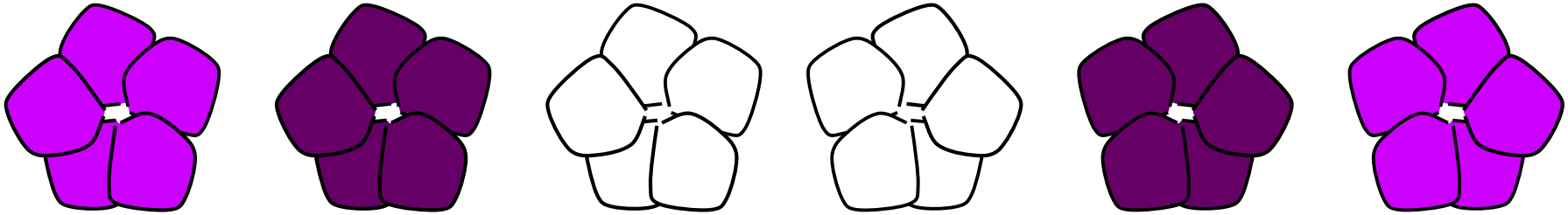
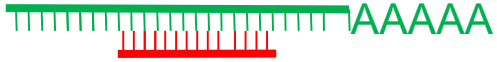
Small RNAs



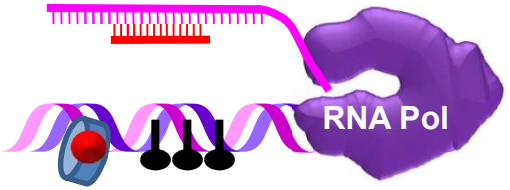
What are small RNAs?

•Small RNAs are a pool of 21 to 24 nt RNAs that generally function in **gene silencing**

•Small RNAs contribute to **post-transcriptional gene silencing** by affecting mRNA translation or stability



•Small RNAs contribute to **transcriptional gene silencing** through epigenetic modifications to chromatin

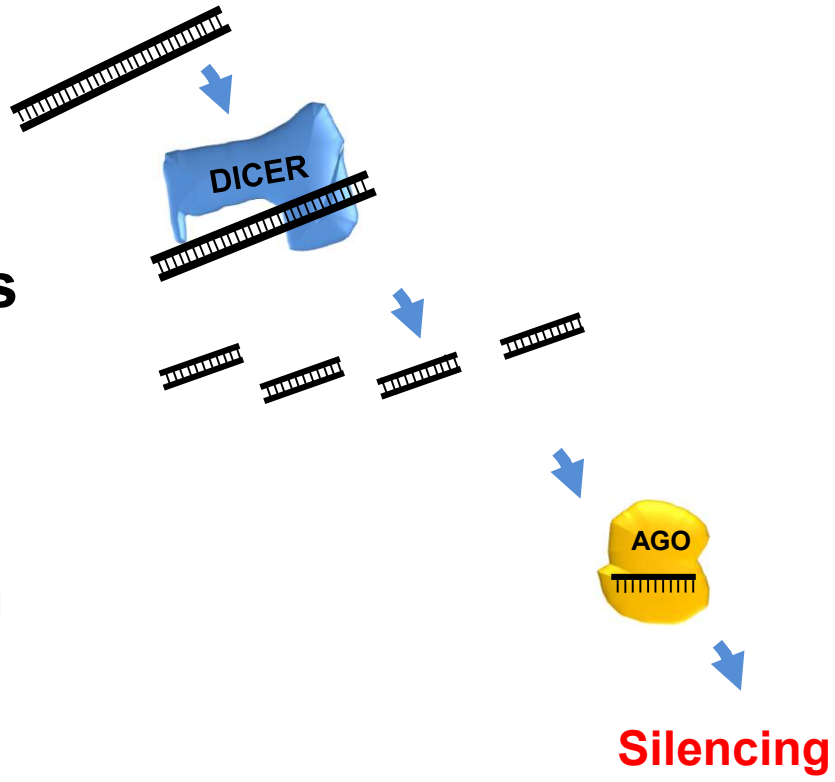


Histone modification, DNA methylation

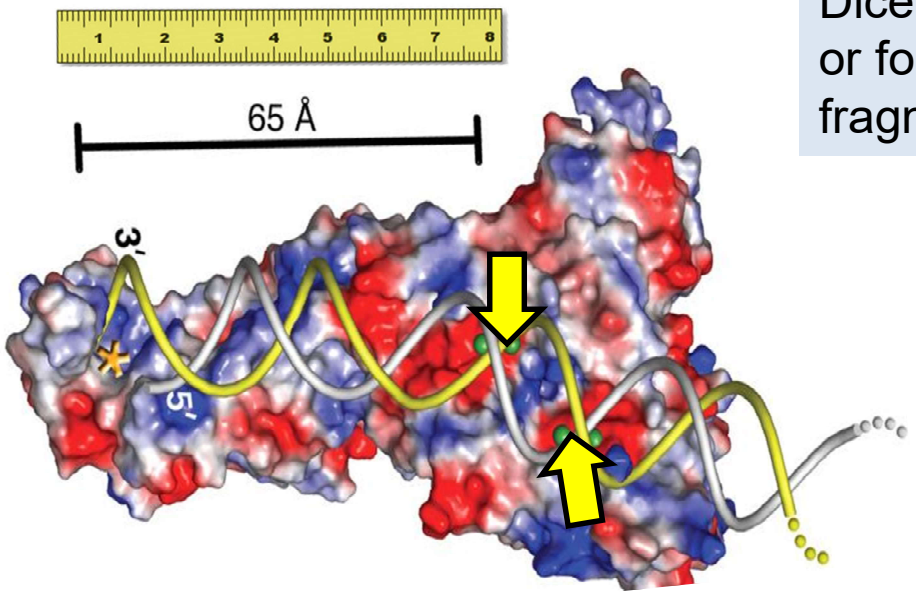
The core of RNA silencing: Dicers and Argonautes

RNA silencing uses a set of core reactions in which **double-stranded RNA (dsRNA)** is processed by **Dicer** or **Dicer-like proteins** into **short RNA duplexes**.

These small RNAs subsequently associate with **ARGONAUTE** proteins to confer silencing.



Dicer and Dicer-like proteins



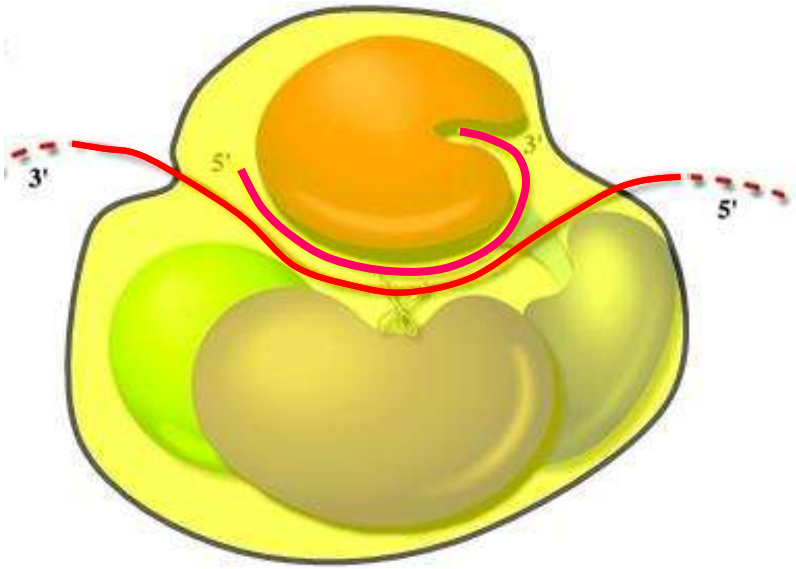
In siRNA and miRNA biogenesis, Dicer or Dicer-like (DCL) proteins cleave long dsRNA or foldback (hairpin) RNA into ~ 21 – 25 nt fragments.

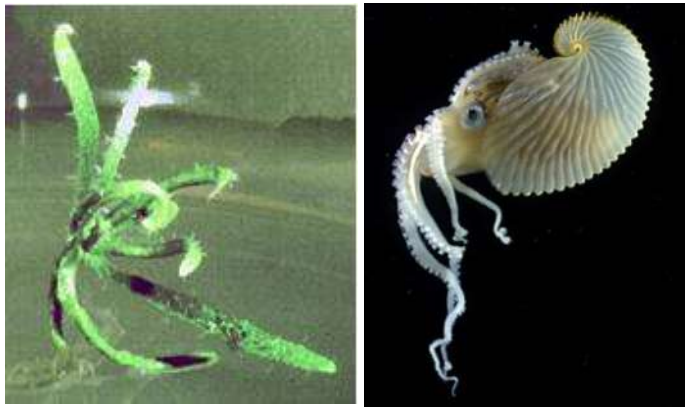
Dicer's structure allows it to measure the RNA it is cleaving. Like a cook who "dices" a carrot, Dicer chops RNA into uniformly-sized pieces.

From MacRae, I.J., Zhou, K., Li, F., Repic, A., Brooks, A.N., Cande, W.., Adams, P.D., and Doudna, J.A. (2006) Structural basis for double-stranded RNA processing by Dicer. Science 311: 195 -198. Reprinted with permission from AAAS. Photo credit: Heidi

Argonaute proteins

ARGONAUTE proteins bind small RNAs and their targets.



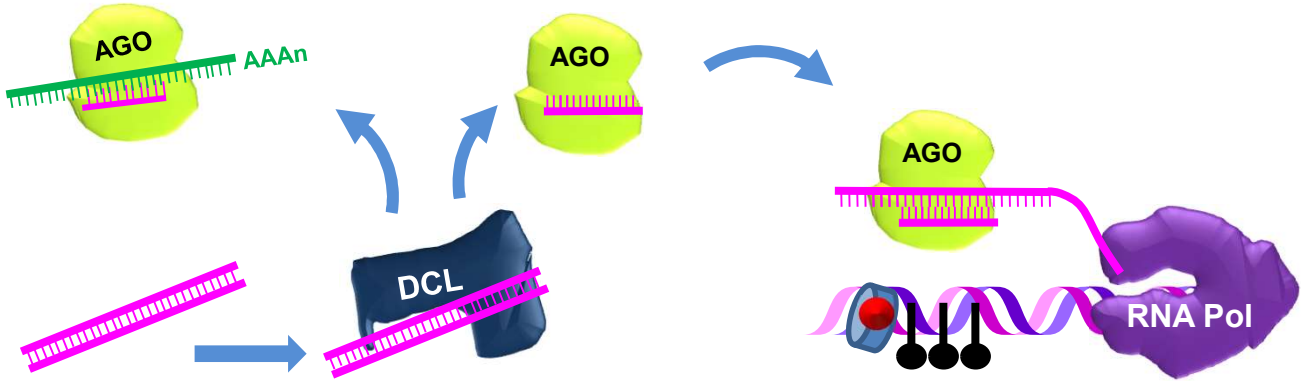


The Arabidopsis *ago1* mutant and the octopus *Argonauta argo*

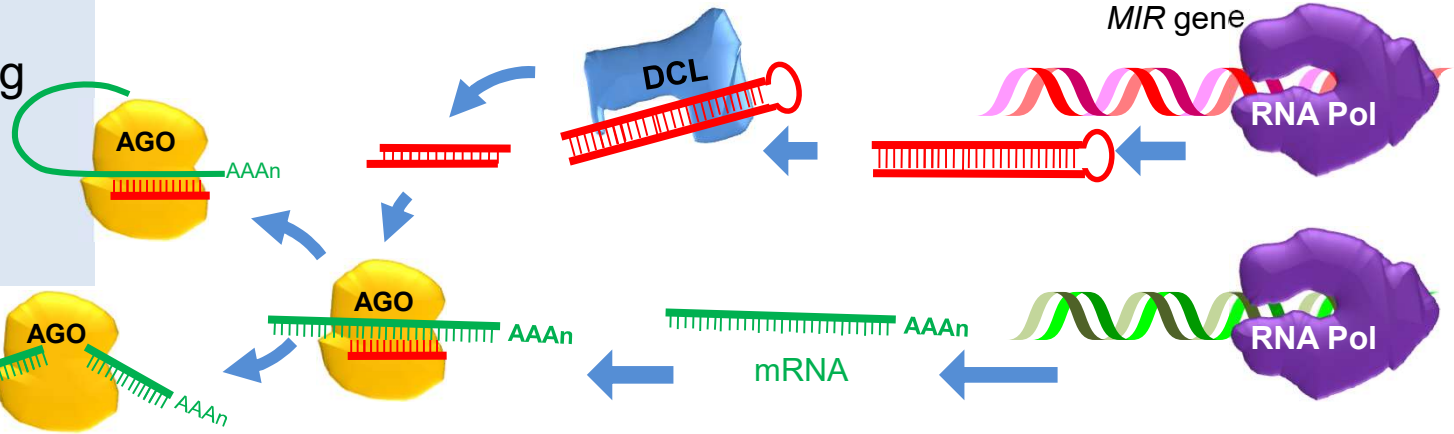
ARGONAUTE proteins are named after the *argonaute1* mutant of Arabidopsis; *ago1* has thin radial leaves and was named for the octopus *Argonauta* which it resembles.

RNA silencing - overview

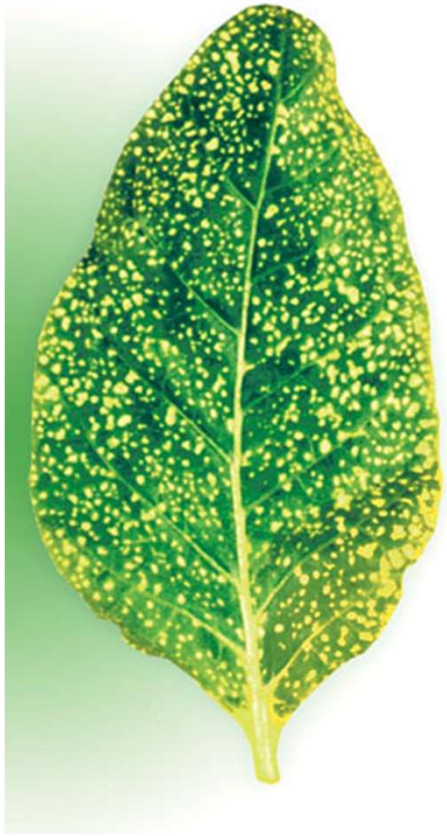
siRNA-mediated silencing via post-transcriptional and transcriptional gene silencing



miRNA - mediated slicing of mRNA and translational repression



siRNAs – Genomic Defenders

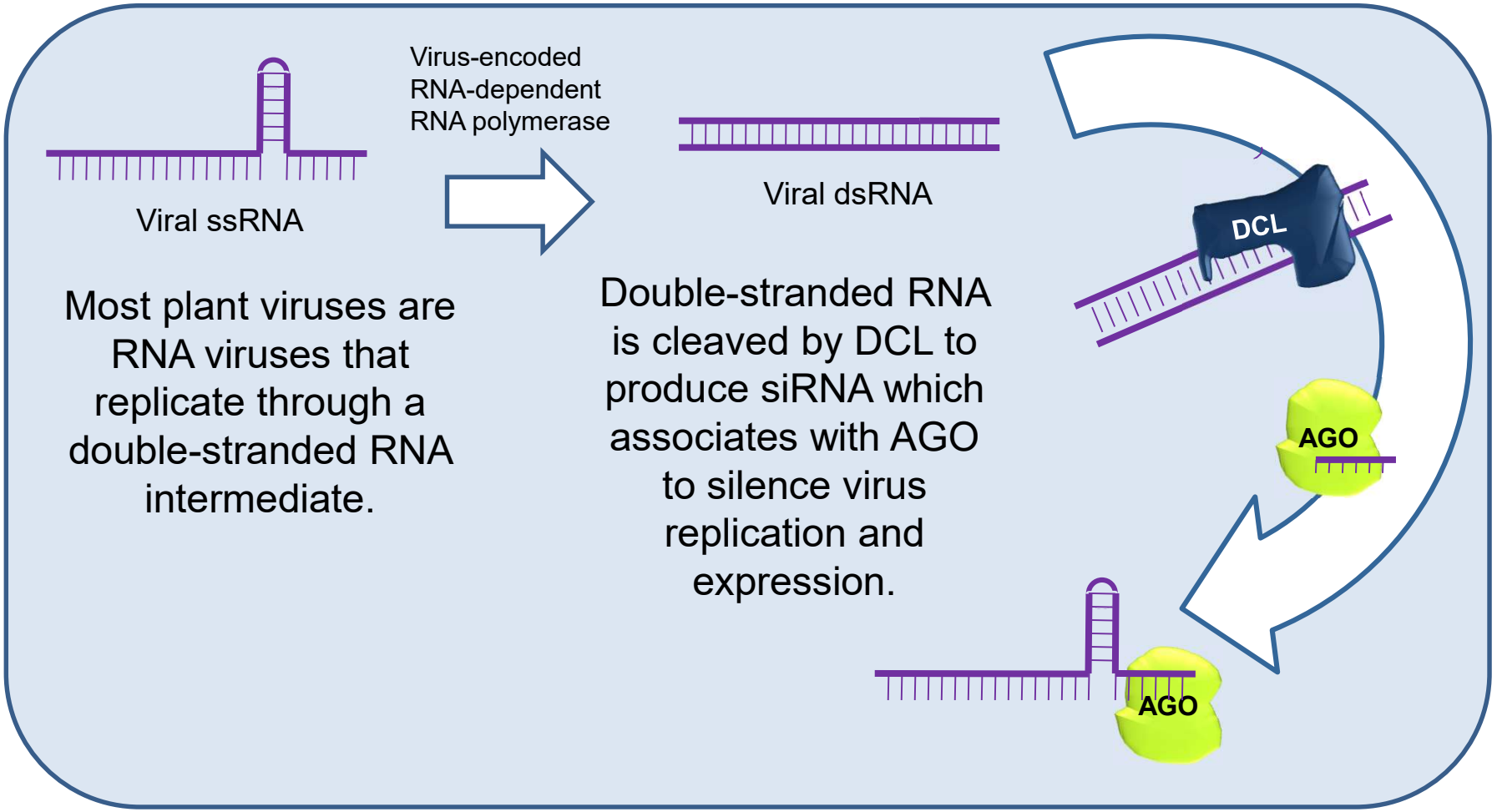


siRNAs protect the genome by

- Suppressing invading viruses
- Silencing sources of aberrant transcripts
- Silencing transposons and repetitive elements

siRNAs also maintain some genes in an epigenetically silent state

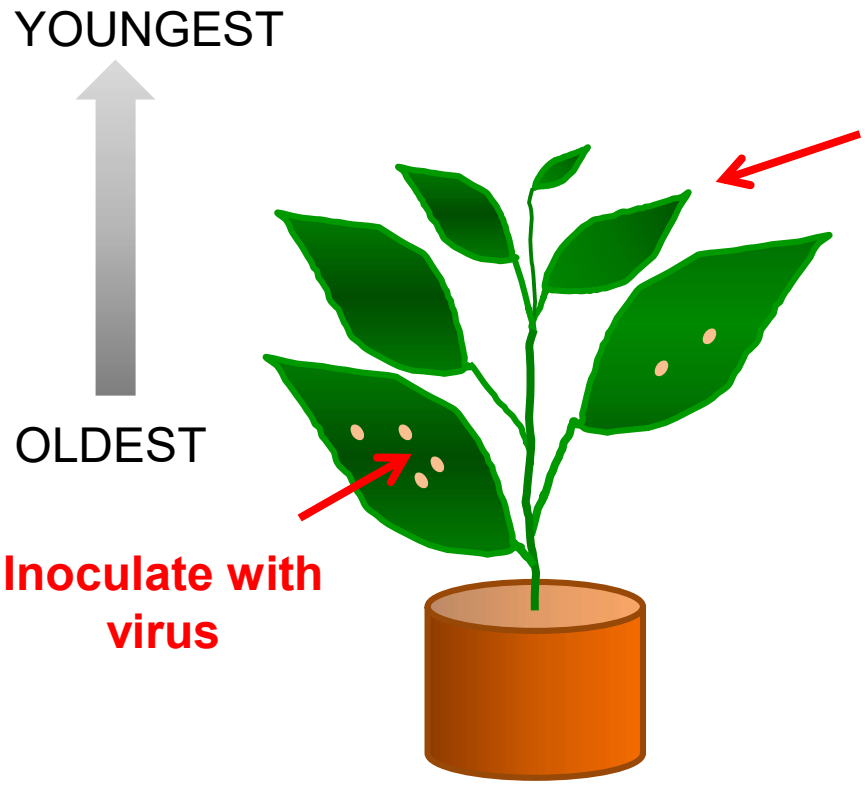
Viral induced gene silencing - overview



Most plant viruses are RNA viruses that replicate through a double-stranded RNA intermediate.

Double-stranded RNA is cleaved by DCL to produce siRNA which associates with AGO to silence virus replication and expression.

Plants can recover from viral infection and become resistant



Younger leaves produced on a virus-infected plant can be symptom-free, indicating that the plant has **recovered** from the infection.

Plants can recover from viral infection and become resistant

OLDEST → YOUNGEST

Leaf inoculated with virus

Leaf inoculated with virus

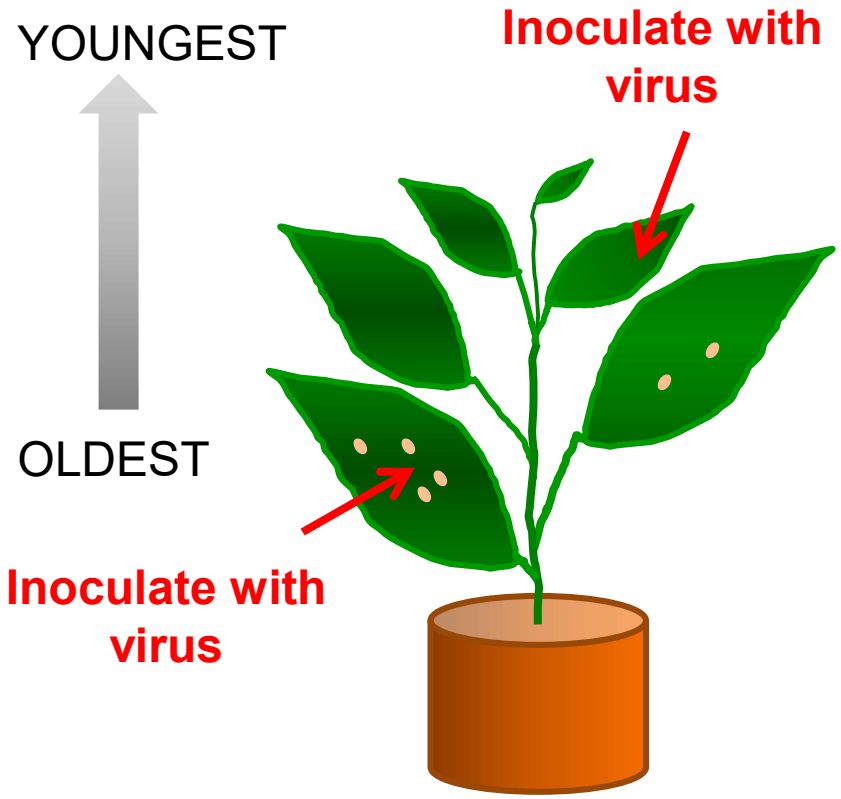
Virus-induced cell death

No symptoms: **recovery**

No symptoms: **resistance**

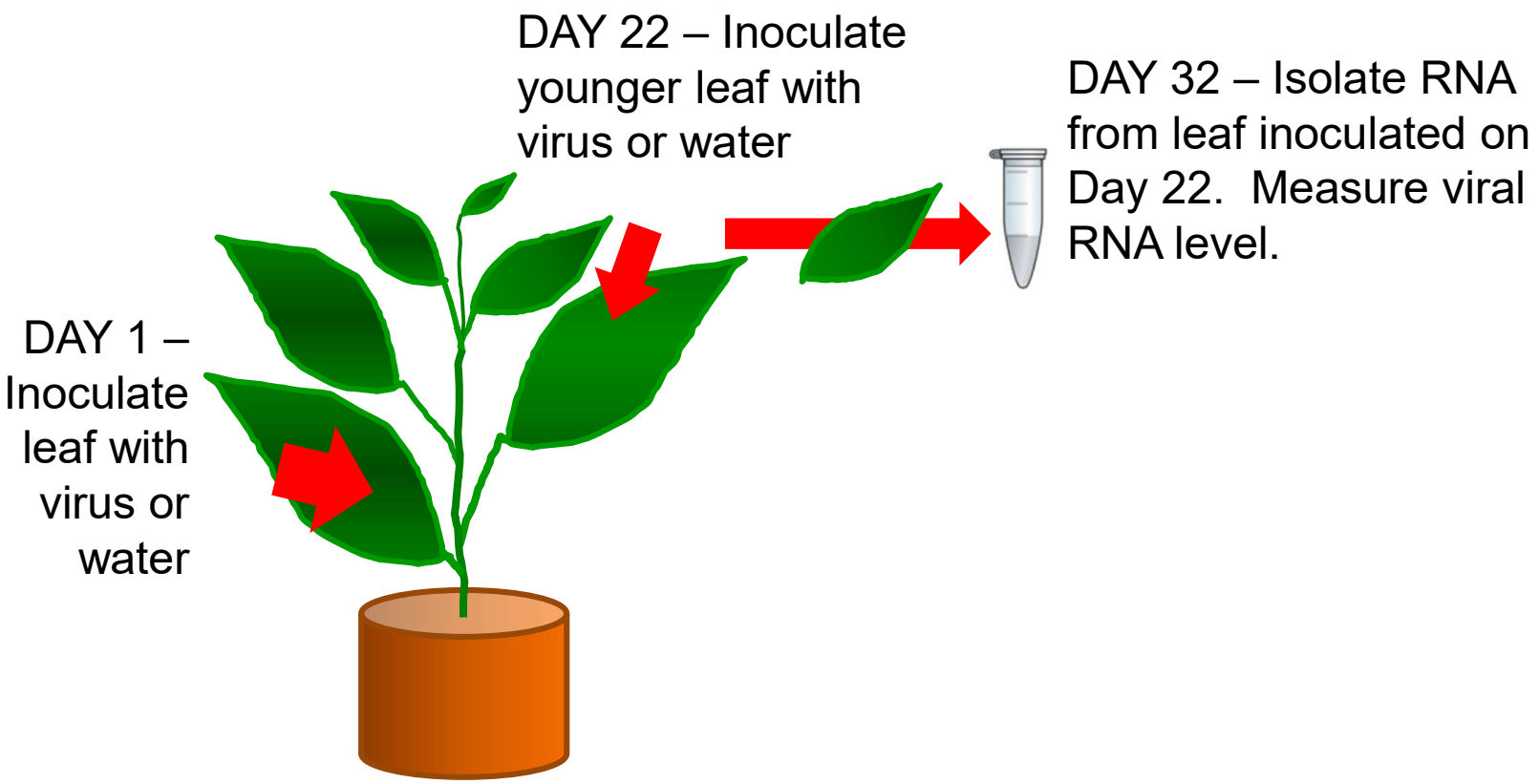
From Ratcliff, F., Henderson, B.D., and Baulcombe, D.C. (1997) A similarity between viral and gene silencing in plants. Science 276: 1558–1560. Reprinted with permission from AAAS.

Plants can recover from viral infection and become resistant



These leaves are also **resistant** to subsequent infection by the same virus.

Viral resistance involves siRNA-mediated silencing

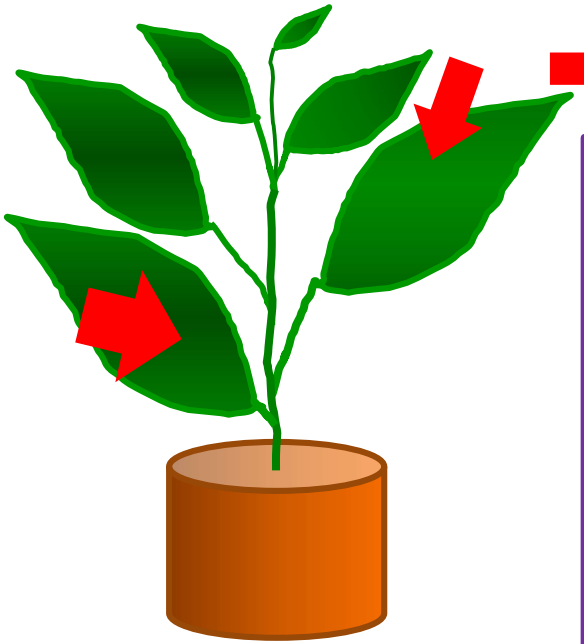


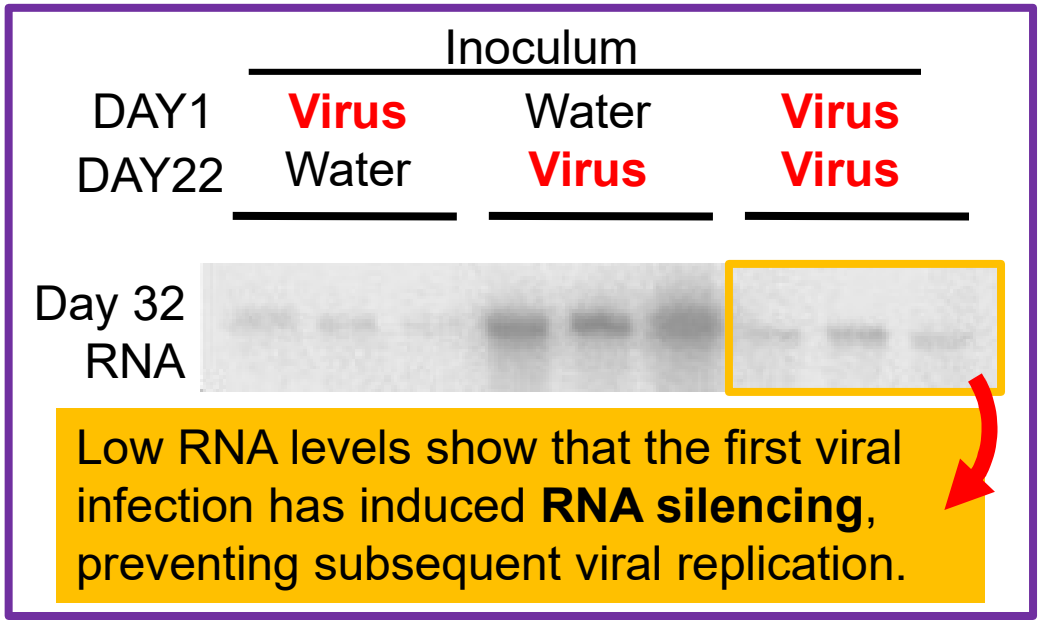
Viral resistance involves siRNA-mediated silencing

DAY 22 – Inoculate younger leaf with virus or water

DAY 32 – Isolate RNA from leaf inoculated on Day 22. Measure viral RNA level.

DAY 1 – Inoculate leaf with virus or water

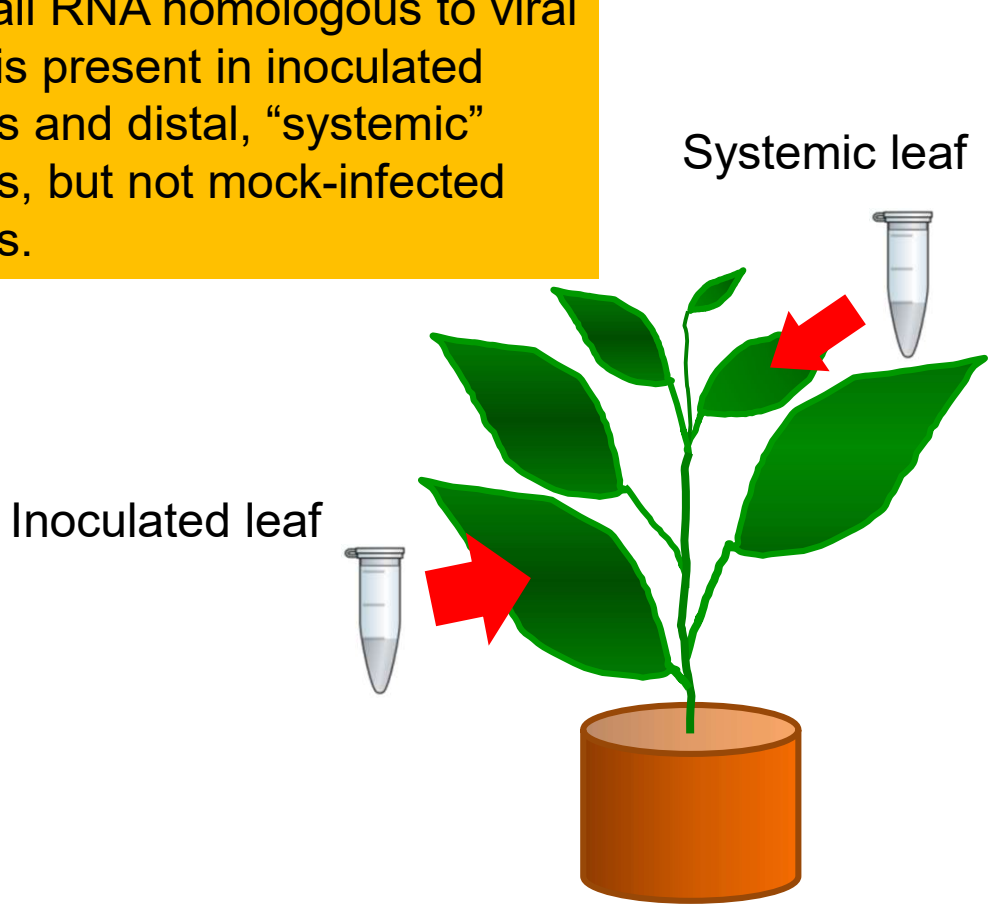


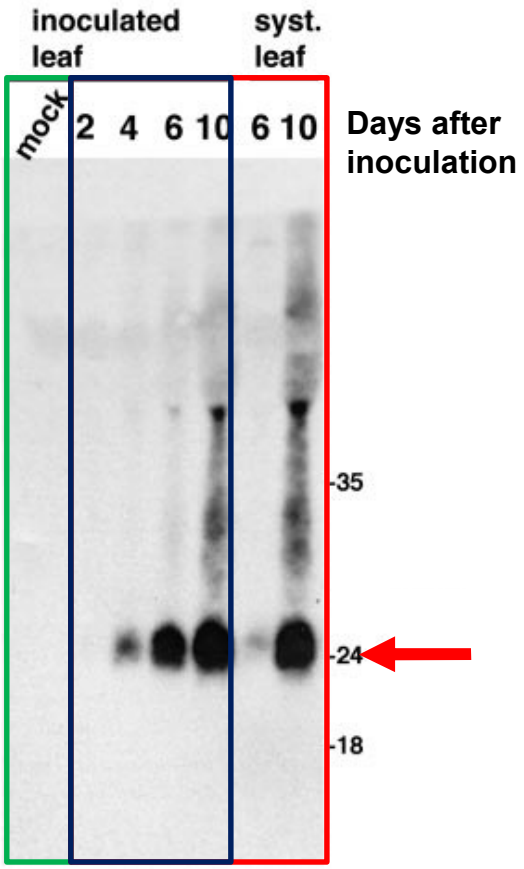


From Ratcliff, F., Henderson, B.D., and Baulcombe, D.C. (1997) A similarity between viral defense and gene silencing in plants. Science 276: 1558–1560. Reprinted with permission from AAAS.

Small RNAs are correlated with viral-induced gene silencing

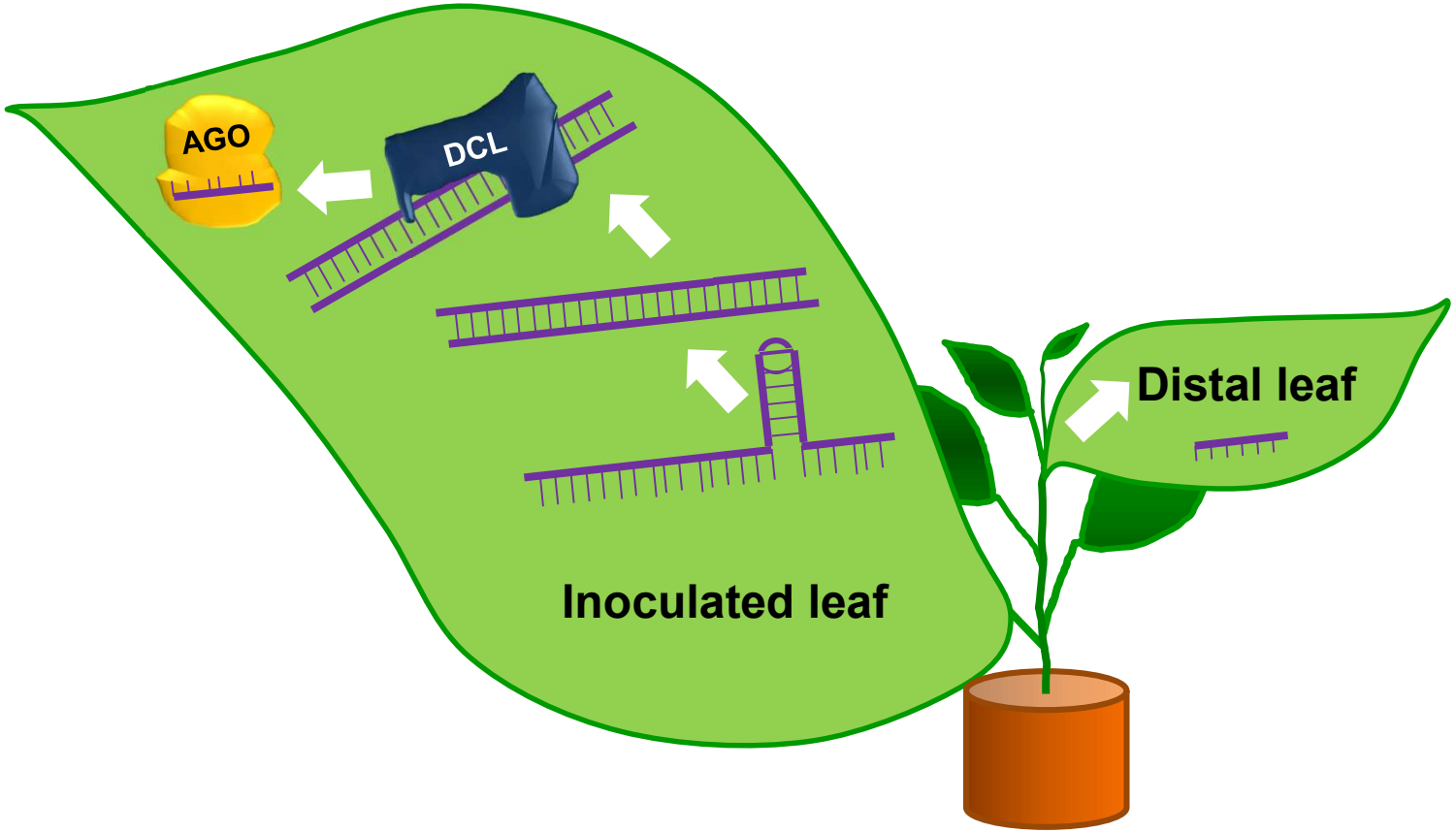
A small RNA homologous to viral RNA is present in inoculated leaves and distal, “systemic” leaves, but not mock-infected leaves.



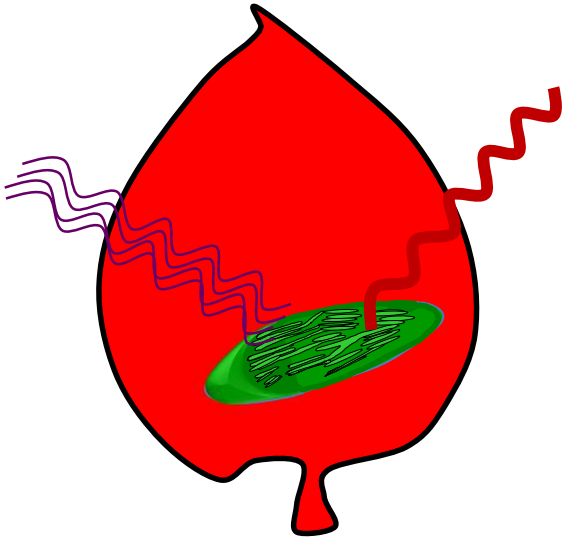


From Ratcliff, F., Henderson, B.D., and Baulcombe, D.C. (1997) A similarity between viral defense and gene silencing in plants. Science 276: 1558–1560. Reprinted with permission from AAAS.

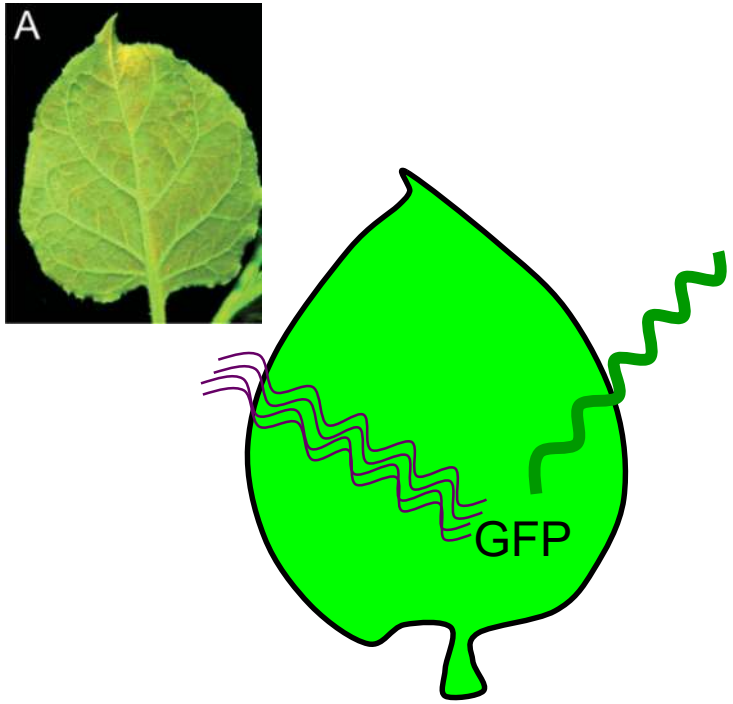
Virus infection causes systemic siRNA accumulation



How does RNA silencing spread systemically???

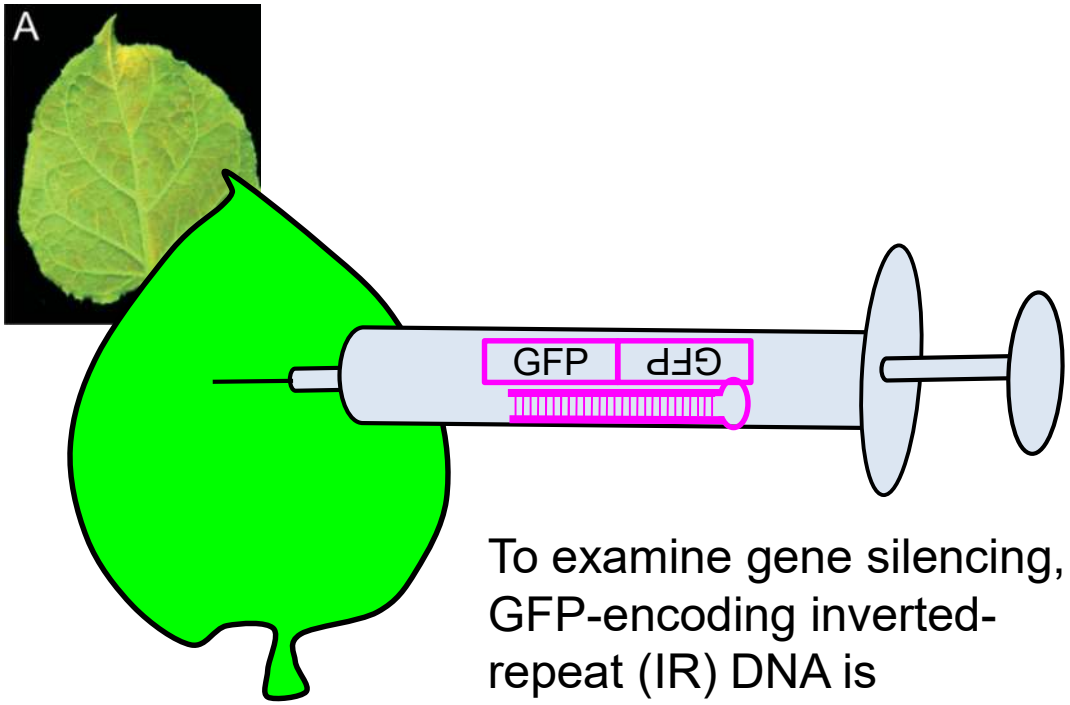


Under UV light, wild-type leaves fluoresce **red**, from chlorophyll in the chloroplasts.



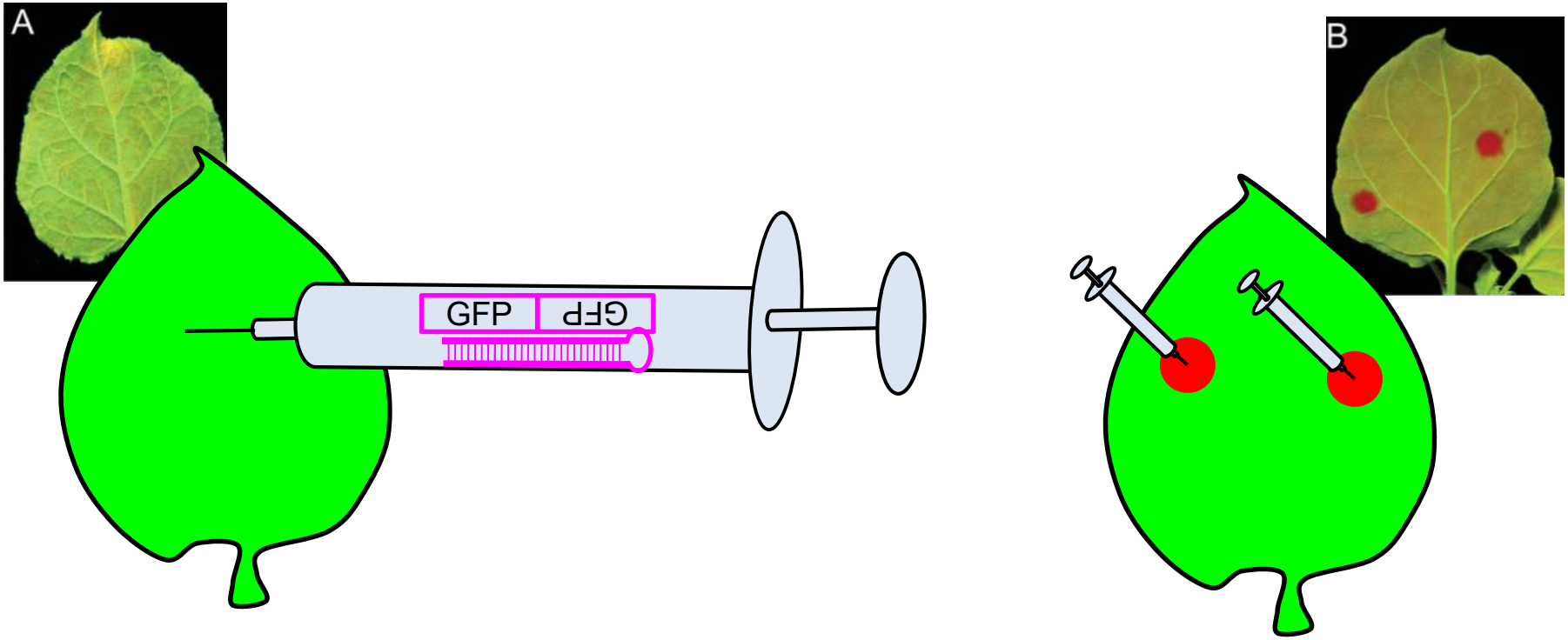
A plant expressing GFP fluoresces **green** under UV light.

Spreading of RNA silencing



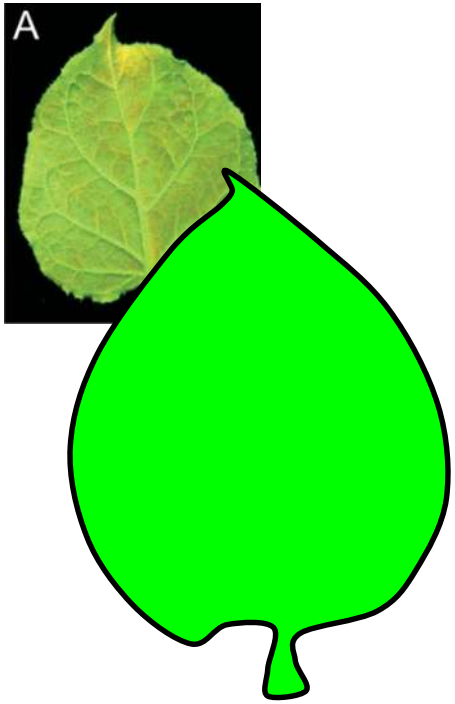
To examine gene silencing, GFP-encoding inverted-repeat (IR) DNA is introduced into the GFP-expressing cells.

Spreading of RNA silencing

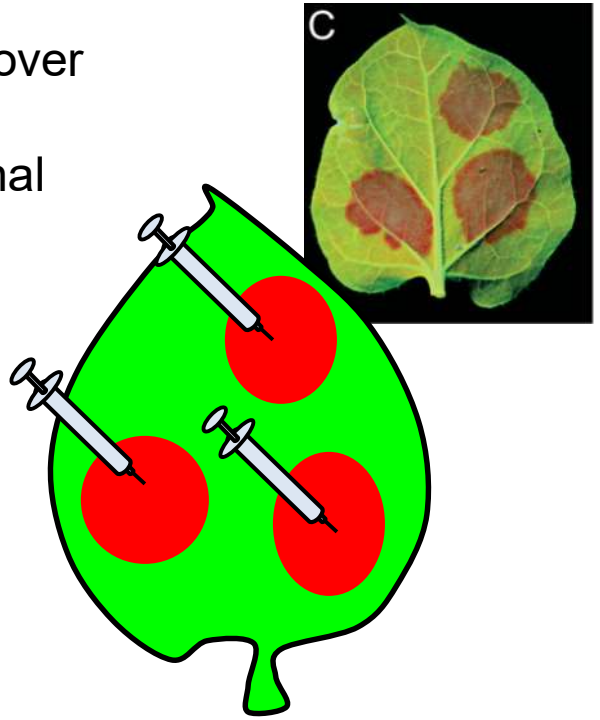


When GFP is silenced, the red chlorophyll fluorescence becomes visible.

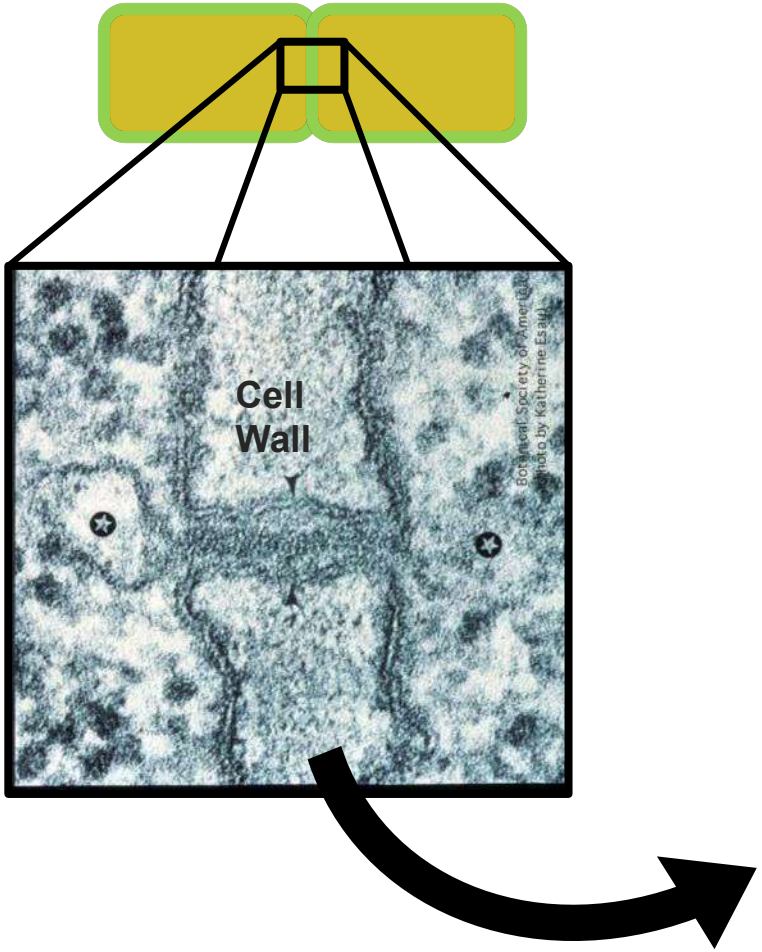
Silencing can spread locally

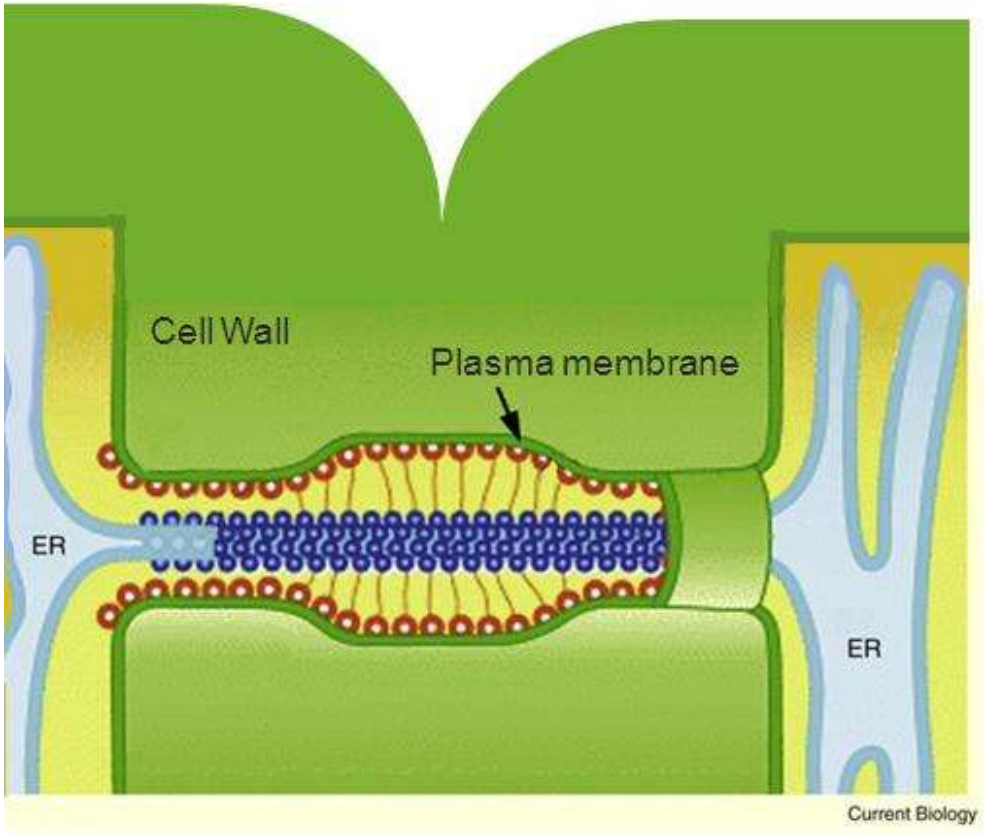


Often the silencing spreads over up to 15 cells, probably by diffusion of the silencing signal through the plasmodesmata.



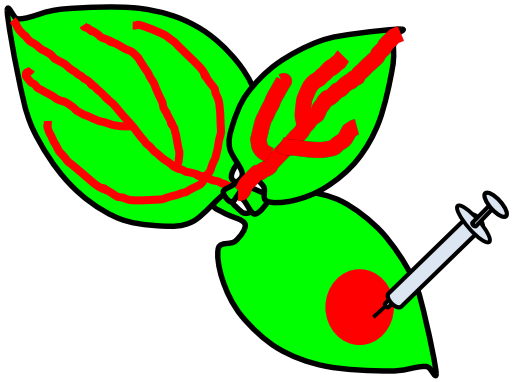
Plasmodesmata are regulated connections between plant cells



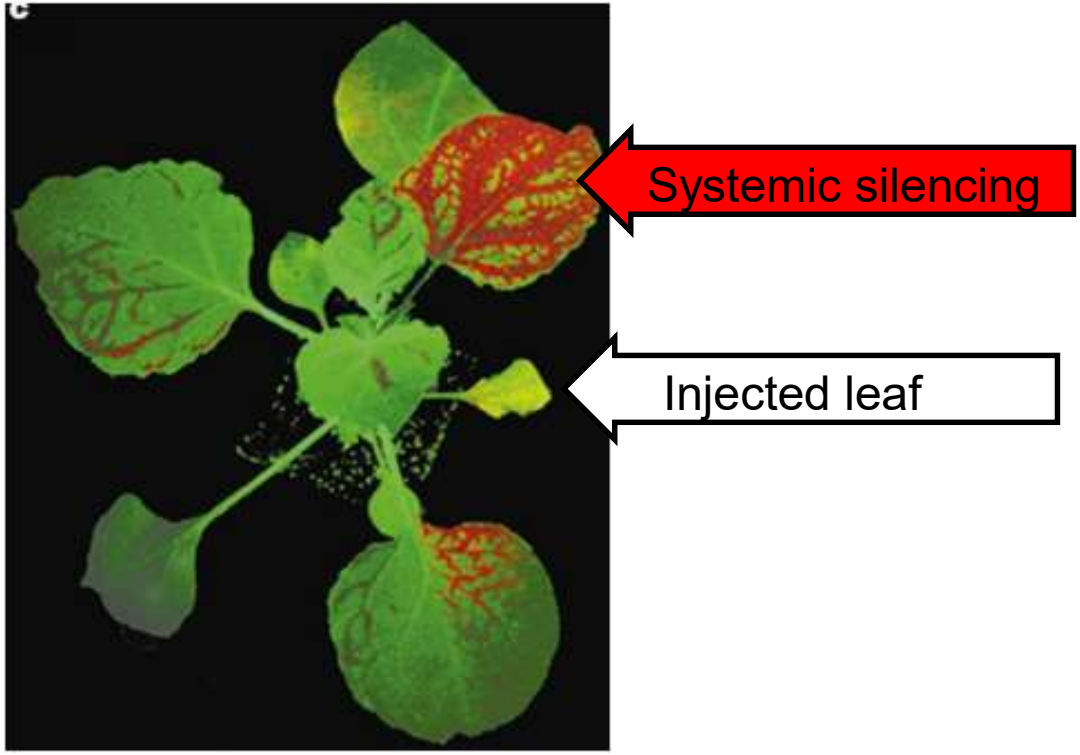


Reprinted from Zambryski, P. (2008) Plasmodesmata. Curr. Biol. 18: R324-325 with permission from Elsevier. TEM image credit BSA Photo by Katherine Esau;

Silencing can spread systemically through the phloem



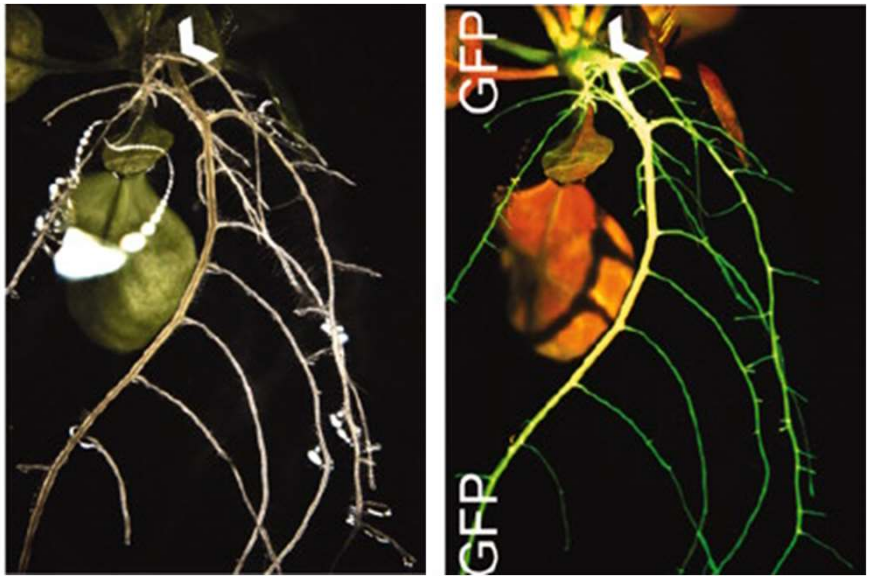
Recent experiments have shed light on the identity of the silencing signal…



Voinnet, O., and Baulcombe, D. (1997) Systemic silencing in gene silencing. Nature 389: 553.

Small RNAs can move from shoot to root in Arabidopsis

Control GFP expressing plant showing GFP in shoot and root



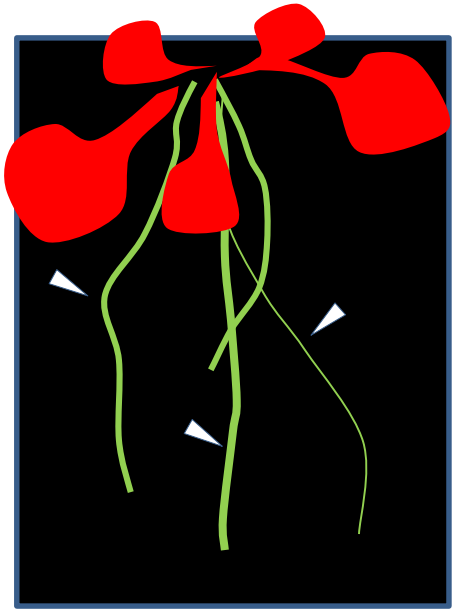
White light Fluorescence

GFP-inverted repeat-expressing shoot grafted onto GFP root – newly formed roots do not express GFP (indicated by arrowheads)

White light Fluorescence

From Molnar, A., Melnyk, C. W., Bassett, A., Hardcastle, T. J., Dunn, R., and Baulcombe, D. C. (2010). Small silencing RNAs in plants are mobile and direct epigenetic modification in recipient cells. Science **328:** 872-875; reprinted with permission from AAAS.

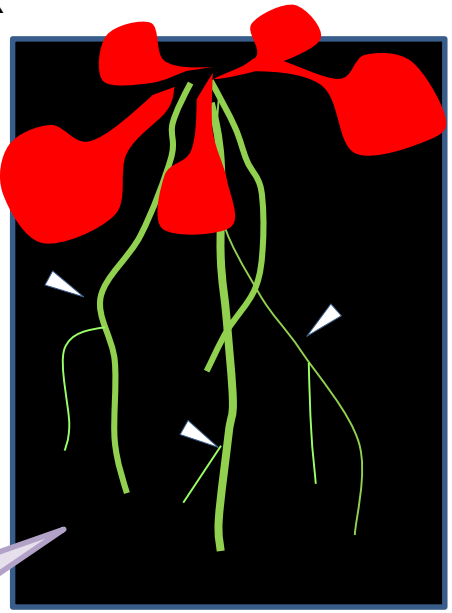
Dicer activity for sRNA production in the shoot is sufficient



GFP-IR

GFP

GFP silenced



GFP-IR

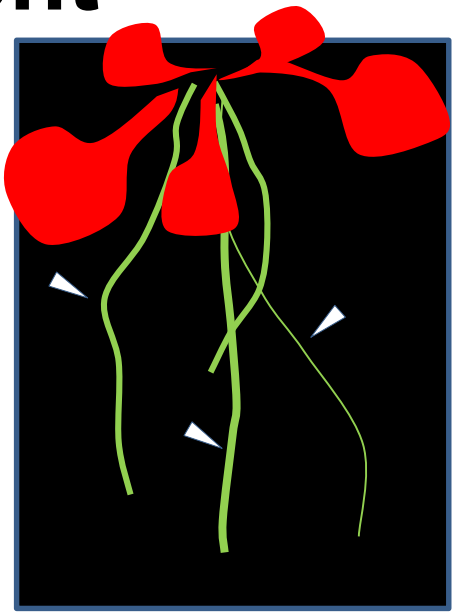
dcl2,3,4

GFP

dcl2,3,4

No silencing; no sRNAs in root

The *dcl2,3,4* mutant cannot make sRNA from dsRNA precursors



GFP-IR

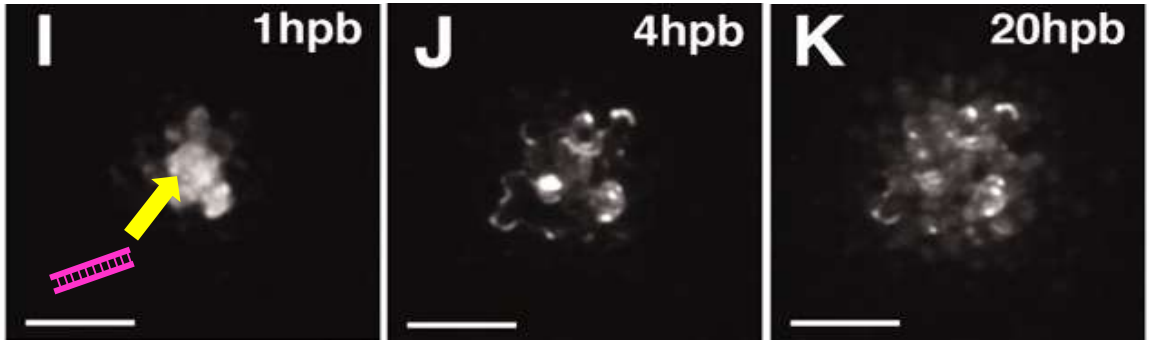
GFP

dcl2,3,4

GFP silenced; sRNAs in root are derived from shoot

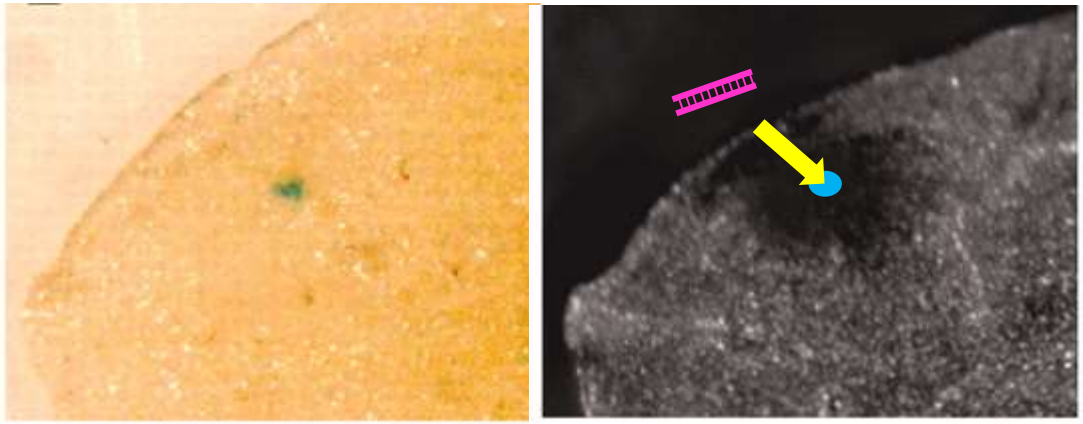
From Molnar, A., Melnyk, C. W., Bassett, A., Hardcastle, T. J., Dunn, R., and Baulcombe, D. C. (2010). Small silencing RNAs in plants are mobile and direct epigenetic modification in recipient cells. Science **328:** 872-875; reprinted with permission from AAAS.

siRNA duplexes move between cells and are sufficient to confer silencing



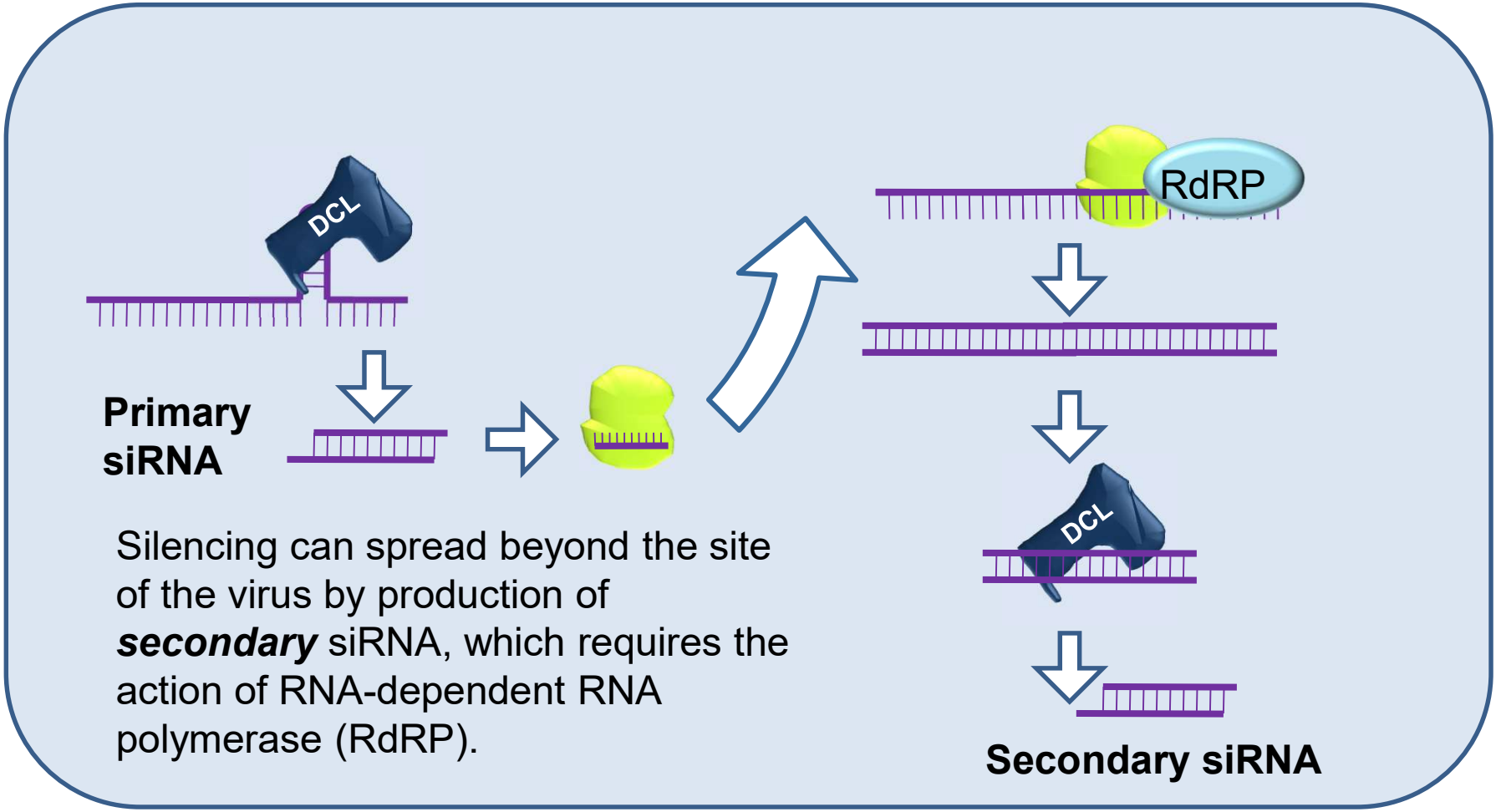
Fluorescently labeled duplex siRNA was bombarded into a single cell. Spreading to adjacent cells is visible at 20 hours post-bombardment.

The duplex siRNA spread from the site of entry (blue dot) and silenced GFP expression in the adjoining cells: it is ***sufficient*** for silencing.



From Dunoyer, P., Schott, G., Himber, C., Meyer, D., Takeda, A., Carrington, J.C. and Voinnet, O. (2010). Small RNA duplexes function as mobile silencing signals between plant cells. Science. 328: 912-916. Reprinted with permission from AAAS.

Systemic silencing is enhanced by signal amplification



Silencing can spread beyond the site of the virus by production of ***secondary*** siRNA, which requires the action of RNA-dependent RNA polymerase (RdRP).

siRNA production mutants are more susceptible to viral disease

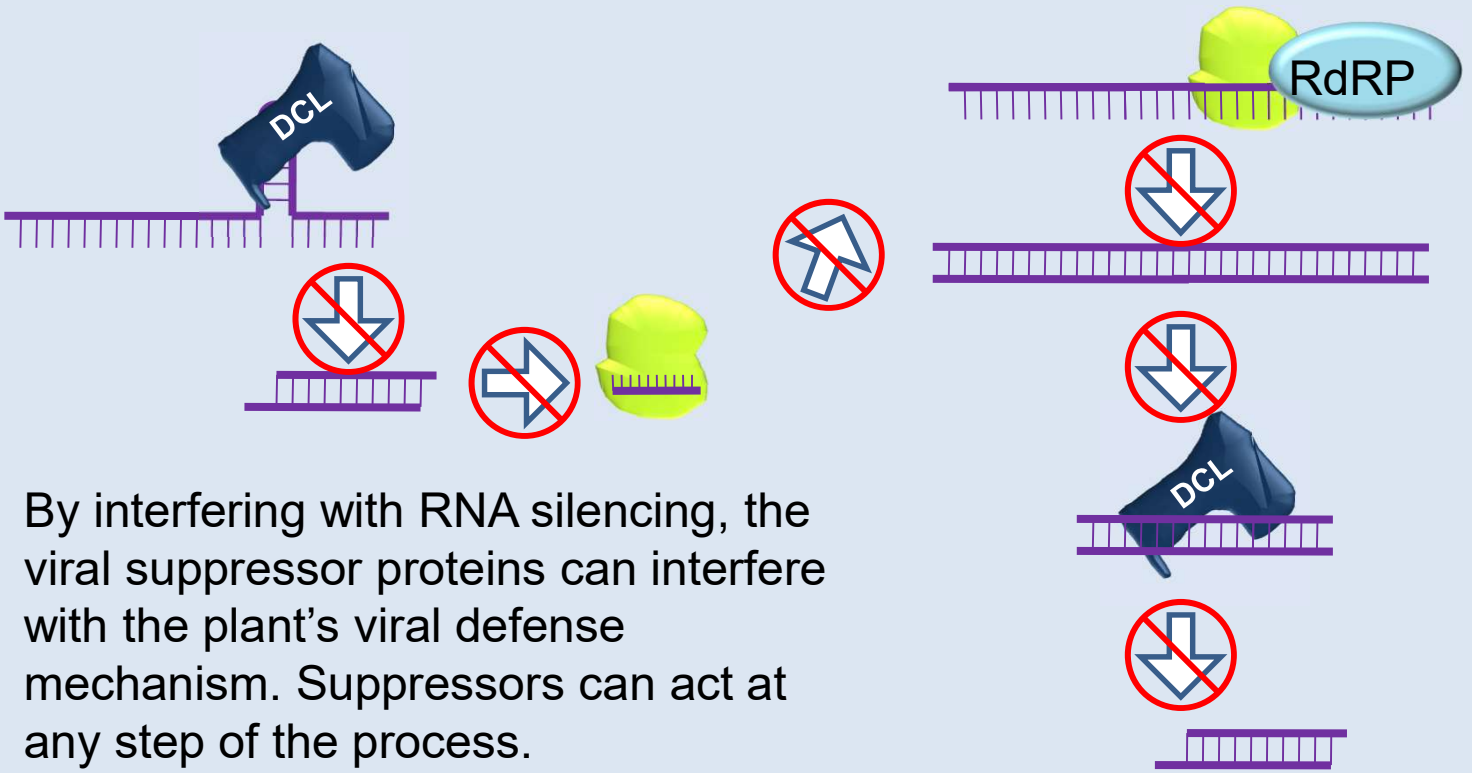
Tobacco Rattle Virus (TRV) silencing in wild-type Arabidopsis plants prevents disease symptoms. Mutants deficient in Dicer activity are unable to suppress viral infection.

WT Arabidopsis inoculated with TRV

Double mutant of *dcl2-dcl4* inoculated with TRV

From Deleris, A., Gallego-Bartolome, J., Bao, J., Kasschau, K., Carrington, J.C., and Voinnet, O. (2006) Hierarchical action and inhibition of plant dicer-like proteins in antiviral defense. Science 313: 68–71. Reprinted with permission from AAAS.

Viruses have suppressor proteins that interfere with RNA silencing



By interfering with RNA silencing, the viral suppressor proteins can interfere with the plant's viral defense mechanism. Suppressors can act at any step of the process.

A viral suppressor protein in action

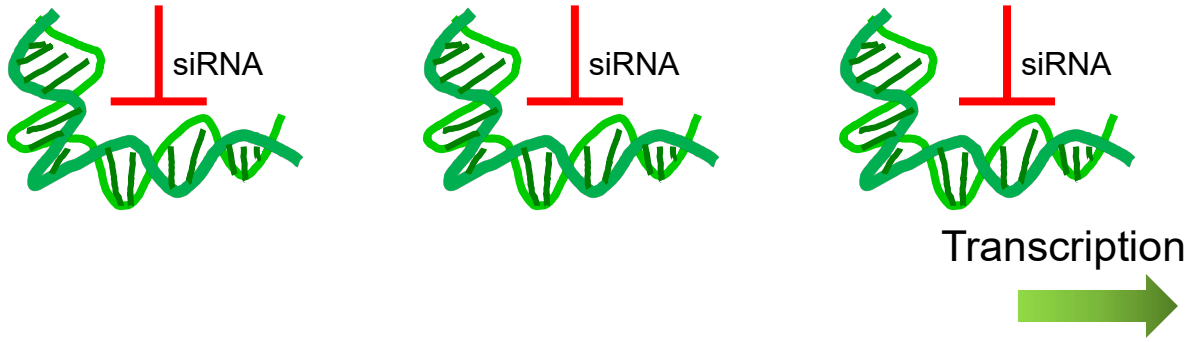
Genes encoding functional, mutant, or no viral suppressor proteins were introduced into plants carrying a silenced GUS gene. The plants were inoculated with a virus expressing GUS. Blue spots indicate GUS expression.

No viral Suppressor: GUS gene silent

Mutant viral suppressor: GUS gene silent

Functional viral suppressor: GUS gene expressed

The plant's RNA silencing efforts are suppressed by the viral protein.



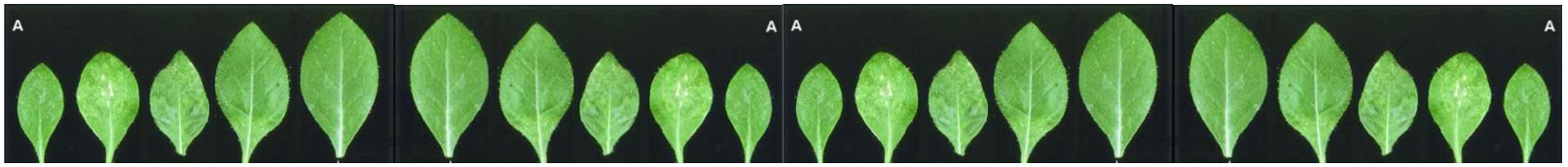
Anandalakshmi, R., Pruss, G.J., Ge, X., Marathe, R., Mallory, A.C., Smith, T.H., and Vance, V.B. (1998). A viral suppressor of gene silencing in plants. Proc. Natl. Acad. Sci. USA 95: 13079–13084.

Small RNAs also protect plants against bacterial pathogens

Wild-type (La-*er*) and small RNA processing mutants (*dcl1-9* and *hen1-1*) inoculated with *Pseudomonas* bacteria. The mutants show more visible disease symptoms and permit more bacterial replication.

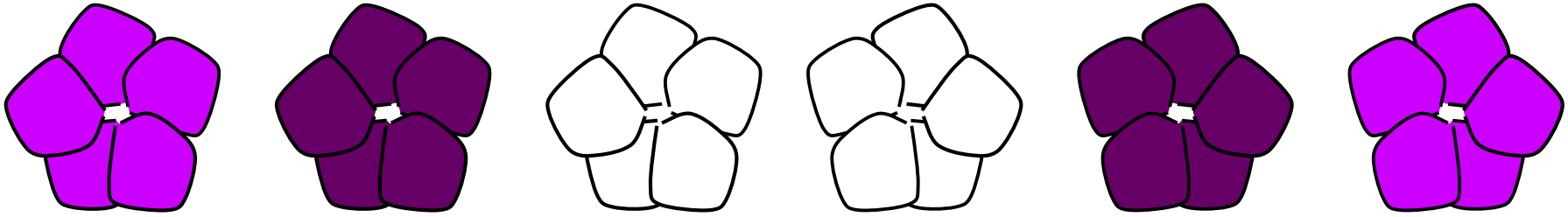
Viral-induced gene silencing summary

- RNA-mediated gene silencing is an important tool in plant defense against pathogens
- siRNAs interfere with viral replication
- siRNAs act systemically to aid in host plant recovery and resistance
- Most viruses produce suppressor proteins that target components of the plant's siRNA defense pathway; these proteins are important tools for dissecting RNA silencing pathways



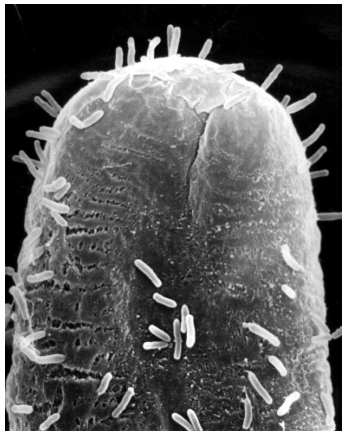
Silencing of transgenes

- Transgenes introduced into plants are frequently silenced by the siRNA pathway
- Silencing can be triggered by:
  - Very high expression levels
  - dsRNA derived from transgene
  - Aberrant RNAs encoded by transgenes
- Transgenes are silenced **post-transcriptionally** and **transcriptionally**



Transgene-induced gene silencing

In the 1980s, scientists developed methods for introducing genes into plant genomes, using the bacterium *Agrobacterium tumefaciens*. The introduced genes are called transgenes.



Agrobacterium tumefaciens on the surface of a plant cell.

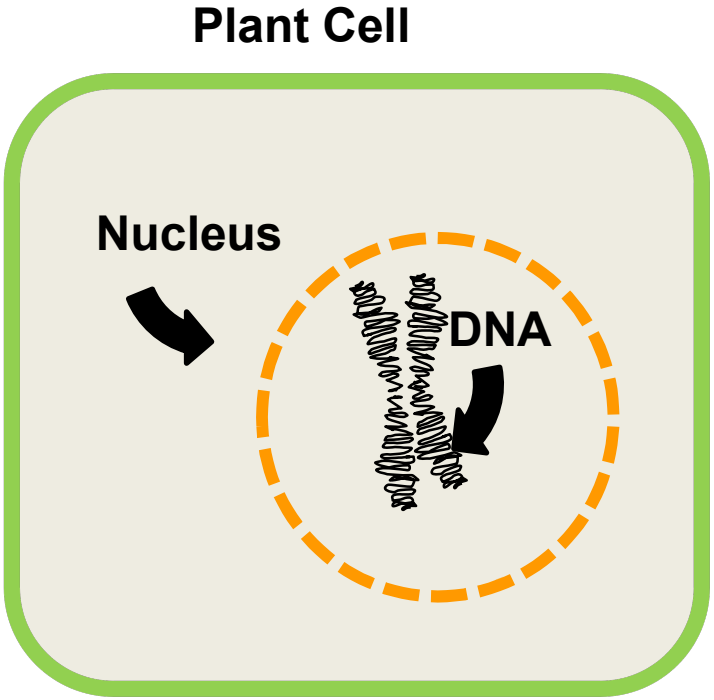
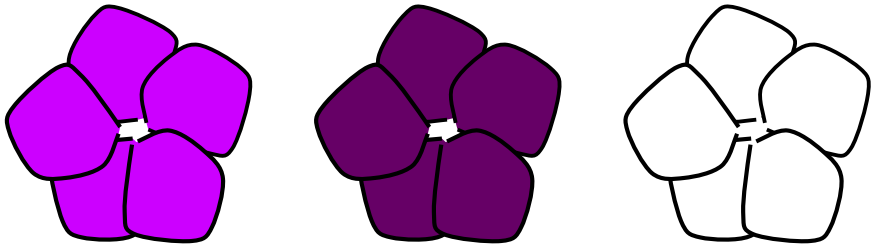


Photo credits: Martha Hawes, University of Arizona

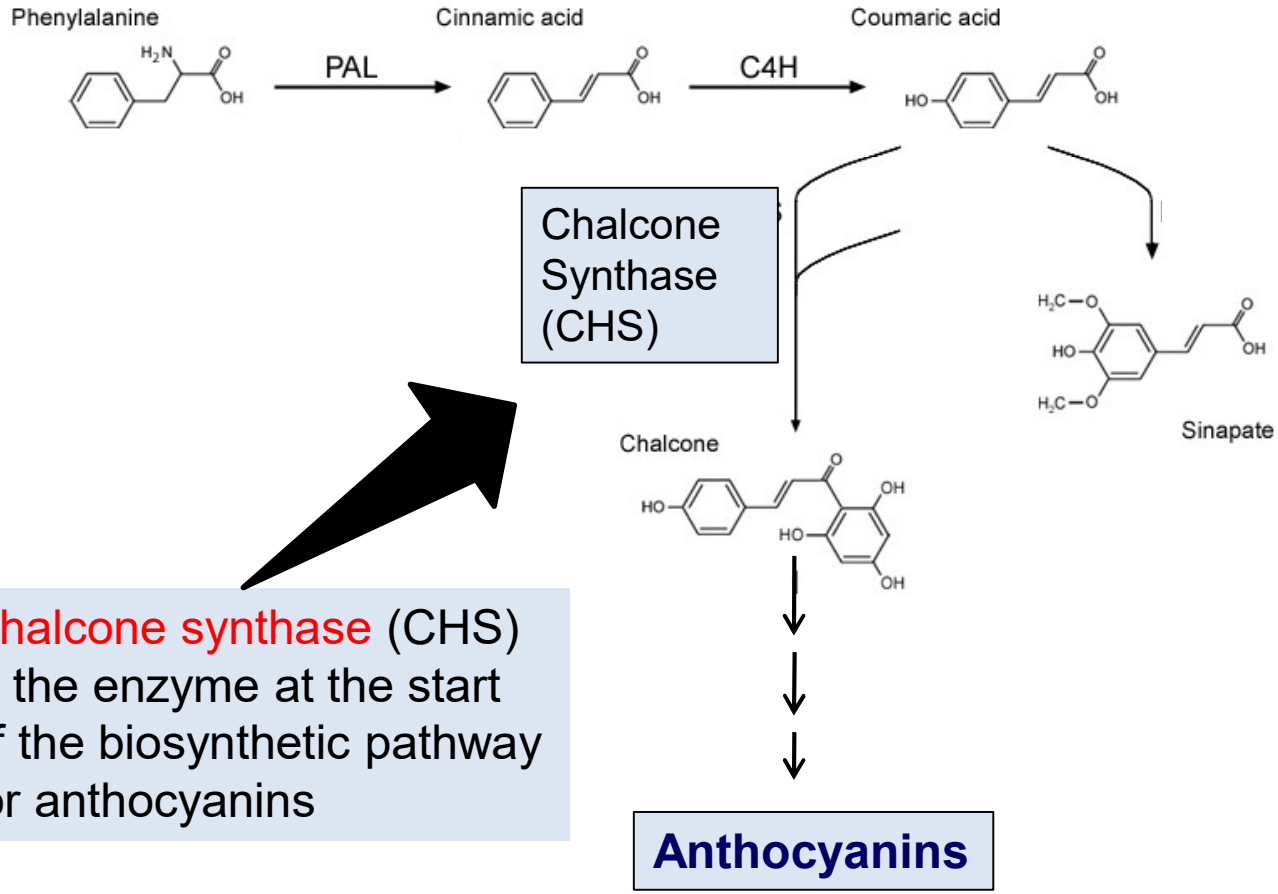
Transgene-induced post-transcriptional silencing



Experiments to modify flower color in petunia gave early evidence of RNA silencing.

Manipulation of chalcone synthase expression to modify pigmentation

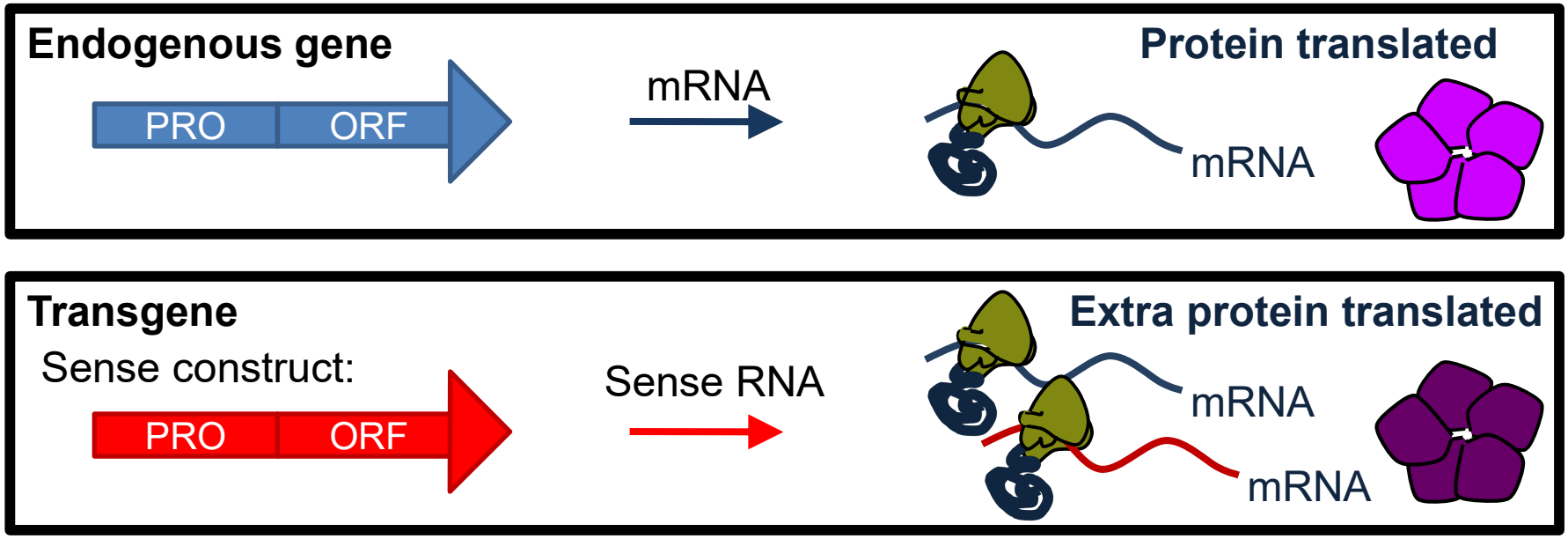
Wild-type petunia producing purple anthocyanin pigments



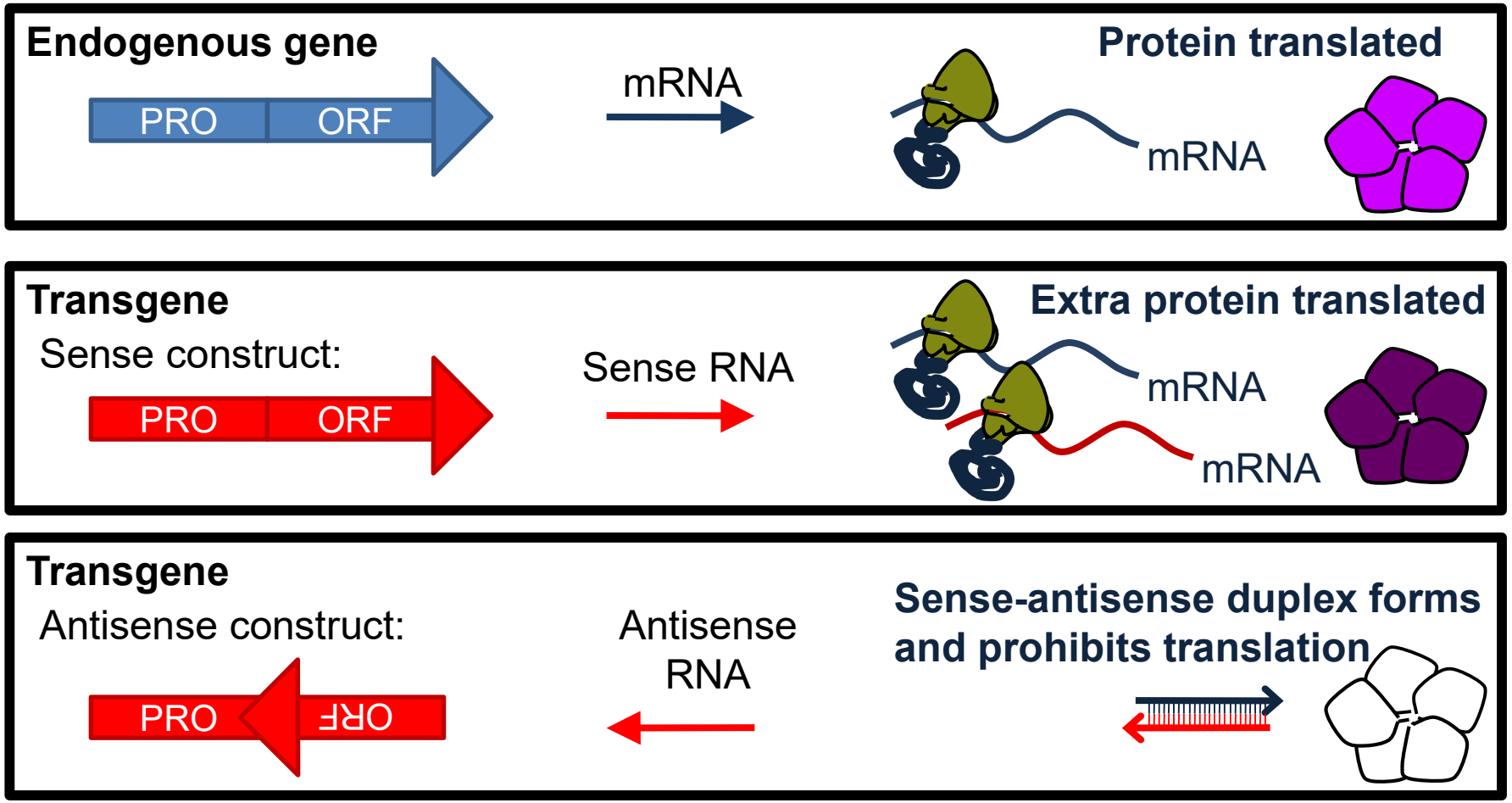
Chalcone synthase (CHS) is the enzyme at the start of the biosynthetic pathway for anthocyanins

Photo credit Richard Jorgensen; Aksamit-Stachurska *et al.* (2008) BMC Biotechnology 8: 25.

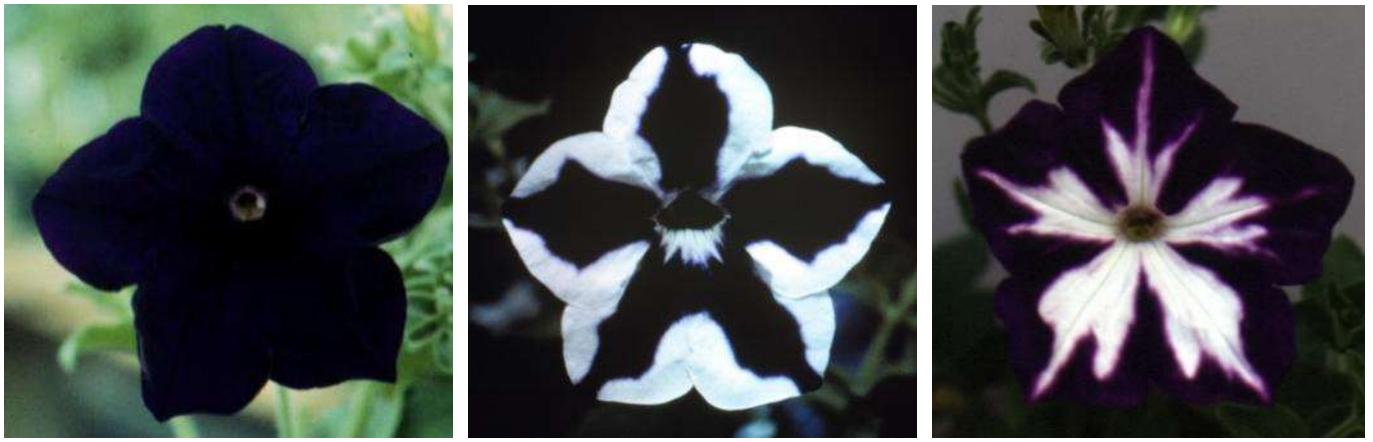
Expectation – sense RNA production would enhance pigmentation...



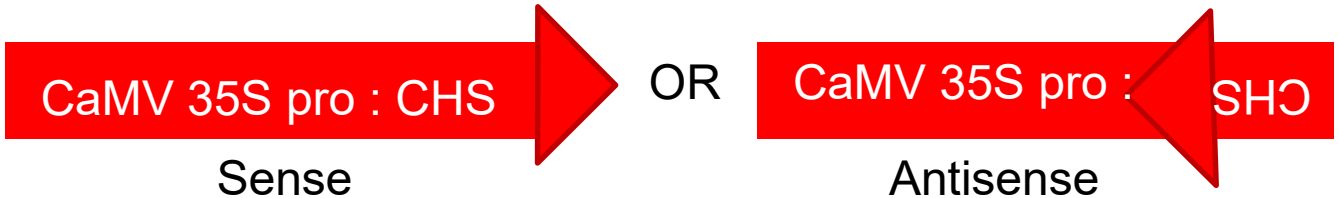
..and antisense RNA production would block pigmentation



Surprisingly, *both* antisense and sense gene constructs can inhibit pigment production



Plants carrying CHS transgene



Sense

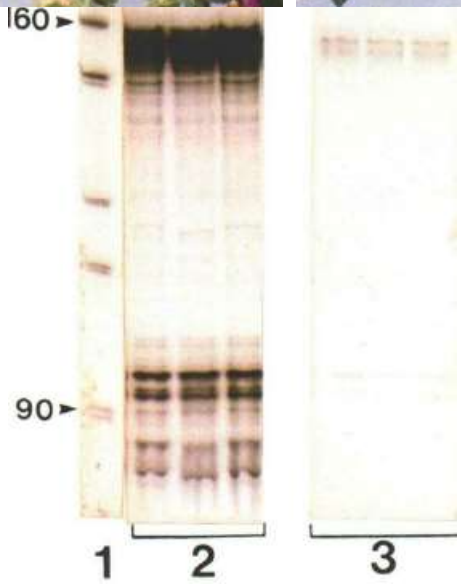
Antisense

Photo credit Richard Jorgense

Silenced tissues do not express endogenous or introduced CHS

Purple flowers

White flowers



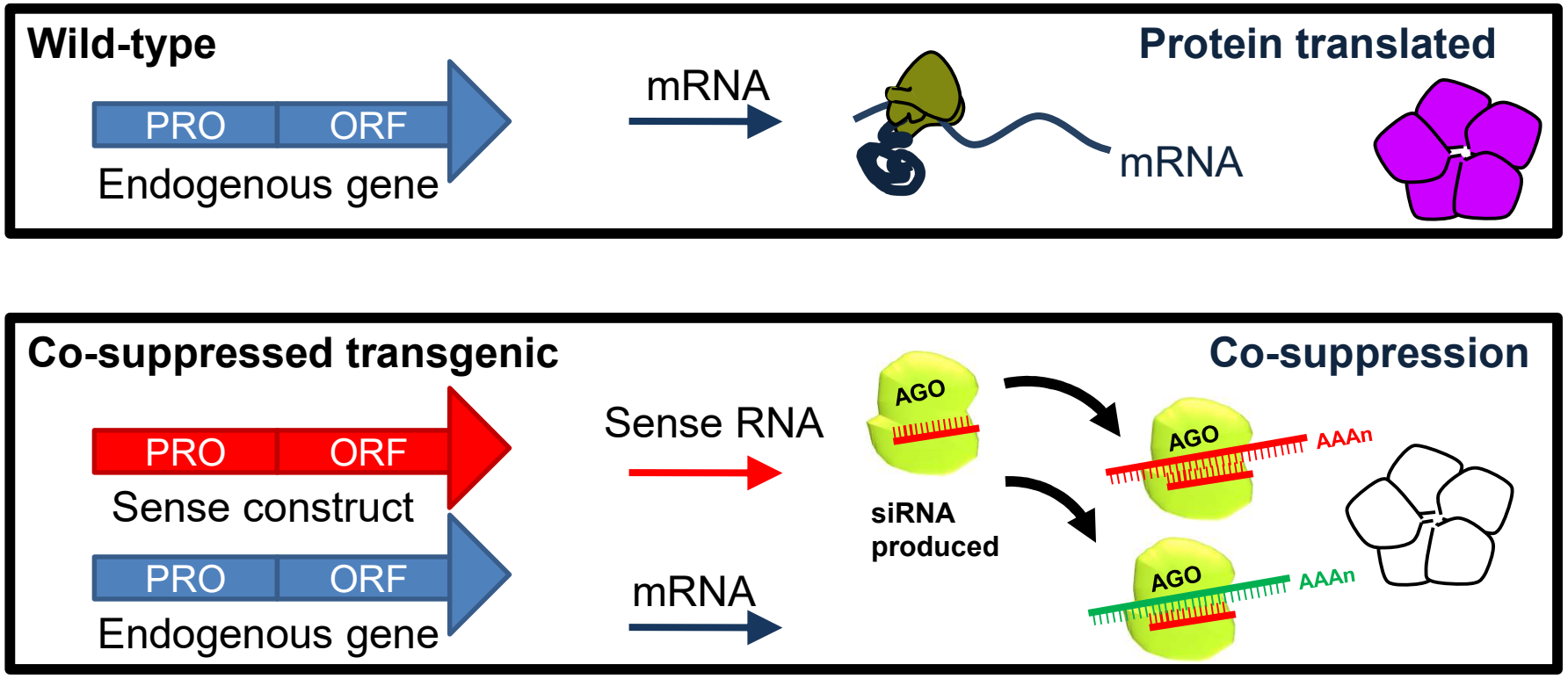
This phenomenon, in which both the introduced gene and the endogenous gene are silenced, has been called “co-suppression”.

Transgene RNA

Endogenous gene RNA

Napoli, C., Lemieux, C., and Jorgensen, R. (1990) Introduction of a chimeric chalcone synthase gene into petunia results in reversible co-suppression of homologous genes *in trans*. Plant Cell **2**: 279–289.

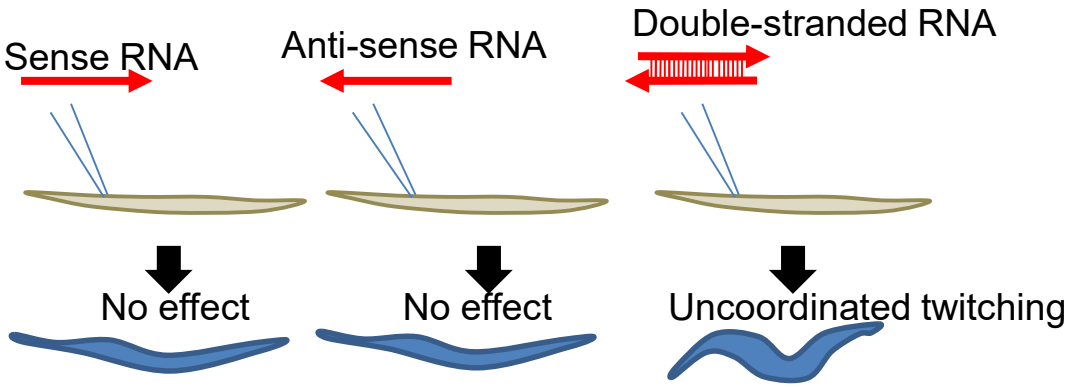
Co-suppression is a consequence of siRNA production



De Paoli, E., Dorantes-Acosta, A., Zhai, J., Accerbi, M., Jeong, D.-H., Park, S., Meyers, B.C., Jorgensen, R.A., and Green, P.J. (2009). Distinct extremely abundant siRNAs associated with cosuppression in petunia. RNA 15: 1965–1970.

Studies of *C. elegans* showed double-stranded RNA is the strongest trigger for gene silencing

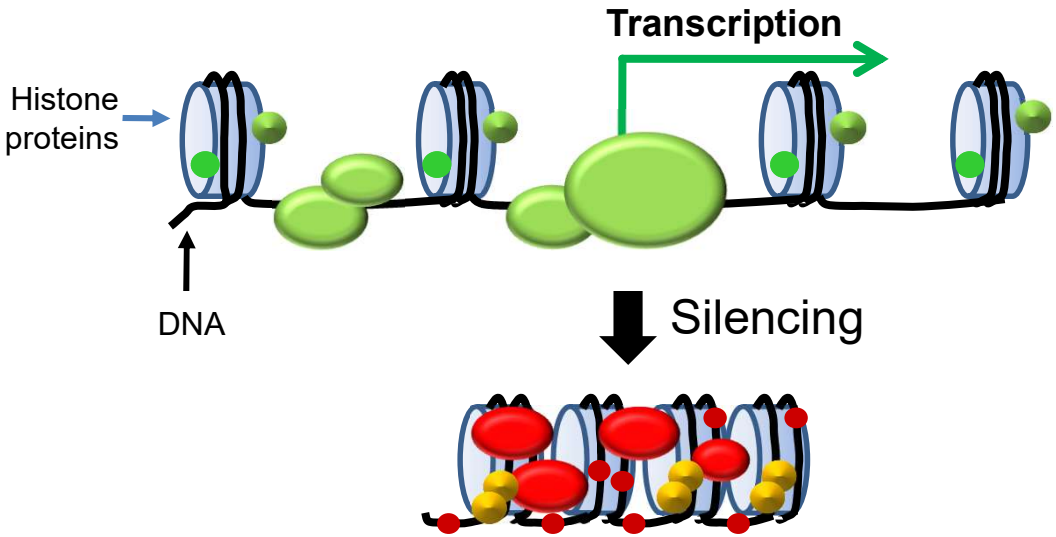
Sense, antisense or double-stranded RNAs homologous to the *unc-22* gene were introduced into worms. Silencing of *unc-22* causes loss of muscle control – hence its name, "*uncoordinated*".



Derived The Nobel Committee based on Fire, A. et al., (1998) Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans.* Nature 391: 806-811.

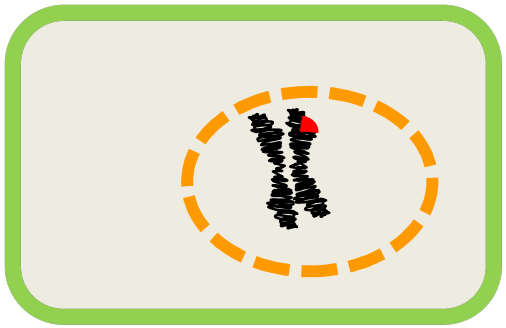
Transcriptional gene silencing

Small RNAs can initiate gene silencing through covalent modifications of the DNA or its associated histone proteins, interfering with transcription.



This form of silencing is frequently associated with stably silenced DNA including centromeres and transposons, but also occurs at genes.

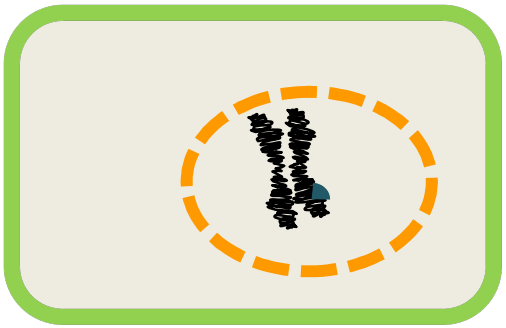
Transcriptional gene silencing



CaMV 35S pro : KAN

Expression of a gene that confers resistance to the antibiotic kanamycin

Transcriptional gene silencing was revealed through experiments to introduce more than one transgene into a plant by genetic crosses.

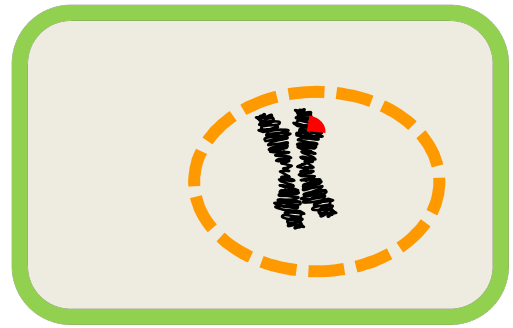


CaMV 35S pro : HYG

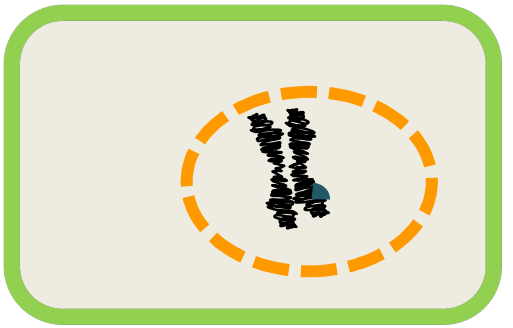
Expression of a gene that confers resistance to the antibiotic hygromycin

Based on Matzke, M., Primig, M., Trnovsky, J., Matzke, A. (1989) Reversible methylation and inactivation of marker genes in sequentially transformed plants. EMBO J. 8: 643-649.

Transcriptional gene silencing



X

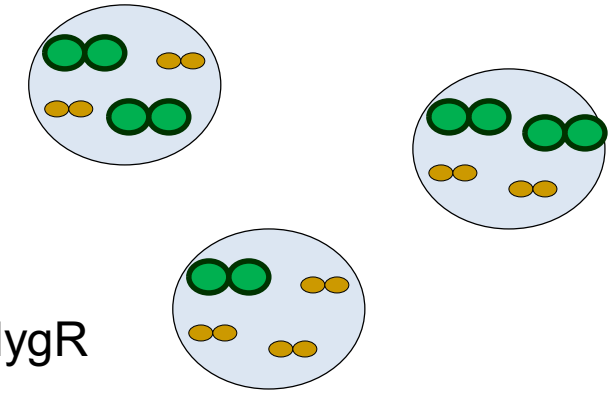


Expected Results

Selection on kanamycin only: 50% KanR

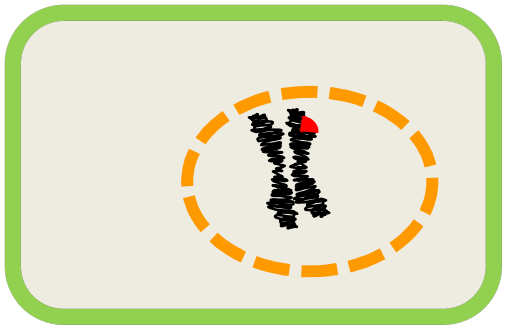
Selection on hygromycin only: 50% HygR

Selection on Kan + Hyg: 25% KanR and HygR

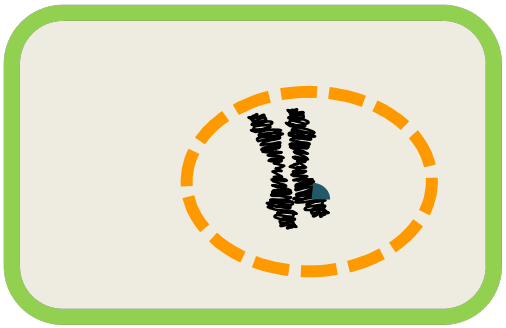


Based on Matzke, M., Primig, M., Trnovsky, J., Matzke, A. (1989) Reversible methylation and inactivation of marker genes in sequentially transformed plants. EMBO J. 8: 643-649.

Transcriptional gene silencing



Sometimes one of the transgenes was silenced in the progeny carrying both genes.

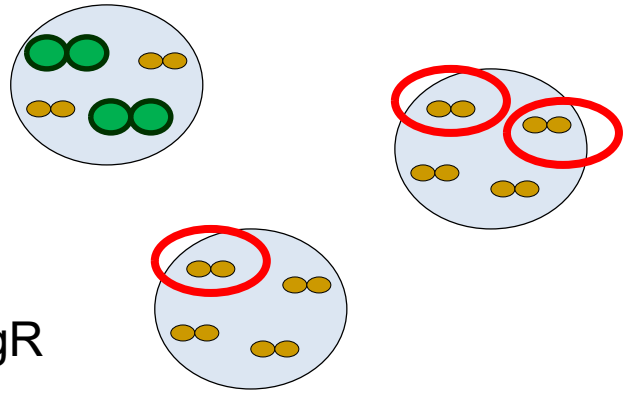


<u>Observed</u> Results

Selection on kanamycin only: 50% KanR

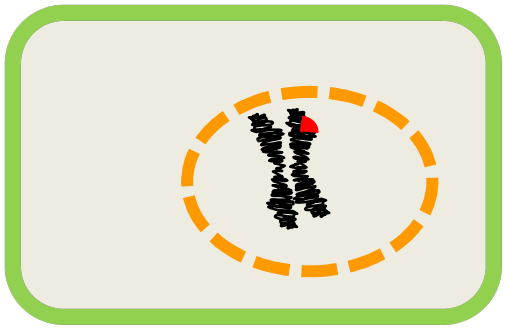
Selection on hygromycin only: 0% HygR

Selection on Kan + Hyg: 0% KanR and HygR



Based on Matzke, M., Primig, M., Trnovsky, J., Matzke, A. (1989) Reversible methylation and inactivation of marker genes in sequentially transformed plants. EMBO J. 8: 643-649.

Transcriptional gene silencing



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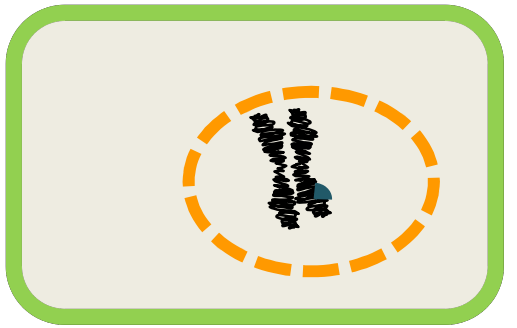
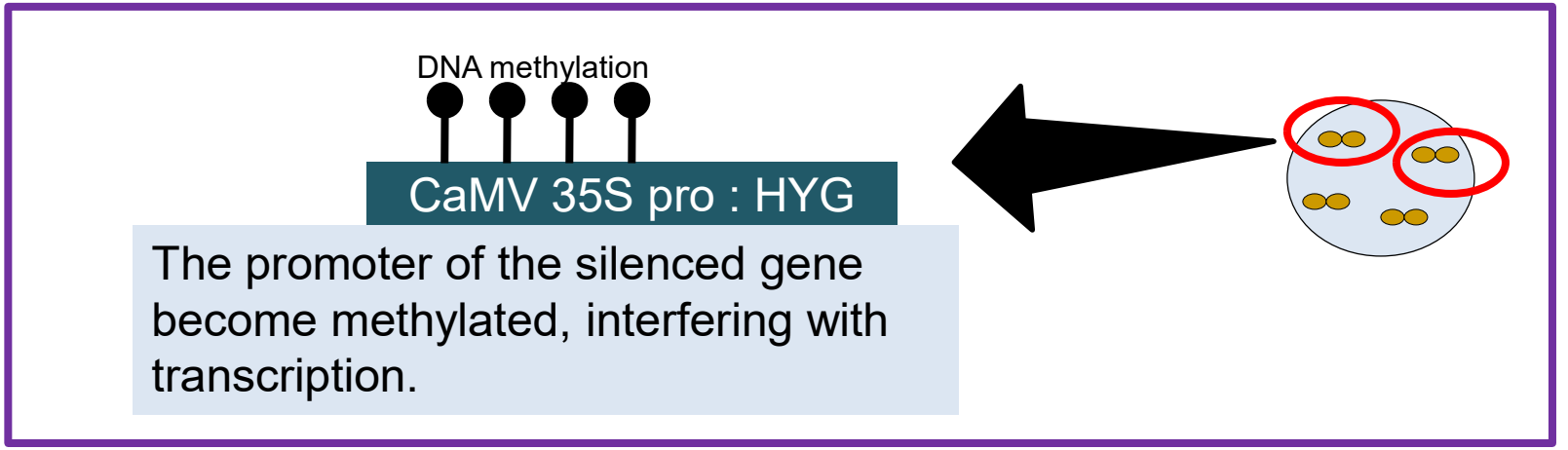
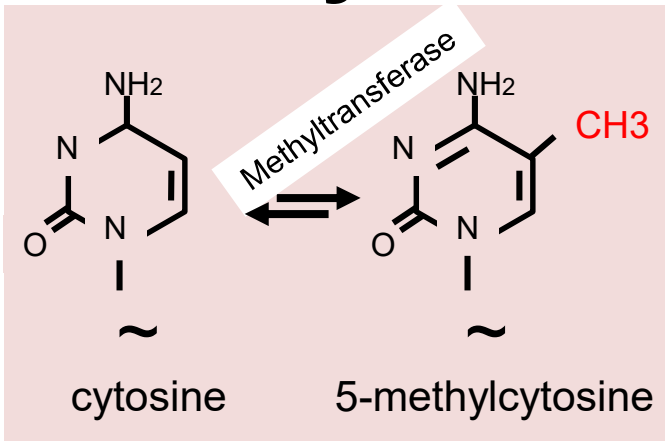




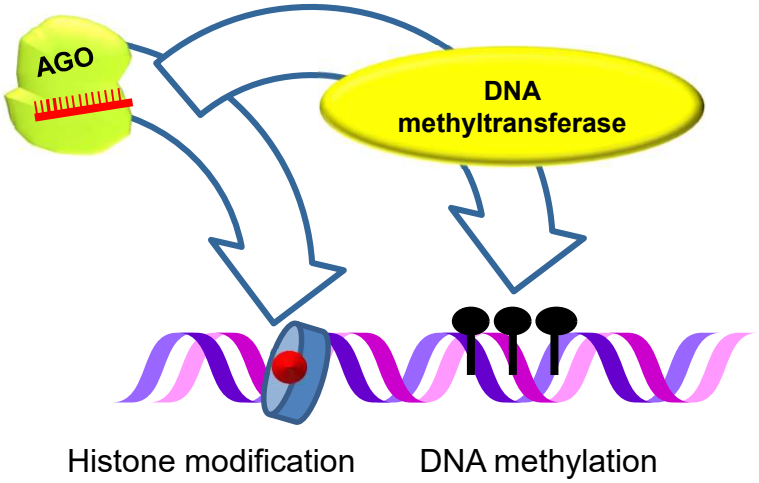
Based on Matzke, M., Primig, M., Trnovsky, J., Matzke, A. (1989) Reversible methylation and inactivation of marker genes in sequentially transformed plants. EMBO J. 8: 643-649.

siRNAs can target DNA for silencing by cytosine methylation or by histone modification



DNA can be covalently modified by cytosine methylation, carried out by DNA methyltransferases.

The precise mechanisms by which siRNAs target DNA for silencing are not known, but involve the action of two plant-specific RNA-polymerase complexes, RNA Polymerase IV (Pol IV) and RNA Polymerase V (Pol V).

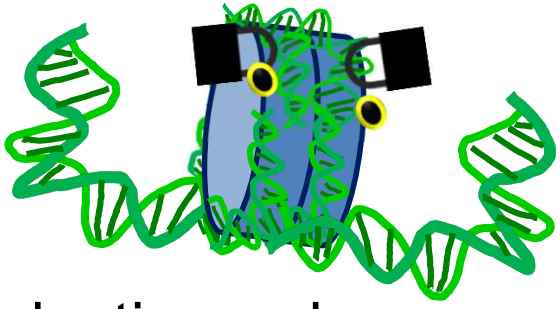


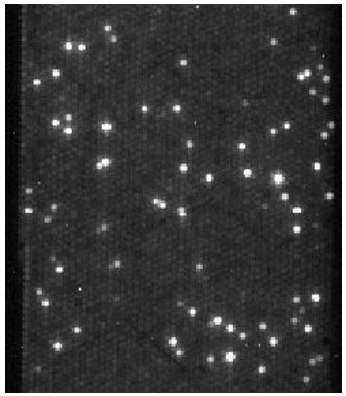
Methods for studying epigenetic modifications

DNA methylation– bisulfite sequencing

Histone modification

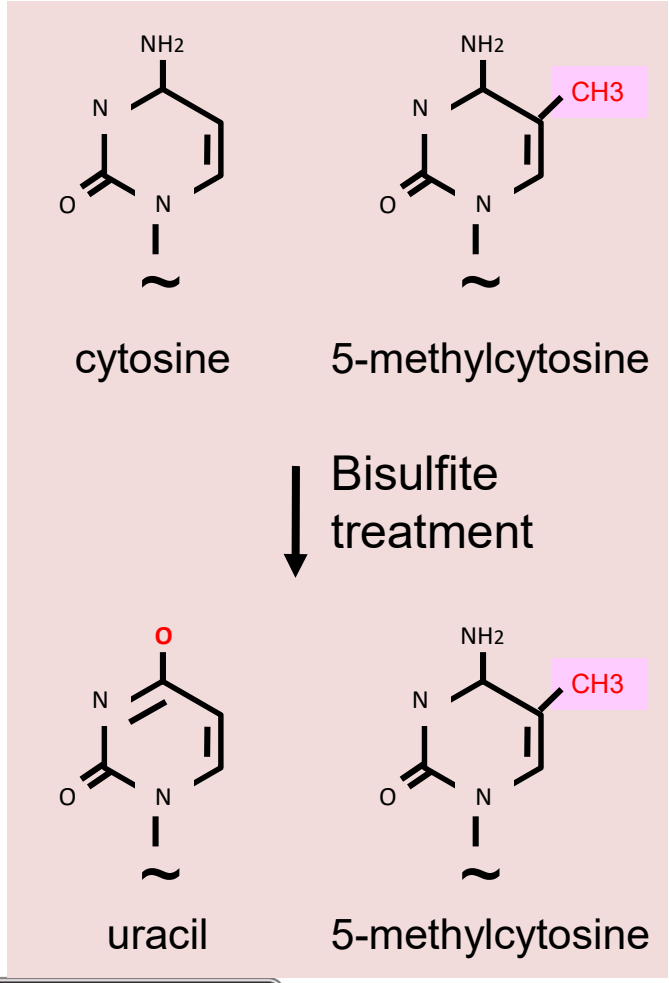
- chromatin immunoprecipitation (ChIP)
- DNA adenosine methylation identification (DamID)

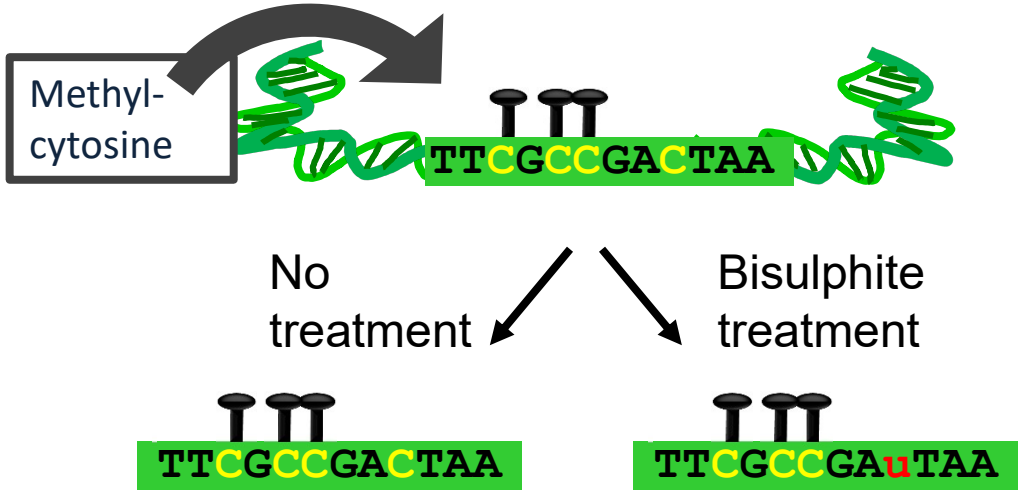




siRNA production – deep sequencing

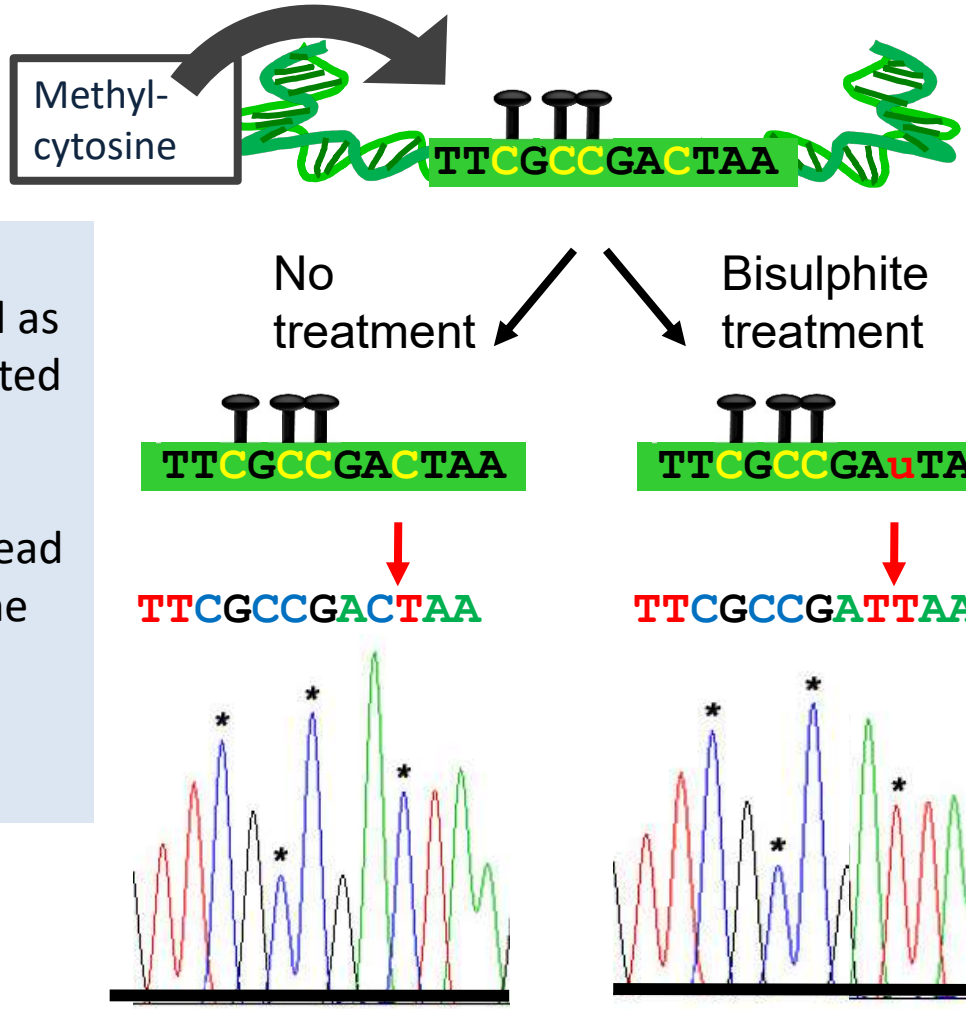
Bisulfite treatment differentiates cytosine and methylcytosine





When DNA is bisulfite treated, unmethylated cytosine is converted to uracil. Methylcytosine is not affected.

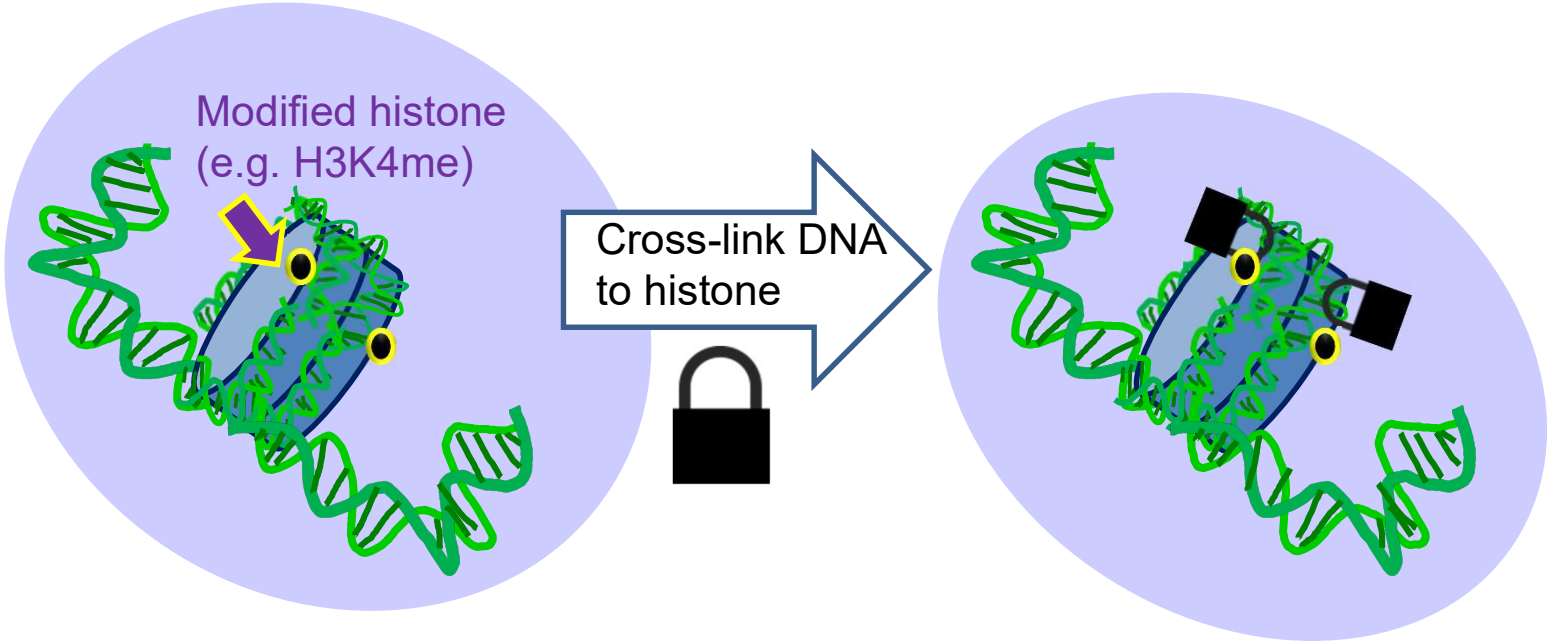
Bisulfite treatment differentiates cytosine and methylcytosine



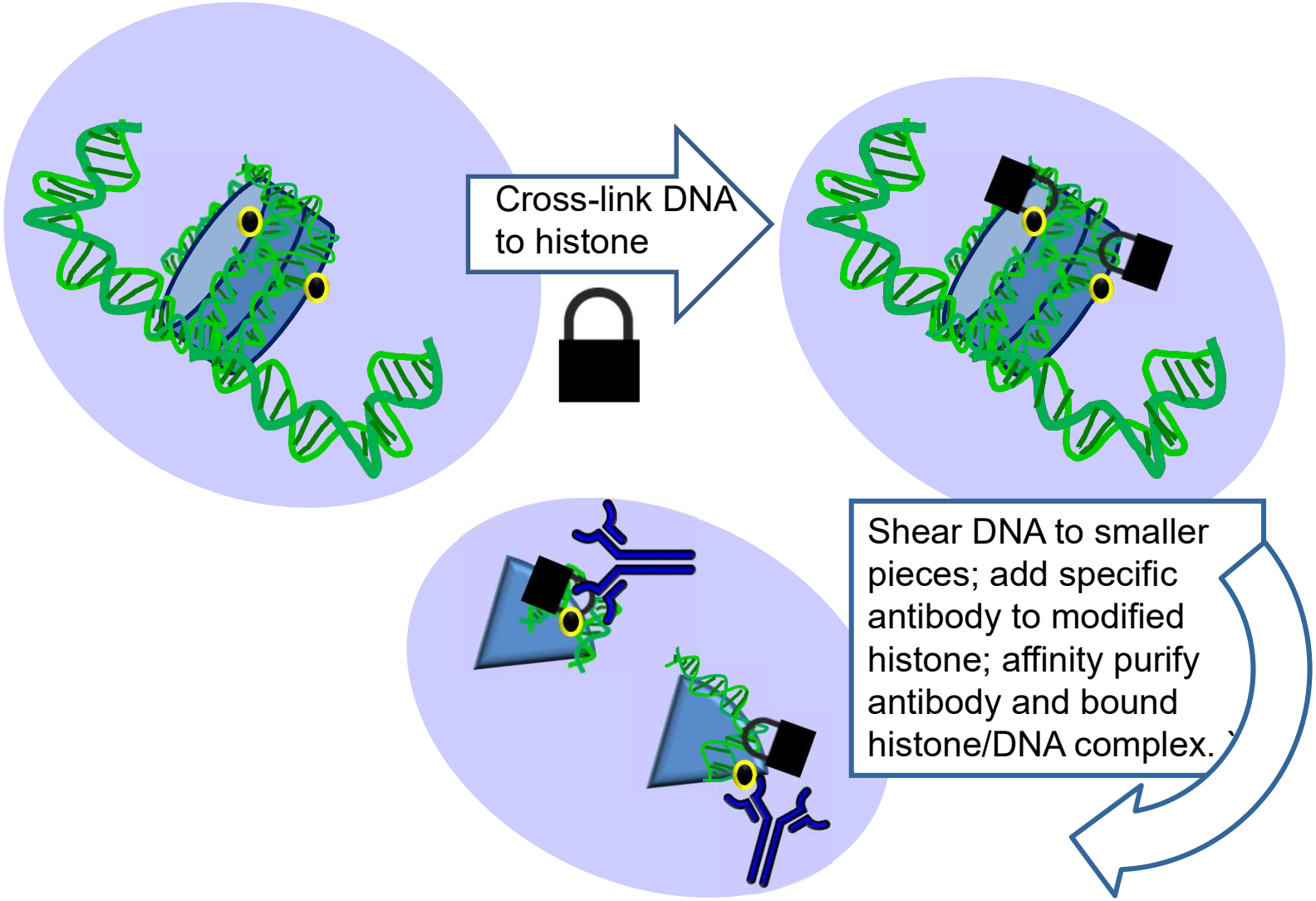
After bisulfite treatment, unmethylated **C**s are read as **T** and so differ in the treated and untreated samples.

By contrast, **methyl-C** is read as **C** and is the same as the reference sequence.

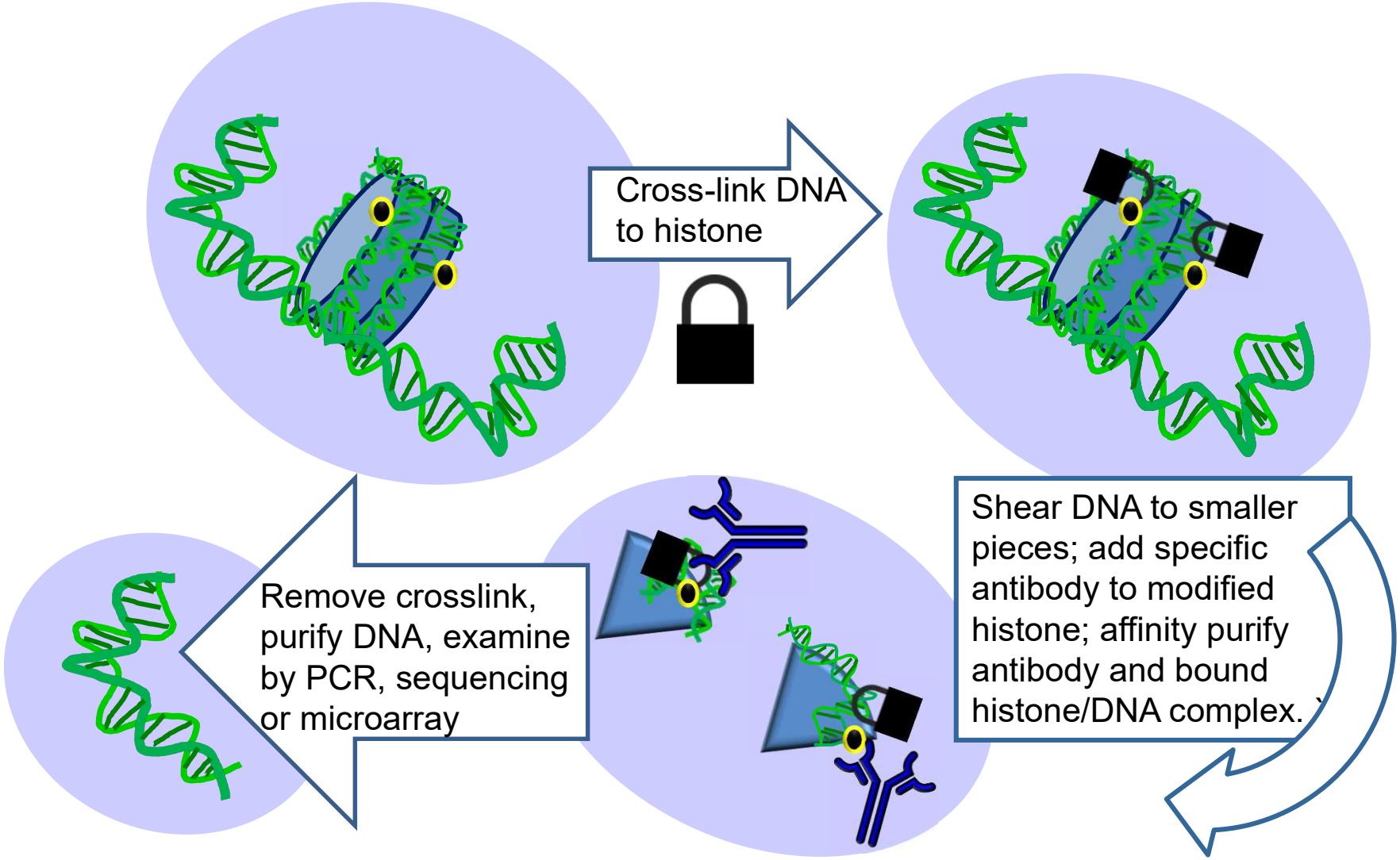
Chromatin Immunoprecipitation (ChiP)



Chromatin Immunoprecipitation (ChiP)

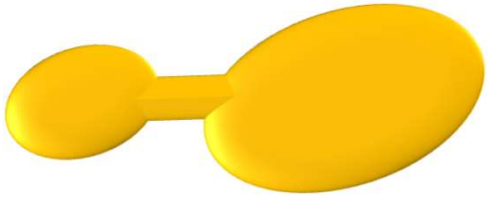


Chromatin Immunoprecipitation (ChiP)



DNA adenine methylation ID (DamID)

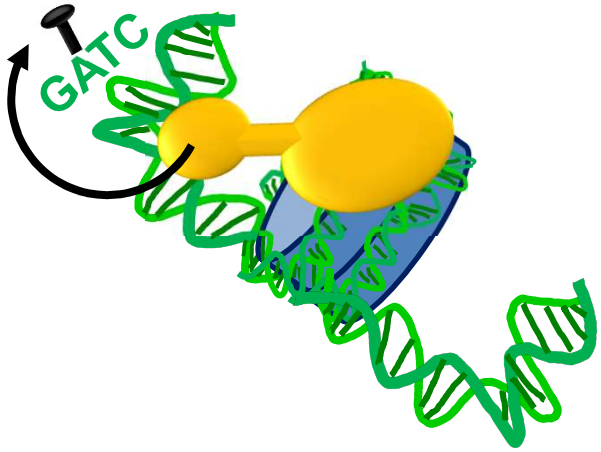
A fusion protein is made of Dam and the protein of interest



Dam methylase from *E. coli*

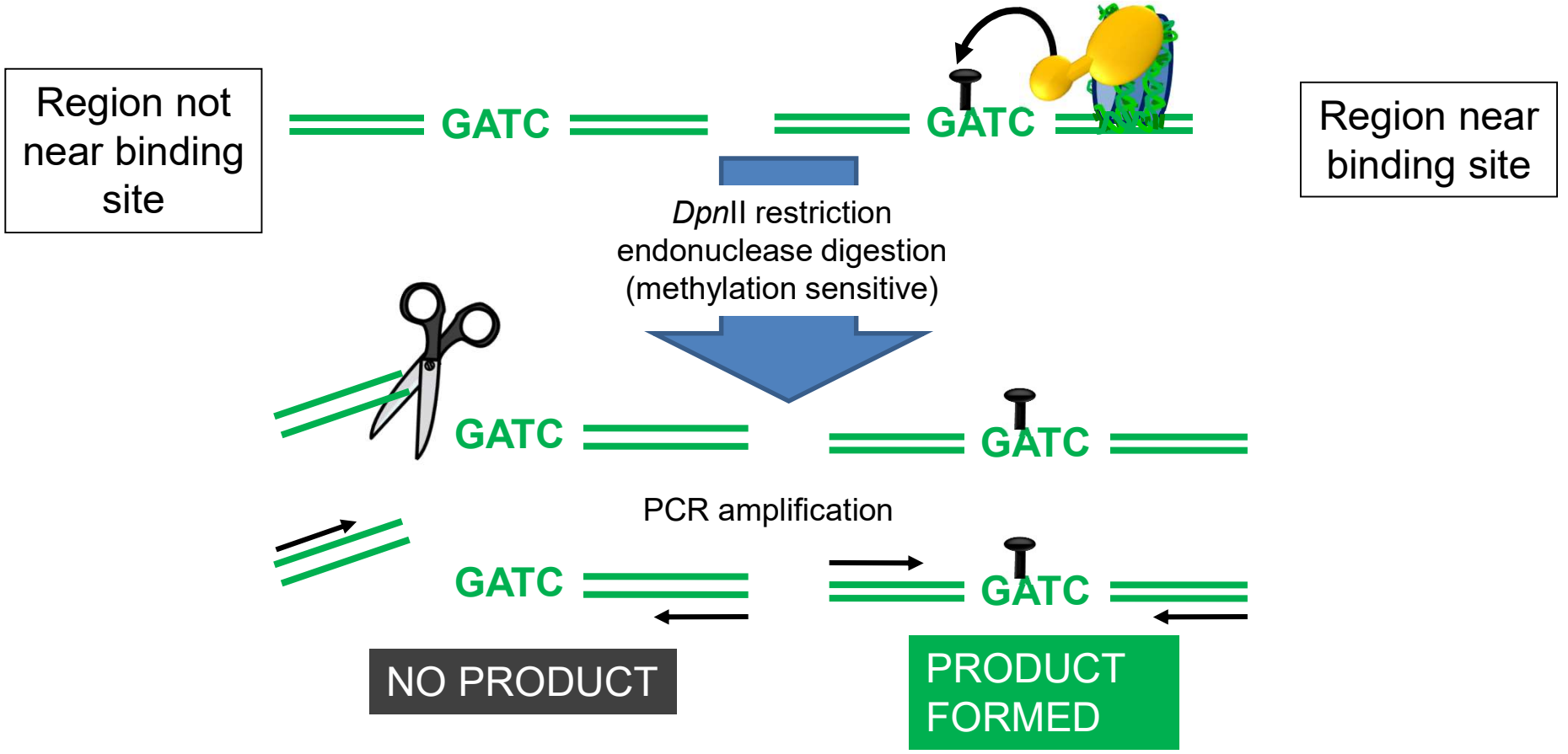
Protein of interest (e.g. LHP1)

Dam is an adenine methyltransferase with specificity for the sequence **GATC**

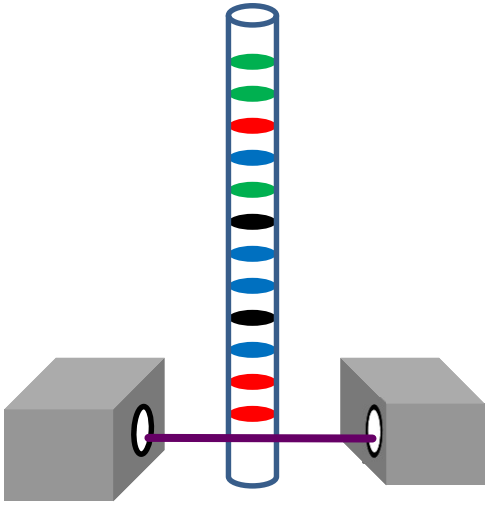


The fusion protein binds to selected regions of chromatin (e.g. H3K27me3) and methylates adenines at nearby GATC sites

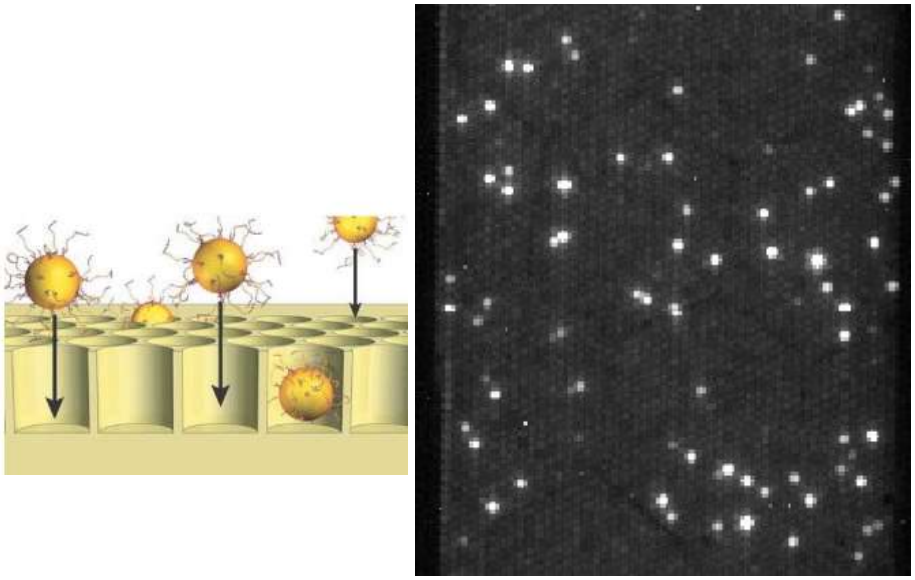
Methylation can be detected by methylation sensitive enzymes



Deep sequencing by "next generation" DNA sequencing methods

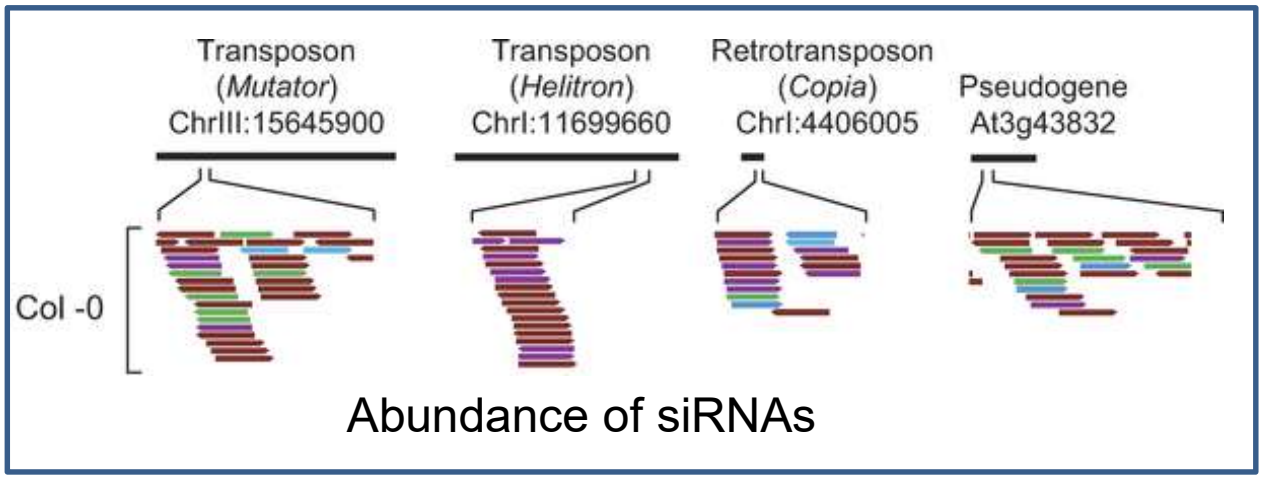


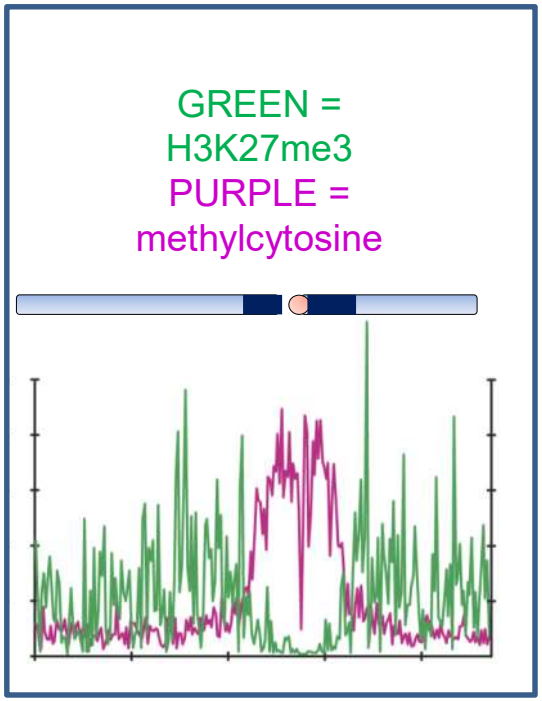
"Classical" DNA sequencing – one molecule examined at a time



"Next generation" DNA sequencing – one million molecules examined at a time

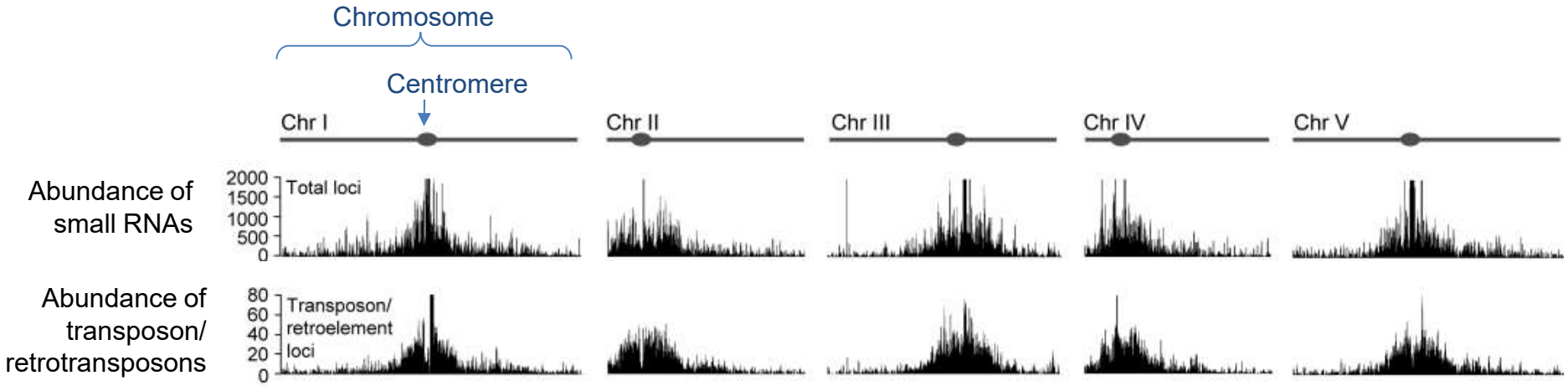
Using next-generation sequencing, epigenetic modifications can be identified genome-wide: EPIGENOMICS





Kasschau KD, Fahlgren N, Chapman EJ, Sullivan CM, Cumbie JS, et al. 2007 Genome-Wide Profiling and Analysis of *Arabidopsis* siRNAs. PLoS Biol 5(3): e57.
Zhang, X., Clarenz, O., Cokus, S., Bernatavichute, Y.V., Pellegrini, M., Goodrich, J., Jacobsen, S.E. (2007) Whole-genome analysis of histone H3 lysine 27 trimethylation in *Arabidopsis*. PLoS Biol. 5: e129.

Most siRNAs are produced from transposons and repetitive DNA



Most of the cellular siRNAs are derived from transposons and other repetitive sequences. In Arabidopsis, as shown above, there is a high density of these repeats in the pericentromeric regions of the chromosome.

Kasschau, K.D., Fahlgren, N., Chapman, E.J., Sullivan, C.M., Cumbie, J.S., Givan, S.A., and Carrington, J.C. (2007) Genome-wide profiling and analysis of *Arabidopsis* siRNAs. PLoS Biol 5(3): e57.

Plants have additional RNA Polymerase complexes that contribute to silencing

Complex	Distribution	Function
RNA Polymerase I	All eukaryotes	Production of rRNA
RNA Polymerase II	All eukaryotes	Production of mRNA, microRNA
RNA Polymerase III	All eukaryotes	Production of tRNA, 5S rRNA
RNA Polymerase IV	Land plants	Production of siRNA
RNA Polymerase V	Angiosperms	Recruitment of AGO to DNA

Loss of function of RNA Pol IV interferes with silencing

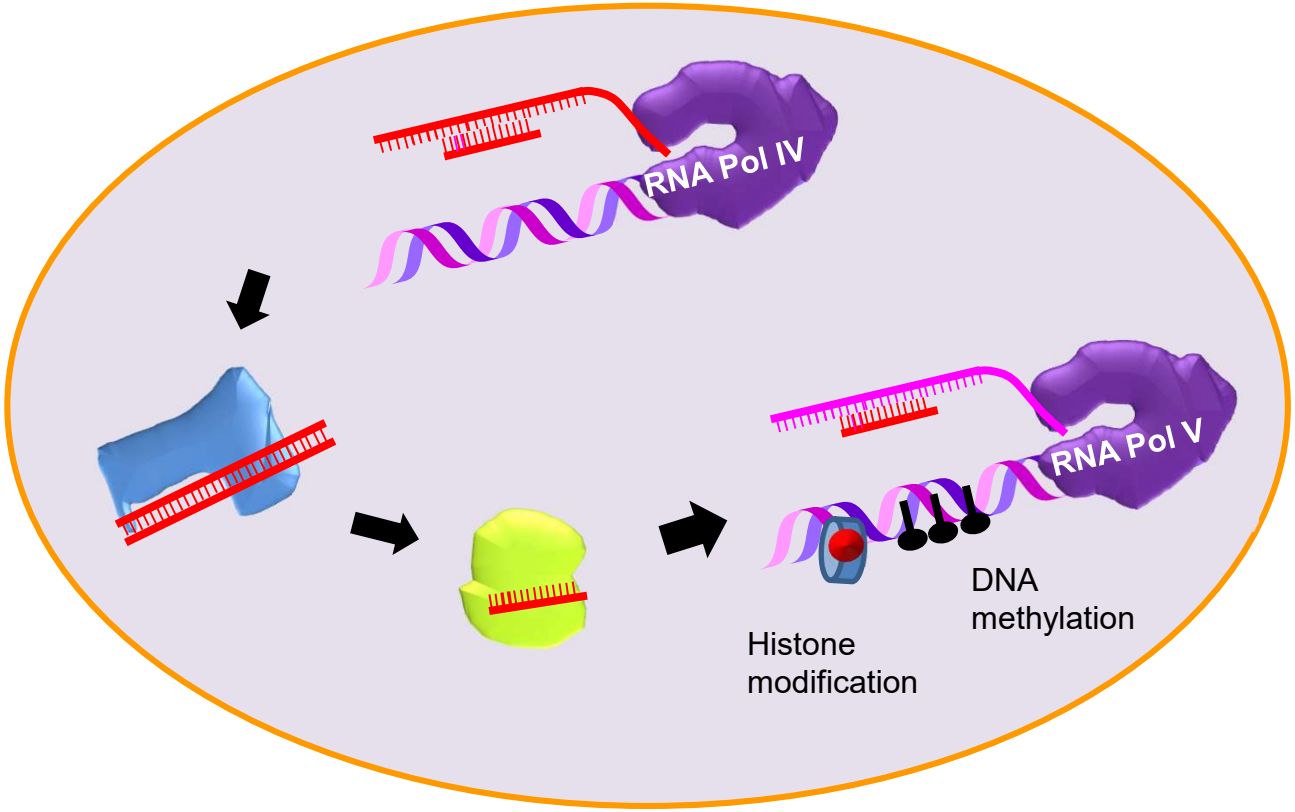
Arabidopsis plant with silenced GFP gene

Loss-of-function mutant *nrpd1a-1*. *NRPD1A* encodes a subunit of RNA Polymerase IV.

Green indicates GFP is expressed, showing that Pol IV is required for gene silencing.

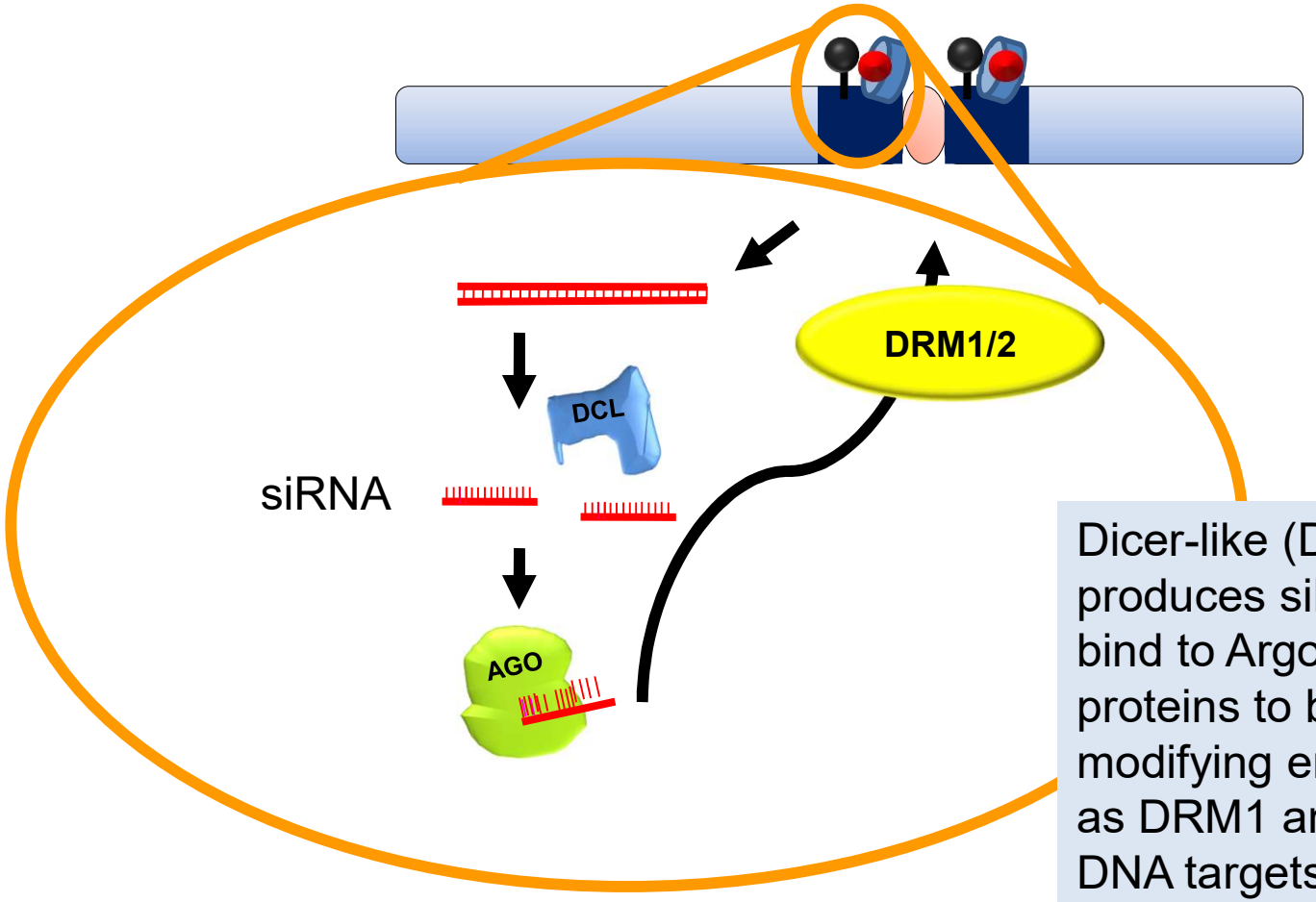
From Herr, A.J., Jensen, M.B., Dalmay, T., and Baulcombe, D.C. (2005) RNA polymerase IV directs silencing of endogenous DNA. Science 308: 118–120. Reprinted with permission from AAAS.

Transcriptional silencing requires RNA Pol IV and V



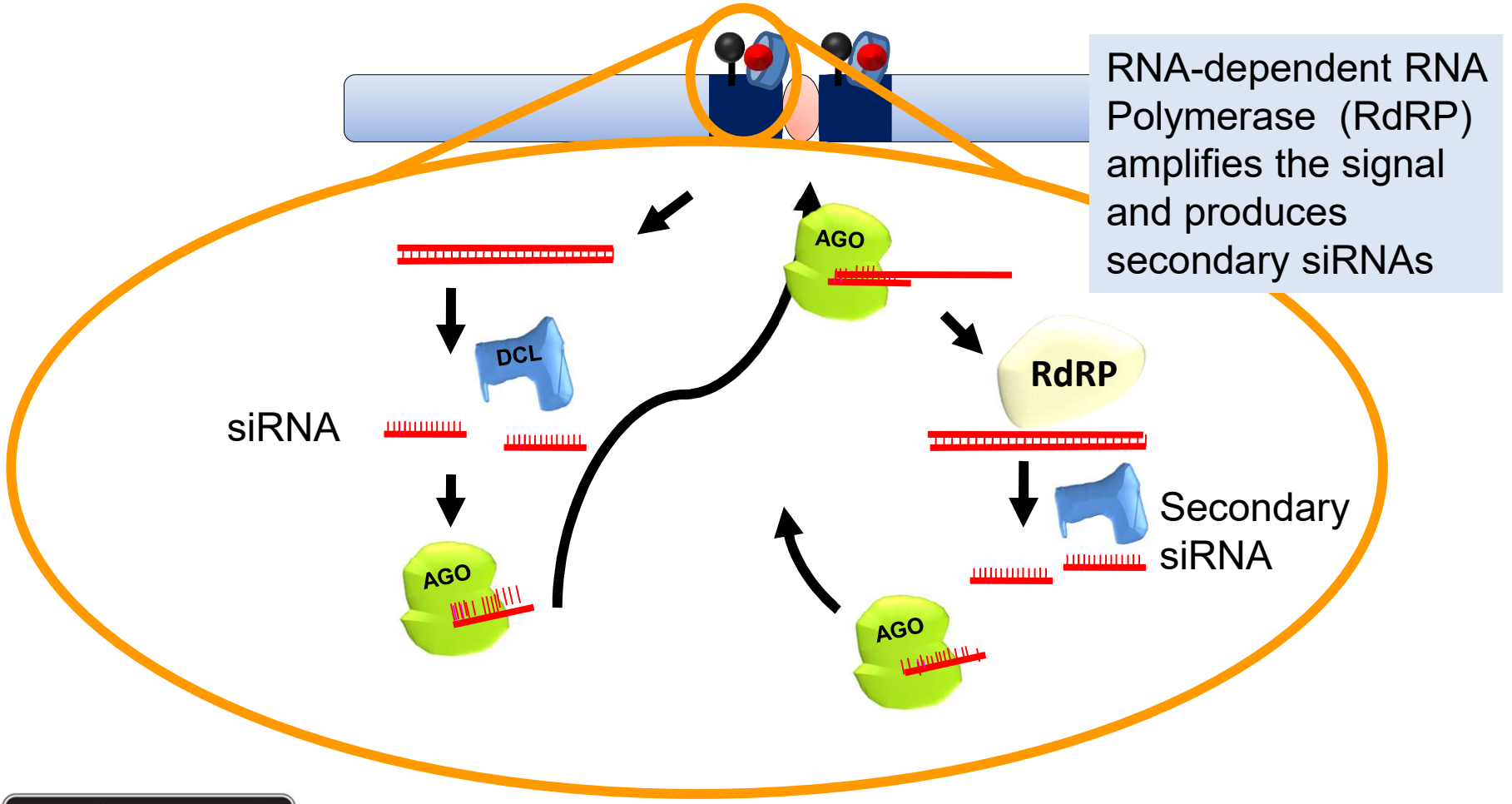
RNA Pol IV contributes to siRNA production. Non-coding RNAs produced by RNA Pol V direct silencing machinery to target sites.

siRNAs recruit DNA methylases and histone-modifying enzymes to targets

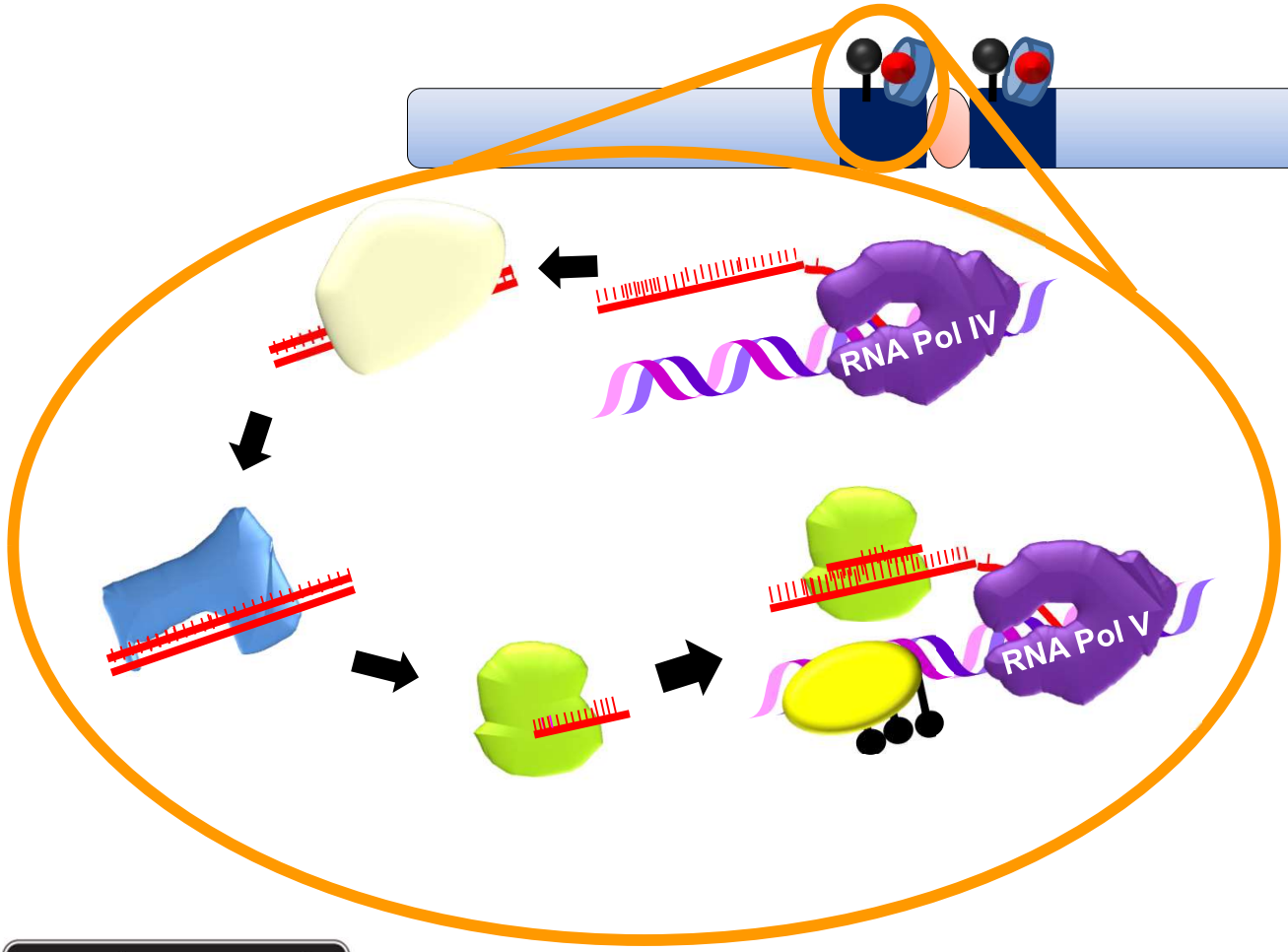


Dicer-like (DCL) nuclease produces siRNAs which bind to Argonaute (AGO) proteins to bring modifying enzymes such as DRM1 and DRM2 to DNA targets.

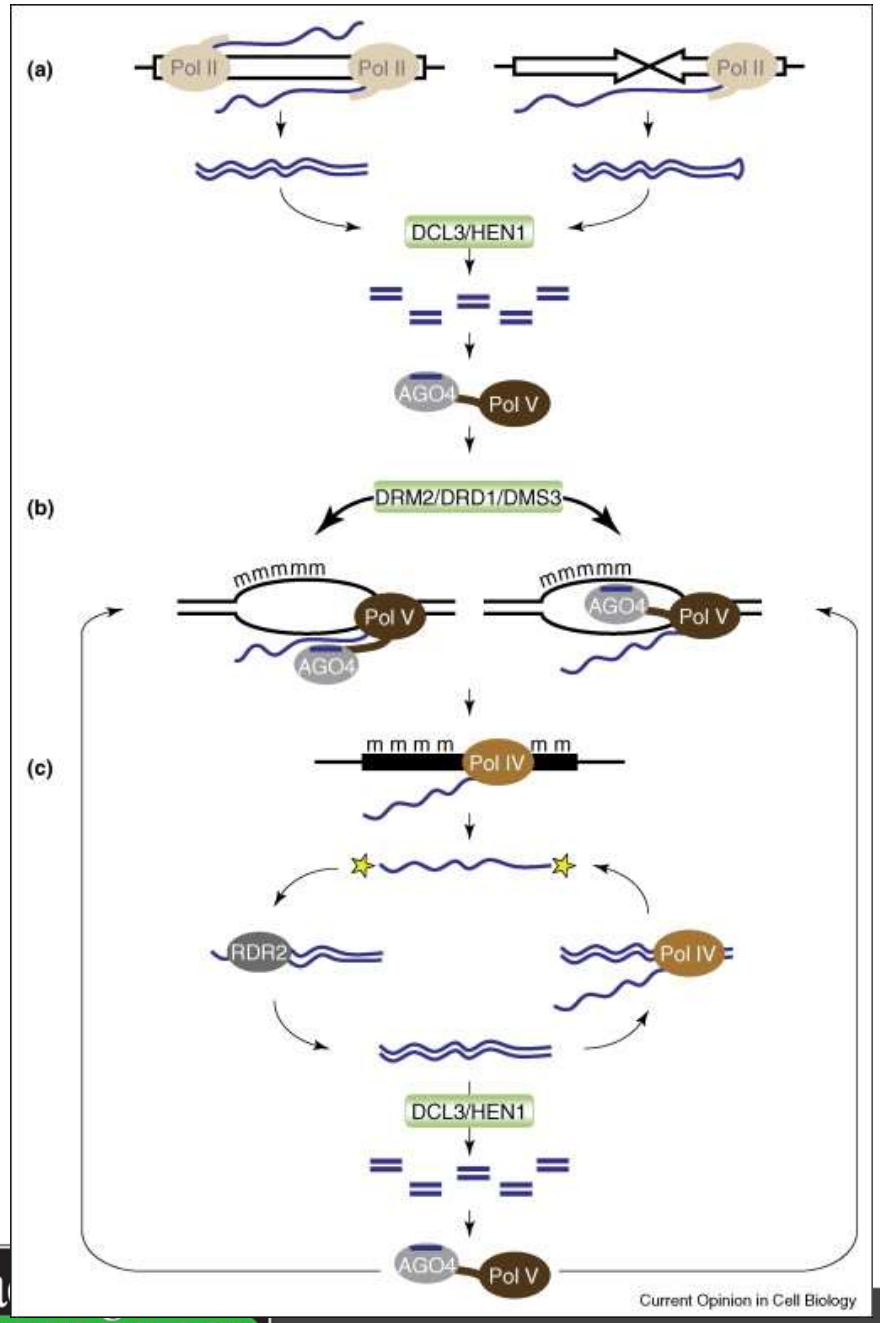
siRNAs recruit DNA methylases and histone-modifying enzymes to targets



siRNAs recruit DNA methylases and histone-modifying enzymes to targets

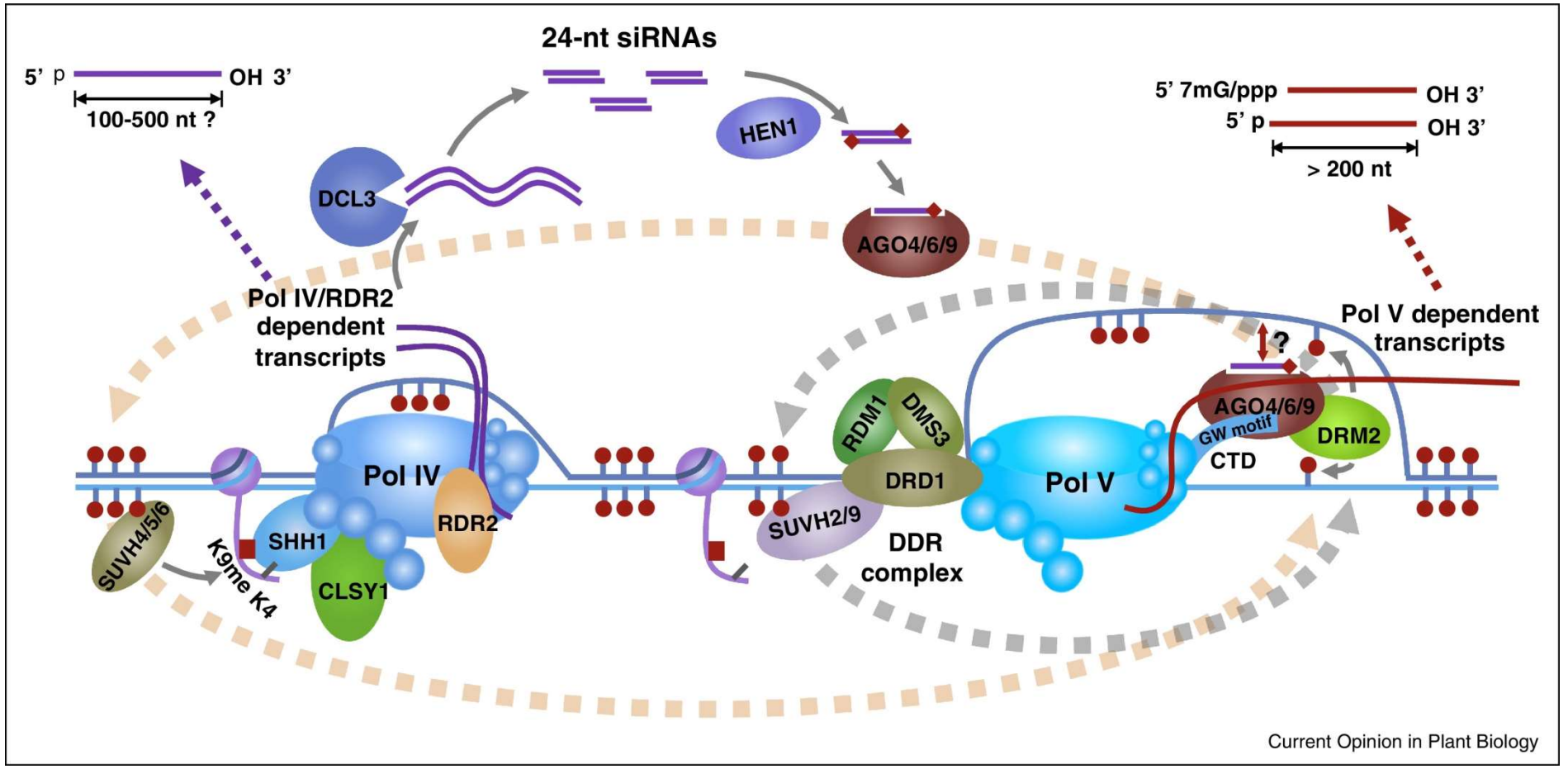


Two plant-specific RNA polymerases, RNA Pol IV and RNA Pol V, contribute to siRNA-mediated silencing.



(a) dsRNA that is independent of Pol IV and Pol V can potentially result from overlapping Pol II transcription (left) or Pol II transcription of inverted repeats (right). Processing by DCL3 produces 24-nt siRNAs that are methylated at their 3' ends by HEN1. One strand is loaded onto AGO4, which interacts with **NRPE1**, the largest subunit of Pol V **(b) Pol V** transcription facilitates DNA *de novo* methylation at the siRNA-targeted site by DRM2, the major *de novo* methyltransferase.

AGO4-bound siRNAs may interact with the nascent RNA (left) or the target DNA (right) to guide methylation. **(c)** To amplify the siRNA trigger, **Pol IV** may directly transcribe the methylated DNA template, producing an aberrant (improperly processed or terminated) RNA (yellow stars) . The aberrant RNA is copied by RDR2 to produce dsRNA precursors of siRNAs that trigger methylation (step B). Pol IV may also transcribe dsRNA in the amplification cycle.

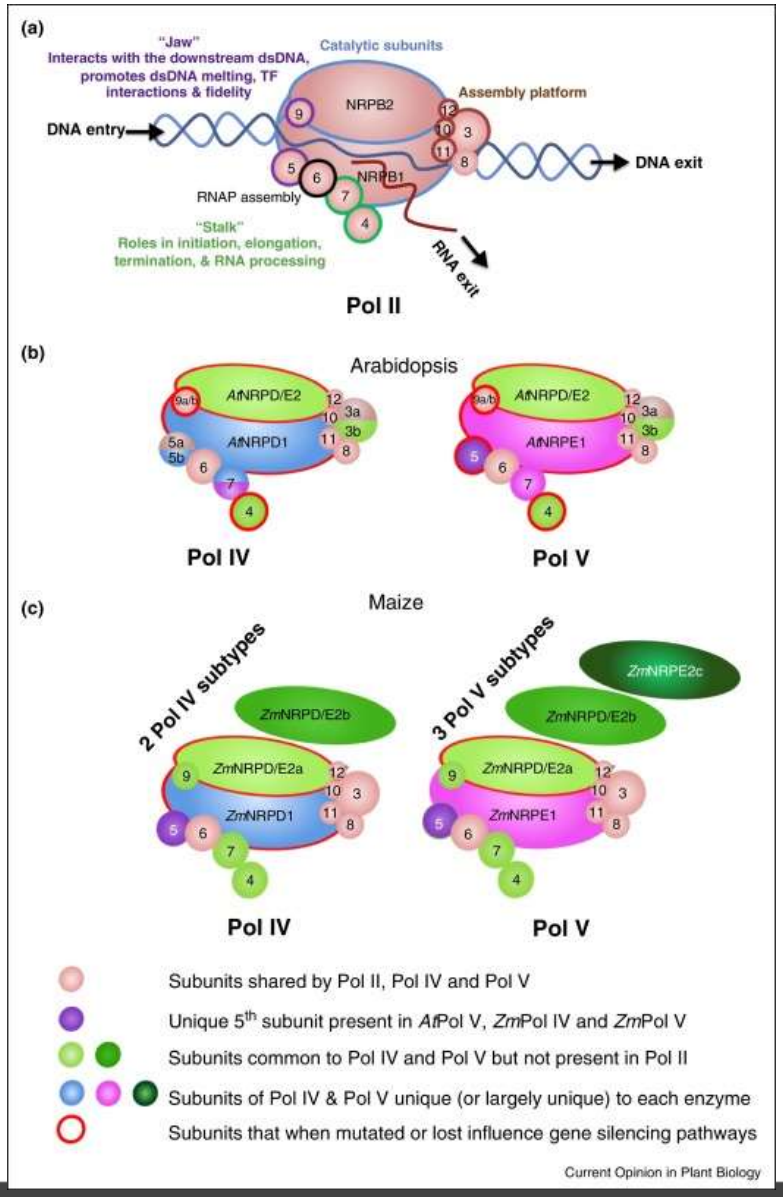


RNA pol V produces **intergenic non-coding (IGN) transcripts** present at low levels in WT plants but reduced in *pol v* mutants. IGN transcripts range in size, but can be up to 200-nt (long noncoding RNAs) Catalytically dead Pol V fails to produce IGN transcripts and to silence adjacent genes.

RNA Pol IV is required for the production of >90% of the *Arabidopsis* 24-nt siRNAs and prevailing models suggest this polymerase generates **long ssRNAs that act as substrates for siRNA production**

Current data suggests that the unique subunits of Pol IV and Pol V arose from their Pol II counterparts via many independent duplication events, starting prior to the evolution of land plants, followed by 'Escape from Adaptive Conflict' subfunctionalization

(escape from adaptive conflict (EAC), in which a single-copy gene is selected to perform a novel function while maintaining its ancestral function. This gene is constrained from improving either novel or ancestral function because of detrimental pleiotropic effects on the other function. After duplication, one copy is free to improve novel function, whereas the other is selected to improve ancestral function)



Plants encode specialized RNA polymerases that act non-redundantly in the *de novo* DNA methylation pathway, differ in their subunit compositions and in the types of noncoding RNAs they generate, and employ unique machinery for their recruitment to chromatin.

Recent advances in our understanding of the evolution and composition of these polymerases has confirmed the existence of specialized Pol IV and Pol V machinery across land plants, and has revealed functional diversity both between and within Pol IV and Pol V subtypes.

The identification of Pol IV and Pol V transcripts *in vitro* and *in vivo* has provided the first clues into the activities of these polymerases and suggests that they are not playing by the same rules governing Pol II activity.

Finally, a quite detailed view of the machinery required to facilitate recruitment of these polymerases to chromatin has emerged, revealing a dependence on previously established chromatin modifications as part of an intricate network of **self-reinforcing loops**.

siRNAs - summary

The siRNA pathway silences foreign DNA, transposons and repetitive elements.

In plants, siRNAs are produced by the action of Dicer-like proteins dicing dsRNA into 24 nt siRNAs

The siRNAs associate with AGO proteins and form silencing complexes

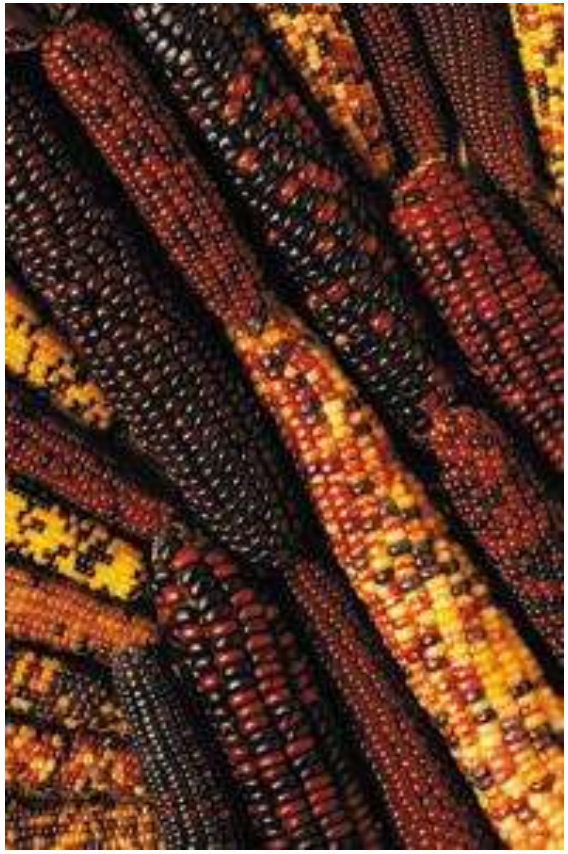
The silencing complexes can act post-transcriptionally on RNA targets, cleaving them or interfering with translation

The silencing complexes can also act on chromatin, silencing their targets by DNA methylation or histone modification

Epigenetic controls in whole-plant processes

- Transposon silencing
- Control of flowering time
- Control of imprinted genes
- Gene silencing *in trans*; paramutation
- Resetting the epigenome

Transposons



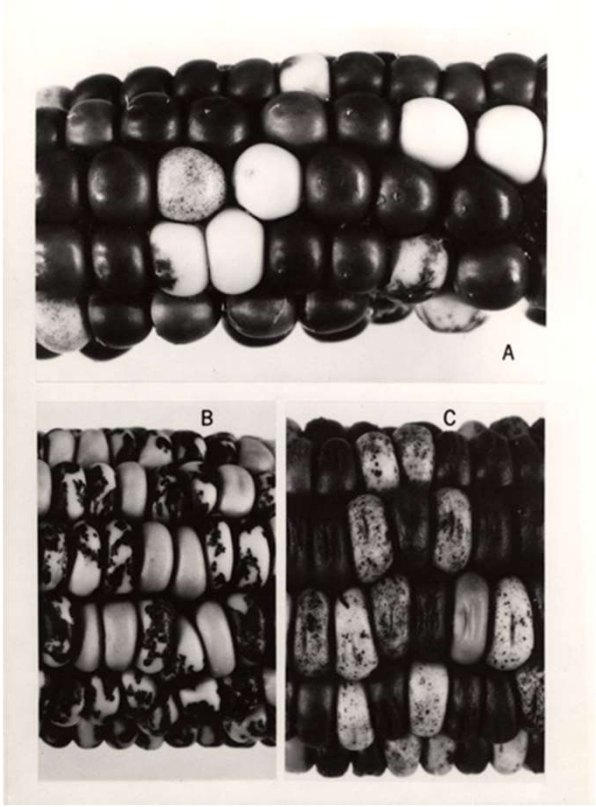
- Fragments of DNA that can insert into new chromosomal locations
- Some copy themselves and increase in number within the genome
- Responsible for large scale chromosome rearrangements and single-gene mutagenic events

Transposons

Barbara McClintock

Transposable elements were discovered in *Zea mays* by Barbara McClintock.

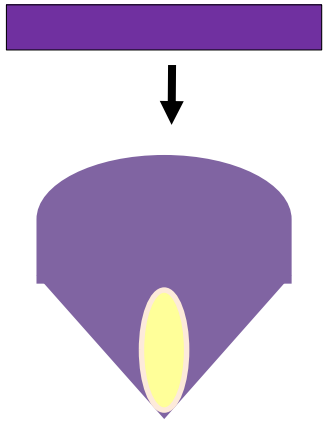
For her discovery, she was awarded the Nobel Prize in Physiology or Medicine in 1983.



Corn kernels showing transposition

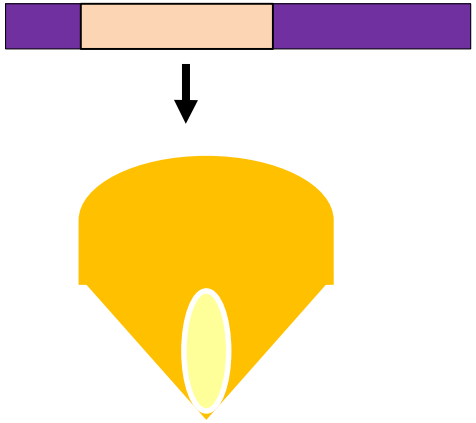
Transposons can cause inactive or unstable alleles

Gene required for pigment biosynthesis



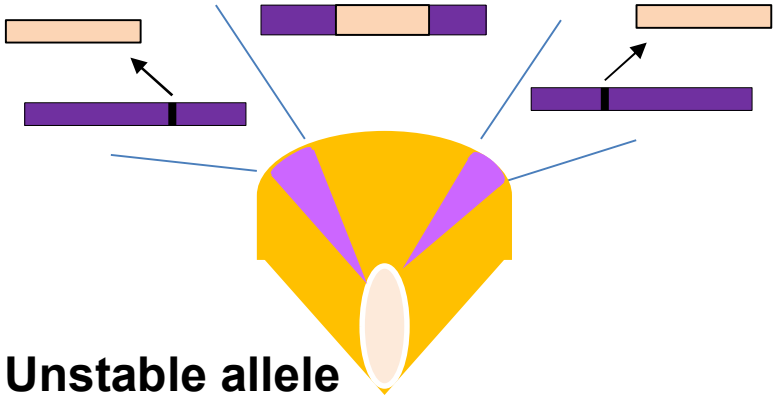
Wild-type allele
Pigmented kernel

Gene interrupted by transposon



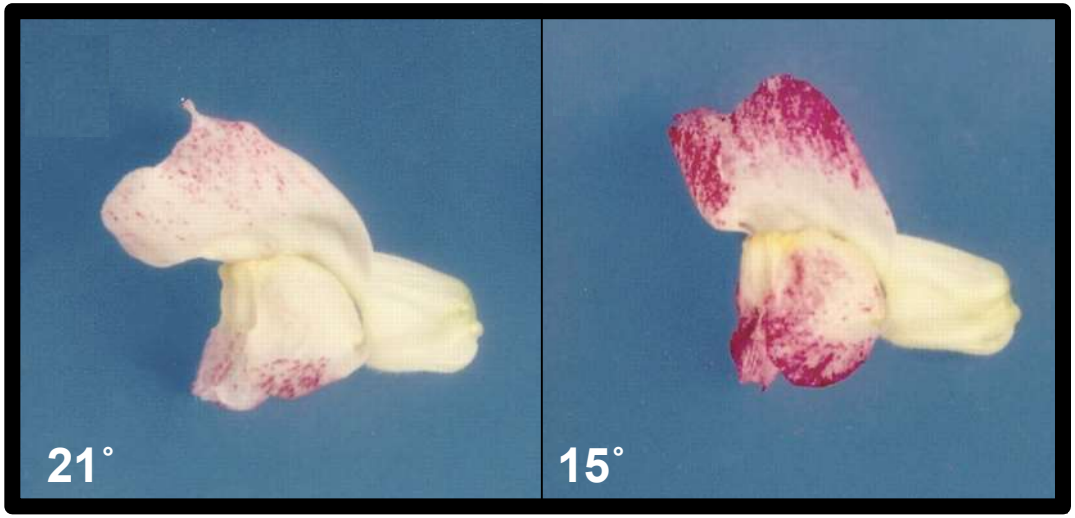
Mutant allele
Unpigmented kernel

Excision of the transposon causes unstable alleles



Unstable allele
Partially pigmented kernel

Naturally occurring transposons are a source of genetic variation



An *Antirrhinum* transposon that is only active at low temperatures.

"Variation is the raw material of evolutionary change"
- Stephen Jay Gould
(1941 – 2002)

Hashida, S.-N. Uchiyama, T., Martin, C., Kishima, Y., Sano, Y., and Mikami, T., (2006) The temperature-dependent change in methylation of the *Antirrhinum* transposon Tam3 is controlled by the activity of its transposase. Plant Cell 18:104-118.

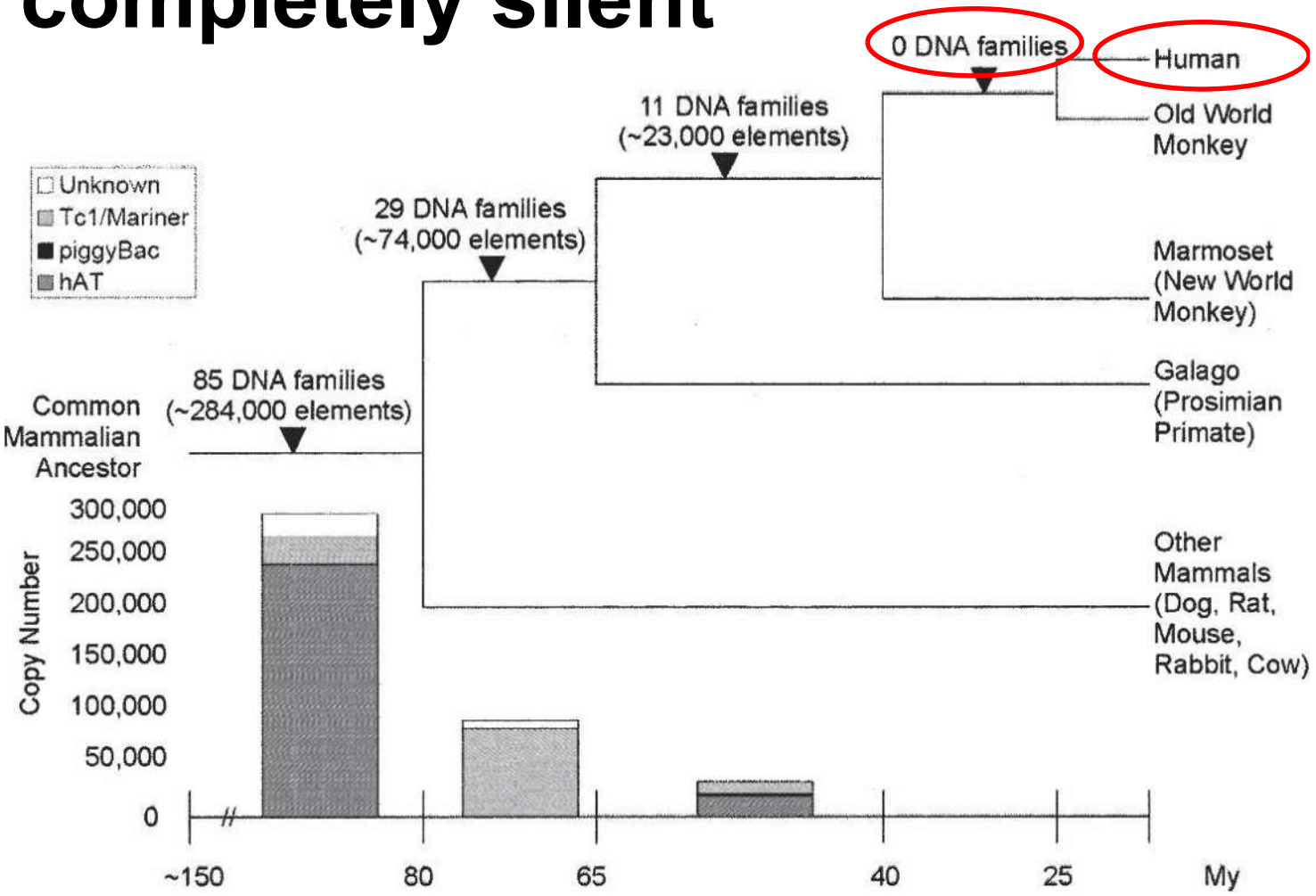
Transposons are abundant

Organism	% of genome derived from transposons
Yeast - *S. cerevisiae*	3%
Nematode - *C. elegans*	6%
Arabidopsis thaliana	14%
Fruitfly - *D. melanogaster*	15%
Rice - *Oryza sativa*	14%
Homo sapiens	44%
Corn - *Zea mays*	60%

Source: Kidwell, M.G. (2002) Genetica 115: 49-63.

Human transposons are almost completely silent

Number of active transposon families through evolutionary time



Pace , J.K., and Feschotte, C. (2007) The evolutionary history of human DNA transposons: Evidence for intense activity in the primate lineage. Genome Res. 17: 422-432

Transposon silencing

By contrast, maize has many active transposons

Epigenetic marks are thought to have evolved to silence foreign DNA (transposons, viruses)

Mutants that interfere with epigenetic silencing release transposons from silencing, and allow mutagenic transposon activity

Photo by Damon Lisch, in Gross, L. (2006) Transposon silencing keeps jumping genes in their place. PLoS Biology 4(10): e353.

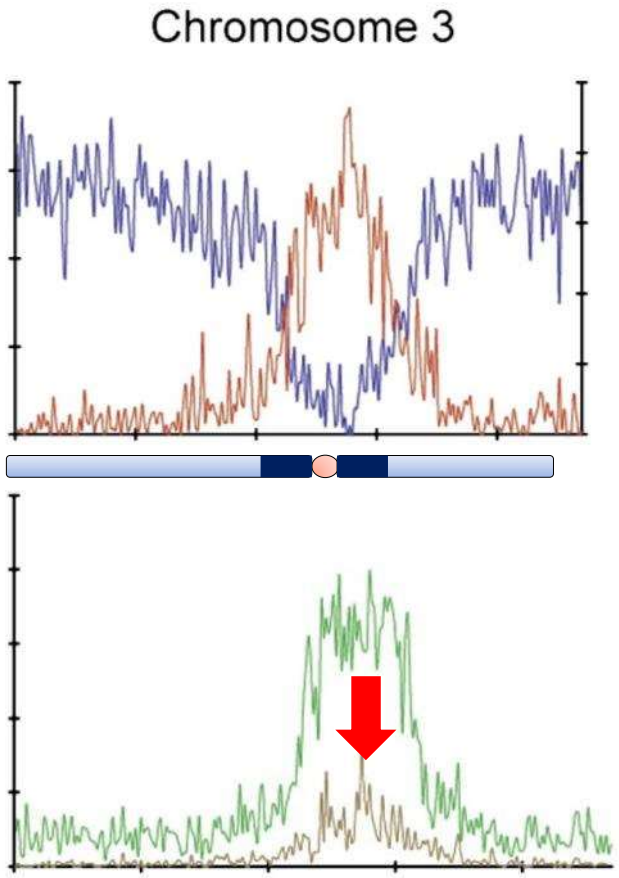
DNA methylation is necessary to silence transposons

Loss-of-function *met1* or *ddm1* (*decrease in DNA methylation1*) mutants have hypomethylated DNA

BLUE = Gene density
RED = Repetitive element density

GREEN = Methylated DNA

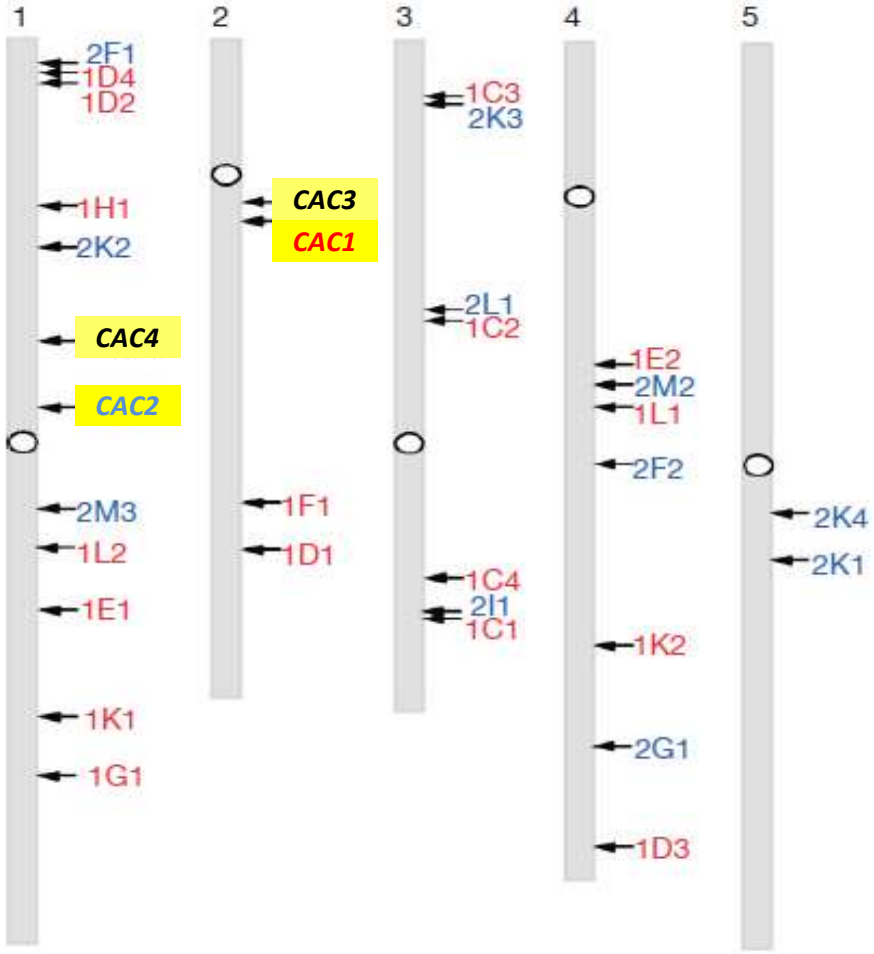
BROWN = Methylated DNA in a *met1* mutant

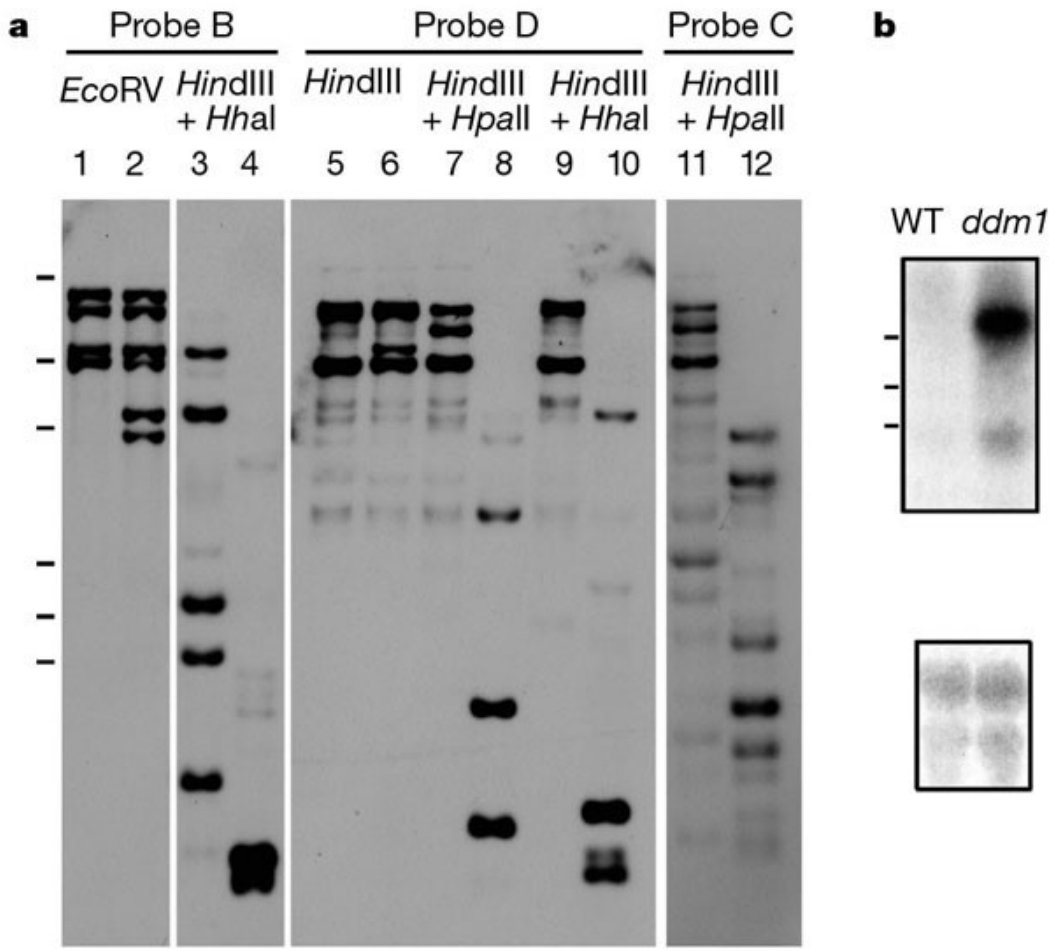


Transposons are activated in *ddm* mutants

Six generations after DNA methylation was reduced by *DDM* inactivation, newly inserted transposons were distributed throughout the genome.

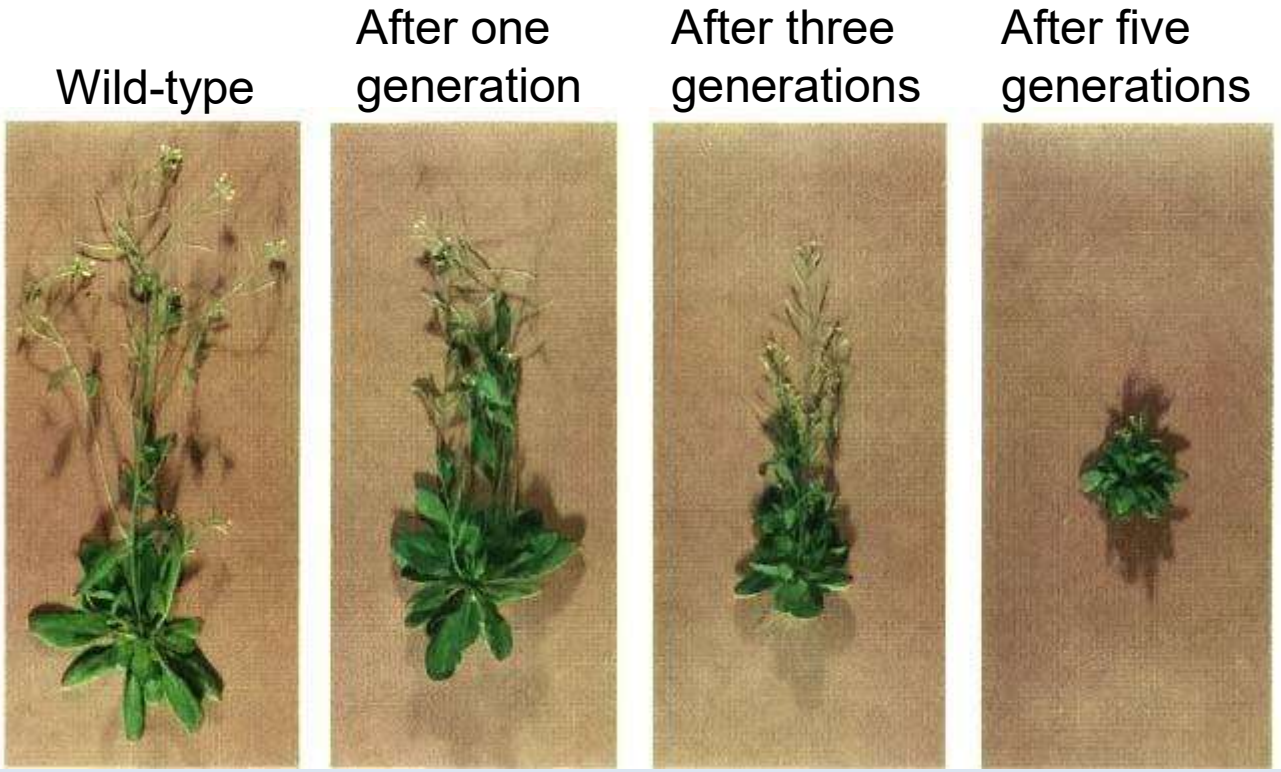
Yellow is site of original insertion, blue and red are new sites of insertion.





Methylation-sensitive restriction enzymes (*Hpa*II or *Hha*I) and probes B, C, D (Fig. 3a) were used to compare the methylation status of *CAC* elements between *ddm1* (even lanes) and Columbia wild-type (odd lanes) plants. The *ddm1* plant is before the repeated self-pollination (four generations before the plant shown in lane 10 of Fig. 3c). It still keeps the donor copies of *CAC* elements (lane 2). The DNA length markers are 19.3, 7.74, 5.53, 3.14, 2.69 and 2.32 kb. b, RNA blot analysis. Probe A (Fig. 3a) was used to detect *CAC* transcript in wild-type and *ddm1* (*clm*) plants. The RNA length markers are 6, 4 and 3 kb. Bottom panel, ribosomal RNA on the filter stained with methylene blue.

Activated transposons induce mutations



After *DDM* inactivation, plants become more and more abnormal as they accumulate transposon-induced mutations.

Kakutani, T., Jeddeloh, J.A., Flowers, S.K., Munakata, K., and Richards, E.J. (1996) Developmental abnormalities and epimutations associated with DNA hypomethylation mutations. PNAS 93: 12406-12411. Copyright (1996) National Academy of Sciences, U.S.A.

Epigenetic silencing of transposons by DNA methylation is necessary to maintain genomic integrity.

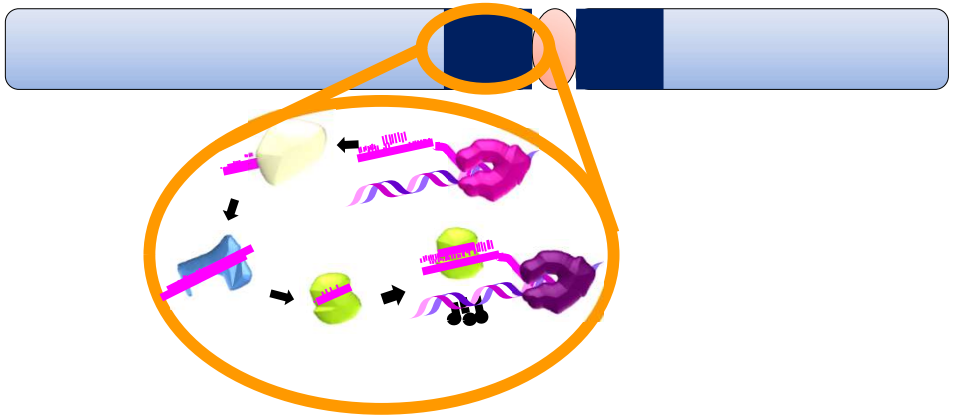
Kakutani, T., Jeddeloh, J.A., Flowers, S.K., Munakata, K., and Richards, E.J. (1996) Developmental abnormalities and epimutations associated with DNA hypomethylation mutations. PNAS 93: 12406-12411. Copyright (1996) National Academy of Sciences, U.S.A.

Initiating and maintaining silencing at repetitive DNA and transposons

How does the genome specifically recognize and silence repetitive elements and transposons?

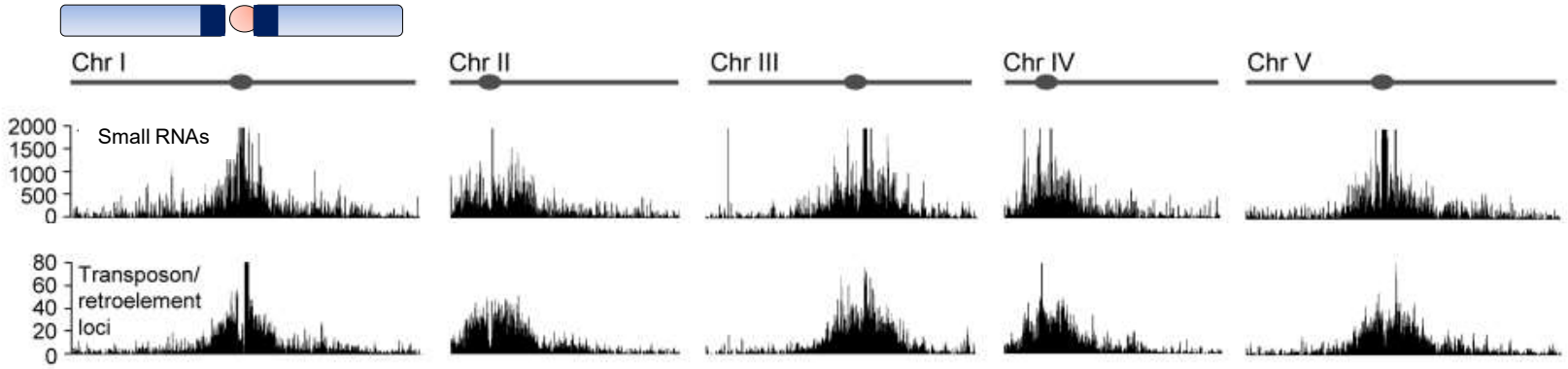
In other words, how does it recognize “self” (genes) from “non-self”? What is the basis for this “genomic immune recognition system”?

Repetitive elements and transposons are actively silenced



Maintaining transposon silencing is an active, dynamic process that requires ongoing siRNA production and epigenetic vigilance.

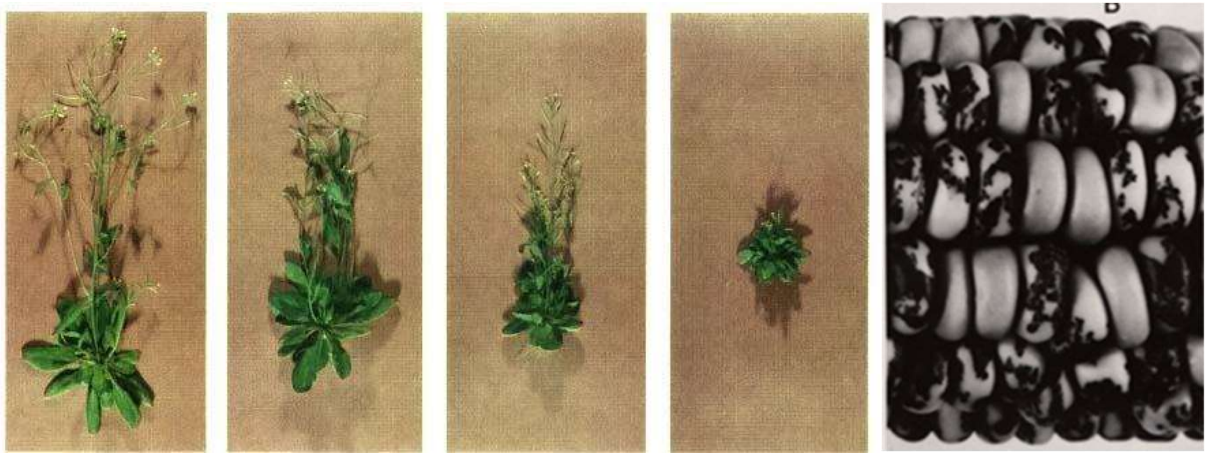
Small interfering RNAs (siRNAs) are preferentially derived from pericentromeric regions



The density of small RNA-homologous loci is highest in the centromeric and pericentromeric regions which contain a high density of repeat sequence classes, such as transposons.

Kasschau, K.D., Fahlgren, N., Chapman, E.J., Sullivan, C.M., Cumbie, J.S., et al. 2007 Genome-wide profiling and analysis of *Arabidopsis* siRNAs. PLoS Biol 5(3): e57.

Epigenetic silencing of transposons and repetitive elements



Transposons must be tightly controlled to prevent widespread mutagenic activity. Epigenetic controls to maintain silencing include DNA methylation, histone modification and siRNA production.