CHAIRE ÉPIGÉNÉTIQUE ET MÉMOIRE CELLULAIRE

Année 2014-2015 :
"Chromatine et Mémoire cellulaire"

16 Février, 2015

Cours III
"Les systèmes de mémorisation liés à la chromatine (2)"

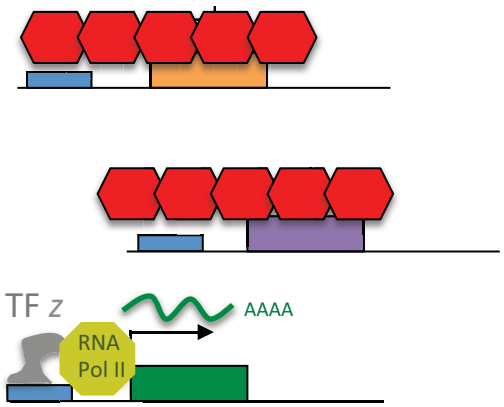
Epigenetics: memorisation of gene activity states

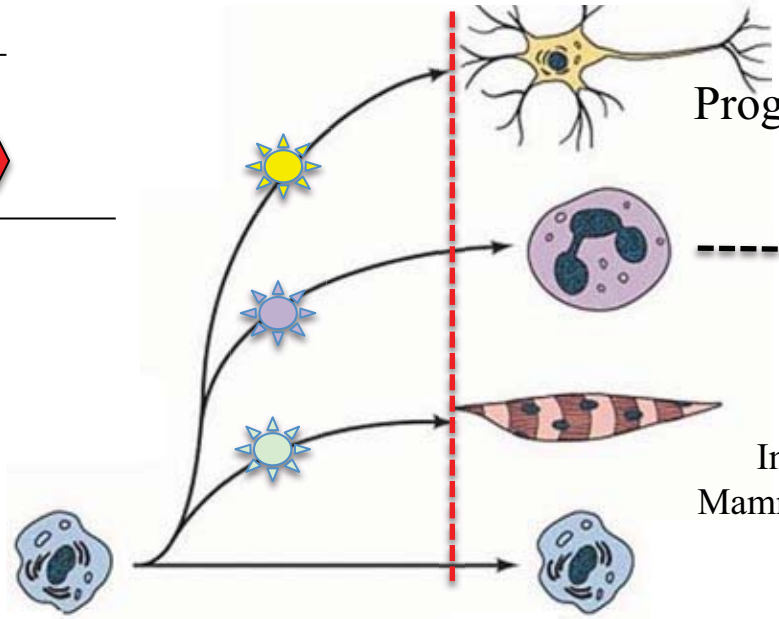
Different cell identities must be maintained throughout life

Developmentally induced

Heritable (somatic) states

(Epigenetic "Barriers")





Programmed or accidentally altered epigenomes

Phenotypic consequences?

Plants: eg vernalisation

Insects: eg social behaviour, life-span

Mammals: physiology, behaviour, disease…?

One Genome

Multiple gene expression & chromatin states (Epigenomes)

DNA sequence-specific Transcription Factors & Signalling pathways

(positional information, cell-cell contacts, growth factors, etc (to establish cell type, patterning, morphogenesis)

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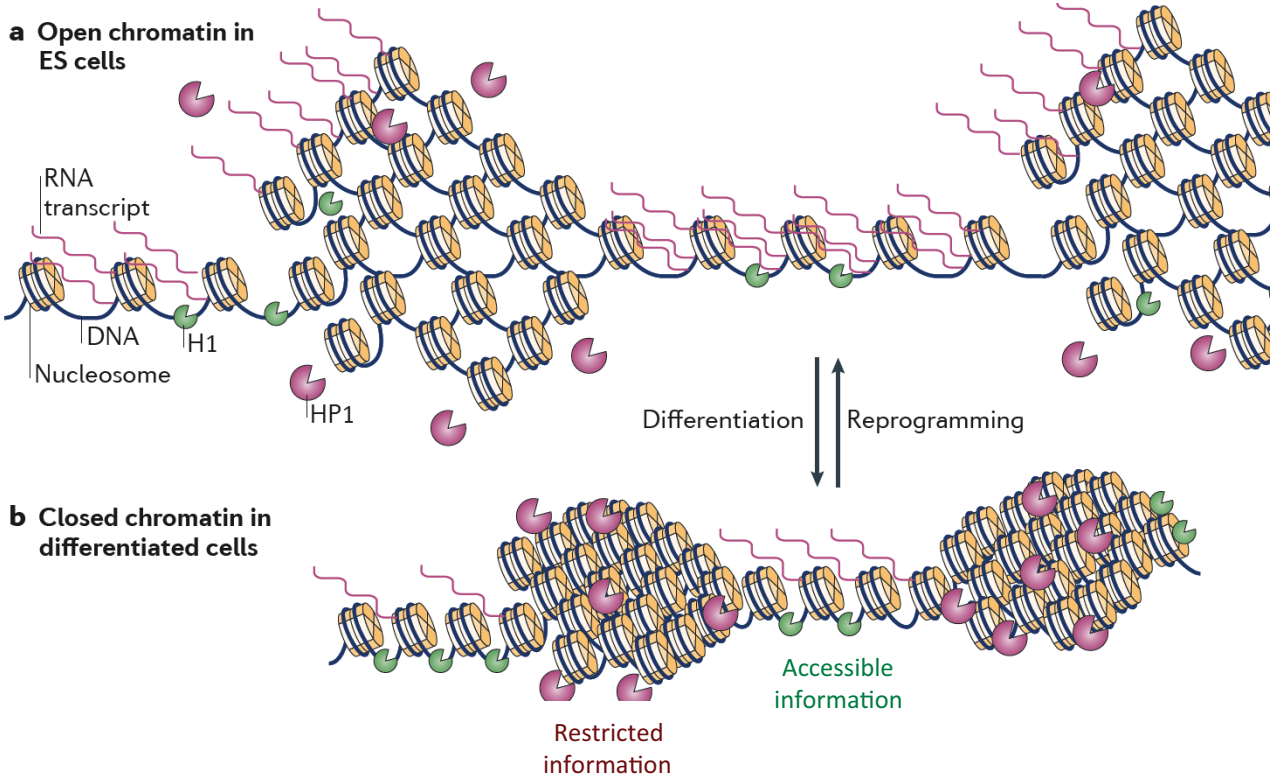
Epigenetics: memorisation of gene activity states



Chromatin states and chromatin compaction change during development and in disease.

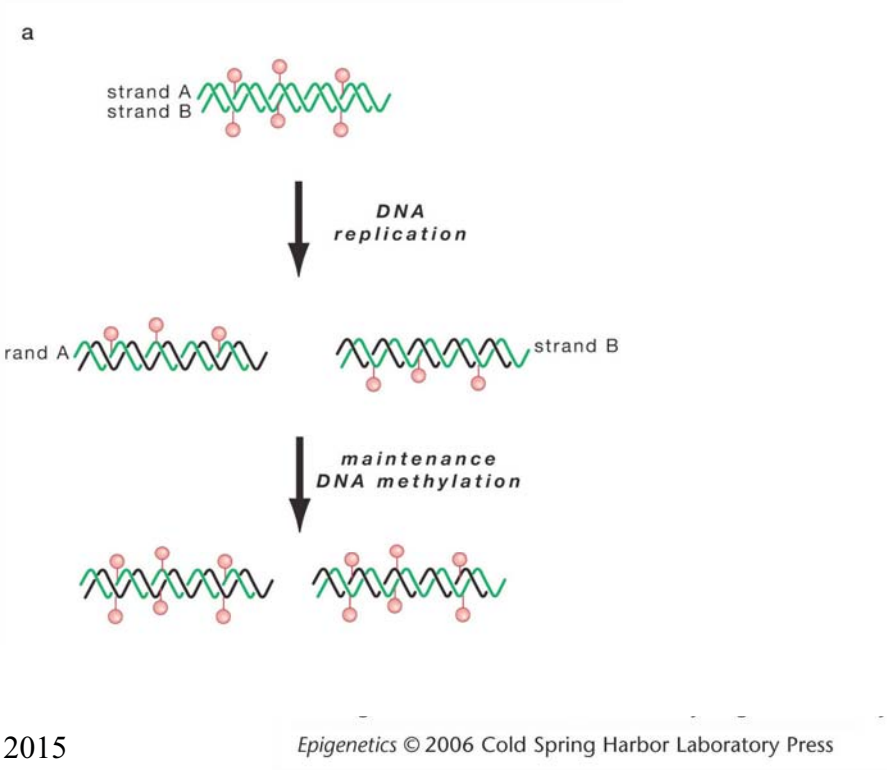
How many of these changes are *memorable* (epigenetic)?
How heritable are changes in chromatin?

Chromatin as the physiological template of the genome and a carrier of cellular memory?

Summary of last week

- **Are chromatin marks truly heritable during cell division?**

- Copying system based on DNA template for DNA Methylation during replication



Epigenetics © 2006 Cold Spring Harbor Laboratory Press

Chromatin as the physiological template of the genome and a carrier of cellular memory?

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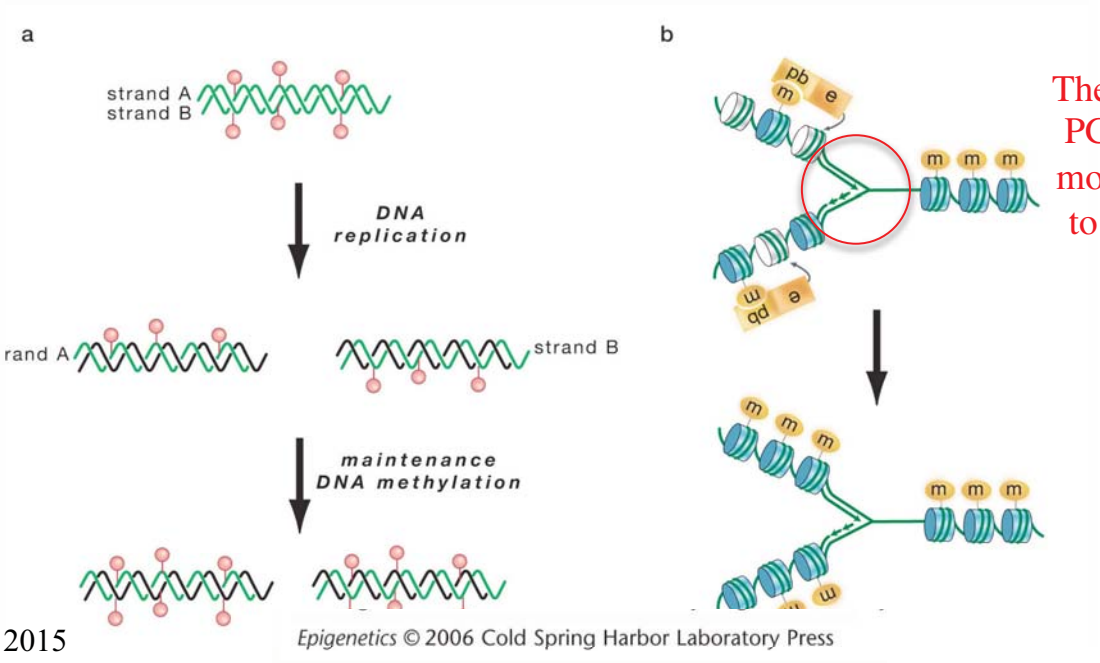

The DNA "replisome" (especially PCNA) recruits some chromatin modifiers and histone chaperones to redistribute nucleosomes and reproduce chromatin states

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- Chromatin dynamics during cell cycle: to maintain states need constant propagation - "**domains**" of chromatin are **required to stably maintain states**

Feed forward loops for epigenetic maintenance

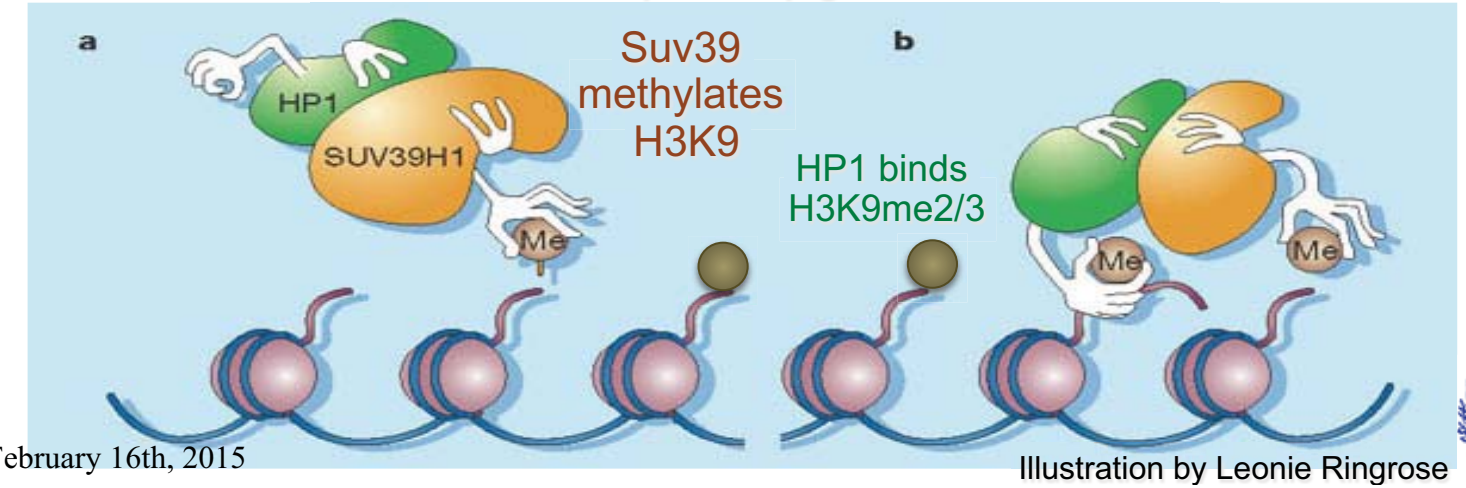

Illustration by Leonie Ringrose

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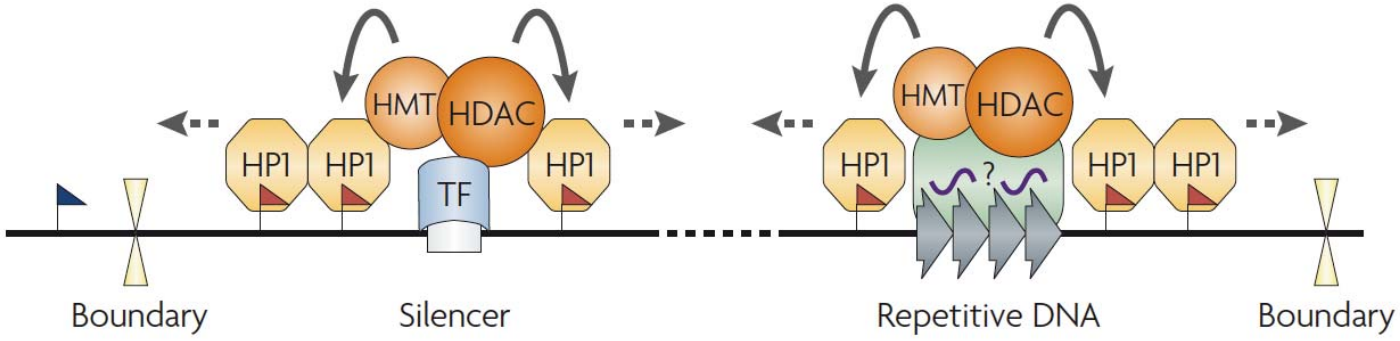

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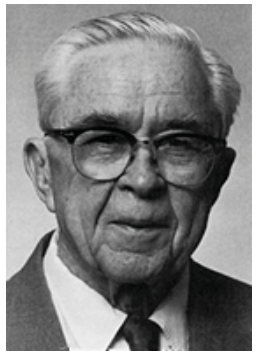
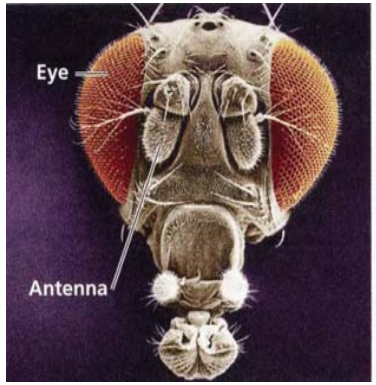
- But they must not spread too far (boundaries?), and must be reversible => constant addition and removal to prevent **aberrant inheritance (H3K9 demethylases)**
- **Replication timing** and **positioning of chromatin in the nucleus** may also be important to propagate memory states…

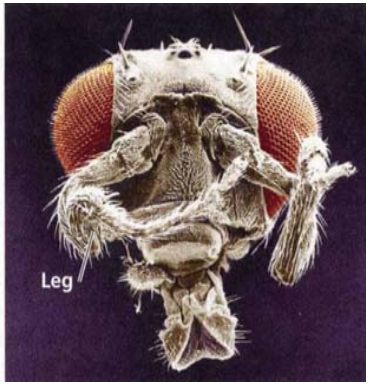
Chromatin memory in maintaining cell identity: Polycomb and Trithorax

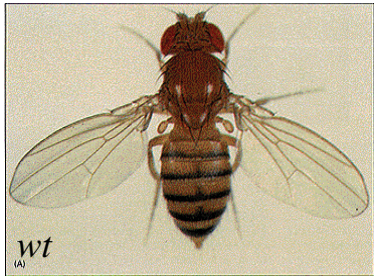
Homeotic Transformations
altered body plans and cell identity



Ed Lewis
(1918-2004)







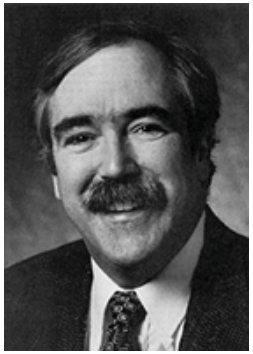
Walter Gehring
(1939-2014)

Genetic analyses identified defects in single genes causing homeotic transformations (cells in one region of the body behave as though they were located in another – from Greek *homeosis*

Hox genes: Transcription Factors that control the correct **timing** and **spatial pattern** of expression of batteries of organogenesis genes during development
(1983: Gehring; Kaufman)

Genetic control of establishment and maintenance of appropriate gene expression patterns during development

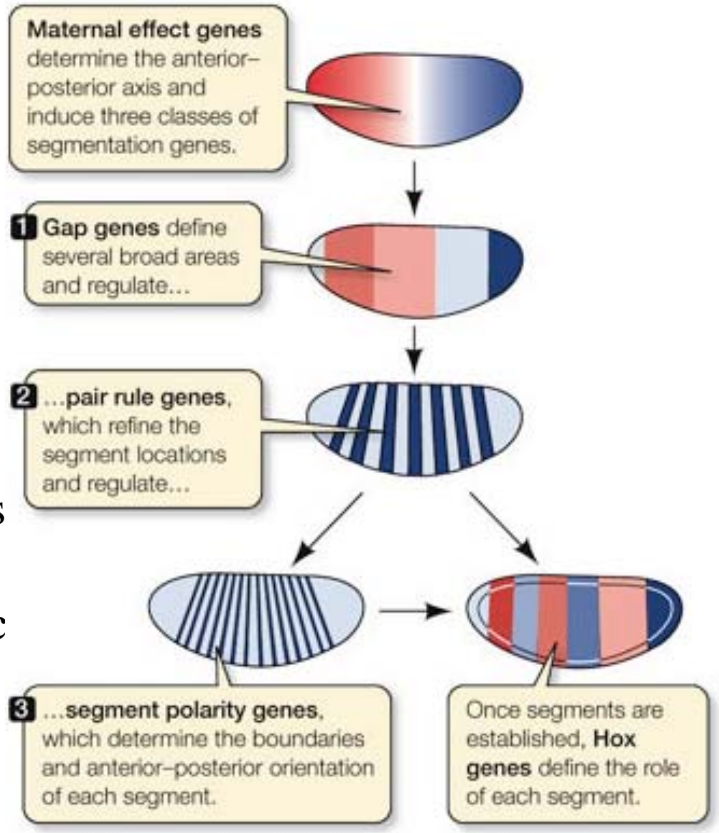
C. Nusslein-Volhard

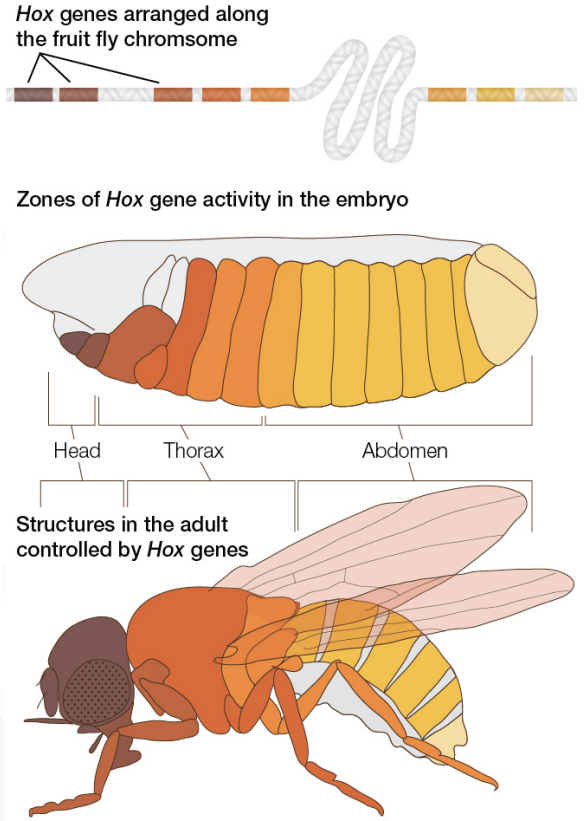


Eric Wieschaus

Maternal transcription factors (eg Gap and Pair-Rule) first establish patterns of homeotic gene expression.

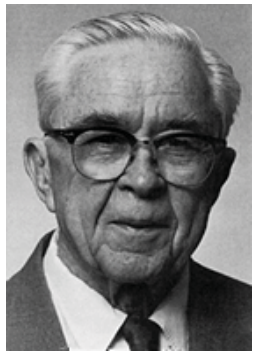
Different homeotic genes are then expressed in different segments giving it a specific identity





Memory of this *positional* information must be conserved up to the adult stage: mis-expression causes developmental defects and homeotic transformations

Polycomb and Trithorax: master controllers of cellular memory during development



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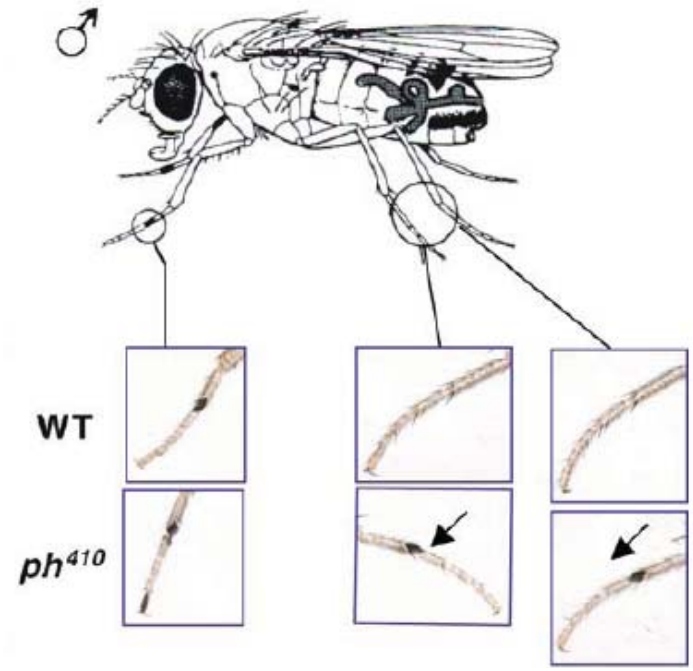
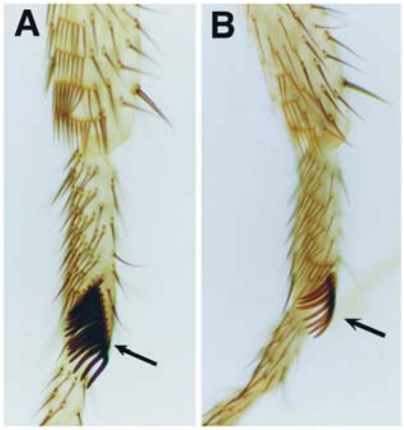


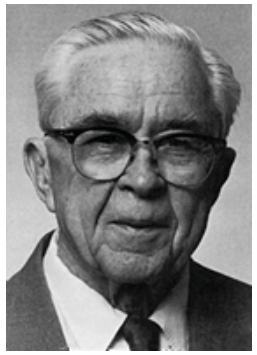
From Grimaud et al, Chrom. Res. 2006



Ed Lewis's genetic analyses also identified mutants not affecting HOX gene *products* but their **spatial control.**

E.B. Lewis (1978) "A gene complex controlling segmentation in Drosophila" Nature, 276: 565–570
I.M. Duncan (1982) Polycomblike: a gene that appears to be required for the normal expression of the bithorax and antennapedia gene complexes of Drosophila melanogaster. Genetics, 102: 49–70

Polycomb and Trithorax: master controllers of cellular memory during development



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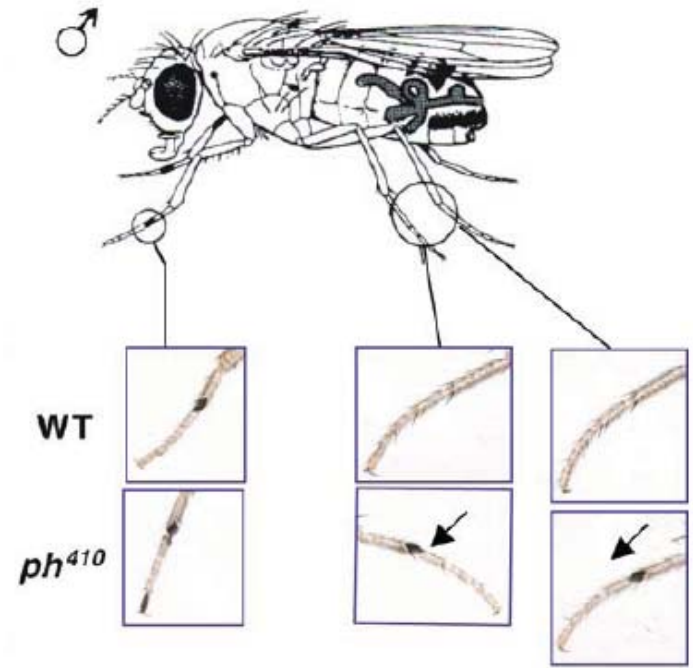

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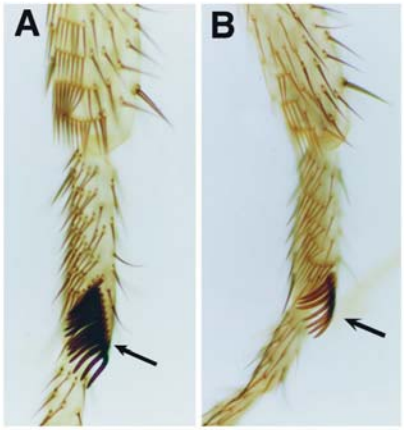

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These regulators (eg Polycomb, extra sex combs) were crucial in **maintaining the fate of individual body segments** determined by the action of the HOX genes.

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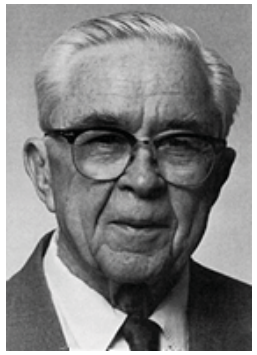

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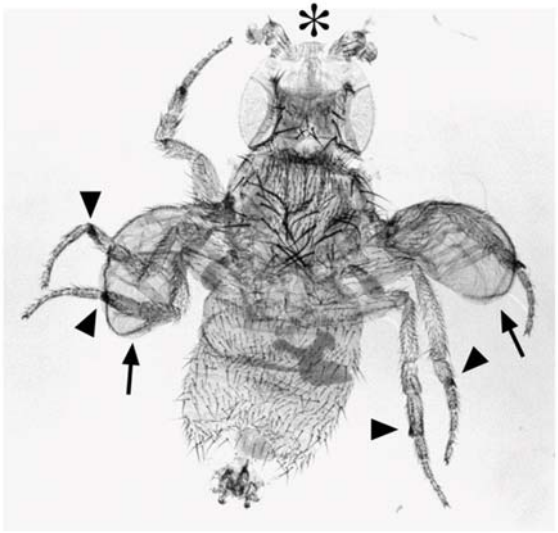
Homeotic transformations in a Su(z)12 mutant pharate adult male.



Su(z)12^{5}*/ Su(z)12*^{3}

Antennae partially transformed into legs

Sex combs present not just on first legs but all legs

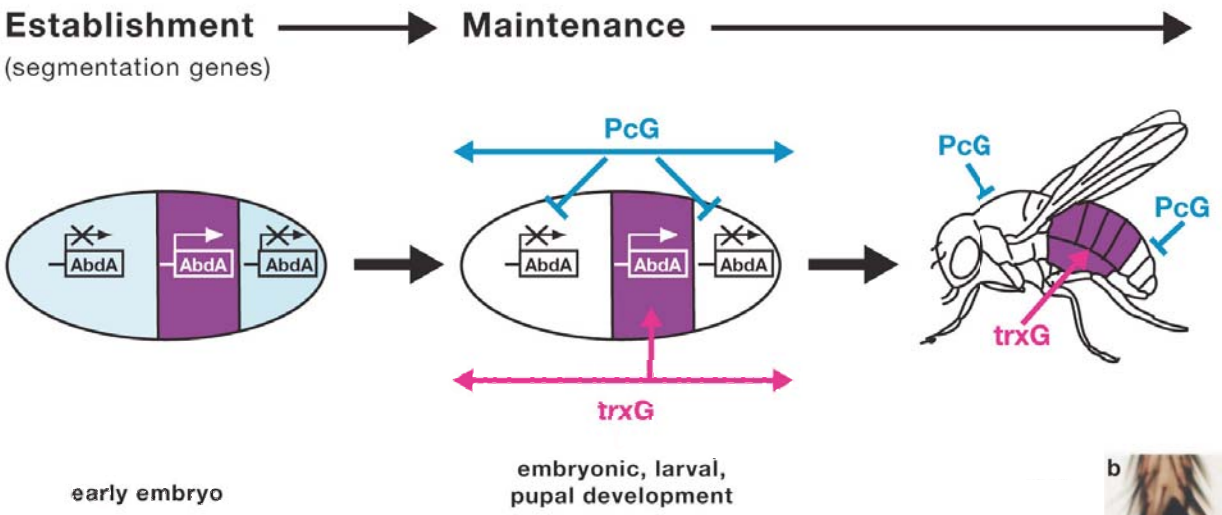
Wings partially transformed into haltere structure

Some of these mutants are also suppressors/enhancers of Position Effect Variegation (PEV)

These regulators (eg Polycomb, extra sex combs) were crucial in **maintaining the fate of individual body segments** determined by the action of the HOX genes.

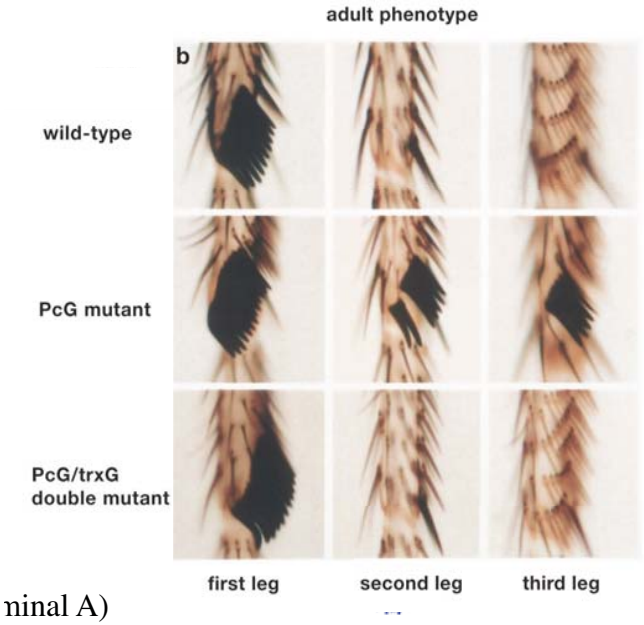
Polycomb and Trithorax: master controllers of cellular memory during development

Mutations in several regulatory genes lead to improper gene expression during development, classified into two antagonistic groups: Polycomb (PcG) & trithorax (trxG)



Trithorax mutations block the derepression of *Hox* genes in Polycomb group mutants (P.W. Ingham)

Myeloid/lymphoid or mixed-lineage Leukemia (MLL) = human homolog



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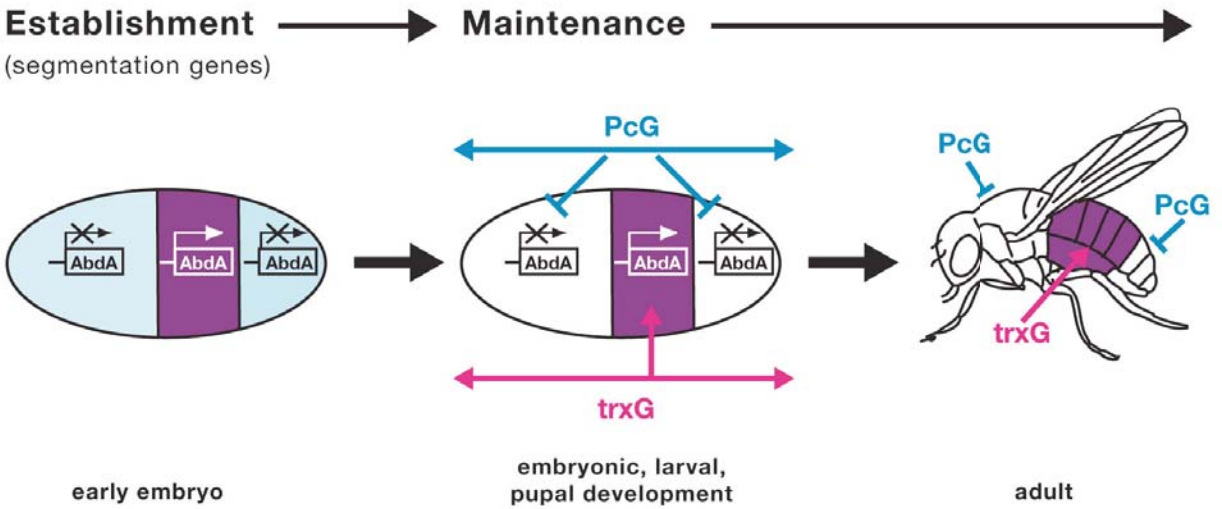
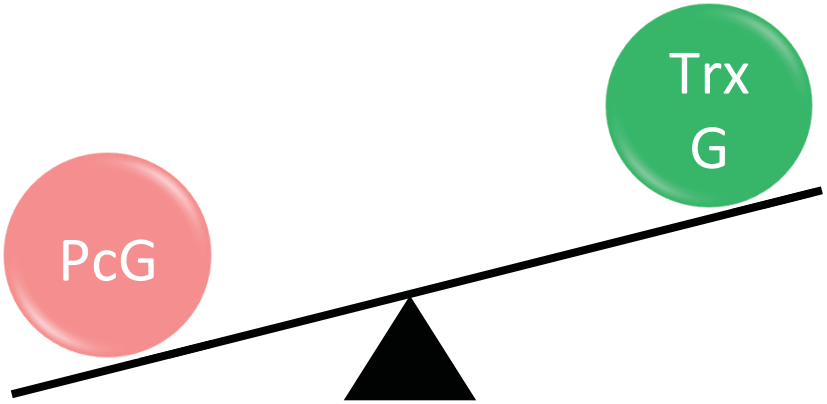
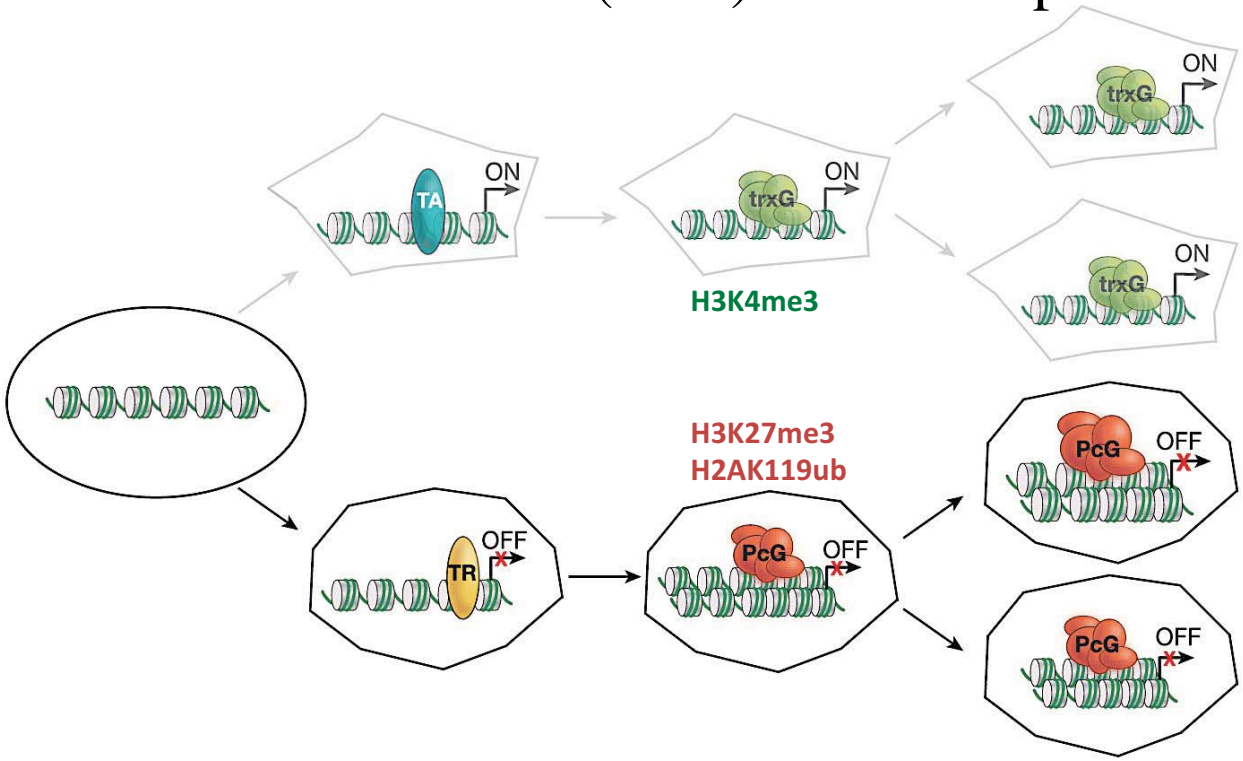




Chromatin as a carrier of cellular memory via Polycomb and Trithorax

Active and inactive states of genes expression established by TFs are **maintained** during cellular differentiation by Polycomb (PcG) and trithorax (trxG) over multiple cell divisions



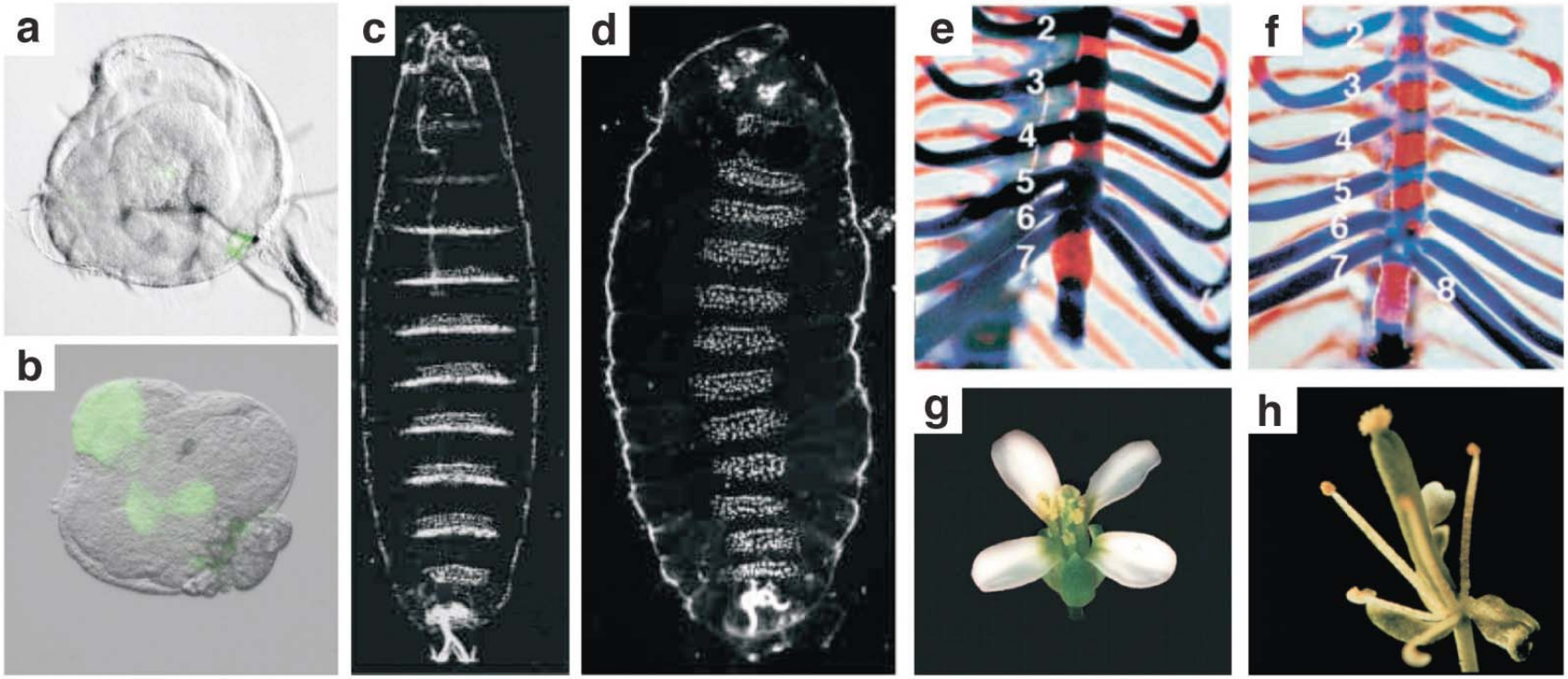
Maintenance of cell identity
Facultative heterochromatin
Stem cell plasticity

Disruption in development => **homeotic transformations, germ line defect**
Disruption in adults => **stem cell defects, cancer…**

In Drosophila studies show that several PcG and trxG components are required *throughout* development to maintain target gene activity.

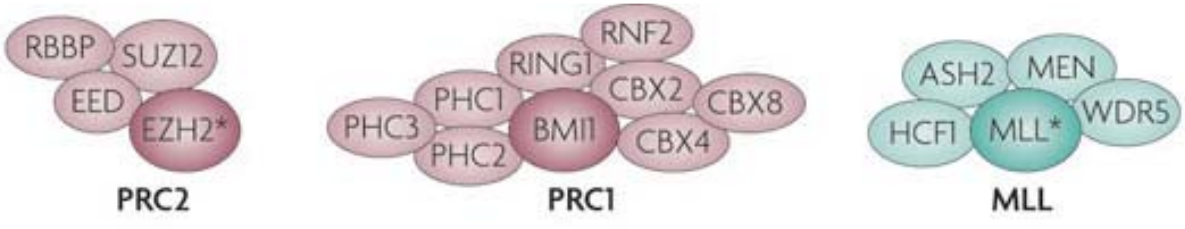
Polycomb and Trithorax are highly conserved through evolution

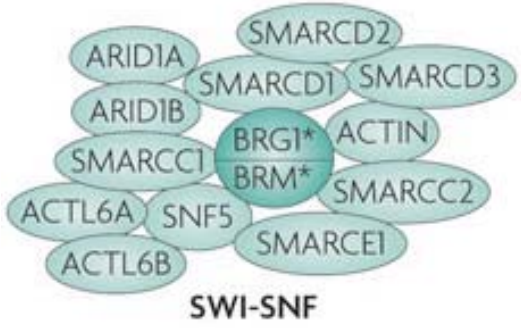
PcG mutations leads to improper gene expression during development and homeotic transformations in flies, mammals, plants…

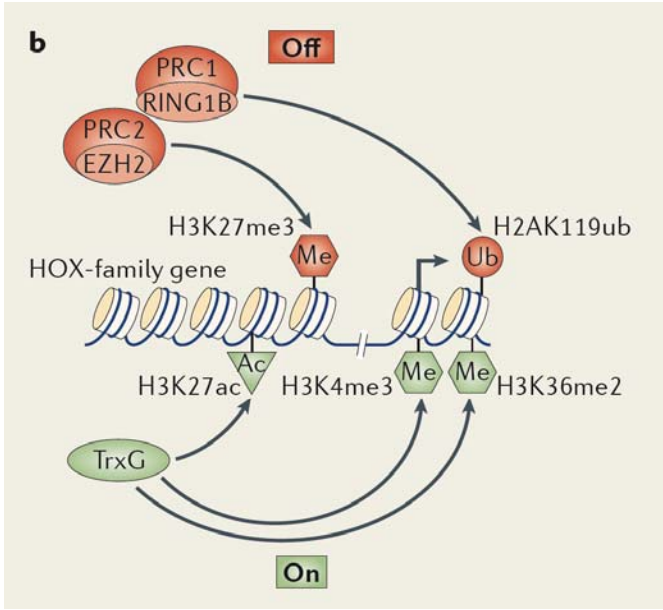


Chromatin as a carrier of cellular memory
Polycomb and Trithorax

Proteins of the PcG and trxG are organized into **large multimeric complexes** that act on their target genes by **modulating chromatin structure**





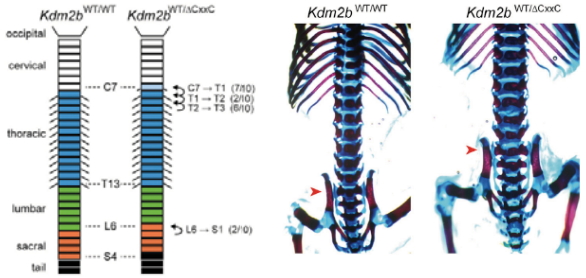


Several histone modifying enzymes (**writers**)
Histone modification binding proteins (**readers**)
Chromatin remodelers (SWI-SNF)

Chromatin as a carrier of cellular memory
Polycomb and Trithorax

PRC2 core component mutants:
Early lethal (postimplantation) and conditional KO show defects in terminal somatic cell differentiation and maintenance of multipotent or progenitor cell states
(Faustet al., 1998;O'Carroll et al., 2001; Pasini et al., 2004; and review Aldiri and Vetter, 2012)

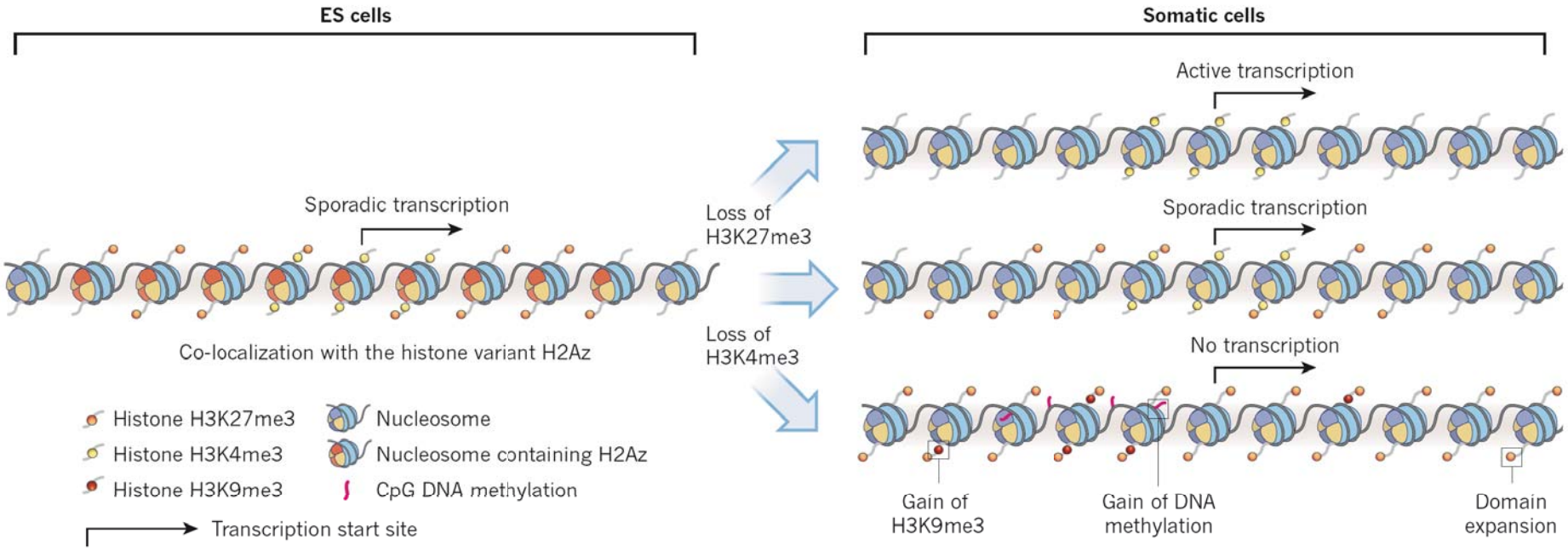
PRC1 components:
Pcgf2, Pcgf4, Cbx2, and Phc1: perinatal lethality and/or homeotic transformations;
Rnf2 is early embryonic lethal
(Akasaka et al., 1996; Core´ et al., 1997; Katoh-Fukui et al., 1998; Takihara et al., 1997; van der Lugt et al., 1994).
Phc2 and Ring1 mutant mice are healthy and fertile with minor homeotic transformations in the anterior–posterior axis (del Mar Lorente et al., 2000; Isono et al., 2005)
Double Ring1/2 KO shows early pre-implantation lethality



TRX components:
Multiple factors – may be required in highly tissue and lineage specific fashion
Several trxG proteins are essential as maternal factors during early mouse development

(Developmental and transgenerational dynamics
COURS IV)

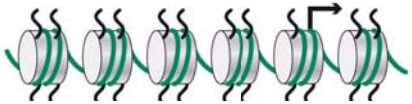
Chromatin properties at PRC2 target genes in mammalian cells before and after differentiation



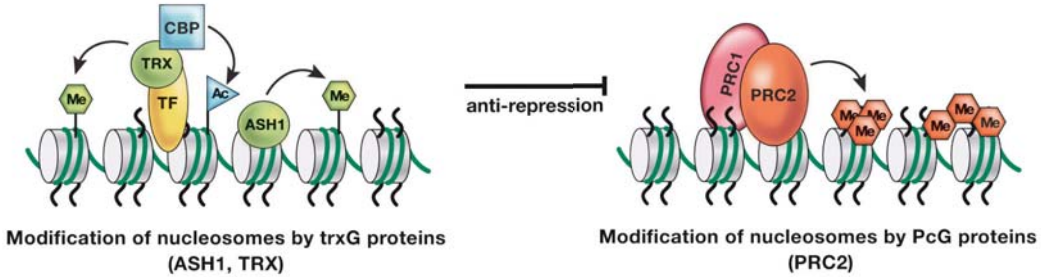
As in flies, Polycomb and Trithorax group proteins appear to participate in changes in cell fate and maintenance of cell identity, as well as stem cell plasticity

Chromatin as a carrier of cellular memory
Polycomb and Trithorax

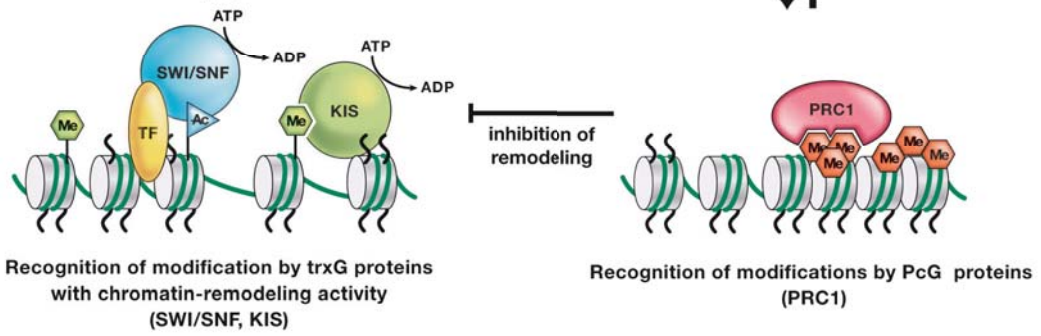
Proteins of the PcG and trxG are organized into **large multimeric complexes** that act on their target genes by **modulating chromatin structure**



How are these complexes recruited?



How do they work?

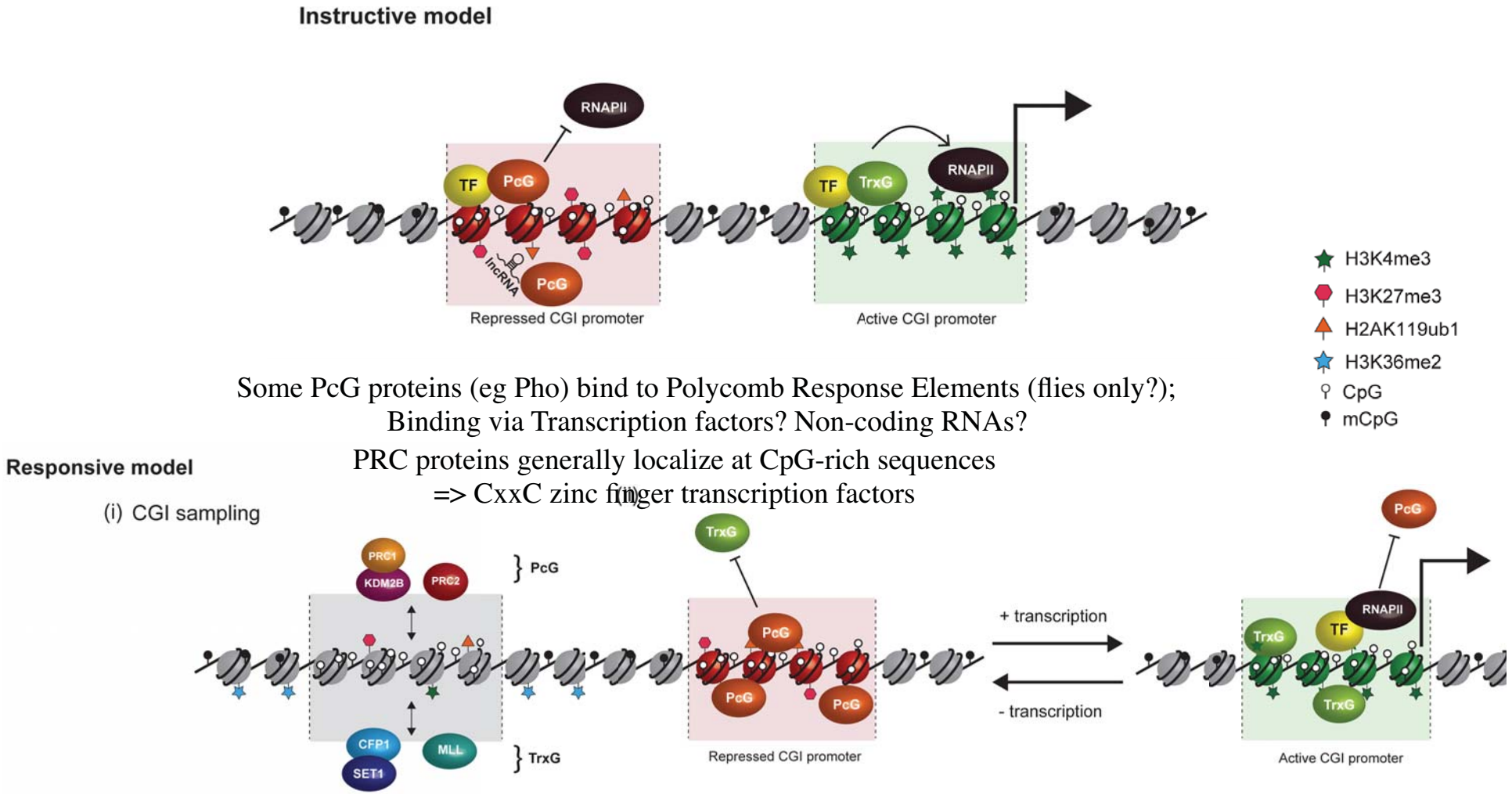


How are they propagated heritably?

active transcription

heritable repression

How are PcG and trxG complexes recruited?



Some PcG proteins (eg Pho) bind to Polycomb Response Elements (flies only?);
Binding via Transcription factors? Non-coding RNAs?

PRC proteins generally localize at CpG-rich sequences
=> CxxC zinc finger transcription factors

Sampling of chromatin state/DNA binding factors
PcG ssociation if a gene is off (no "active" histone marks)
trxG association at active genes?

E. Heard, February 16th, 2015

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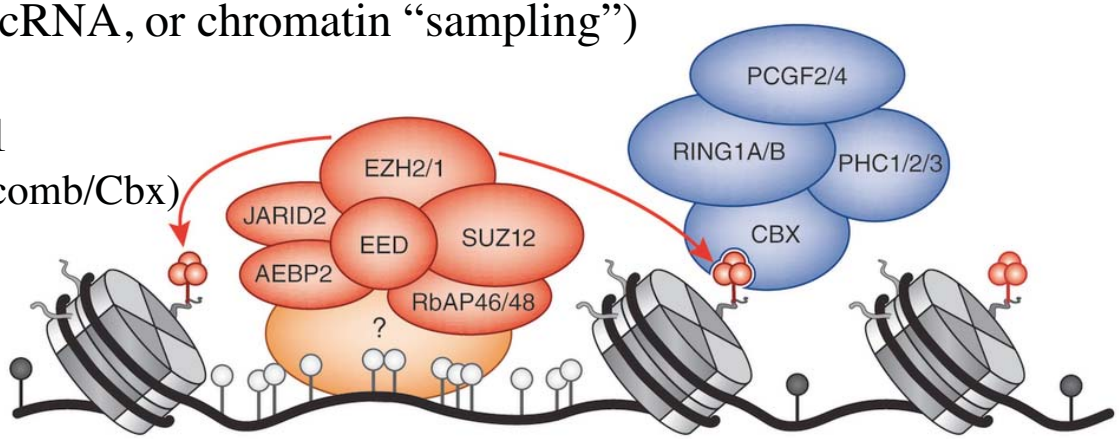
How are the two (or more) Polycomb complexes recruited?

PRC2 first, PRC1 second:

1. PRC2 recruited (via TF, ncRNA, or chromatin "sampling")
2. Ezh2 methylates H3K27
3. H3K27me3 recruits PRC1

(via the chromodomain of Polycomb/Cbx)



R. Paro, D.S. Hogness (1991) The Polycomb protein shares a homologous domain with a heterochromatin-associated protein of *Drosophila*. PNAS 88: 263–267

How are the two (or more) Polycomb complexes recruited?

Revolution in the Polycomb hierarchy

(Comet and Helin, 2014 for review)

PRC2 first, PRC1 second:

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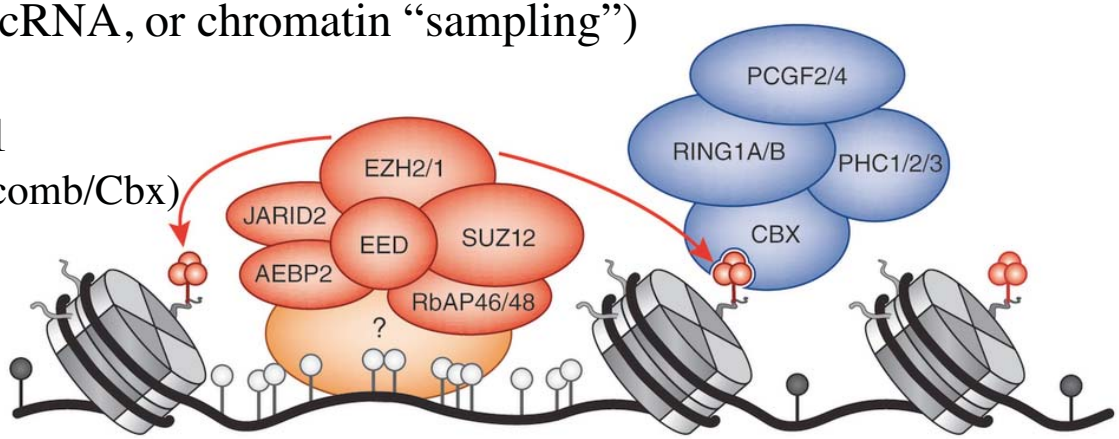
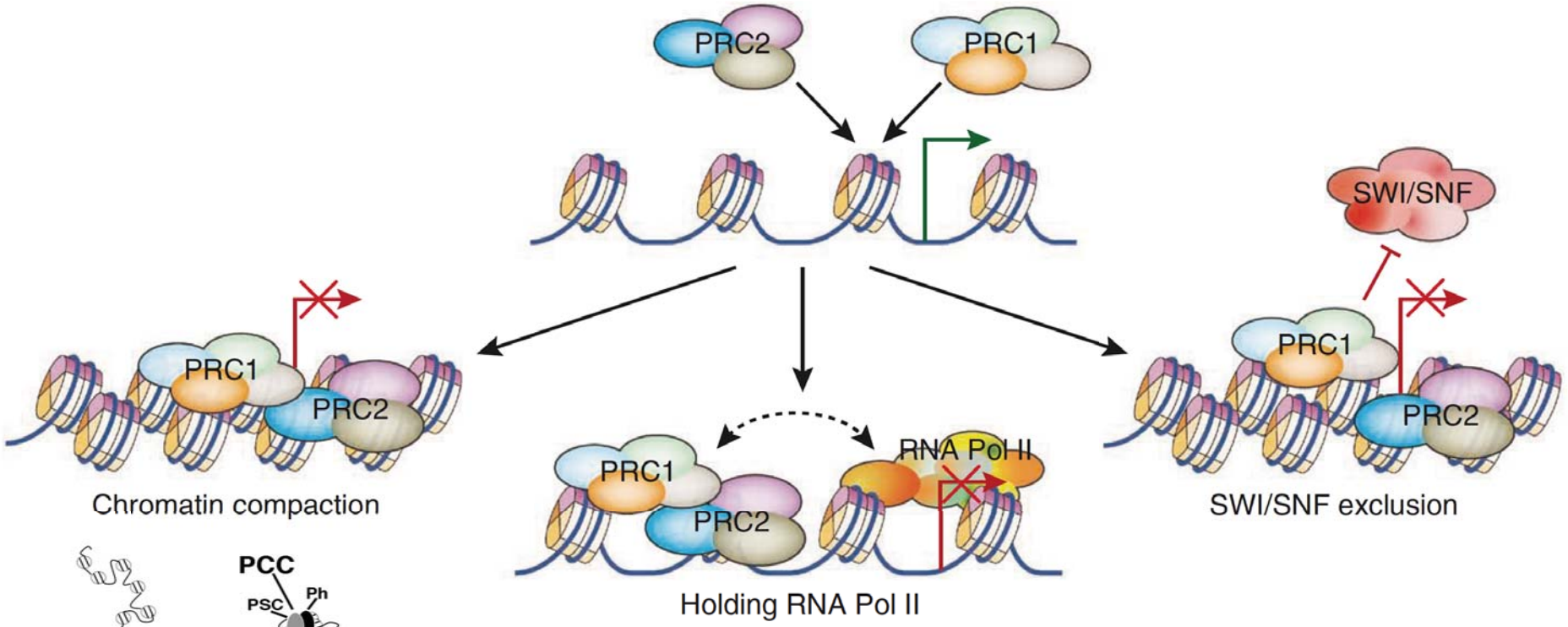


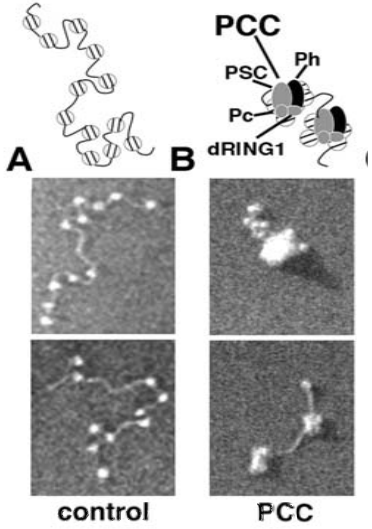
Blackledge et al. (2014). Variant PRC1 complex-dependent H2A ubiquitylation drives PRC2 recruitment and polycomb domain formation. Cell 157, 1445–1459.

Cooper et al (2014) Targeting Polycomb to Pericentric Heterochromatin in ES Cells Reveals a Role for H2AK119u1 in PRC2 Recruitment. Cell Reports 7, 1456–1470

R. Paro, D.S. Hogness (1991) The Polycomb protein shares a homologous domain with a heterochromatin-associated protein of *Drosophila*. PNAS 88: 263–267

How do they work?
Actions of PRC1 and PRC2 Complexes





Nucleosomes but not histone tails are important for compaction by a PcG complex (Psc subunit of PRC1)

(Francis et al, 2004)

E. Hea

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How are Polycomb and Trithorax chromatin states replicated?

- Unlike HP1/H3K9me3/Suv39h – so far, no evidence for direct binding of PRC1/ PRC2 to DNA Replisome (PCNA, Uhrf1, CAF-1 etc)

How are Polycomb and Trithorax chromatin states replicated?

- Unlike HP1/H3K9me3/Suv39h – so far no evidence for direct binding of PRC1/PRC2 to DNA Replisome (PCNA, Uhrf1, CAF-1 etc)
- In Drosophila embryos, H3K27me3 modification is **lost during replication** – and in mammalian cells also, levels are far **lower** on new histones in nascent chromatin than on old histones.
- But PRC-silenced genes are **not reactivated** during S-phase (DNA replication)…

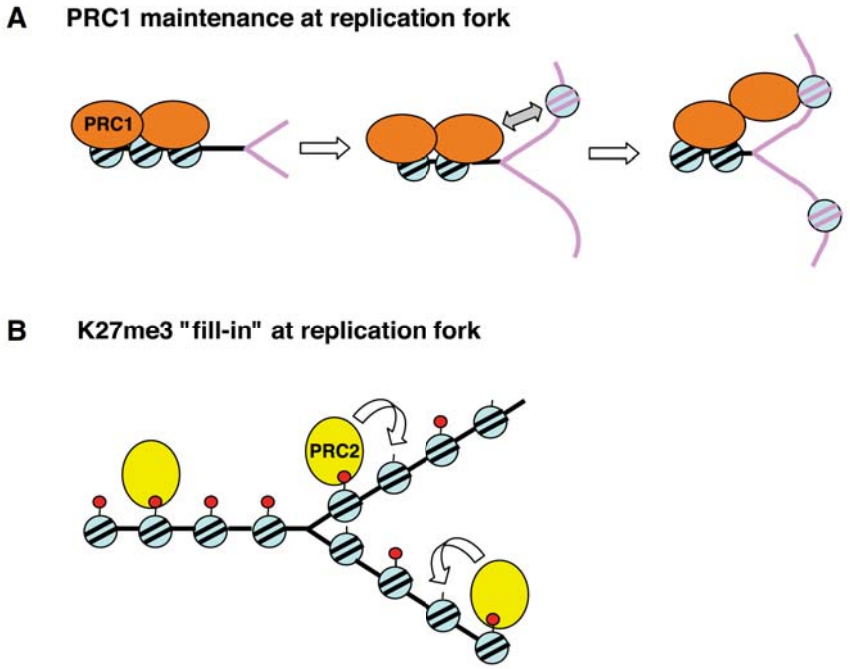
Why?
Buffering of repressed chromatin states?
Thanks to **domains** of modified chromatin not single nucleosomes:
PcG-associated histone marks distribute over large chromatin domains, facilitating their re-establishment (cf H3K9me - COURS II)

Model of imprecise copying/re-establishment:

"Nucleosomes are the basic units of chromatin but are not necessarily the basic units for gene repression."

(Huang, Xu and Zhu, Phil Trans R Soc, 2013)

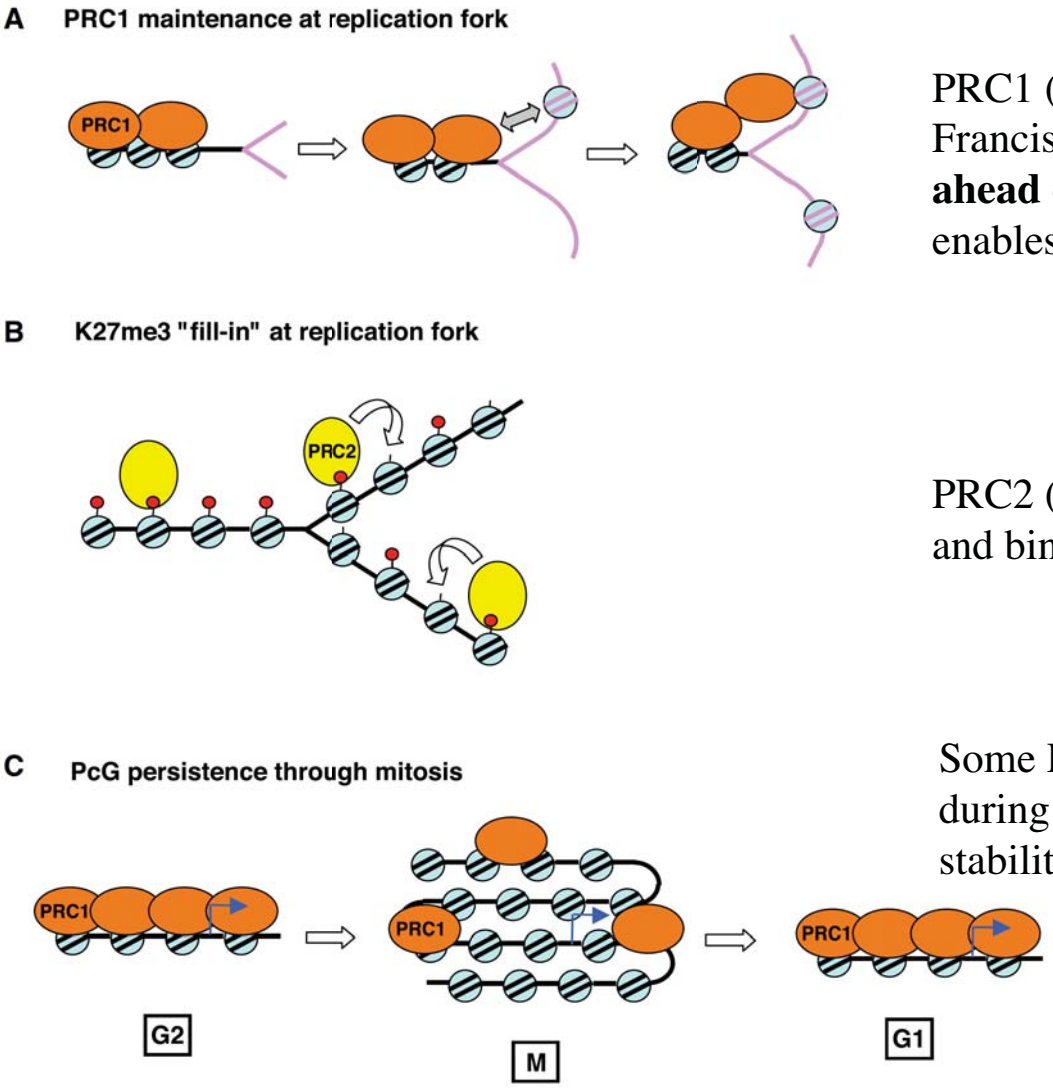
How are Polycomb and Trithorax chromatin states replicated?



PRC1 (Psc – capable of compacting chromatin, Francis et al 2004) remains associated **behind and ahead** of replication fork => looping/bridging enables epigenetic inheritance?

PRC2 (and PRC1) can fill in unmodified histones and bind chromatin following replication

How are Polycomb and Trithorax chromatin states replicated?



PRC1 (Psc – capable of compacting chromatin, Francis et al 2004) remains associated **behind and ahead** of replication fork => looping/bridging enables epigenetic inheritance?

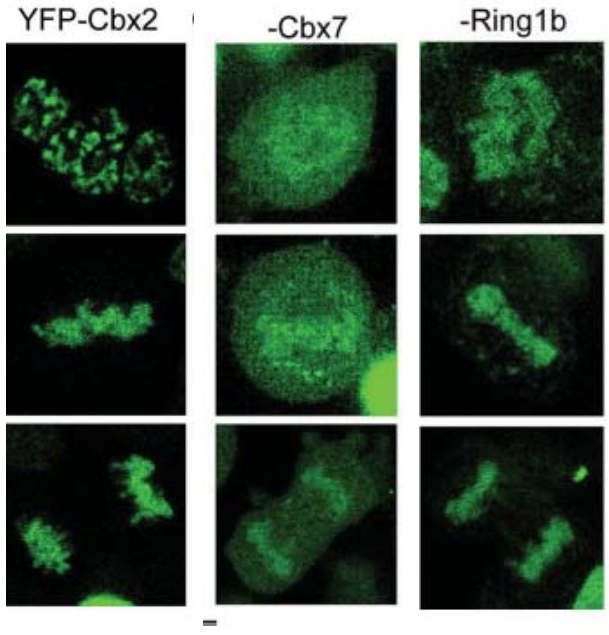
PRC2 (and PRC1) can fill in unmodified histones and bind chromatin following replication

Some PRC2 and PRC1 components can remain during mitosis => Mitotic bookmarking reinforces stability of repressed state in daughter cells

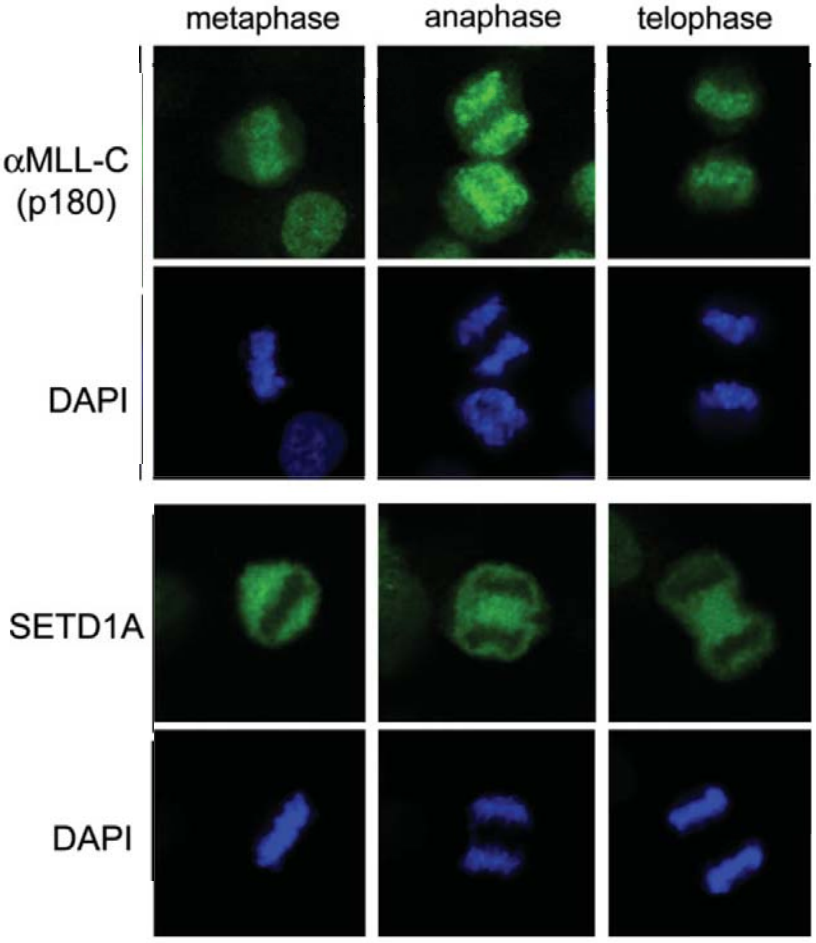
Some trxG and PcG proteins remain associated during Mitosis

Mitotic Bookmarking

Cbx2 stably associates with mitotic chromosomes via a PRC2- or PRC1-independent mechanism and is needed for recruiting PRC1 complex to mitotic chromosomes.
(Zhen et al, MBoC 2014)

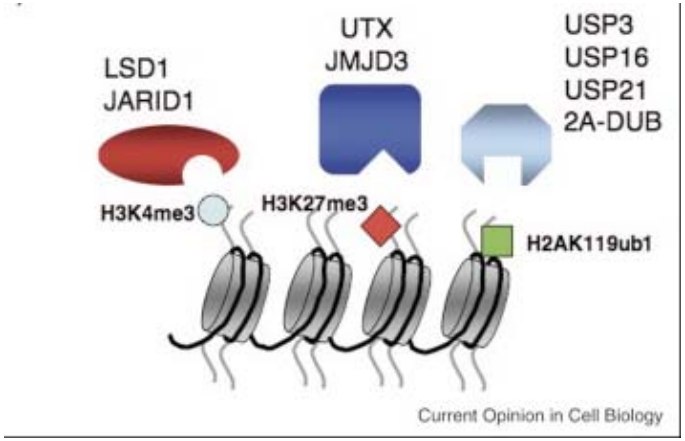


MLL bookmarking at gene promoters during M phase allows rapid transcriptional reactivation following M phase
(Blobel et al, Mol. Cell, 2009)



Removing PcG and trxG chromatin marks

1. Passive loss (absence of maintenance mechanisms)
2. Active loss (enzymatic removal of histone modifications, histone exchange, nucleosome eviction, chromatin remodelling, etc)



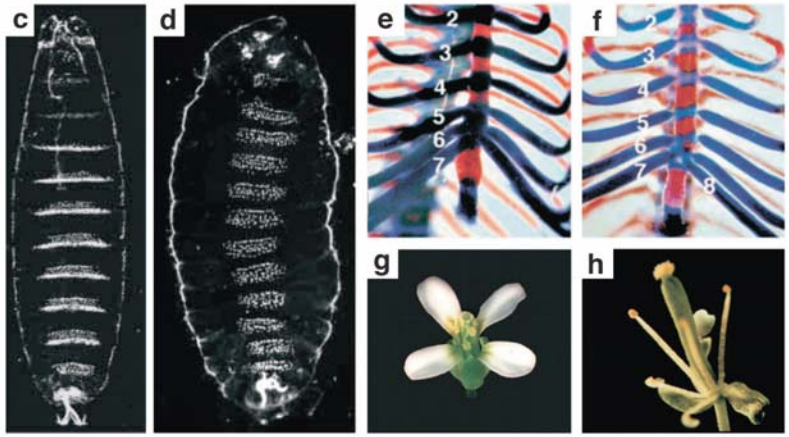
Chromatin is highly dynamic
Yet states of gene activity can be stably propagated over hundreds of cell divisions in many cases
⇒ Synergy between chromatin, RNA-based, DNA methylation-based, nuclear organization and other mechanisms?

Reprogramming may involve both active and passive removal.
Occurs in a developmental context
May also occur accidentally (sporadic loss), or after DNA damage, or with ageing– and may lead to epimutation and disease.

(COURS IV et V)

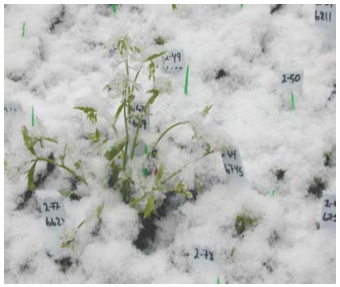
Examples of Polycomb dependent heritable gene silencing reset at every generation

Development



X-chromosome inactivation
Stable monoallelic expression

Epigenetic regulation
as Environmental Memory



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X-Chromosome Inactivation

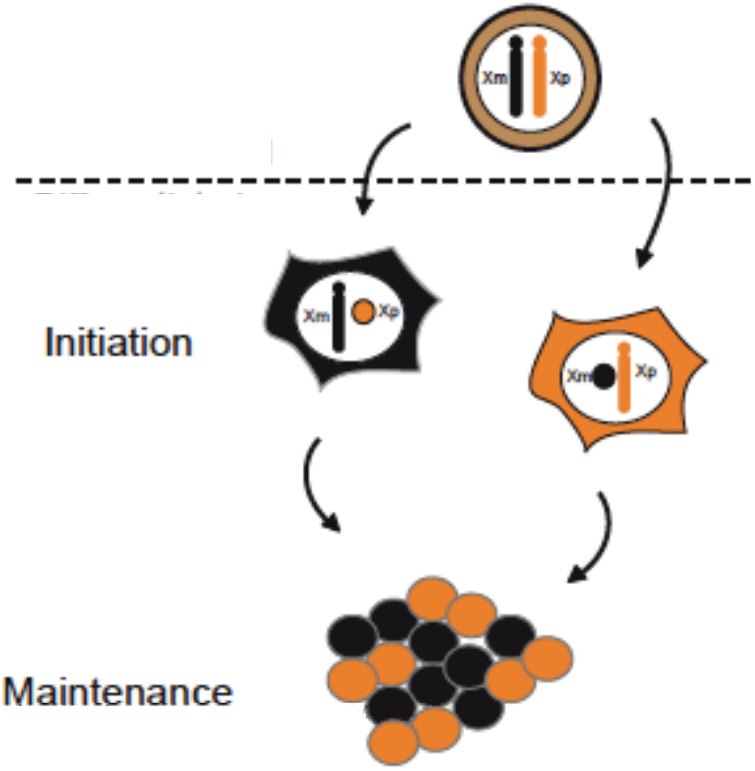
Mary Lyon
(1929-2014)

Lyon Hypothesis (1961)

(1)Heteropyknotic X chromosome is genetically inactivated (Xi)

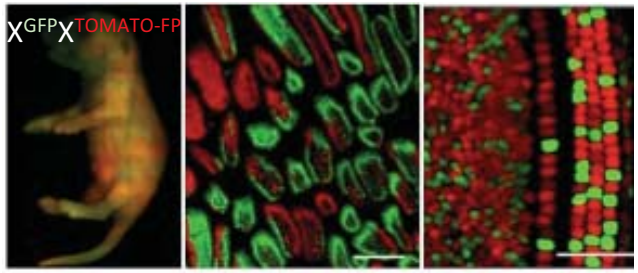
(2) Either paternal or maternal Xi in different cells of the same animal

(3) X inactivation must occur early in embryonic development, and once established be **stably maintained: HOW?**



Random XCI

Females are mosaics



Lyon, M. F. (1961), Gene Action
(Mus musculus L.) Nature. 190 (

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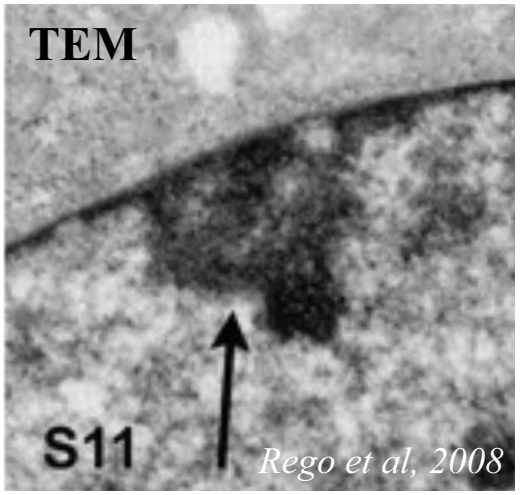
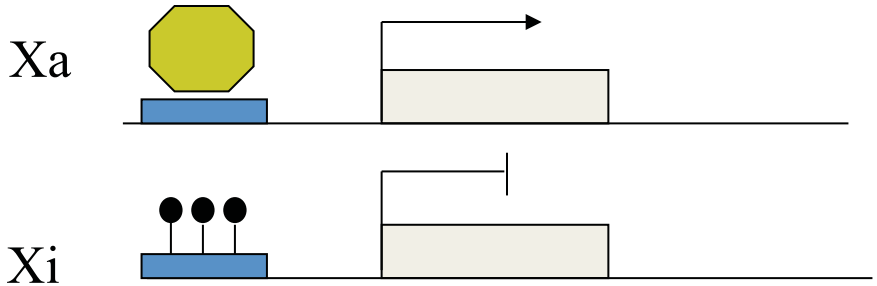
1530

X-Chromosome Inactivation

Identical DNA sequences
Opposite gene activity states
Mitotically heritable





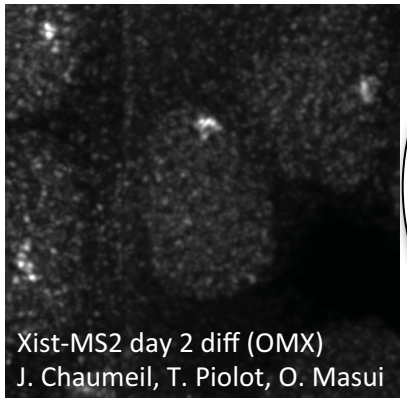
Epiallele : epigenetically marked regulatory sequence

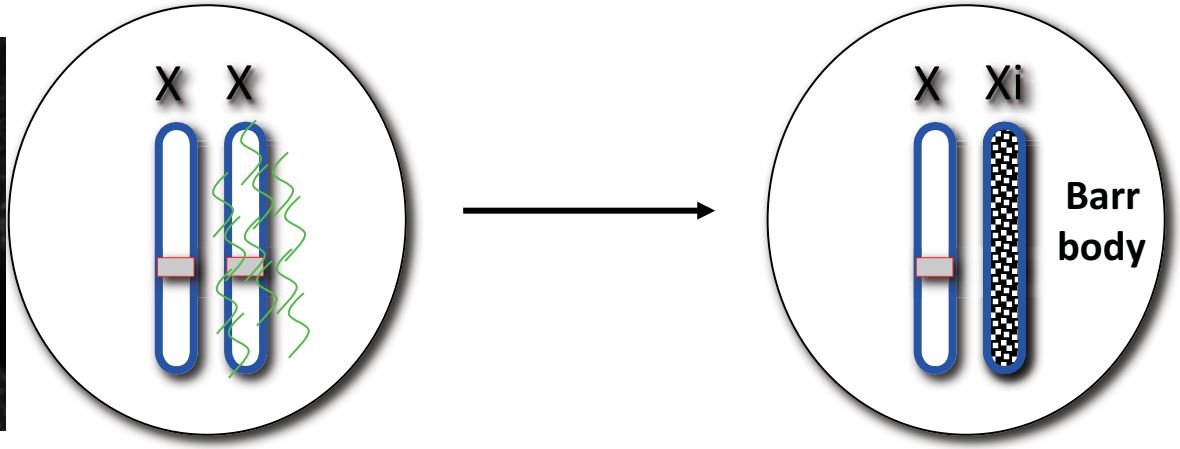
DNA methylation of the promoter regions of X-linked genes:
classic example of stable epigenetic marking

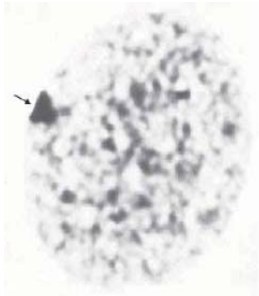
However promoter methylation occurs **late** during the X-inactivation process
And is even **absent** in some mammals (marsupials)!

X-Chromosome Inactivation

Differential treatment of identical DNA sequences in the same nucleoplasm





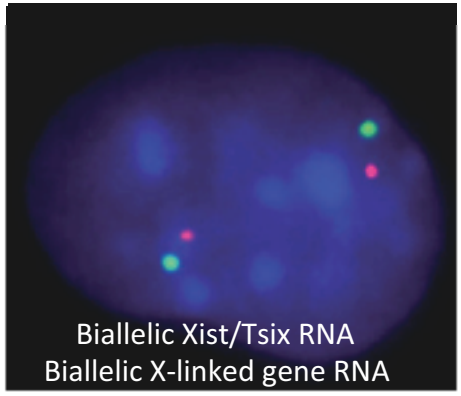


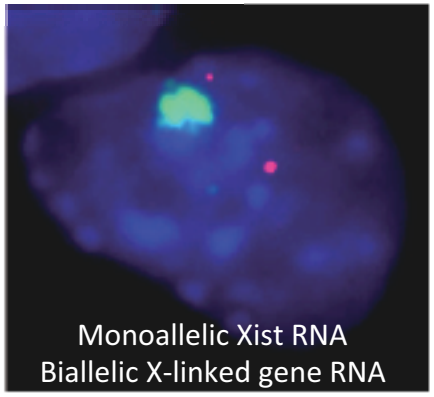
Xist RNA triggers X inactivation

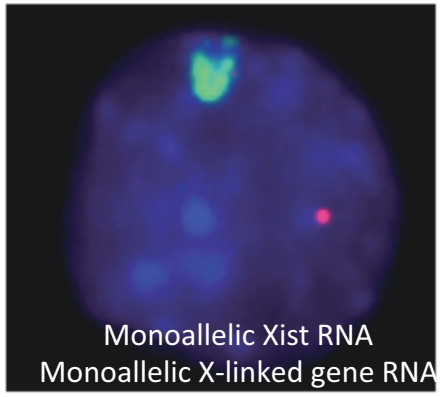
A multitasking RNA: gene silencing, chromatin changes, chromosome reorganisation

Maintenance

Polycomb complexes, DNA methylation, nuclear organisation, asynchronous replication

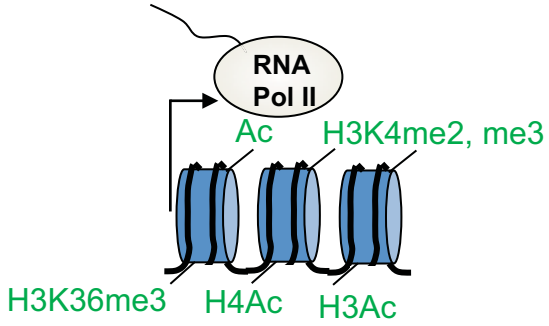




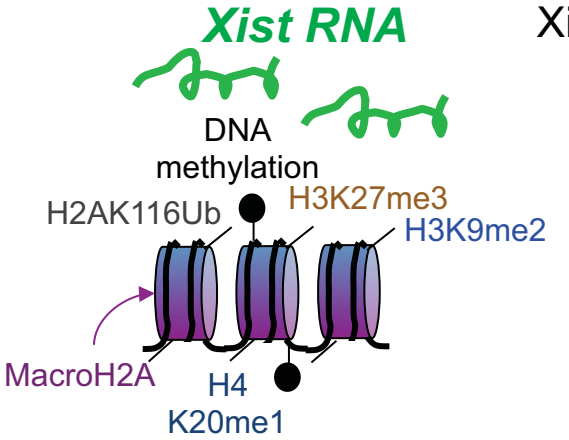


X-Chromosome Inactivation

Identical DNA sequences
Opposite gene activity states
Mitotically heritable



Active X chromatin

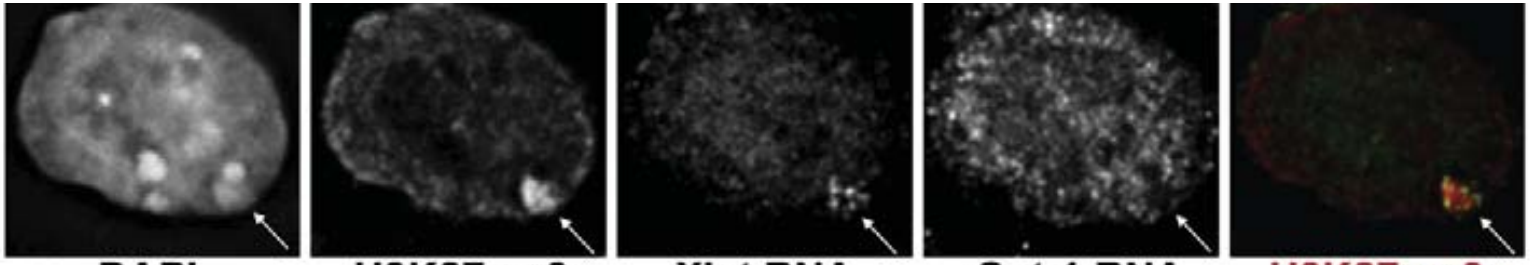


Inactive X chromatin

Xi-associated proteins:

SAF-A
macroH2A
PRC2
PRC1
ATRX
SMCHD1
CDYL

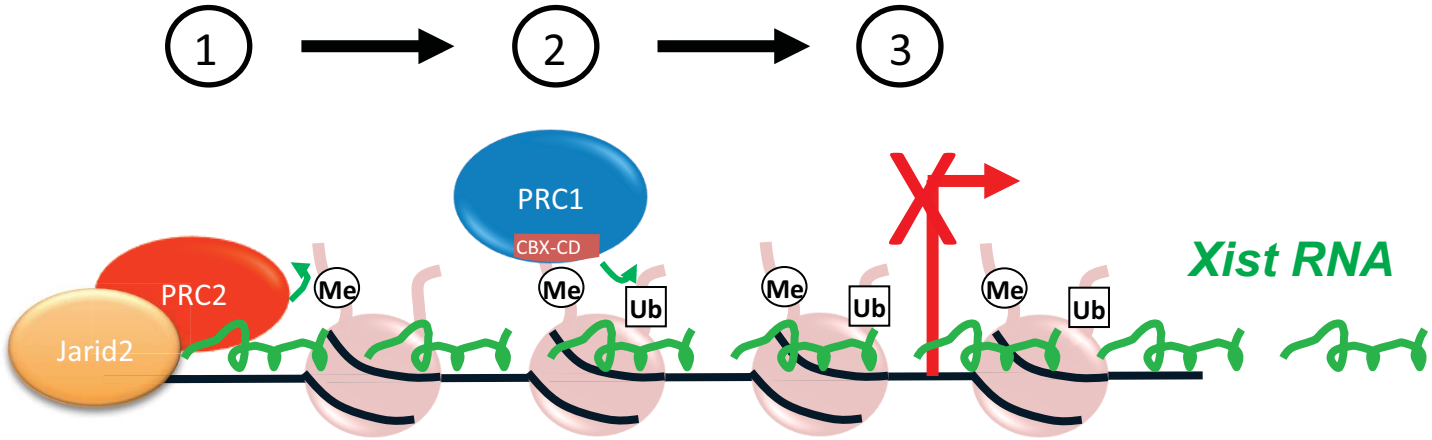
How are PRC1 & PRC2 complexes recruited to the Xi?
How are they maintained? What is their role (if any)?



DAPI | H3K27me3 | Xist RNA | Cot-1 RNA | H3K27me3 Xist

Establishment and maintenance of Polycomb silencing in X chromosome inactivation

"Classical" model for Polycomb action



HOWEVER

- PRC1 recruitment to Xi appears to be unaffected in absence of PRC2 and Jarid2
- Jarid2 can bind H2AK119ub1 in flies and mammalian cells
- PRC2-mediated recruitment of canonical PRC1 complexes fails to catalyze significant levels of H2AK119ub1
- So far no evidence for direct association between Xist RNA with Jarid2 or PRC2 *in vivo*

=> New Models Required!

For reviews see Escamilla et al, 2011; Gendrel and Heard, 2014

Courtesy of N. Brockdorff (2015)

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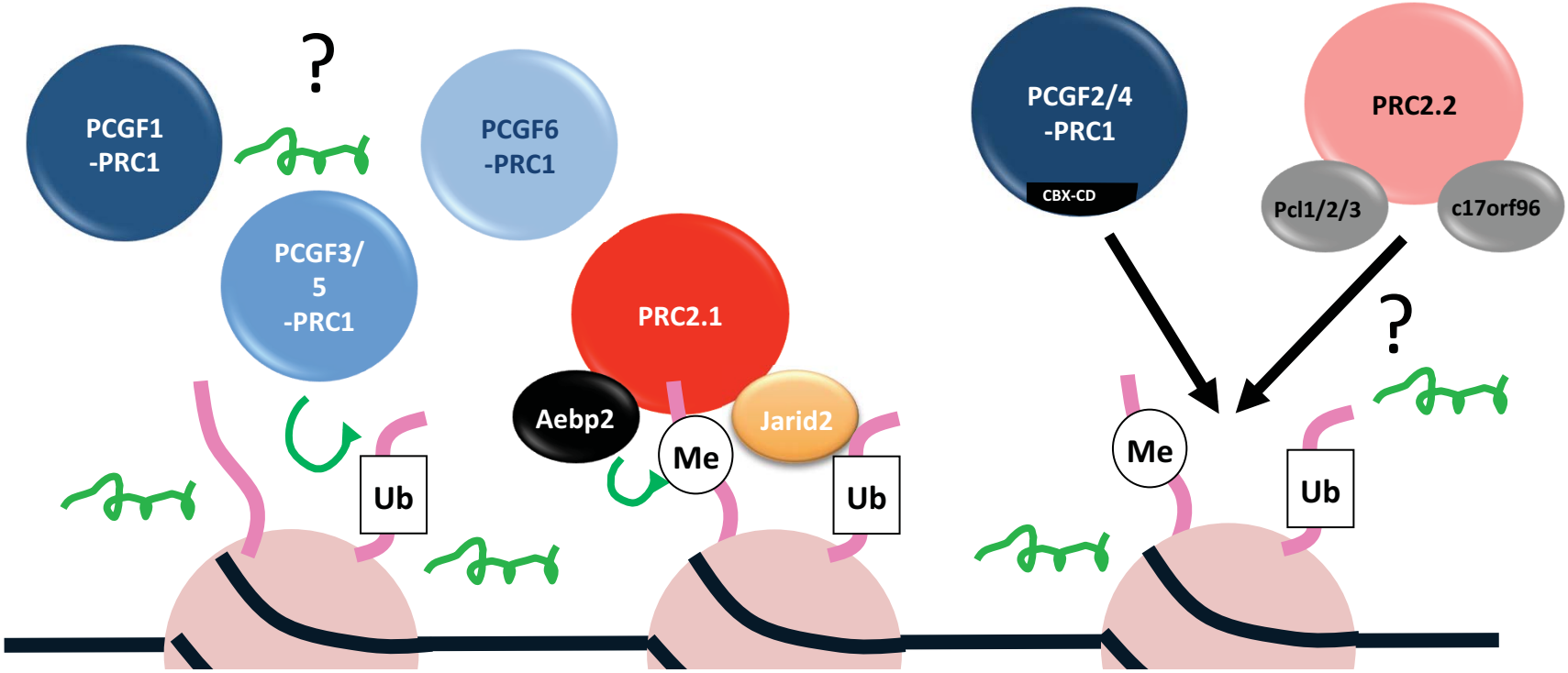
Establishment and maintenance of Polycomb silencing in X chromosome inactivation

A new model for Polycomb action in XCI?

PRC1 is recruited first (how?)

Then PRC2 recruited via Jarid2 binding to H2AK119Ub?

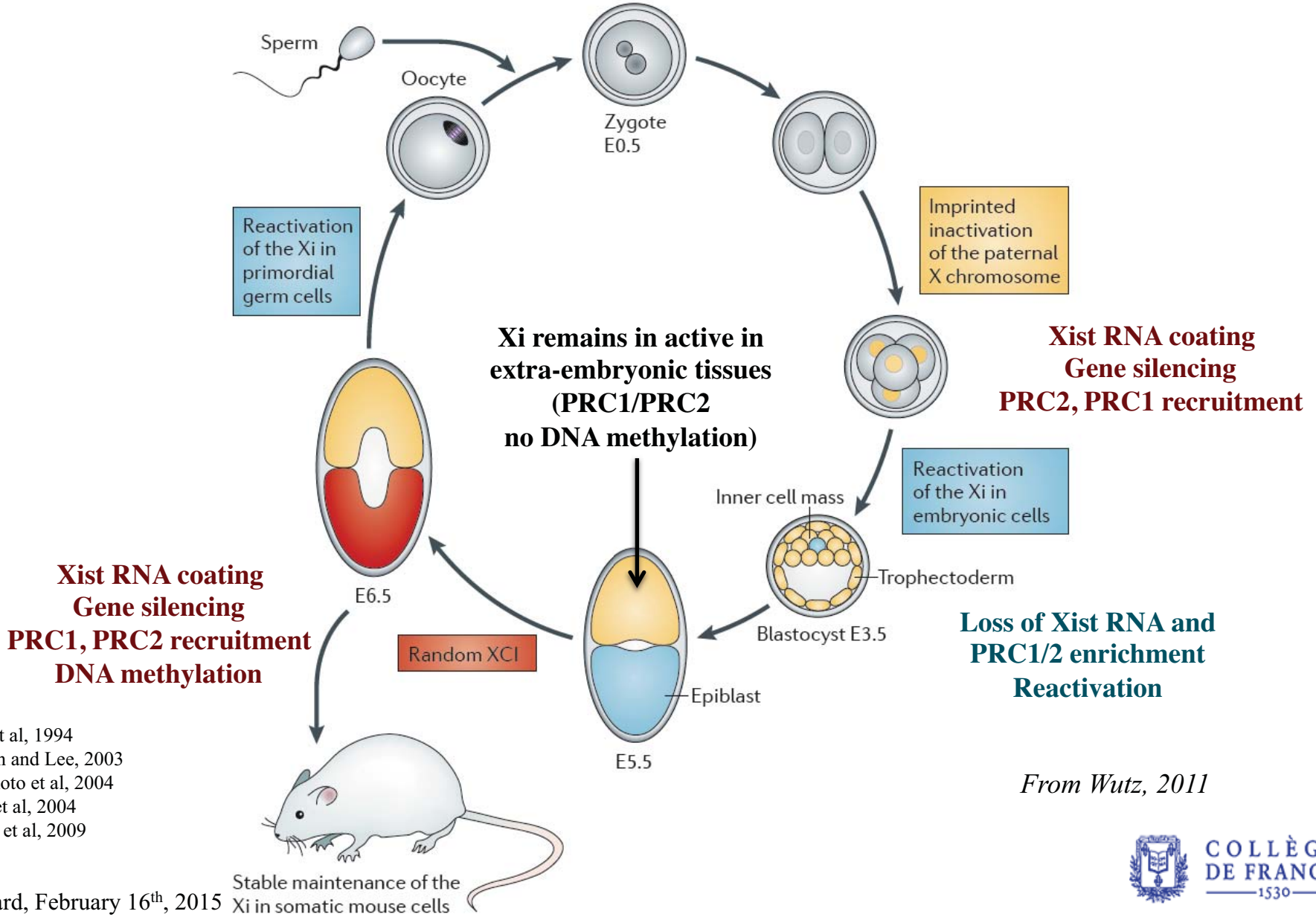


Does it matter?

Deletion of PRC2 and PRC1 during random XCI

So far no obvious defects…

Two waves of X inactivation with different epigenetic requirements during mouse development



Kay et al, 1994
Huyhn and Lee, 2003
Okamoto et al, 2004
Mak et al, 2004
Patrat et al, 2009

From Wutz, 2011

A role for PRC2 in the maintenance of silencing on the Xi in extra-embryonic tissues

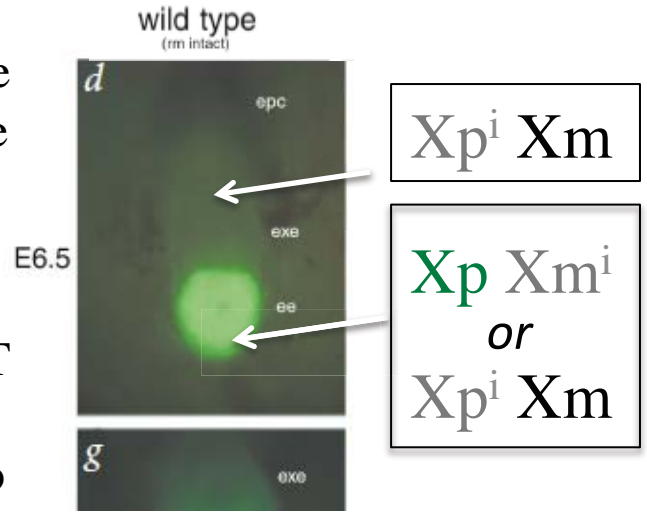
Imprinted X inactivation maintained by a mouse *Polycomb* group gene
Wang et al (2001) Nat. Genet. 28: 371-375.

In Polycomb (*Eed*) mutant (hypomorph) embryos, GFP reactivation is observed in some extraembryonic cells – but only at later stages of development
⇒ **Polycomb is involved in maintaining the inactive state of the paternal X chromosome in these tissues but not initiation.**

Embryos derived from $X^{gfp}Y$ male mated with a XX female

Imprinted XCI in extraembryonic tissues
=> no GFP+ cells in WT

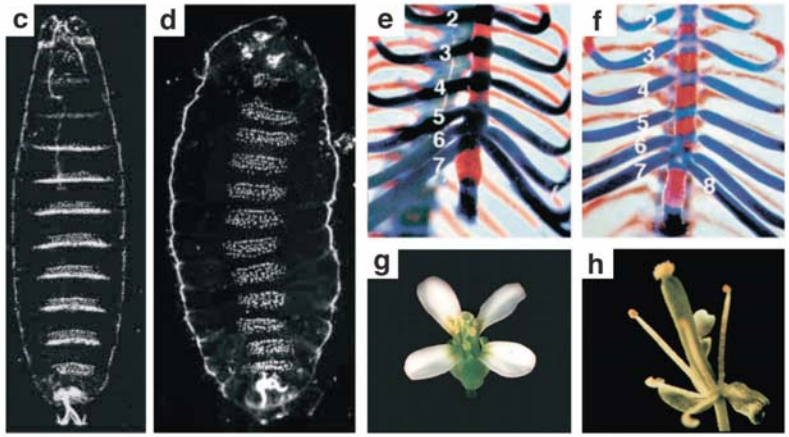
Random XCI in embryo



To do: PRC1+2 mutant embryos and conditional knock outs, to define time window when PcG maintenance is critical *in vivo*

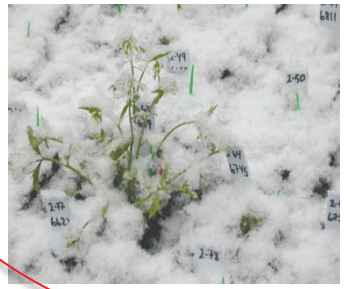
Examples of Polycomb dependent heritable gene silencing reset at every generation

Development



X-chromosome inactivation
Stable monoallelic expression

Epigenetic regulation
as Environmental Memory



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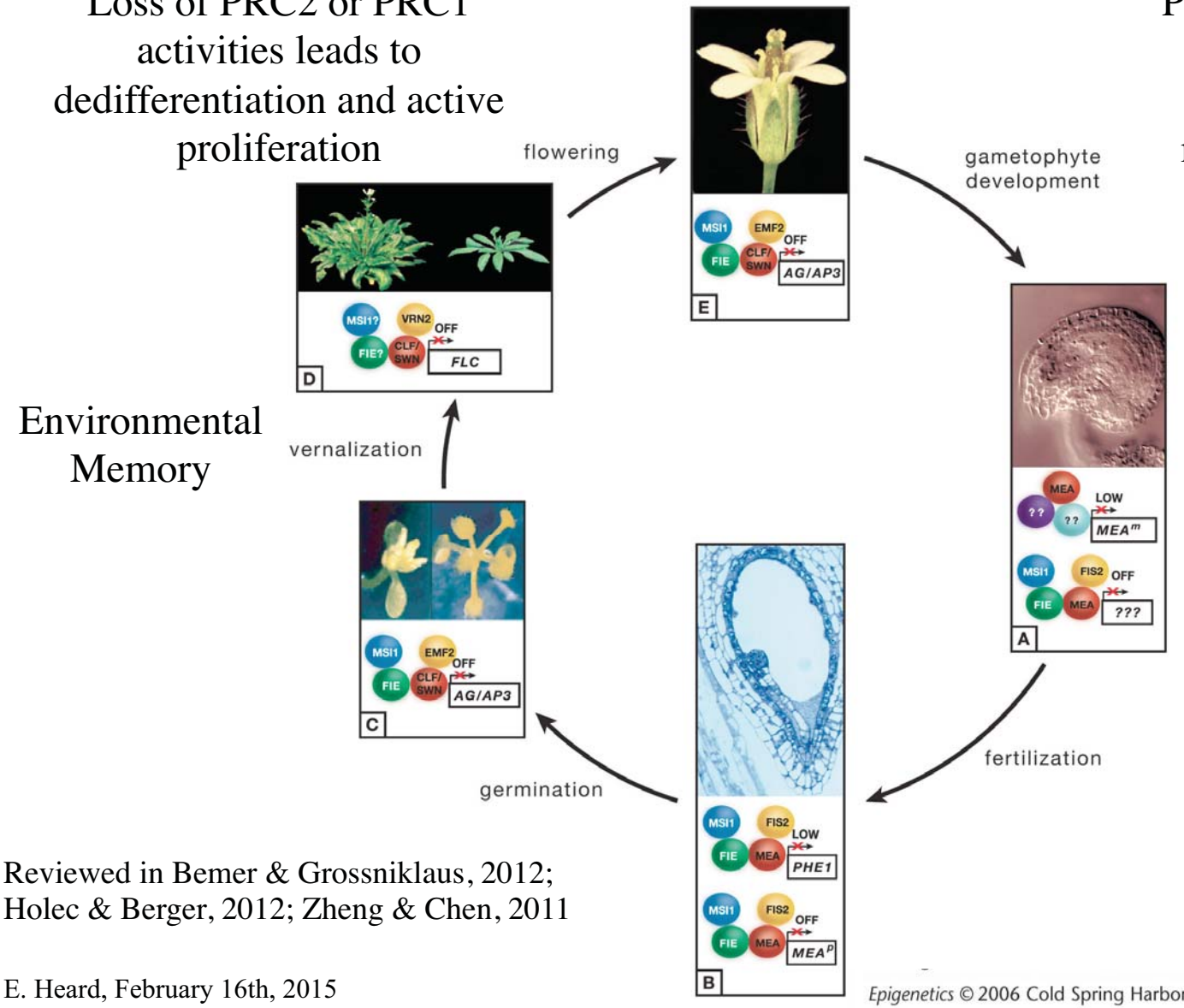
Roles of Polycomb group complexes in Plants

PRC2-mediated gene repression affects most developmental transitions in Arabidopsis

Loss of PRC2 or PRC1 activities leads to dedifferentiation and active proliferation

PcG proteins are central regulators of cell fate acquisition and maintenance in plants

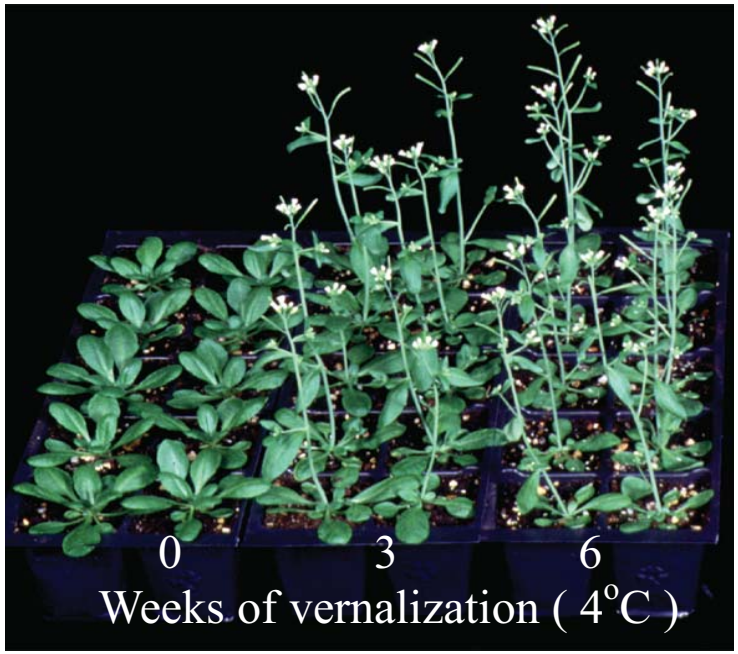


Reviewed in Bemer & Grossniklaus, 2012; Holec & Berger, 2012; Zheng & Chen, 2011

Vernalization in plants:
Memory of the cold mediated by Polycomb

From Latin: vernus, of the spring

Acquisition of a plant's ability to flower in the spring by a chilling treatment (exposure to the prolonged cold of winter)

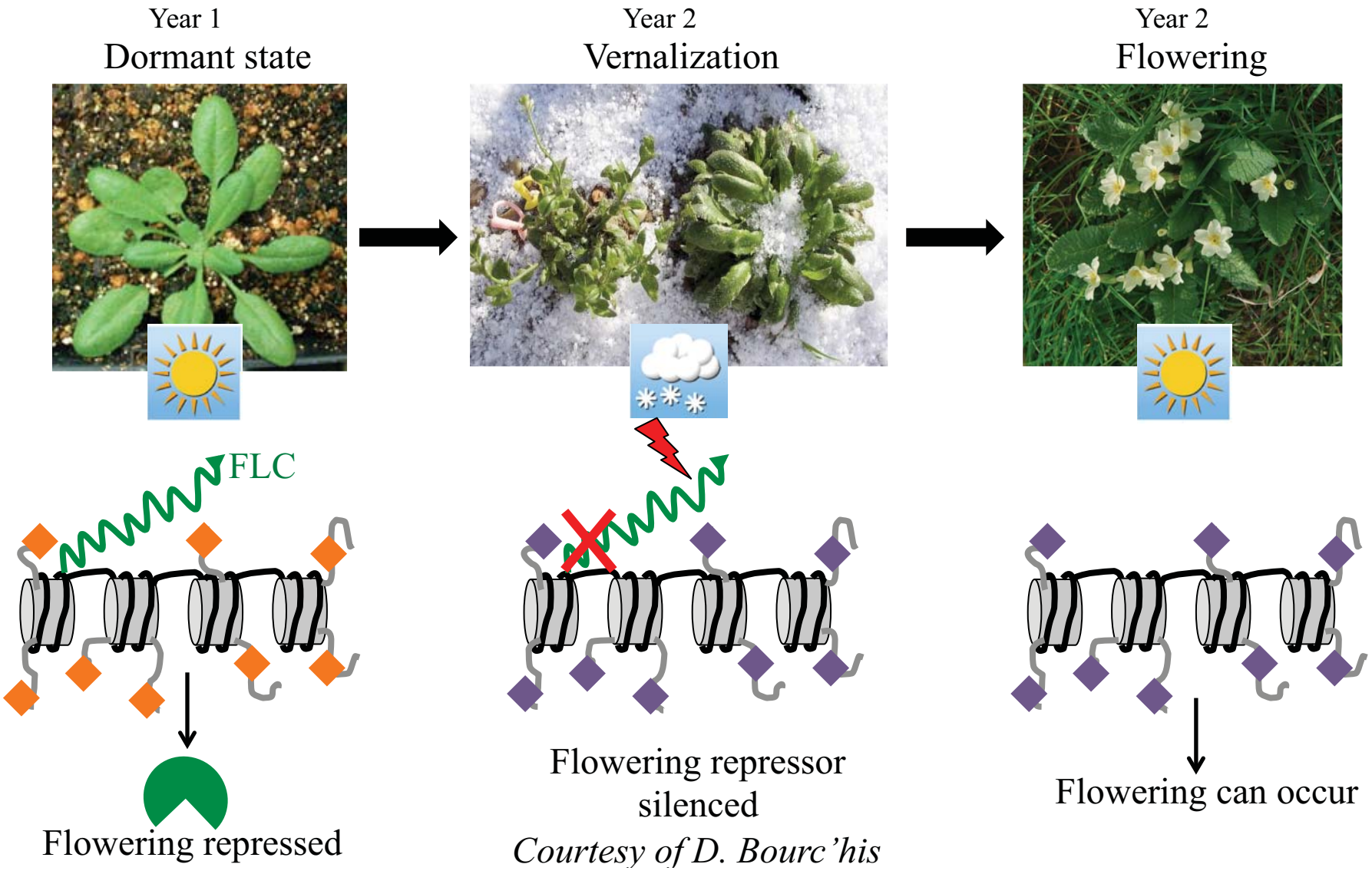


- quantitative
- reversible
- perceived by dividing cells
- not graft transmissible
- mitotically stable
- reset at every generation

After vernalization, plants have acquired ability to flower, but require additional seasonal cues or weeks of growth before they actually flower.

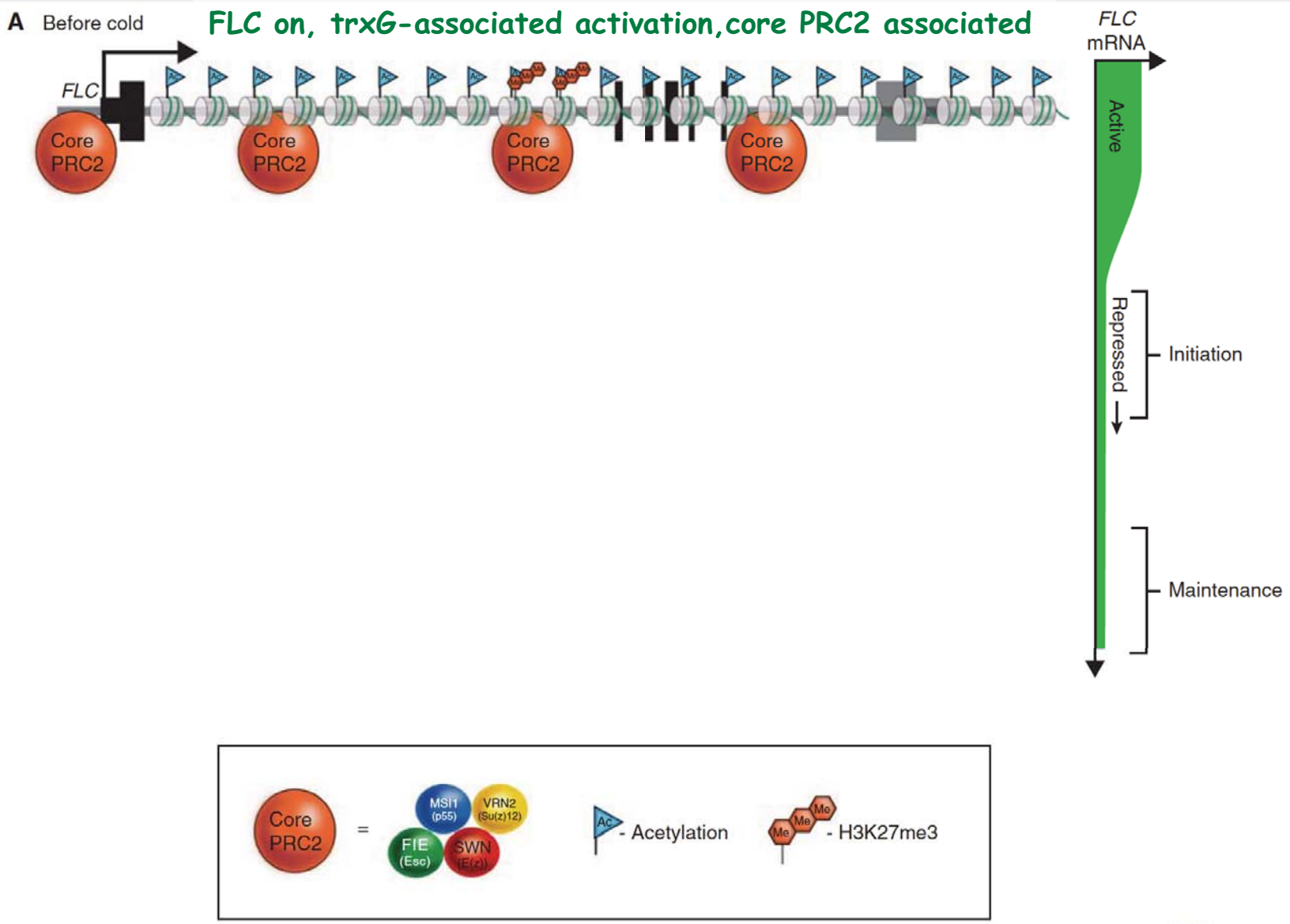
Vernalization

Vernalization causes a gradual reduction in expression of the floral repressor *FLC*



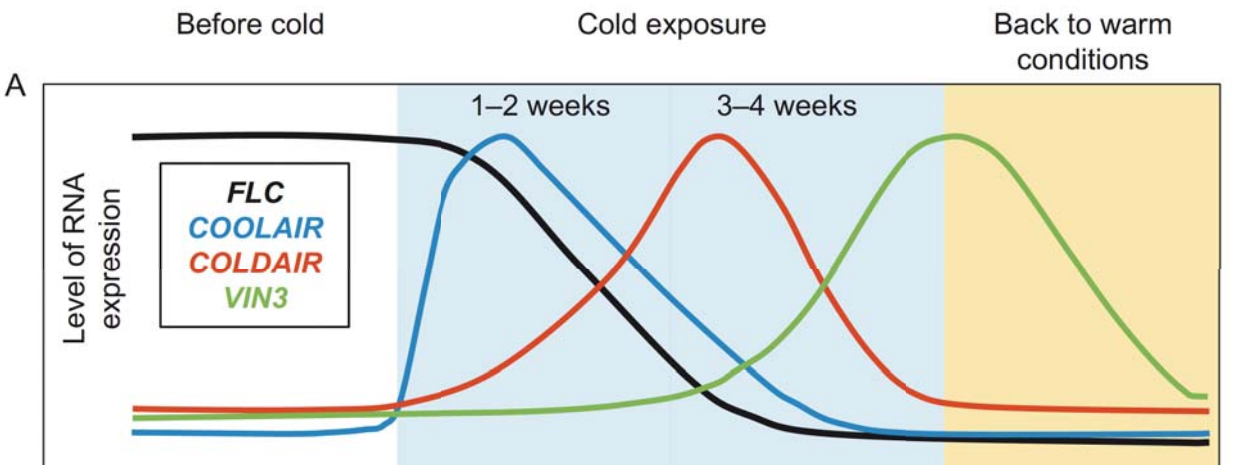
Courtesy of D. Bourc'his

Polycomb group protein repression at the *FLC* locus



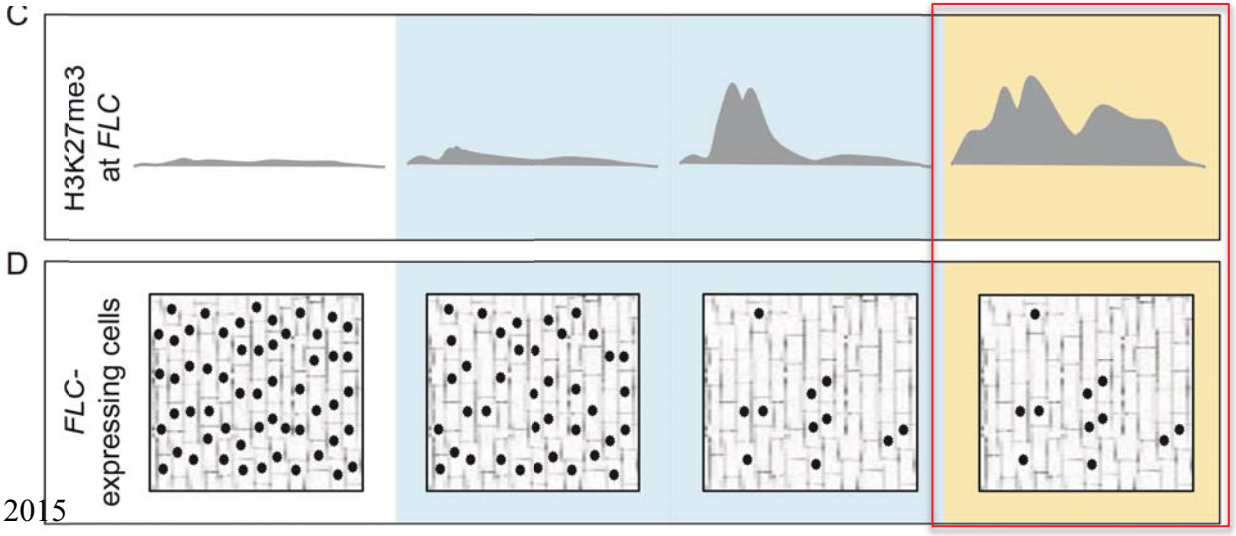
For review, see Dean and Baulcombe, CSH Perspectives, 2014

Polycomb group protein repression at the *FLC* locus



After cold, nucleated H3K27me3 causes some cells to switch to a silenced state with high levels of H3K27me3 blanketing the gene (NB requires cell division)

The great the number of cells with H3K27me3, the more likely FLC will remain repressed & flowering will occur



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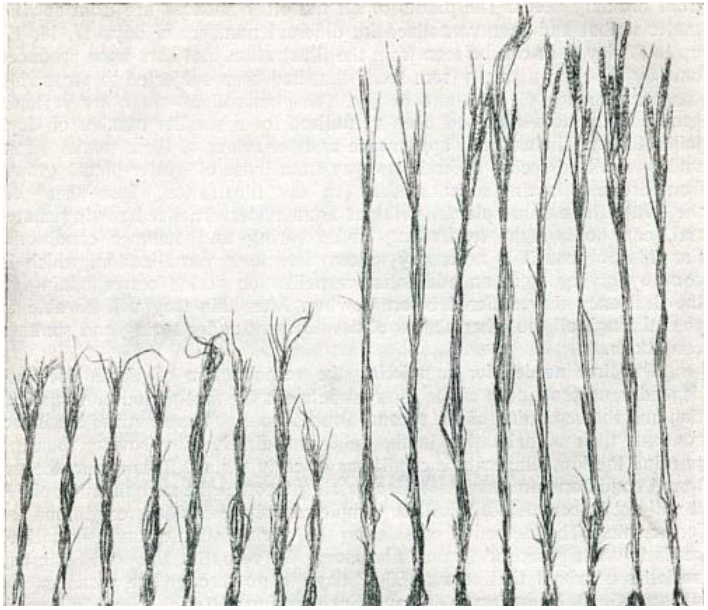
Vernalization in plants:
Memory of the cold mediated by Polycomb

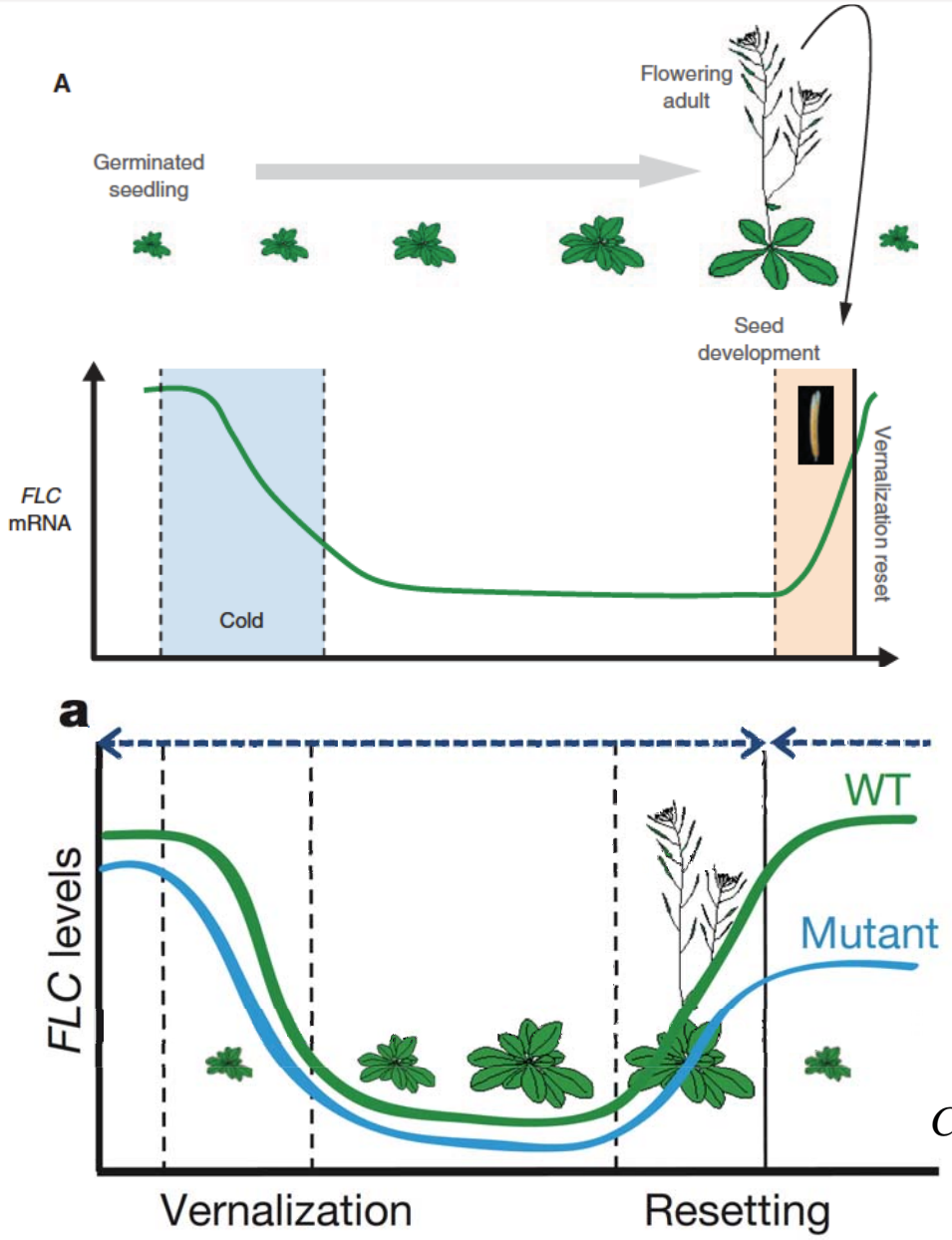
Trofim Lysenko
(1898-1976)

In 1930's Soviet union, Lysenko proposed the use of vernalization to make seeds of winter cereals behave like spring cereals – putting an end to famine resulting from forced collectivization – earning him the support of Stalin



Lysenko rejected the laws of Mendel and genetics, supported Lamarckism: idea that offspring inherit traits their parents acquired in their lifetimes - favored by Stalin, who felt that Mendelian biology rendered life unacceptably deterministic…

Polycomb group protein repression at the *FLC* locus



Resetting
(germ line or embryogenesis)

Partial resetting
Transmission to nex generation…?

Crevillén et al, Nature, 2014

CHAIRE ÉPIGÉNÉTIQUE ET MÉMOIRE CELLULAIRE

Année 2014-2015 :
"Chromatine et Mémoire cellulaire"

23 Février, 2015

Cours IV
"La mémoire chromatinienne au cours du developpement et à travers les generations"

Seminaire:
DEBORAH BOURC'HIS (Institut Curie, Paris)

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