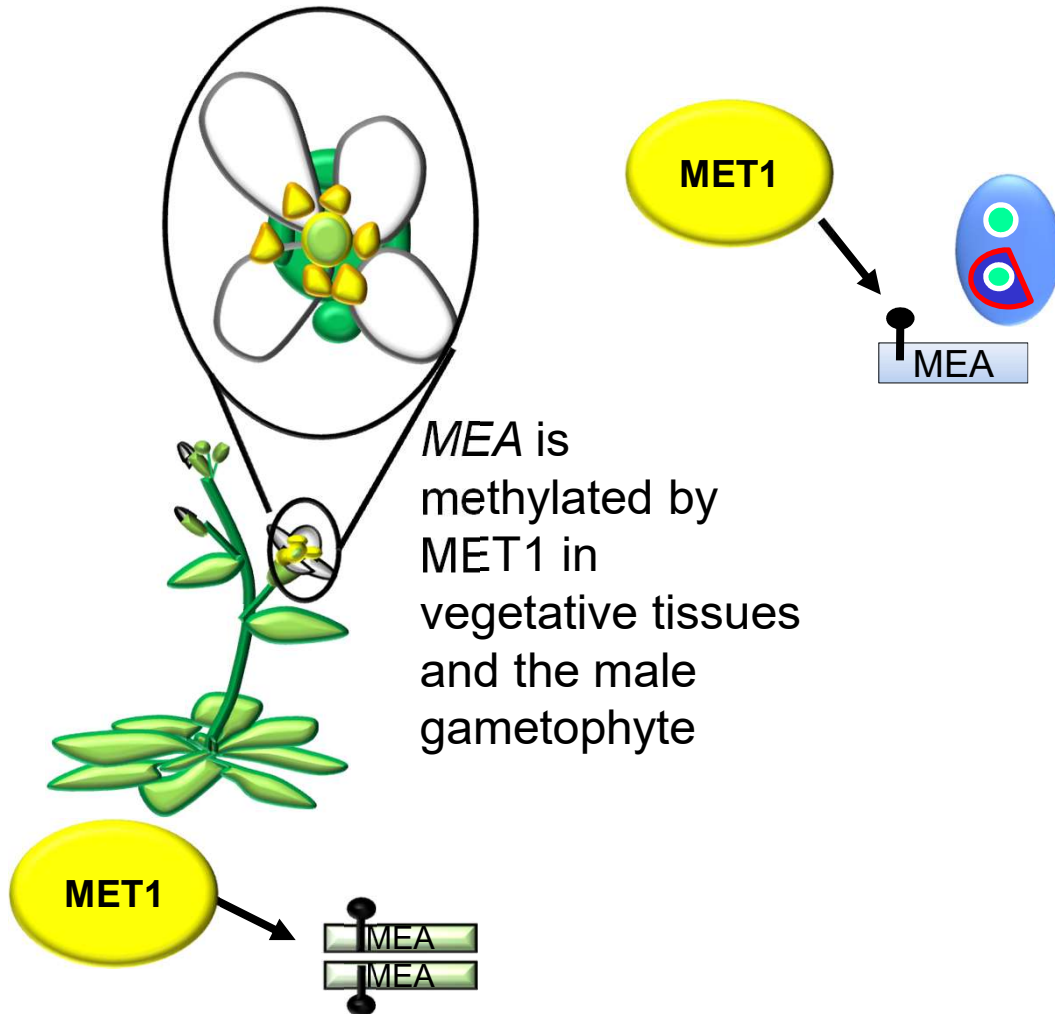
The *MEDEA* (*MEA*) gene is imprinted



The paternal allele is silent.

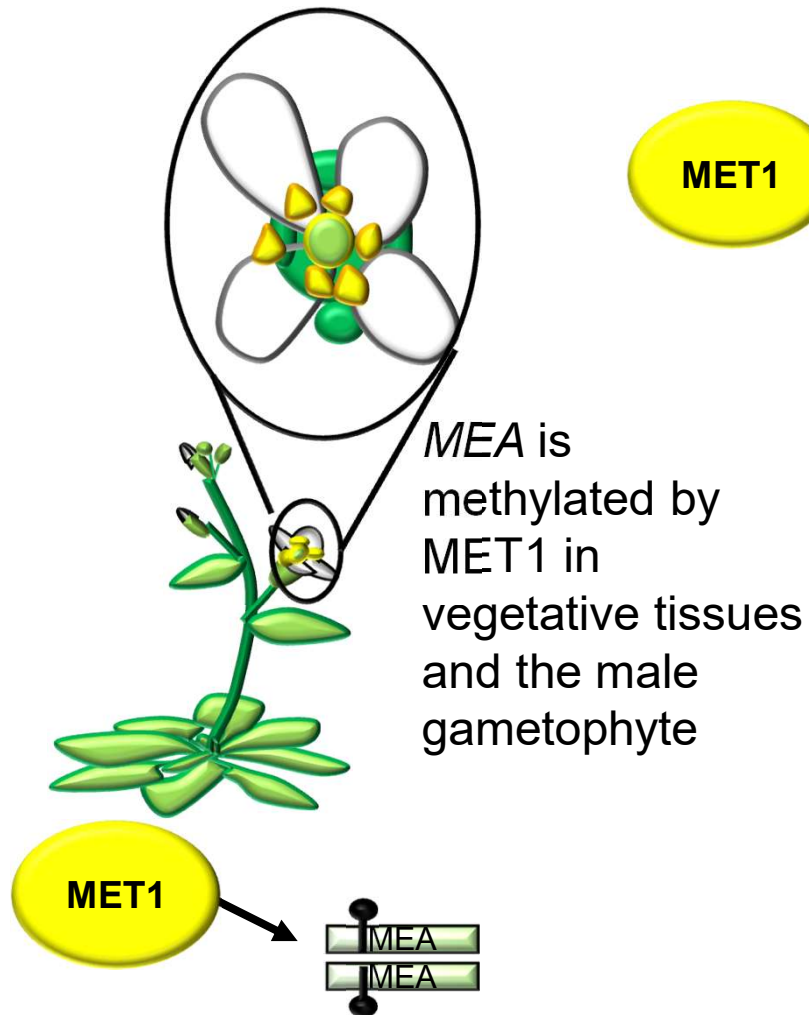
The phenotype of the progeny is based on the maternal genotype only.

MEA is silenced in most cells

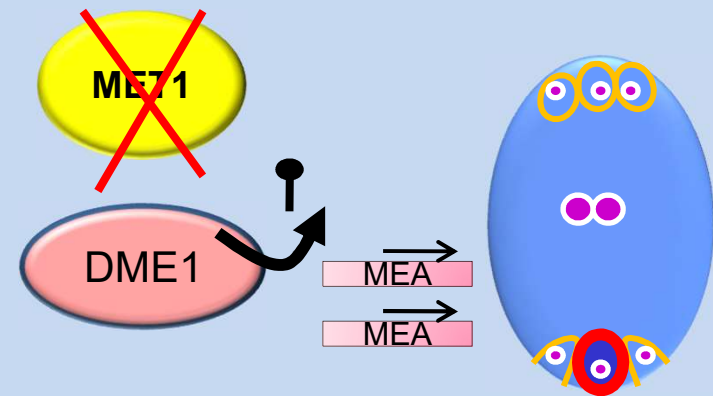


The *MEA* allele inherited from the father is ***silent***.

MEA is silenced in most cells

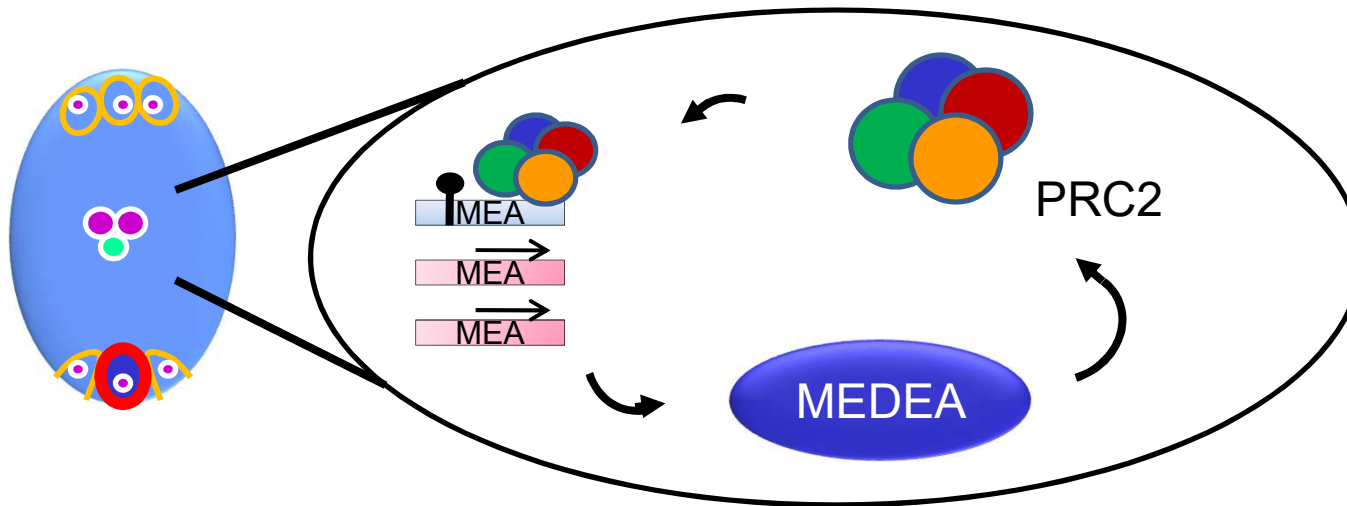


MEA is demethylated and expressed in the female gametophyte.

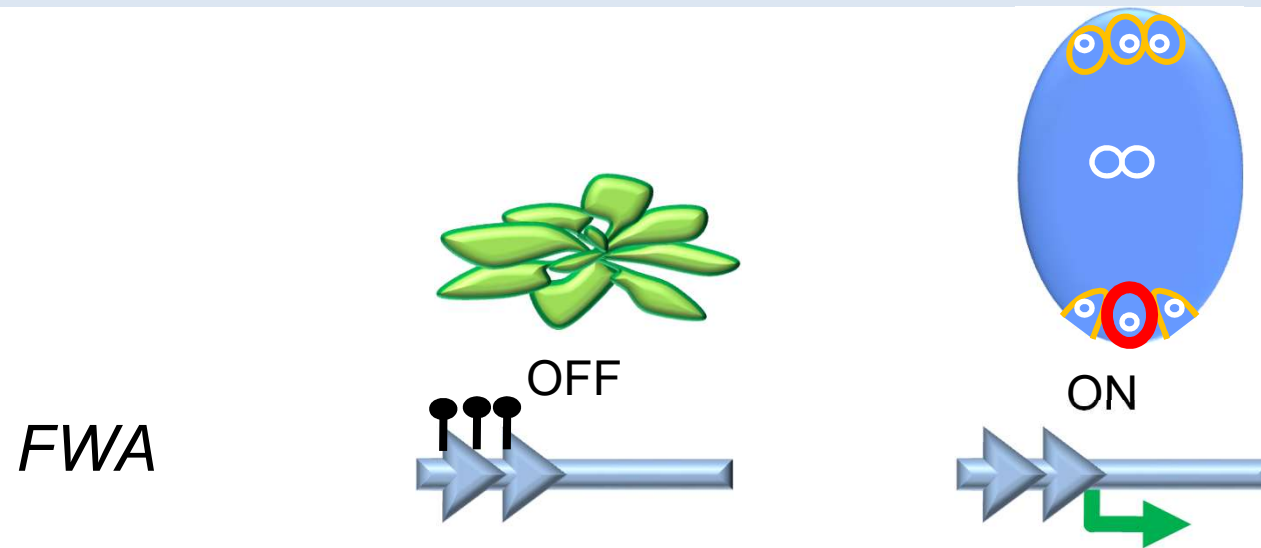


MEA regulates its own imprinting

- MEA encodes a component of PRC2.
- In the triploid endosperm, continued silencing of the parental allele requires histone modification by PRC2.

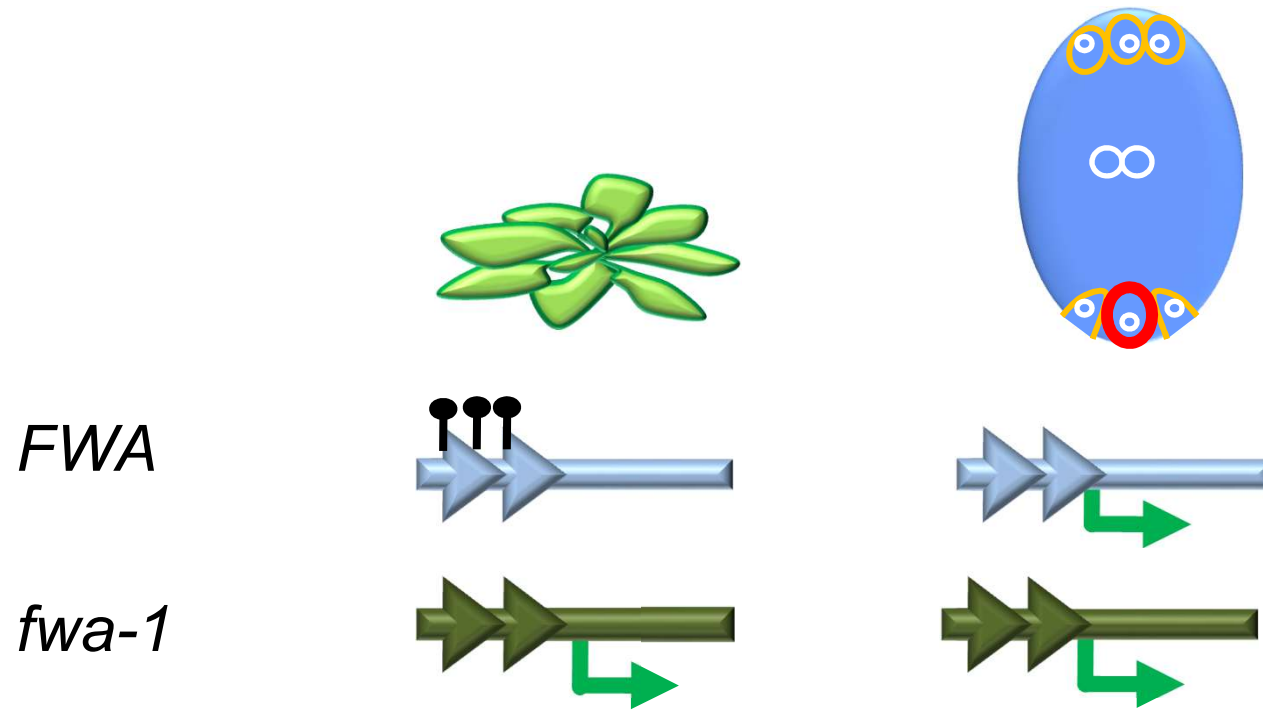


Silencing *in trans*: silencing of *FWA*



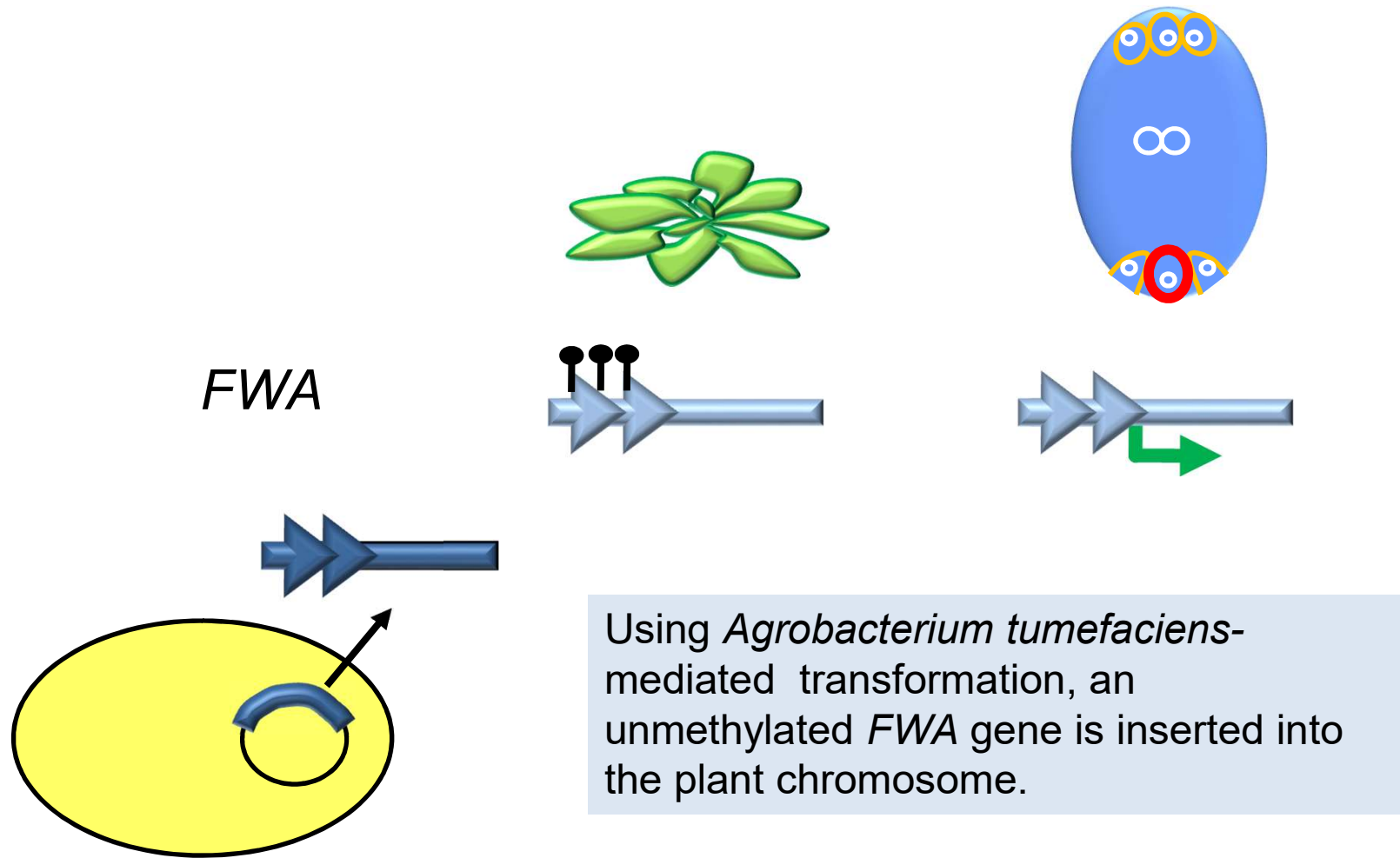
FWA is another imprinted gene. It is expressed in the female gametophyte, like *MEA*.

Silencing *in trans*: silencing of *FWA*



The *fwa-1* epiallele is hypomethylated and expressed in vegetative tissues.

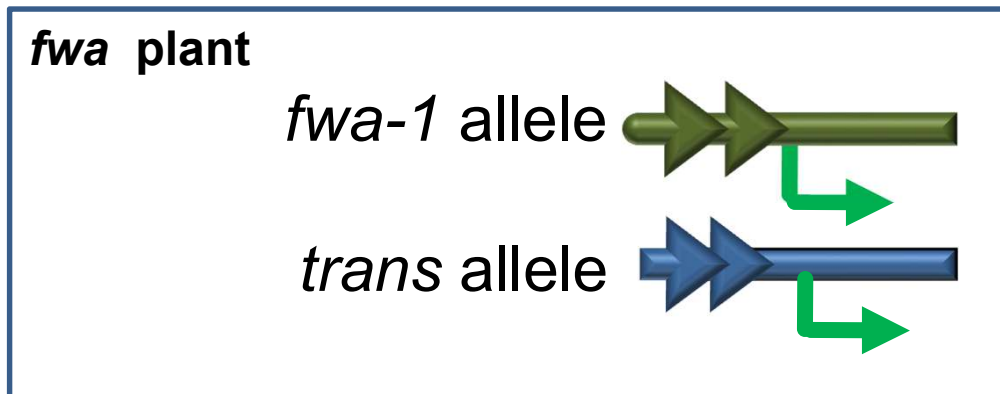
Silencing *in trans*: silencing of *FWA*



Silencing *in trans*: silencing of *FWA*

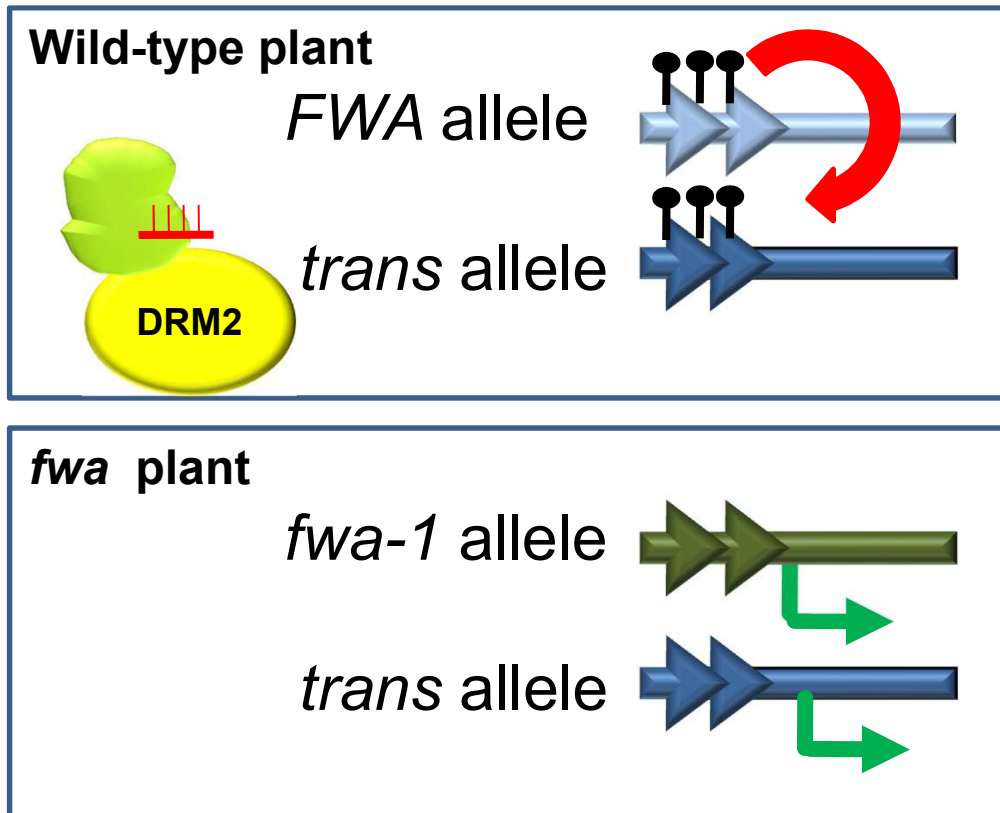


In plants with the wild-type *FWA* allele, the *trans* allele is silenced.



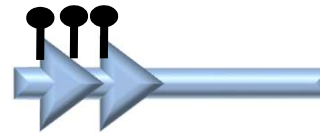
In plants with the *fwa-1* allele, the *trans* allele is active.

Silencing *in trans*: silencing of *FWA*

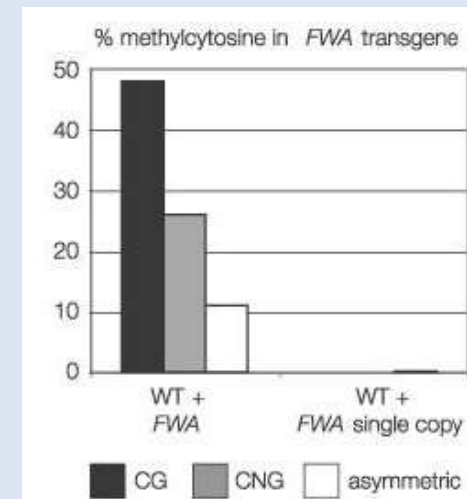


In plants with the wild-type *FWA* allele, methylation is maintained by siRNA and DRM2, which is capable of also methylating the *FWA* gene introduced *in trans*.

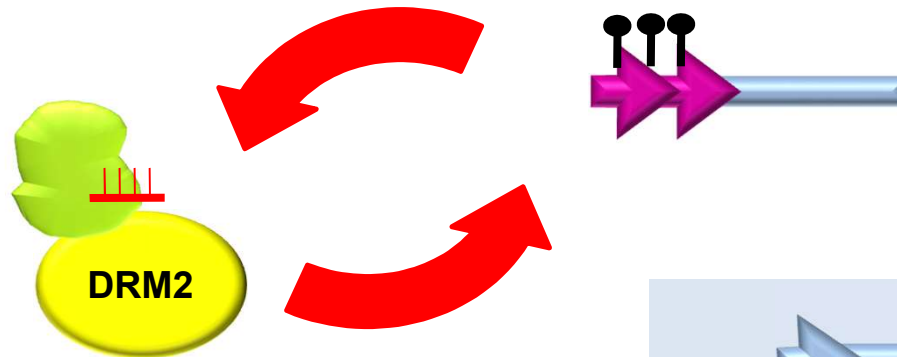
Silencing *in trans*: silencing of *FWA*



A *FWA* gene with one repeat deleted does not promote DNA methylation.



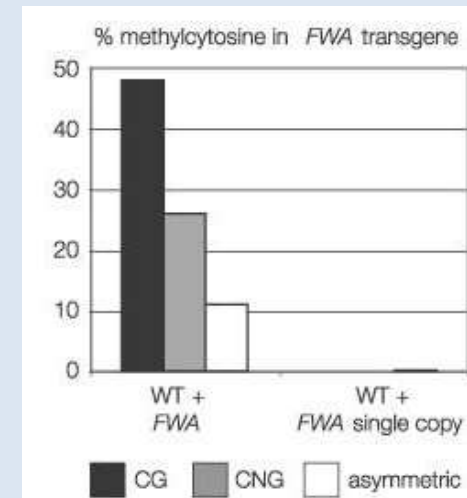
Silencing *in trans*: silencing of *FWA*



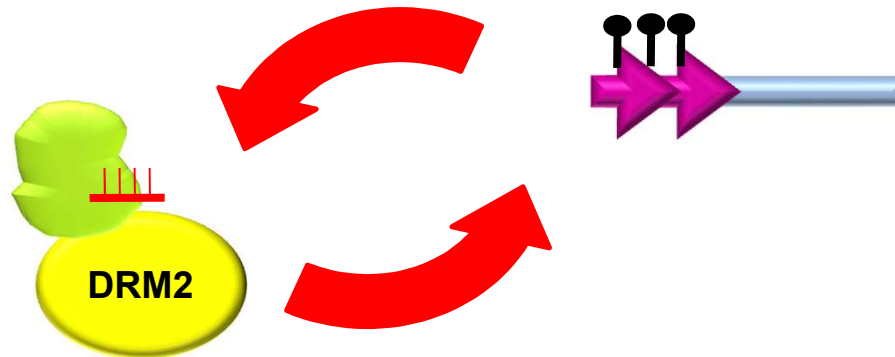
The **tandem repeat** upstream of the *FWA* gene promotes siRNA production and is necessary for methylation and silencing.



A *FWA* gene with one repeat deleted does not promote DNA methylation.



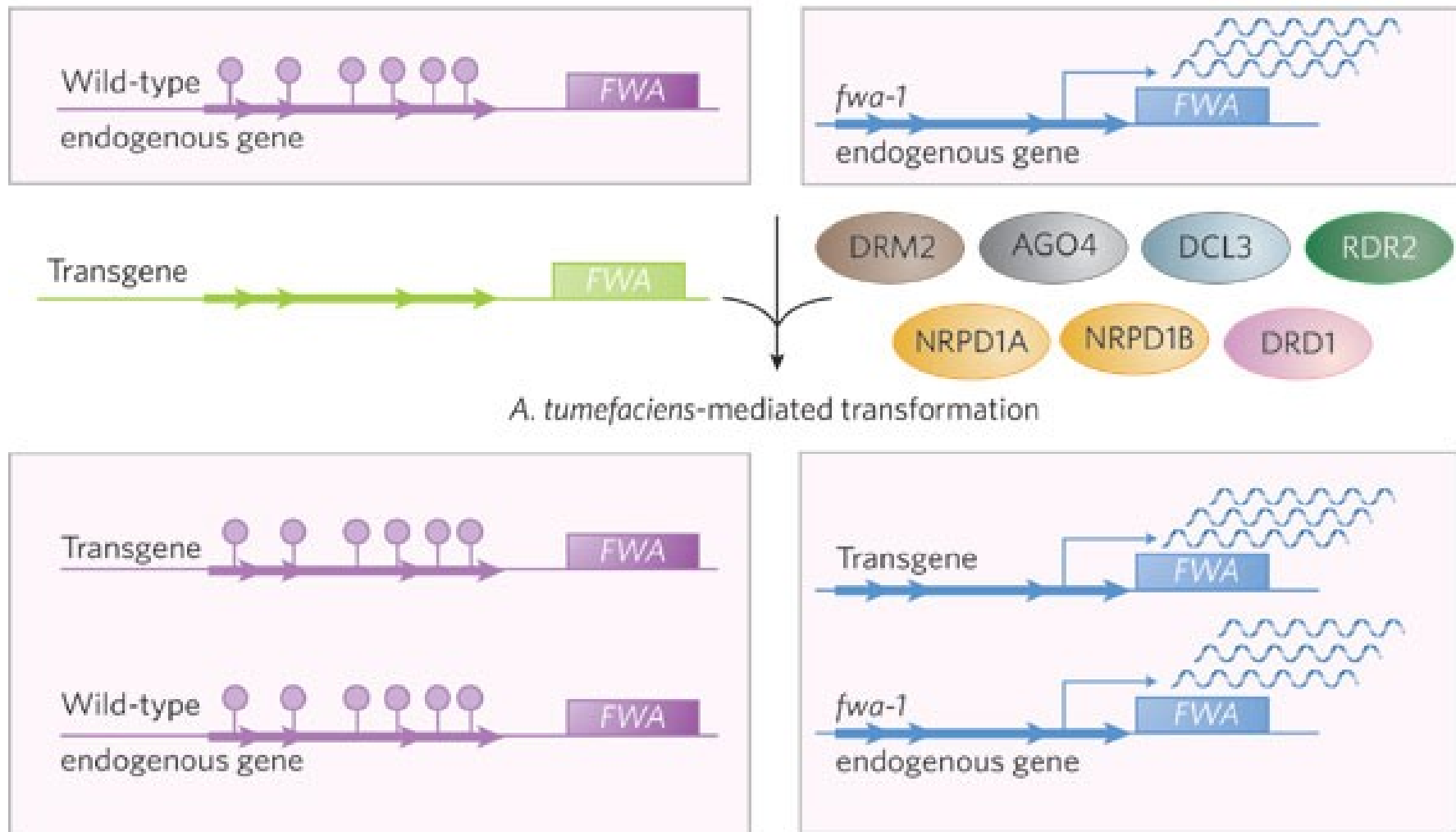
Silencing *in trans*; silencing of *FWA*



The **tandem repeat** upstream of the *FWA* gene promotes siRNA production and is necessary for methylation and silencing.

Tandem repeats are common features of transposons and repetitive elements. The presence of the tandem repeats triggers the epigenetic mechanism that plants use to silence potentially “foreign” DNA.

b



Silencing *in trans*: paramutation

Paramutation: The interaction of two alleles of the same locus, resulting in a heritable change of one allele that is induced by the other allele. The maize *b1* locus has a paramutagenic allele.

Silencing *in trans*: paramutation

B-1 allele
(highly active)



Plants with an active B-1 allele have a lot of purple anthocyanin pigmentation.



Paramutation: The interaction of two alleles of the same locus, resulting in a heritable change of one allele that is induced by the other allele. The maize *b1* locus has a paramutagenic allele.

Silencing *in trans*: paramutation

B-I allele
(highly active)



B' allele
(weakly active)



The B' allele is weakly active, and plants are pale.

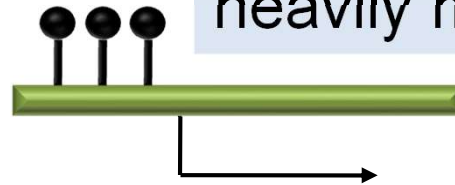


Silencing *in trans*: paramutation

B-I allele
(highly active)



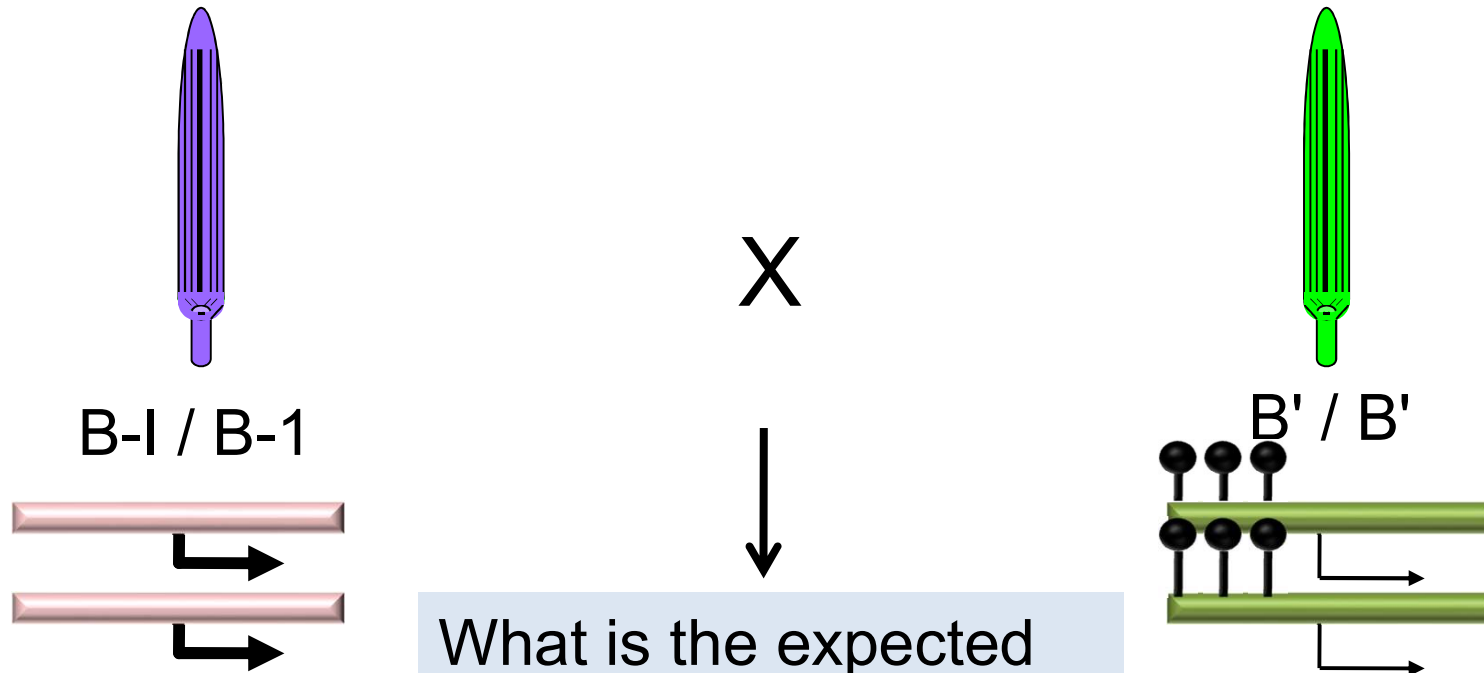
B' allele
(weakly active)



The B' allele is heavily methylated.

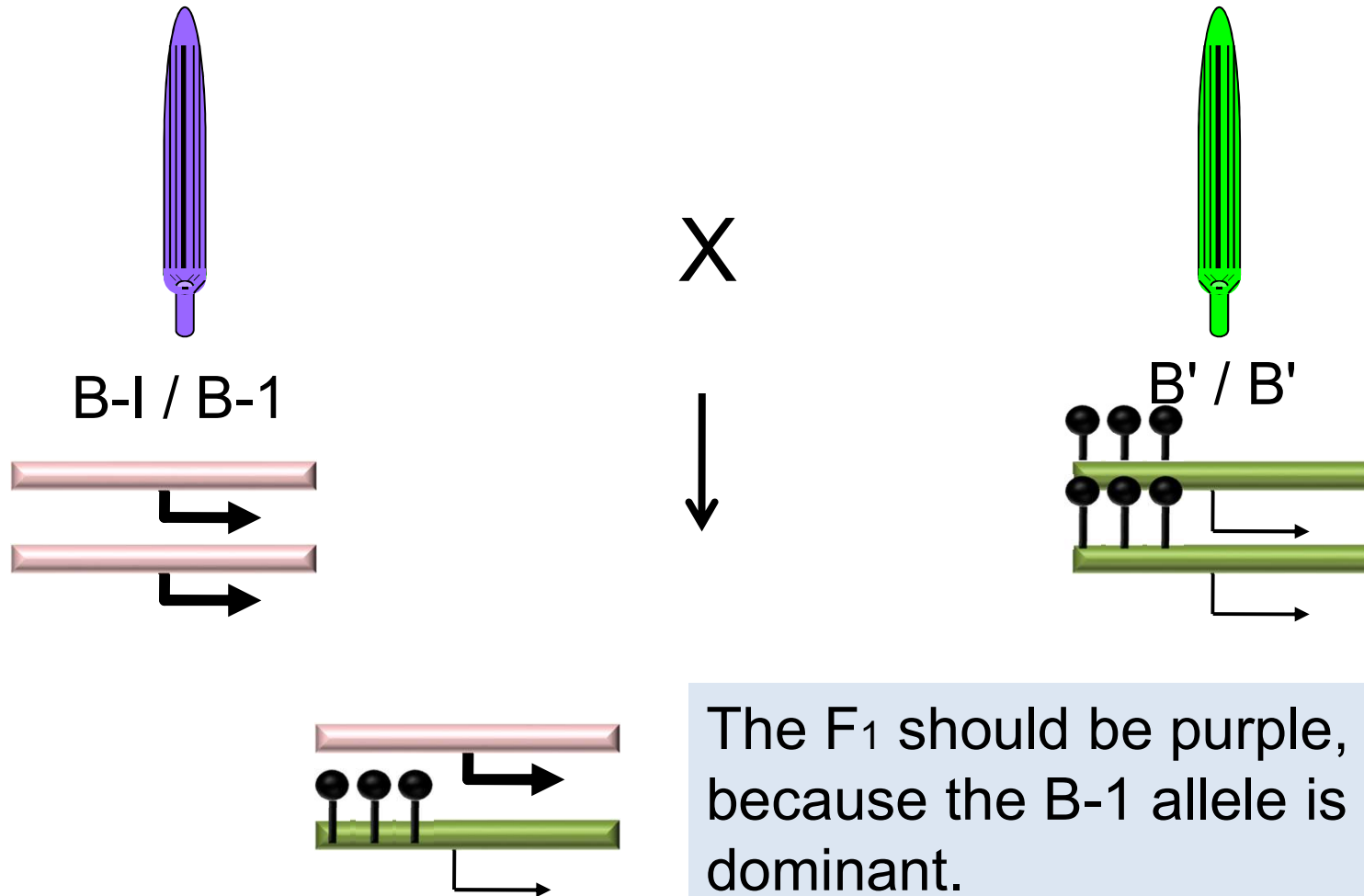


Silencing *in trans*: paramutation

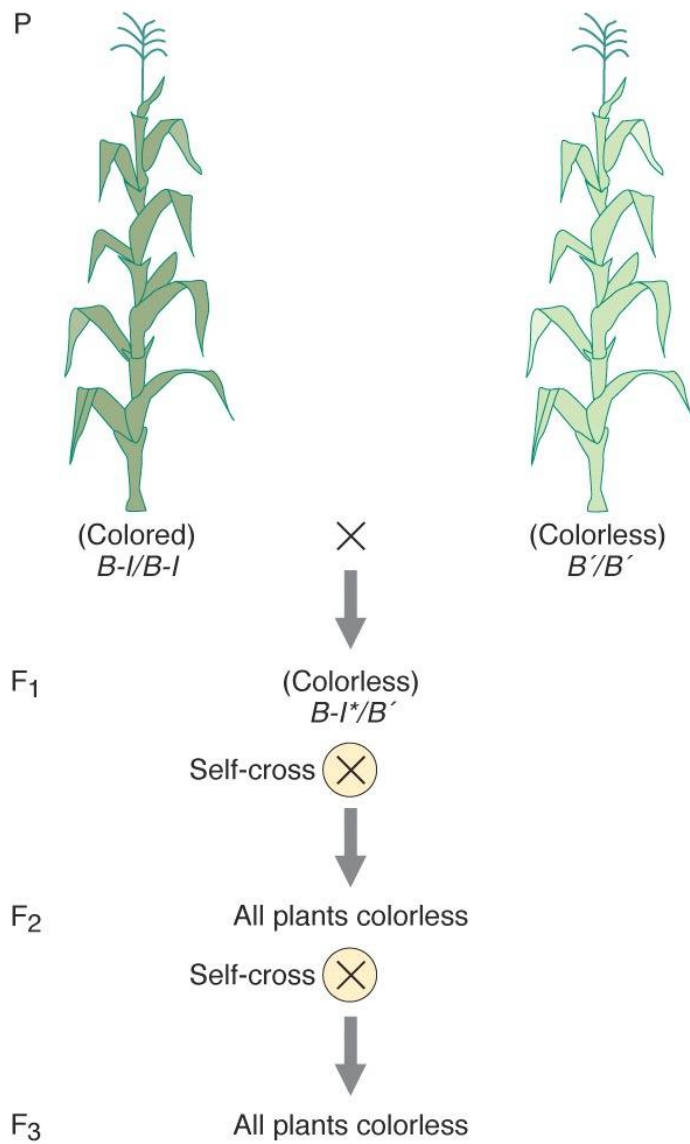


What is the expected phenotype of the progeny of a cross between these two homozygous parents?

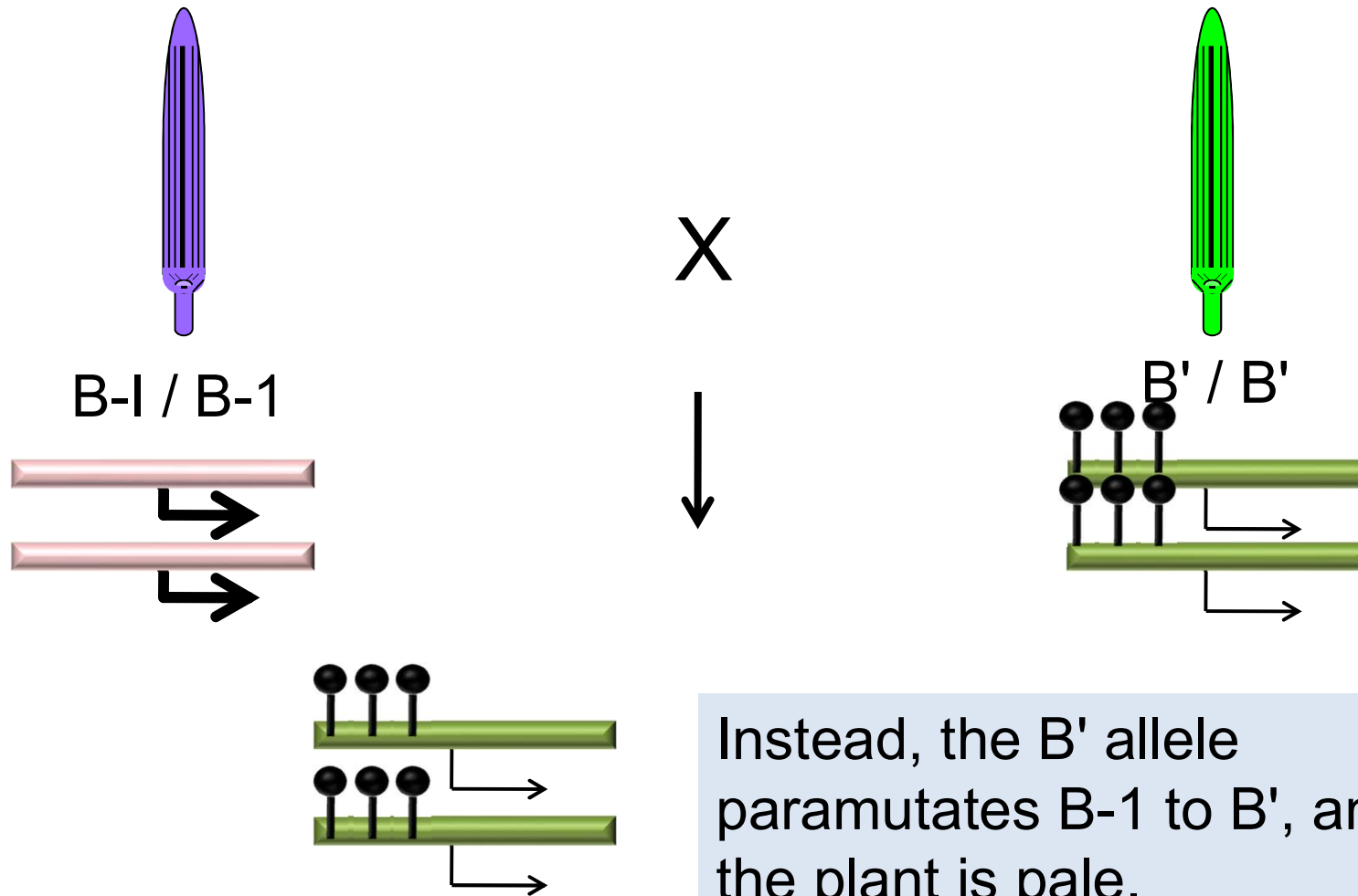
Silencing *in trans*: paramutation



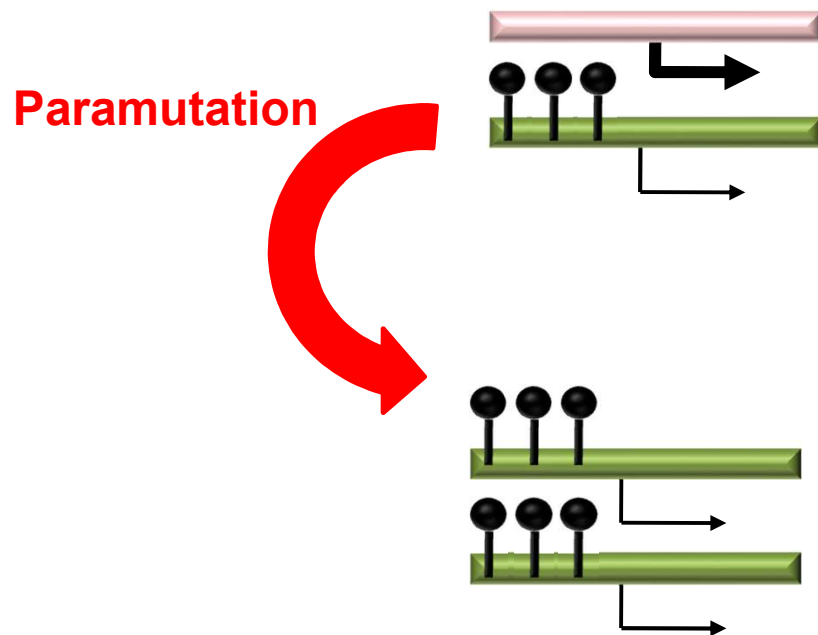
The F₁ should be purple, because the B-1 allele is dominant.



Silencing *in trans*: paramutation

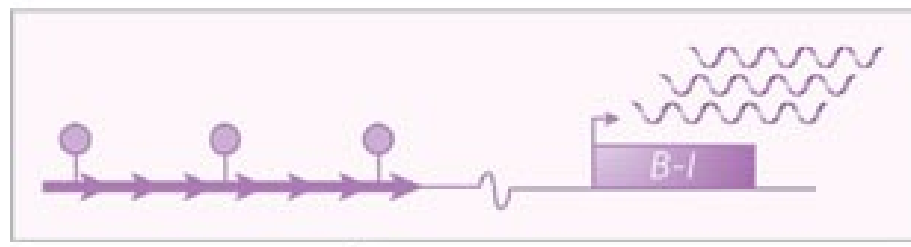


Silencing *in trans*: paramutation

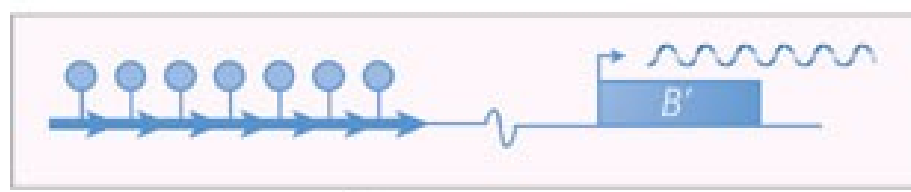


This system provides a straightforward genetic screen for mutations that interfere with paramutation in maize. Such studies have shown that siRNA acting *in trans* is the basis for paramutation.

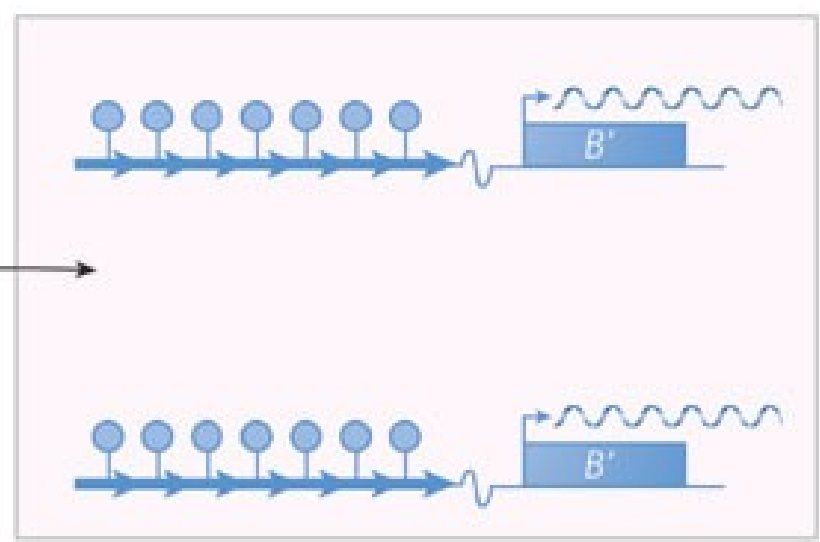
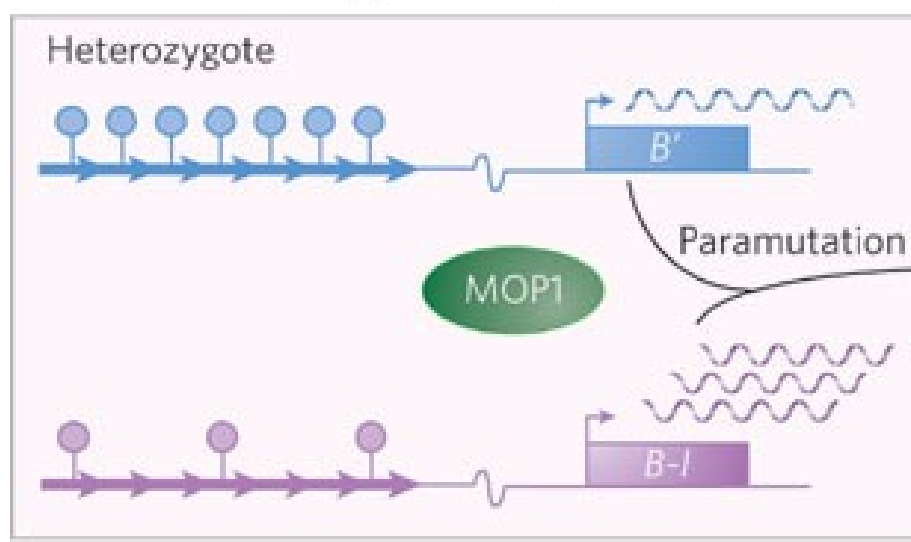
a



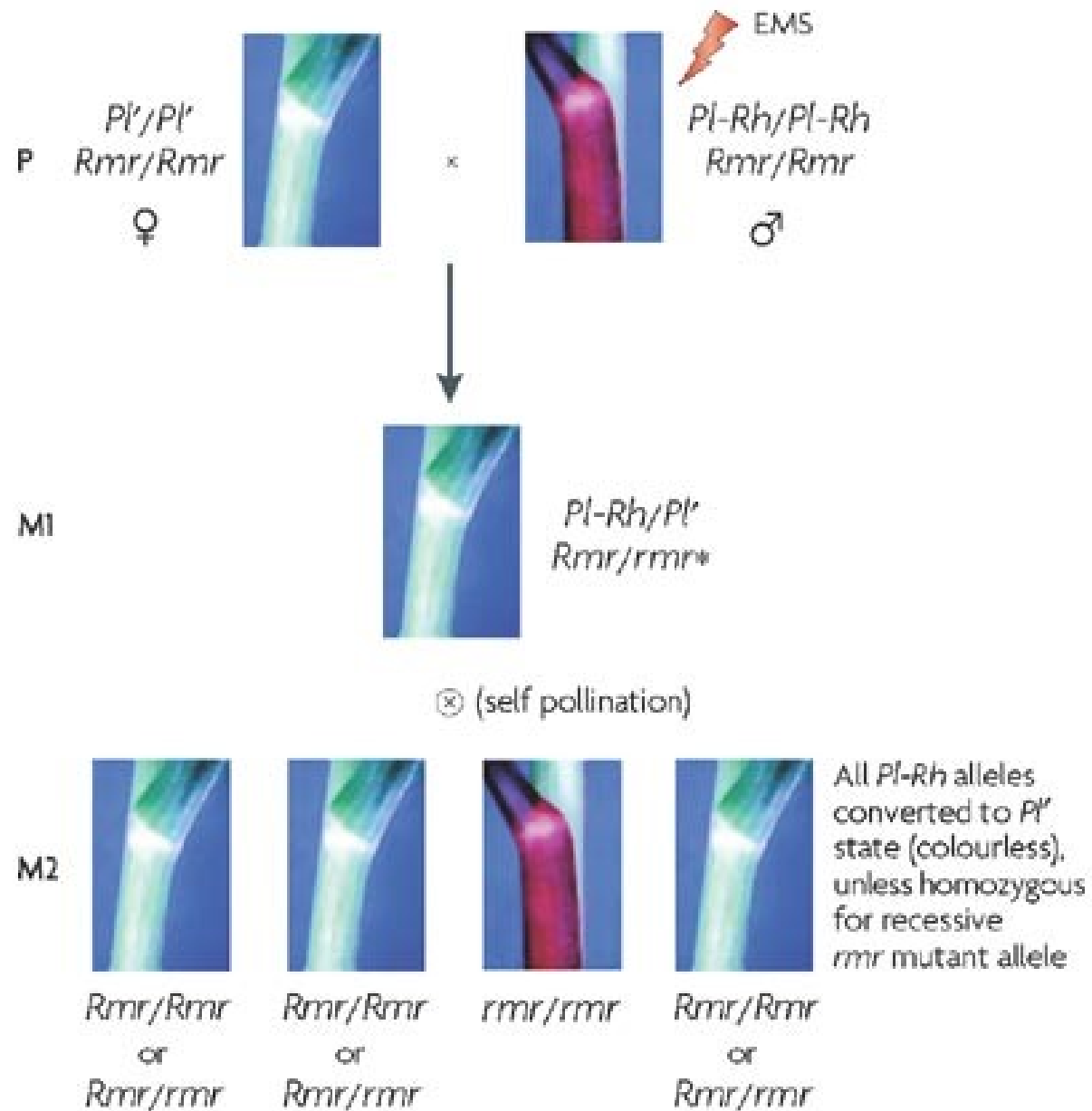
↓ -10% Spontaneous conversion



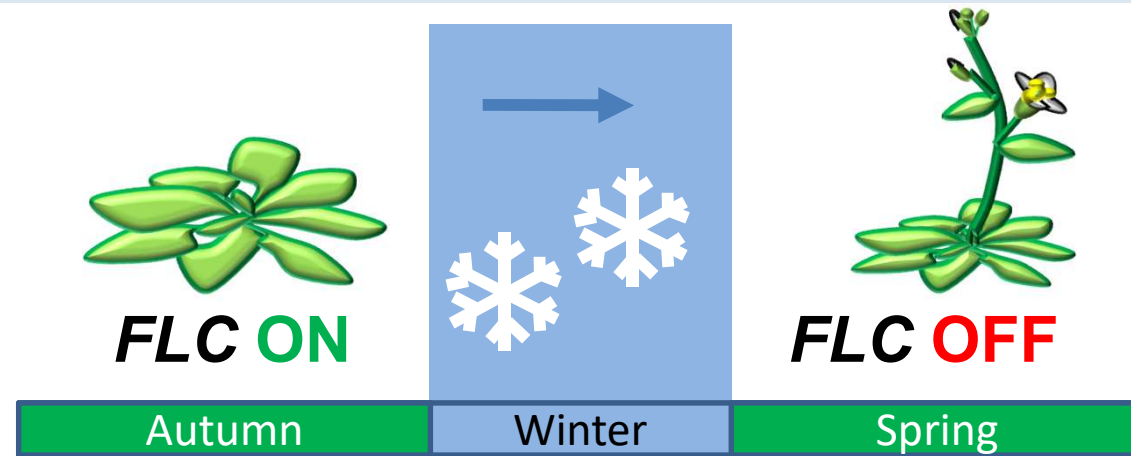
↓ Crossing B' with B-l



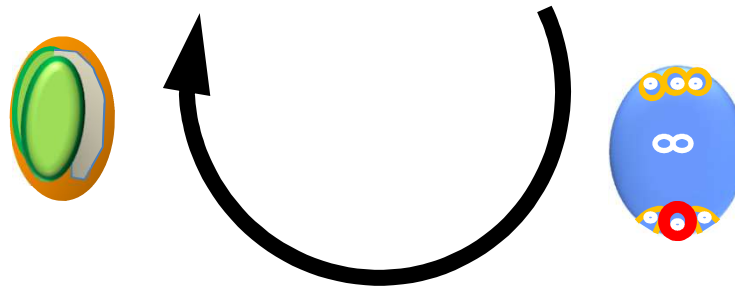
a M2 screen for *rnr* mutants



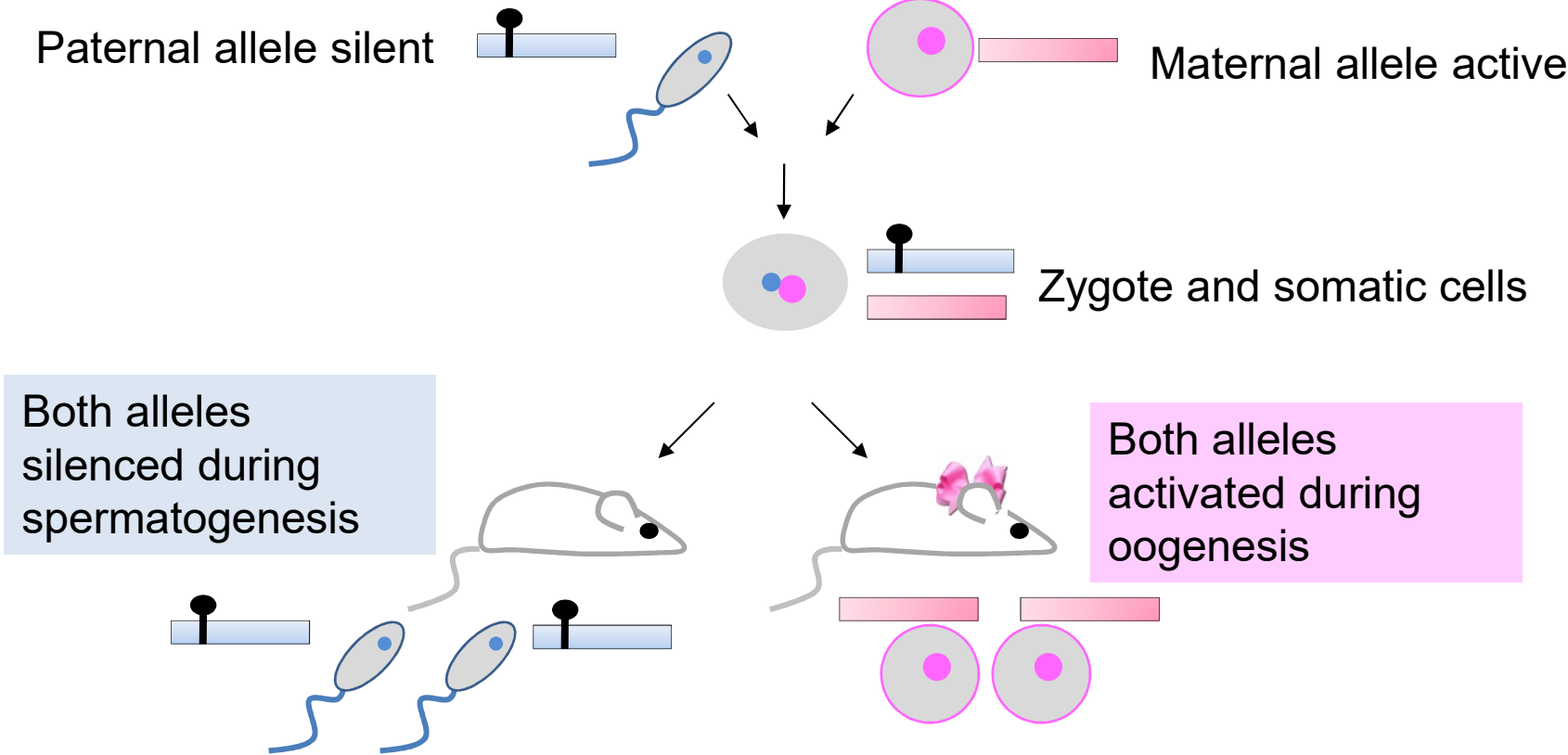
Resetting the epigenome



When does FLC switch on again between generations?

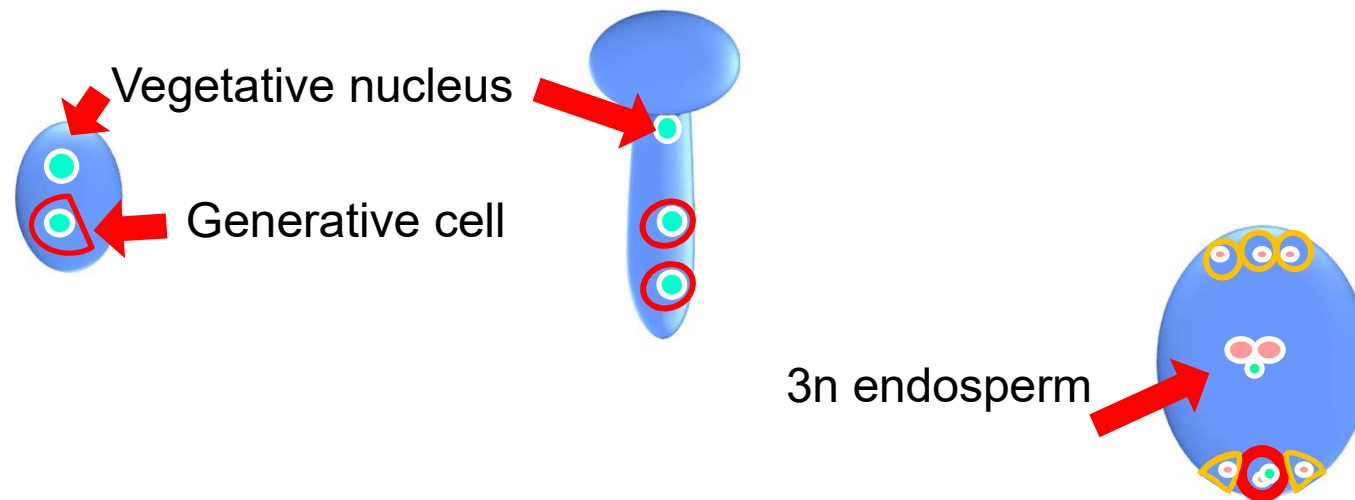


In animals, imprinted genes are reset during gametogenesis

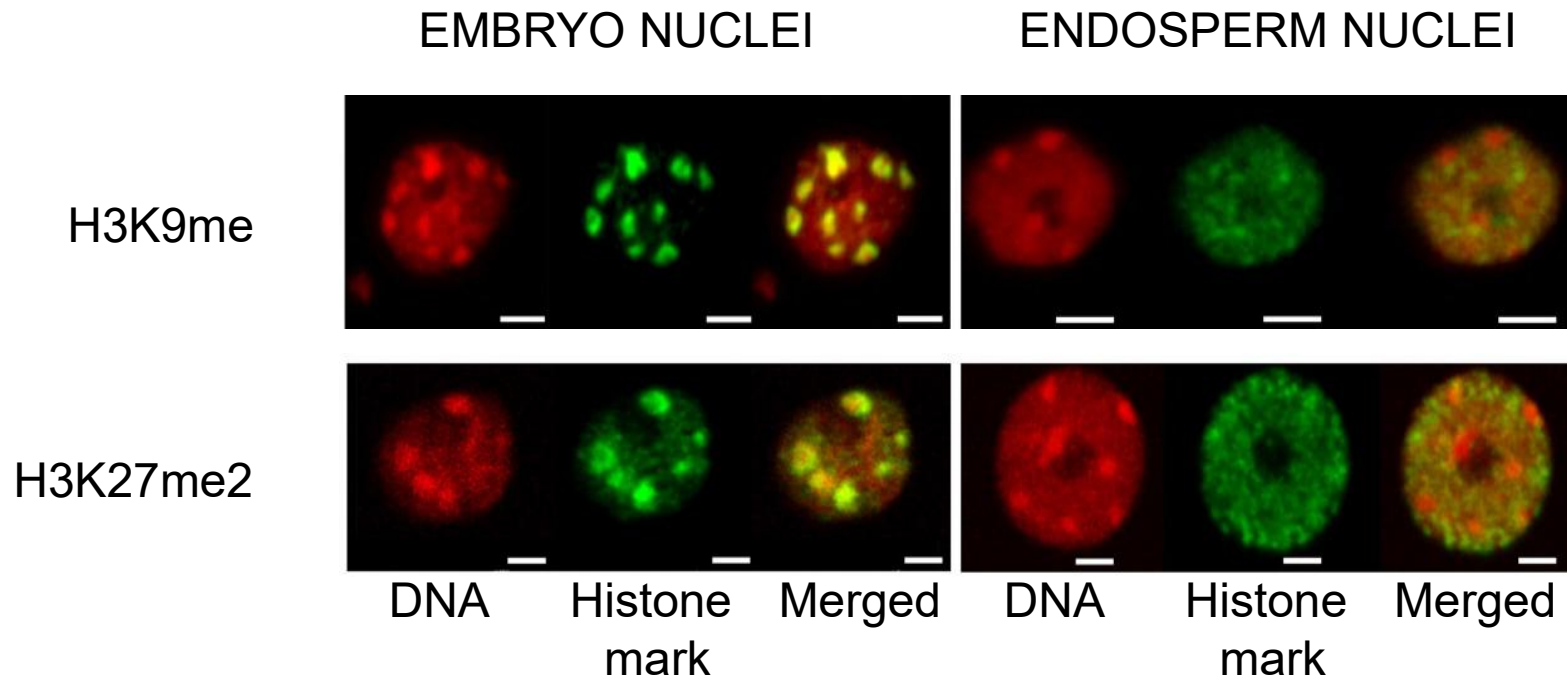


Epigenetic reprogramming in plants

Large-scale epigenetic changes have been observed in the endosperm and in the vegetative nucleus of the male gametophyte.

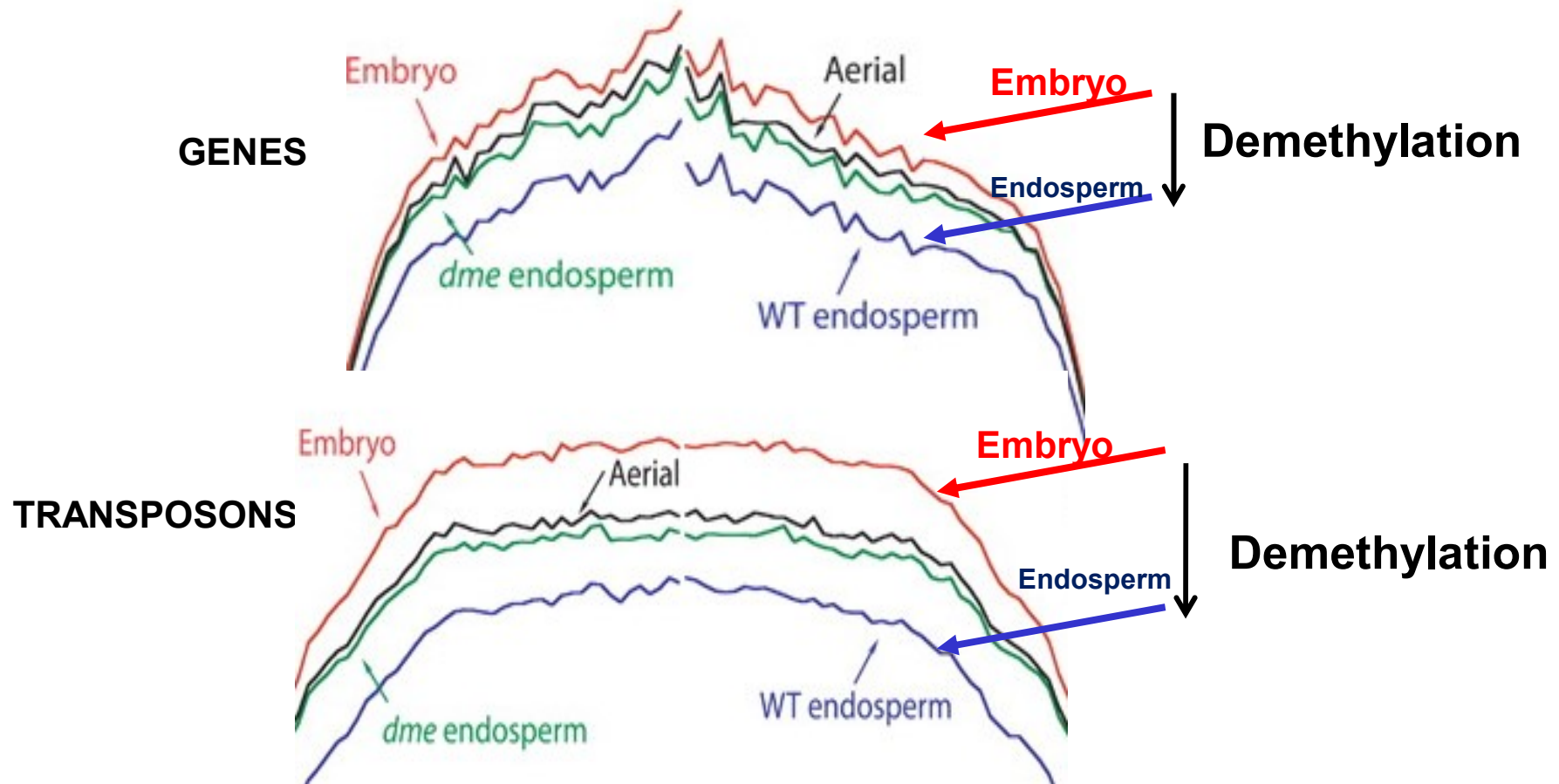


The heterochromatin fraction is reduced in endosperm



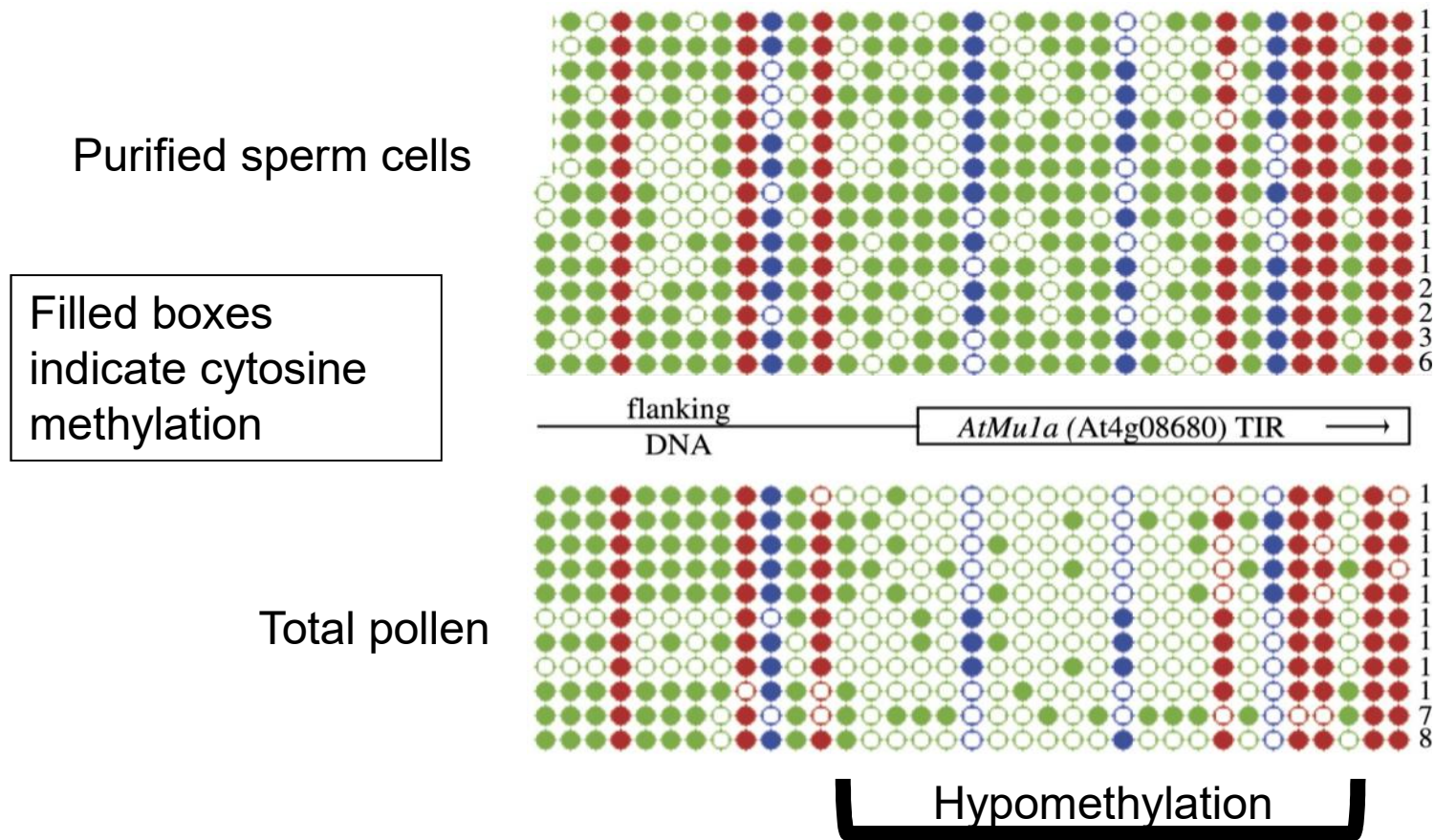
In endosperm nuclei, heterochromatin marks such as H3K9me and K3K27me2 disperse into euchromatin, suggesting genome-wide epigenetic changes.

DNA in endosperm is demethylated as compared to embryo



From Hsieh, T.-F., Christian A. Ibarra, C.A., Silva, P., Zemach, A., Eshed-Williams, L., Fischer, R.L., and Zilberman, D. (2009) Genome-wide demethylation of *Arabidopsis* endosperm. *Science* 324: [1451-1454](#). Reprinted with permission from AAAS.

Transposons in pollen vegetative cells are hypomethylated



Reprinted from Slotkin, R.K., Vaughn, M., Borges, F., Tanurdžić, M., Becker, J.D., Feijó, J.A., and Martienssen, R.A. (2009) Epigenetic reprogramming and small RNA silencing of transposable elements in pollen. *Cell* 136: [461-472](#). Copyright 2009, with permission from Elsevier.

Transposons are specifically activated in pollen

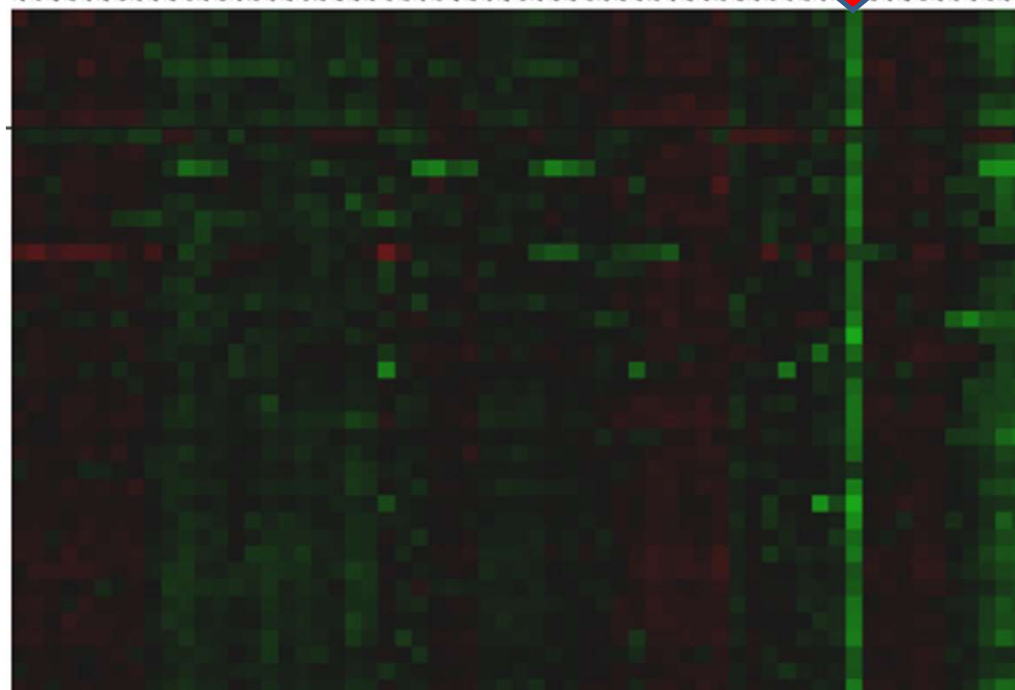
POLLEN RNA



Rows indicate transcript levels for different transposons.

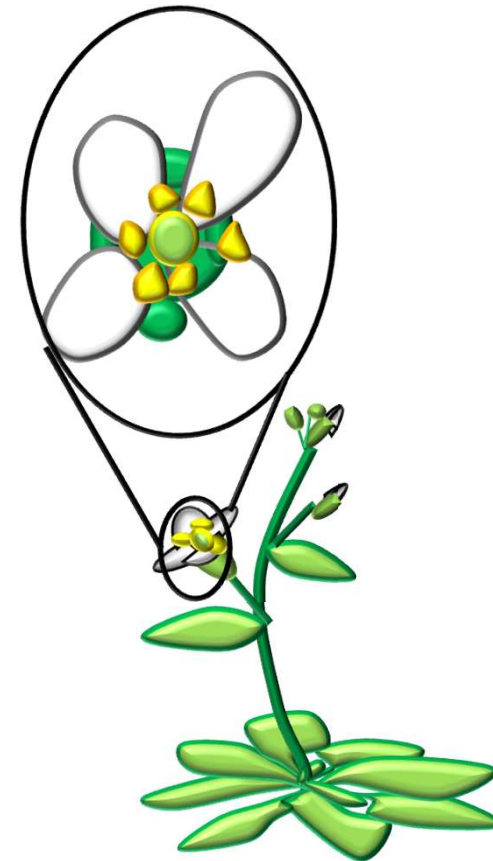
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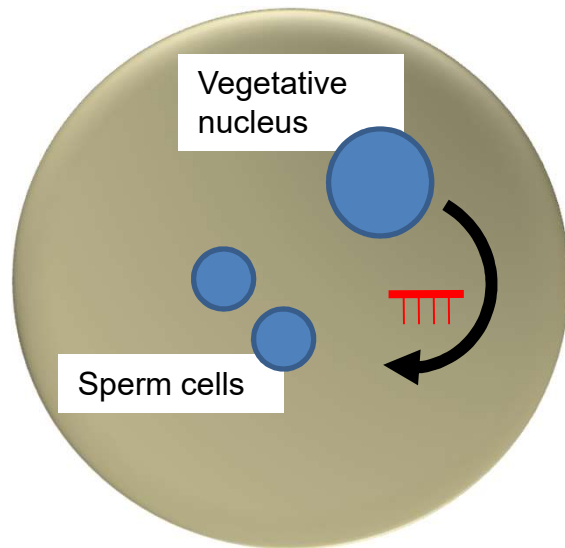


Resetting of epigenetic marks during development

- These results suggest that resetting epigenetic marks occurs during angiosperm reproduction
- Activation of silenced genes can activate RNA-mediated DNA methylation pathways



Resetting of epigenetic marks in non-germ cells?



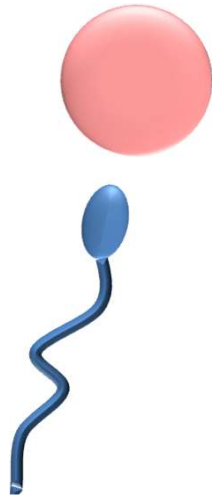
siRNAs generated by this process could move into the generative tissues to ensure proper epigenetic reprogramming, without the mutagenic potential of activating transposons in the germ cells themselves.

Summary

- Expression of DNA is controlled by epigenetic marks including DNA methylation and histone modifications.
- siRNAs contribute to epigenetic programming
- Epigenetic programming silences transposons and controls the timing of many genes that control plant development.

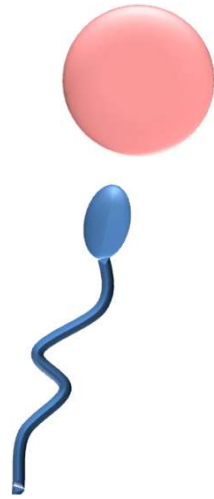


Genomic imprinting



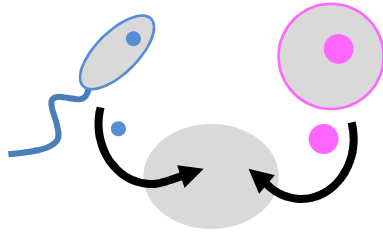
- The zygote receives two copies of each gene, one from the mother's genome and one from the father's.
- At most loci, both copies are active.

Genomic imprinting



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- At most loci, both copies are active.
- Some loci, **imprinted** loci, show a "parent of origin effect".
- Expression of these loci is controlled by epigenetic factors.

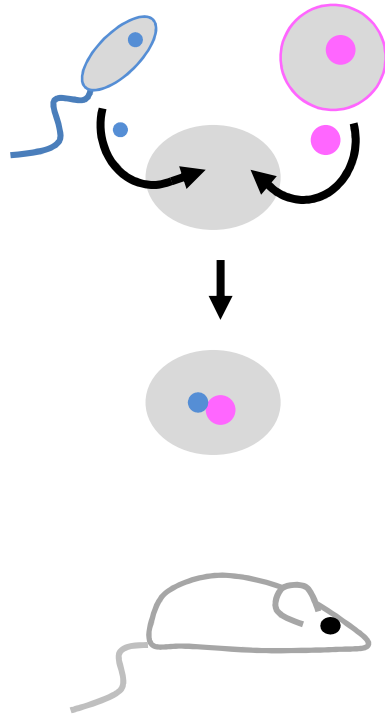
Imprinting – nuclear transplant experiment



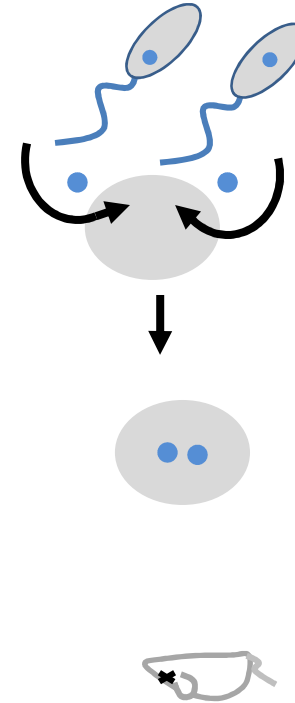
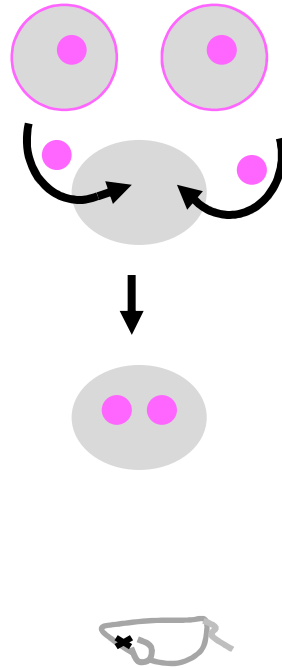
Placing a sperm and an egg nucleus into an enucleated fertilized cell leads to a normal embryo.



Imprinting – nuclear transplant experiment

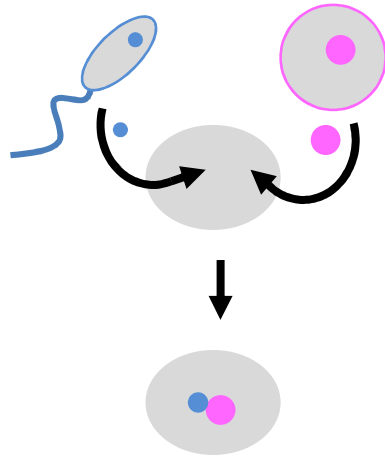


experiment



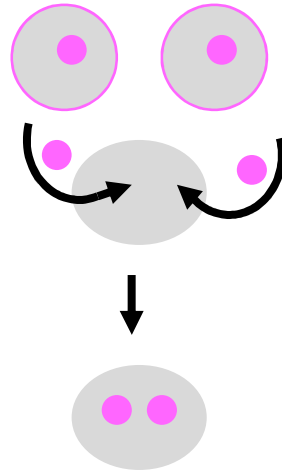
Zygotes that receive only maternal or only paternal nuclei do not survive.

Imprinting – nuclear transplant experiment

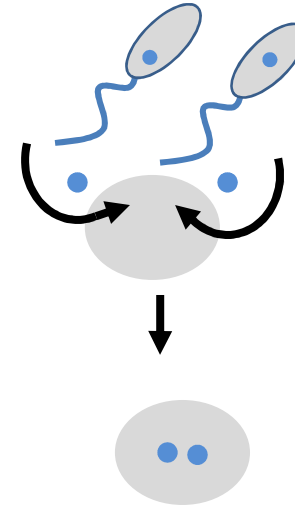


The two parental genomes are not equivalent

experiment



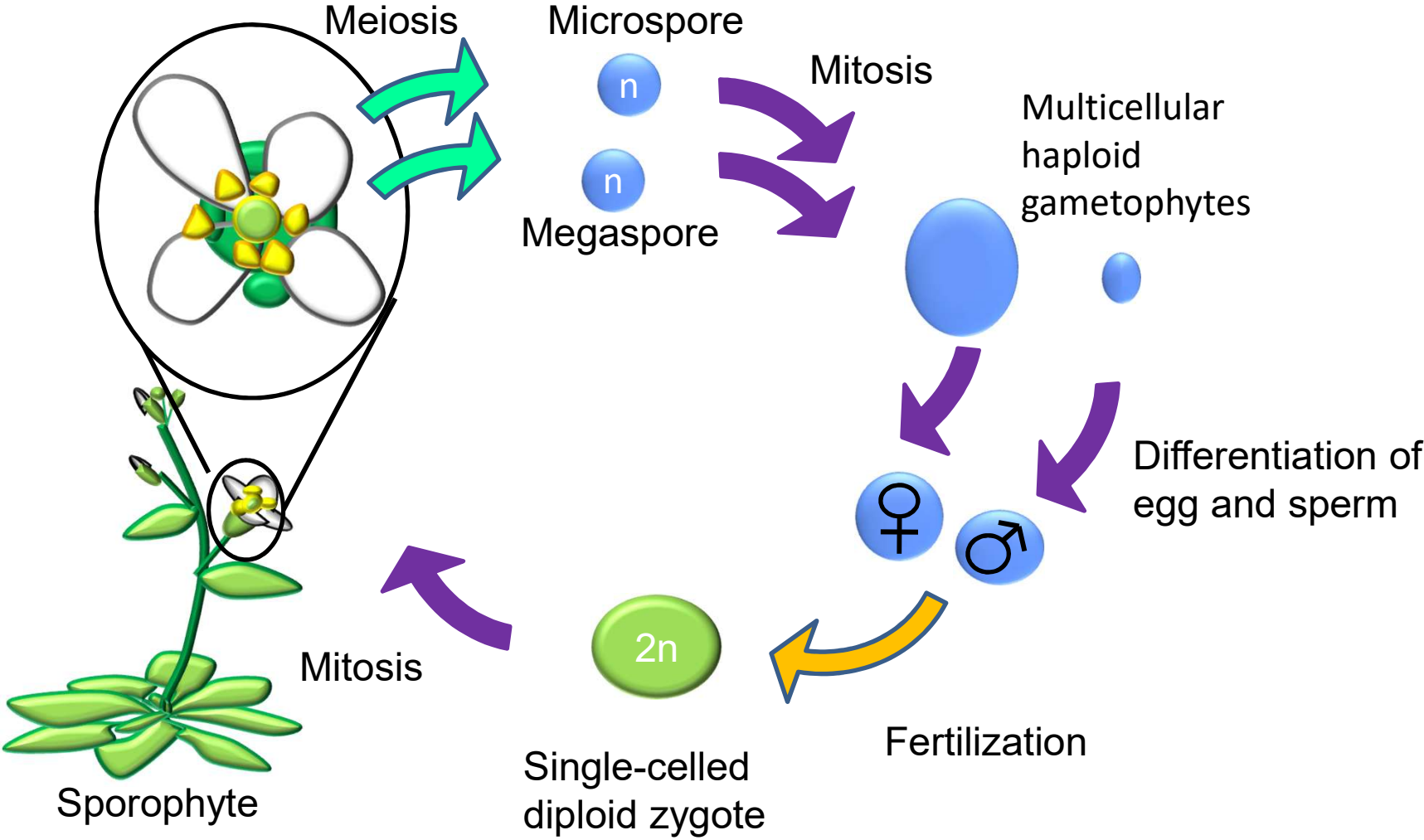
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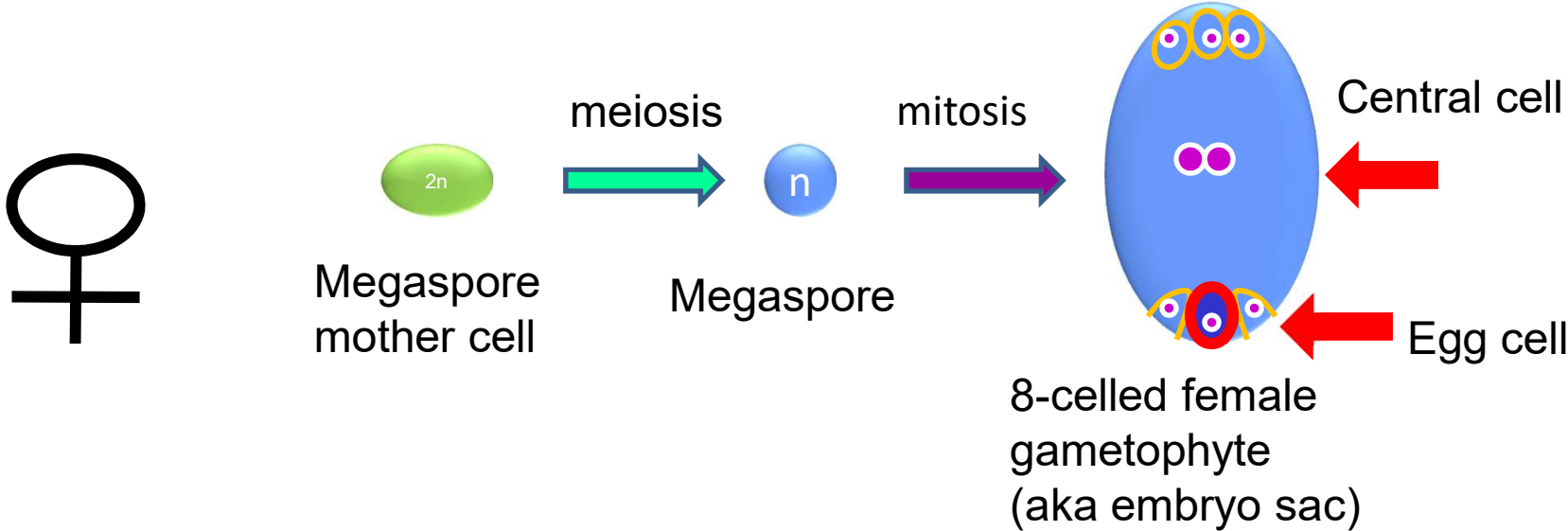
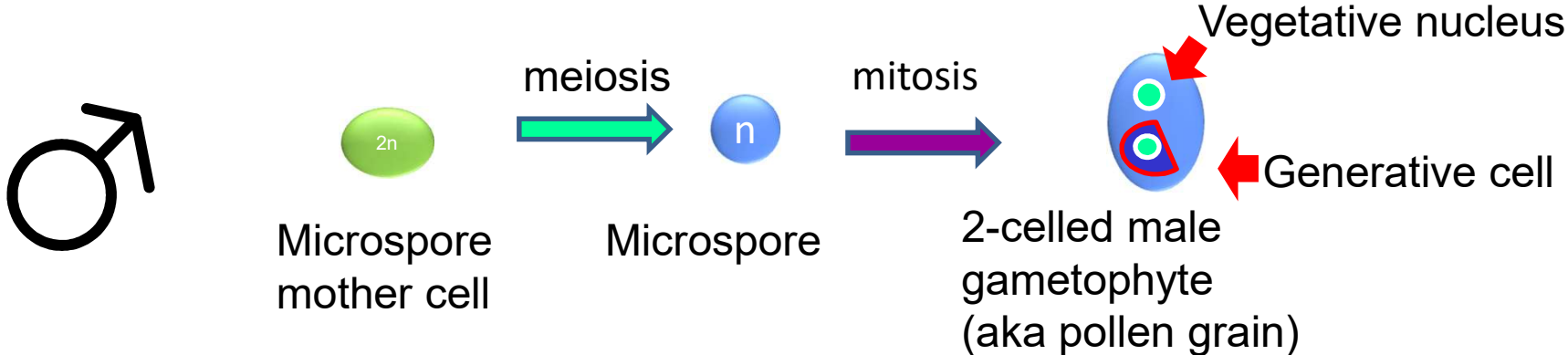
Imprinting in angiosperms

- Angiosperms show genomic imprinting too, but their reproduction is a bit more complicated than that of animals.....

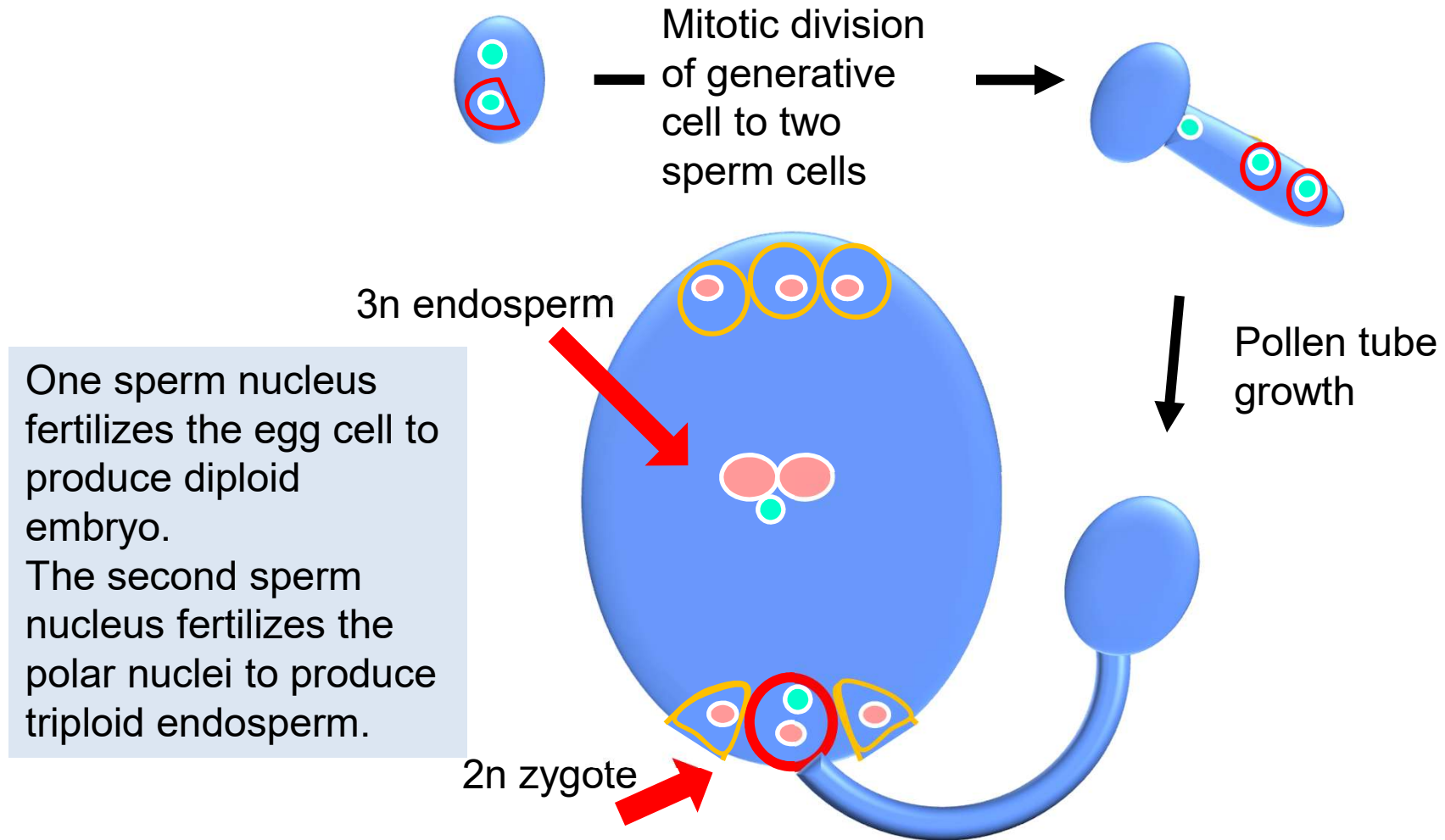
Angiosperm reproduction



Development of male and female gametophyte

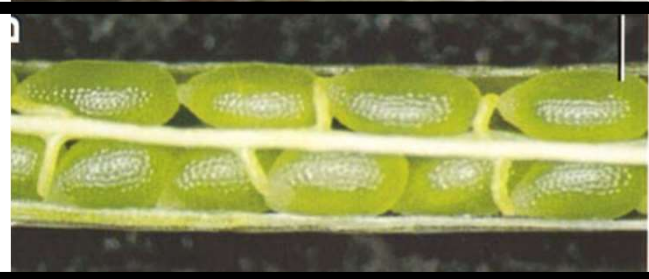


Double Fertilization



The *MEDEA* (*MEA*) gene is imprinted

♂ *MEA/mea* x ♀ *MEA/MEA*
All seeds viable

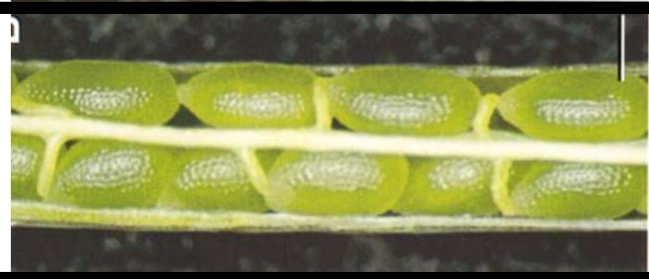


♂ *MEA/MEA* x ♀ *MEA/mea*
50% of seeds abort



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All seeds viable



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50% of seeds abort



In the second cross, 50% of the seeds receive the mutant *mea* allele from their mother.

These seeds abort, even though they also have a wild-type *MEA* allele inherited from their father.

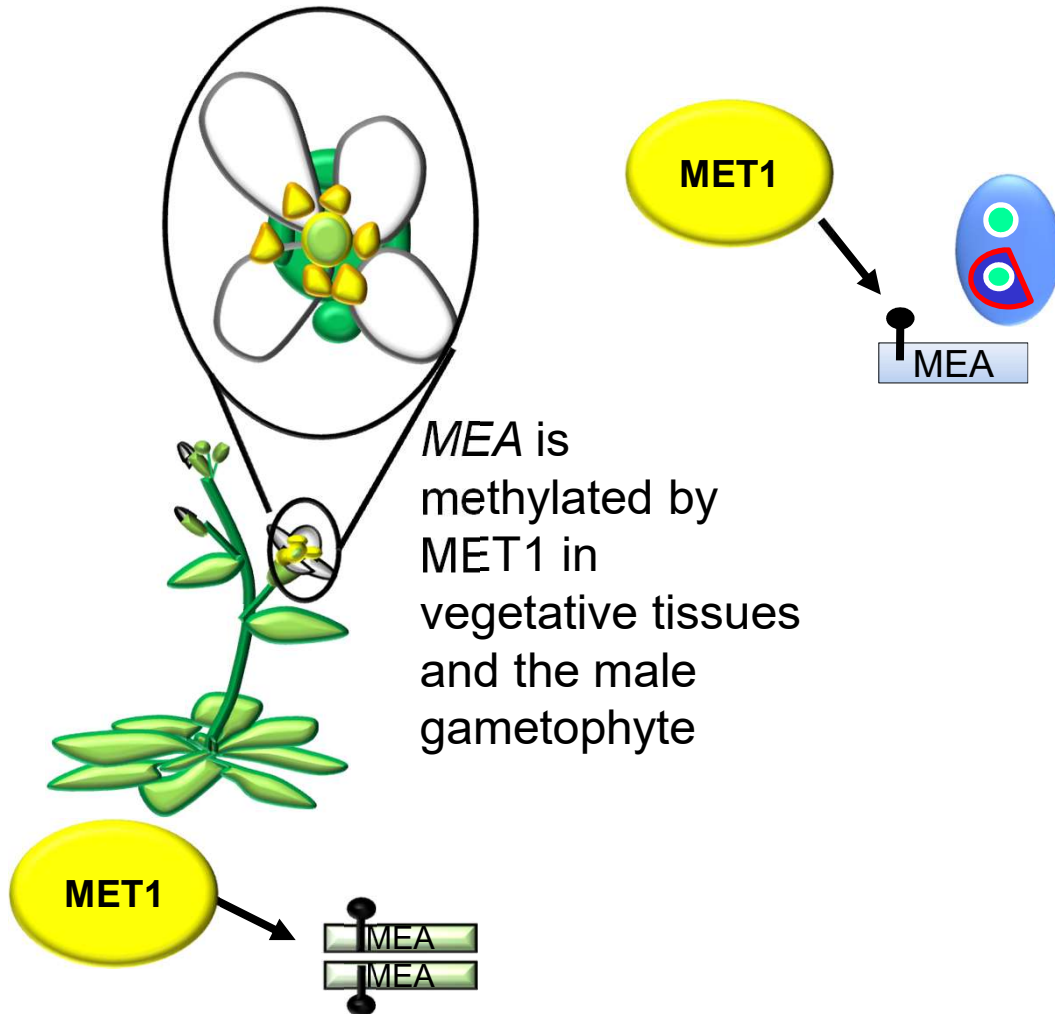
The *MEDEA* (*MEA*) gene is imprinted



The paternal allele is silent.

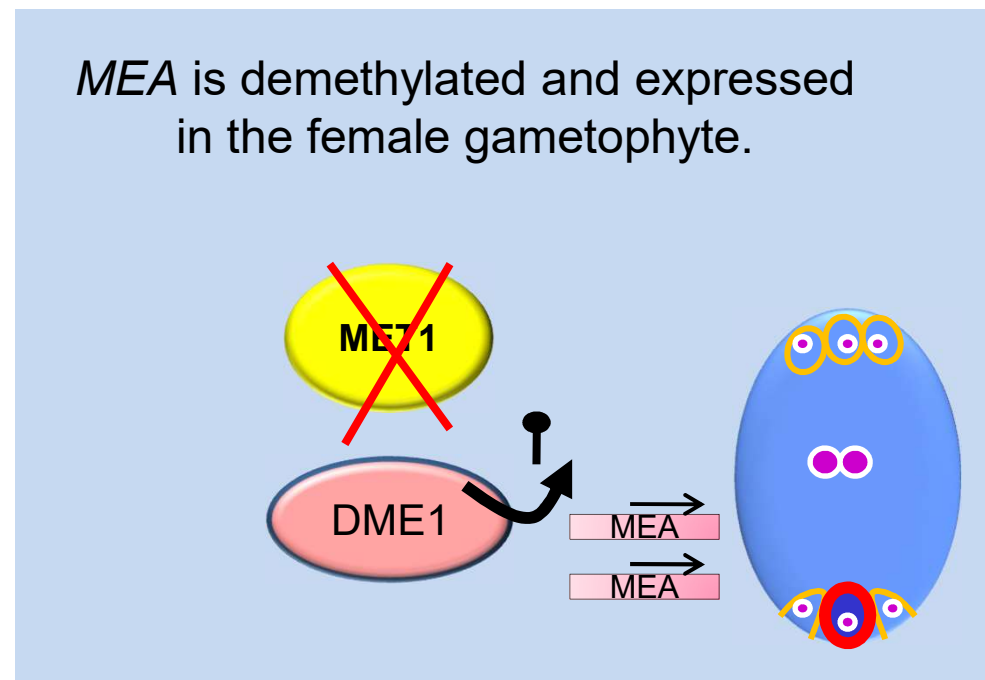
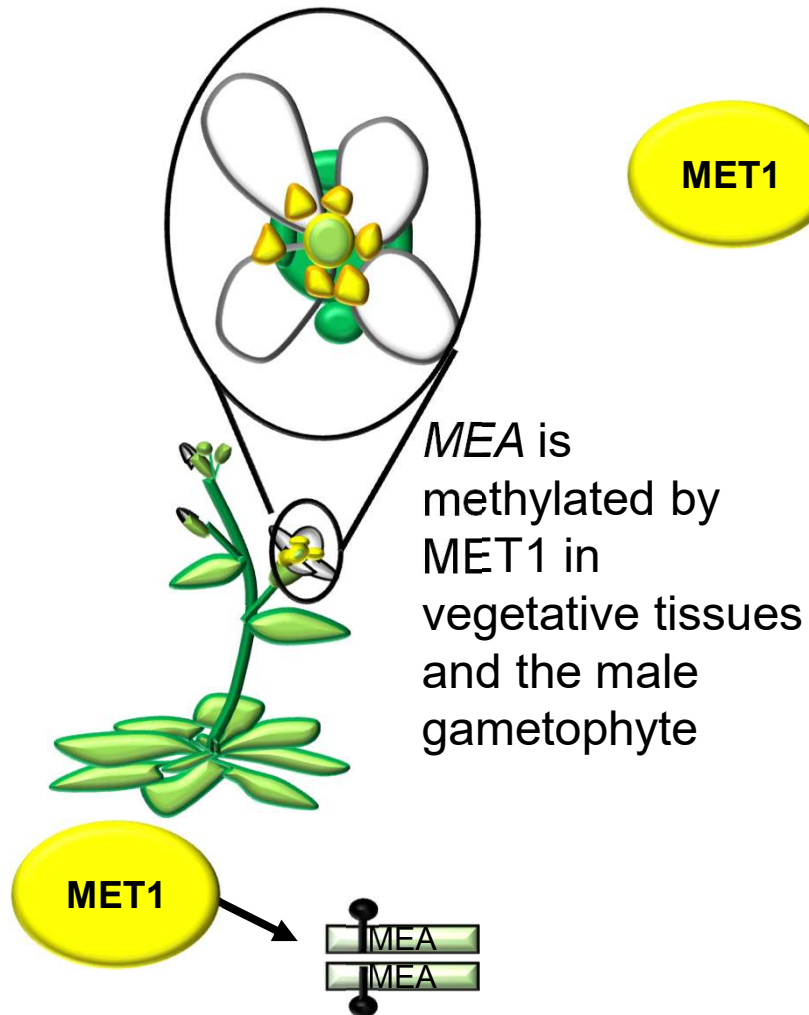
The phenotype of the progeny is based on the maternal genotype only.

MEA is silenced in most cells



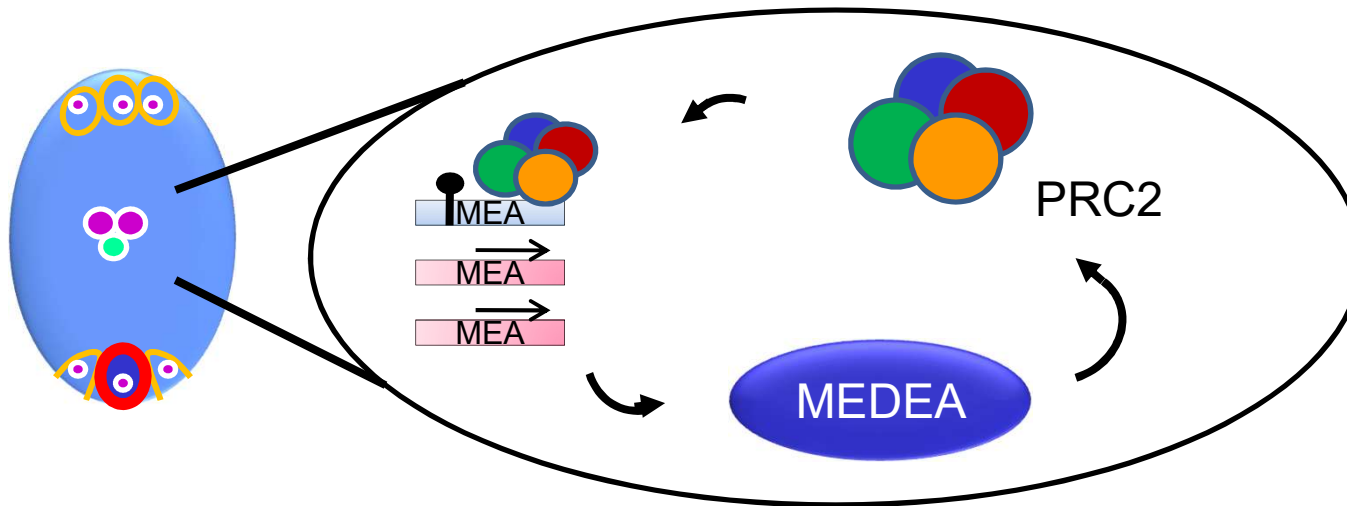
The *MEA* allele inherited from the father is ***silent***.

MEA is silenced in most cells

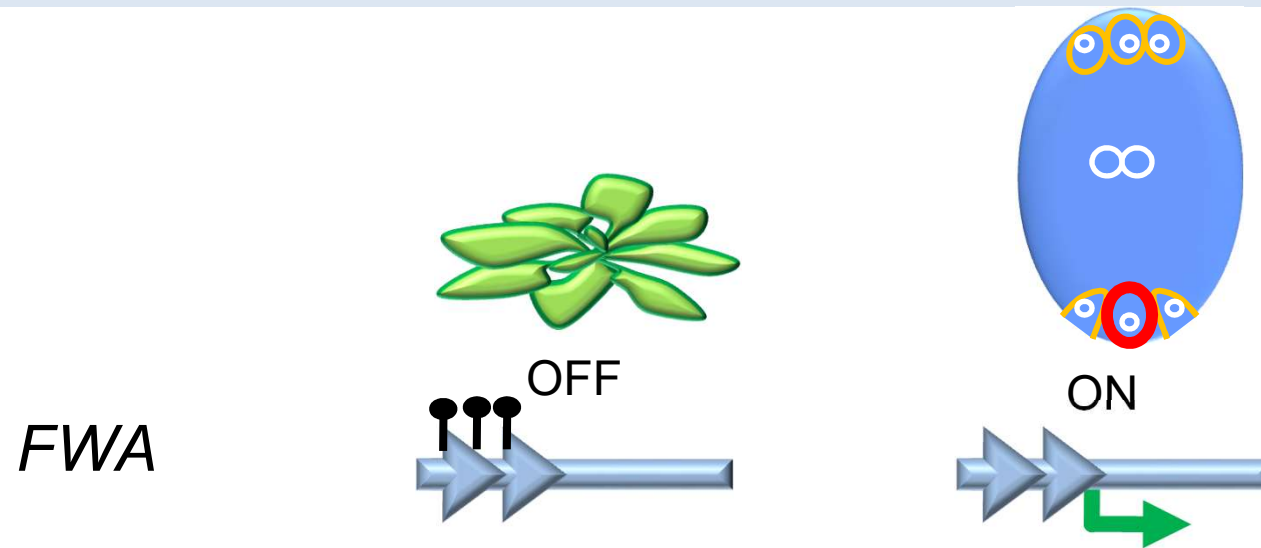


MEA regulates its own imprinting

- MEA encodes a component of PRC2.
- In the triploid endosperm, continued silencing of the parental allele requires histone modification by PRC2.

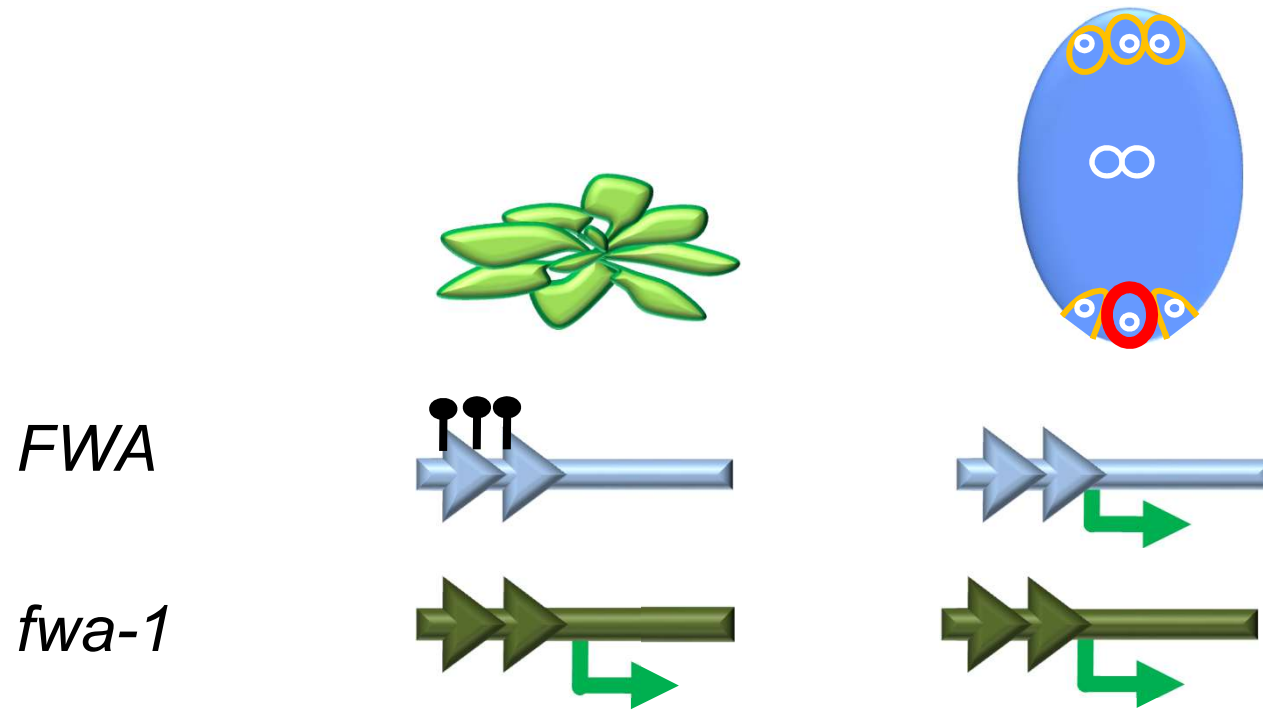


Silencing *in trans*: silencing of *FWA*



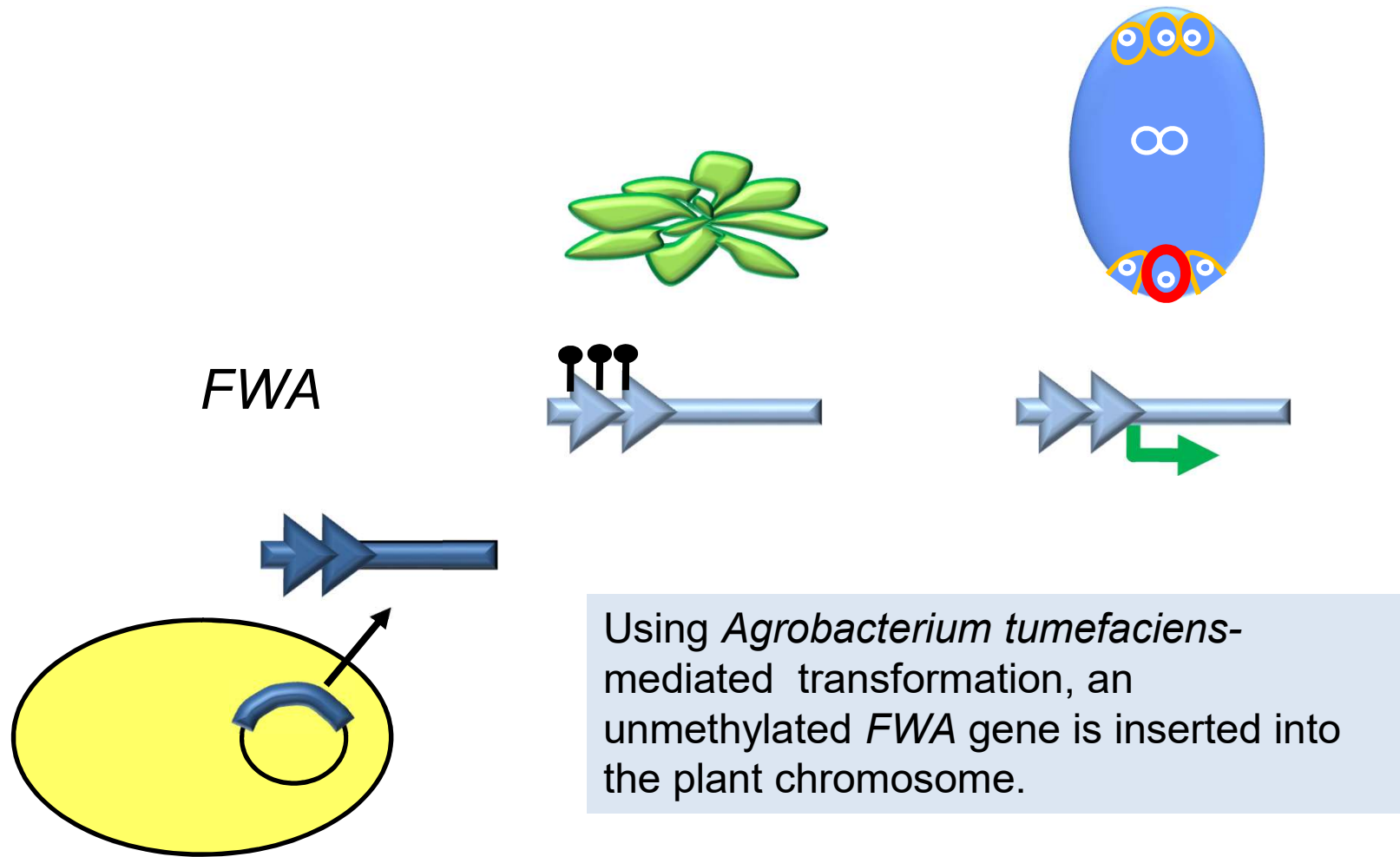
FWA is another imprinted gene. It is expressed in the female gametophyte, like *MEA*.

Silencing *in trans*: silencing of *FWA*



The *fwa-1* epiallele is hypomethylated and expressed in vegetative tissues.

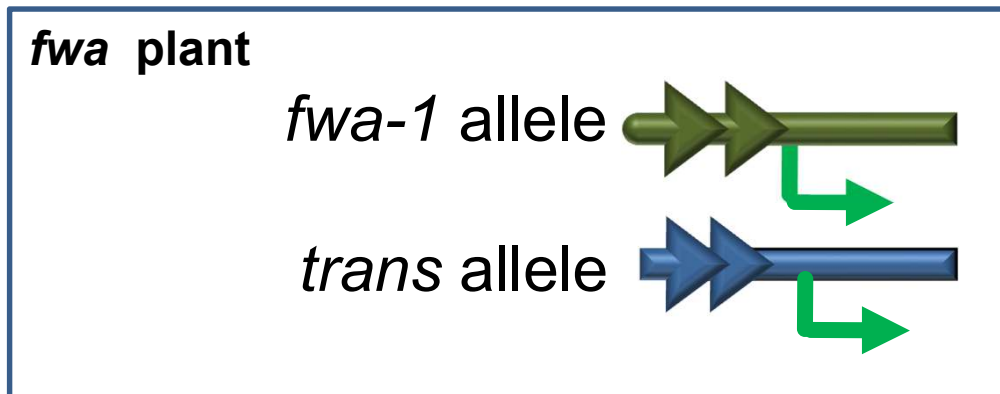
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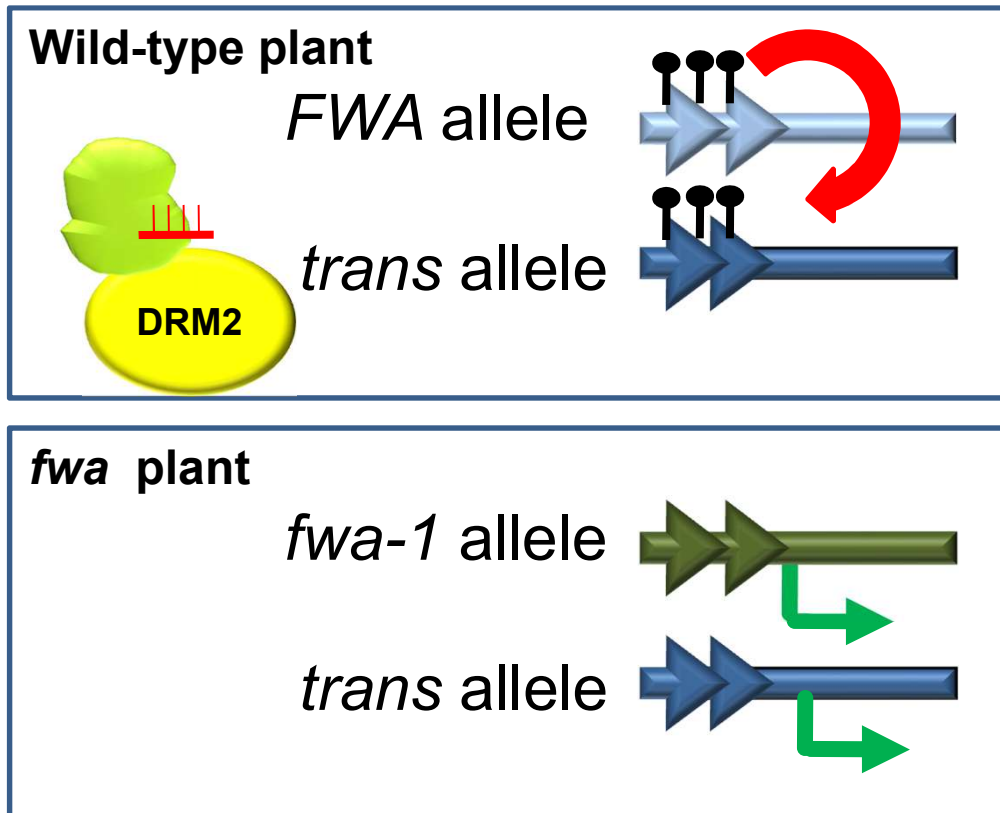


In plants with the wild-type *FWA* allele, the *trans* allele is silenced.



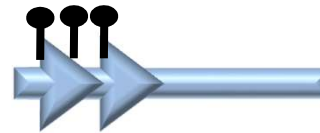
In plants with the *fwa-1* allele, the *trans* allele is active.

Silencing *in trans*: silencing of *FWA*

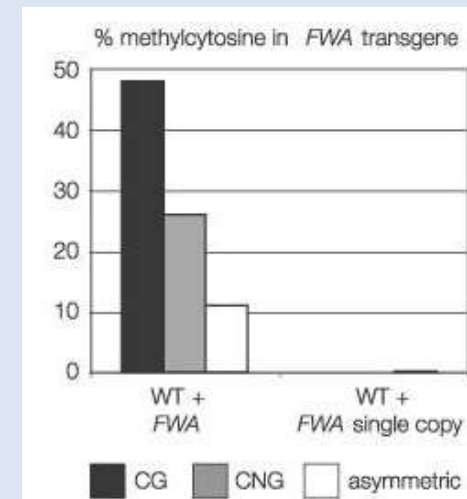


In plants with the wild-type *FWA* allele, methylation is maintained by siRNA and DRM2, which is capable of also methylating the *FWA* gene introduced *in trans*.

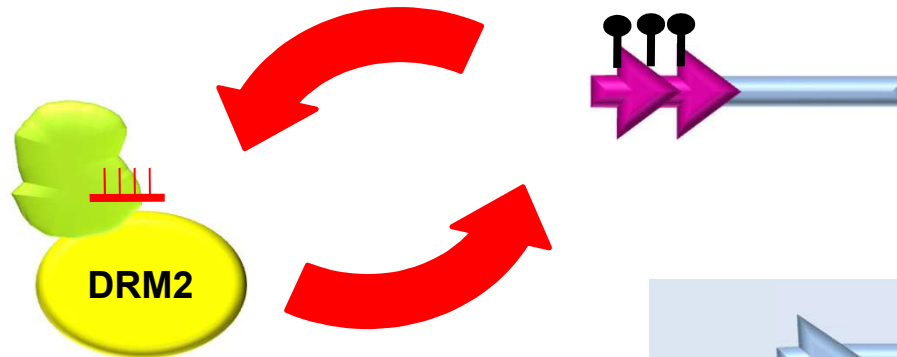
Silencing *in trans*: silencing of *FWA*



A *FWA* gene with one repeat deleted does not promote DNA methylation.



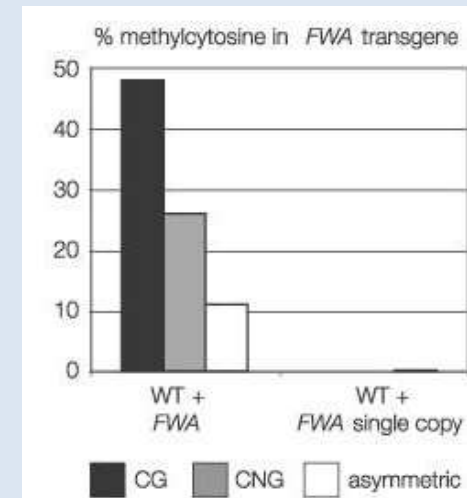
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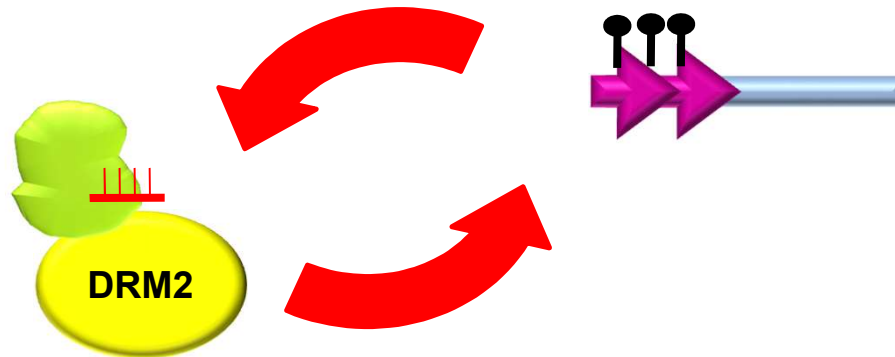
The **tandem repeat** upstream of the *FWA* gene promotes siRNA production and is necessary for methylation and silencing.



A *FWA* gene with one repeat deleted does not promote DNA methylation.



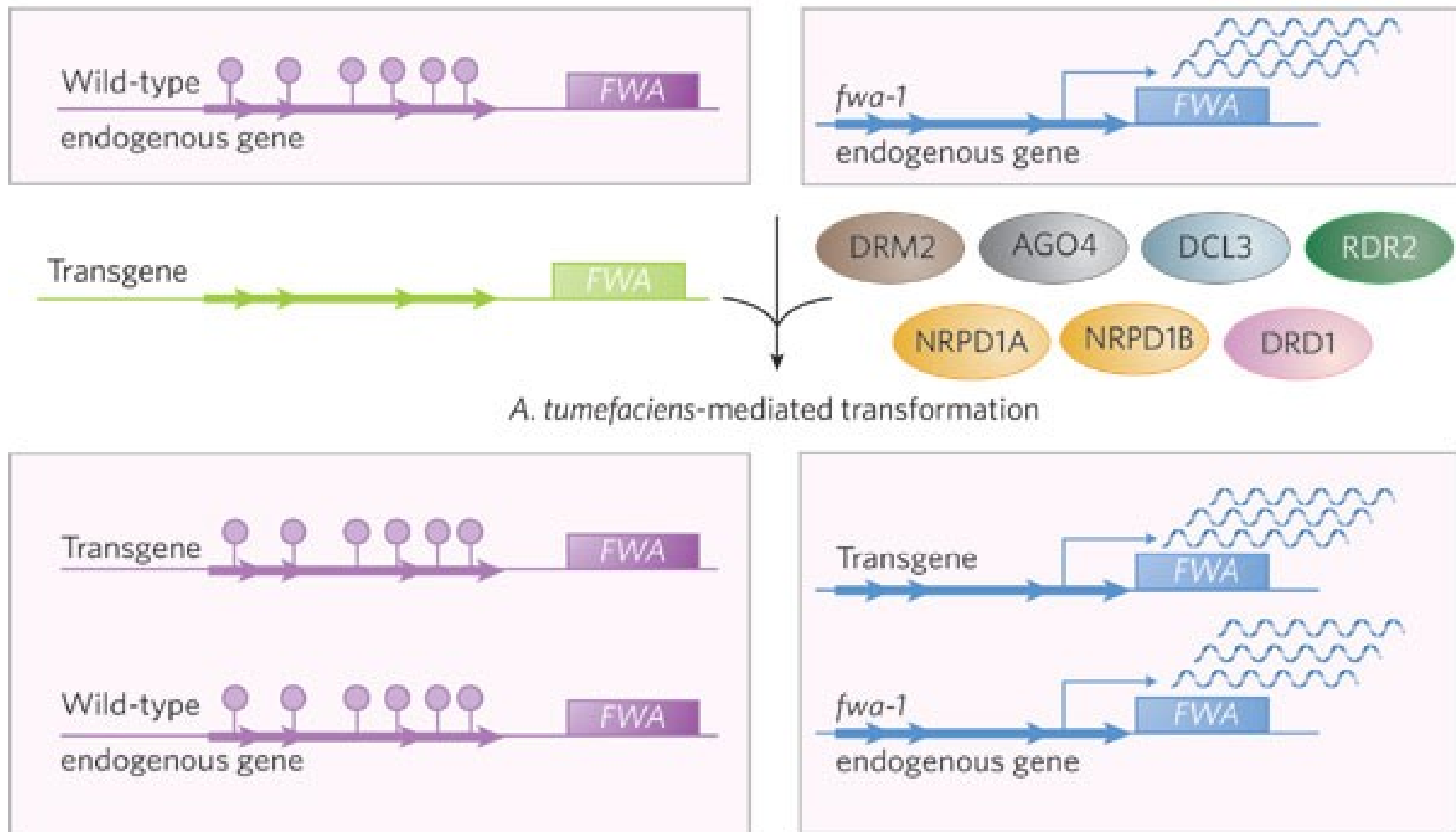
Silencing *in trans*; silencing of *FWA*



The **tandem repeat** upstream of the *FWA* gene promotes siRNA production and is necessary for methylation and silencing.

Tandem repeats are common features of transposons and repetitive elements. The presence of the tandem repeats triggers the epigenetic mechanism that plants use to silence potentially “foreign” DNA.

b



Silencing *in trans*: paramutation

Paramutation: The interaction of two alleles of the same locus, resulting in a heritable change of one allele that is induced by the other allele. The maize *b1* locus has a paramutagenic allele.

Silencing *in trans*: paramutation

B-1 allele
(highly active)



Plants with an active B-1 allele have a lot of purple anthocyanin pigmentation.



Paramutation: The interaction of two alleles of the same locus, resulting in a heritable change of one allele that is induced by the other allele. The maize *b1* locus has a paramutagenic allele.

Silencing *in trans*: paramutation

B-I allele
(highly active)



B' allele
(weakly active)



The B' allele is weakly active, and plants are pale.

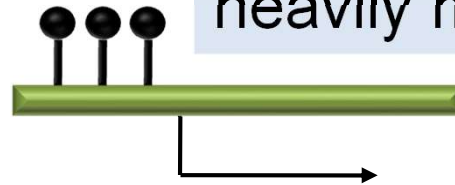


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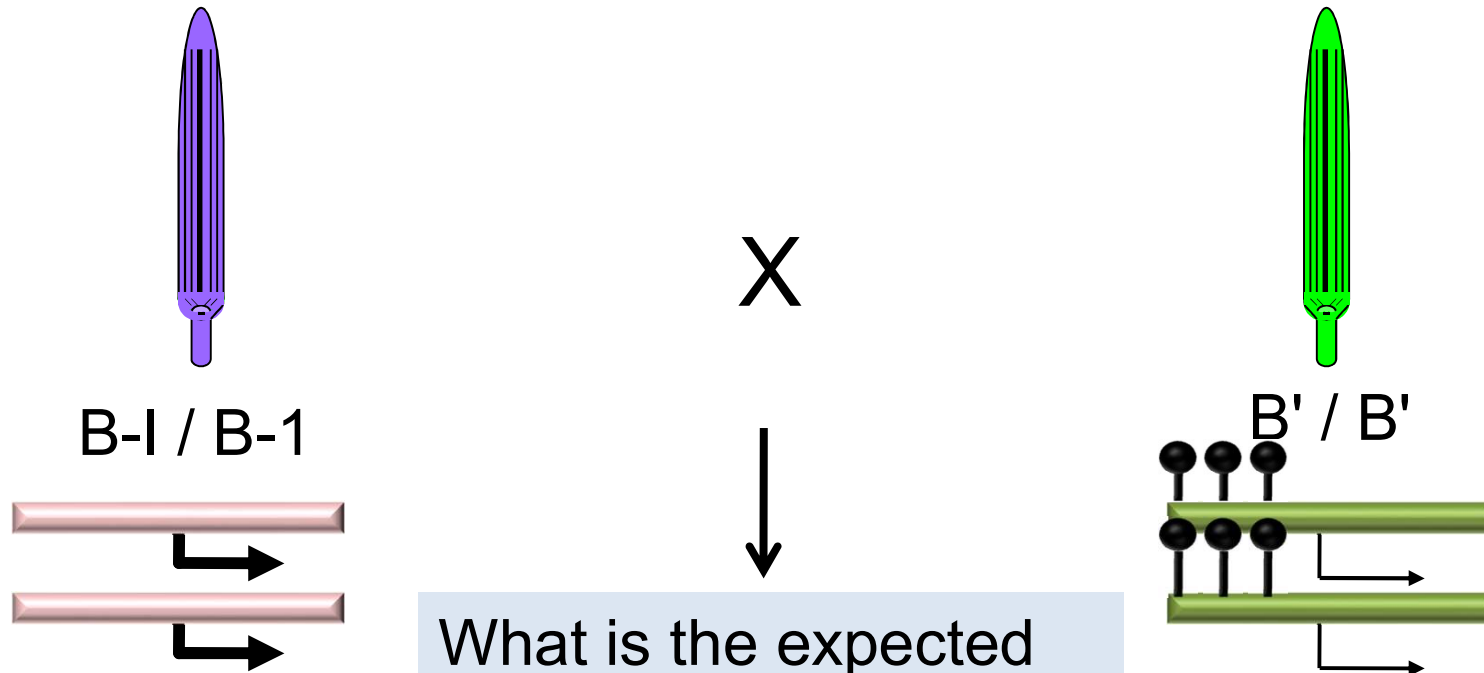
B' allele
(weakly active)



The B' allele is heavily methylated.

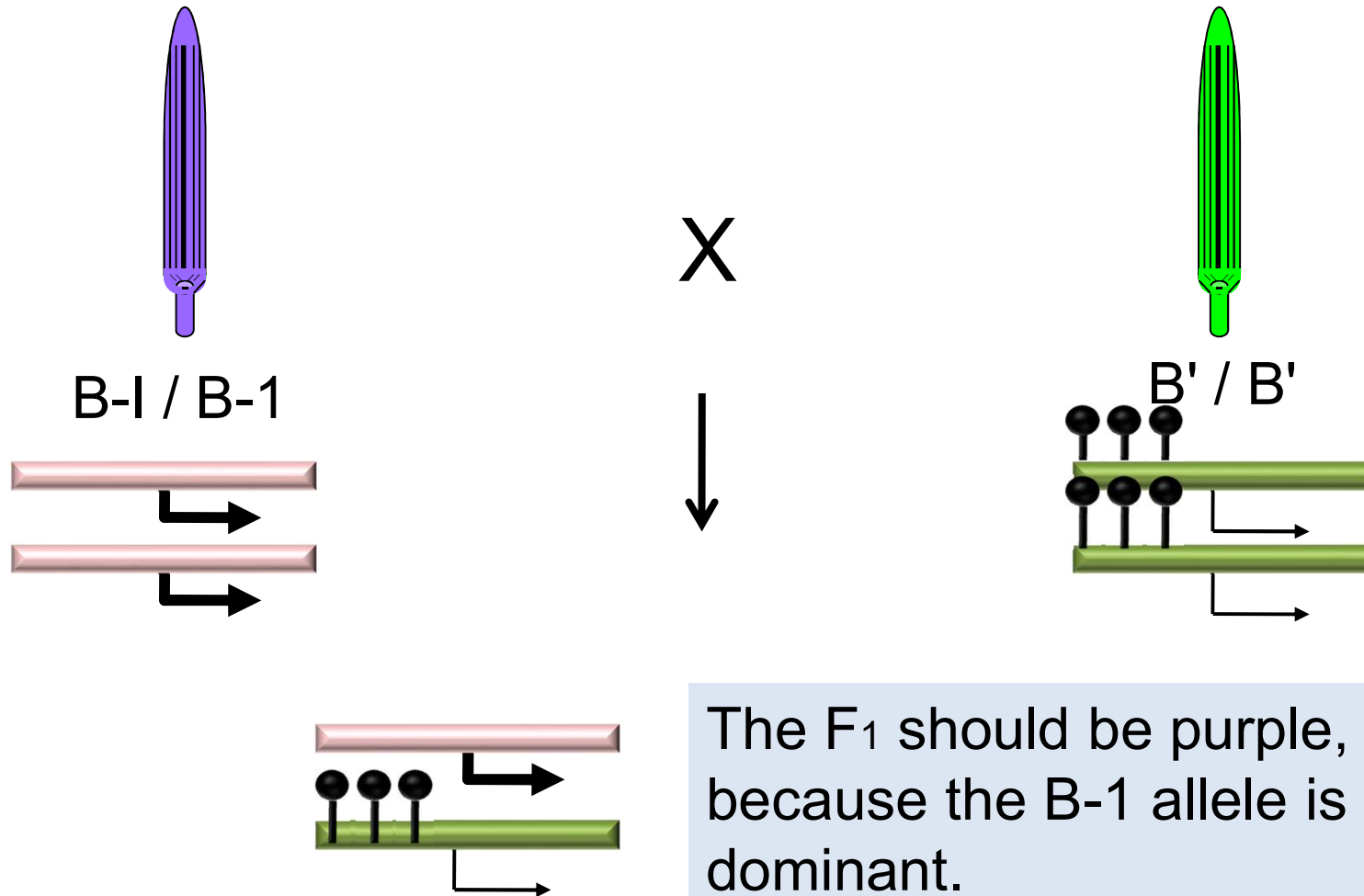


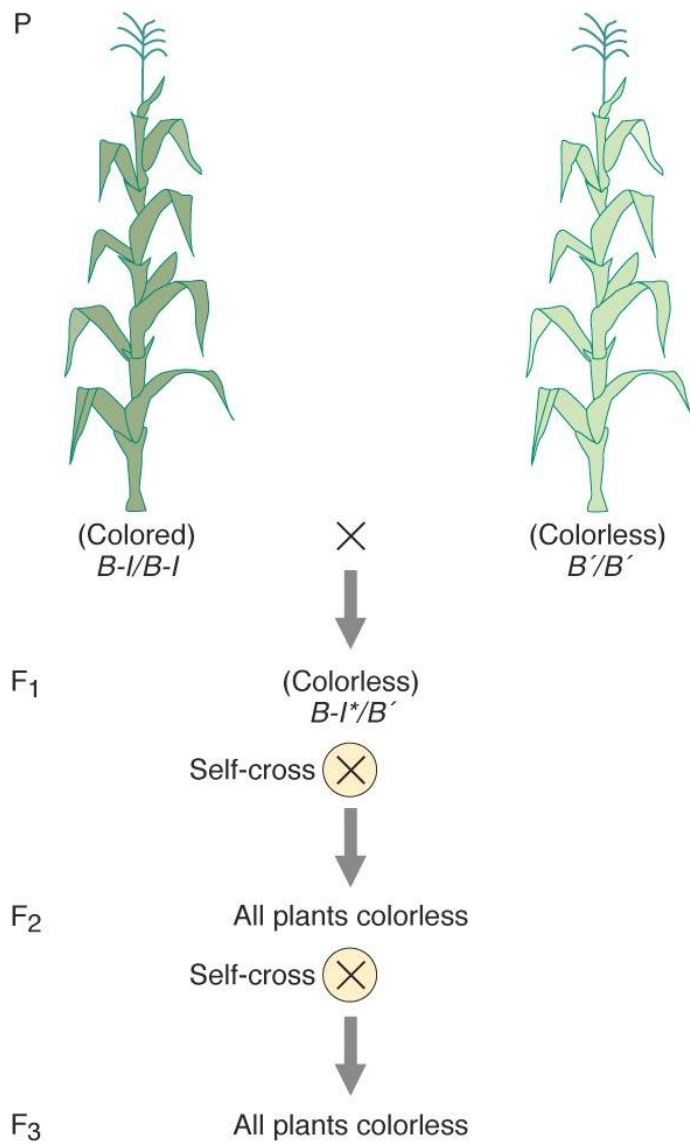
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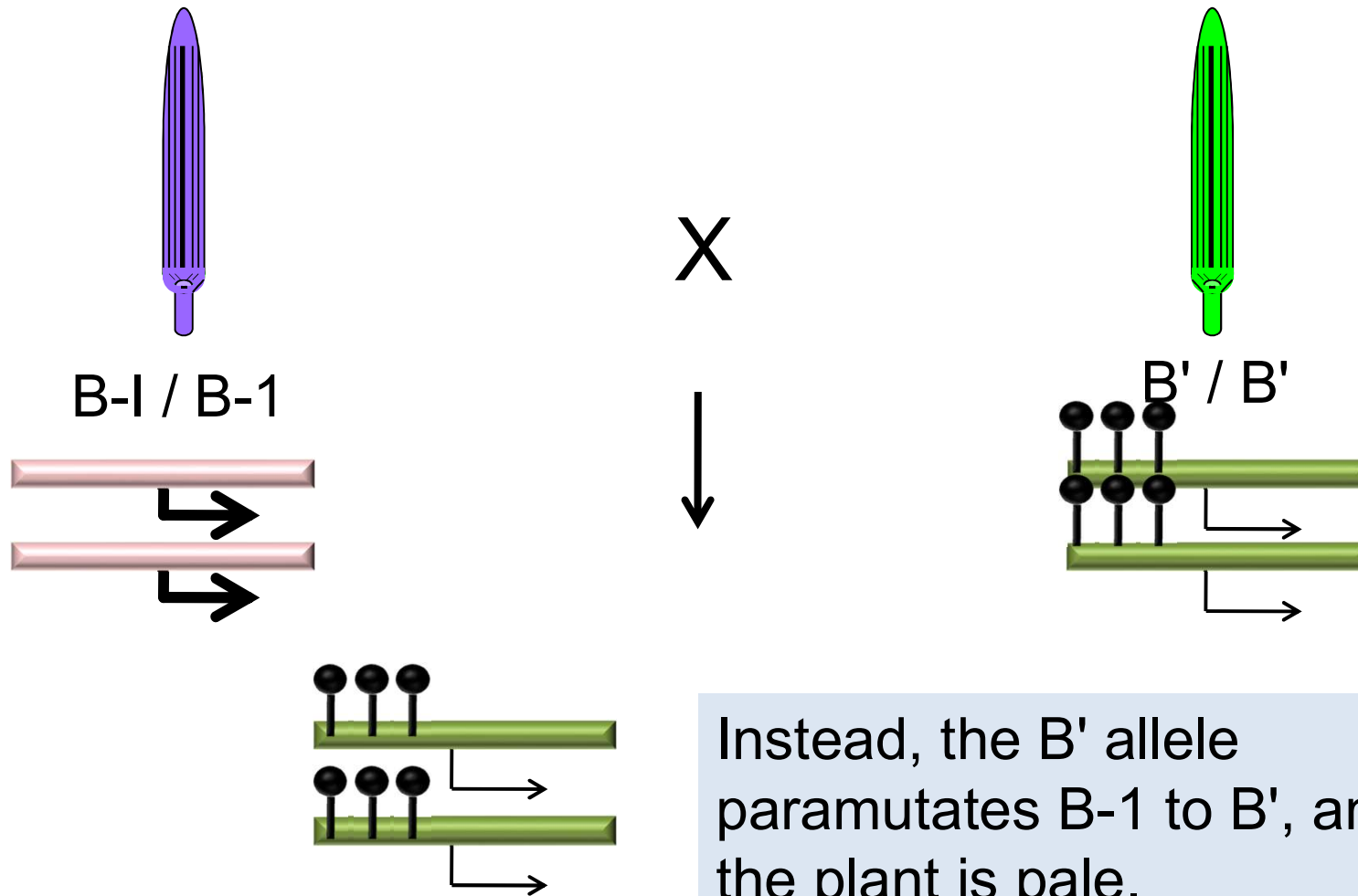
What is the expected phenotype of the progeny of a cross between these two homozygous parents?

Silencing *in trans*: paramutation

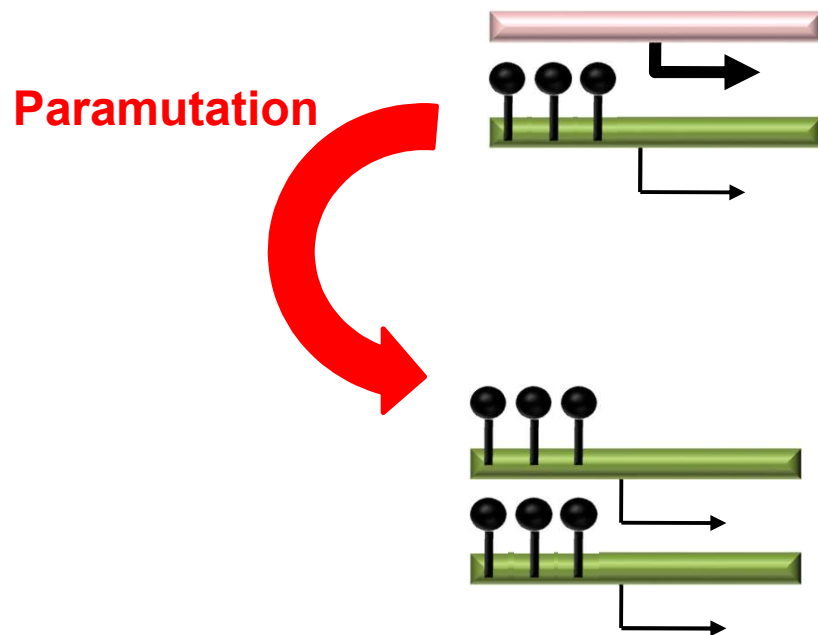




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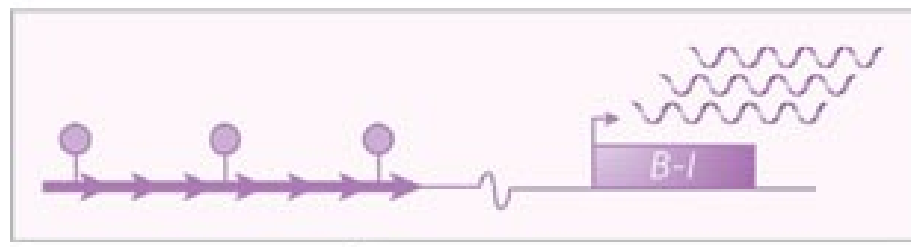


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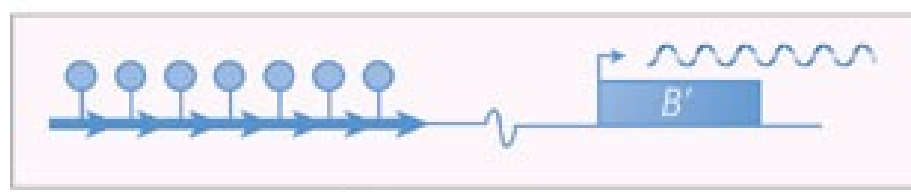


This system provides a straightforward genetic screen for mutations that interfere with paramutation in maize. Such studies have shown that siRNA acting *in trans* is the basis for paramutation.

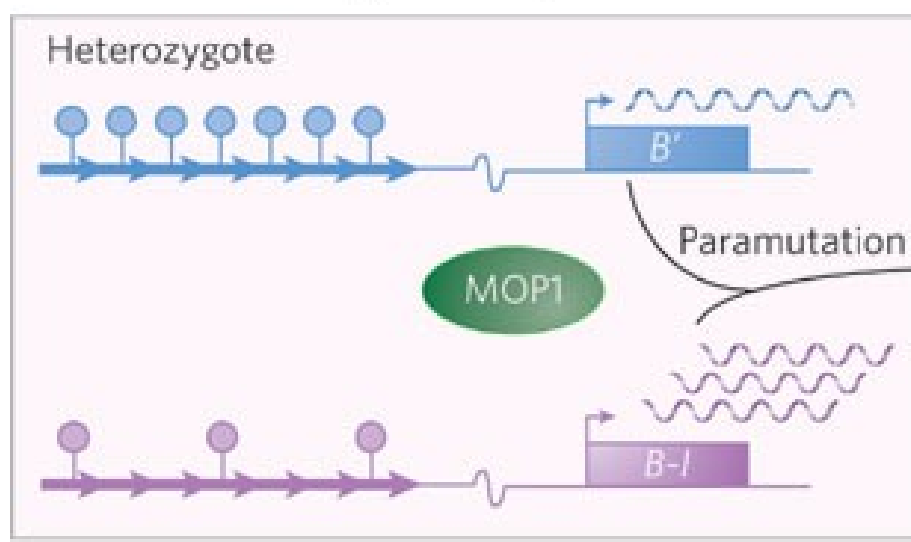
a



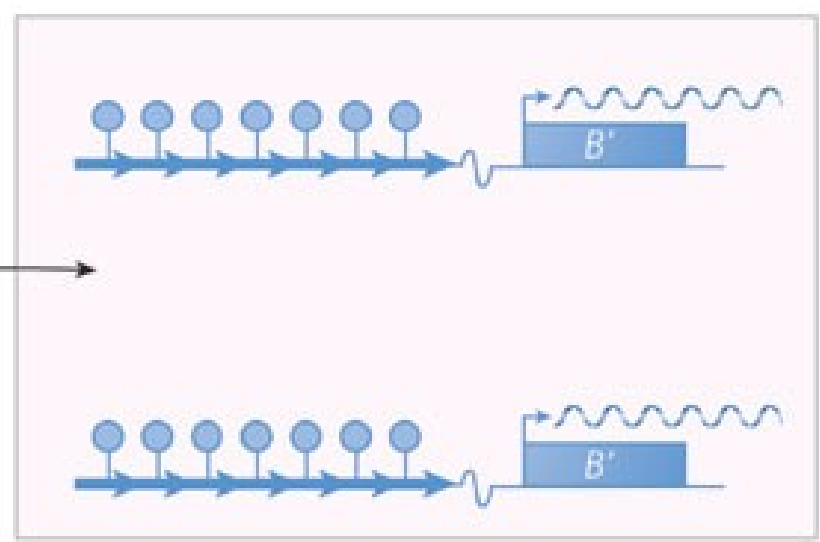
↓ -10% Spontaneous conversion



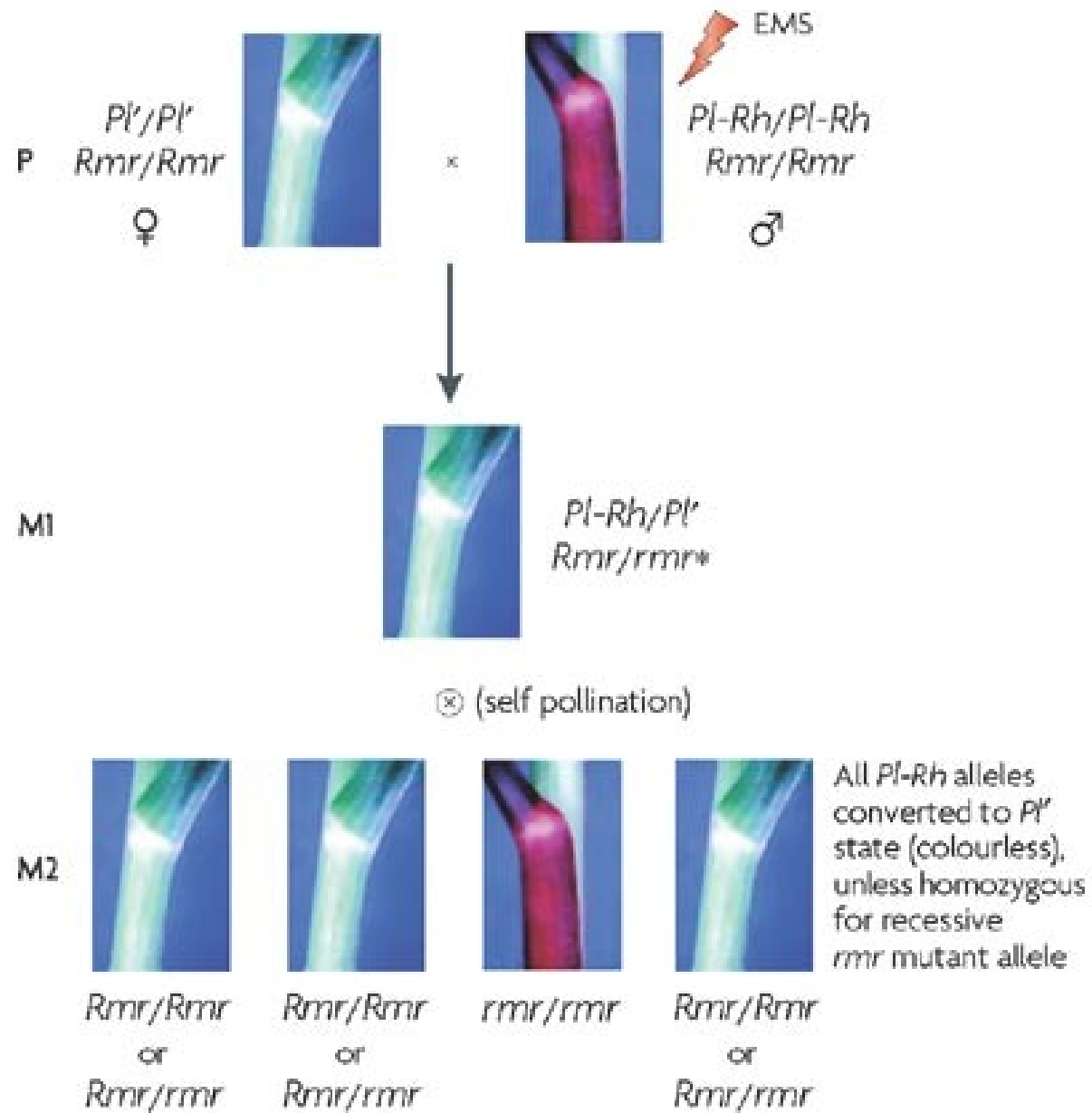
↓ Crossing B' with B-l



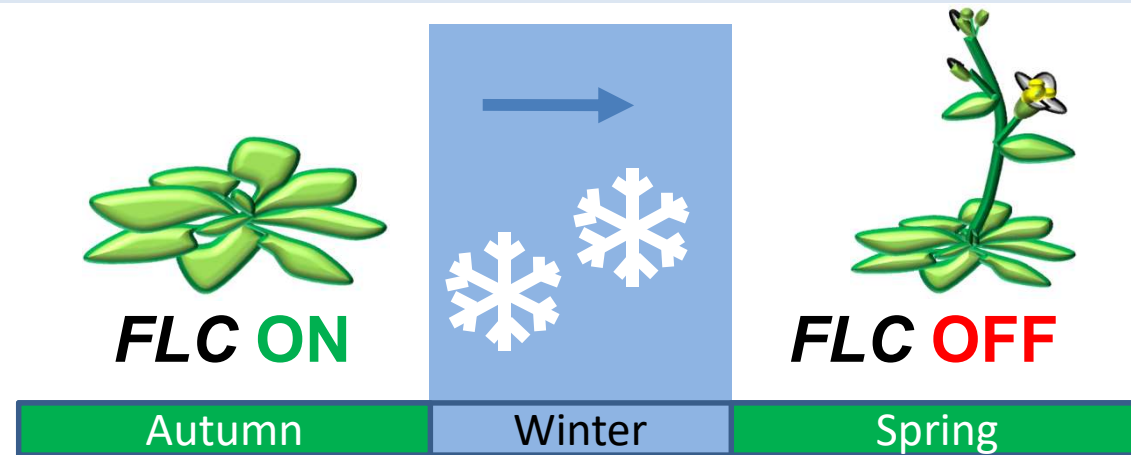
Paramutation



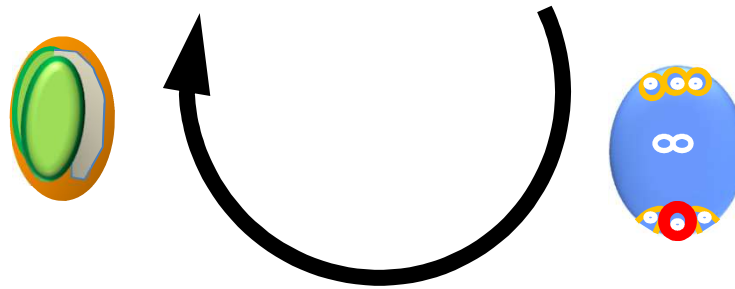
a M2 screen for *rnr* mutants



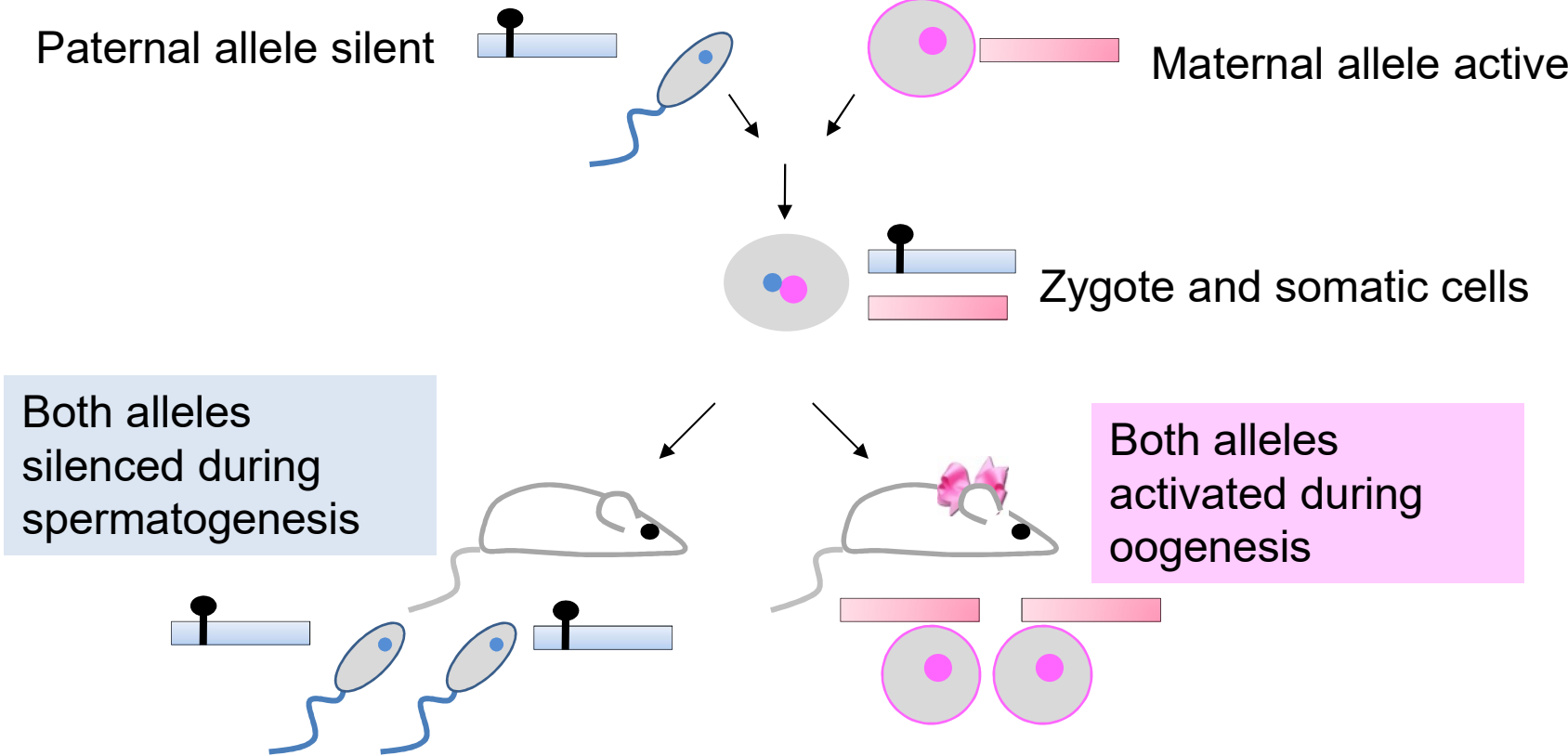
Resetting the epigenome



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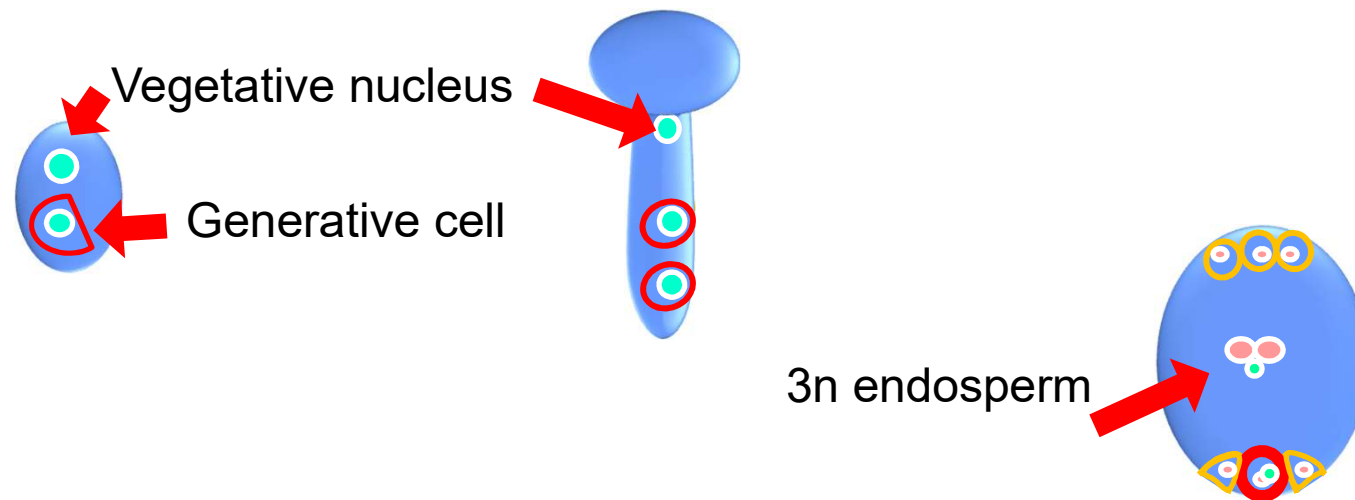


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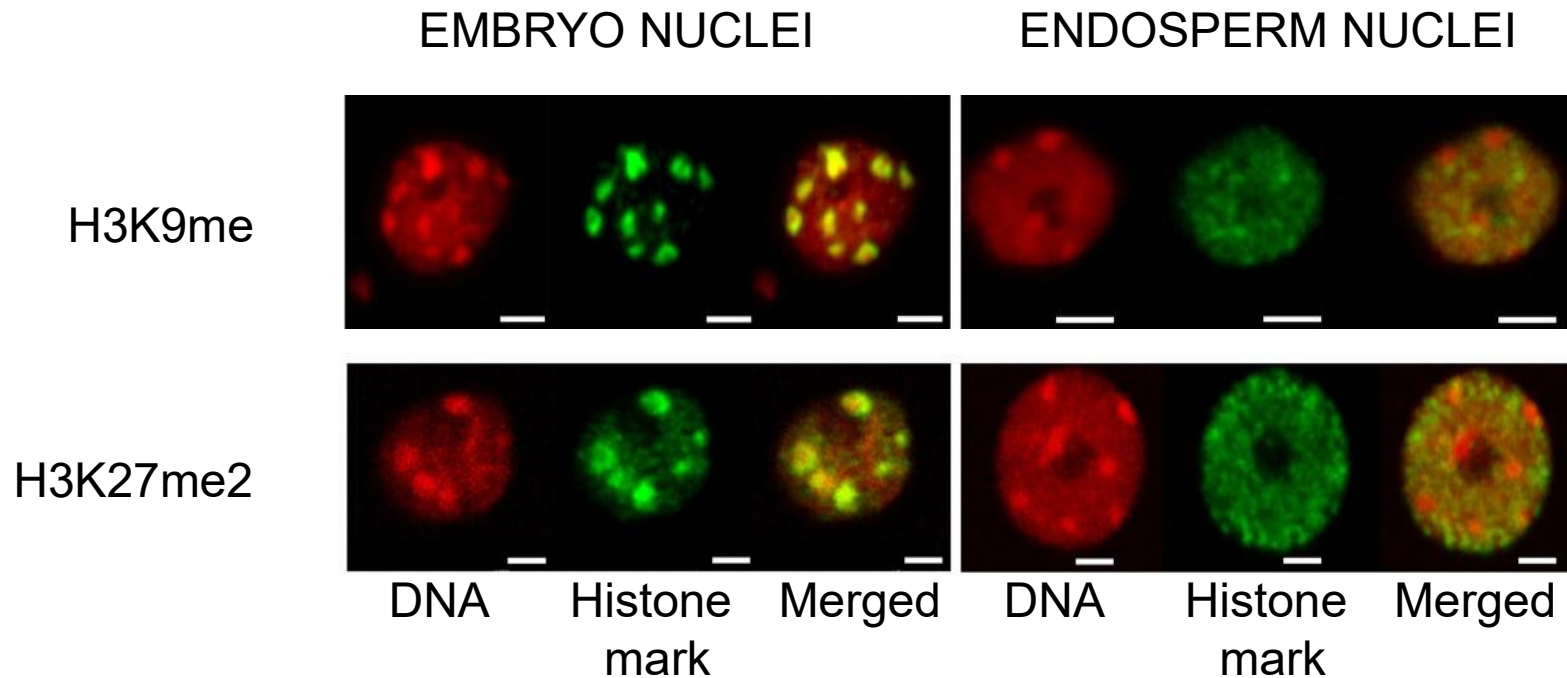


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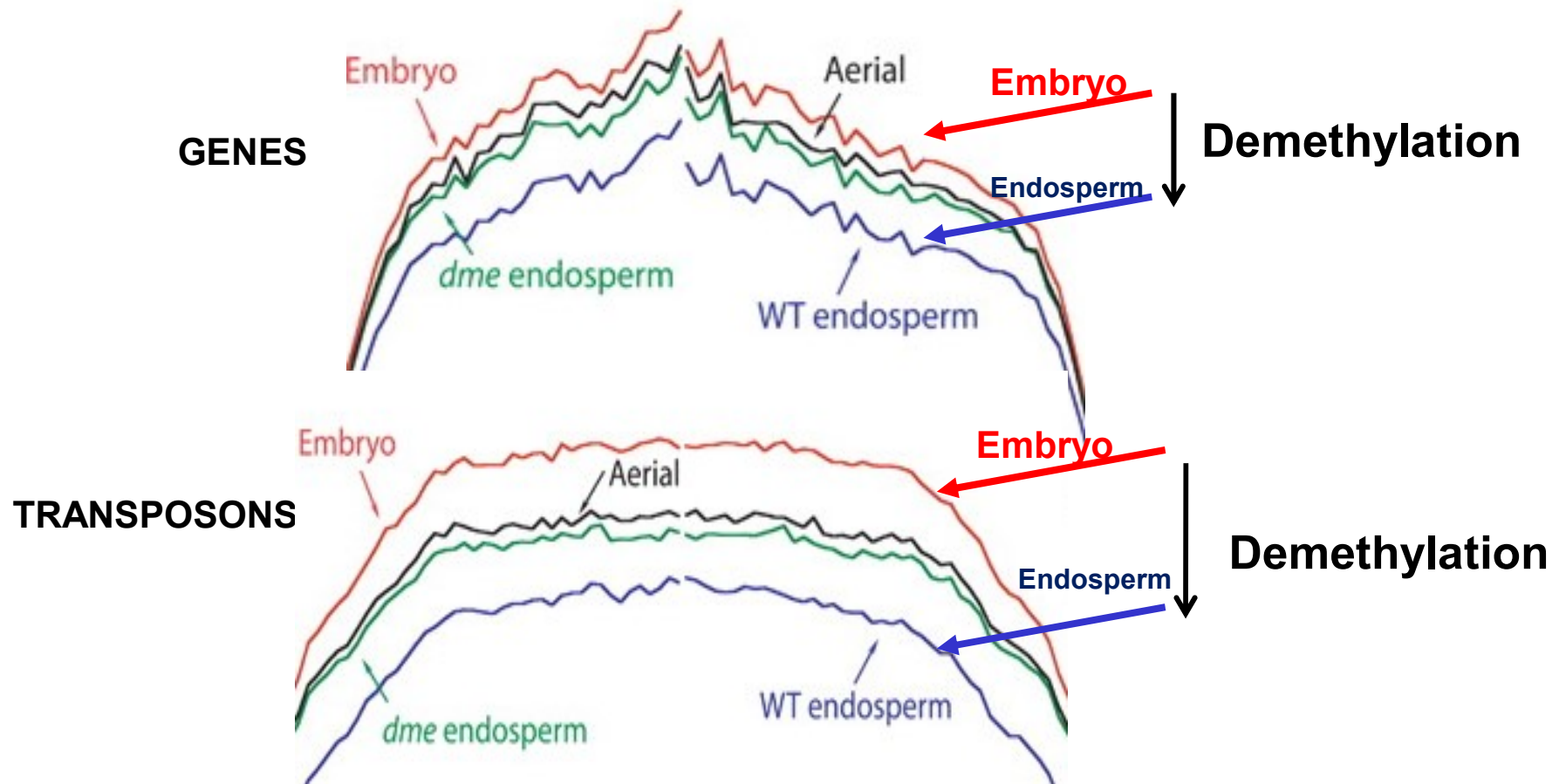


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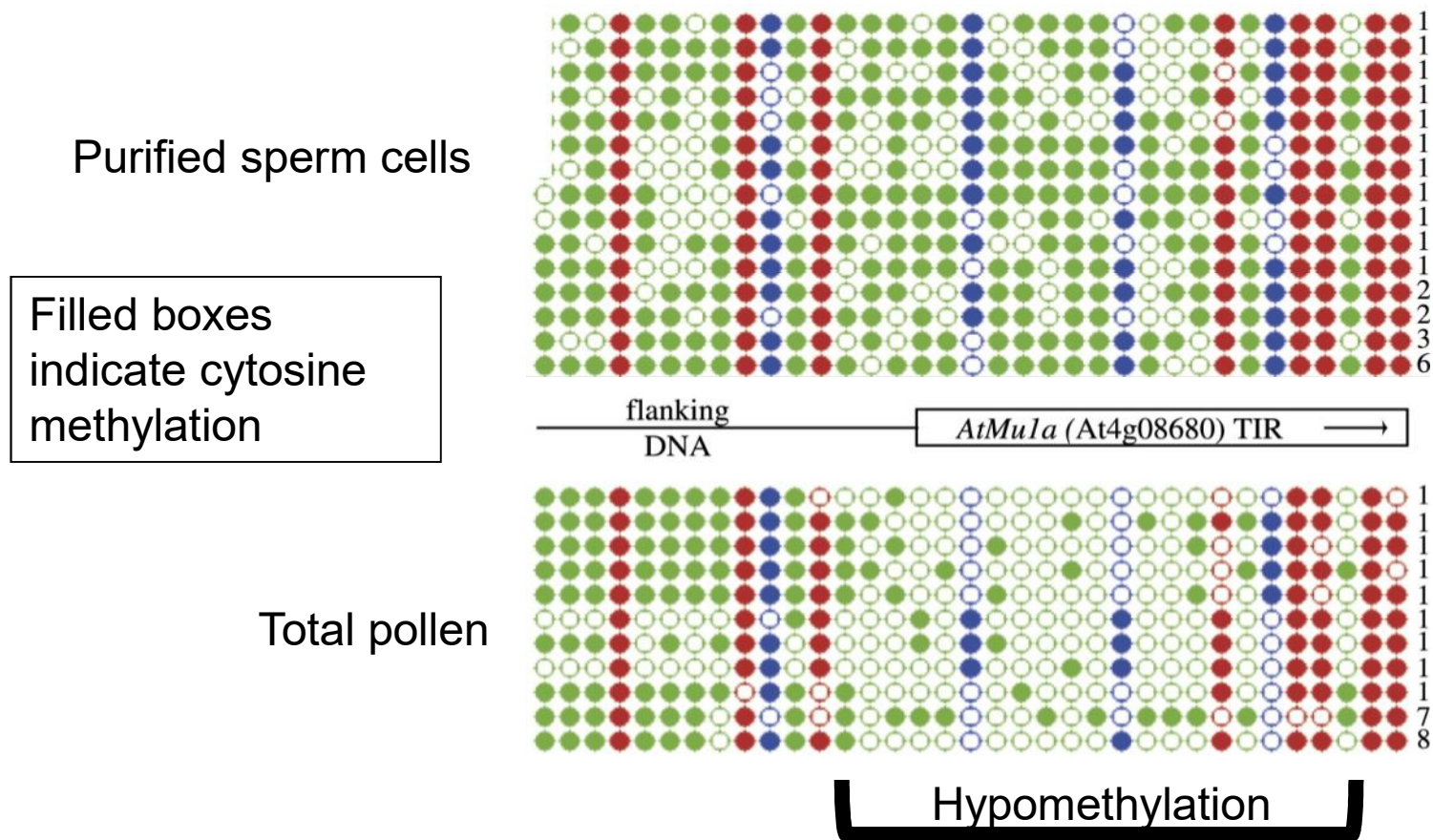
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Transposons in pollen vegetative cells are hypomethylated



Reprinted from Slotkin, R.K., Vaughn, M., Borges, F., Tanurdžić, M., Becker, J.D., Feijó, J.A., and Martienssen, R.A. (2009) Epigenetic reprogramming and small RNA silencing of transposable elements in pollen. *Cell* 136: [461-472](#). Copyright 2009, with permission from Elsevier.

Transposons are specifically activated in pollen

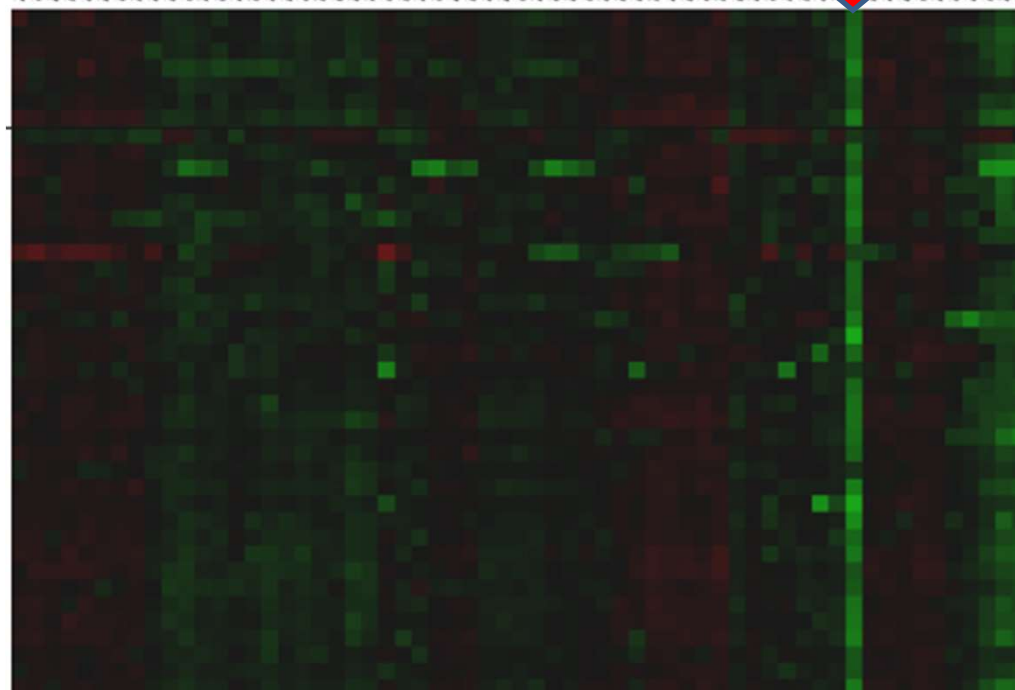
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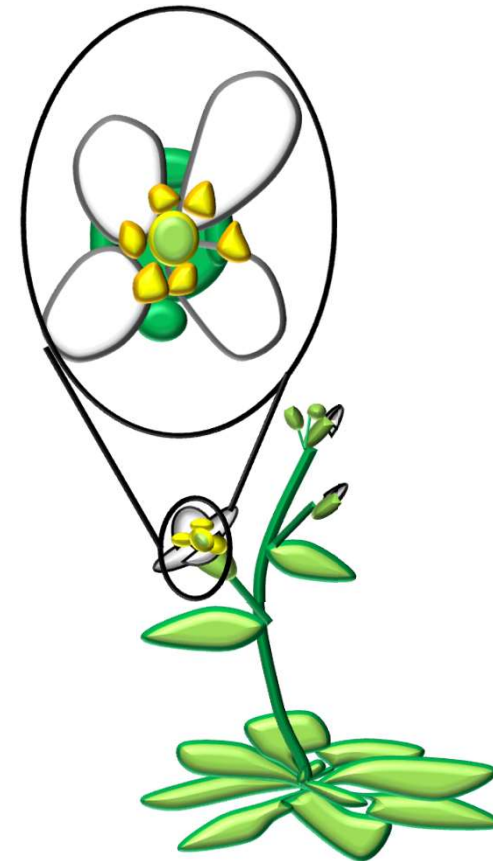
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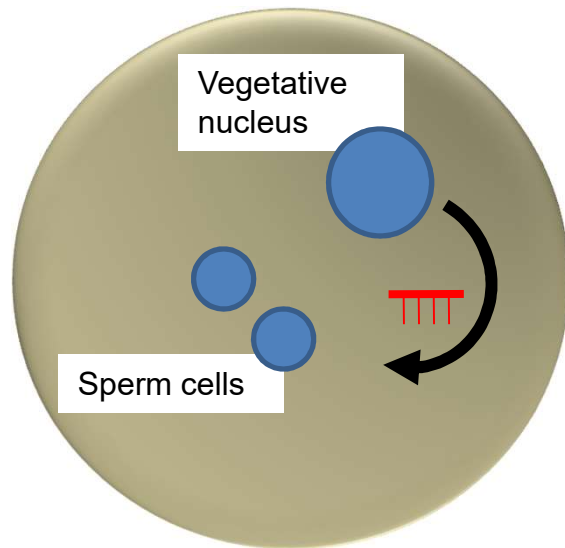


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Resetting of epigenetic marks in non-germ cells?



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