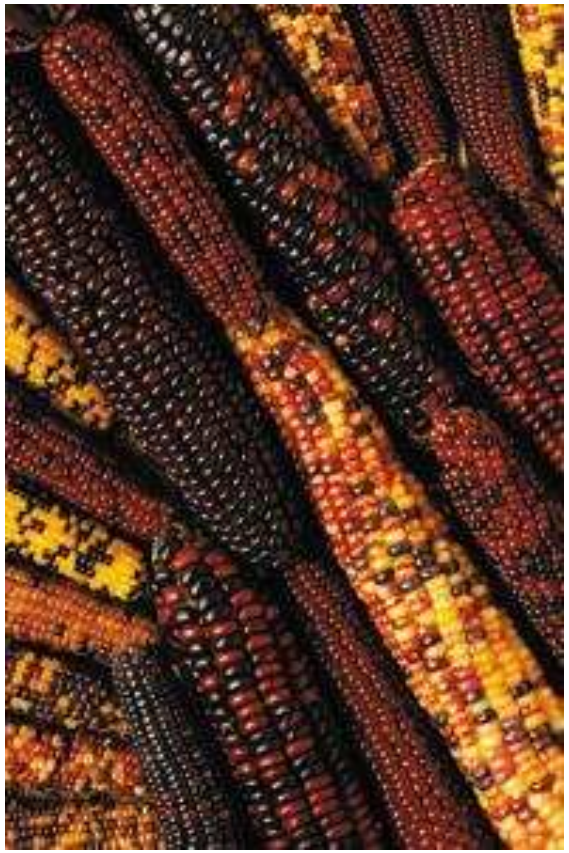
Epigenetic controls in whole-plant processes

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

Transposons



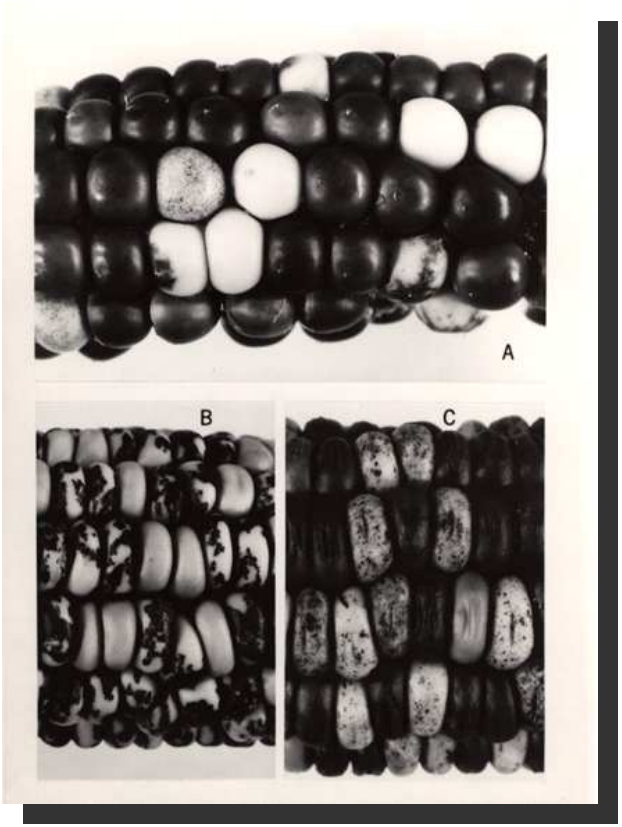
- Fragments of DNA that can insert into new chromosomal locations
- Some copy themselves and increase in number within the genome
- Responsible for large scale chromosome rearrangements and single-gene mutagenic events

Transposons

Barbara McClintock

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

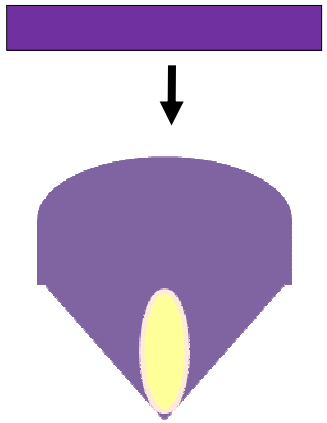
For her discovery, she was awarded the Nobel Prize in Physiology or Medicine in 1983.



Corn kernels showing transposition

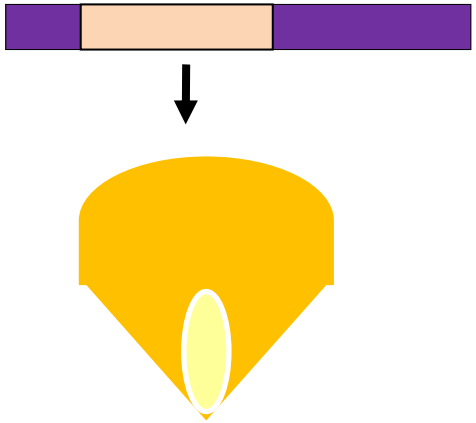
Transposons can cause inactive or unstable alleles

Gene required for pigment biosynthesis



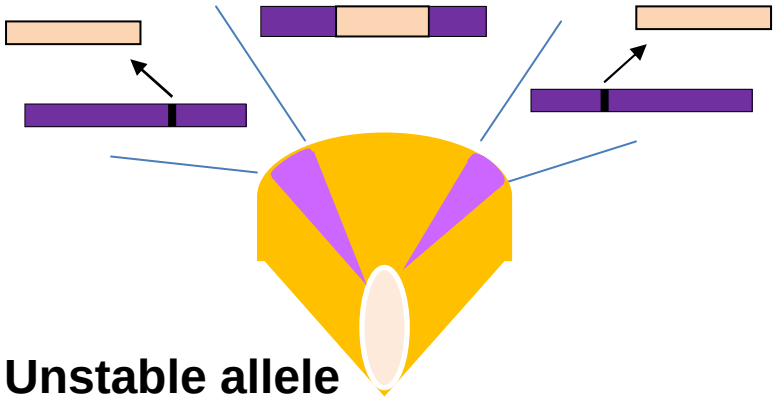
Wild-type allele
Pigmented kernel

Gene interrupted by transposon



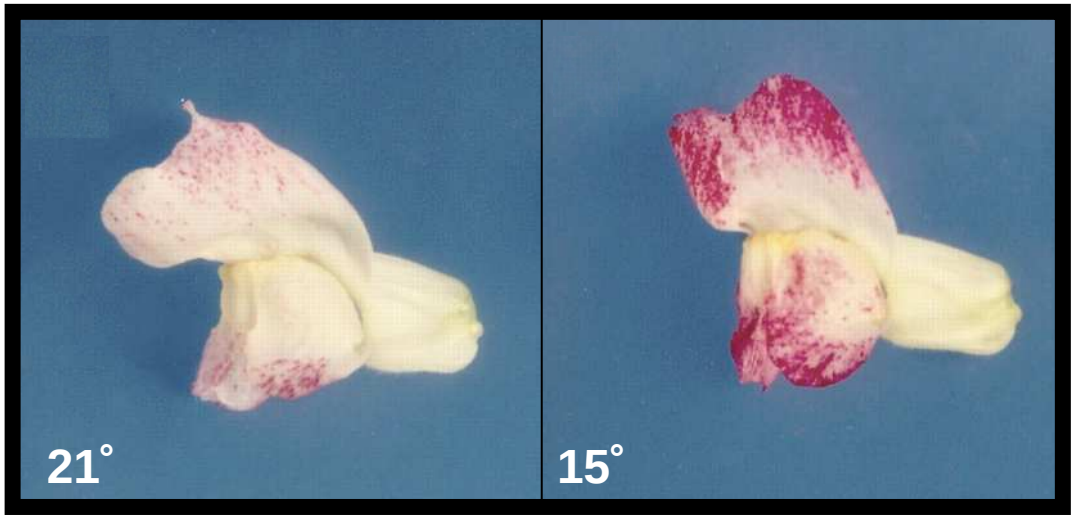
Mutant allele
Unpigmented kernel

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.

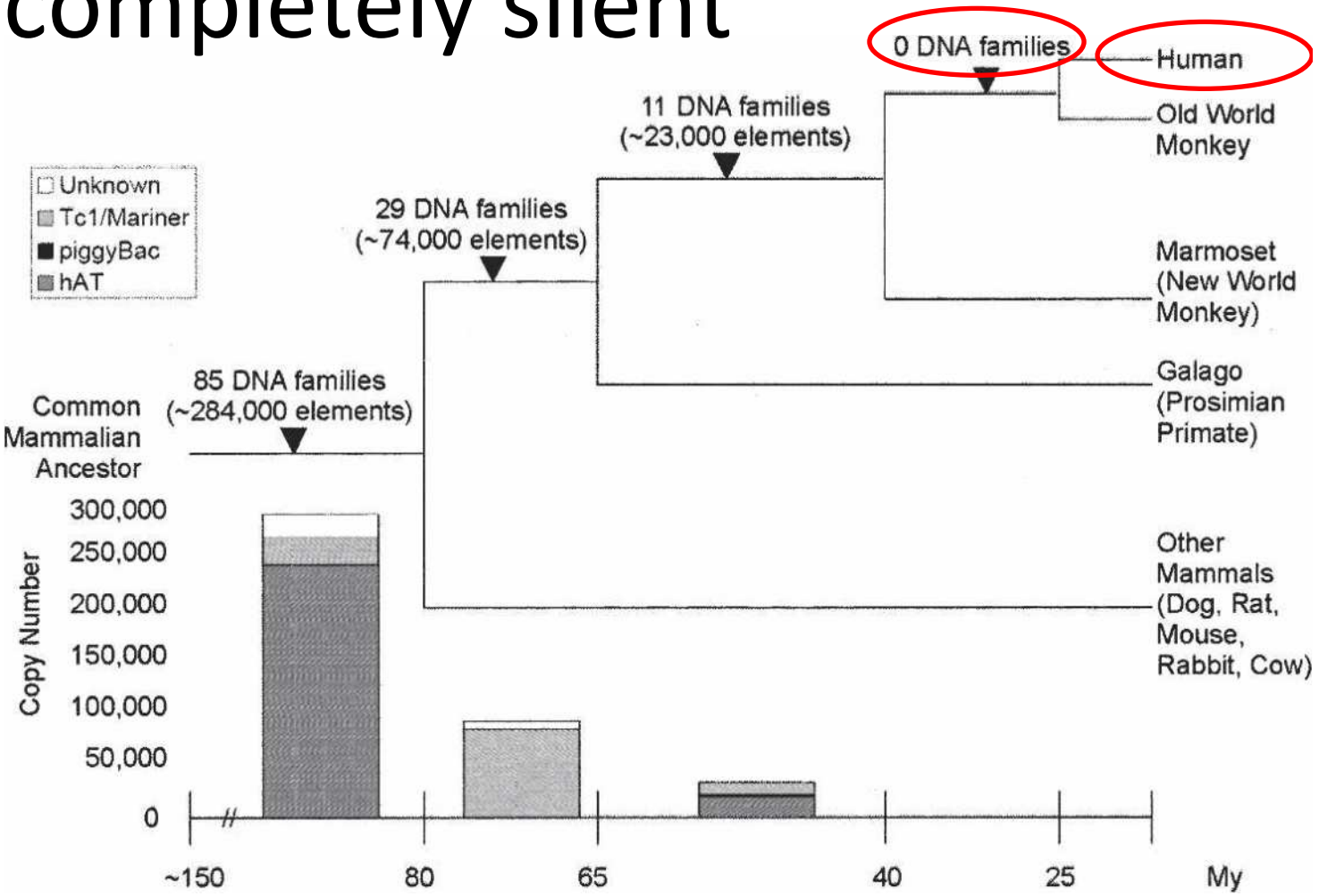
Transposons are abundant

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.

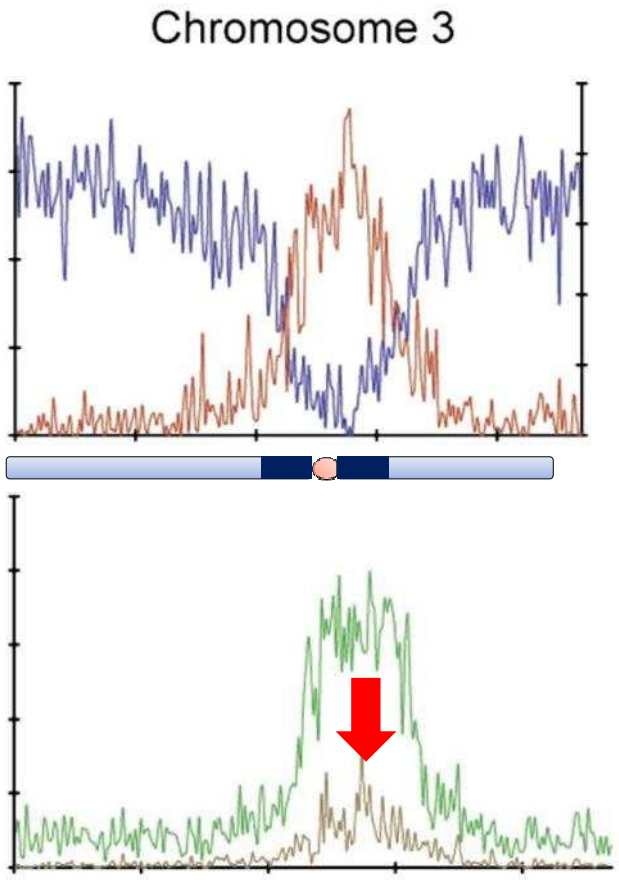
DNA methylation is necessary to silence transposons

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

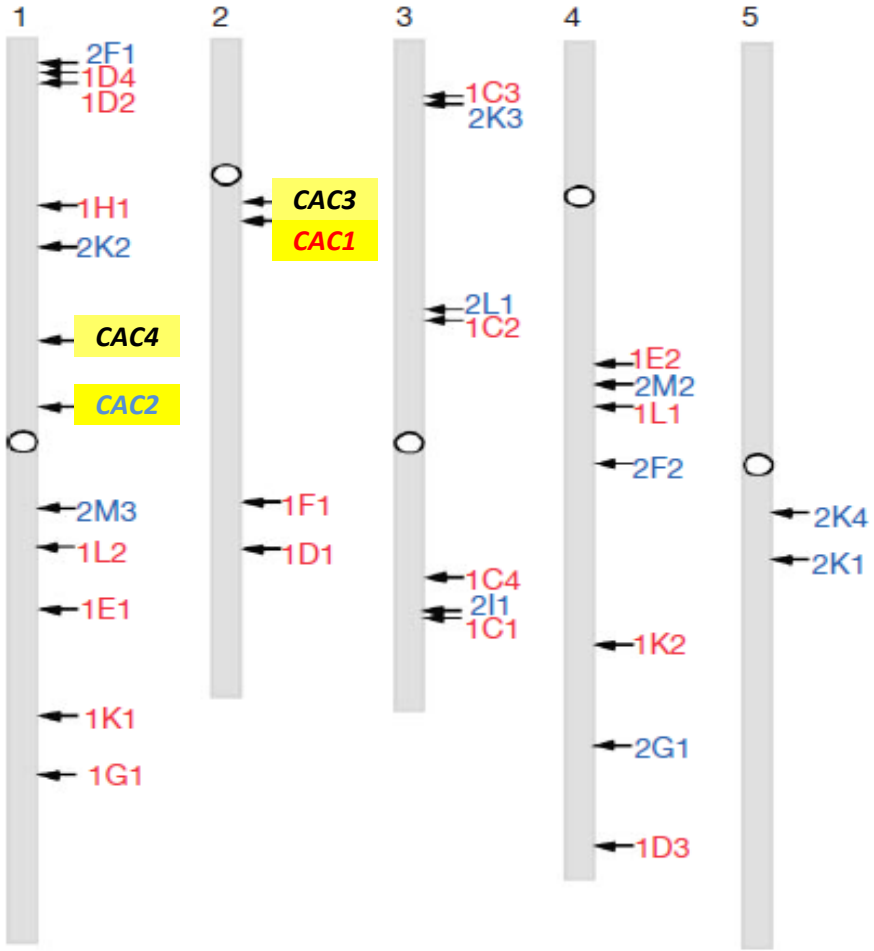


Reprinted from Zhang, X., Yazaki, J., Sundaresan, A., Cokus, S., Chan, S.W.-L., Chen, H., Henderson, I.R., Shinn, P., Pellegrini, M., Jacobsen, S.E., and Ecker., J.R. (2006) Genome-wide high-resolution mapping and functional analysis of DNA methylation in *Arabidopsis.* Cell 126: 1189–1201. With permission from Elsevier.

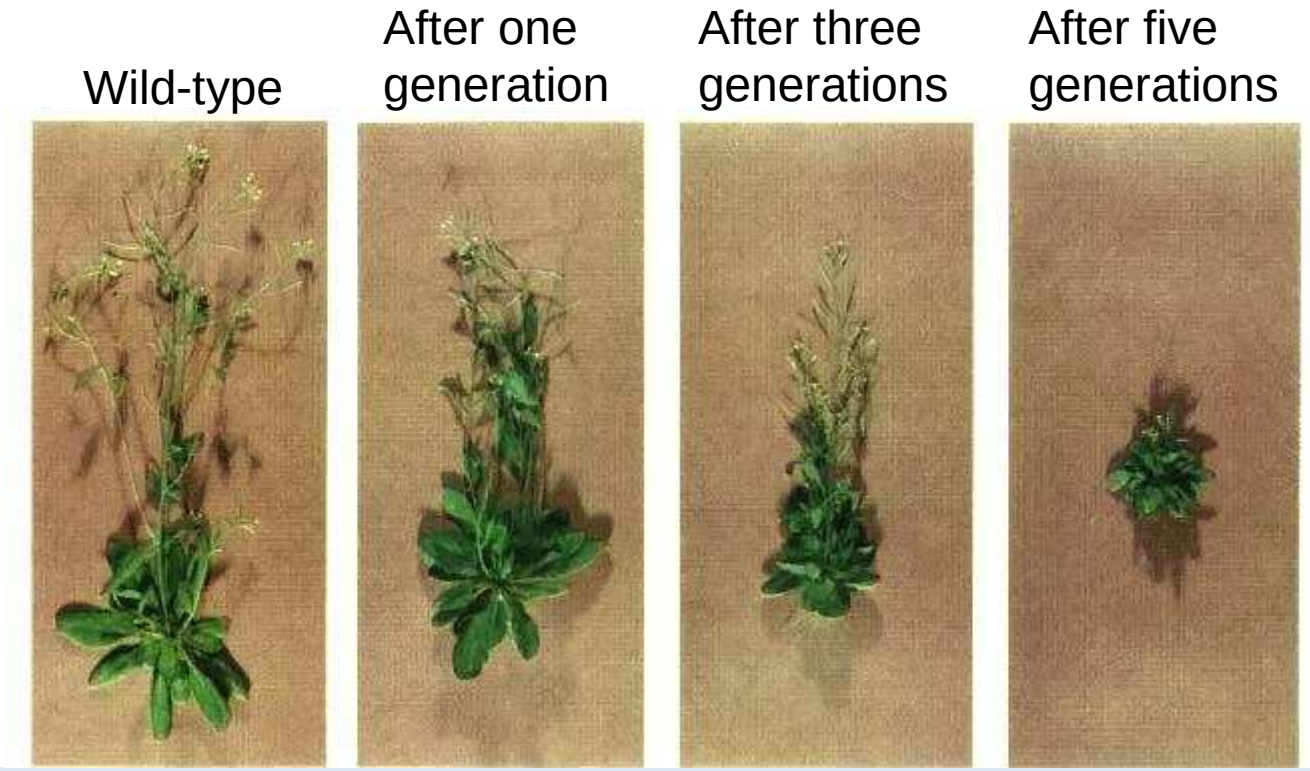
Transposons are activated in *ddm* mutants

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.

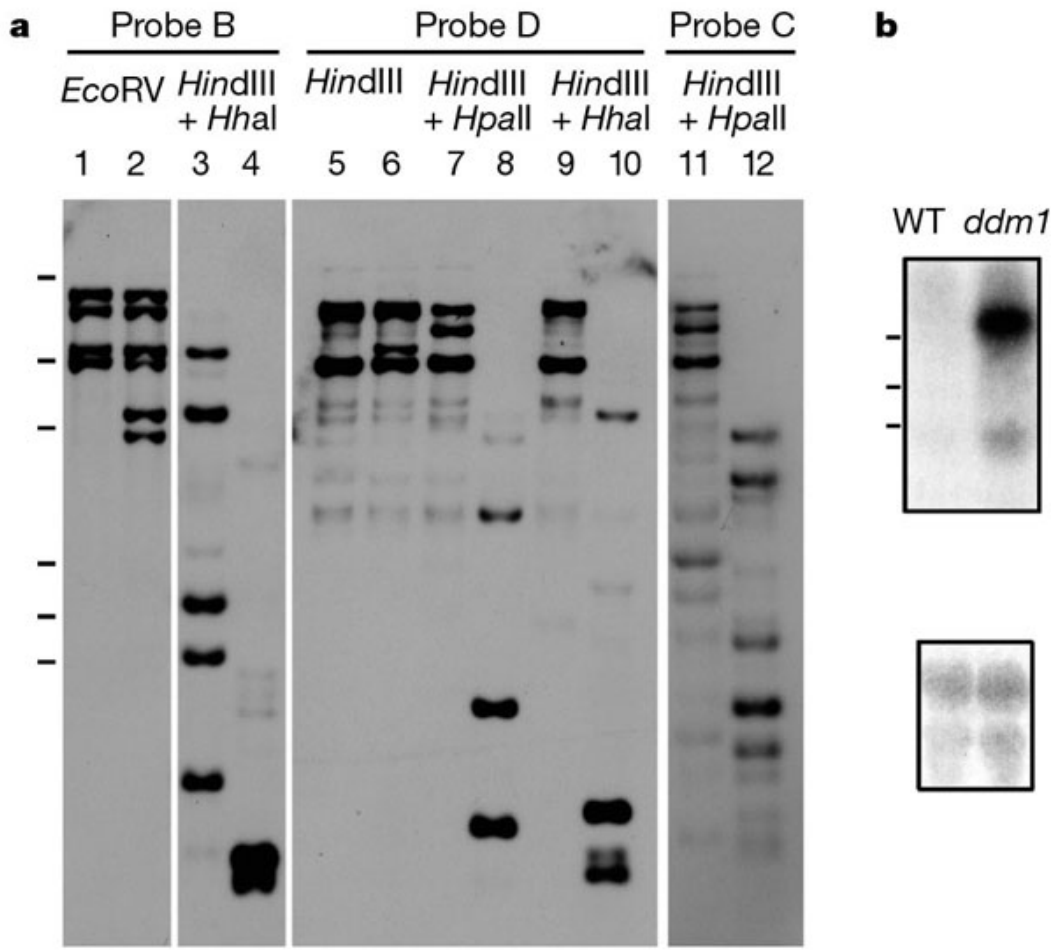


Activated transposons induce mutations

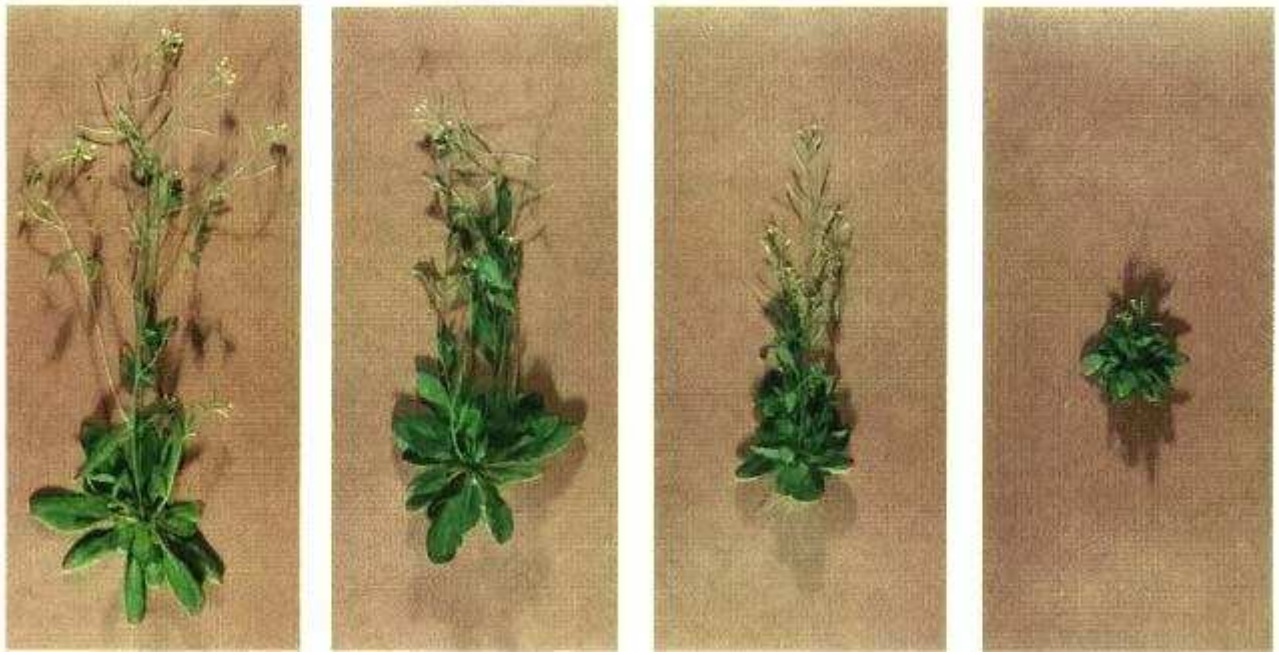


After *DDM* inactivation, plants become more and more abnormal as they accumulate transposon-induced mutations.

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.



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.



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

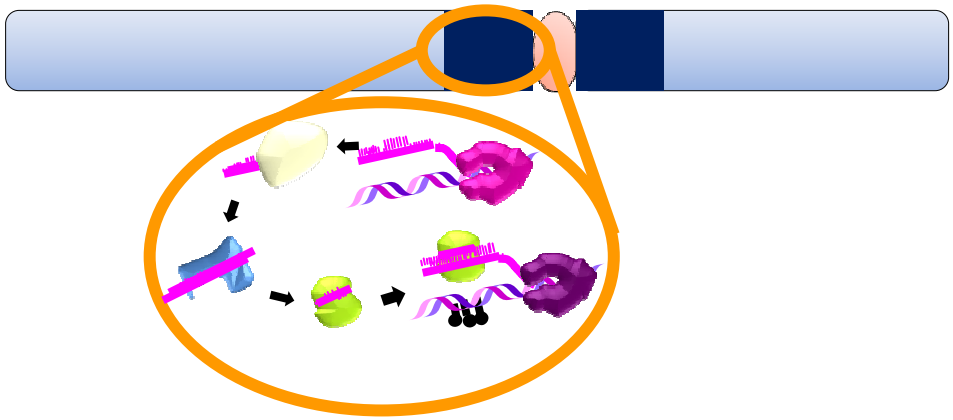
Kakutani, T., Jeddeloh, J.A., Flowers, S.K., Munakata, K., and Richards, E.J. (1996) Developmental abnormalities and epimutations associated with DNA hypomethylation mutations. PNAS 93: 12406-12411. Copyright (1996) National Academy of Sciences, U.S.A.

Initiating and maintaining silencing at repetitive DNA and transposons

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

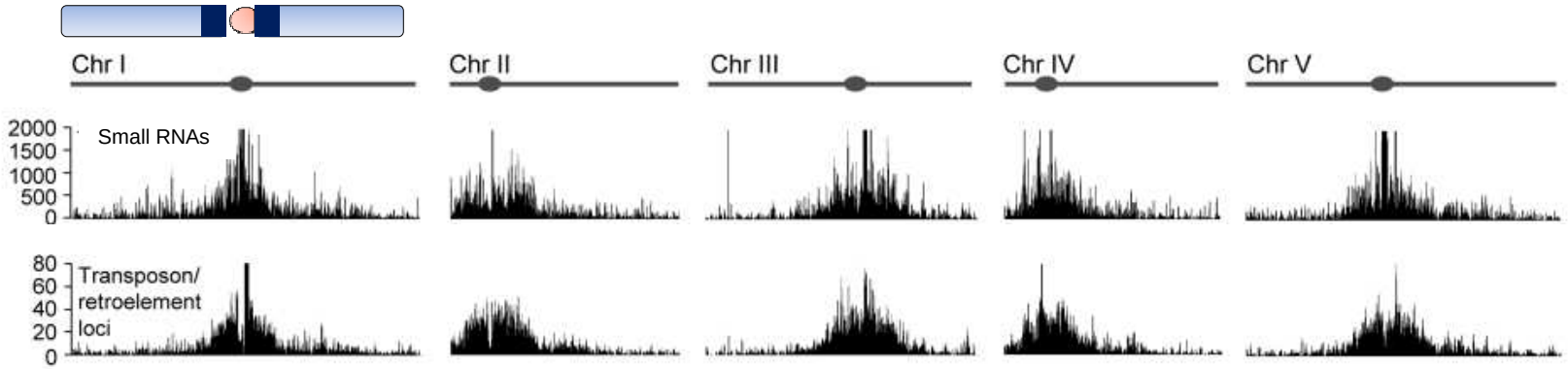
In other words, how does it recognize “self” (genes) from “non-self”? What is the basis for this “genomic immune recognition system”?

Repetitive elements and transposons are actively silenced



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

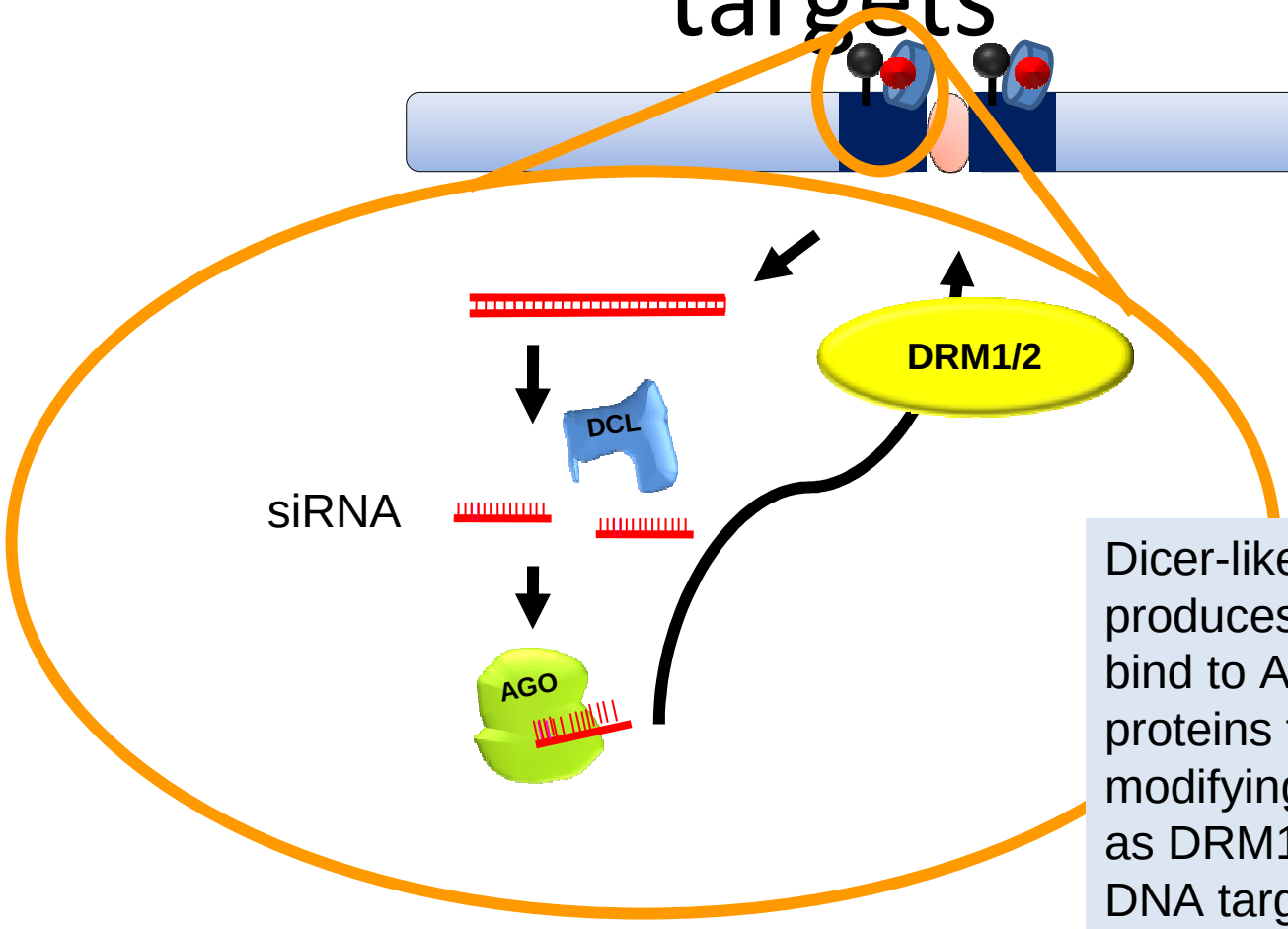
Small interfering RNAs (siRNAs) are preferentially derived from pericentromeric regions



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.

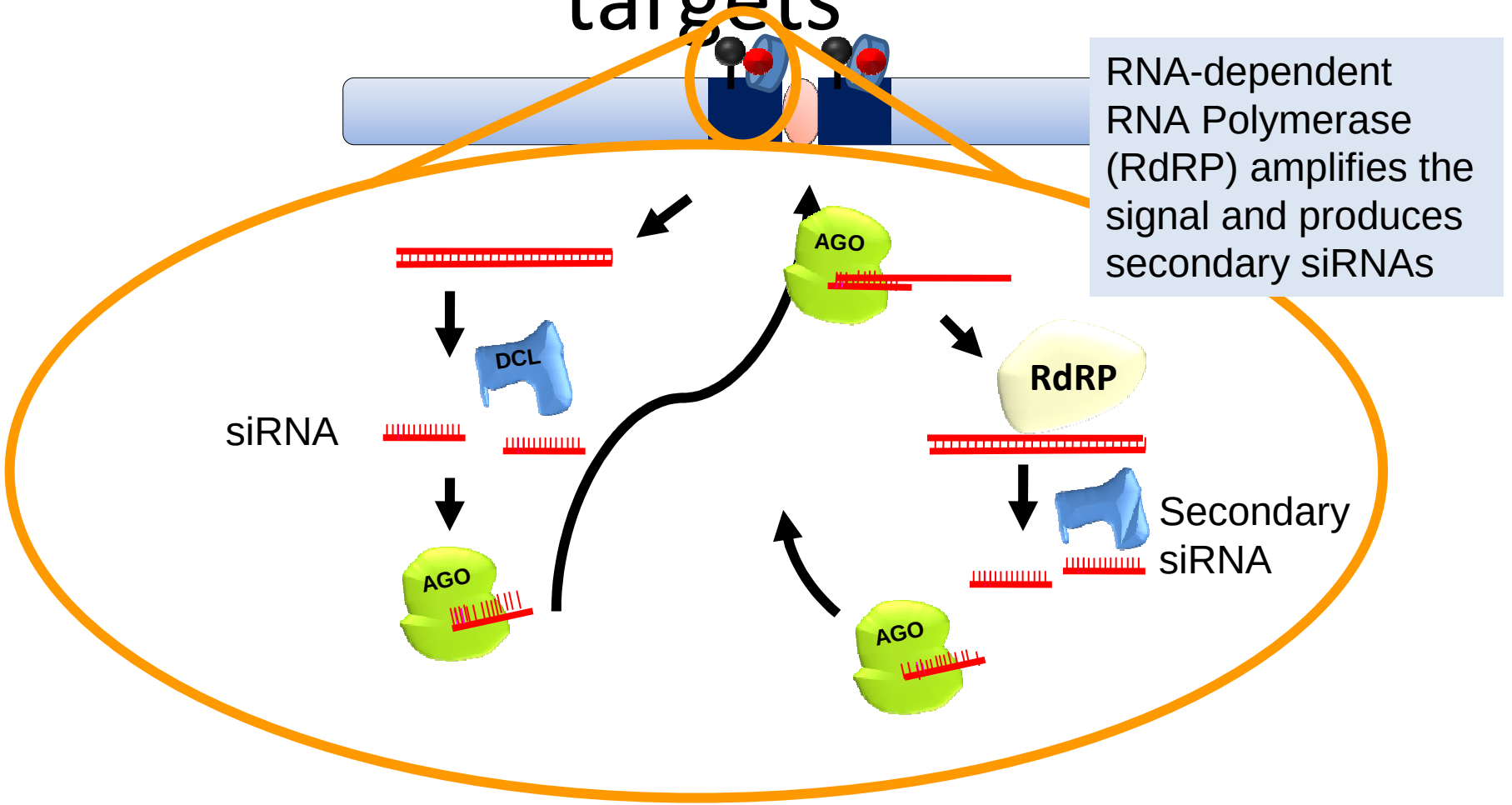
Kasschau, K.D., Fahlgren, N., Chapman, E.J., Sullivan, C.M., Cumbie, J.S., et al. 2007 Genome-wide profiling and analysis of *Arabidopsis* siRNAs. PLoS Biol 5(3): e57.

siRNAs recruit DNA methylases and histone-modifying enzymes to targets

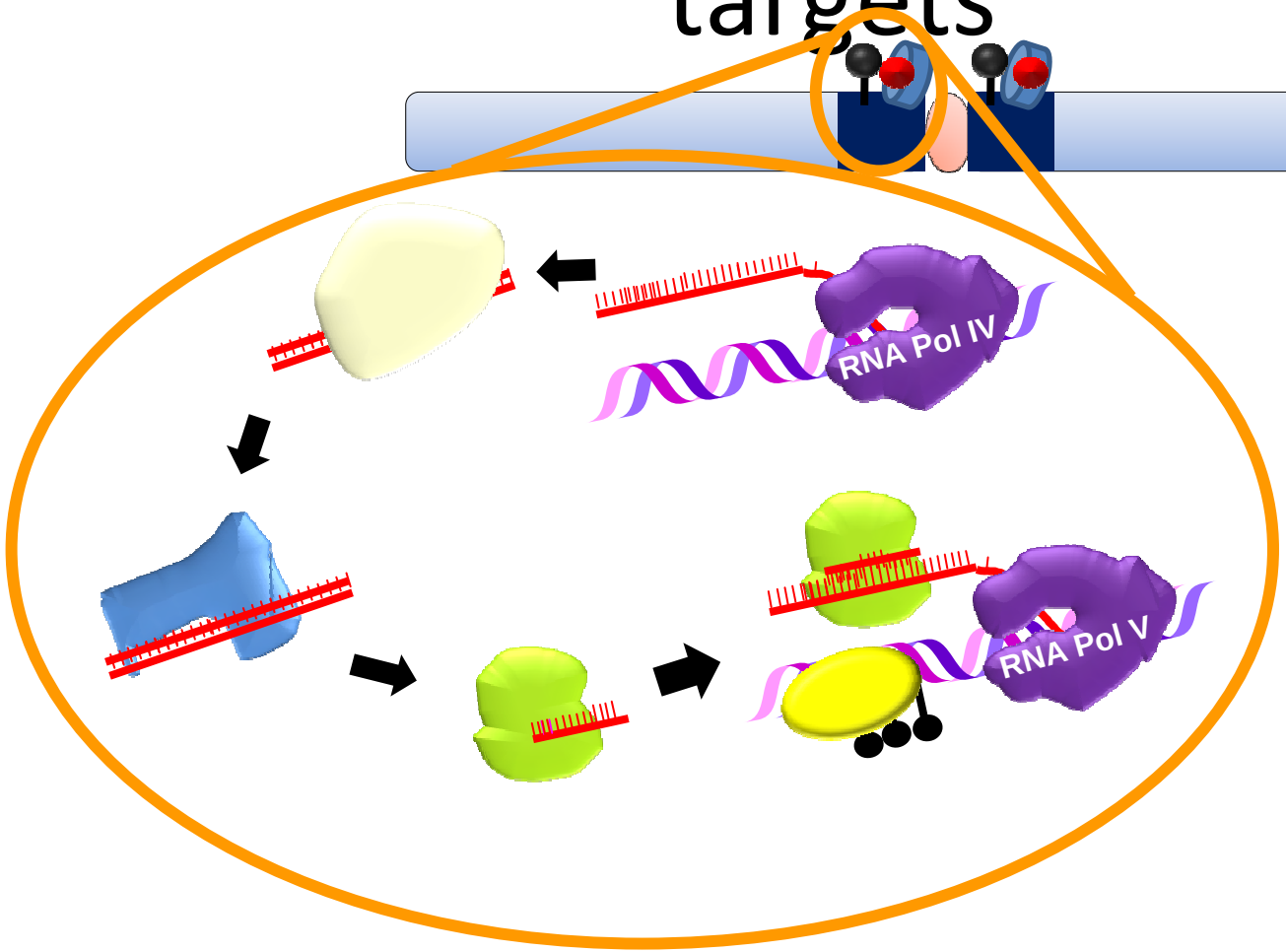


Dicer-like (DCL) nuclease produces siRNAs which bind to Argonaute (AGO) proteins to bring modifying enzymes such as DRM1 and DRM2 to DNA targets.

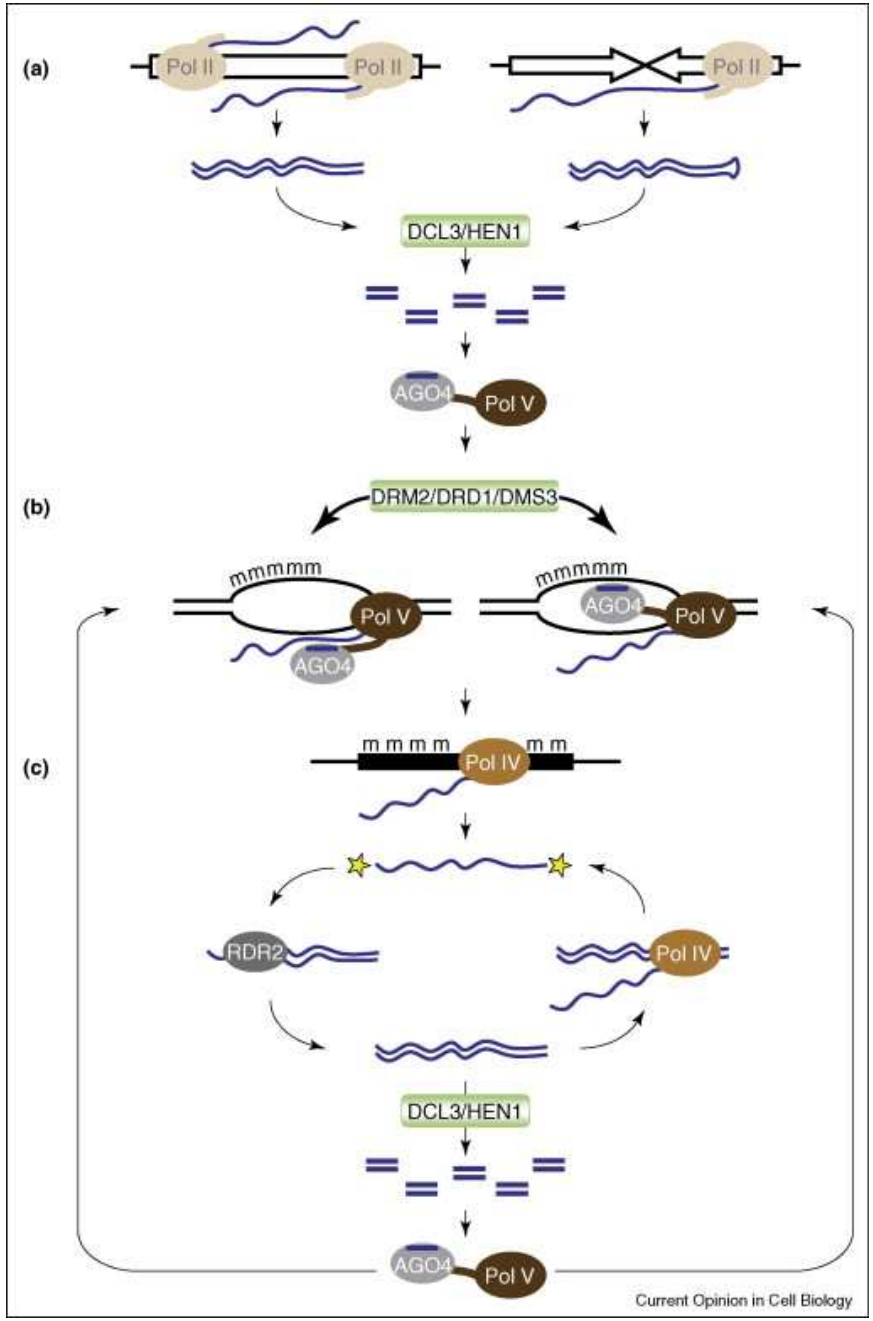
siRNAs recruit DNA methylases and histone-modifying enzymes to targets



siRNAs recruit DNA methylases and histone-modifying enzymes to targets



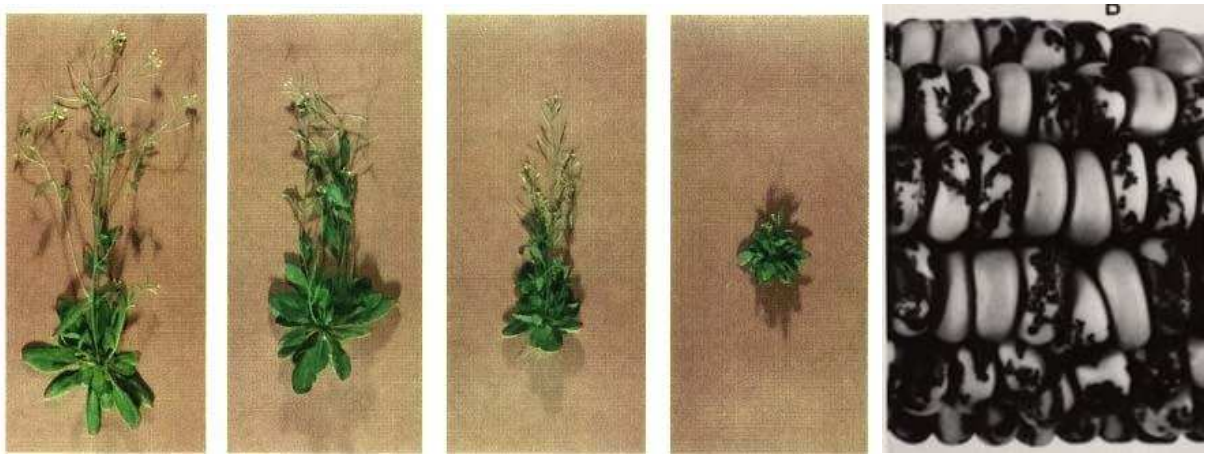
Two plant-specific RNA polymerases, RNA Pol IV and RNA Pol V, contribute to siRNA-mediated silencing.



(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.

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.