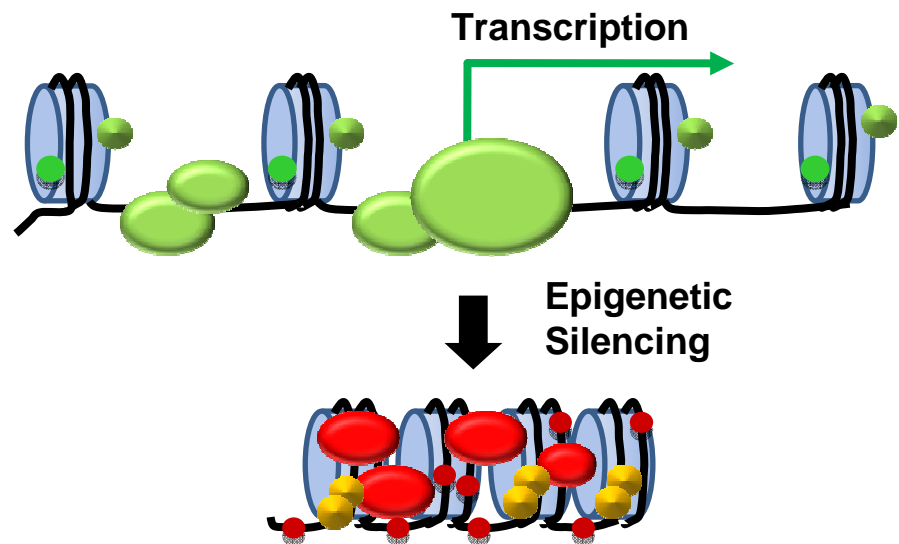


# Epigenetics

Any potentially stable and heritable change in gene expression that occurs without a change in DNA sequence

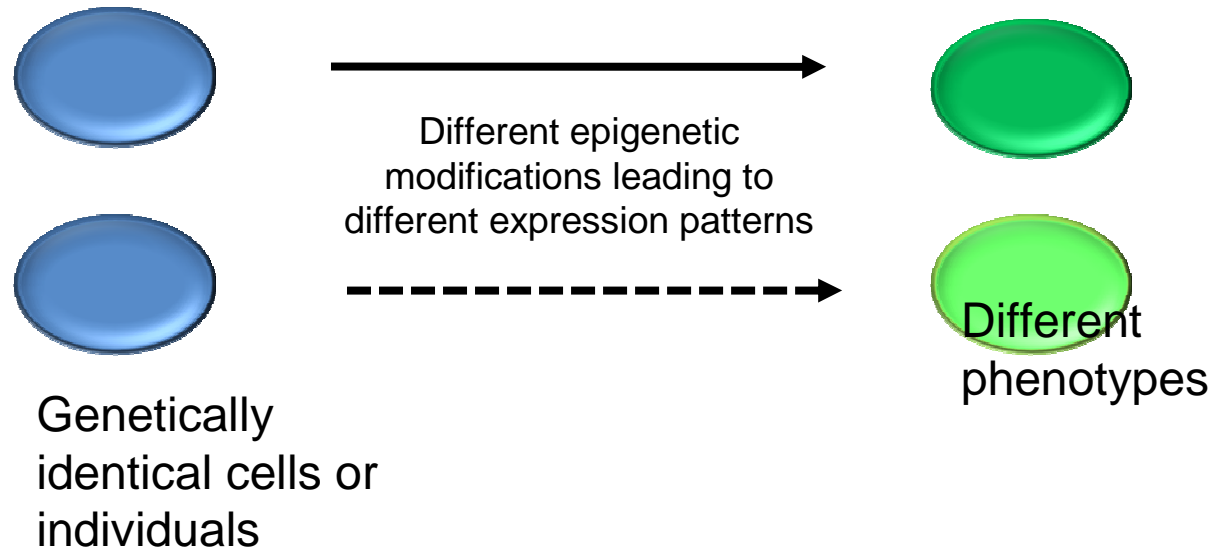
# What does “epigenetics” mean?

- **Literally**, epigenetics means above, or on top of, genetics.
- Usually this means information coded beyond the DNA sequence, such as in covalent modifications to the DNA or modifications to the chromatin structure.

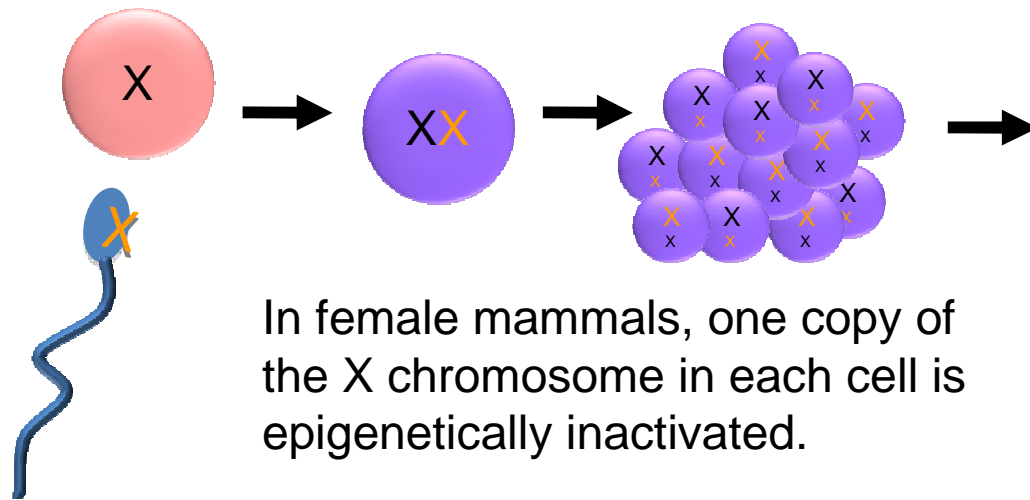


# What does “epigenetics” mean?

- **Practically**, epigenetics describes phenomena in which genetically identical cells or organisms express their genomes differently, causing phenotypic differences.



# X chromosome inactivation involves epigenetic silencing



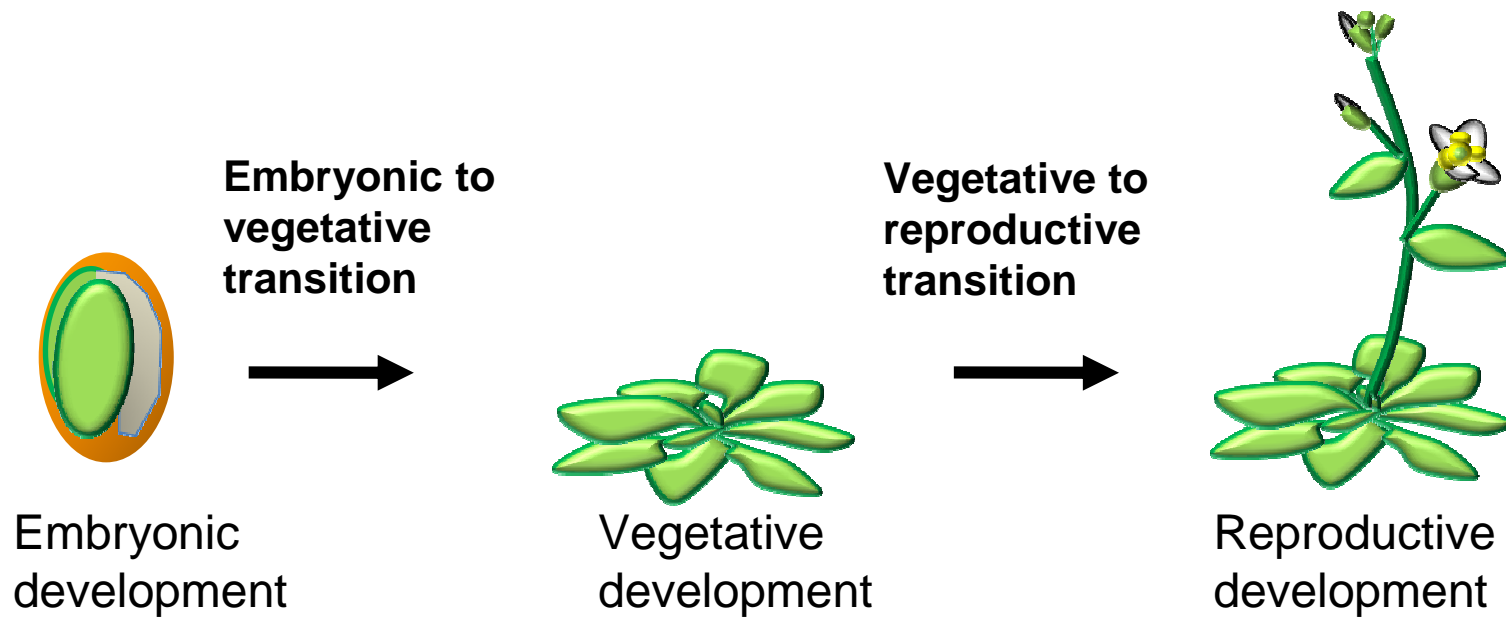
Fur color in cats is determined in part by *orange*, an X-linked gene. A female cat that is heterozygous for the *orange* gene shows black and orange patches, corresponding to which X chromosome is active.

# Epigenetic programming in plants helps silence transposons and maintain centromere function

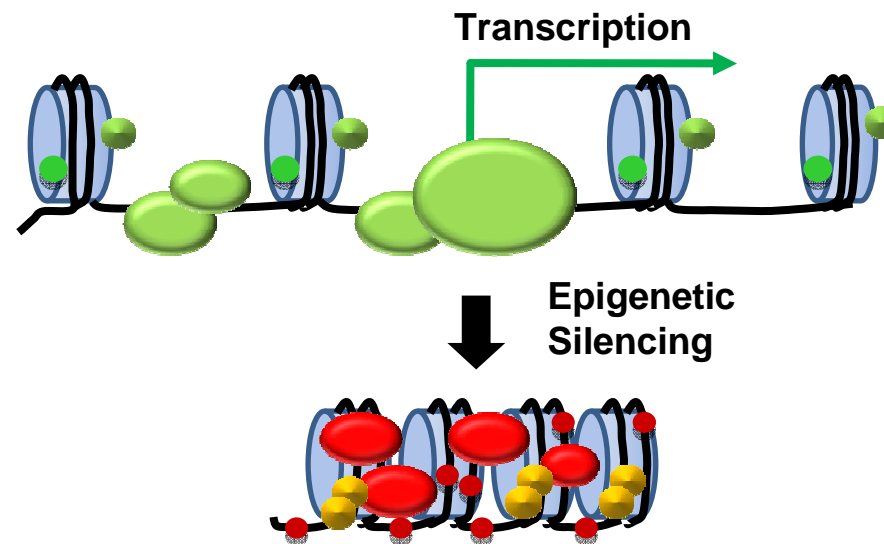


Photo by Damon Lisch, in Gross, L. (2006) Transposon silencing keeps jumping genes in their place. *PLoS Biology* 4(10): [e353](#).  
Photos courtesy of the [Barbara McClintock Papers](#), American Philosophical Society. Zhang, W., et al. (2008) *Plant Cell* 20: [25-34](#).

# Epigenetic programming in plants helps control developmental transitions

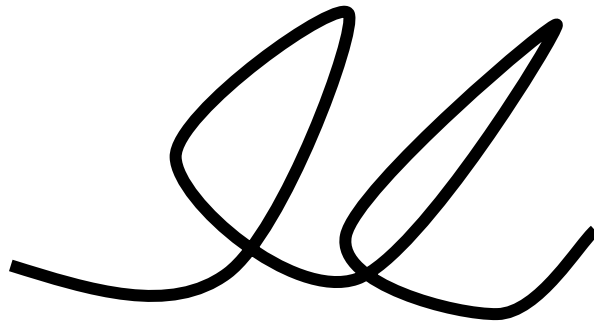
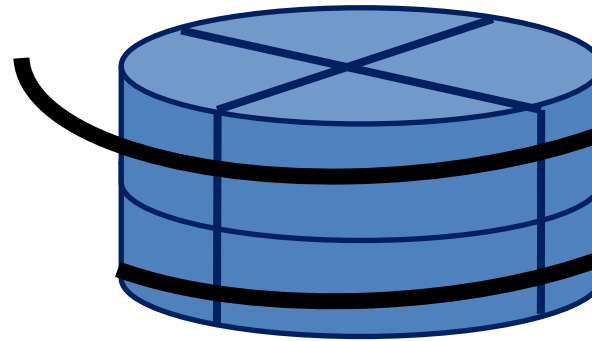


# Epigenetic marks and their maintenance



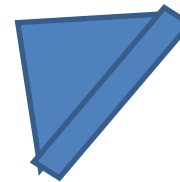
# Eukaryotic DNA is packaged in nucleosomes

Approximately 147 base pairs of DNA wrapped around a histone octamer



~ 147 bp DNA

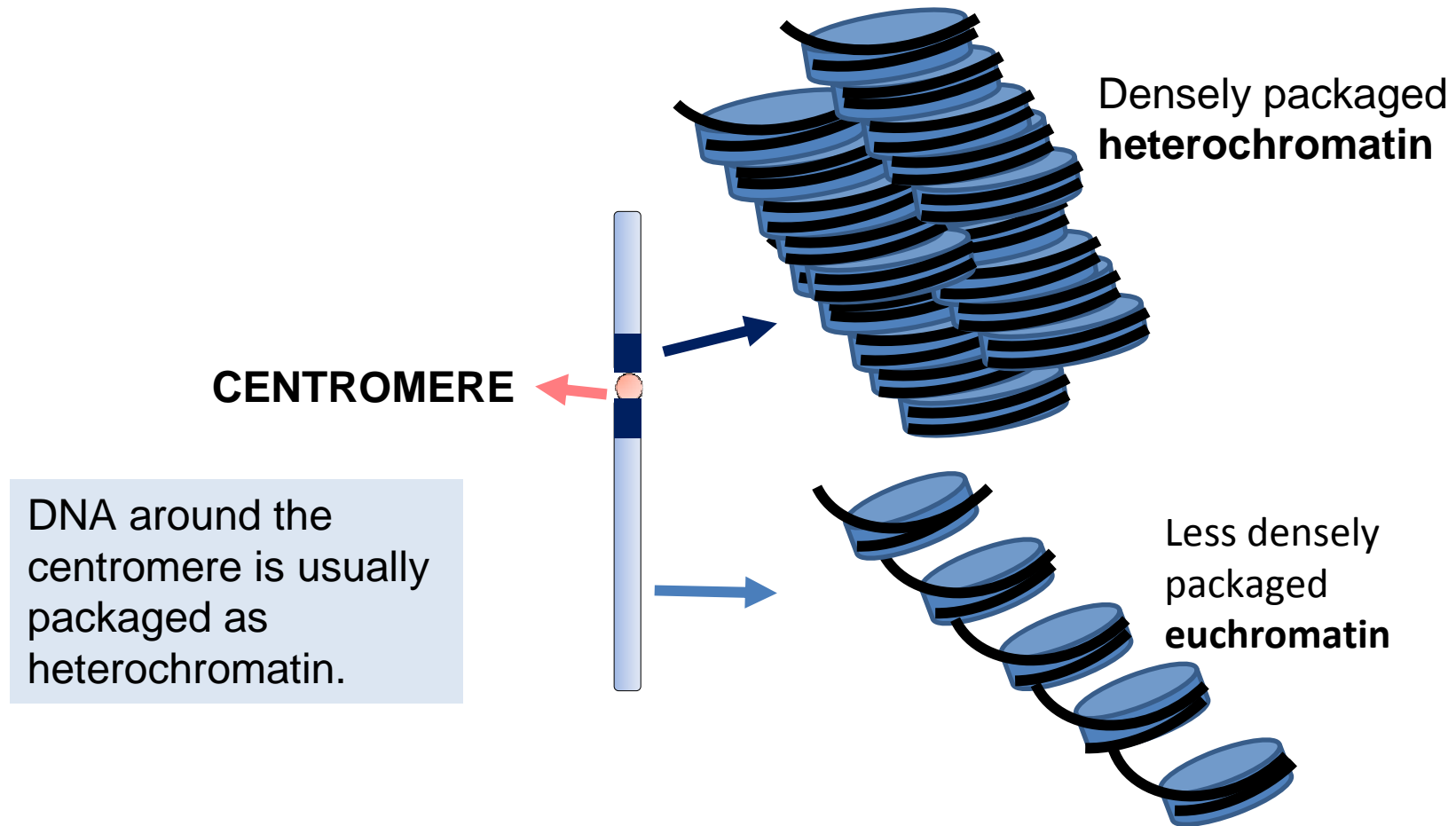
+



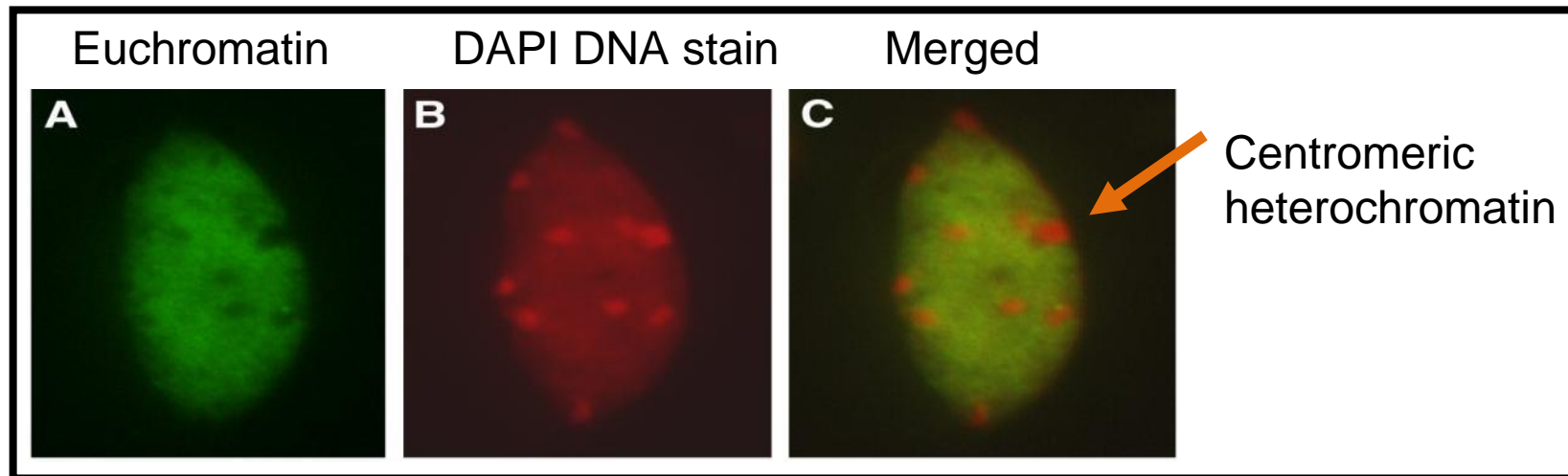
8 histones:  
2 each H2A  
H2B  
H3  
H4



# Chromosomes consist of heterochromatin and euchromatin

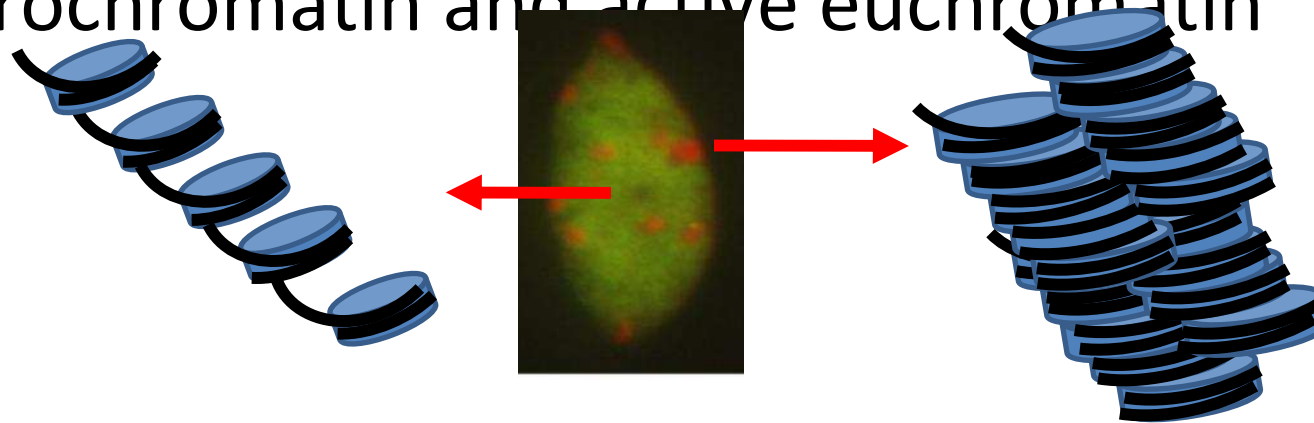


# Heterochromatin and euchromatin form distinct domains in nuclei

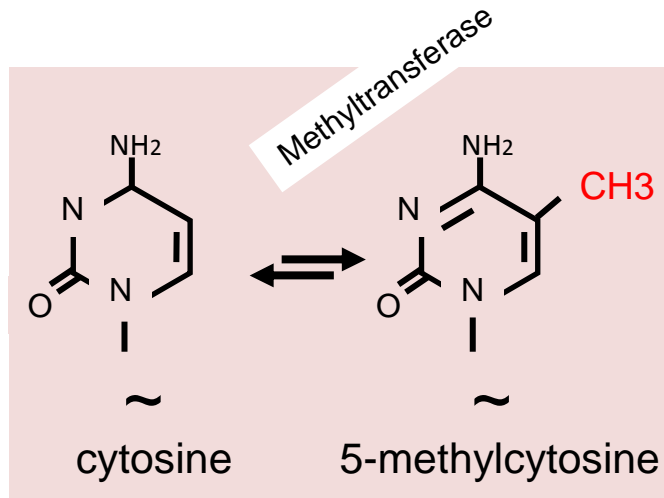


# Epigenetic modifications

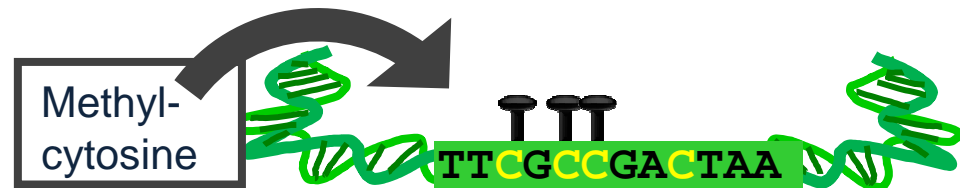
- Epigenetic modifications include:
  - Cytosine methylation of DNA
  - Histone modifications
- Collectively, these changes contribute to the distribution of DNA into silent, heterochromatin and active euchromatin



# DNA methylation



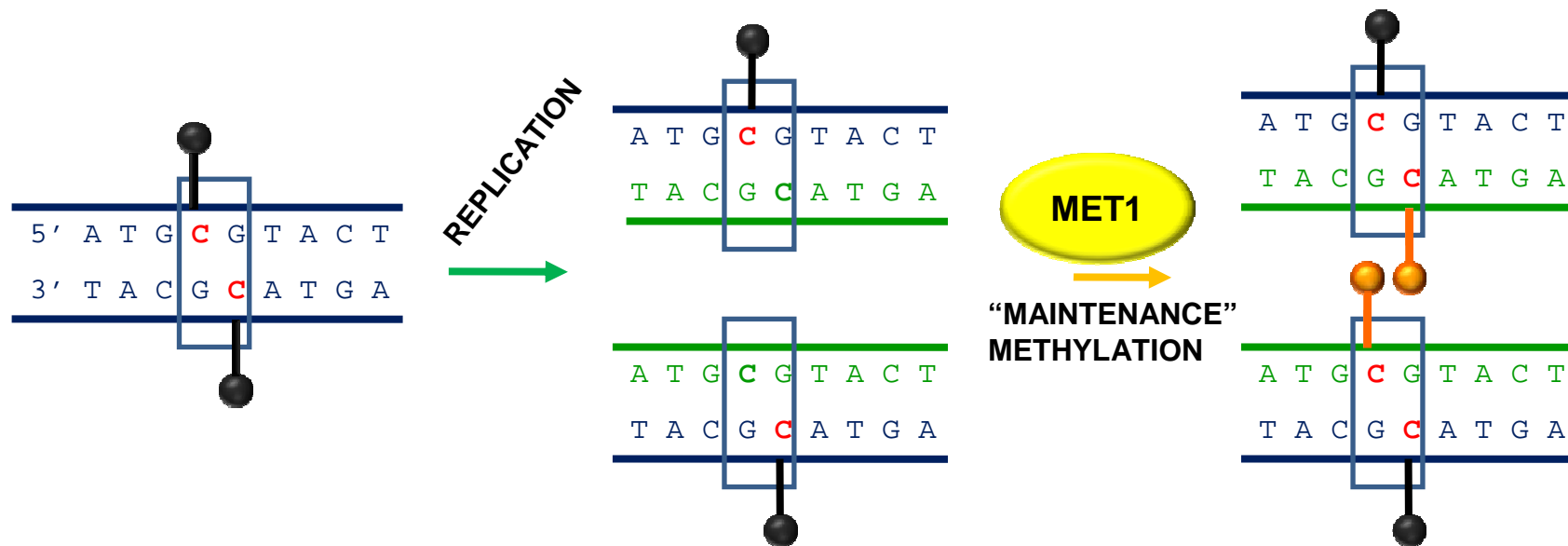
DNA can be covalently modified by cytosine methylation.



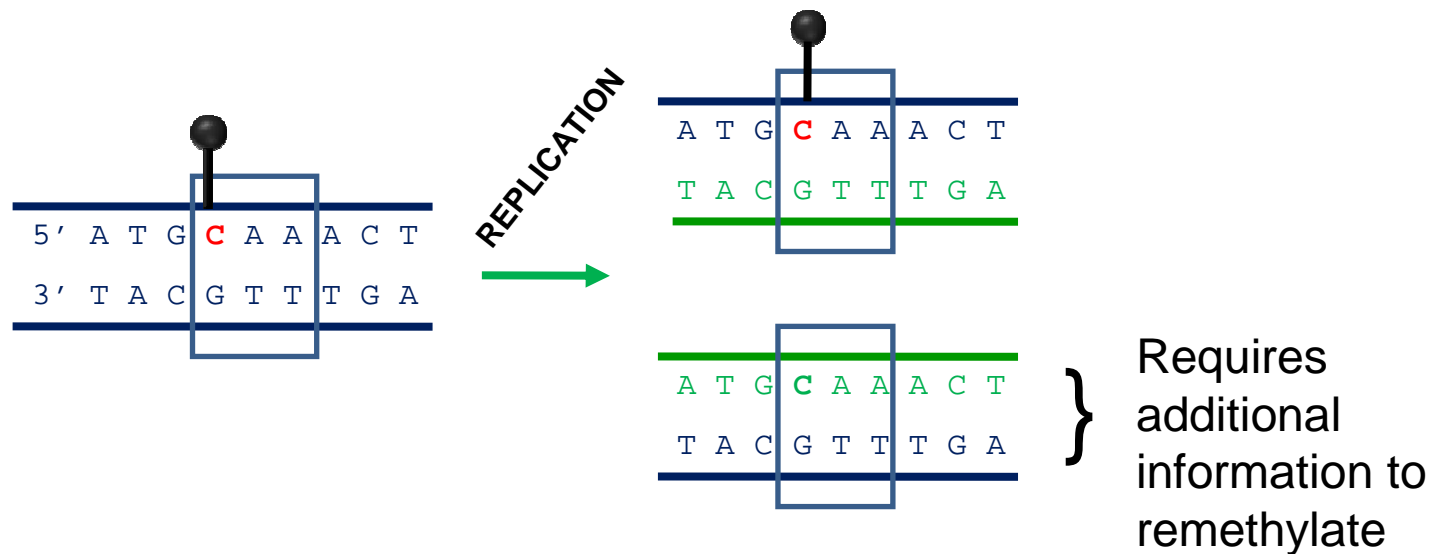
# DNA methyltransferases in Arabidopsis

- MET1 (METHYLTRANSFERASE1) – 5'-CG-3' sites
  - Silencing of transposons, repetitive elements, some imprinted genes
  - Homologue of mammalian DNMT1
- DRM1 and DRM 2 (DOMAINS REARRANGED 1 and 2) - 5'-CHH-3' sites (H= A, C or T)
  - DRM2: homologue of mammalian DNMT3
  - Primarily targets repetitive elements
  - Requires the active targeting of siRNAs
- CMT3 (CHROMOMETHYLASE3) – 5'-CHG-3' sites
- Uniquely found in plants
  - Interacts with histone mark H3K9me2 (transposons, repetitive regions)

# CG methylation can be propagated during DNA replication

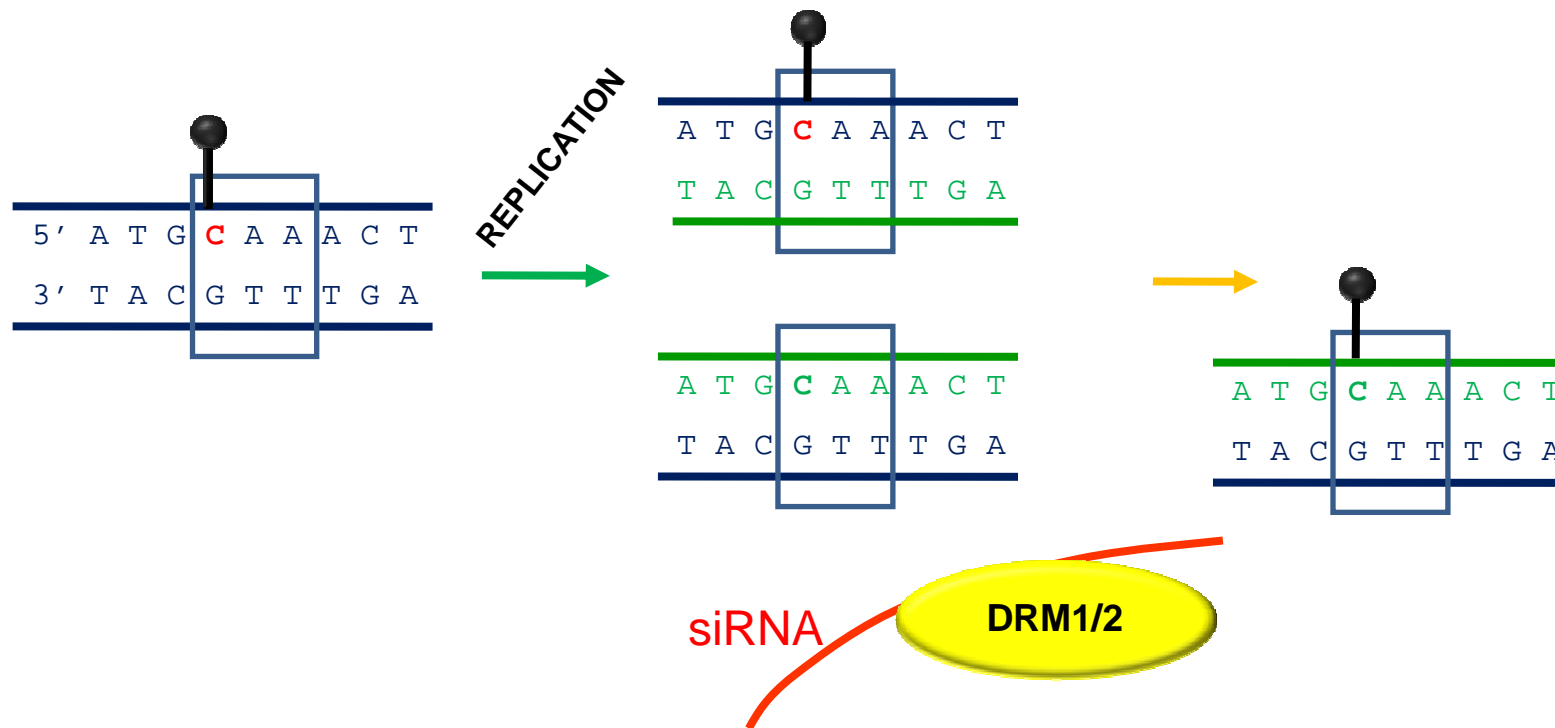


# Asymmetric methylation sites require additional information



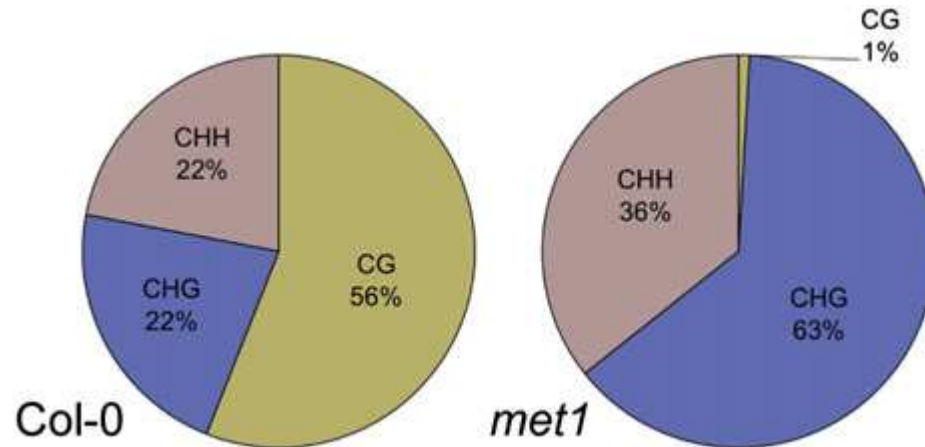
Asymmetric methylation sites are maintained (and initiated) by information on associated histones, and an RNA-based mechanism, RNA-directed DNA Methylation (RdDM), that directs DNA methylases to these sites.

# Some sites are maintained by small interfering RNAs (siRNAs)



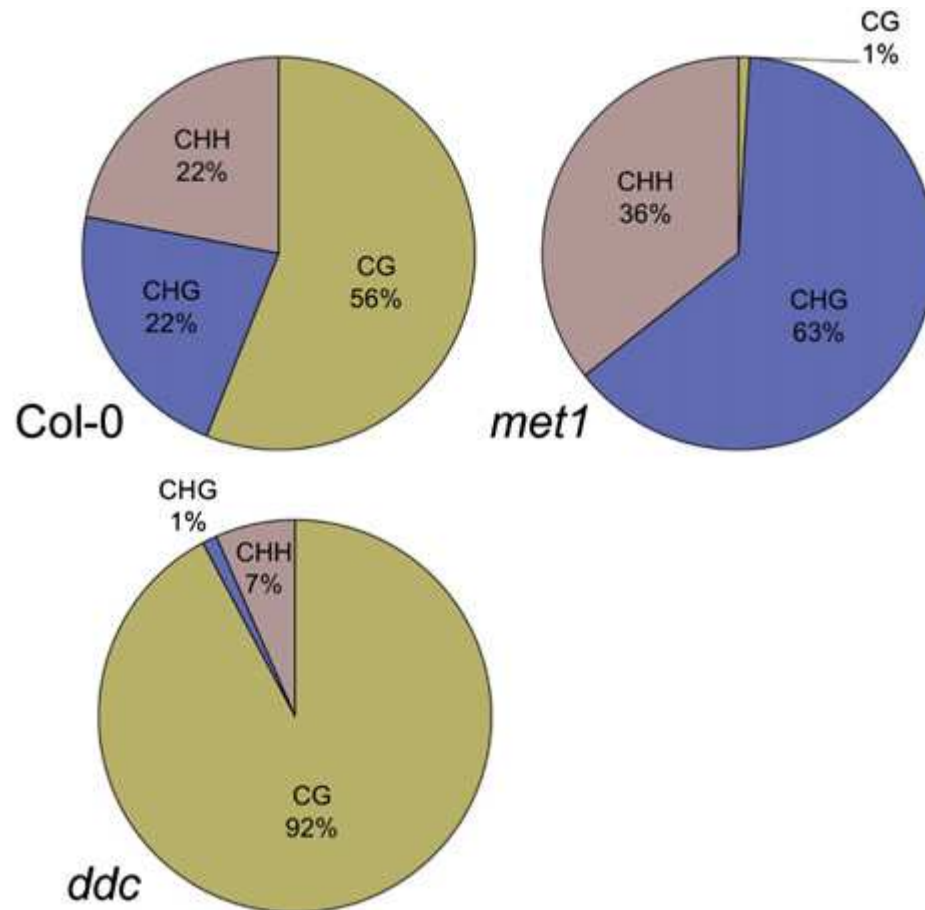


# Different DNA methylases act on different sites



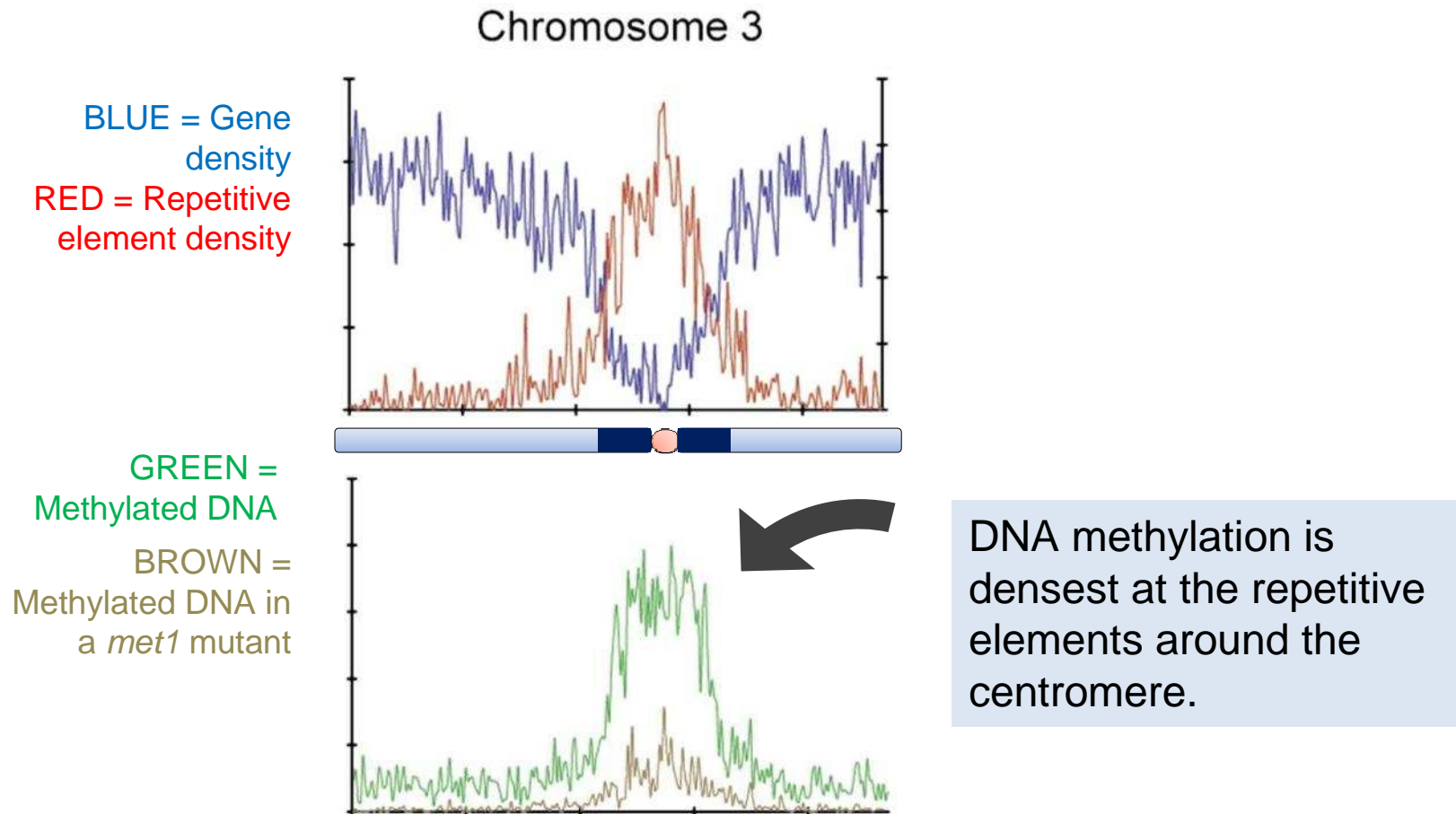
In the *met1* mutant, CG methylation is abolished, revealing that MET1 is solely responsible for CG methylation

# Different DNA methylases act on different sites



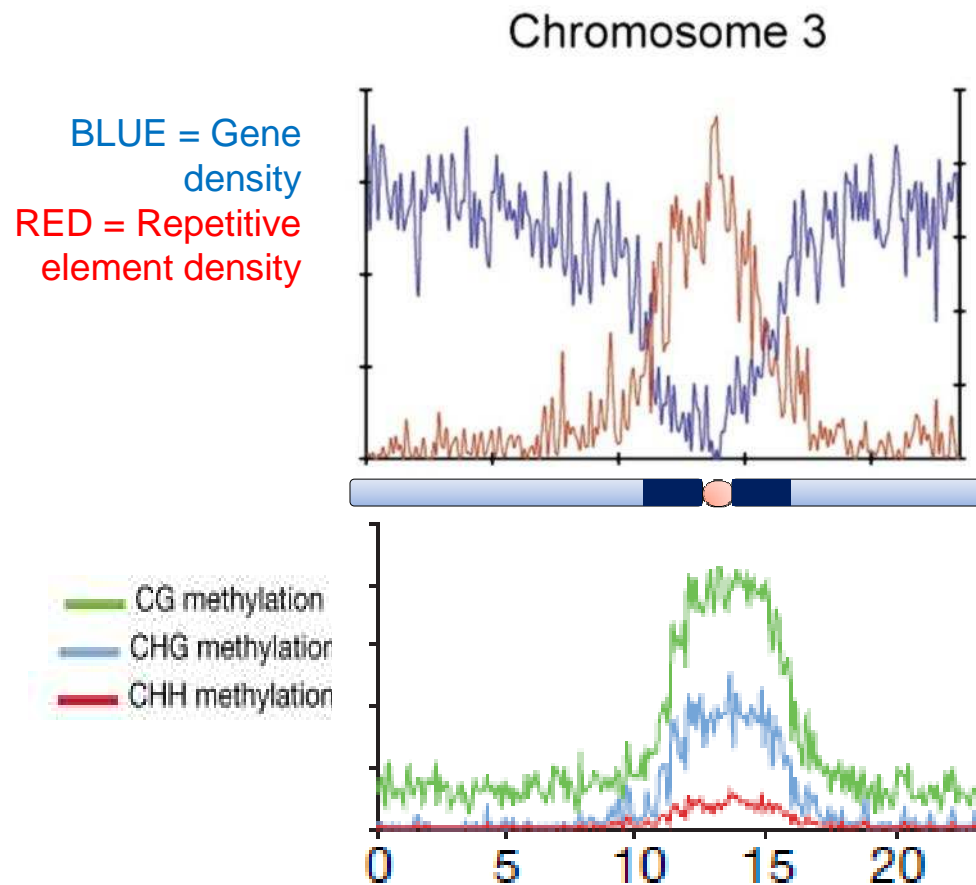
By contrast, in the triple mutant of *drm1*, *drm2* and *cmt3*, (*ddc*) nearly all non-CG methylation is abolished, revealing the roles of these genes in non-CG methylation.

# Heterochromatin DNA is highly methylated



Reprinted from Zhang, X., et al. (2006) Genome-wide high-resolution mapping and functional analysis of DNA methylation in *Arabidopsis*. Cell 126: [1189–1201](#) with permission from Elsevier.

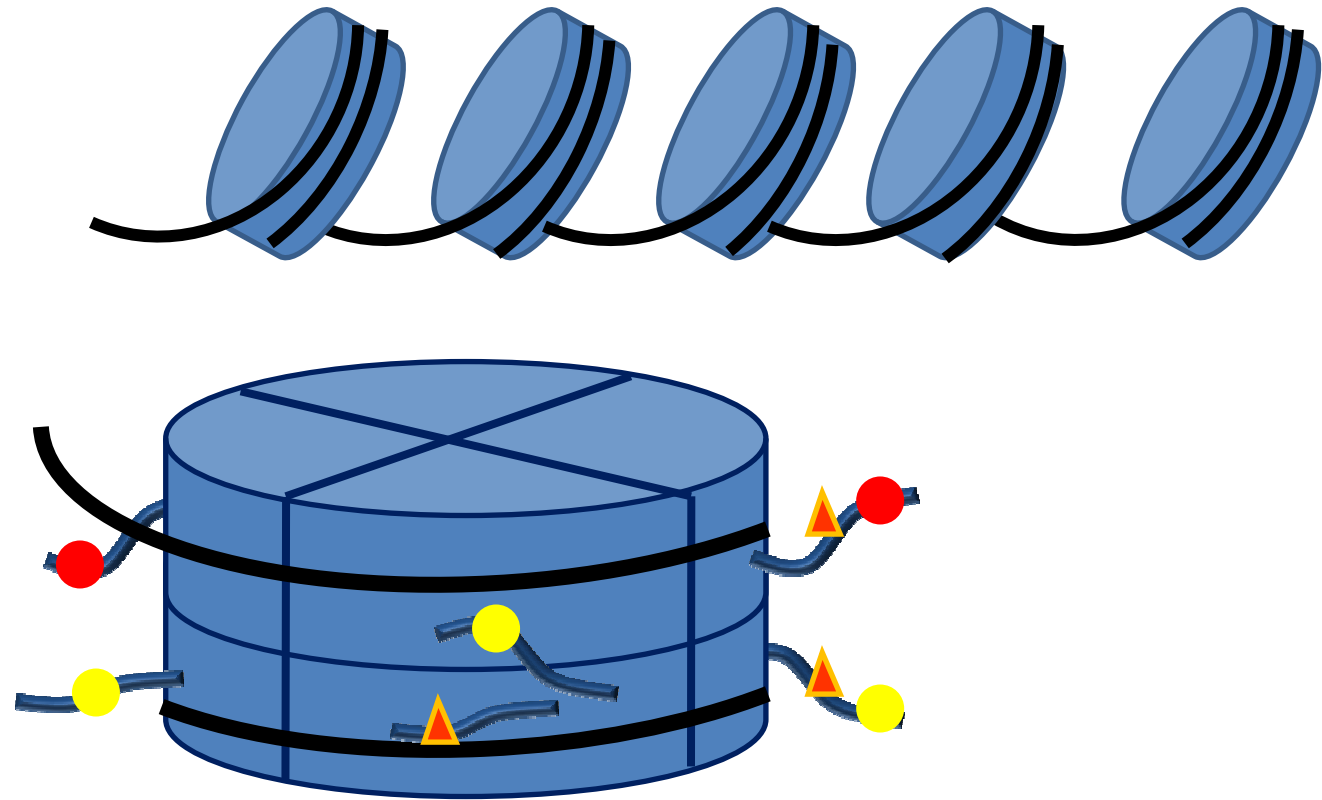
# Heterochromatin DNA is highly methylated



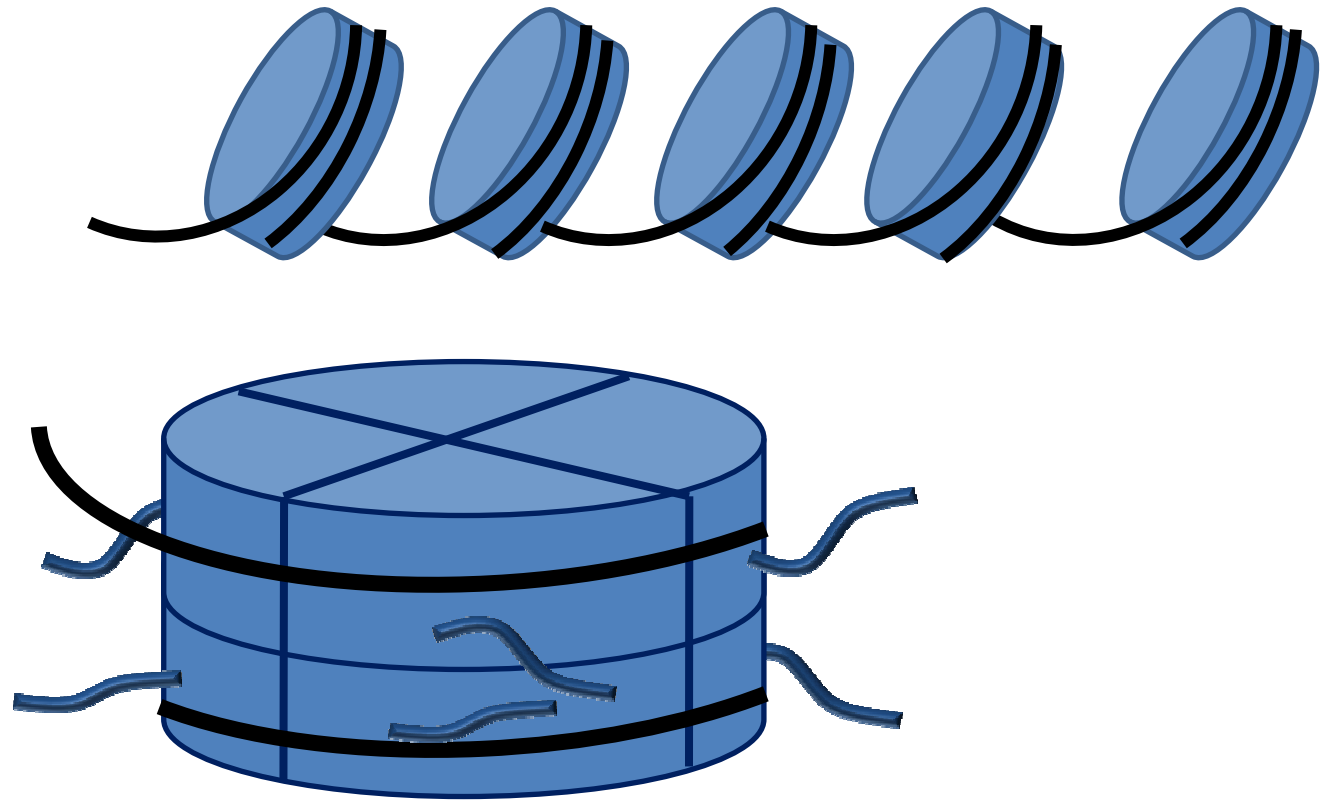
Although CG methylation is more abundant in pericentromeric regions, a higher proportion of CHH methylation is found there.

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.; Cokus et al., (2008) Shotgun bisulphite sequencing of the *Arabidopsis* genome reveals DNA methylation patterning *Nature* 452: [215-219](#).

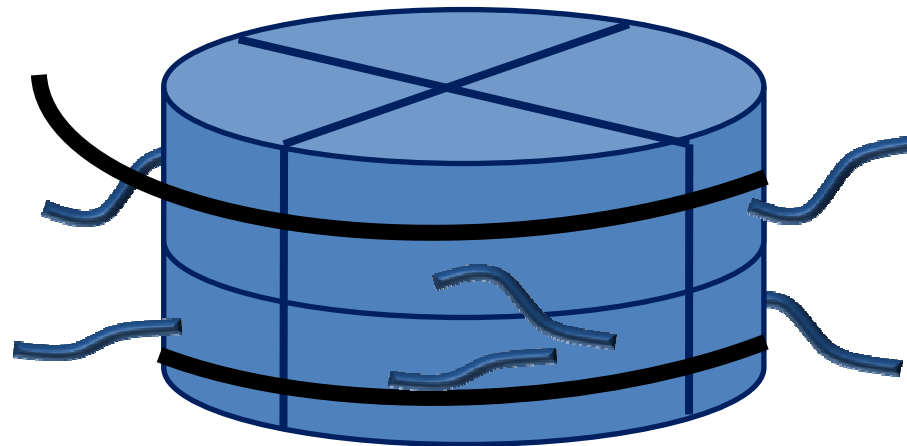
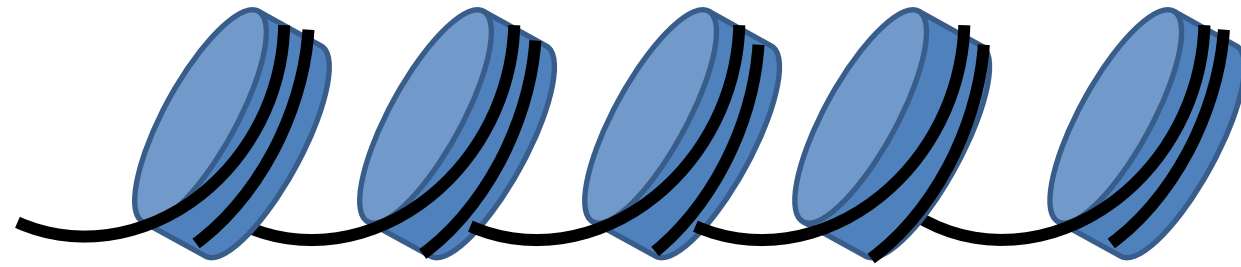
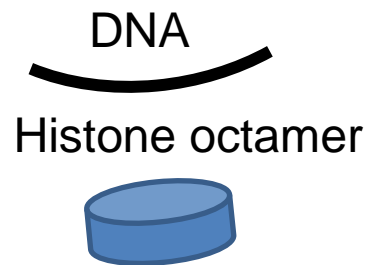
# Histone modification



Histone proteins can be modified  
to affect chromatin structure



# Histone proteins can be modified to affect chromatin structure



NUCLEOSOME

The amino terminal regions of the histone monomers extend beyond the nucleosome and are accessible for modification.

# The Histone Code

- Histones can be modified by
  - Acetylation (Ac)
  - Ubiquitination (Ub)
  - Methylation (Me)
  - Phosphorylation (P)
  - Sumoylation (Su)
- Depending on their position, these can contribute to transcriptional activation or inactivation.



# Example – H3 modifications

**H3**

	Me		Me P		Ac		Me Ac		Ac		Me Me P
	A R T <b>K</b> Q T A R	<b>K</b> <b>S</b> T G G	<b>K</b> A P	<b>R</b> <b>K</b> Q L A T	<b>K</b> A A	<b>R</b> <b>K</b> <b>S</b>					
	4	9 10	14	17 18	23	26 27 28					

The amino terminus of H3 is often modified at one or more positions, which can contribute to an activation or inhibition of transcription.

# Example – H3 modifications

**H3**

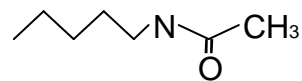
	Me		Me P		Ac		Me Ac		Ac		Me Me P
	A R T <b>K</b> Q T A R	<b>K</b> <b>S</b> T G G	<b>K</b> A P	<b>R</b> <b>K</b> Q L A T	<b>K</b> A A	<b>R</b> <b>K</b> <b>S</b>					
	4	9 10	14	1718	23	262728					

The amino terminus of H3 is often modified at one or more positions, which can contribute to an activation or inhibition of transcription.

## Lysine can be acetylated, or mono-, di-, or tri-methylated



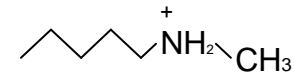
Lysine (K)



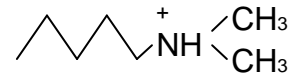
Acetylated lysine (KAc)

### Methylated lysine

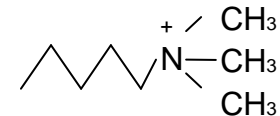
Mono (Kme1)



Di (Kme2)



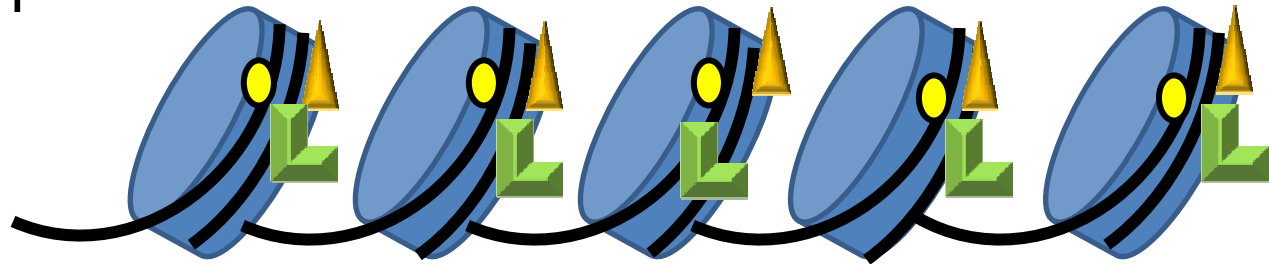
Tri (Kme3)



# Histone modification affects chromatin structure

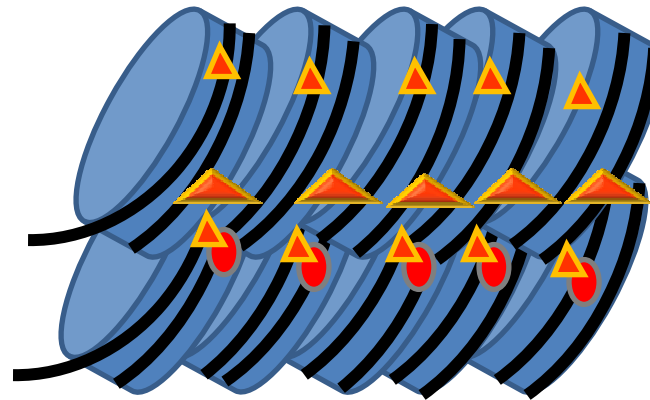
Open configuration

H3	Me	P	Ac
	K4	S10	K14

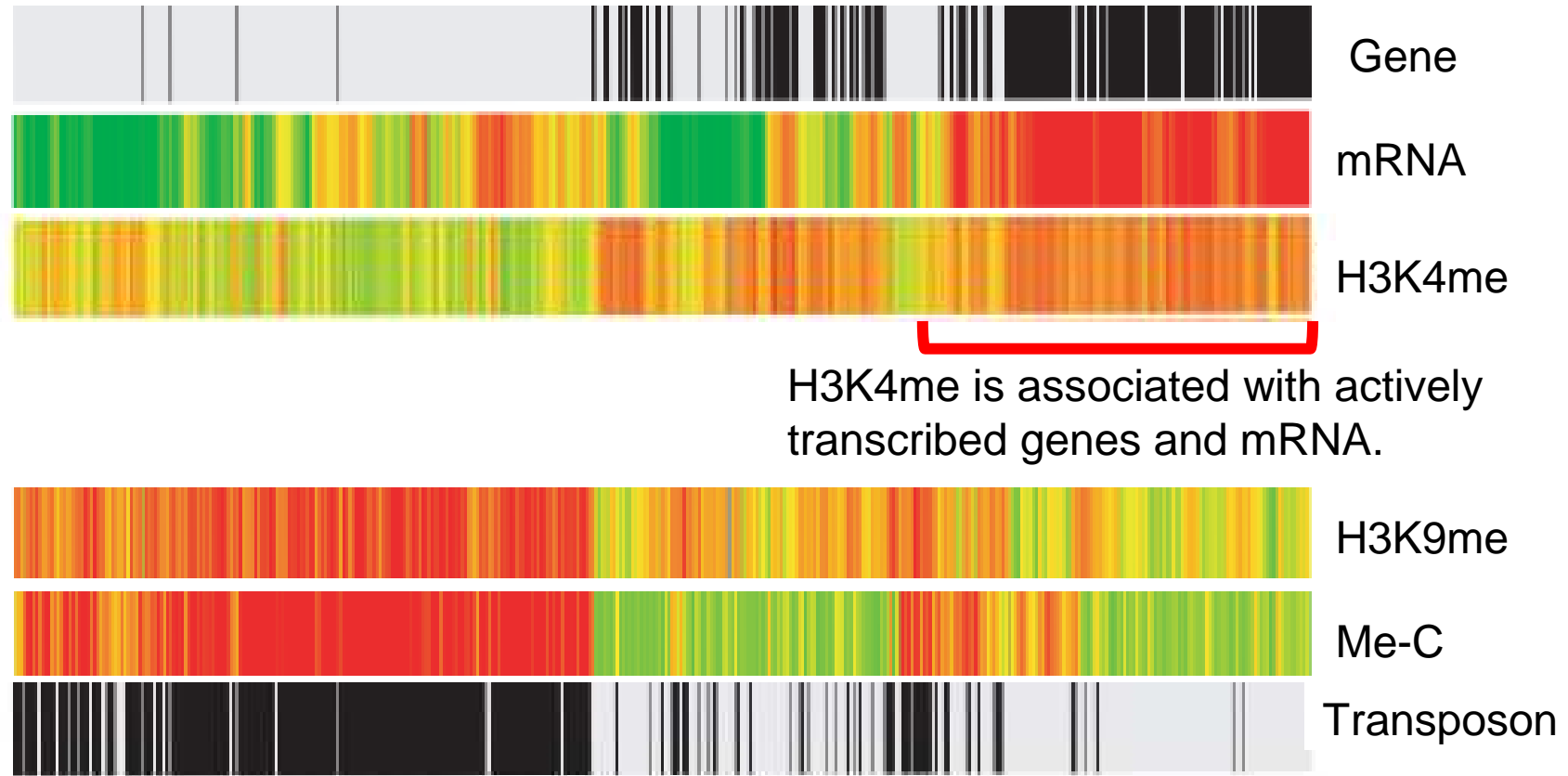


Closed configuration

H3	Me	Me P
	K9	K27 S28



# Different histone modifications are associated with genes and transposons

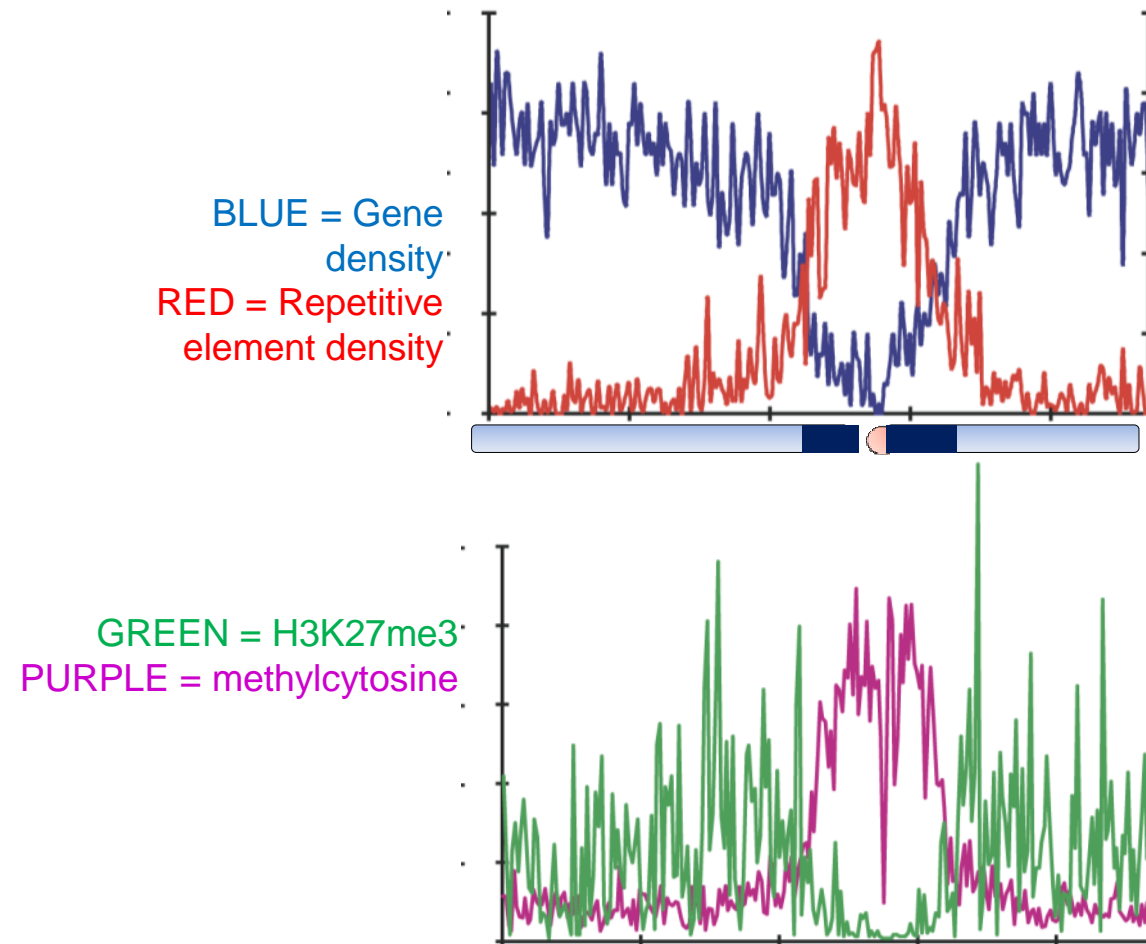


H3K4me is associated with actively transcribed genes and mRNA.

H3K9me is associated with methylated DNA (Me-C) and transposons.

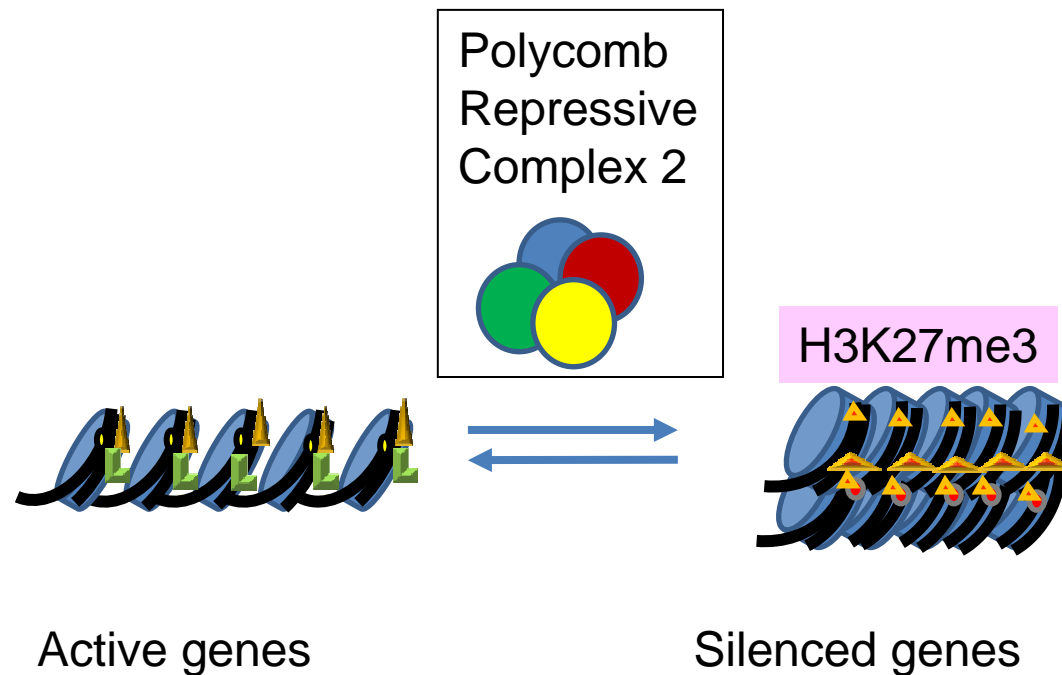
Red = high correlation  
Green = low correlation

# H3K27me3 is associated with genes



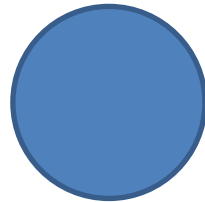
H3K27me3 in Arabidopsis is present within the gene-rich region, not the repeat-rich region.

# H3K27me3 methylation is conferred by Polycomb Repressive Complex 2



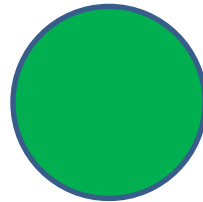
# PRC2 core proteins in Drosophila

Drosophila  
PRC2 has a  
conserved core  
of four proteins



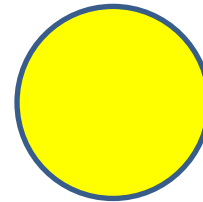
E(Z)

Enhancer of zeste (E(Z))



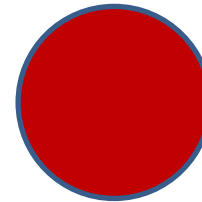
ESC

Extra sex comb (ESC)



SU(Z)12

Suppressor of zeste 12 (SU(Z)12)

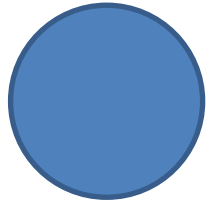


NURF55

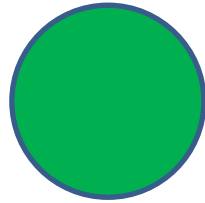
NURF55

# Arabidopsis has multiple forms of three of the core PRC2 proteins

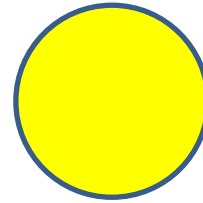
## Drosophila PRC2



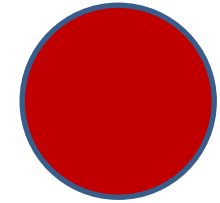
E(Z)  
(methylase)



ESC



SU(Z)12



NURF55

## Arabidopsis PRC2

CURLY LEAF (CLF)

MEDEA (MEA)

SWINGER (SWN)

FERTILIZATION  
INDEPENDENT  
ENDOSPERM  
(FIE)

FERTILIZATION-  
INDEPENDENT SEED 2  
(FIS2)

EMBRYONIC FLOWER 2  
(EMF2)

VERNALIZATION 2 (VRN2)

MULTICOPY  
SUPPRESSOR  
OF IRA1  
(MSI1,2,3,4,5)



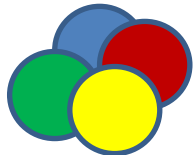
# Plants make multiple PRC2 complexes with different targets



MEA + FIS2 complex



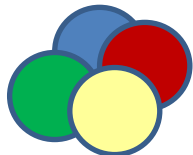
Seed development



CLF/SWN + VRN2 complex



Transition to flowering

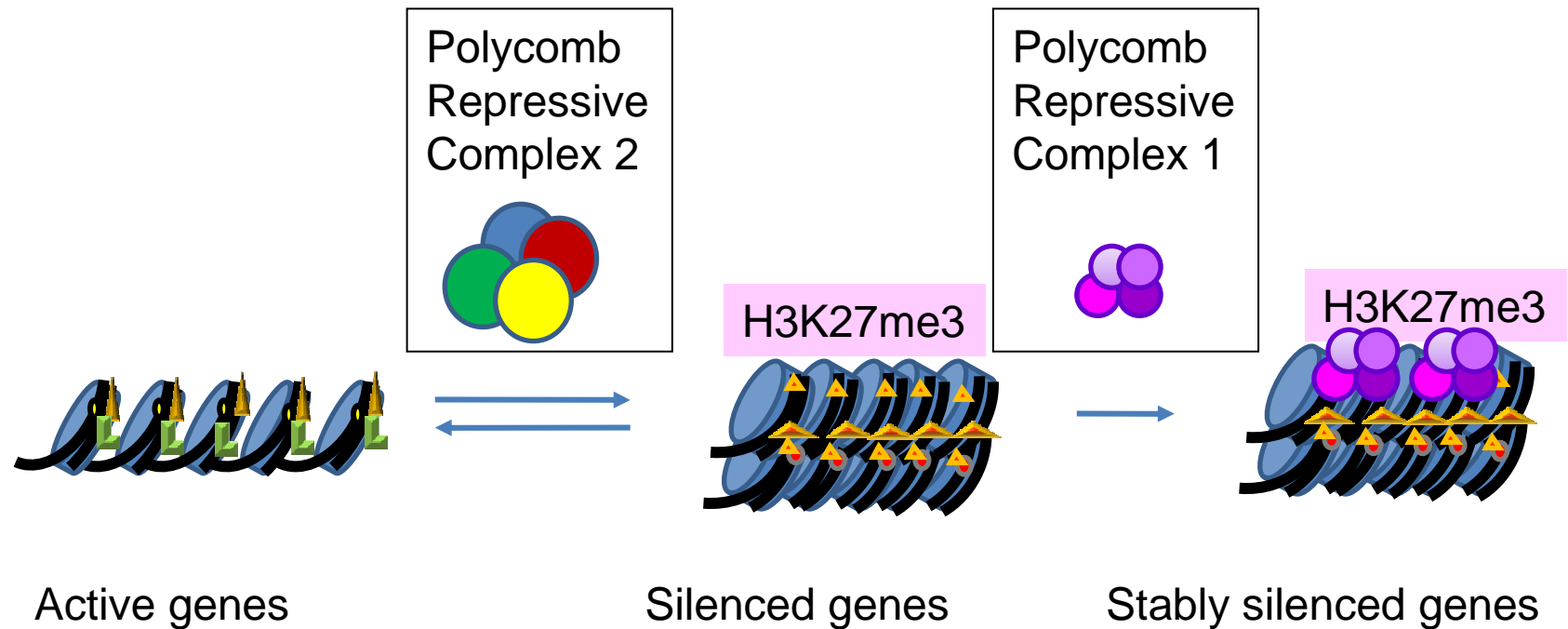


CLF/SWN + EMF2 complex

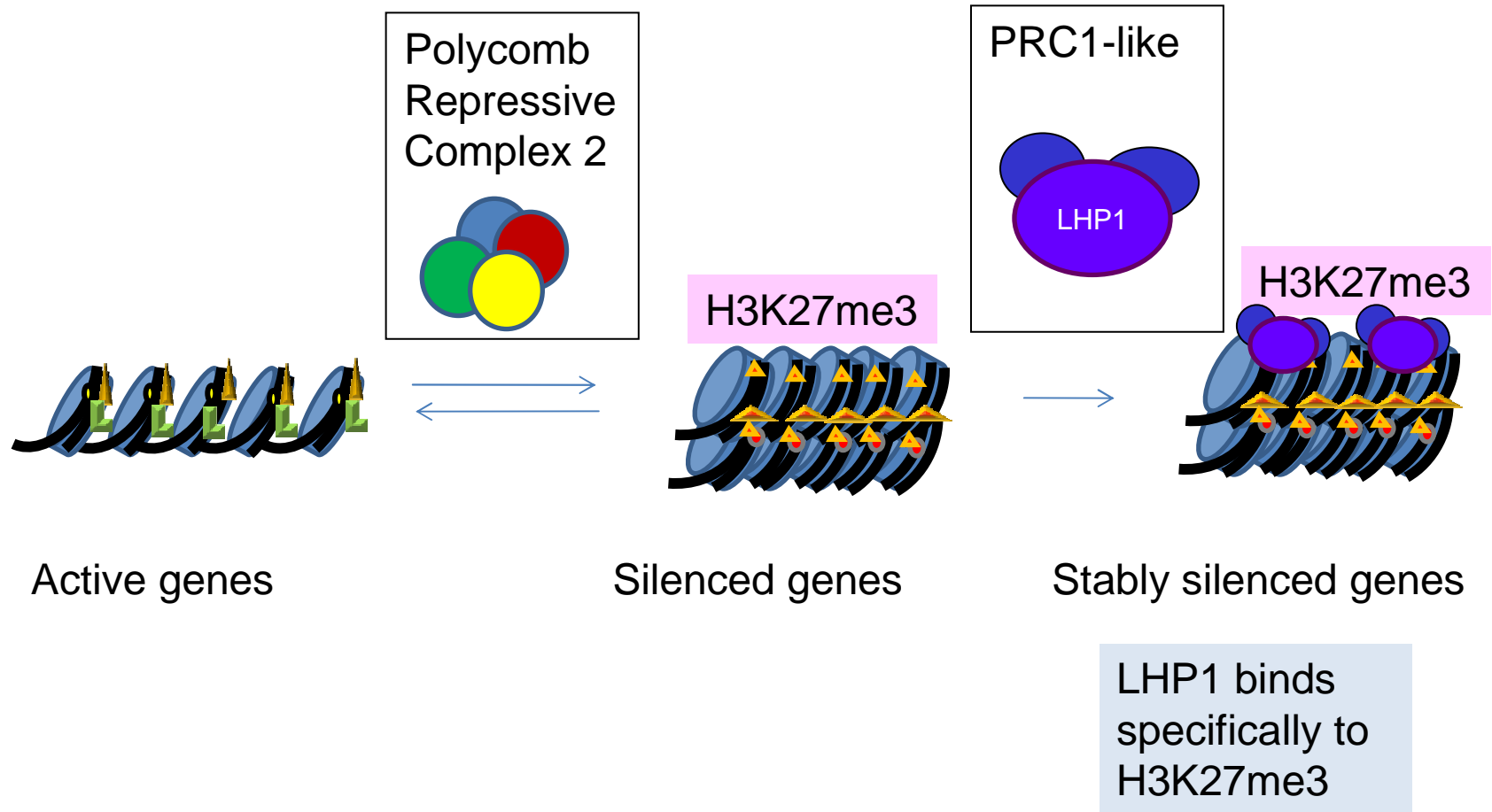


Floral organogenesis

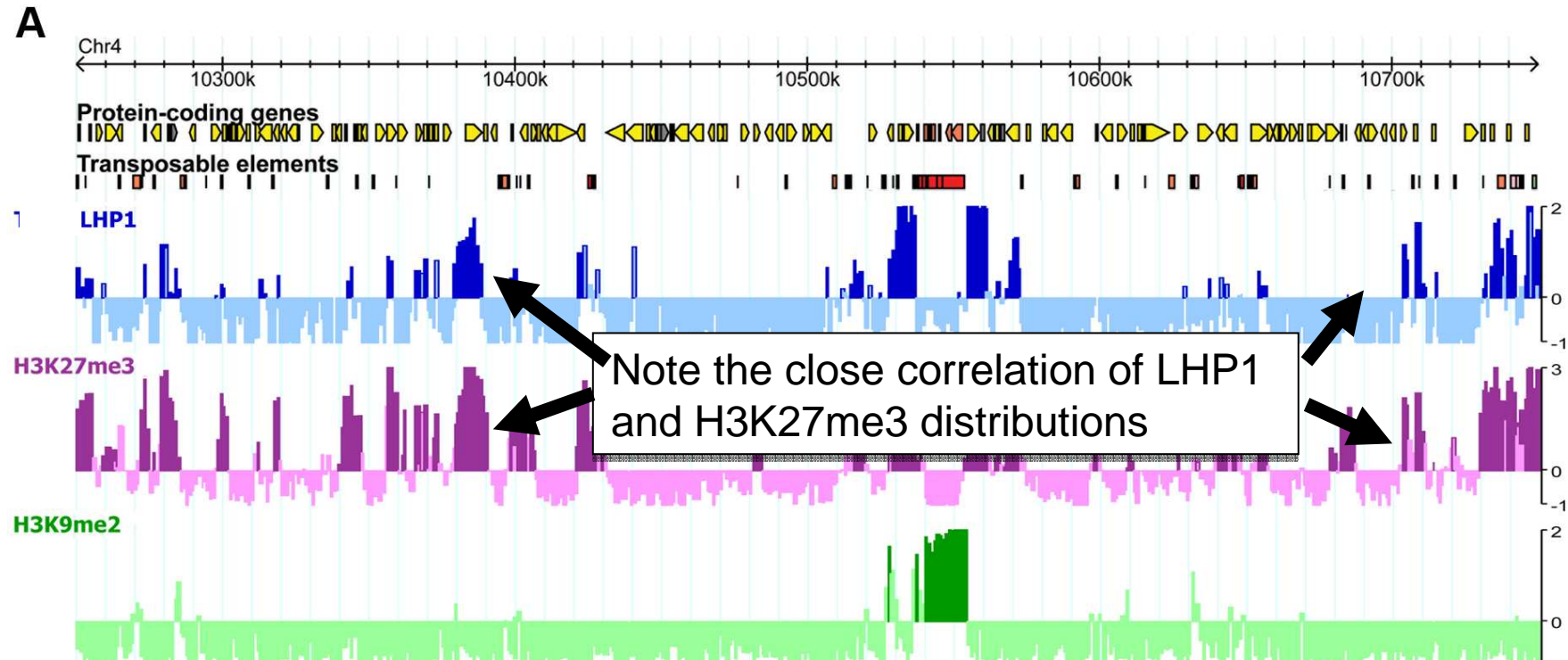
# In animals, H3K27me3 silencing is *maintained* by PRC1



# In plants, PRC1-like functions similarly

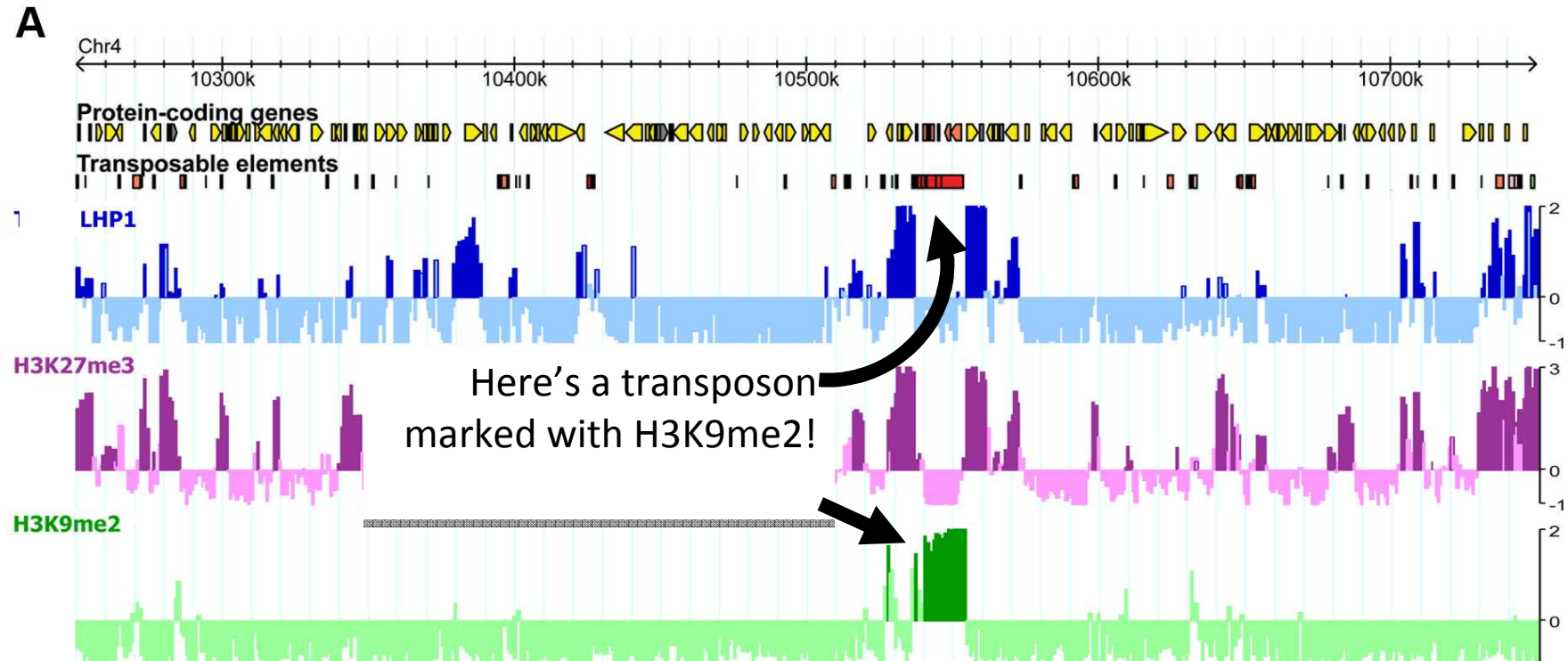


# LHP1 co-localizes with H3K27me3



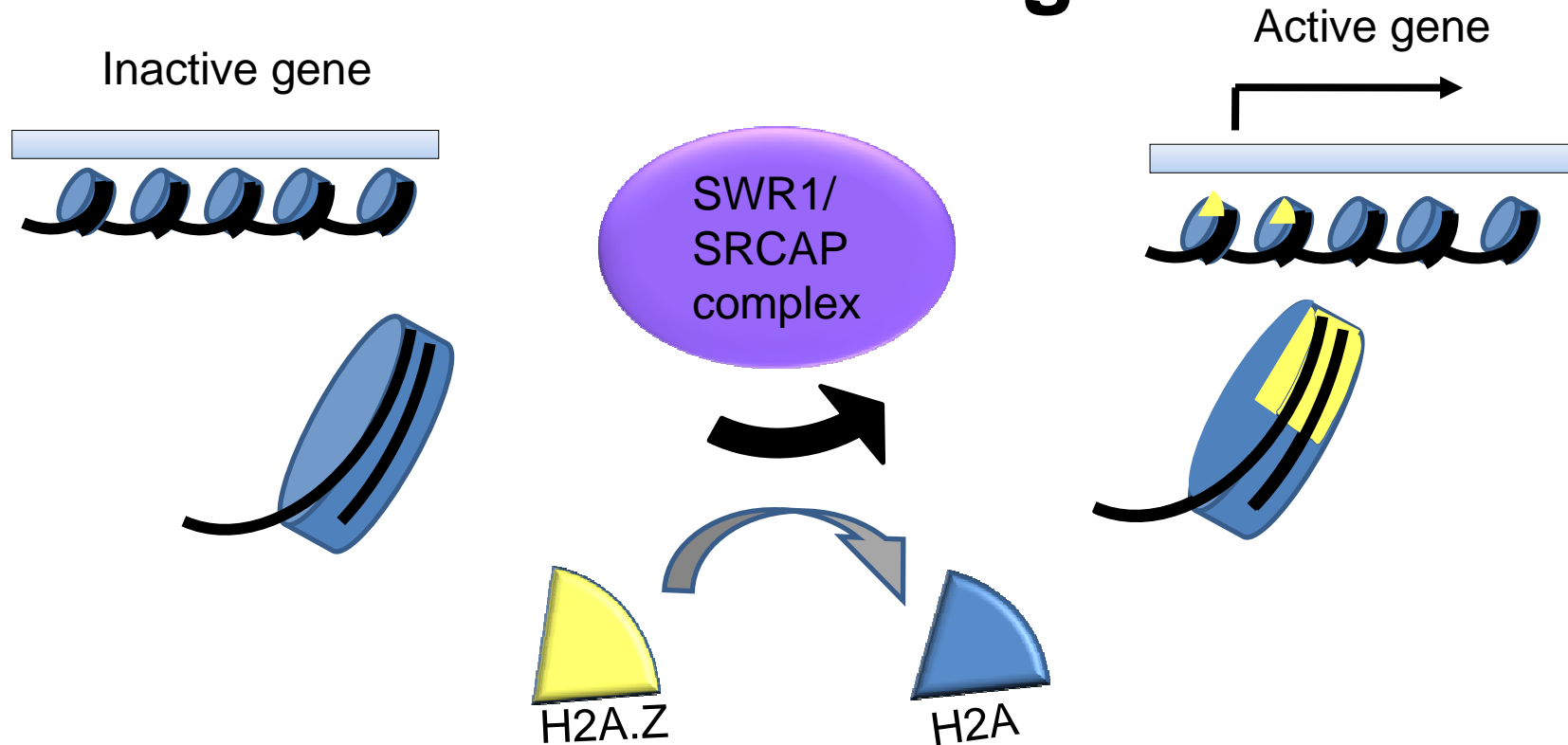
Turck F, Roudier F, Farrona S, Martin-Magniette M-L, Guillaume E, et al. 2007 Arabidopsis TFL2/LHP1 specifically associates with genes marked by trimethylation of histone H3 lysine 27. *PLoS Genet* 3(6): [e86](#).

# LHP1 co-localizes with H3K27me3



Turck F, Roudier F, Farrona S, Martin-Magniette M-L, Guillaume E, et al. 2007 Arabidopsis TFL2/LHP1 specifically associates with genes marked by trimethylation of histone H3 lysine 27. PLoS Genet 3(6): [e86](#).

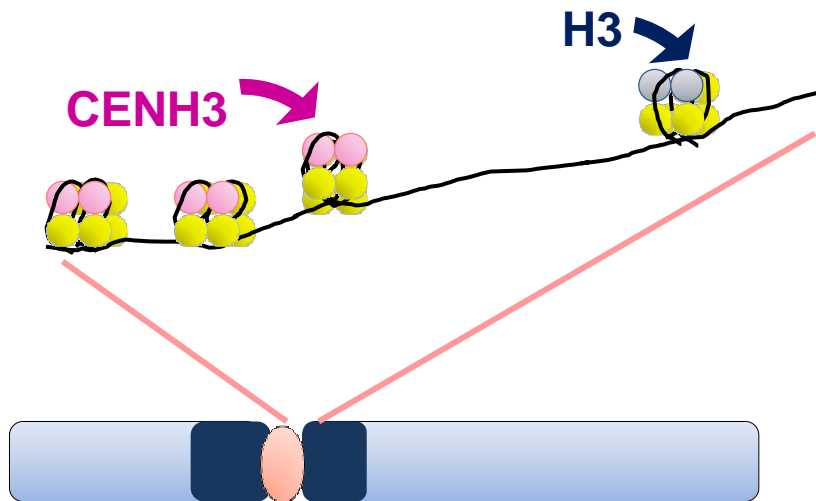
# An H2 variant, H2A.Z is associated with some active genes



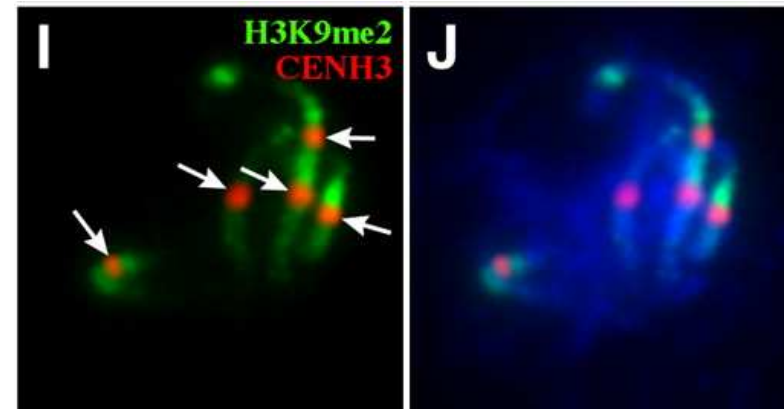
The histone variant H2A.Z promotes transcription and is swapped into the nucleosome by the SWR1/SRCAP complex.

SWR1 complex mediates the ATP-dependent exchange of histone H2A for the H2A variant H2A.Z leading to transcriptional regulation of selected genes by chromatin remodeling.

# An H3 variant, CENH3, specifies centromeres



Nucleosomes at the centromere incorporate an H3 variant called CENH3.



The **CENH3**-containing centromere is flanked by the **H3K9me2**-enriched pericentromere.

# Summary – epigenetic marks

