Ch 2 - L2.2

Programming and reprogramming 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

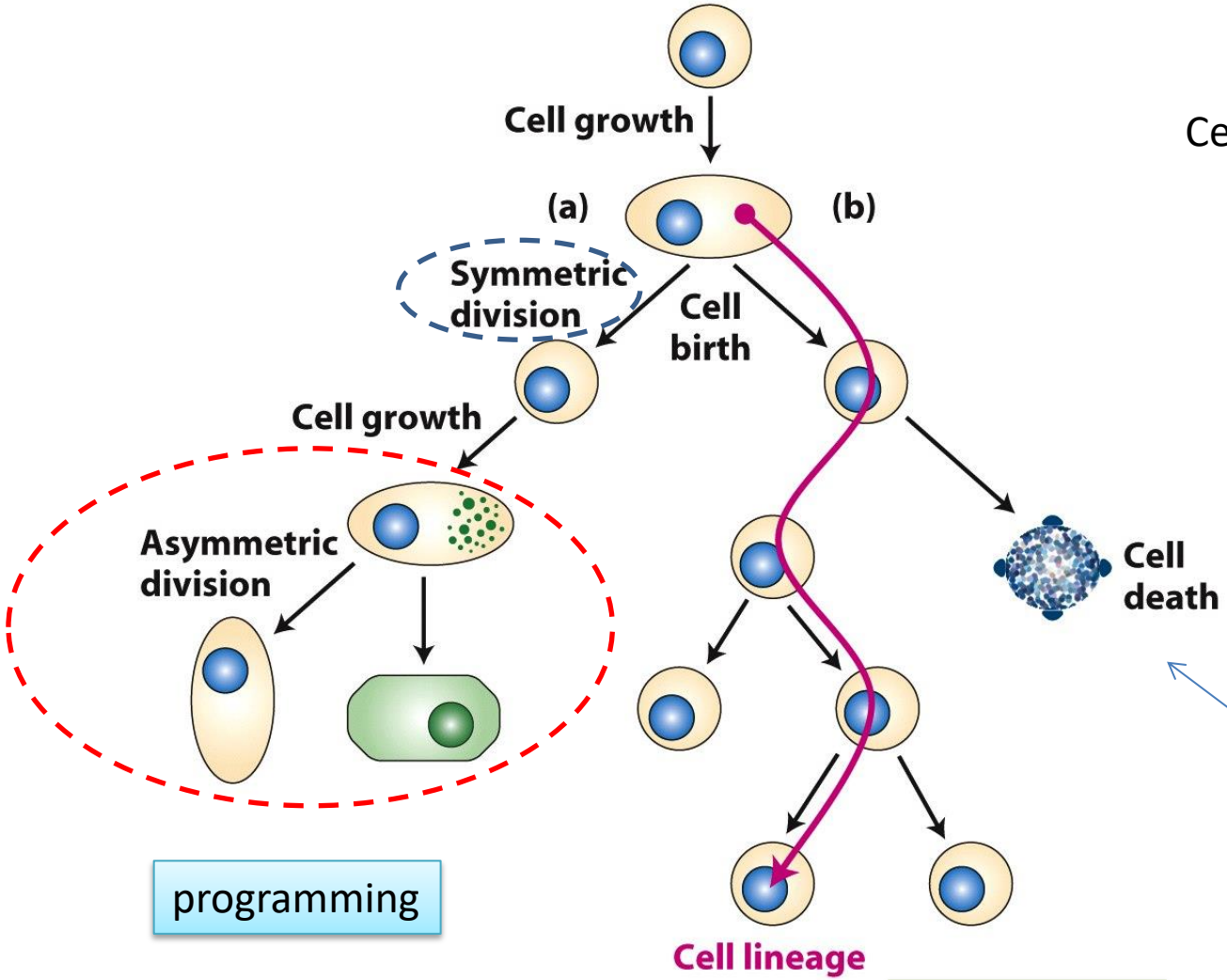
nature structural & molecular biology

Epigenetic programming and reprogramming during development

Irene Cantone & Amanda G Fisher

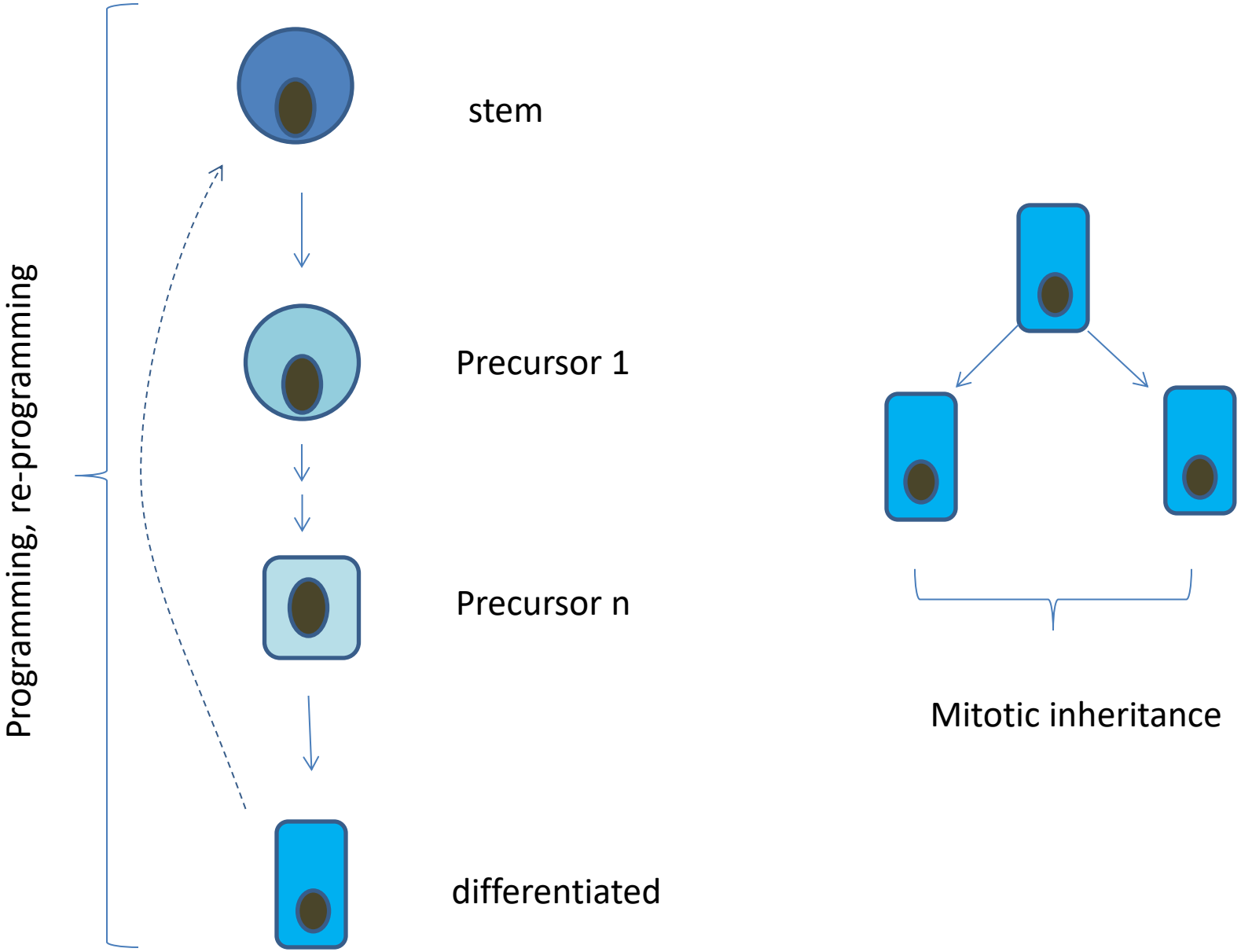
Cell identity is determined by specific gene expression patterns that are conveyed by interactions between transcription factors and DNA in the context of chromatin. In development, epigenetic modifiers are thought to stabilize gene expression and ensure that patterns of DNA methylation and histone modification 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

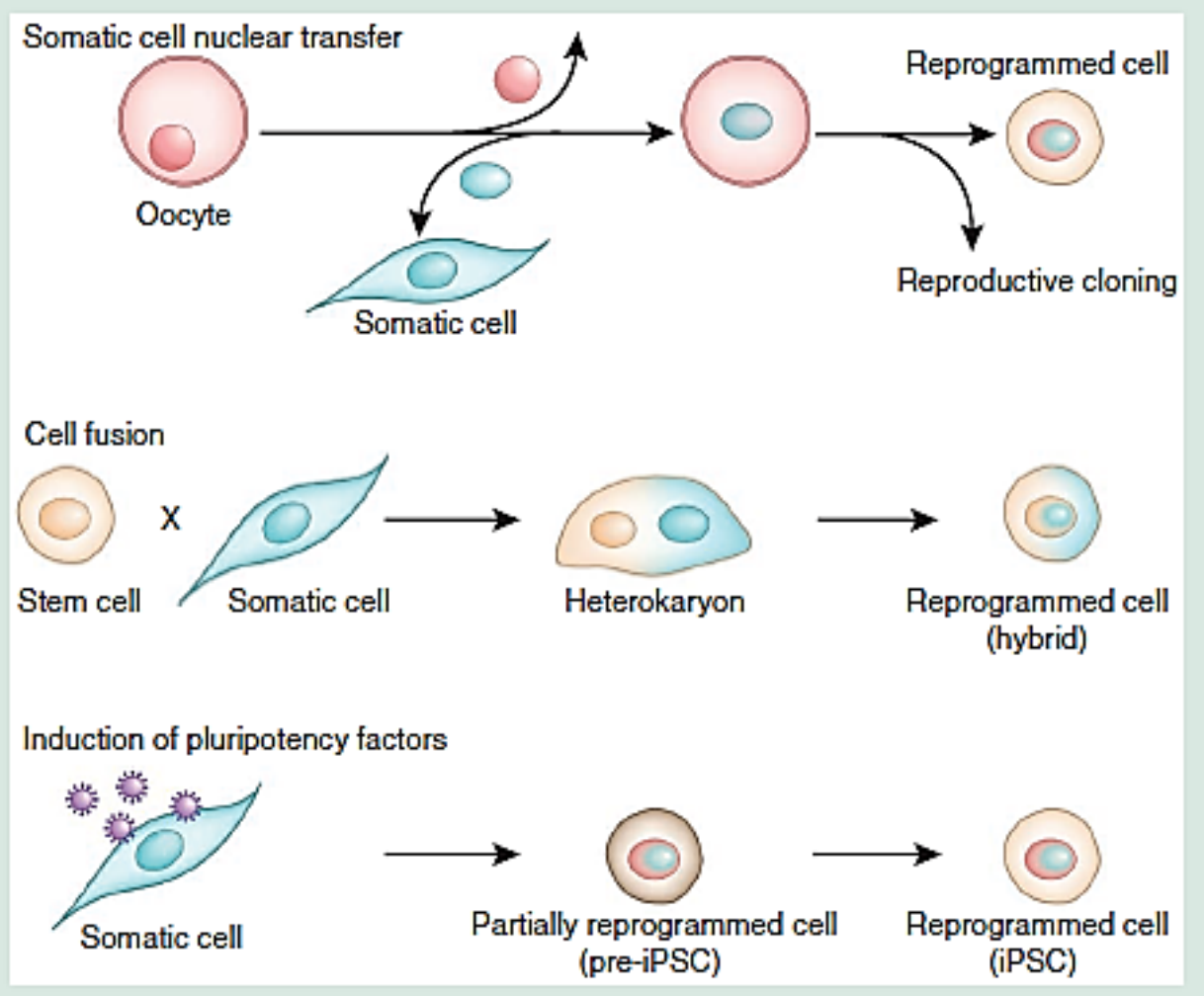


Apoptosis

Figure 21-1
Molecular Cell Biology, Sixth Edition


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

Re-programming



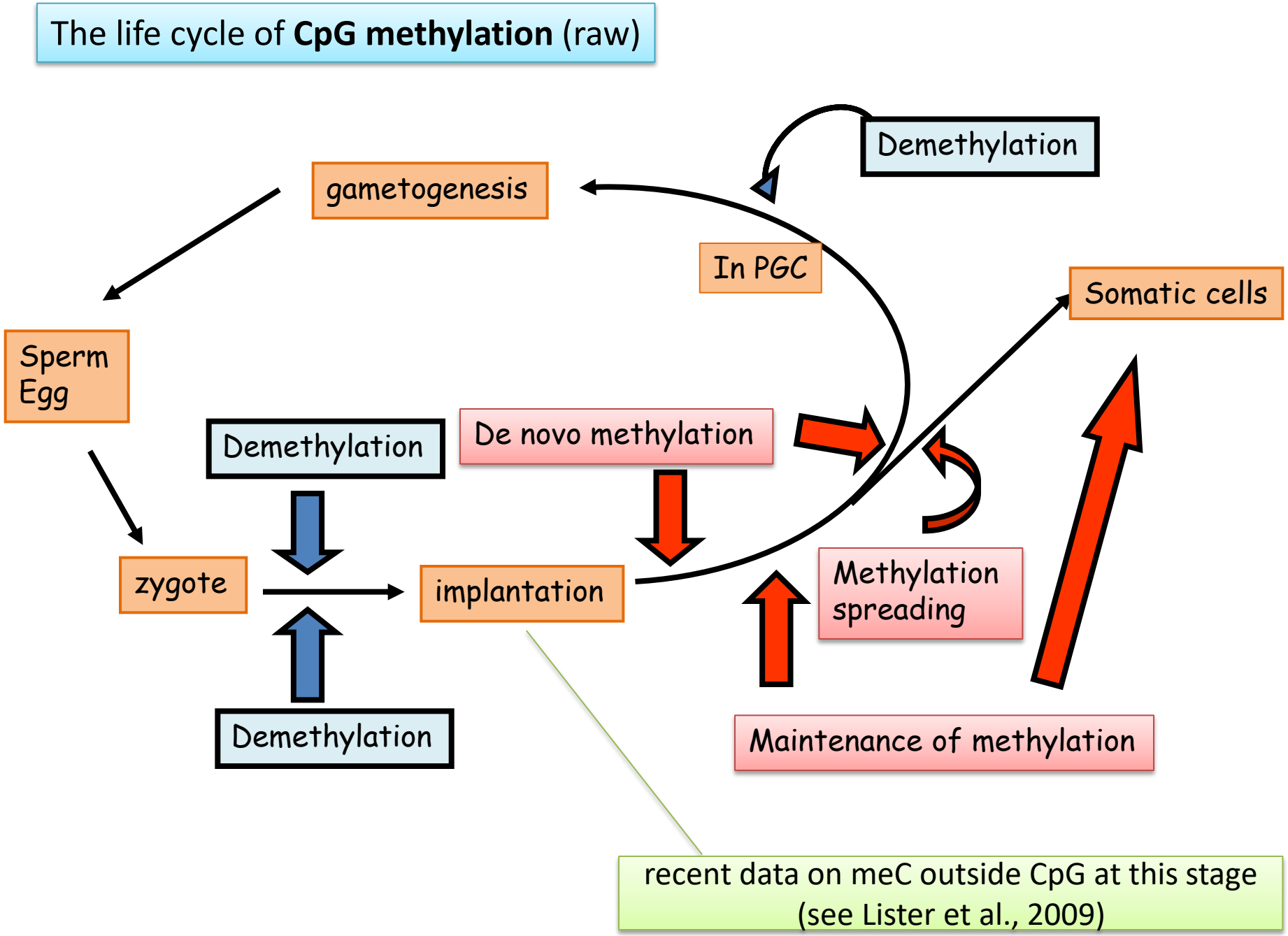
Cantone & Fisher, 2013

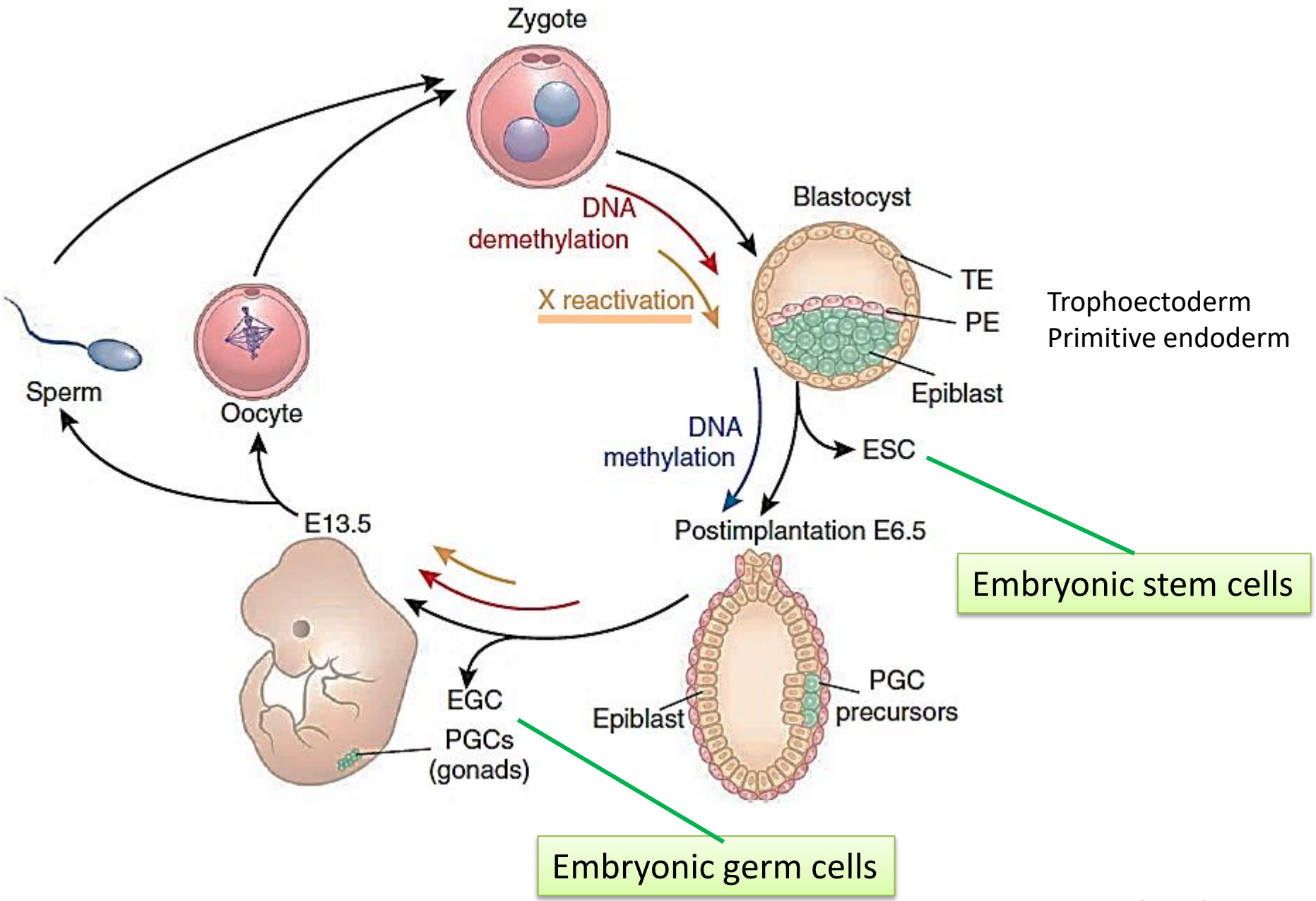
Transcription factors (sequence-specific DNA binding proteins) can induce re-programming of chromatin domains and partial stemness

exploring chromatin determination in the life cycle of Mammals

Essentially in the mouse

First point explored: DNA CpG methylation

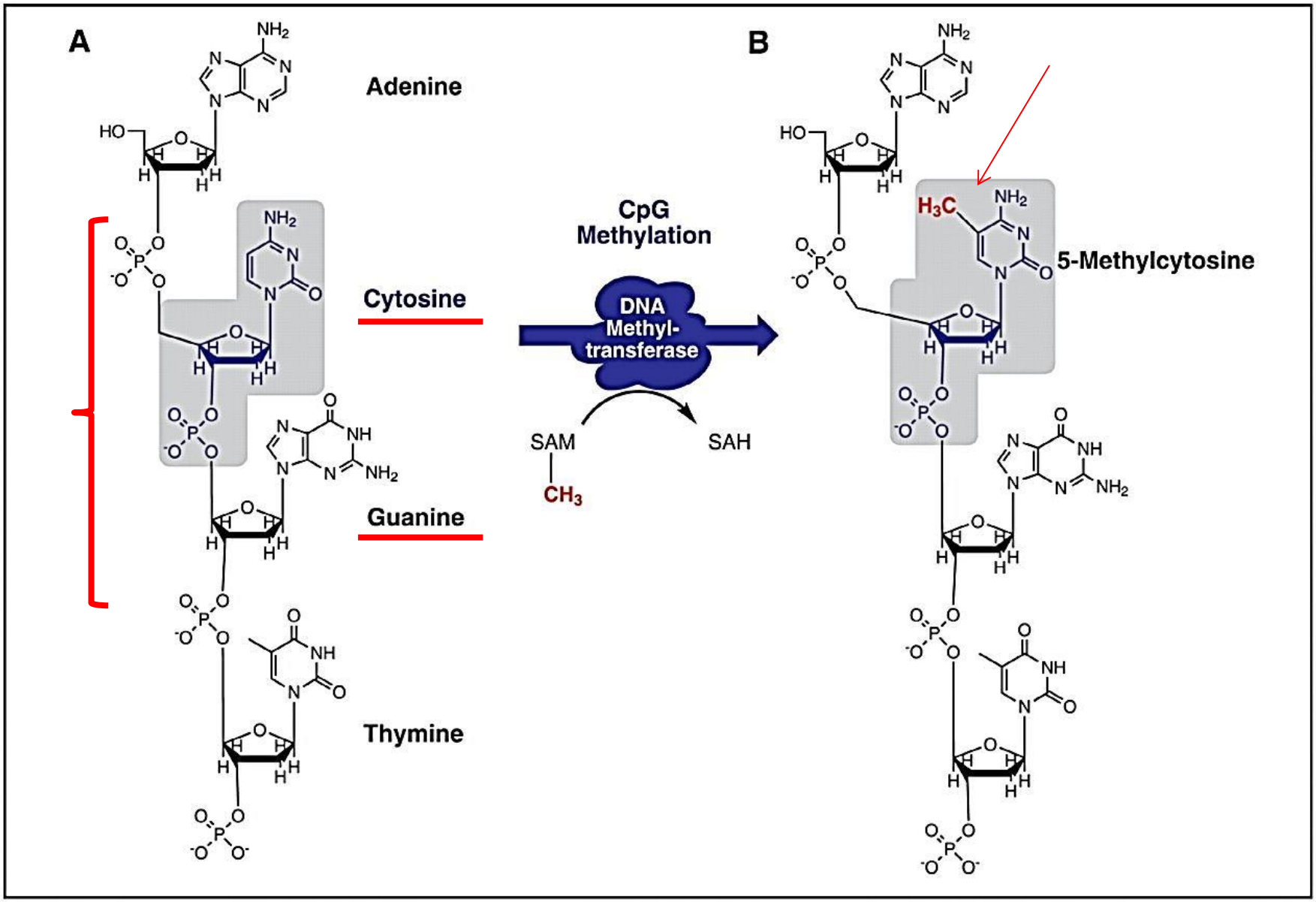

The life cycle of **CpG methylation** (raw)
Demethylation
gametogenesis
In PGC
Somatic cells
Sperm
Egg
Demethylation
De novo methylation
zygote
implantation
Methylation
spreading
Demethylation
Maintenance of methylation
recent data on meC outside CpG at this stage
(see Lister et al., 2009)



Cantone & Fisher, 2013

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

CpG methylation



CpG - Cytosine methylation

is a dynamic mark

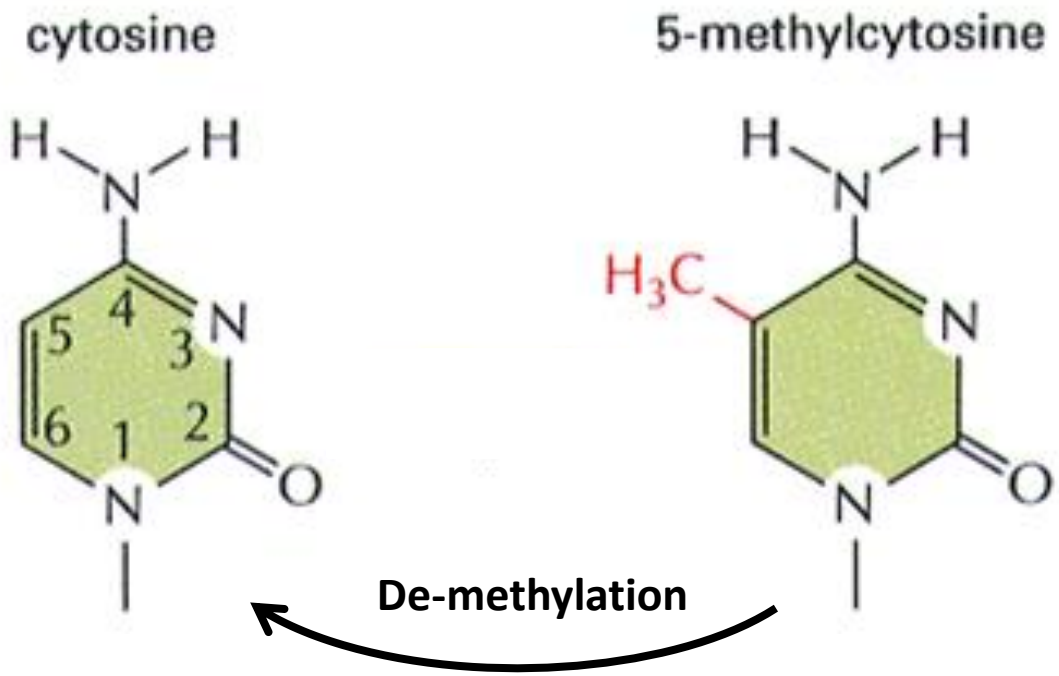
Enzymes that catalyze methylation are SAM-dependent enzymes:

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.

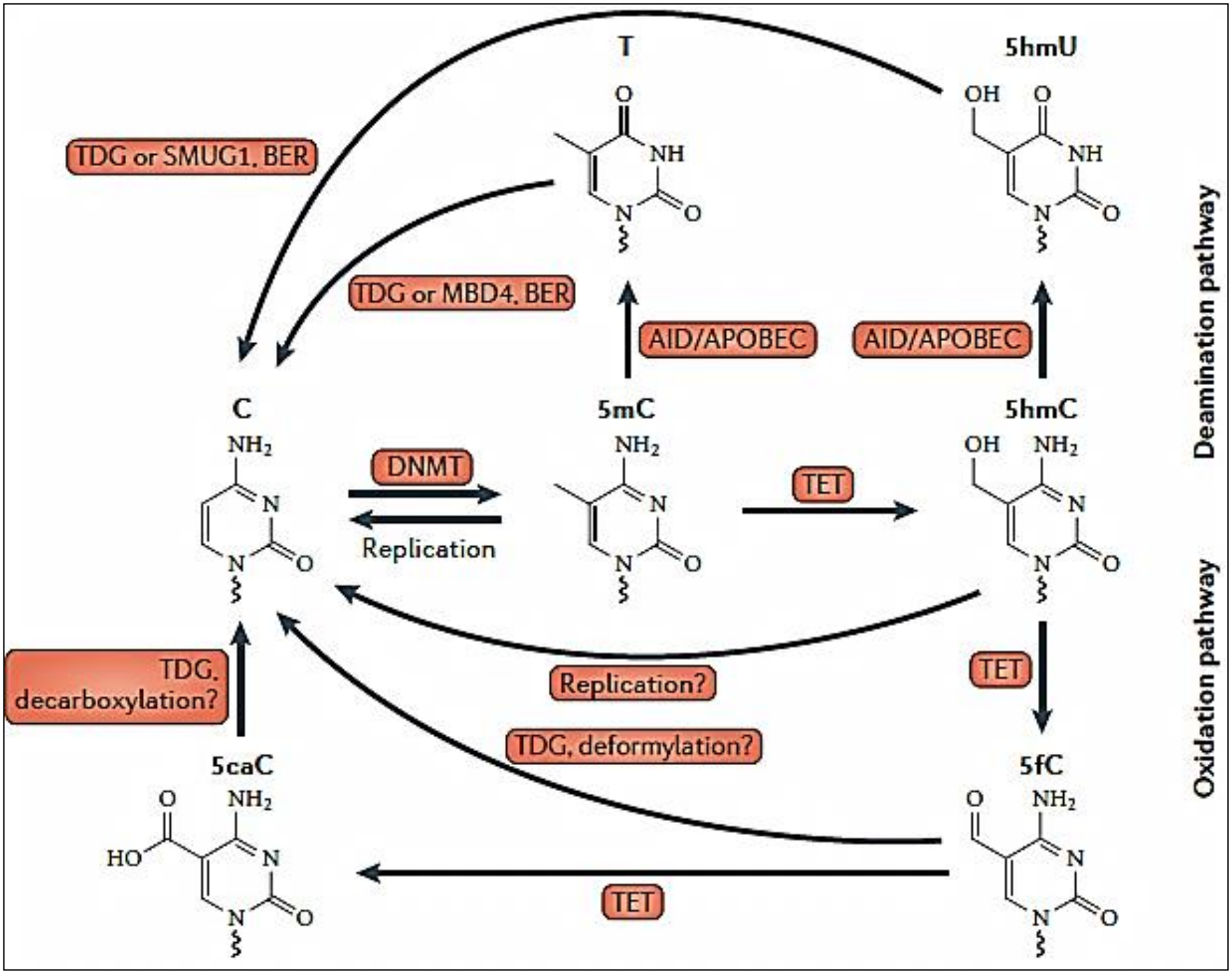
De-methylation can follow three ways: oxydation, dilution, repair



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

DNA repair

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 that in turn recruit HDACs

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

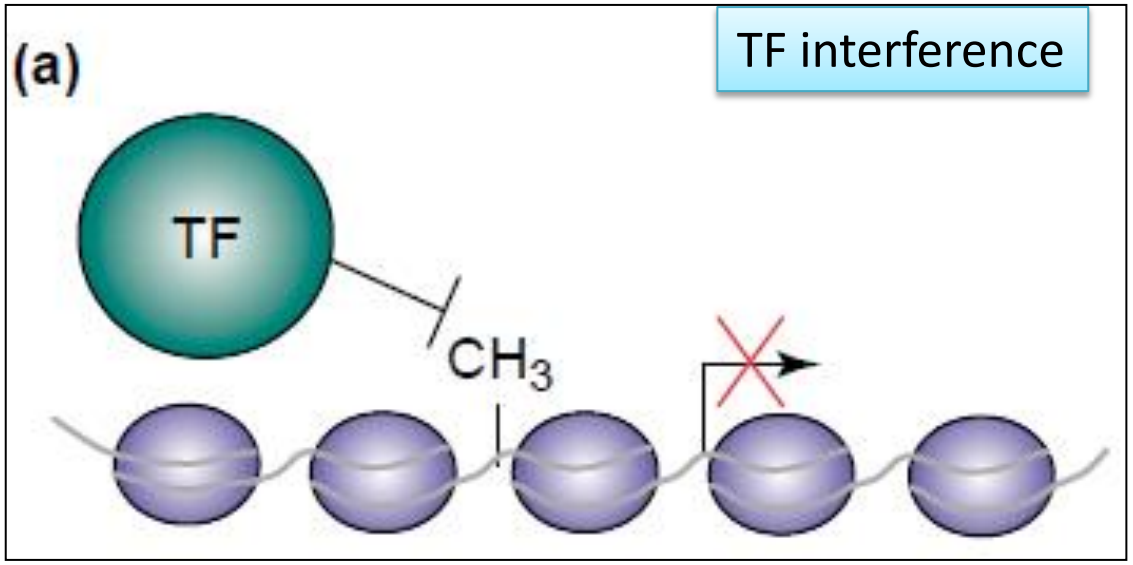


Figure 2. Mechanisms of DNA-methylation-mediated repression.
(**a**) DNA methylation in the cognate DNA-binding sequences of some (*not many, ndr*) transcription factors (TF) can result in inhibition of DNA binding. By blocking activators from binding targets sites, DNA methylation directly inhibits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

From Klose & Bird, 2006

Methyl-CpG binding proteins

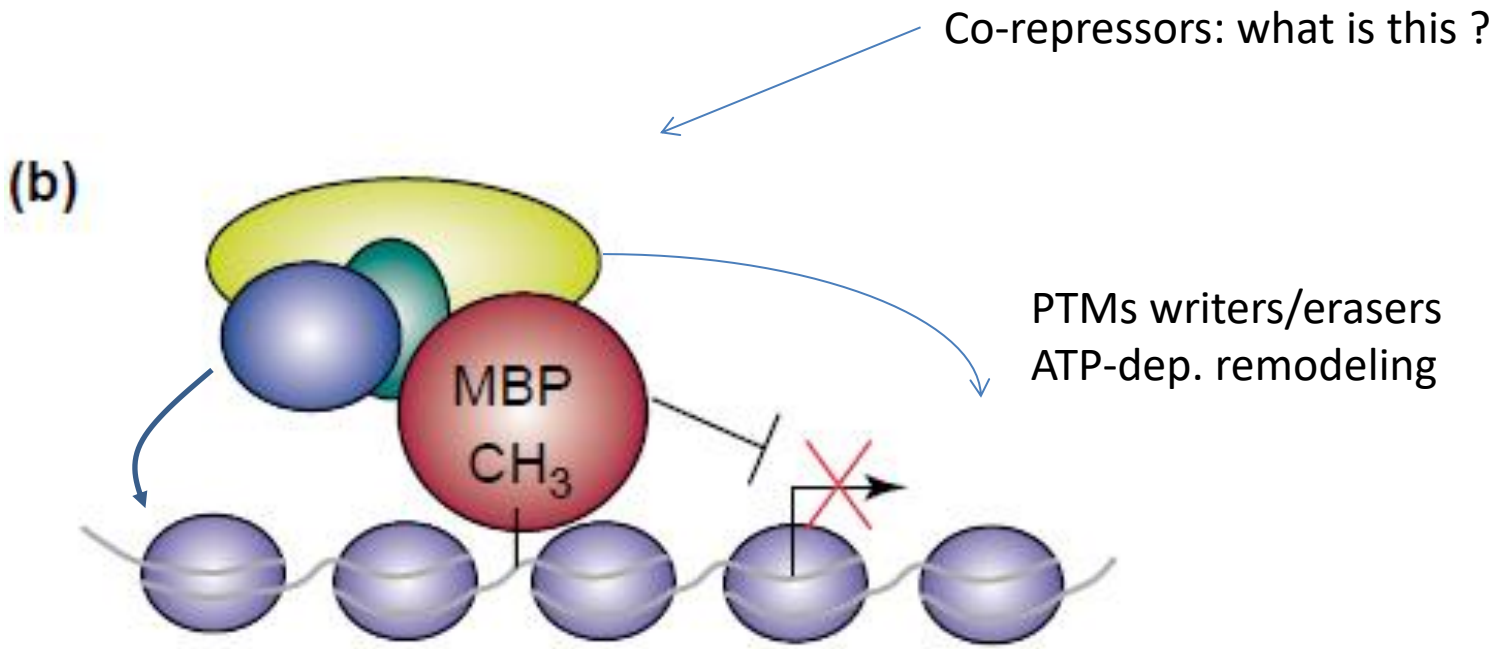
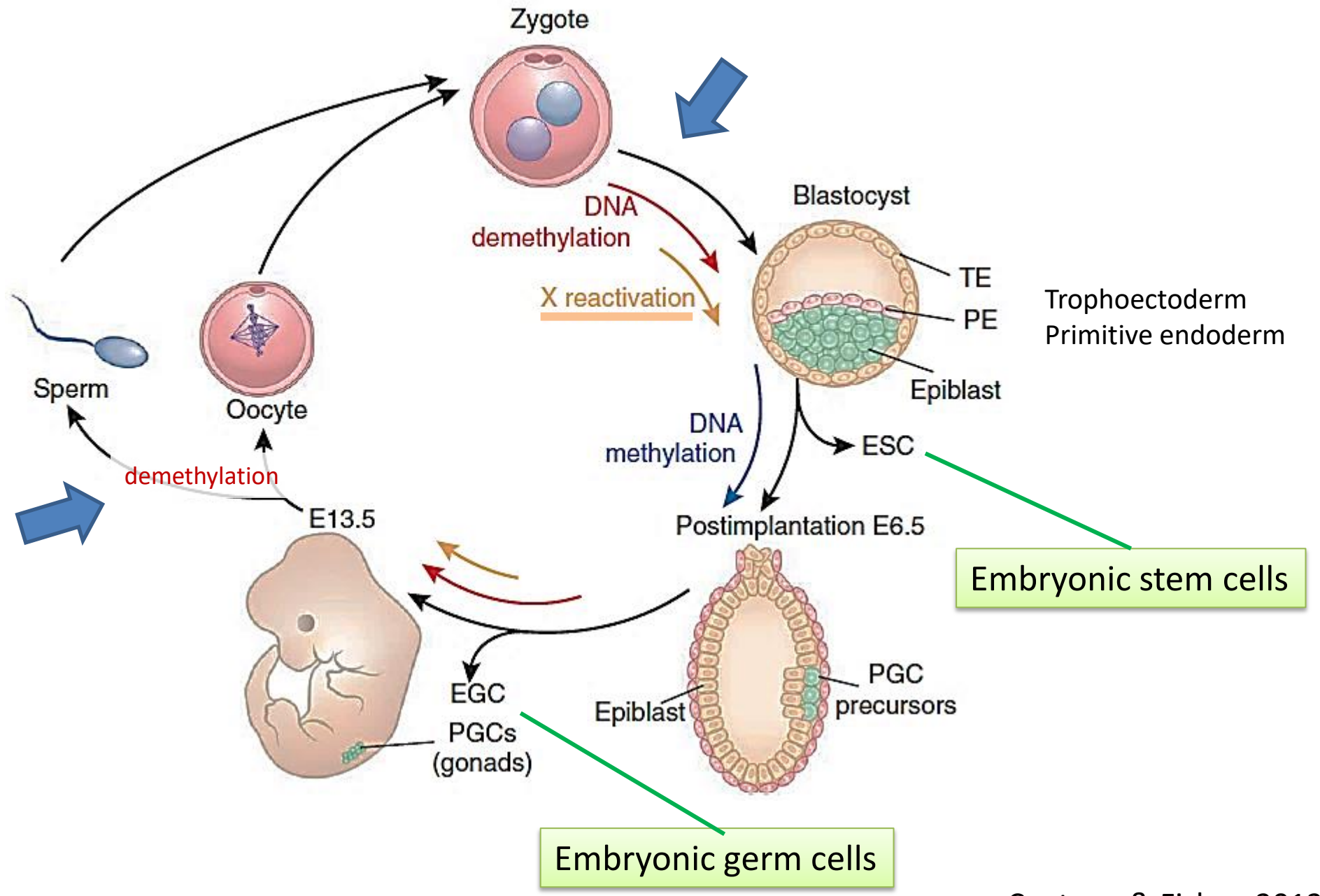


Figure 2. Mechanisms of DNA-methylation-mediated repression.
(**b**) Methyl-CpG-binding proteins (MBPs) directly recognize methylated DNA and recruit **co-repressor molecules** to silence transcription and to modify surrounding chromatin

From Klose & Bird, 2006

Back to our early embryo ....



Cantone & Fisher, 2013

Cantone & Fisher, 2013

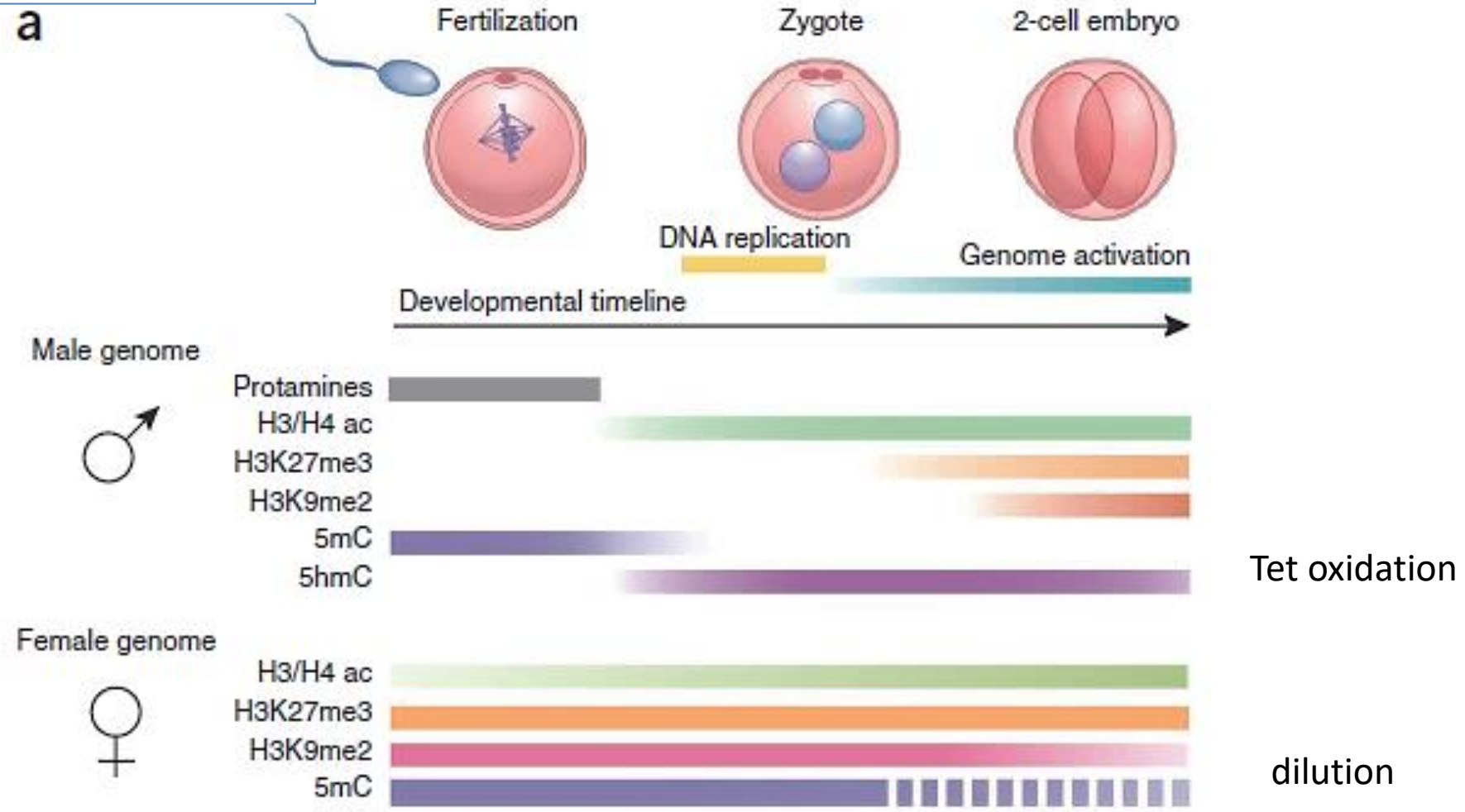
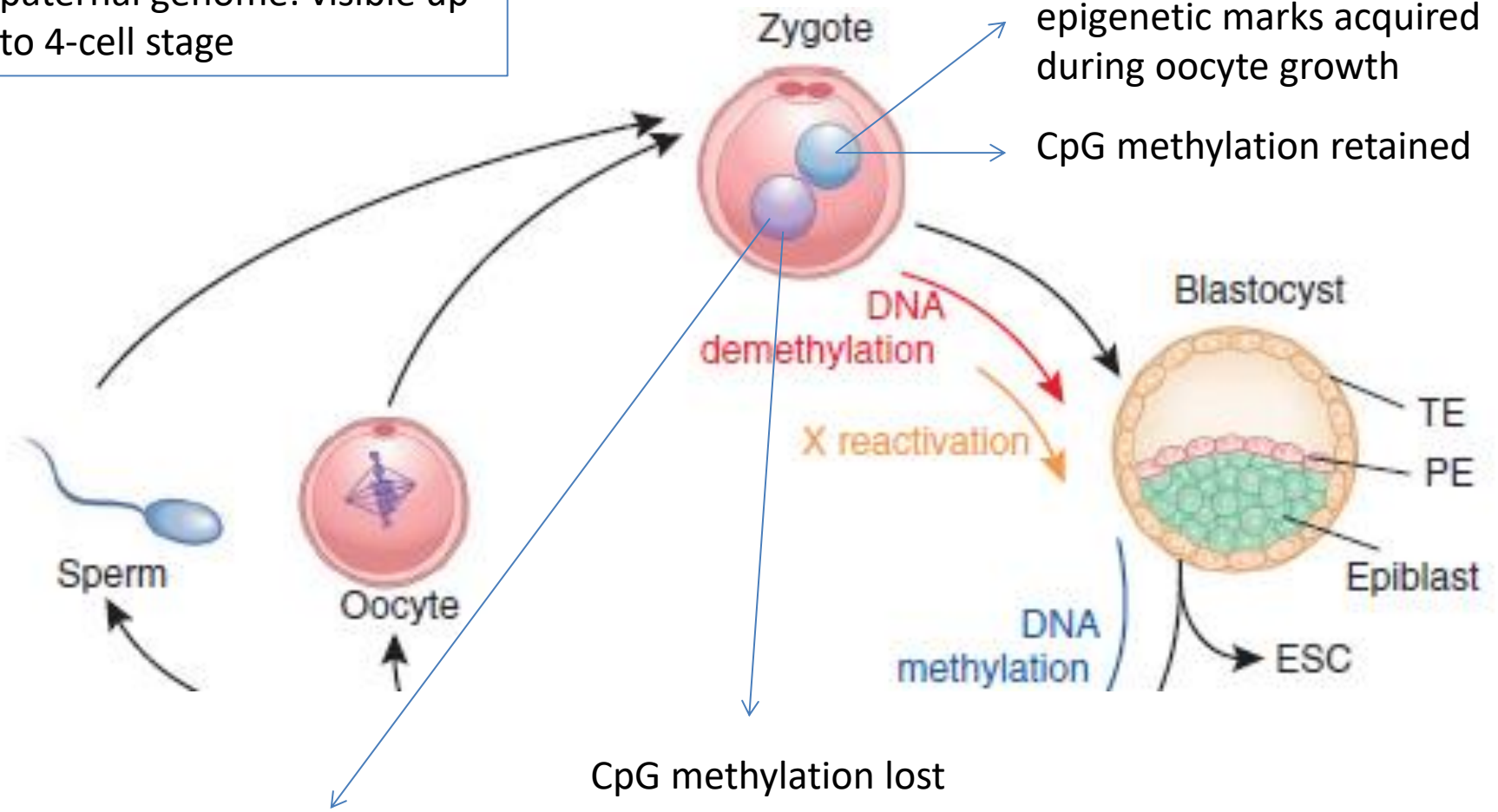


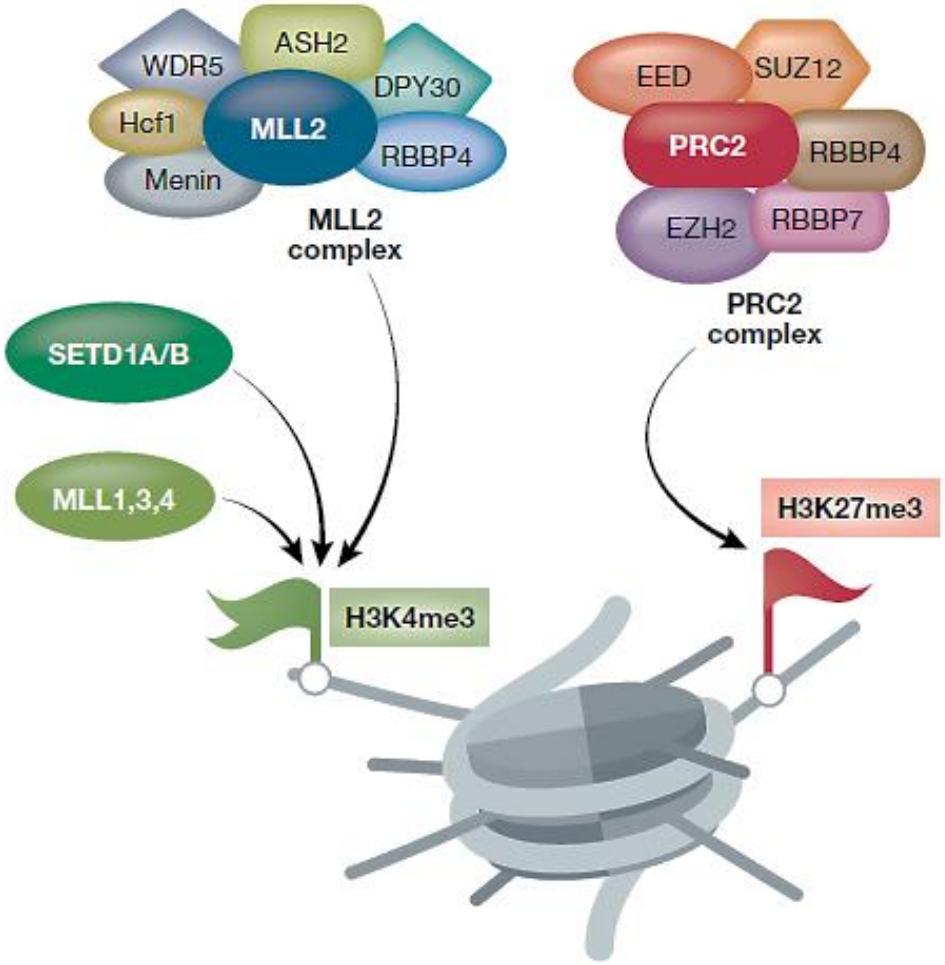
Figure 2 Epigenetic changes during *in vivo* reprogramming. (**a**) Schematic of global DNA and histone modifications that lead to transcriptional activation of the embryonic genome between the late zygote (paternal genome only) and the 2-cell stage. Gamete genomes undergo different epigenetic programs 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



before pronuclei fusion

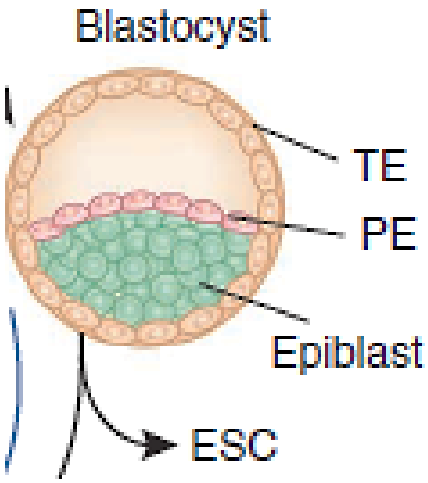
In **ESC** (but not only there) there are many «**bivalent**» chromatin domains. This kind of domain are also found in precursors and in general in cells that are not completely differentiated.



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.

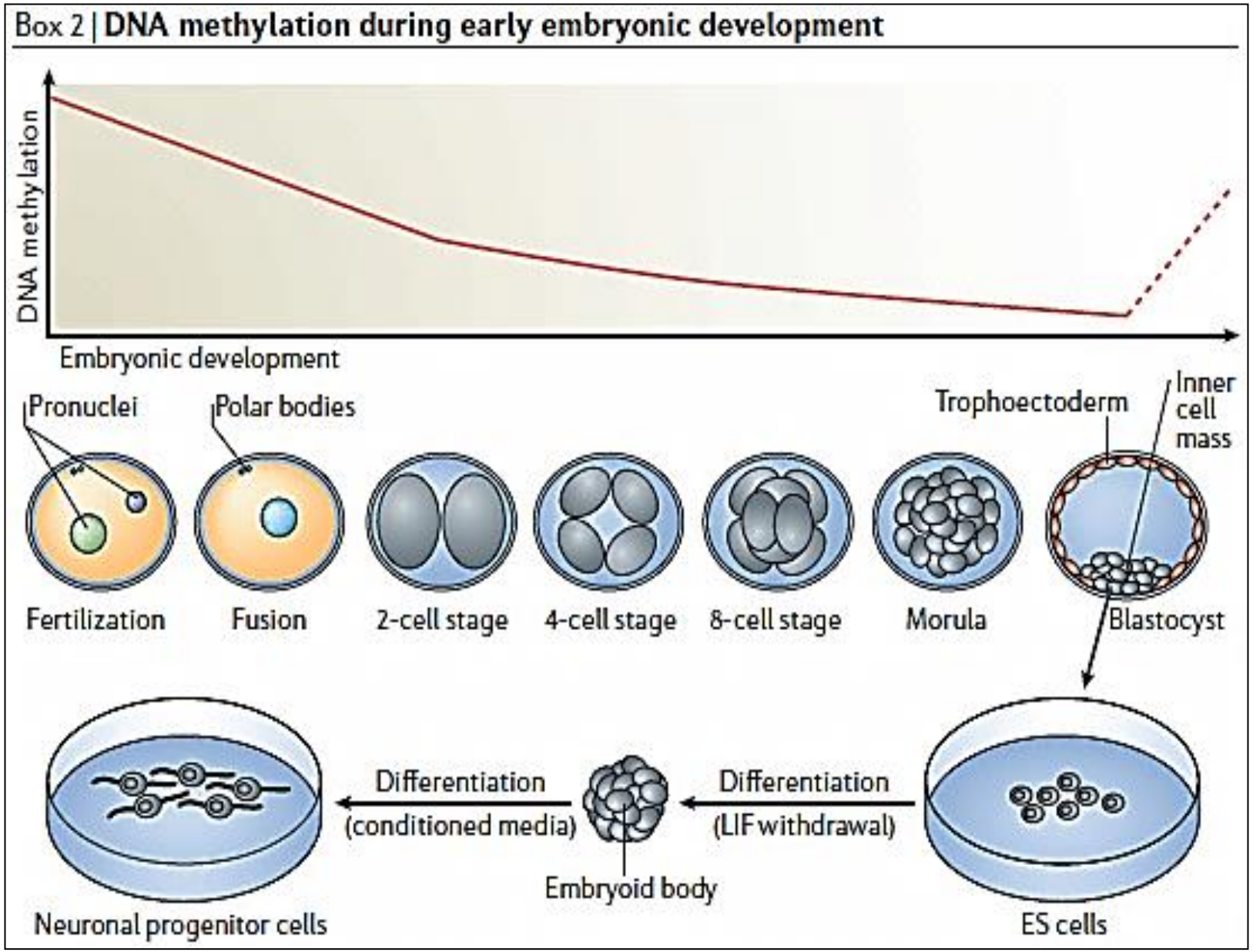
From Harikumar & Meshorer, 2015



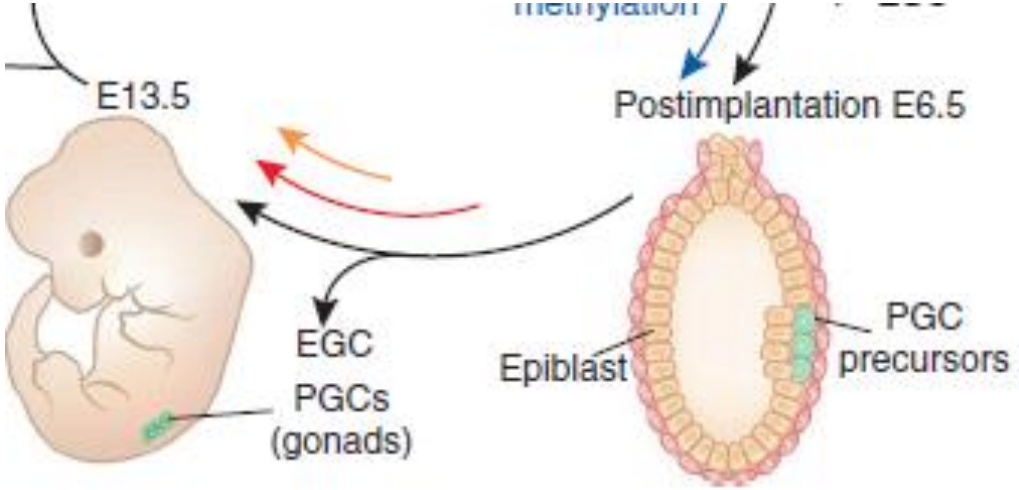
TE= trophoectoderm
PE=primitive endoderm

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

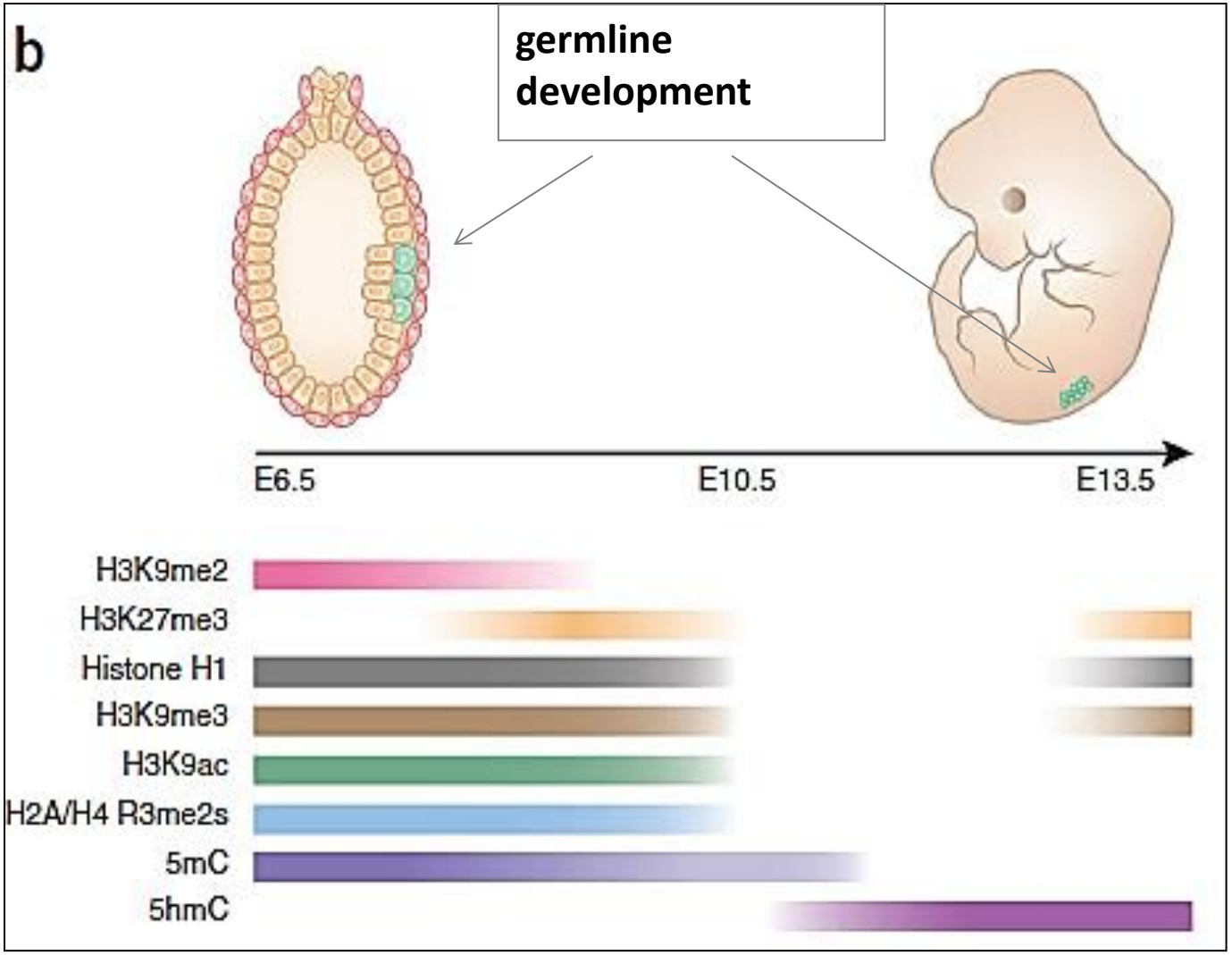

Box 2 | DNA methylation during early embryonic development
DNA methylation
Embryonic development
Pronuclei
Polar bodies
Trophoectoderm
Inner cell mass
Fertilization
Fusion
2-cell stage
4-cell stage
8-cell stage
Morula
Blastocyst
Differentiation (conditioned media)
Differentiation (LIF withdrawal)
Embryoid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



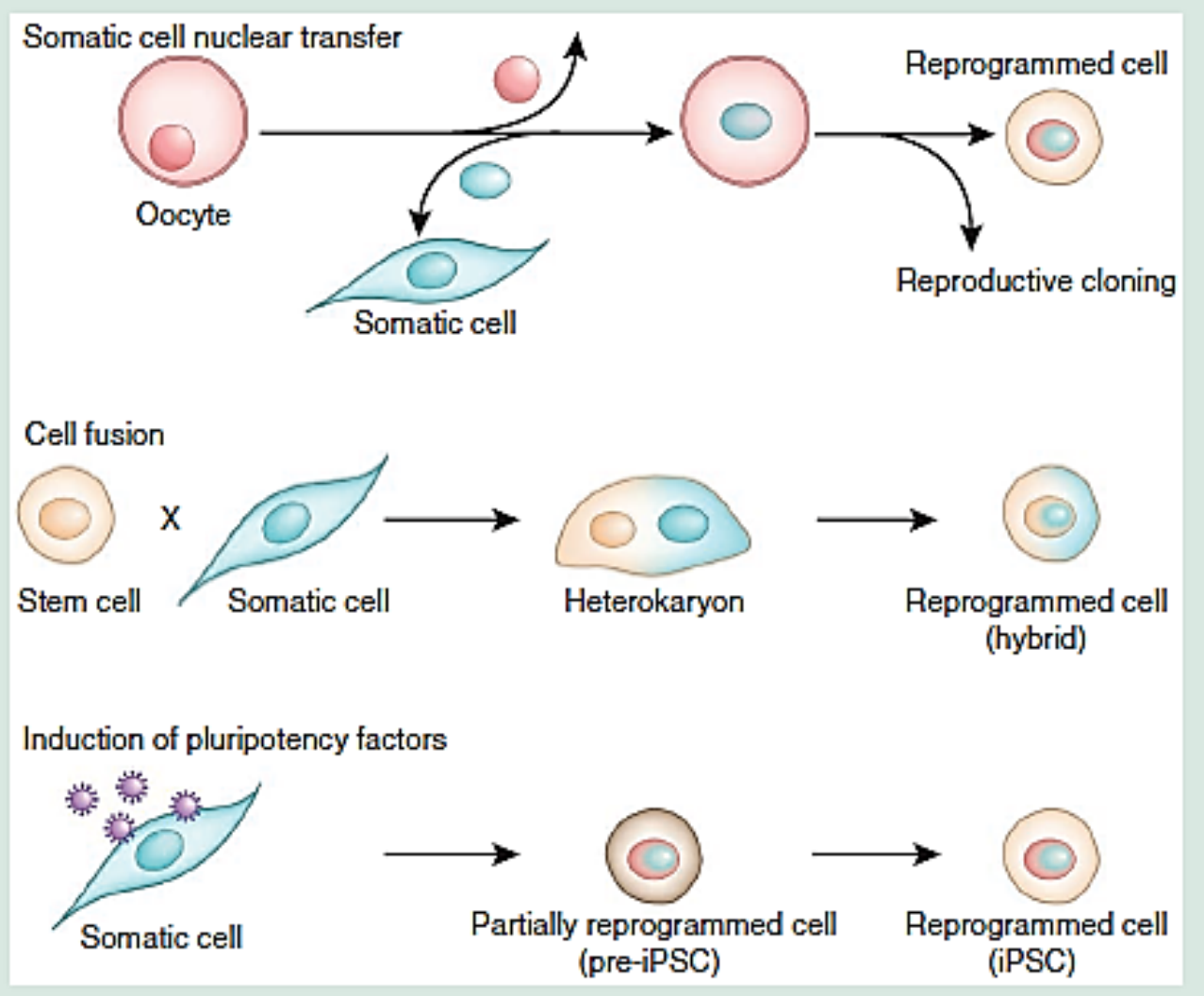
Cantone & Fisher, 2013

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