L2.2

Epigenetic inheritance

While this one contains an important discussion on trans-generational transmission of epigenetic characters.

This is not relevant to what we discuss today, but it will become very important for Lesson 2.4.

Box 1. Transgenerational inheritance; considering caveats and alternative mechanisms

Non-chromatin based mechanisms likely contribute to transgenerational inheritance. For example, some of these phenotypes might arise from cryptic genetic variation given that inbred strains, nearly identical clones or even neighboring cells in the same organism may possess marked genetic differences [108]. Such genetic variation could be passed on to offspring or arise *de novo* (e.g., transposable elements, mutations) and account for differences. Unfortunately, these alternatives are seldom examined in transgenerational studies. Furthermore, establishing transgenerational inheritance in its purest sense is often confounded by maternal care, social transmission, or other variables that may propagate a phenotype without requirement for epigenetic memory *per se*. Indeed recent studies suggest that maternal care may play a significant role even in the transmission of phenotypes originating from the father [109].

Even if a phenotype is transmitted in a transgenerational epigenetic fashion, chromatin events may not always be responsible for their propagation. Transcriptional loops are one example [110]. As in somatic tissue, noncoding RNAs such as siRNA, piRNAs as well as miRNA contribute to inheritance and might function independently of changes at the level of chromatin (recently reviewed by [63]). In fact, a recent study showed that miRNAs are important for transmitting the experience of trauma to progeny through the paternal lineage [65]. Studying the importance of these varied contributions to transgenerational inheritance is important in understanding whether they are truly epigenetic.

From Lesson 2.1, we have learnt that the epigenetic marks to histones, as well as CpG methylation, are reproduced over the new DNA (chromatin) after DNA replication in a period of time that can extend to the next G1.

Copying pre-existing PTMs to new «naif» nucleosomes implies a general property of chromatin that we can call: « spreading »

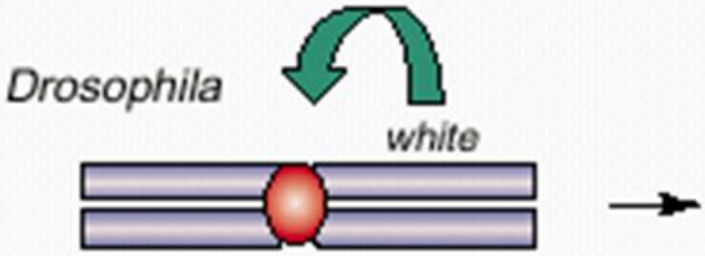
Is heterochromatin spreading a general principle ?

We have nice examples from older genetical research....

The first data concerns the historical concept in Genetics of the so-called «positional effect variegation» (**PEV)** in *D. melanogaster (…and other model organisms).*

Drosophila ?
Please note that Insects present very low levels of cytosine methylation. It has been mainly explained for transposon silencing.

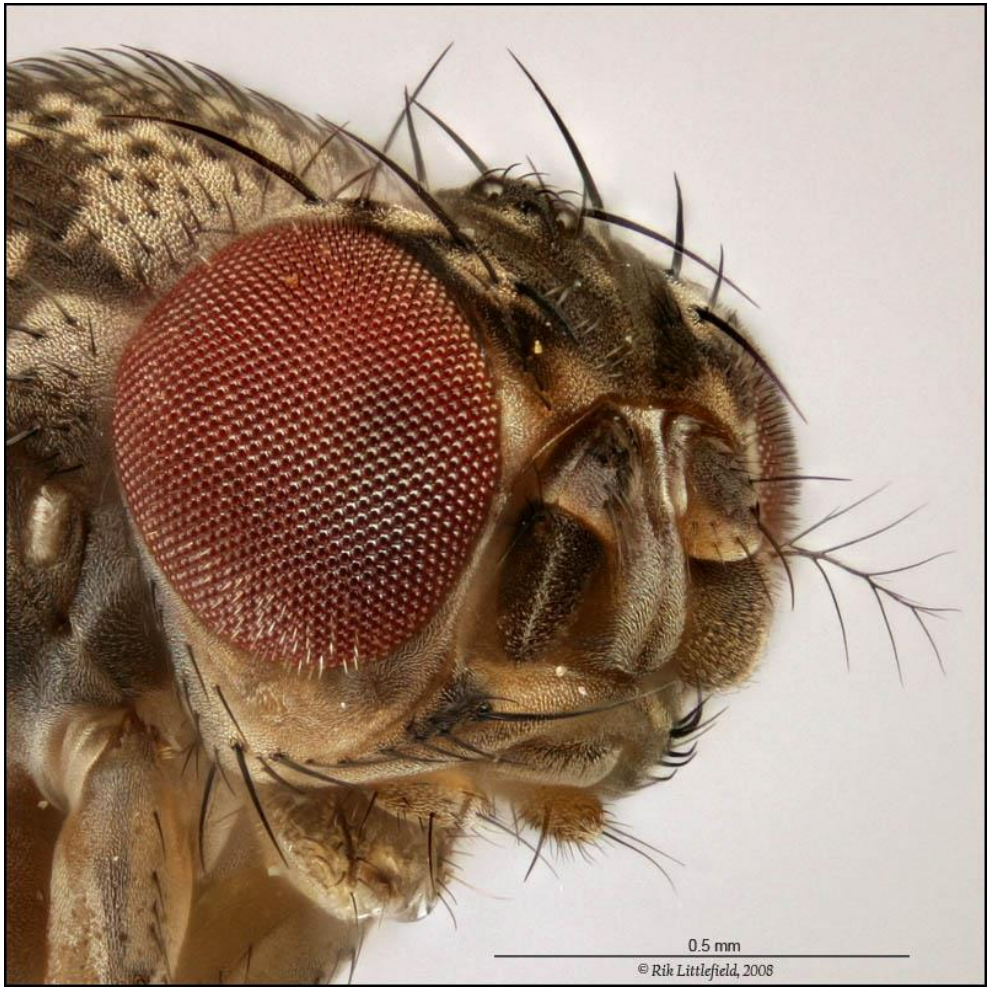
PEV = Positional Effect Variegation Silencing effects of heterochromatin in different organisms.



Translocation of the Drosophila *white* gene close to the centromere results in mosaic (variegated) expression in the eye.

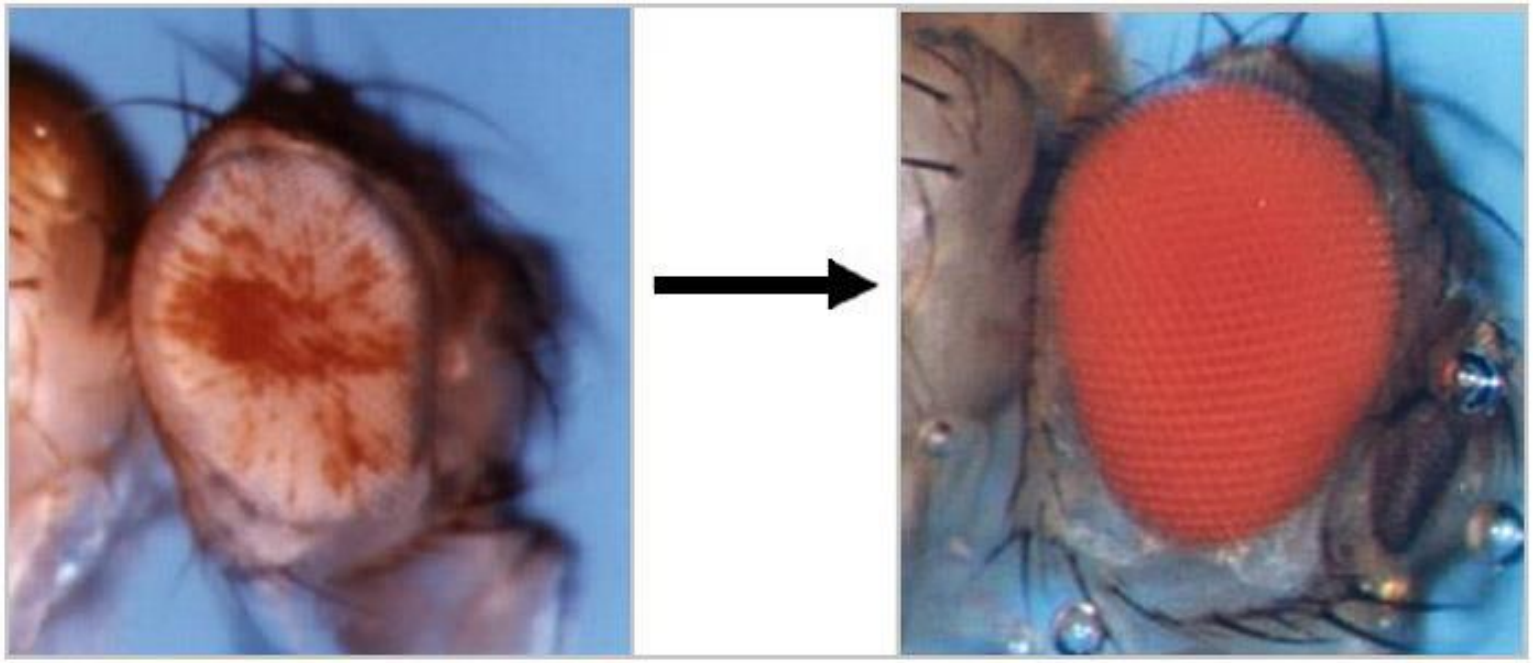
Insertion of transgenes into peri-centromeric regions in mice gives expression in a proportion of cells from expressing tissues. Expression is clonally stable through multiple cell divisions but also undergoes stochastic switching between the active and inactive states.

Insertion of the Ade2 gene into the telomeric region of Saccharomyces cerevisiae causes metastable silencing of the gene resulting in sectored colonies containing white (expressing) and red (nonexpressing) cells.


0.5 mm
© Rik Littlefield, 2008

Variegated

Actually, looking at the eyes of these animals, we observe patches of pigmented cells among a general white color of other cells.



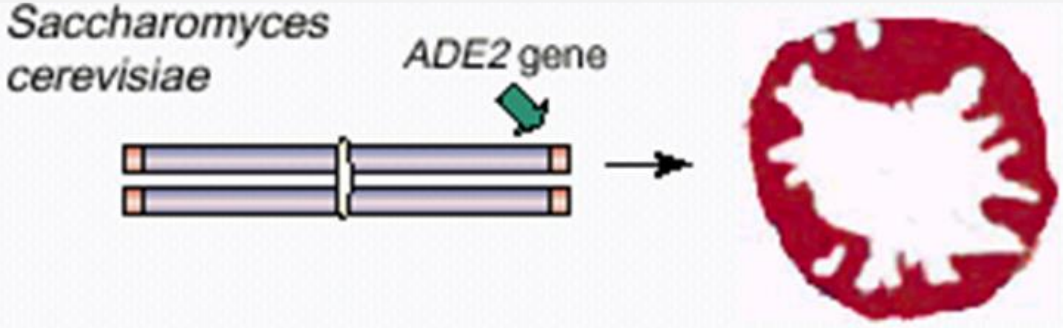
Washington University Department of Biology - Mutations in HP1 lead to a loss of silencing, a suppression of PEV.

Mendez et al, 2011

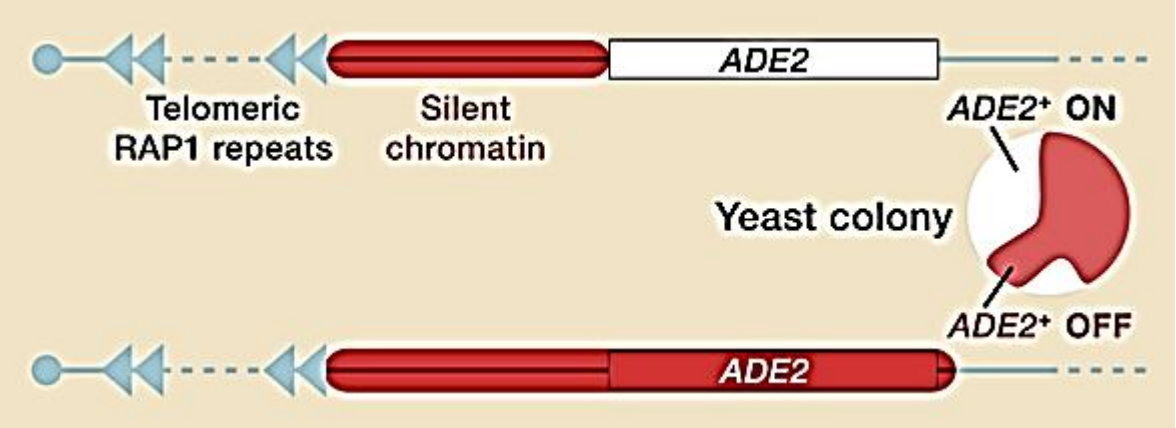
PEV = Positional Effect Variegation Silencing effects of heterochromatin in different organisms.

Translocation of the Drosophila *white* gene close to the centromere results in mosaic (variegated) expression in the eye.

Insertion of transgenes into peri-centromeric regions in mice gives expression in a proportion of cells from expressing tissues. Expression is clonally stable through multiple cell divisions but also undergoes stochastic switching between the active and inactive states.

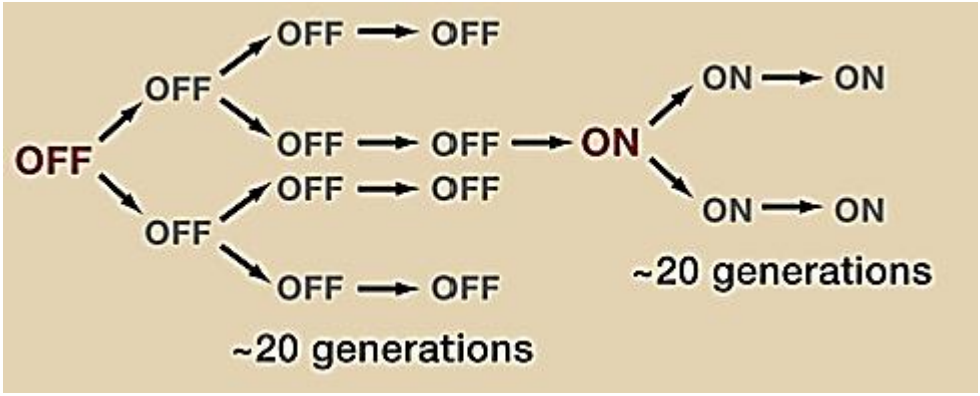


Insertion of the Ade2 gene into the telomeric region of Saccharomyces cerevisiae causes **metastable** silencing of the gene resulting in sectored colonies containing white (expressing) and red (nonexpressing) cells.

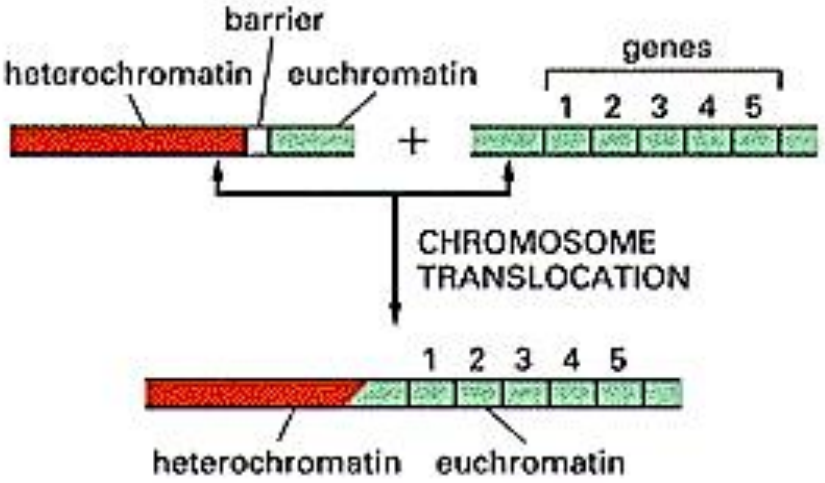


PEV

This variegation results from stochastic loss and re-establishment of silent chromatin and indicates that following a switch in gene expression, the daughters of the switching cell have **a memory of the expression state** of the mother cell.

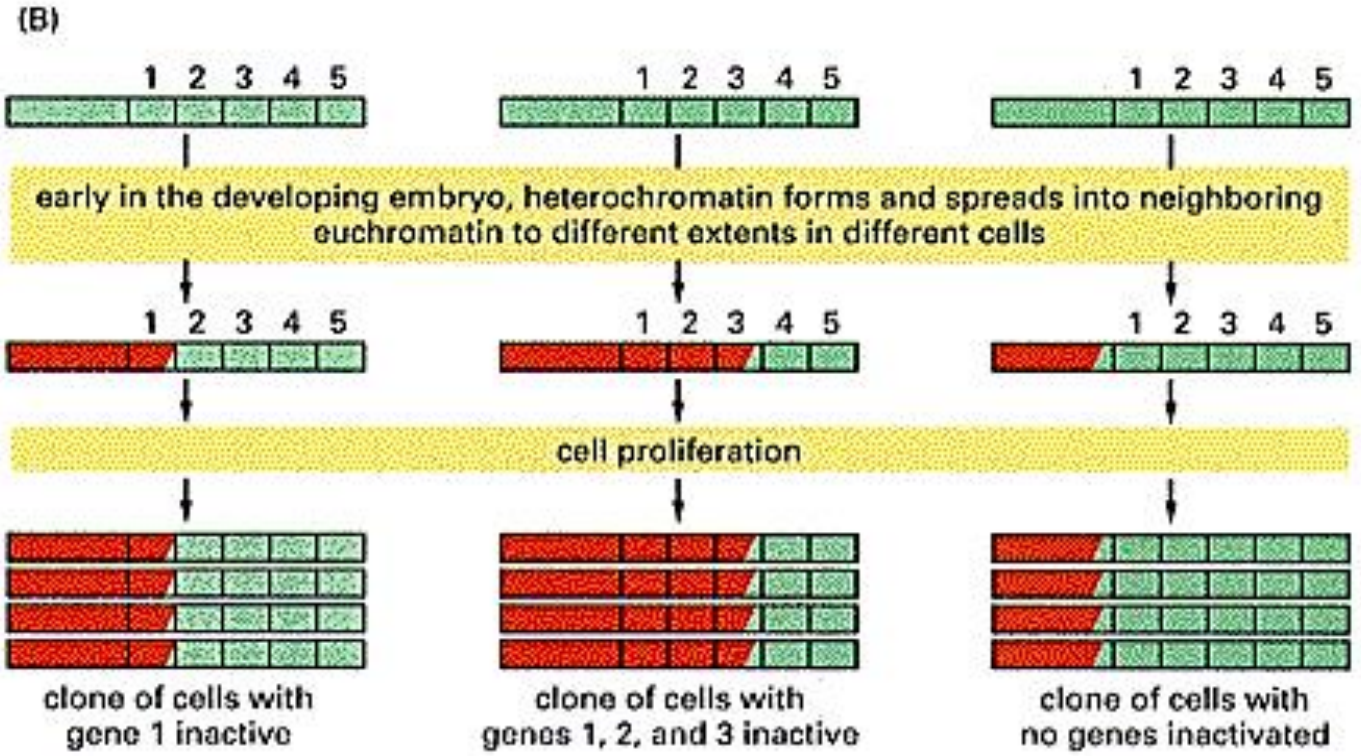


In normal situation, a «barrier» exist between heterochromatic and euchromatic domains
We call it «insulator».



Establishement →

Maintenance →



Where does heterochromatin start from ?

In other words, what is the original signal or event that will start heterochromatin formation ?

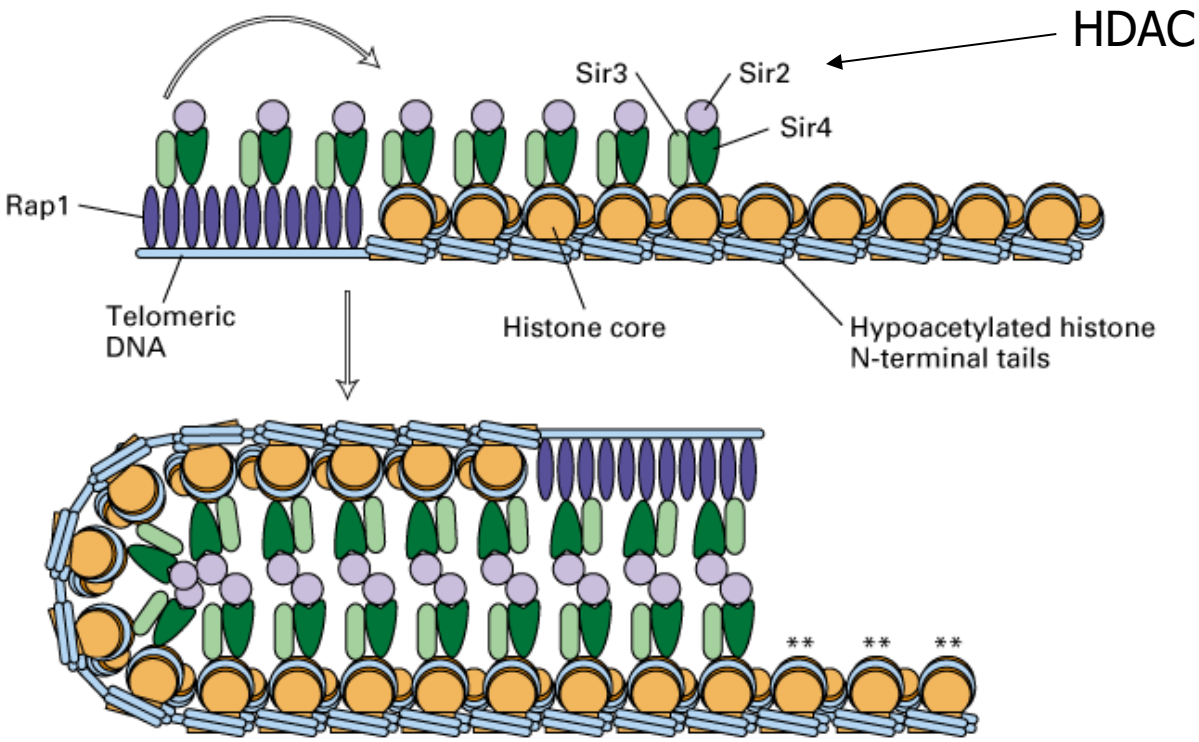
What are the **determinants** ?

cis & *trans*

Again, hints derive from older studies on the Yeasts (simpler, smaller genomes, less regulators on the plot, and *high rates of homologous recombination*!)

Most studied models:

- Telomeric heterochromatin (S. cerevisiae)
- MAT locus (S. cervisiae)
- Centromeric heterochromatin (S. pombe)



budding yeast

Linear chromosome replication

Telomere structure

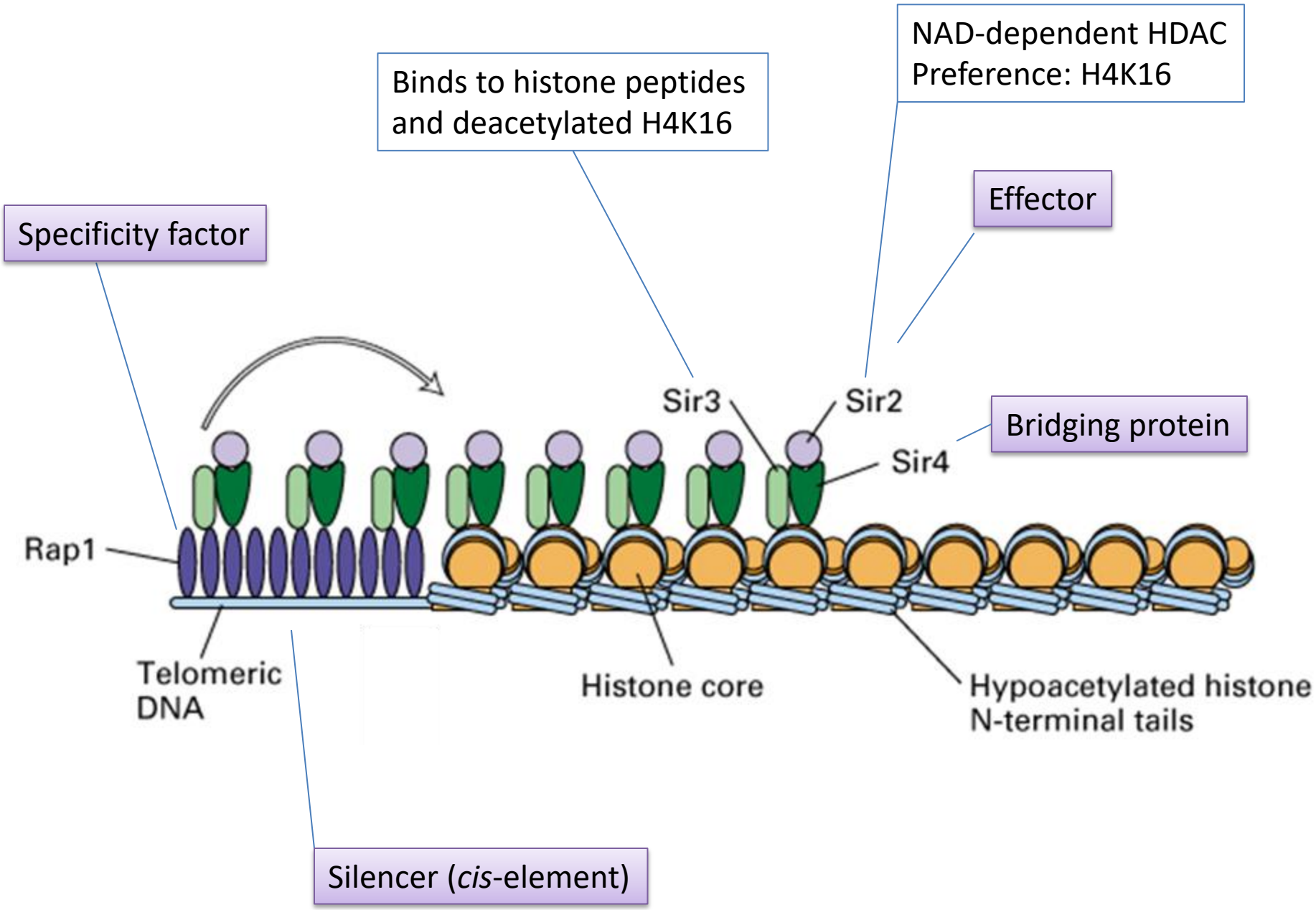
Telomerase

Model for the formation of telomeric heterochromatin. Black lines wrapped around nucleosomes represent DNA.

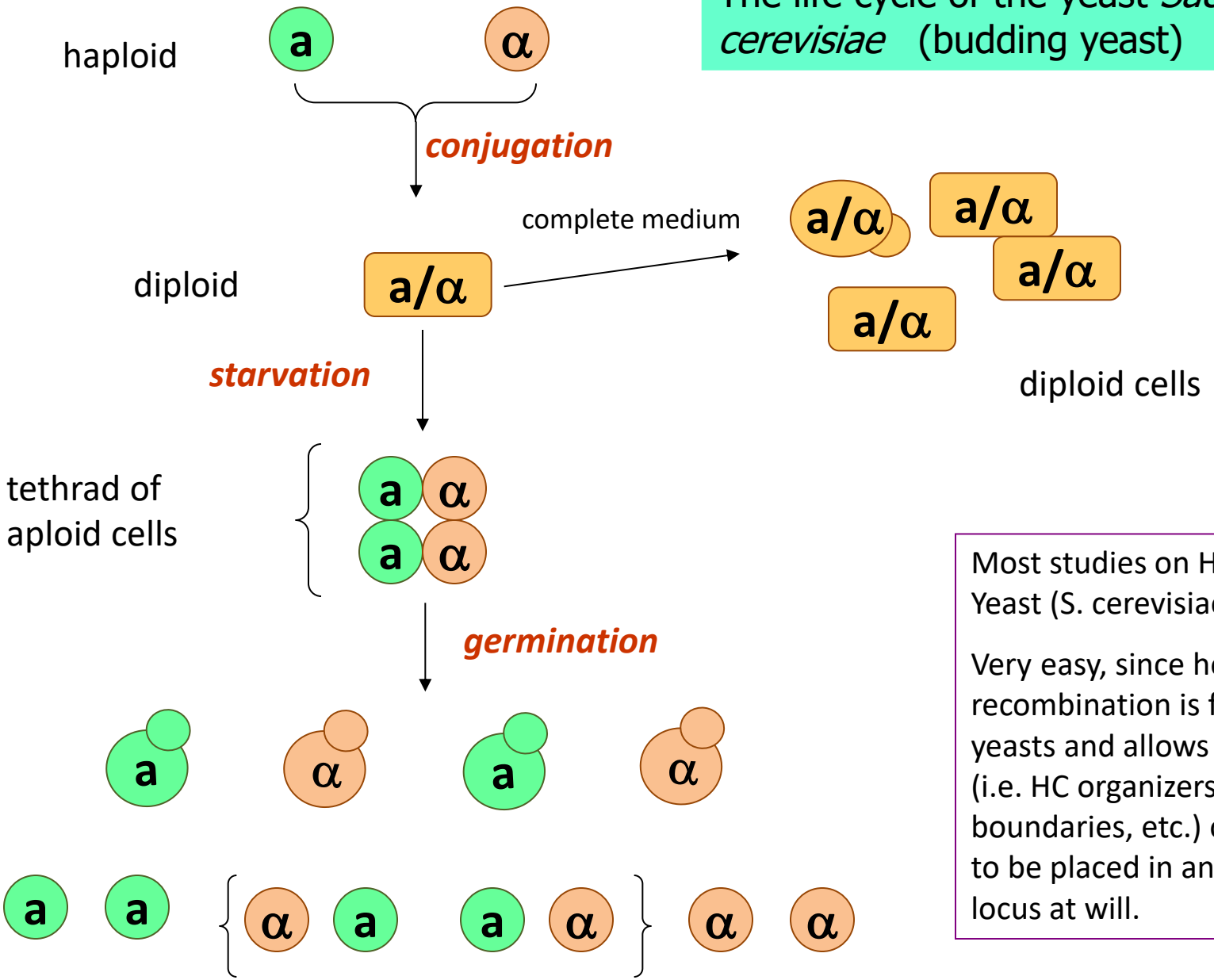
(a) Core telomeric heterochromatin in wild-type cells containing only a single genomic copy of SIR3. It is proposed that the RAP1-containing telosome folds back onto subtelomeric regions. In this manner, RAP1–SIR–histone interactions are all required for stability of the complex.

(b) Upon SIR3 overexpression, telomere position effect and the presence of SIR3 is extended up to some 16–20 kb from the telomere. SIR3 overexpression causes loss of some SIR4 and most SIR2 from the complex. Due to the interdependence of RAP1–SIR3–H4 interactions, and because all three SIR proteins are required for extension of heterochromatin by SIR3, it is proposed that the complex necessary for the initiation of heterochromatin formation requires RAP1, the SIR proteins and H4.

S. Cerevisiae telomere HC



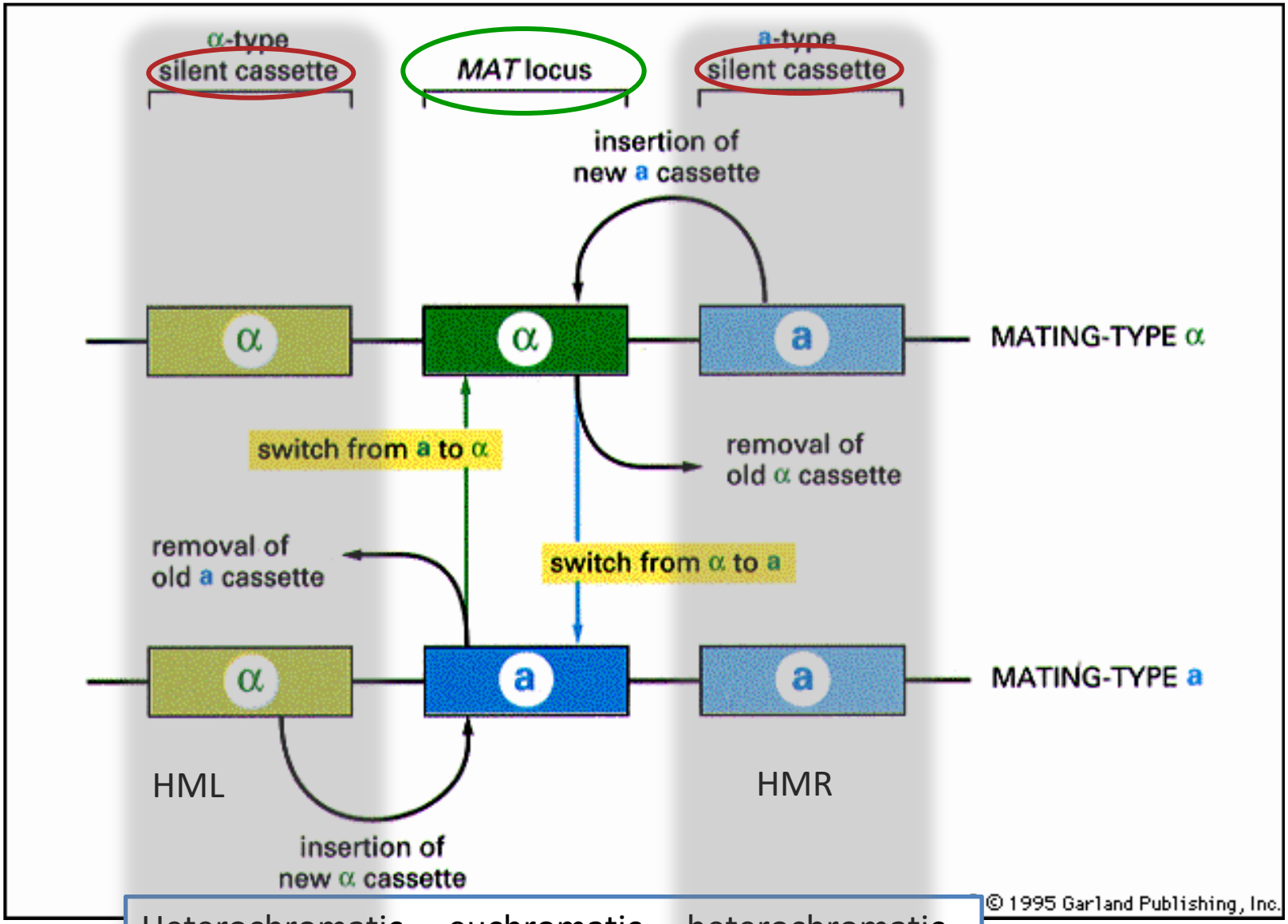
The life cycle of the yeast *Saccaromyces cerevisiae* (budding yeast)



Most studies on HC made in the Yeast (S. cerevisiae / S. pombe)

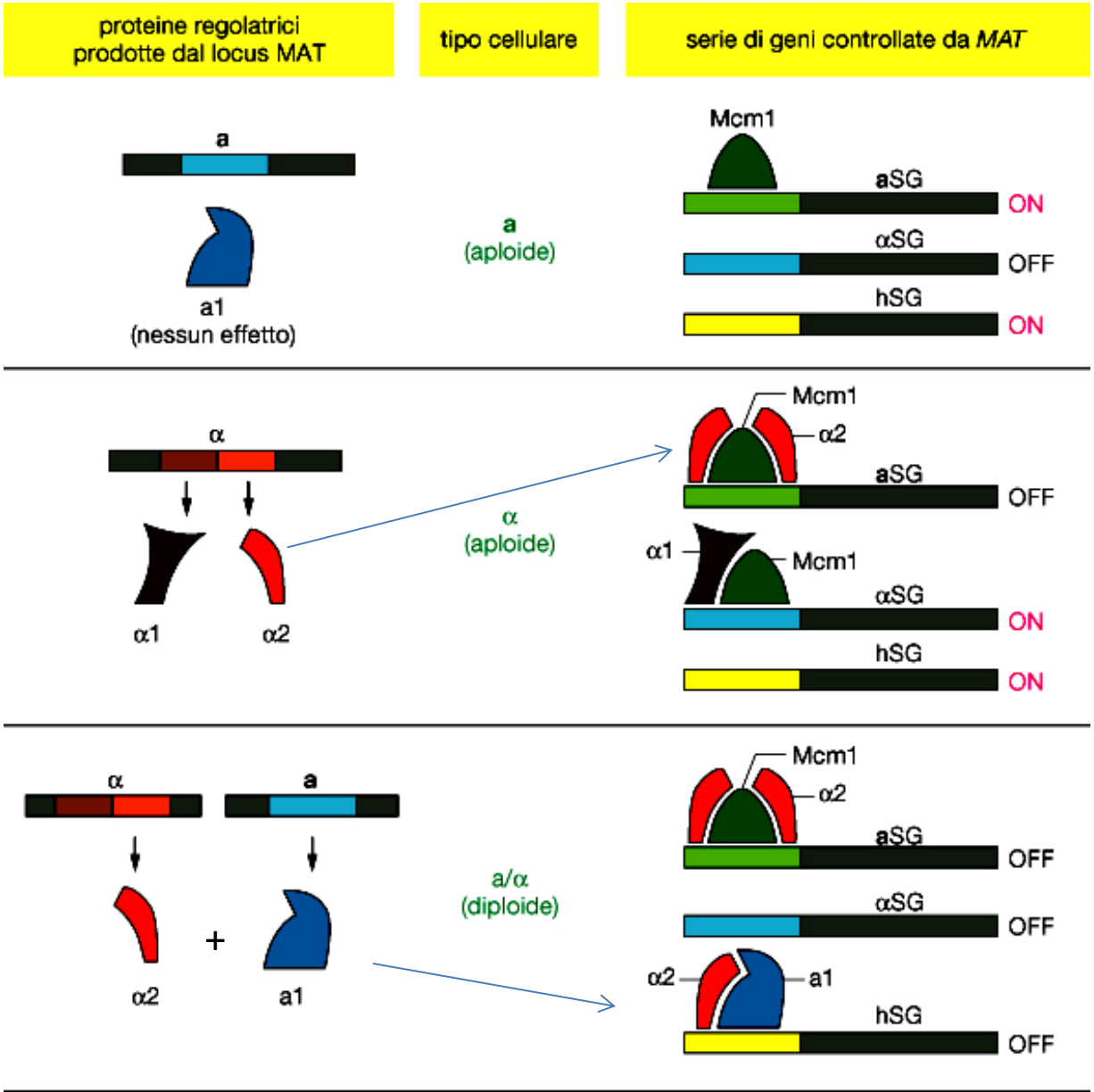
Very easy, since homologous recombination is favored in yeasts and allows HC elements (i.e. HC organizers, silencers, boundaries, etc.) or reporters to be placed in any genomic locus at will.

HMR/HML loci in yeast have heterochromatic features, e.g. are resistant to endonuclease digestion and **silence constructs placed within**



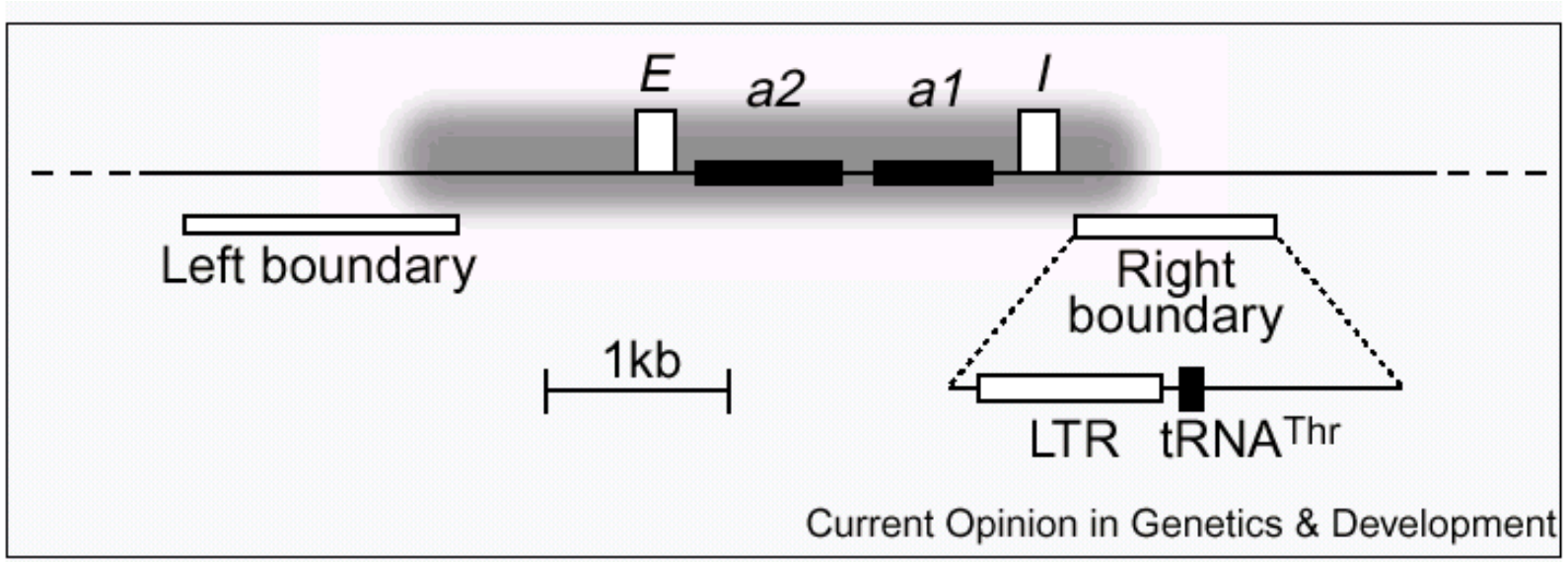
Heterochromatic euchromatic heterochromatic

A and α cassettes encode transcription factors that regulate aploid and mating type-specific genes



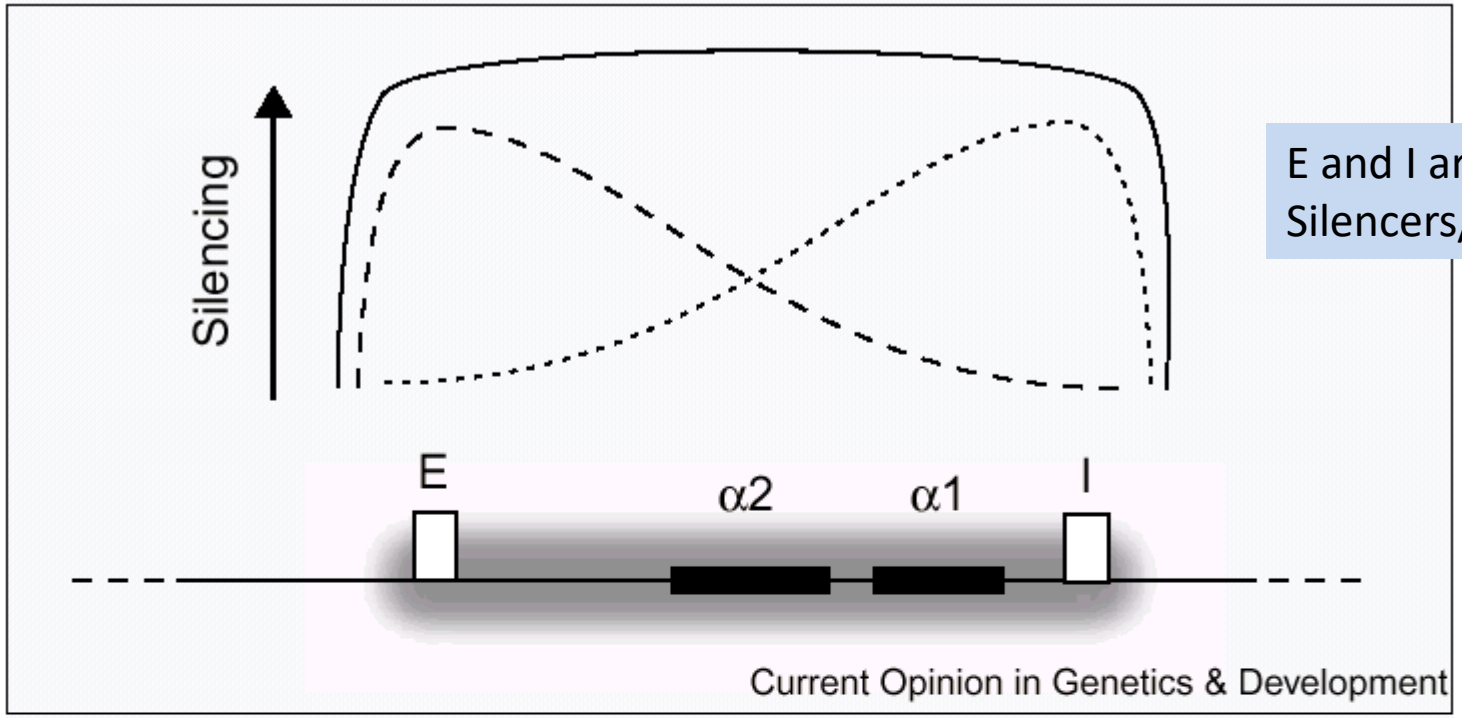
Mcm1 is constitutive

a-specific genes (aSG)
α-specific genes (αSG)
haploid-specific genes (hSG)



HMR

Organization of heterochromatin barriers surrounding *HMR*. The *HMR* locus is diagrammed, showing the location of the mating type genes *a1* and *a2*, the *E* and *I* heterochromatin organizing centers (silencers) and the left and right heterochromatin barriers as defined in [1••]. Background shading indicates the extent of the repressed domain. An expansion of the right barrier shows the location of a Ty1 LTR and the gene for $tRNA^{Thr}$. Earlier evidence suggested that both these elements contribute to barrier activity [1••] but more recent data indicate that the $tRNA^{Thr}$ gene is necessary and sufficient for full barrier activity

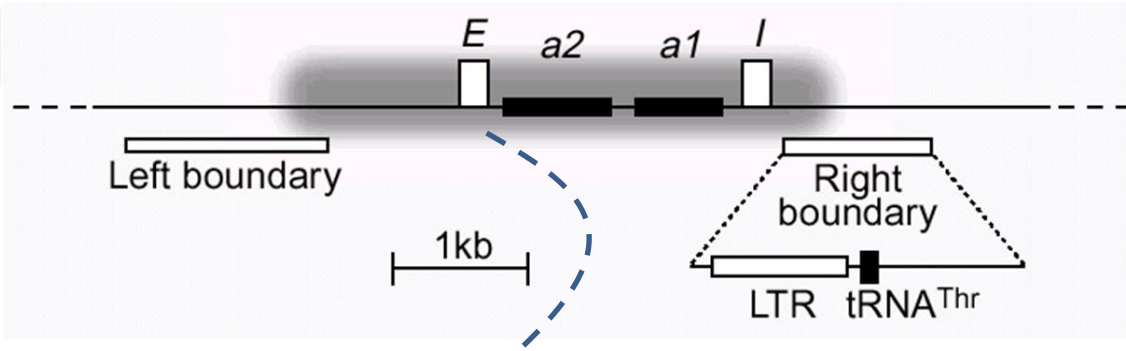


E and I are mixed
Silencers/barriers

HML

Domain organization by directional initiation of heterochromatin. The *HML* locus is diagrammed, showing the location of the mating type genes, α*1* and α*2*, and the *E* and *I* heterochromatin organizing centers (silencers). Background shading indicates the extent of the repressed domain. Repression emanating from *E* is represented as a dashed line, whereas that emanating from *I* is represented as a dotted line. The sum of the effects of the two organizing centers, shown as a solid line, results in uniformly high repression between the two centers with repression dropping off sharply outside the domain. This model emerges from studies on the domain organization of HML presented in [35•].

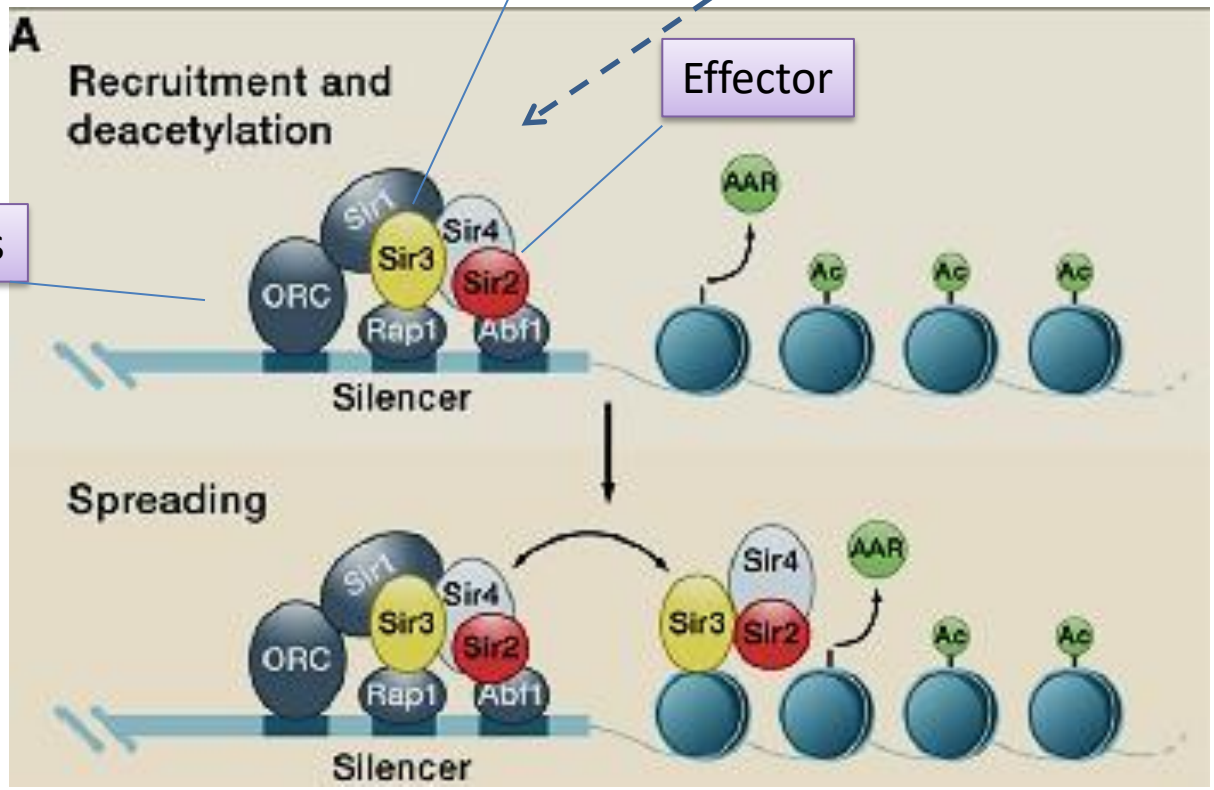
S. Cerevisiae MAT locus HC



Bridging protein

Effector

Specificity factors

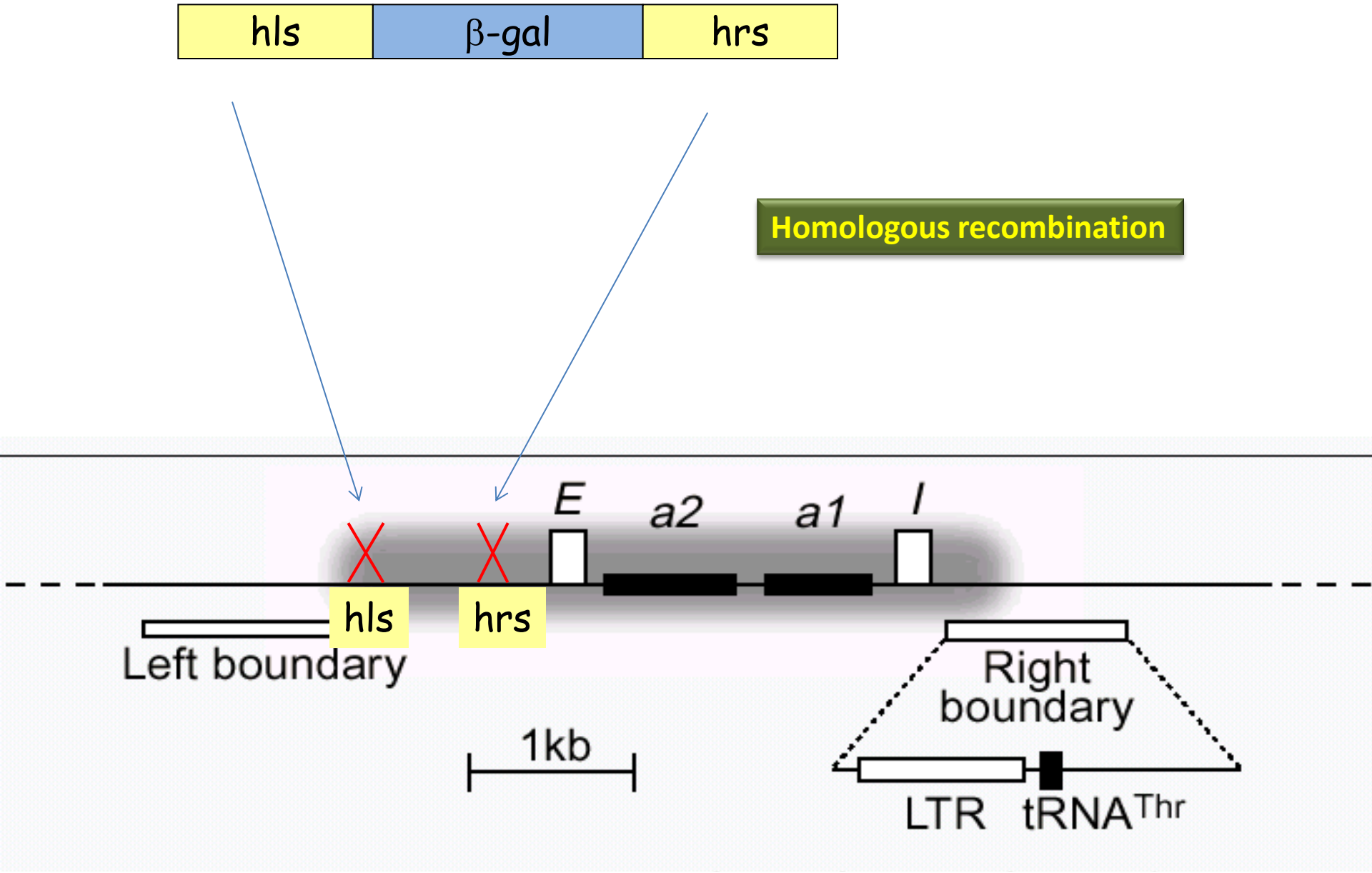


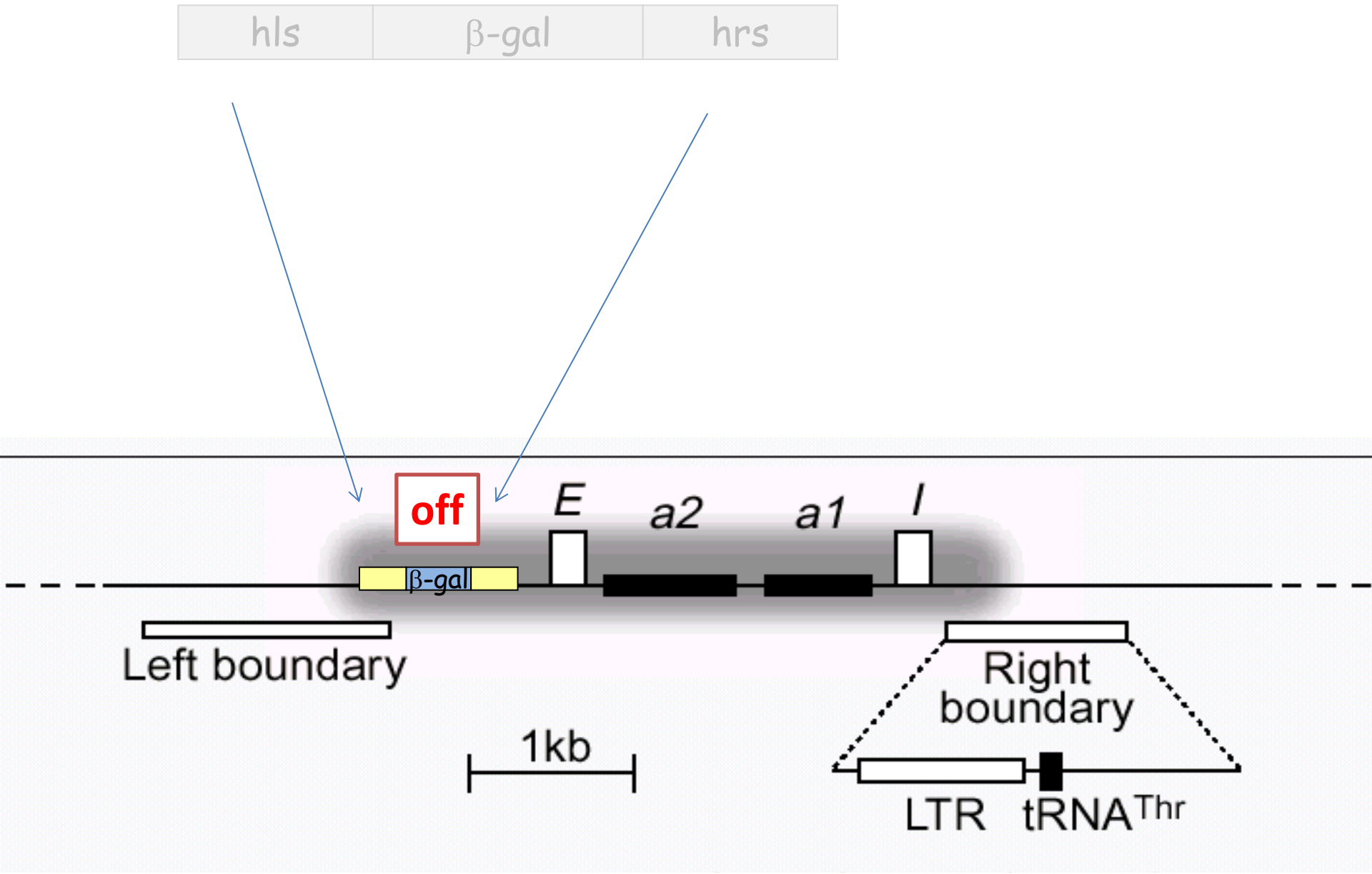
Silencer (*cis*-element)

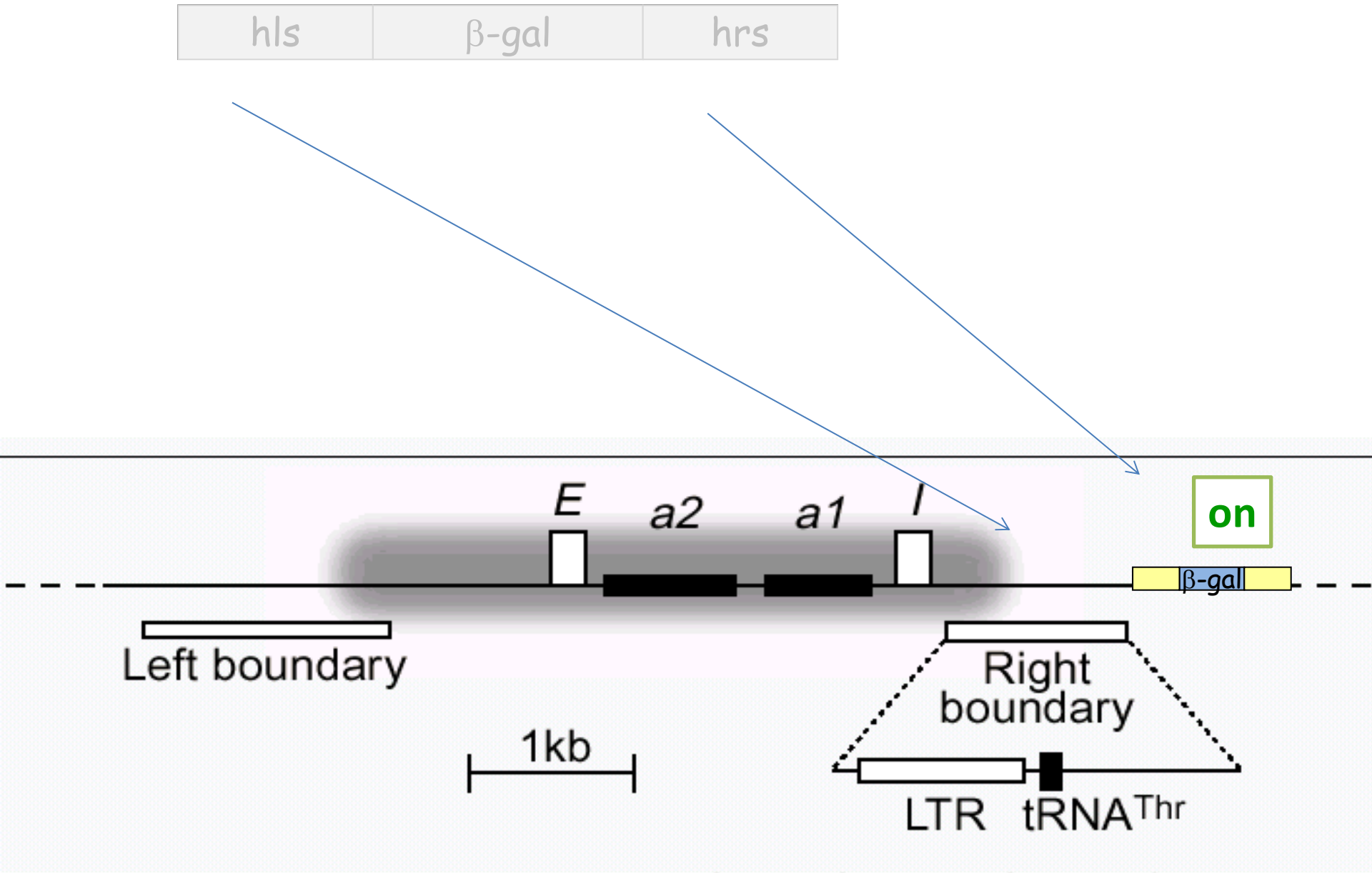
Methodology

High efficiency of Homologous Recombination in Yeast makes easy to study the effects of Heterochromatizatin / euchromatization, using reporter genes such as:

- Beta-galactosidase (blue color)
- Ura4 (S. pombe) or Ura3 in S.cerevisiae, is required to grow in absence of uracil and renders cells sensitive to toxicity of 5-FOA
- …other


hls
β-gal
hrs
Homologous recombination
E
a2
a1
I
hls
hrs
Left boundary
1kb
Right boundary
LTR
tRNA Thr


hls
β-gal
hrs
off
E
a2
a1
I
β-gal
Left boundary
Right boundary
1kb
LTR
tRNA Thr


hls
β-gal
hrs
E
a2
a1
I
on
β-gal
Left boundary
Right boundary
1kb
LTR
tRNA Thr

Looking to these model systems, we can conclude:

- A DNA element that we can call «silencer» (talking heterochromatin)
- A molecule recognizing this DNA element, called «specificity factor»
- A bridging protein
- A chromatin competent enzyme

Can we guess anything about chromatin establishment in animals ?

- A DNA element that we can call «silencer» (talking heterochromatin)
- A molecule recognizing this DNA element, called «specificity factor»
- A bridging protein
- A chromatin competent enzyme

TF binding sites

Transcription Factors

co-regulator

PRC2 ?

Chromatin establishment, programming and re-programming during development

Mechanisms :

1) How are different chromatin domain established ?
   - pre-existing epigenetic marks
   - DNA sequence
   - Stage- and tissue-specific Transcription Factors

programming

2) How are chromatin domains maintained ?
   - Epigenetic marks inheritance
   - Local spreading

maintenance

1) How can chromatin domains be re-programmed ?
   - Specific combination of Transcription Factors
   - Erasure of histone marks and CpG methylation

reprogramming

Suggested readings, for those of you who are development adepts…

REVIEW FOCUS ON EPIGENETIC DYNAMICS

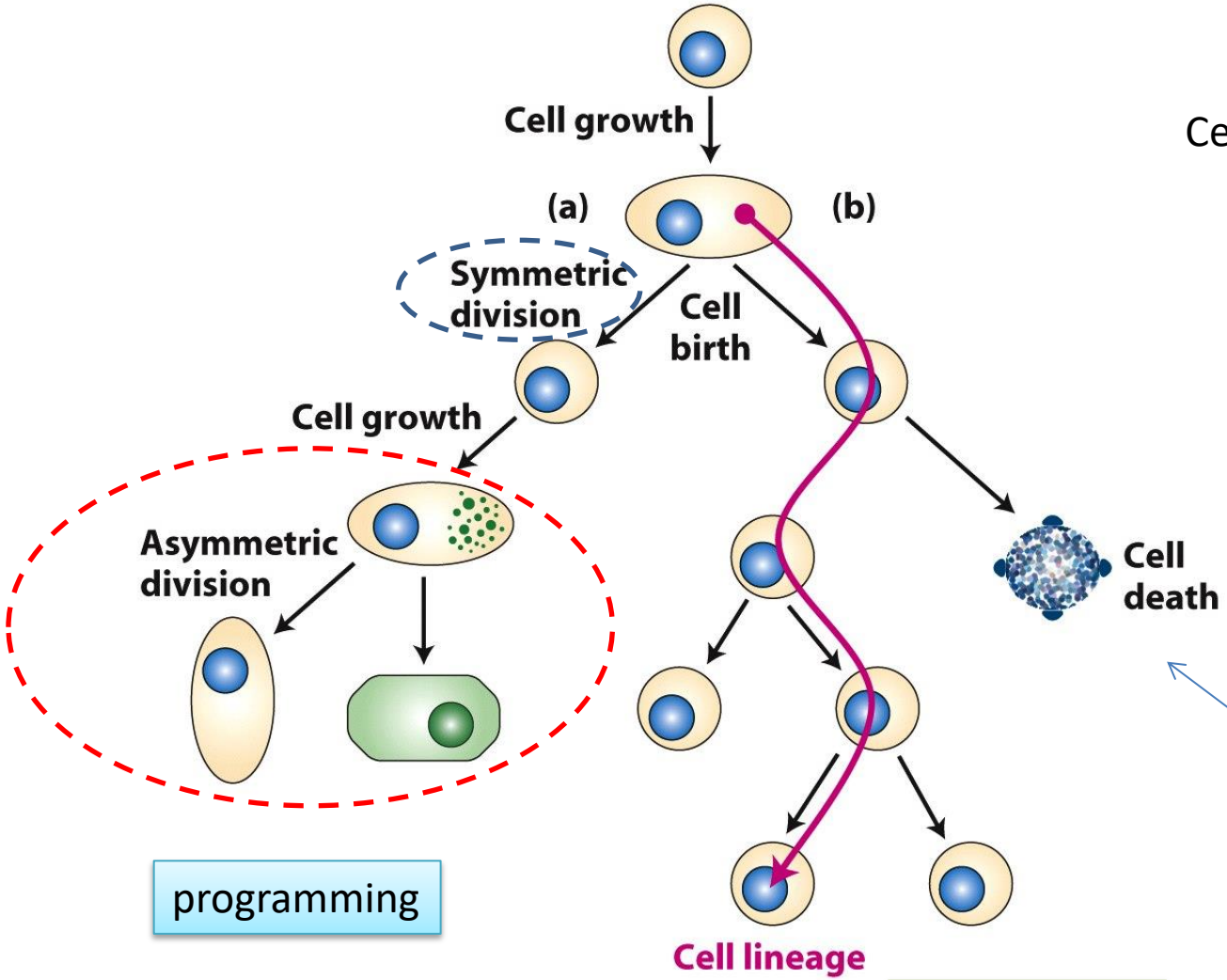
nature structural & molecular biology

Epigenetic programming and reprogramming during development

Irene Cantone & Amanda G Fisher

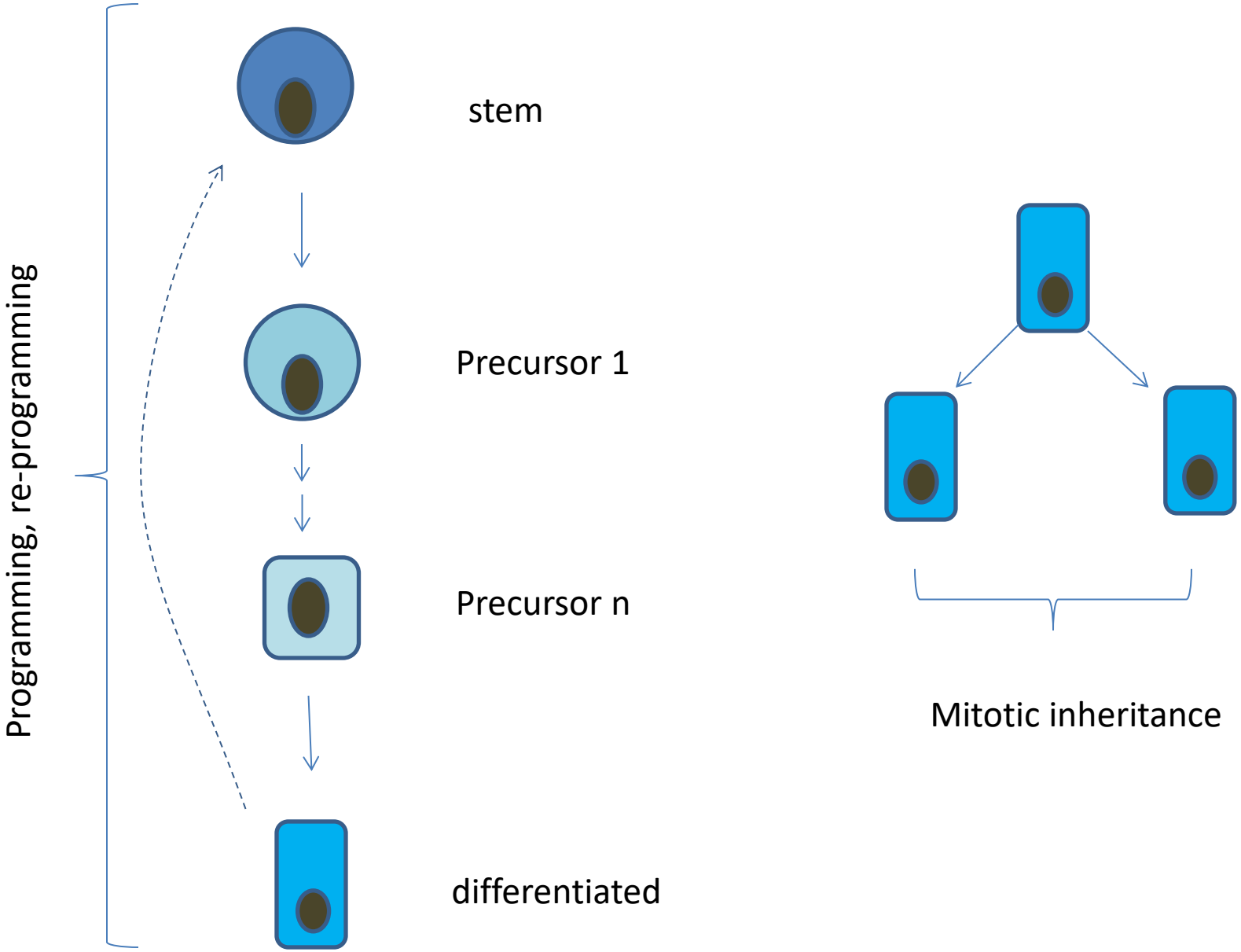
Cell identity is determined by specific gene expression patterns that are conveyed by interactions between transcription factors and DNA in the context of chromatin. In development, epigenetic modifiers are thought to stabilize gene expression and ensure that patterns of DNA methylation and histone modification are reinstated in cells as they divide. Global erasure of epigenetic marks occurs naturally at two stages in the mammalian life cycle, but it can also be artificially engineered using a variety of reprogramming strategies. Here we review some of the recent advances in understanding how epigenetic remodeling contributes to conversion of cell fate *in vivo* and *in vitro*. We summarize current models of epigenetic erasure and discuss the various enzymes and mechanisms that may operate in cellular reprogramming.

Cellular genealogy

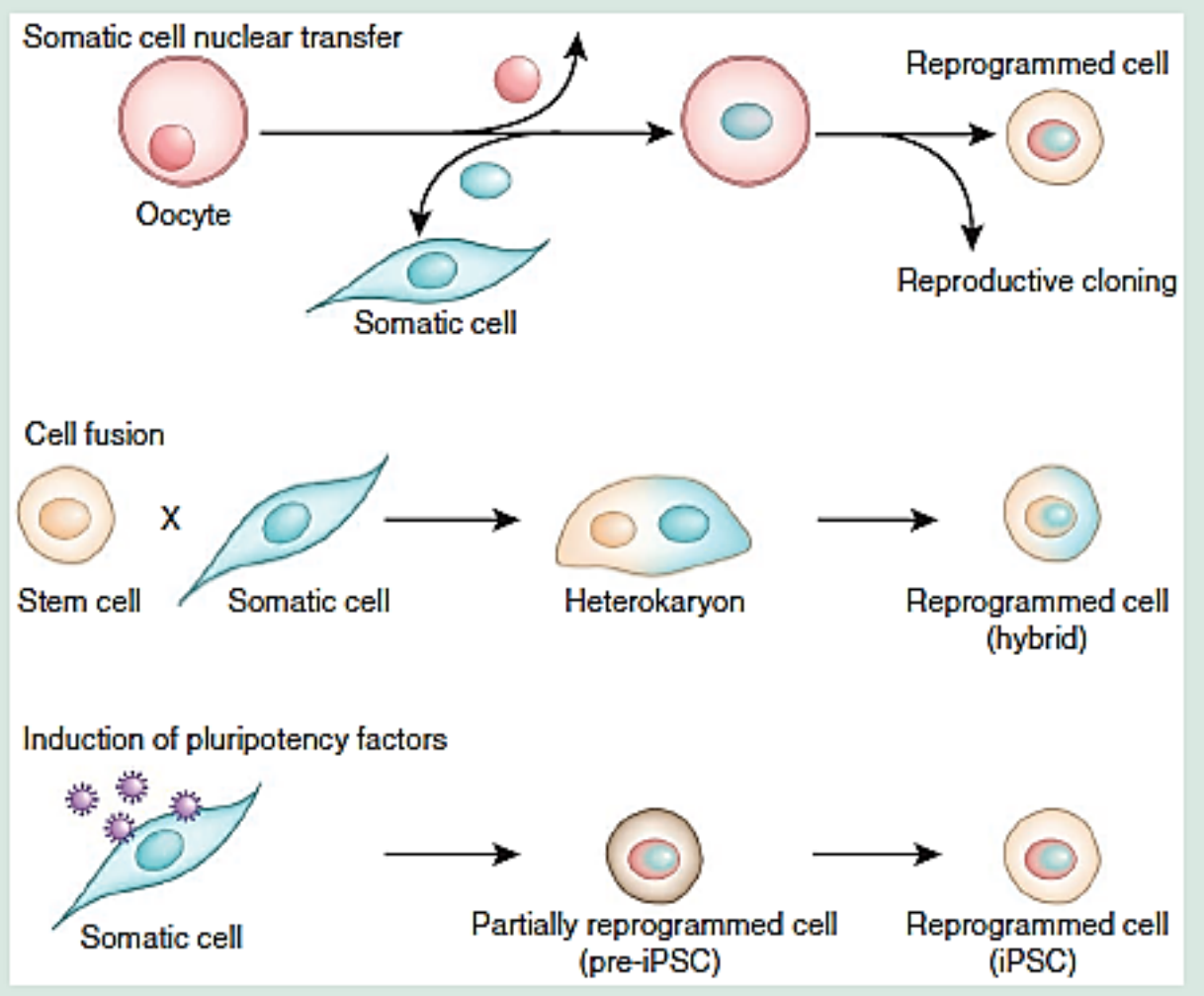


Apoptosis

Figure 21-1
Molecular Cell Biology, Sixth Edition


Programming, re-programming
stem
Precursor 1
Precursor n
differentiated
Mitotic inheritance

Re-programming



Cantone & Fisher, 2013

Transcription factors (sequence-specific DNA binding proteins) can induce re-programming of chromatin domains and partial stemness

http://www.genecards.org/cgi-bin/carddisp.pl?gene=NPM1

ES cells

neuronal progenitor cells

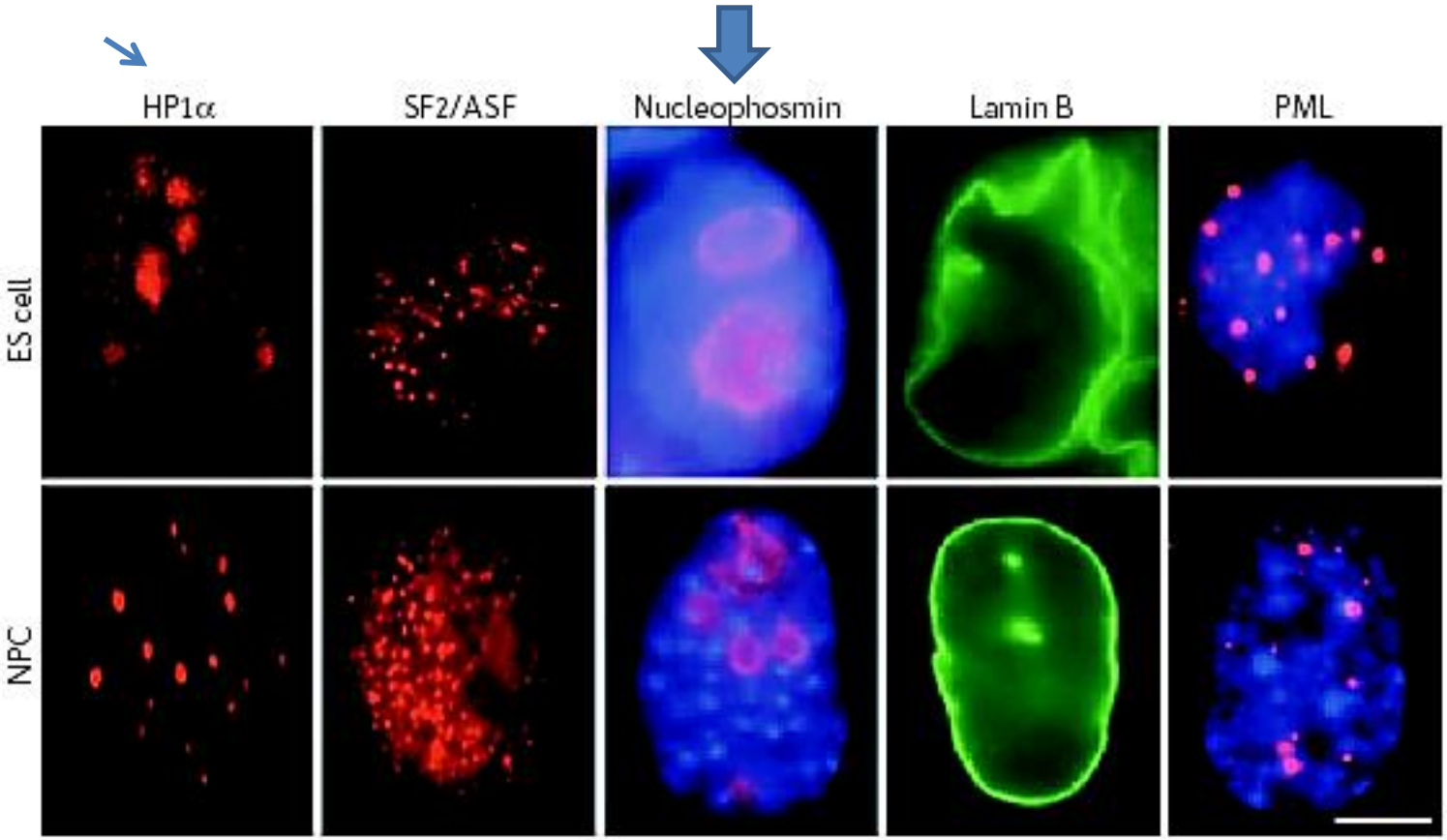


Figure 1 | **Nuclear architecture in ES cells and differentiating ES-derived cells.** Nuclear domains in an undifferentiated embryonic stem (ES) cell (top) and a differentiating ES-derived neuronal progenitor cell (NPC, bottom). From left to right: heterochromatin, as detected with an anti-HP1α antibody, is confined to fewer and larger foci in ES cells compared with NPCs; nuclear speckles, as detected with an anti-SF2/ASF antibody, appear as small, dispersed foci in ES cells and become more conspicuous in NPCs; nucleoli, as identified with an anti-nucleophosmin antibody, appear larger in ES cells compared with NPCs; the ill-defined nuclear lamina in ES cells, stained with an anti-lamin B antibody, becomes round and distinct in NPCs; promyelocytic leukaemia (PML) bodies labelled with an anti-PML antibody show similar patterns in ES cells and NPCs. DAPI, blue. Scale bar, 5 μm.

(from Meshorer & Misteli, 2006)

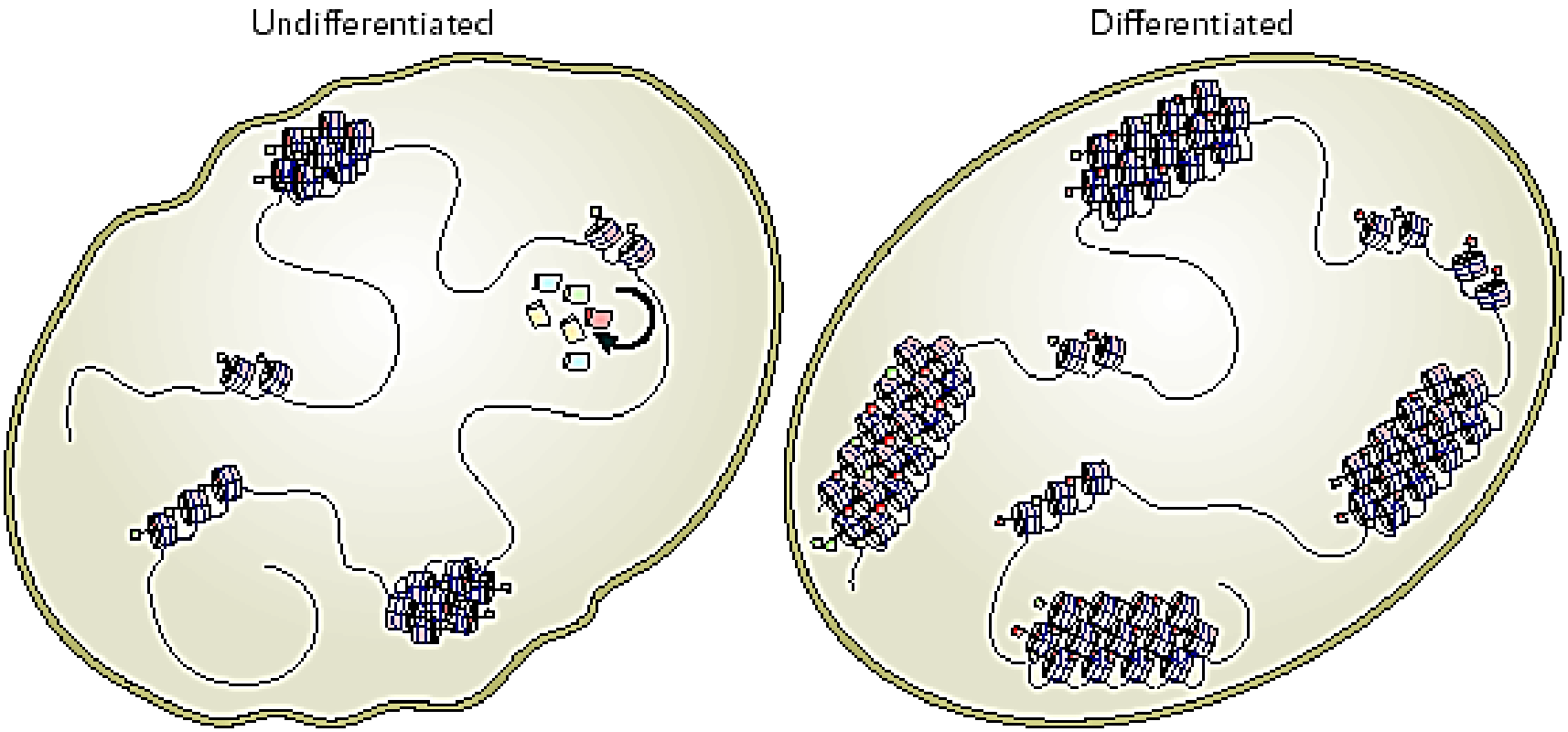
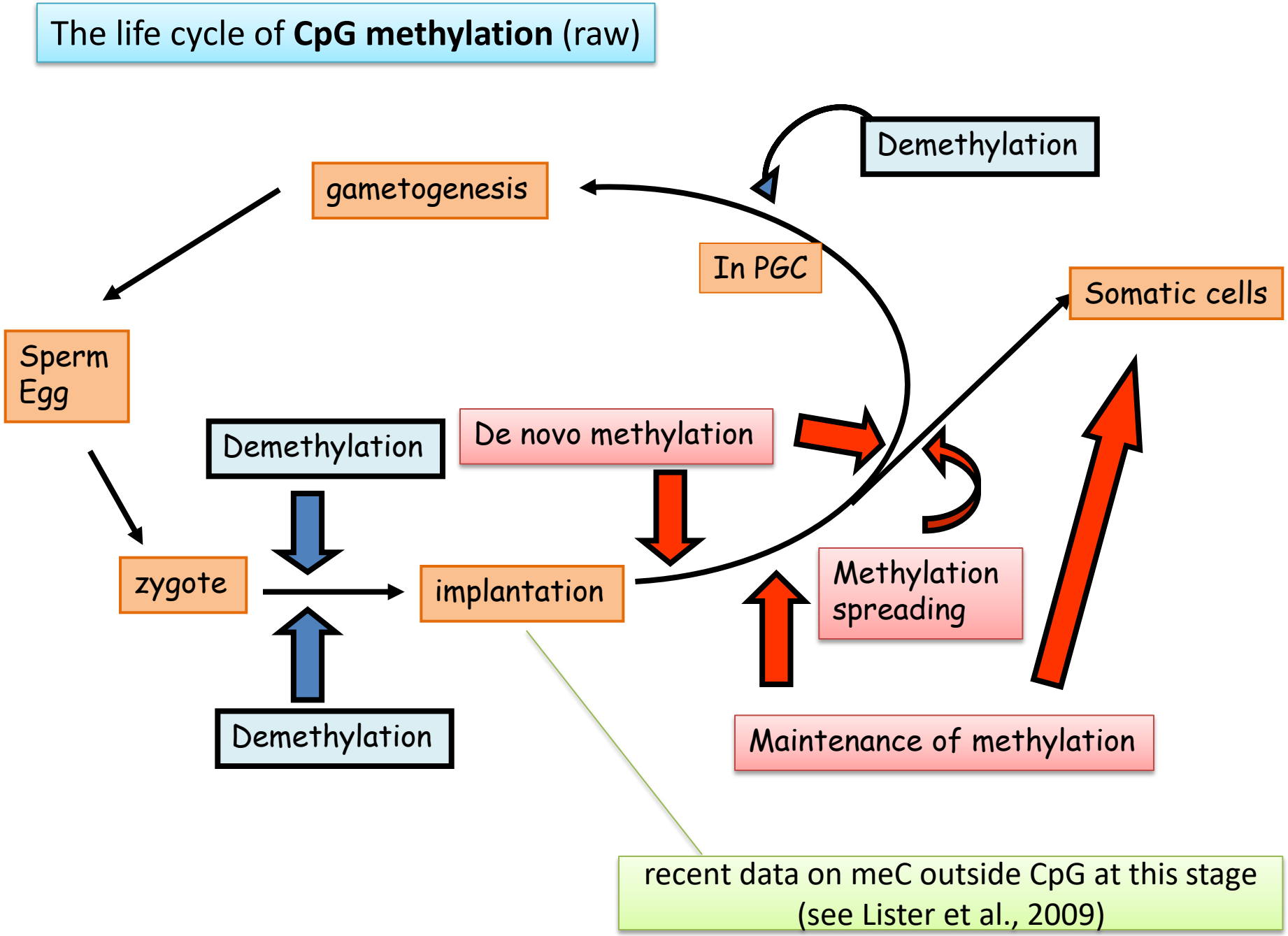
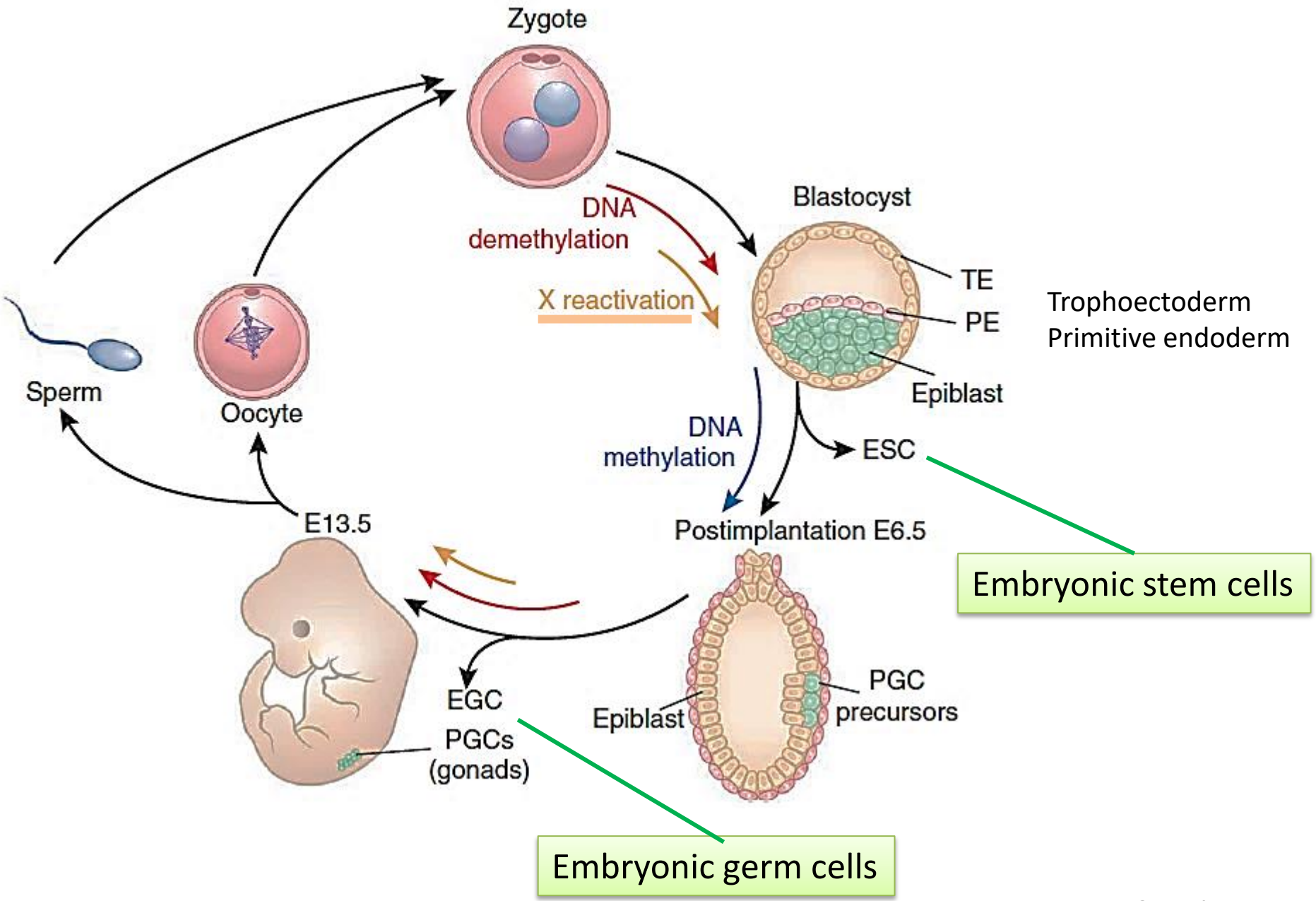


Figure 2 | **Chromatin during ES-cell differentiation.** In pluripotent embryonic stem (ES) cells (left), chromatin is globally decondensed, enriched in active histone marks (green circular tags), and contains a fraction of loosely bound architectural chromatin proteins. As cells differentiate (right), regions of condensed heterochromatin form, silencing histone marks (red circular tags) accumulate, and structural chromatin proteins become more stably associated with chromatin.

(from Meshorer & Misteli, 2006)

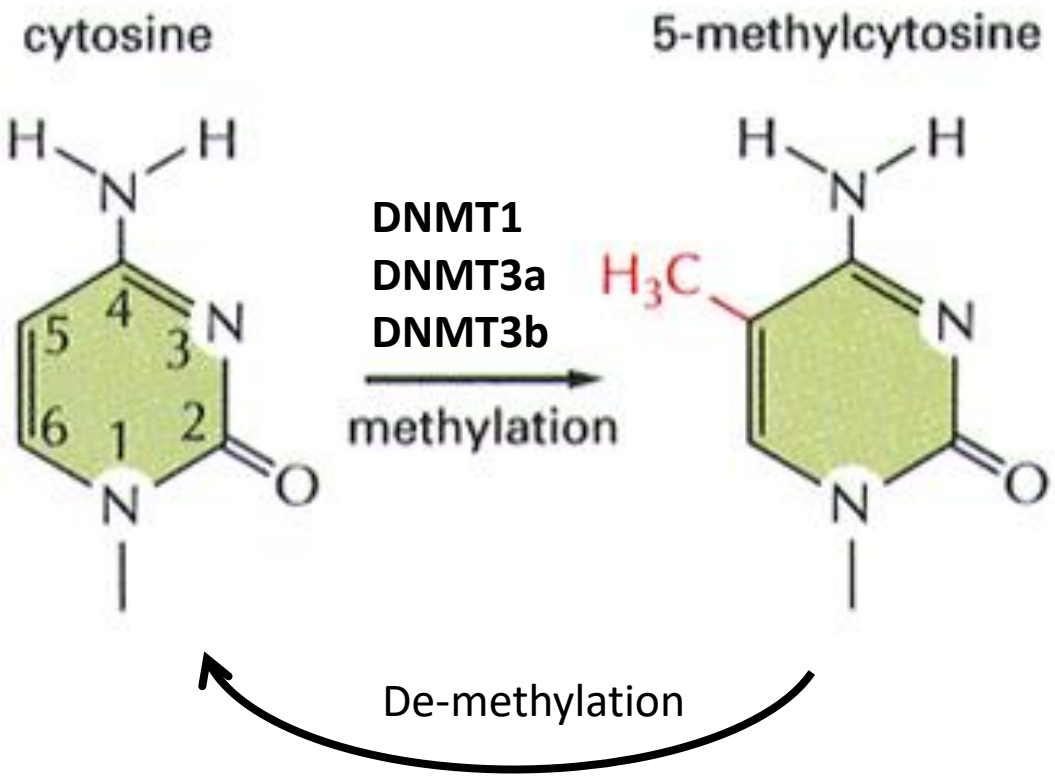
exploring chromatin determination in the life cycle of Mammals


The life cycle of **CpG methylation** (raw)
Demethylation
gametogenesis
In PGC
Somatic cells
Sperm
Egg
Demethylation
De novo methylation
zygote
implantation
Methylation
spreading
Demethylation
Maintenance of methylation
recent data on meC outside CpG at this stage
(see Lister et al., 2009)



Cantone & Fisher, 2013

Before proceeding, let us consider again some aspects of CpG methylation biochemistry



1. **Oxidative pathway (Tet enzymes)**
2. **Dilution (DNMT1 off during repeated cell division)**
3. **BER**

CpG - Cytosine methylation

is a dynamic mark

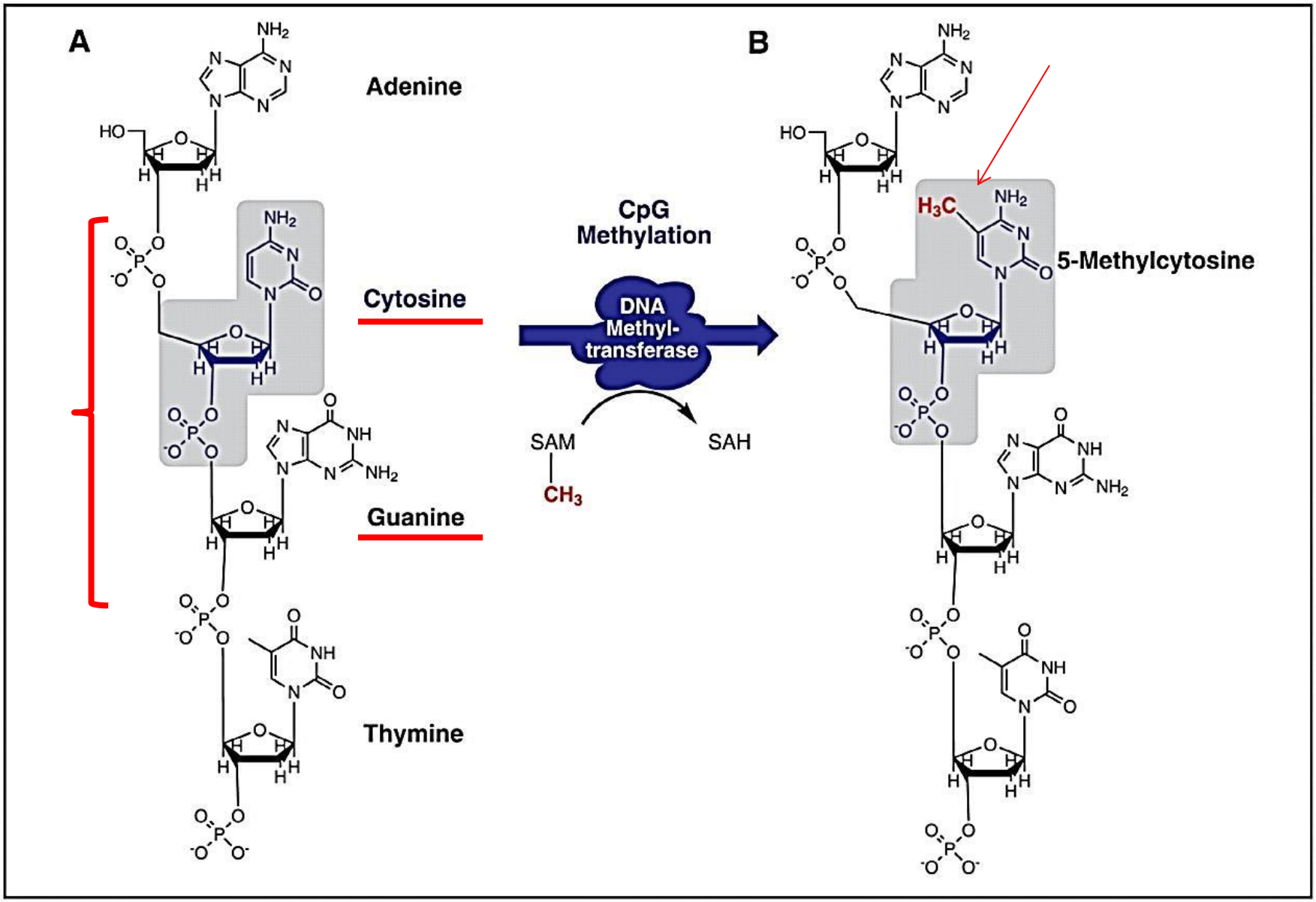
Enzymes that catalyze methylation are SAM-dependent enzymes:

DNMT1 – maintenance enzyme
DNMT3a -
DNMT3b - } *de novo* methyl-transferases

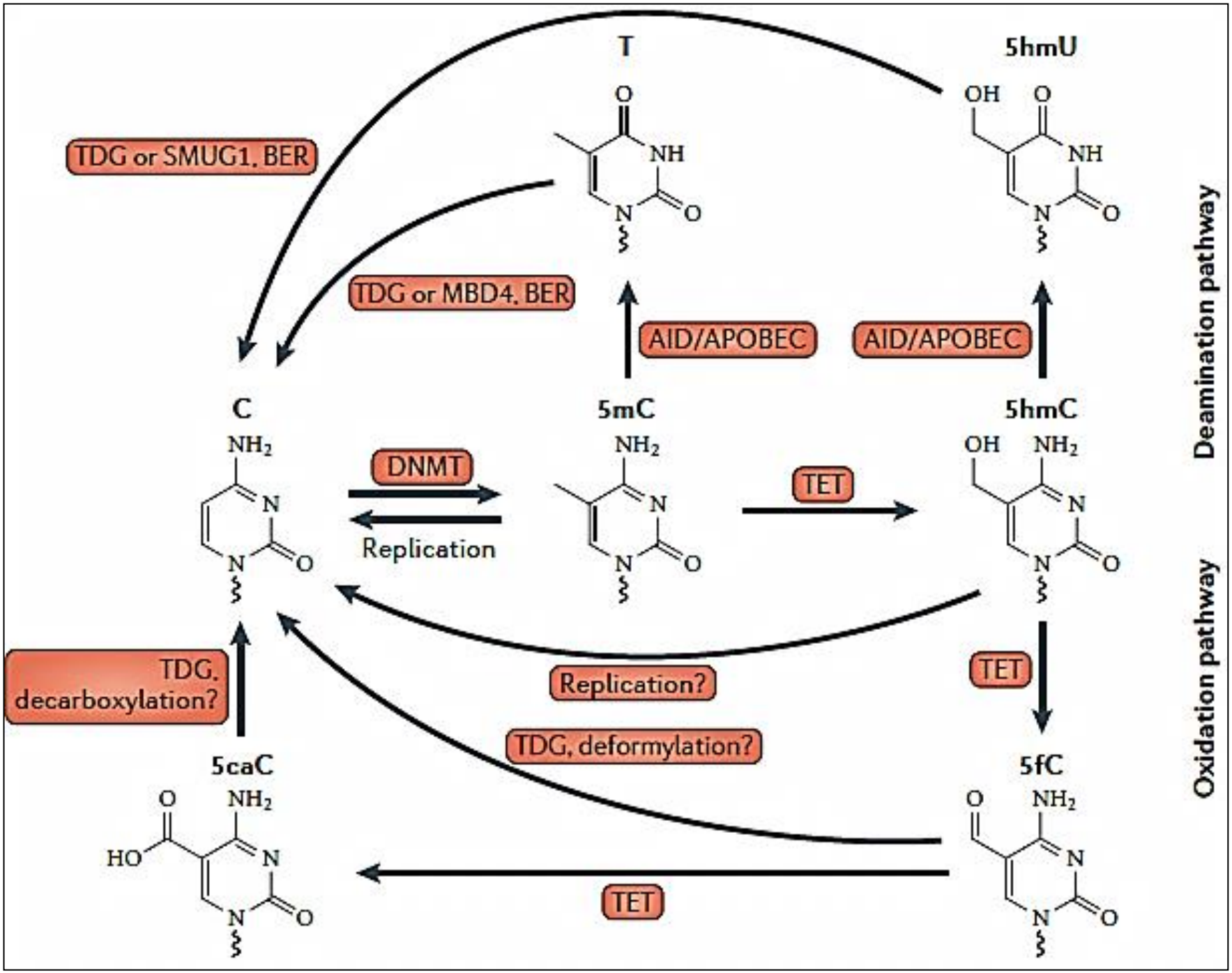
DNMT3L is a similar gene that encodes a nonenzymatic protein, that has cofactor and regulator roles. It binds to unmethylated H3K4 .
DNMT3L also interacts with HDAC1.

A fourth homologue DNMT2 has little activity toward DNA, higher with RNA.

CpG methylation



Branco et al., Nat Rev Genet. (2012) 13: 7-13.



Remember that CpG methylation is usually repressive. It can:

- occlude regions to certain TFs
- recruit MBD-containing proteins

Finally, keep in mind that CpG metabolism enzymes are usually associated with chromatin-competent WERs such as HDACs and HMTs

CpG methylation (hypermethylation, meaning that a region containing several CpG dinucleotides show several methylations) is usually associate with **silencing.** Possible mechanisms:

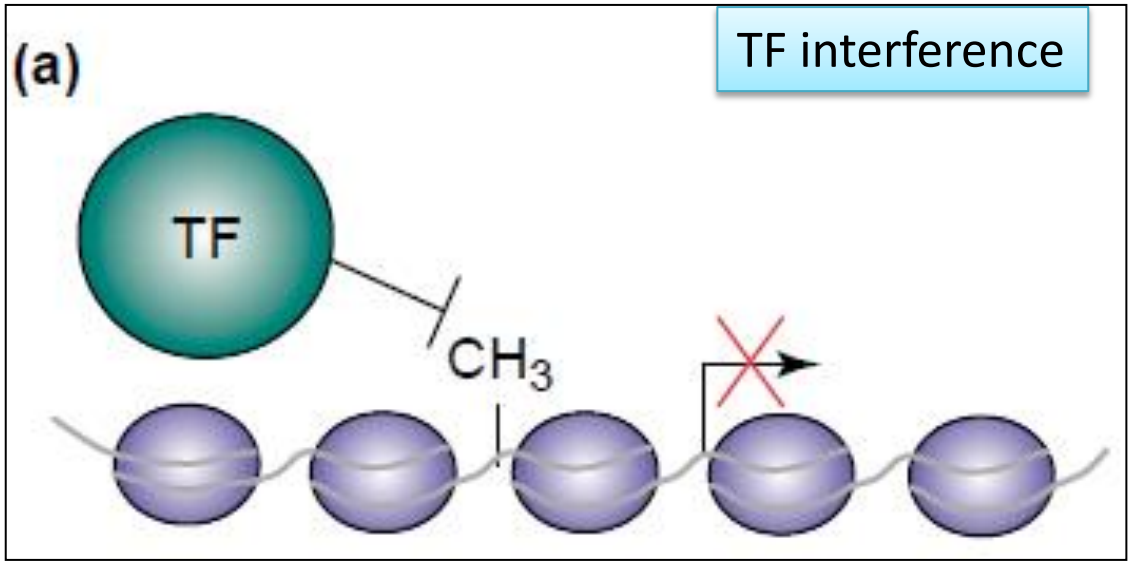


Figure 2. Mechanisms of DNA-methylation-mediated repression.
(**a**) DNA methylation in the cognate DNA-binding sequences of some (*not many, ndr*) transcription factors (TF) can result in inhibition of DNA binding. By blocking activators from binding targets sites, DNA methylation directly inhibits transcriptional activation

From Klose & Bird, 2006

Methyl-CpG binding proteins

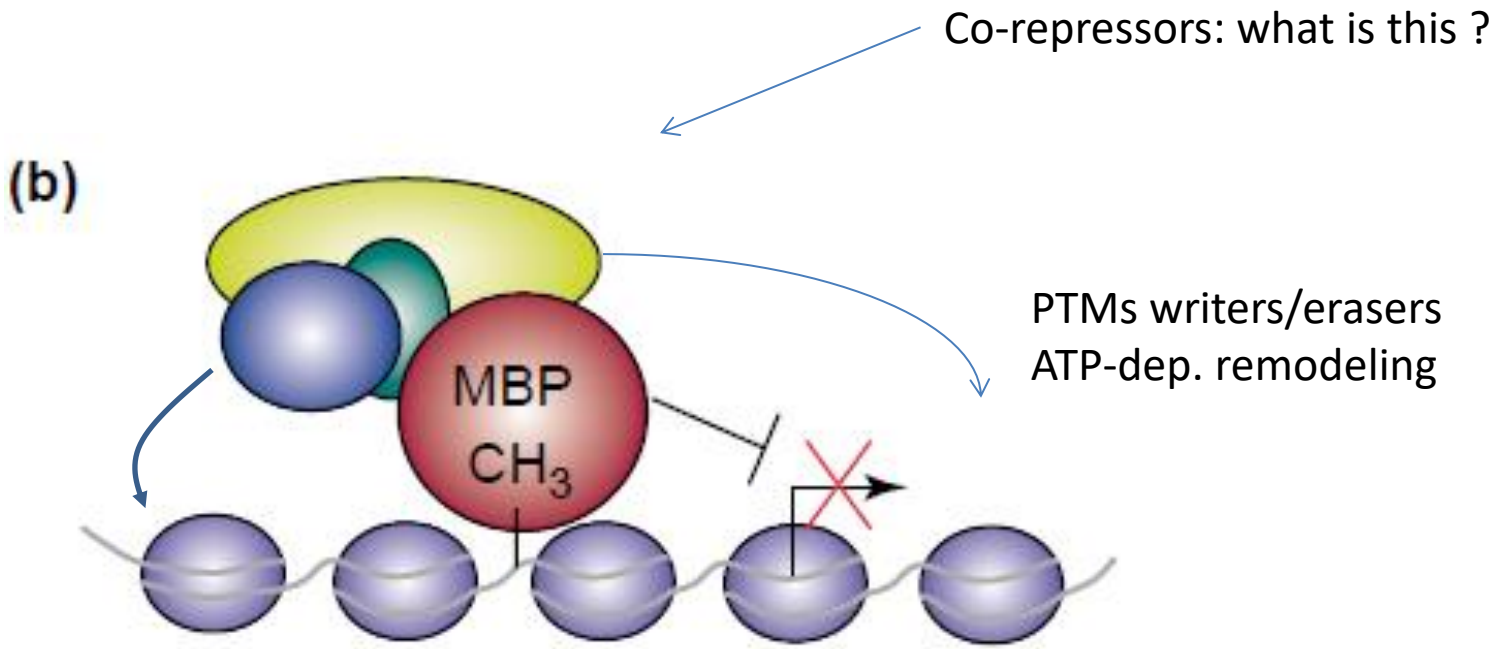


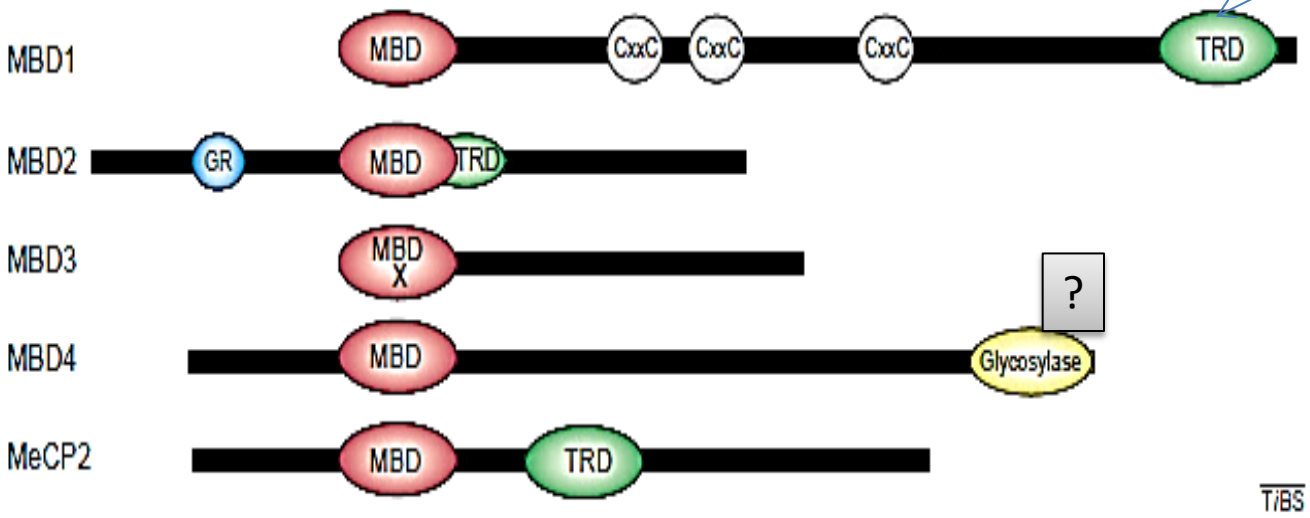
Figure 2. Mechanisms of DNA-methylation-mediated repression.
(**b**) Methyl-CpG-binding proteins (MBPs) directly recognize methylated DNA and recruit **co-repressor molecules** to silence transcription and to modify surrounding chromatin

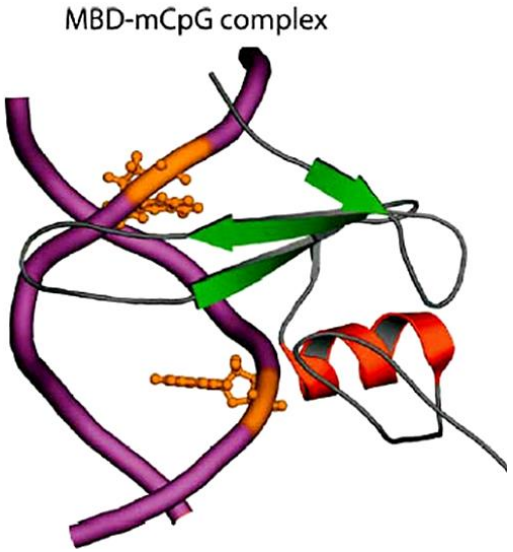
From Klose & Bird, 2006

Readers

Methyl DNA binding proteins have common and specific domains

How do we define a «transcriptional repressor domain ?»





A family of **methyl-CpG-binding proteins** (MBPs). Six mammalian MBPs have been characterized so far:

1. MBD1 uses its methyl-binding domain (MBD) to bind methylated DNA sequences. In addition, MBD1 contains three zinc-binding domains (CxxC), one of which binds specifically to non-methylated CpG dinucleotides, and a C-terminal transcriptional repression domain (TRD).
2. MBD2 possesses an MBD that overlaps with its TRD domain, and a GR repeat at its N terminus.
3. MBD3 contains a well-conserved MBD domain that does not recognize methylated DNA owing to crucial amino acid changes.
4. MBD4 binds methylated DNA through an MBD domain and has a C-terminal glycosylase domain that is important for is function in DNA repair.
5. MeCP2 is the founding member of the MBD protein family and contains a conserved MBD domain and an adjacent TRD domain.
6. The sixth protein, Kaiso, is unrelated and is not shown here.

From Klose & Bird, 2006, TiBS 31: 89-97.

Back to our early embryo ....

Cantone & Fisher, 2013

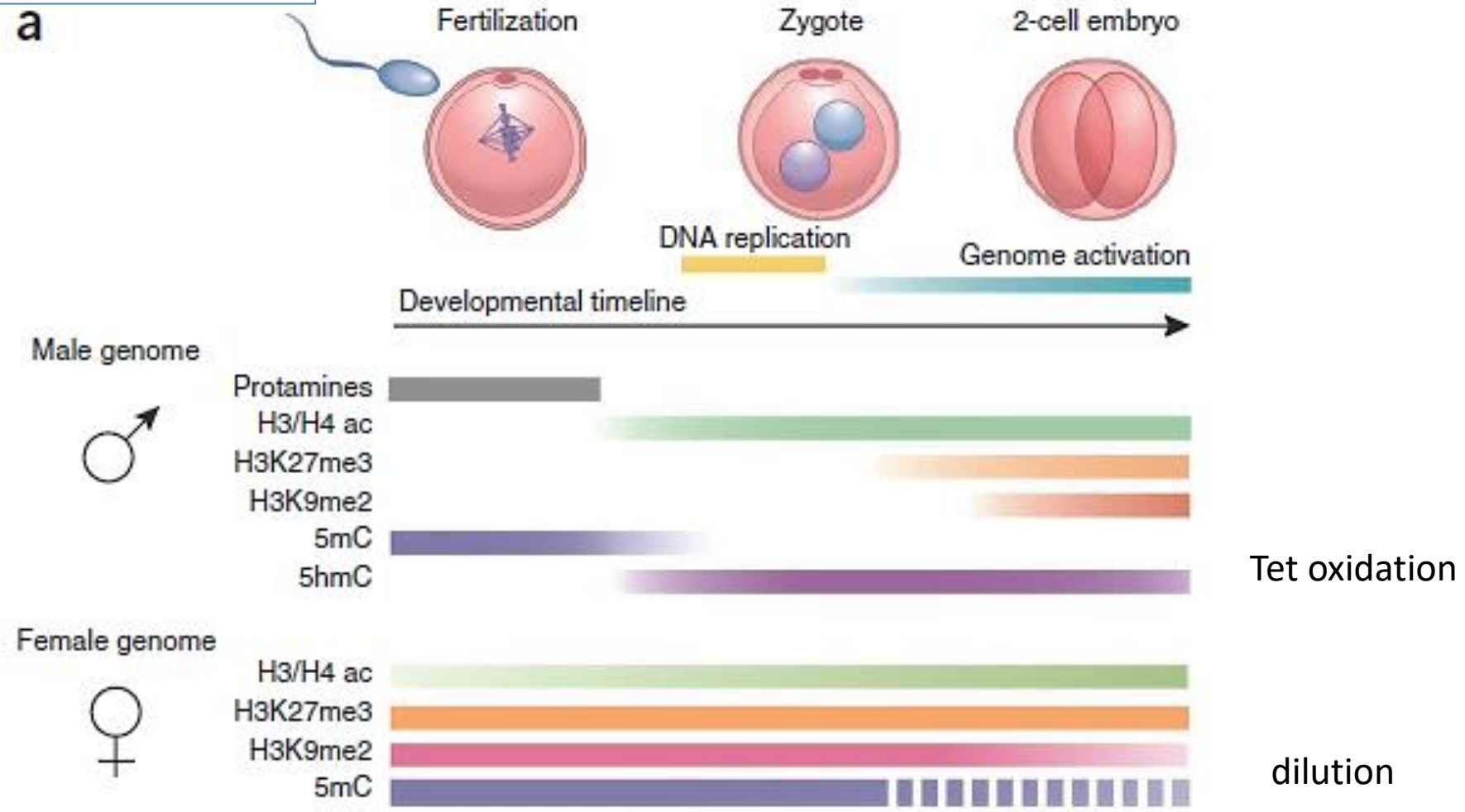
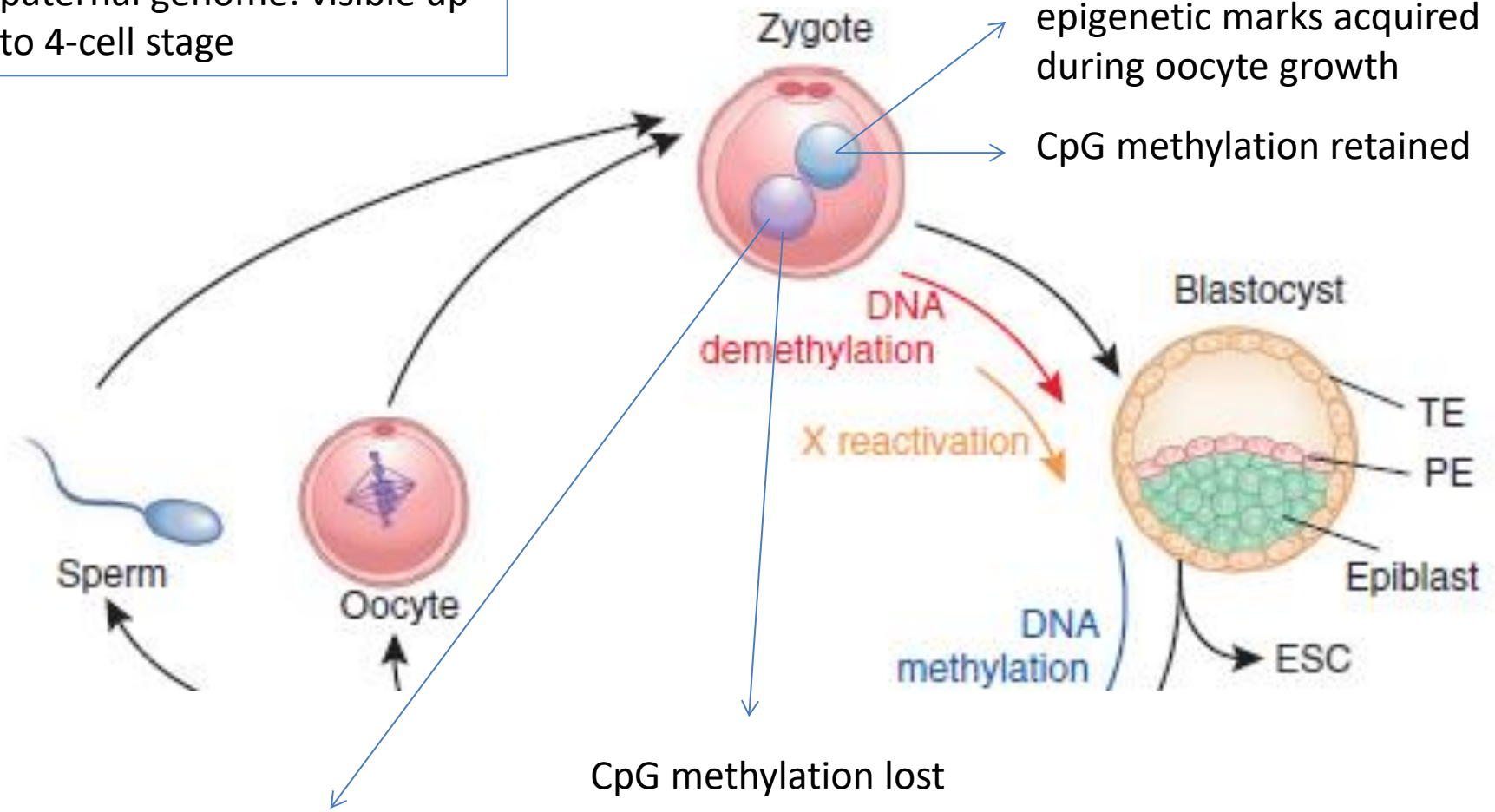


Figure 2 Epigenetic changes during *in vivo* reprogramming. (**a**) Schematic of global DNA and histone modifications that lead to transcriptional activation of the embryonic genome between the late zygote (paternal genome only) and the 2-cell stage. Gamete genomes undergo different epigenetic programs after fertilization with the paternal genome being mostly subject to epigenetic remodeling at the zygote stage and the maternal genome gradually losing repressive modifications during the subsequent cleavage divisions.

Asymmetry in maternal and paternal genome: visible up to 4-cell stage

Maternal genome: maintains epigenetic marks acquired during oocyte growth

CpG methylation retained

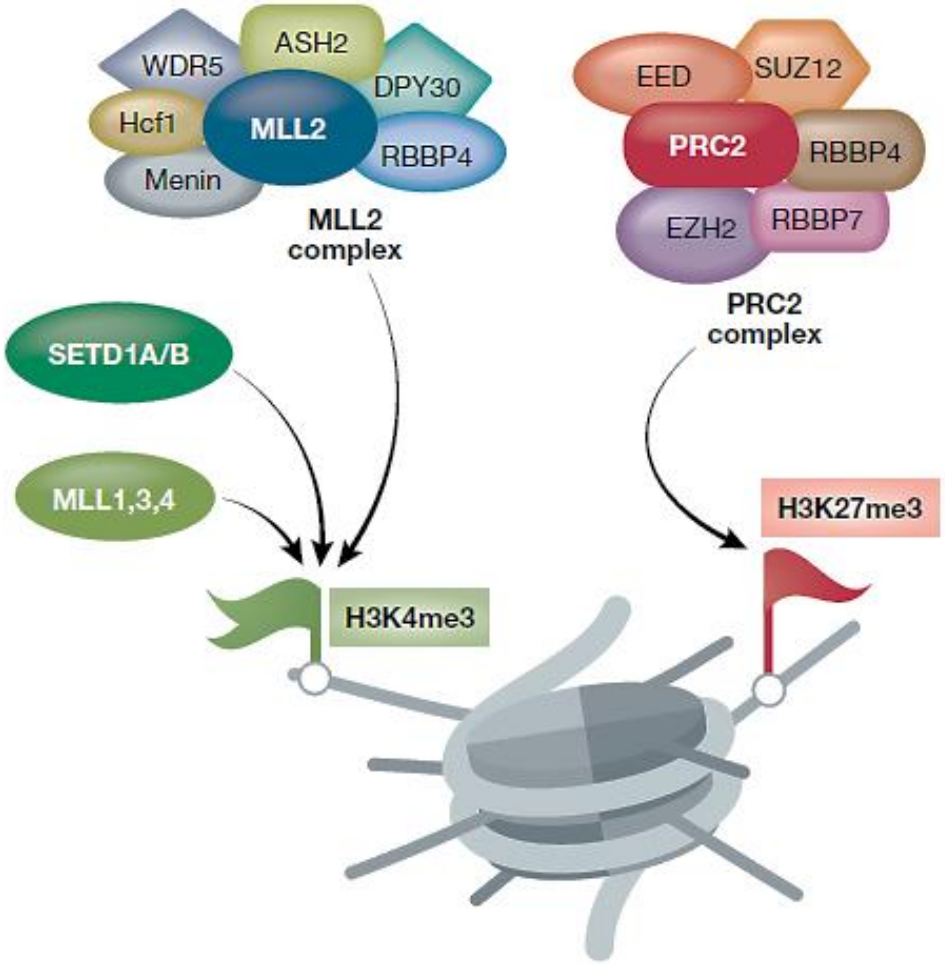


CpG methylation lost

Paternal genome: exchanges protamines with new histones coming from maternal cytoplasm (H4K5ac + H4K12ac).

before pronuclei fusion

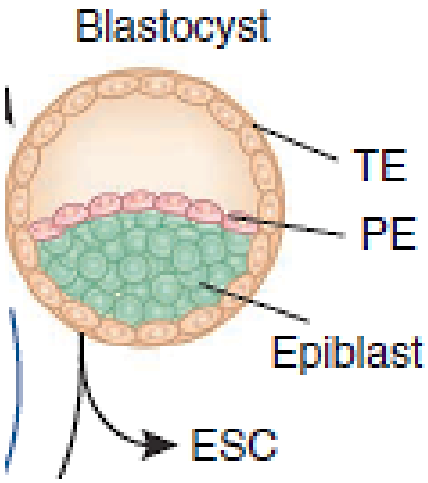
In **ESC** (but not only there) there are many «**bivalent**» chromatin domains. This kind of domain are also found in precursors and in general in cells that are not completely differentiated.



Drosophila nomenclature is Tritorax and Polycomb group proteins.
In Human, they are called MLL2 complex and PCR2.

Tritorax (TrxG) and MLL2 complex are responsible of H3K4me3.
Polycomb group proteins (PcG) and PCR2 write H3K27me3.

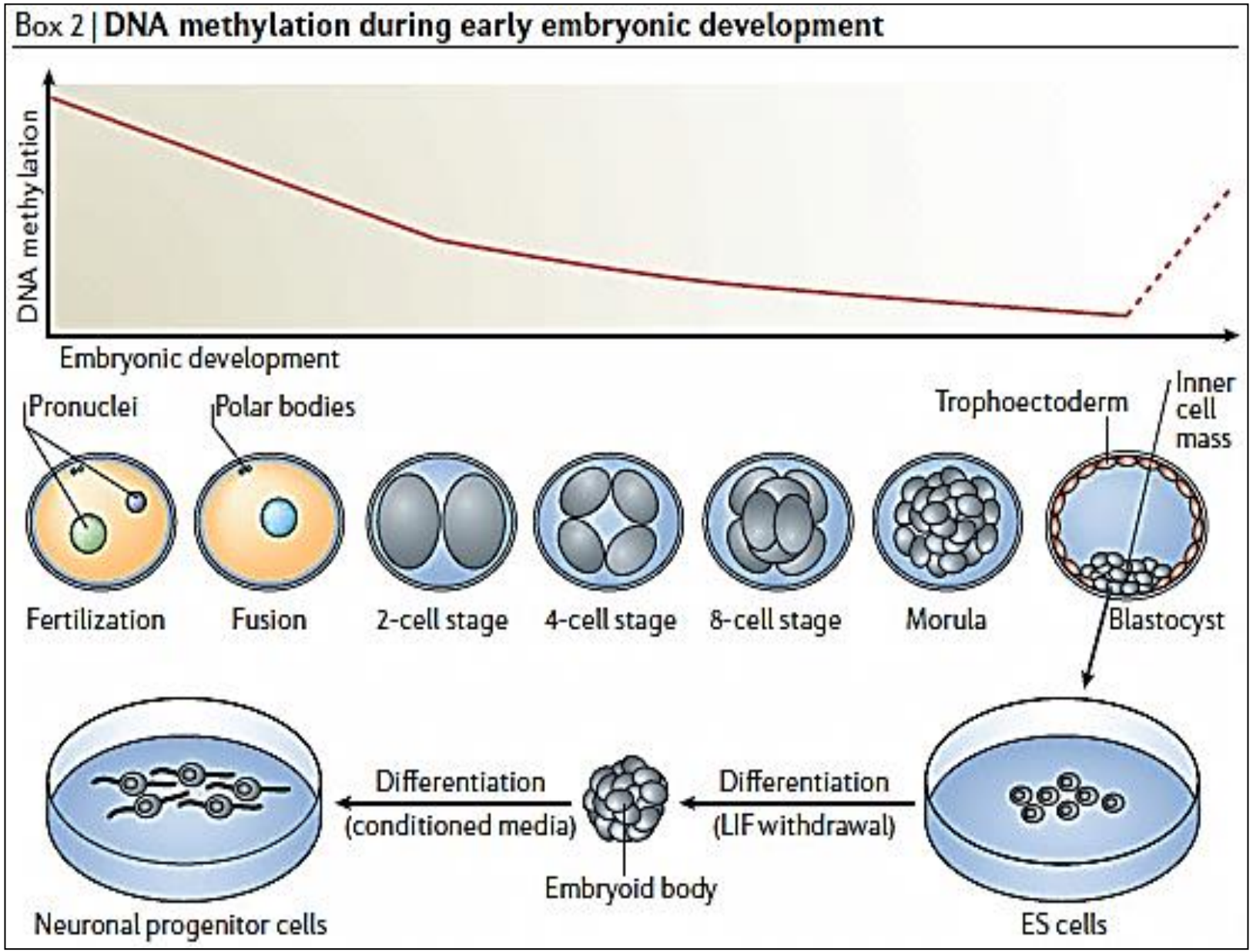
From Harikumar & Meshorer, 2015



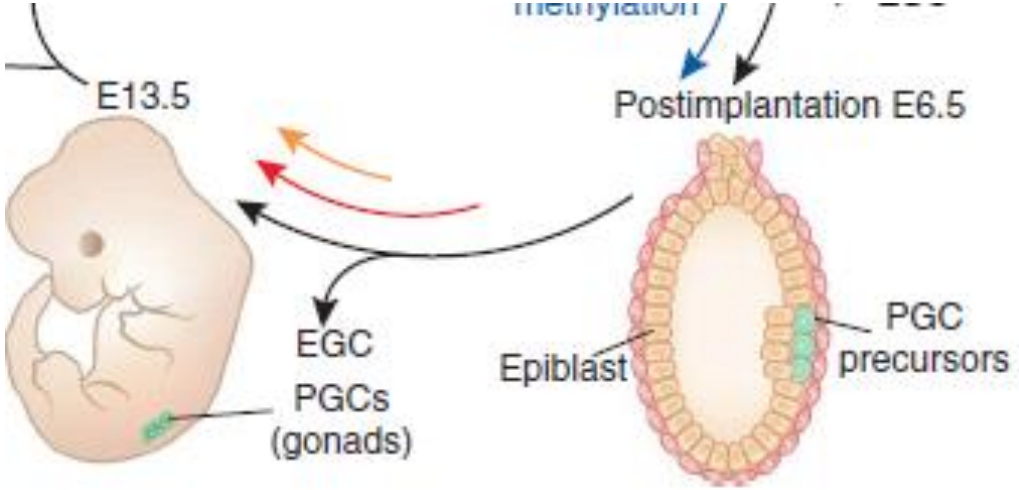
TE= trophoectoderm
PE=primitive endoderm

Embryonic stem cells

Since we can culture ES cells and differentiate in vitro, there are many studies on chromatin dynamics in this model.
Be aware that ESC represent an «in vitro» model system

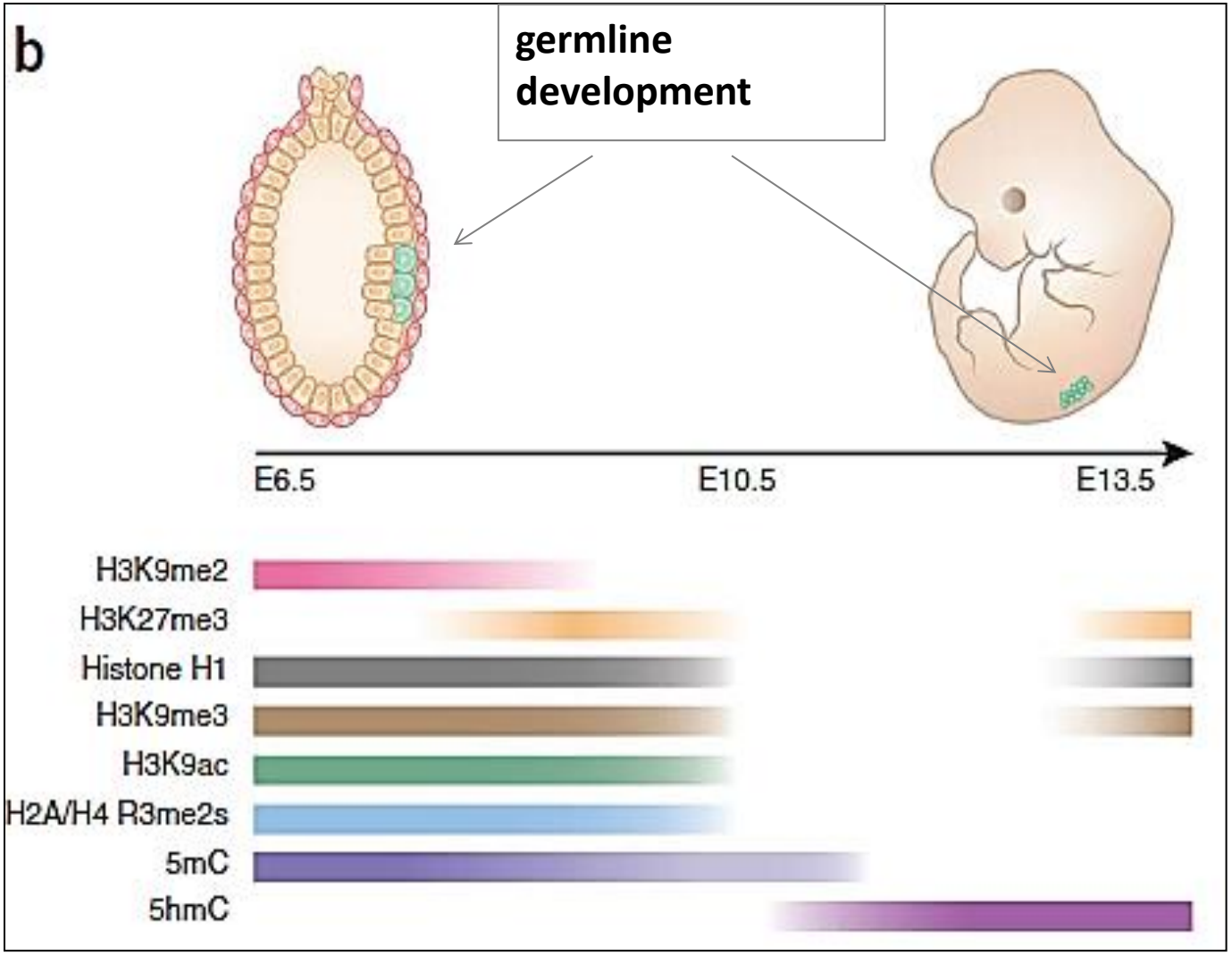

Box 2 | DNA methylation during early embryonic development
DNA methylation
Embryonic development
Pronuclei
Polar bodies
Trophoectoderm
Inner cell mass
Fertilization
Fusion
2-cell stage
4-cell stage
8-cell stage
Morula
Blastocyst
Differentiation (conditioned media)
Differentiation (LIF withdrawal)
Embryoid body
Neuronal progenitor cells
ES cells

Re-programming



Primordial Germ Cell (PGC) precursors will start to be re-programmed around E6.5 AT this stage they **are already marked as somatic**, but as soon as they migrate to destination they **start loosing H3K9me2 and acquiring H3K27me3**.

Note that when PGC enter the gonads (E11.5-E12.5) there is rapid and extensive **CpG demethylation** reaching complete at day E13.5.



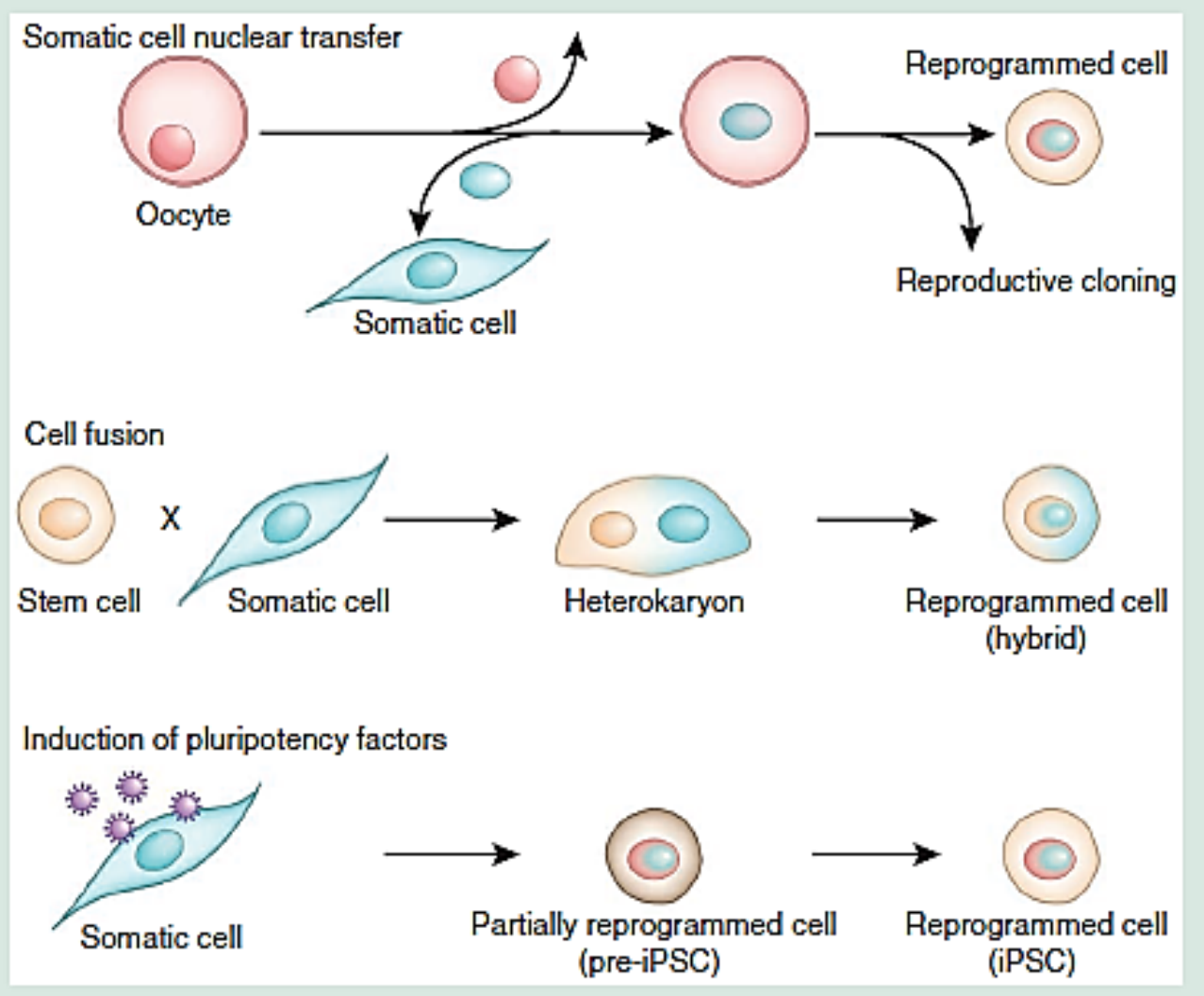
Cantone & Fisher, 2013

Tet enzymes

Figure 2 Epigenetic changes during *in vivo* **reprogramming**

(**b**) Global epigenetic changes during germline development from PGC specification (E6.5) to the mitotic/meiotic arrest at E13.5. Two major reprogramming phases can be distinguished during PGC migration toward the genital ridges (E7.5–E10.5) and upon their arrival into the gonads (E10.5–E12.5).

Re-programming



Cantone & Fisher, 2013

In vitro re-programming (iPSC). Many experimental studies.

PCR2 action required, as well as «Tritorax» components

De-methylation fo CpG required

Any action reducing heterochromatin favors reprogramming

e.g. valproic acid is a HDAC inhibitor: treating somatic cells with VA will increase efficiency of reprogramming using Transcription Factor mix transfection.

This is thought to be an effect of increased TF accessibility.

Commenting...

To date, most of the histone modification analyses in the preimplantation embryo and PGCs have been done by immunofluorescence, and detection of gross changes might obscure more distinct and perhaps important locus-specific events. In the next years, it will therefore be key to adapt genome-wide mapping techniques to the nanoscale to characterize the different combinations of histone and DNA modifications in small reprogramming populations or even in single cells.

Cantone & Fisher, 2013