

# Lecture I-6\_2018

Epigenetic control of development  
and cell fate

# Development and epigenetics

The formation of the >200 embryonic and adult somatic cell types requires a coordinated sequence/order of reprogramming (erasure) and programming (setting) events

Coordinated development of genetic and epigenetic control

# Todays topics

Early development – phases of genome wide reprogramming

Resetting, establishment and maintenance of epigenetic marks to control gene dosage (genomic imprinting and X-inactivation)

Epigenetic control of mature cell differentiation (T-cells)

# Early development and pluripotency

At earliest stages of development totipotent and pluripotent cell programs are established to form the basis for a complete development.

Totipotency = capacity to develop in all cell types

pluripotency = capacity to develop in many cell types

The development of totipotency and pluripotency comes with a substantial genome epigenetic (re-)programming.

# Early development – phases of reprogramming

- „in vivo“ in early cells of the embryo to obtain toti- and pluripotent stem cells (e.g. embryonic stem cells).
- „in vivo“ in early germ cells = primordial germ cells (PGCs) to reset cells to an erased ground state.
- „in vitro“ in induced pluripotent stem cells (iPS) after over-expression of 4 stem cell factors (Oct4, Sox2, c-Myc, Klf4) to generate stem cells with a pluripotent capacity.

Embryo and somatic cells

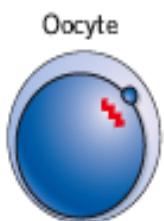
DNA methylation

H3K27 methylation

H3K4 methylation

Pluripotency-associated genes

Developmental genes



Zygote



Sperm

Inner cell mass



ES cells



Embryonic germ cells



Germ cells

DNA methylation

H3K27 methylation

Pluripotency-  
associated genes

Developmental genes

# Control of <sup>epi</sup>genetic programs of stem cells

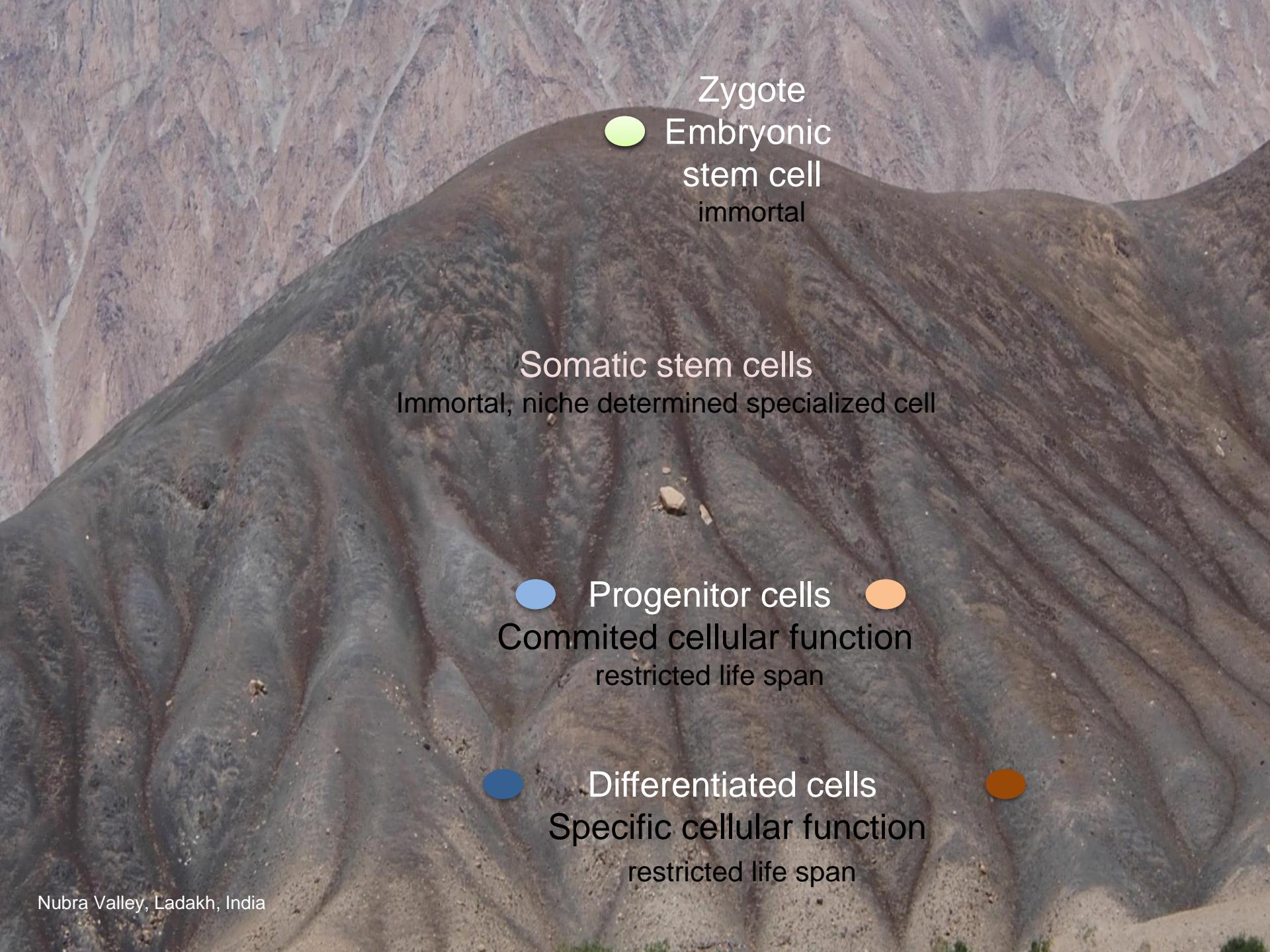
**Stem cells** are undifferentiated biological cells that can divide (unlimited) through mitosis and produce more stem cells.

**Stem cells** can differentiate in various types of specialized cells

The differentiation is controlled by changes in gene expression and controlled in a temporal and cell type specific manner.

In the fetus and the adult most cells are programmed to execute specific „differentiated“ programs.

Some cells remain a restricted differentiation and self renewal potential as „somatic stem cells“ (e.g. in hematopoiesis).



Zygote

Embryonic  
stem cell  
immortal

Somatic stem cells

Immortal, niche determined specialized cell

Progenitor cells

Committed cellular function  
restricted life span

Differentiated cells

Specific cellular function  
restricted life span

# Stem cells

- Natural stem cells are classified as **pluripotent** (e.g. embryonic stem cells) or **multipotent** (somatic stem cells, e.g. hematopoietic stem cells).

Stem cells:

maintain a functional plasticity

have the capacity to undergo (unlimited) self renewal

can differentiate in various cell types

Pluripotent embryonic stem cells can be cultivated indefinitely (immortal) and can generate a complete new animal (mouse)

Somatic stem cells have limited cultivation potential (*ex vivo*) with the exception of induced pluripotent stem cells.

# Development of stem cell programs

Epigenetic „ground states“ are naturally reached in early cells of the embryo and during germ cell development..

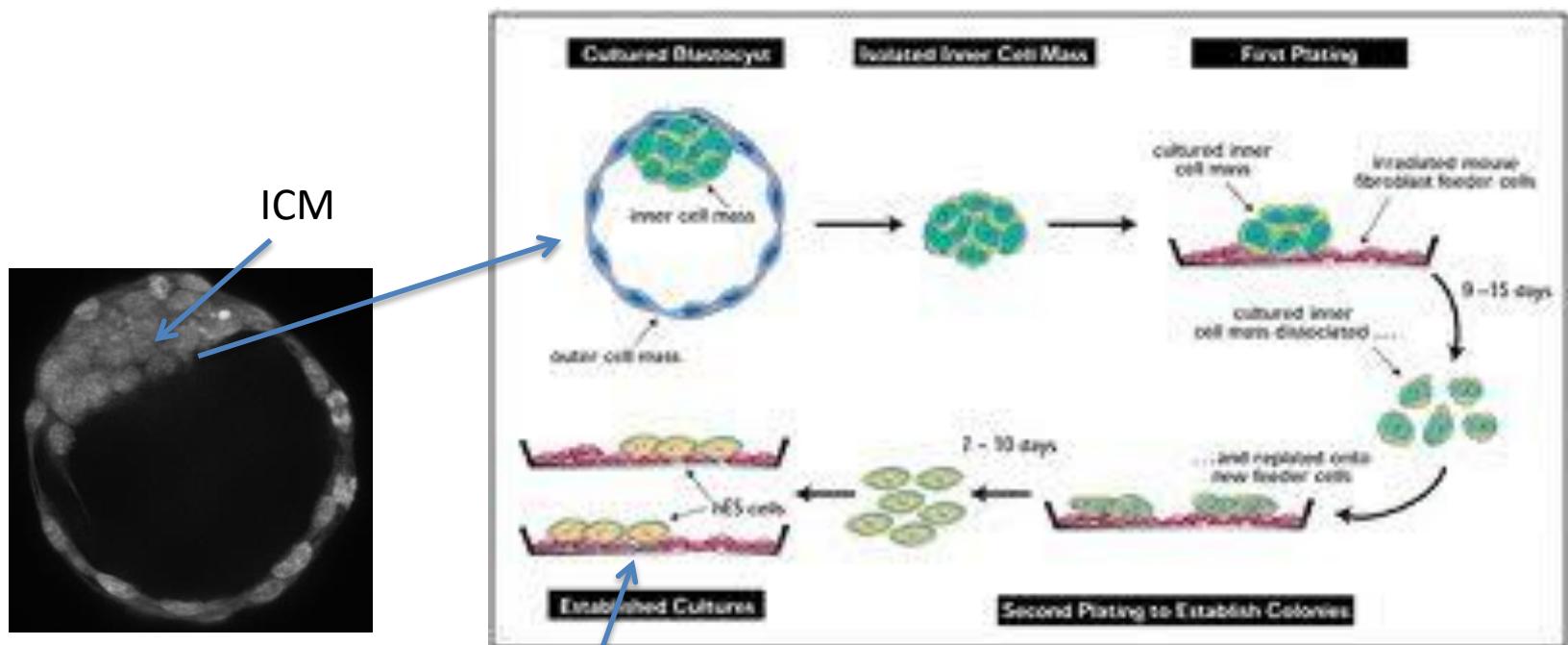
Cells of this „ground state“ can be isolated and cultivated

New manipulation technologies (iPS, SCNT =„Cloning“) allow to induce a ground state development in nearly each somatic cell by „forced“ re-expressing stem cell programs.

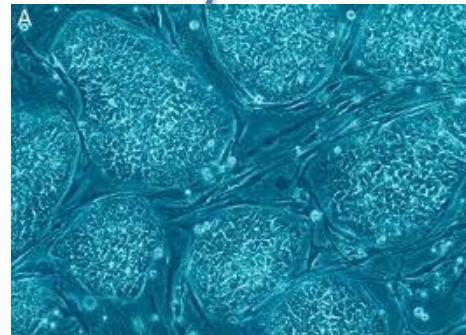
The mechanisms of this stem cell reprogramming are only partially understood- they include the resetting of the epigenome and the reinforcement of stable stem cell like regulatory circuits.

Reprogramming involves the resetting of epigenetic modifications:  
Histone modifications, DNA-methylation

# Embryonic stem cells are derived from the inner cell mass of the blastocyst



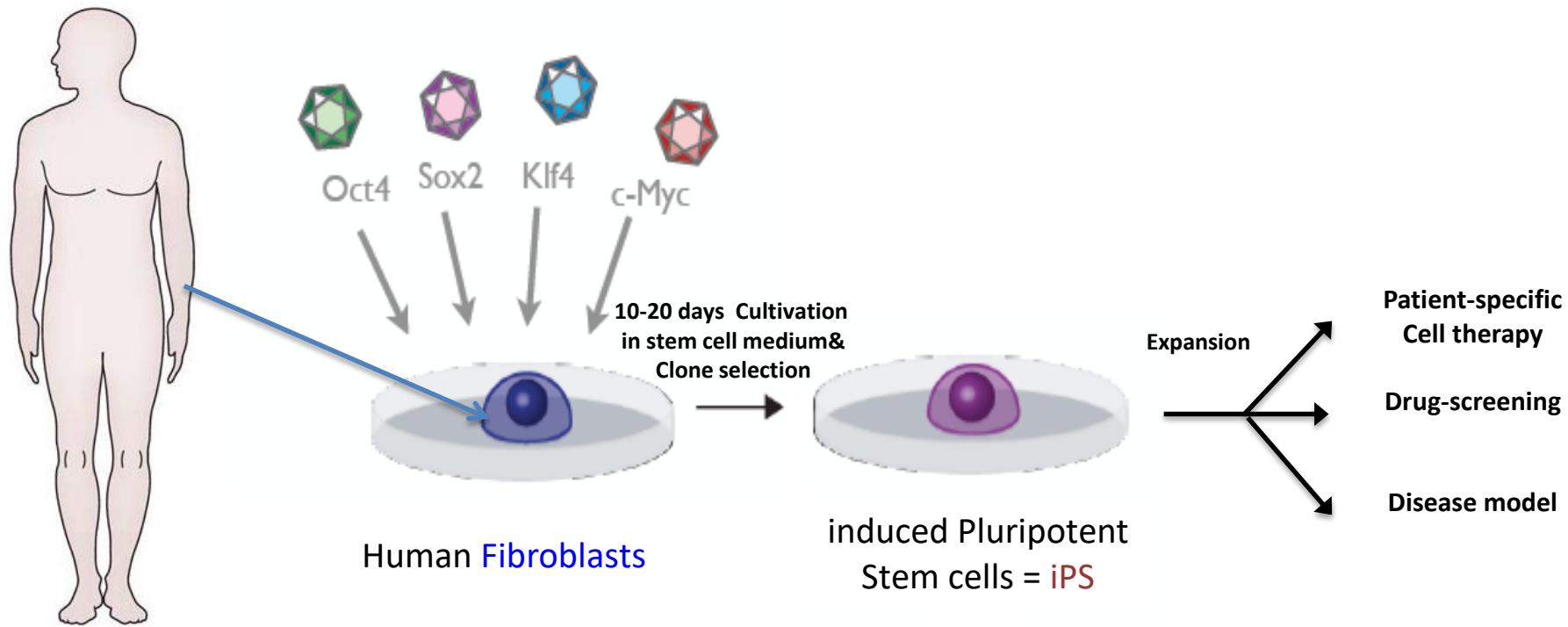
<http://www.biology.iupui.edu/biocourses/Biol540/11hEScellsfull09.html>



Explanted  
and cultivated  
in specific media

# Induced pluripotent stem cells = iPS

Grow human primary cells and transfect with gene cassettes expressing four transcription factors



The overexpression of „four stem cell factors“ induces the activation of endogenous stem cell programs: expression of Oct4, Sox2, Nanog, Lin28, ....  
The „reprogramming“ into this stem cell state requires extensive epigenetic reprogramming

# Developmental control of <sup>epi</sup>genetic programs

The development of stem cells and differentiation of somatic cells is accompanied by controlled genetic and epigenetic changes.

In early stem cells genetic and epigenetic programs are reset to a pluripotent/totipotent ground state.

This resetting of gene expression programs includes/requires the establishment of a low (ground) level of heterochromatic modifications (H3K9me2/3; DNA-methylation).

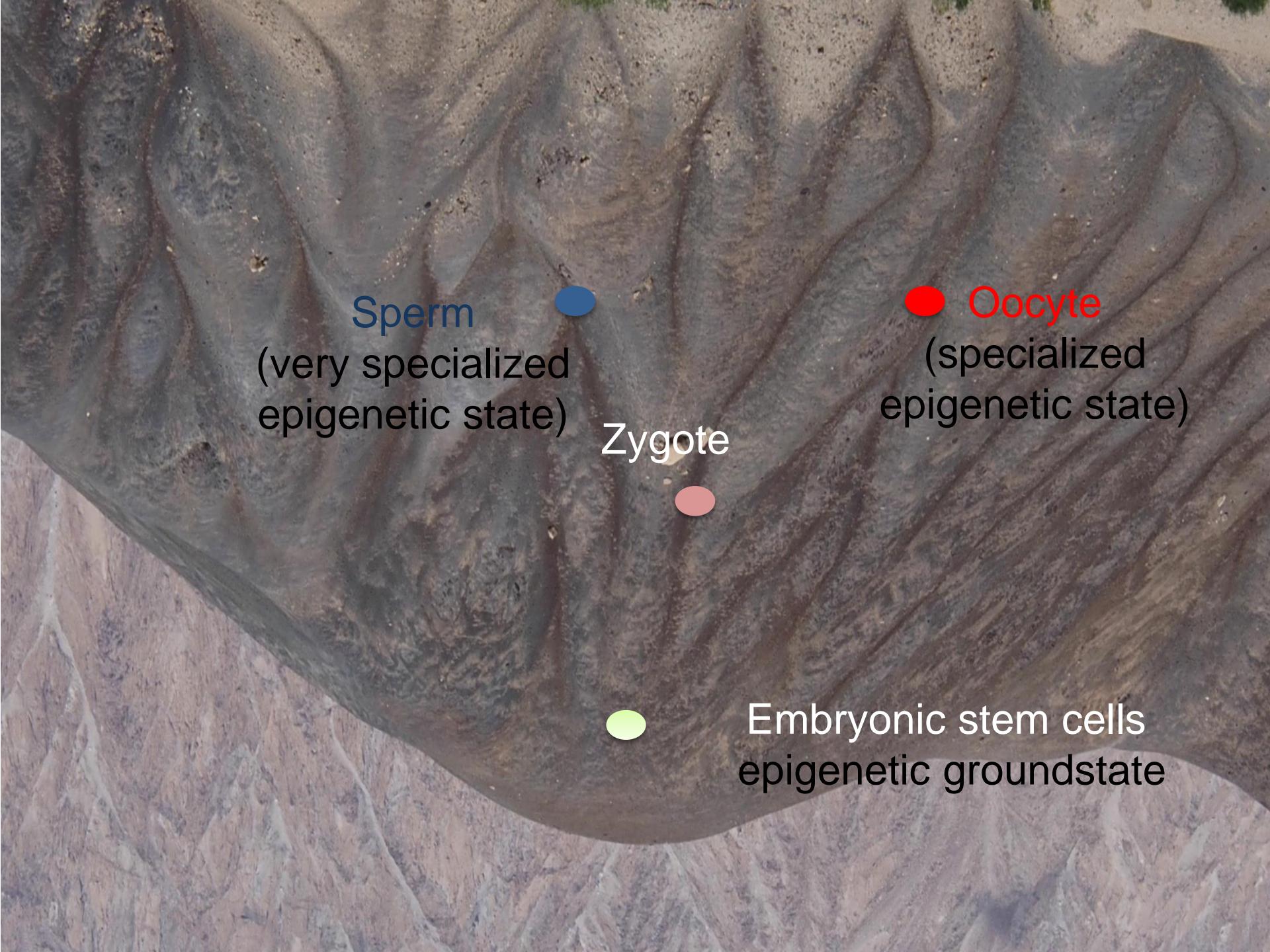
# Development of stem cell programs

At the beginning of a new life the genetic program is restarted to allow the formation of all cell types from one (few) cells (epigenesis)

Development involves the ordered temporal and cell specific execution of genetic programs following the establishment of an epigenetic ground state (state of totipotency)

To reach the ground state histone modifications and DNA-methylation are reset (erased) in the zygote and during the first cellular cleavage stages.

The mechanisms of this epigenetic resetting are still to be understood – they are of extreme importance for stem cell biology



Sperm  
(very specialized  
epigenetic state)

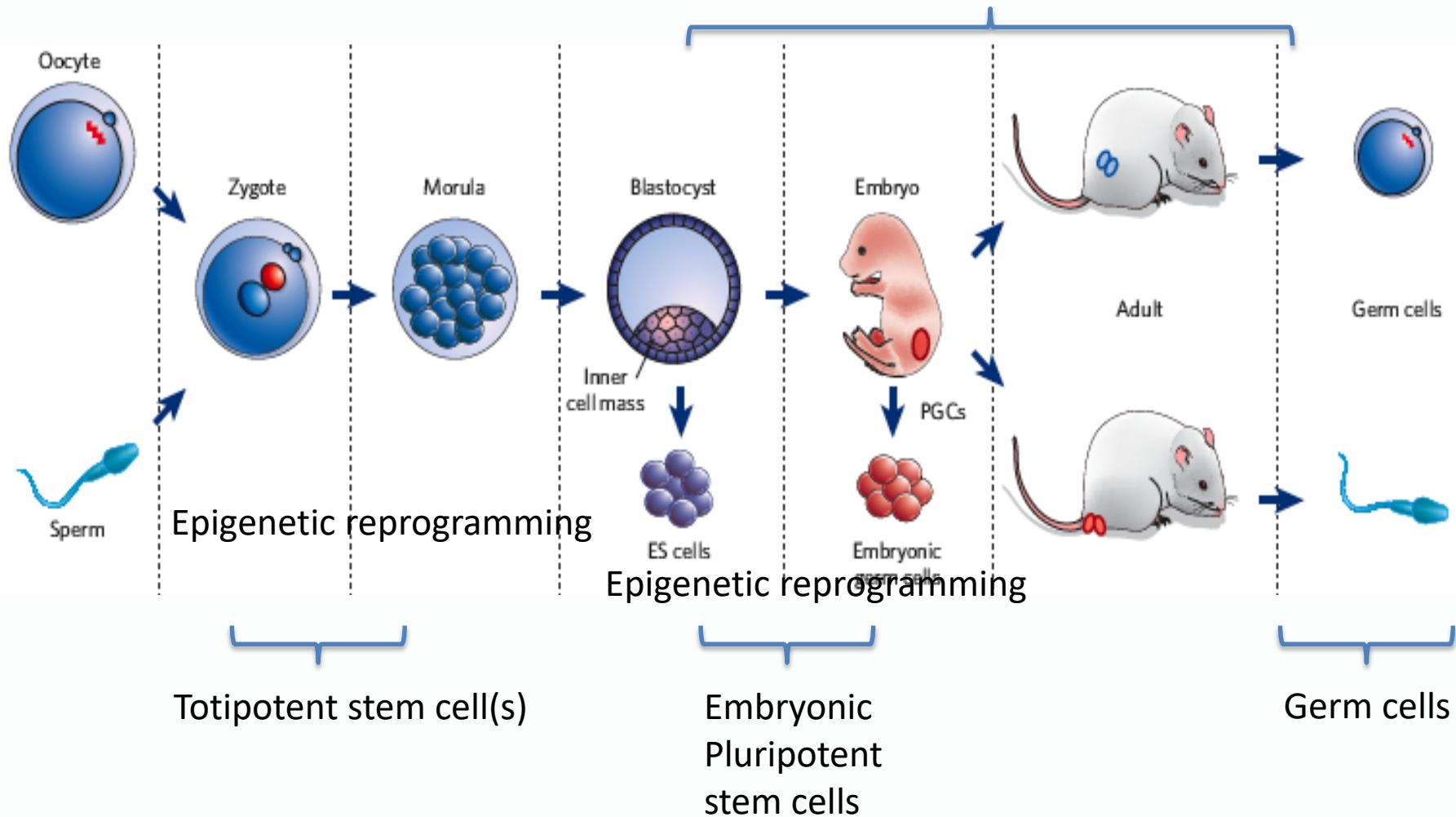
Oocyte  
(specialized  
epigenetic state)

Zygote

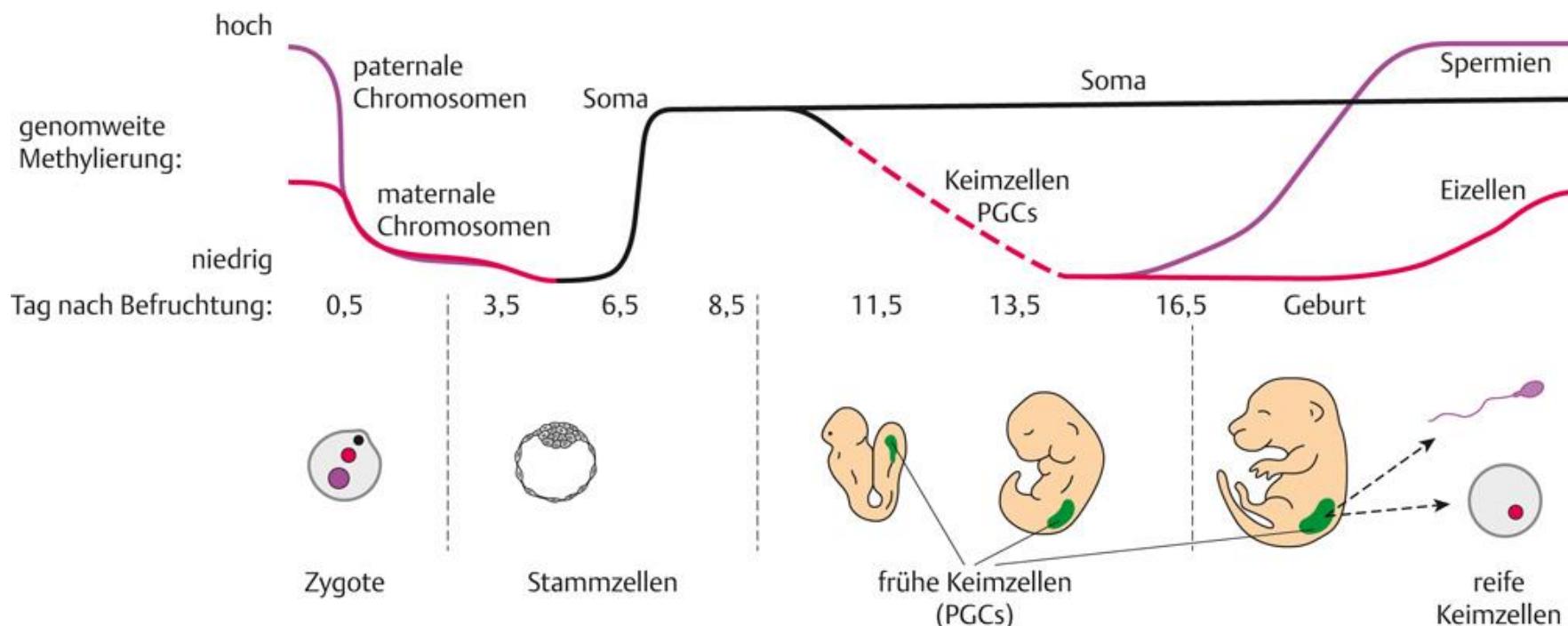
Embryonic stem cells  
epigenetic groundstate

# Development and epigenetic reprogramming

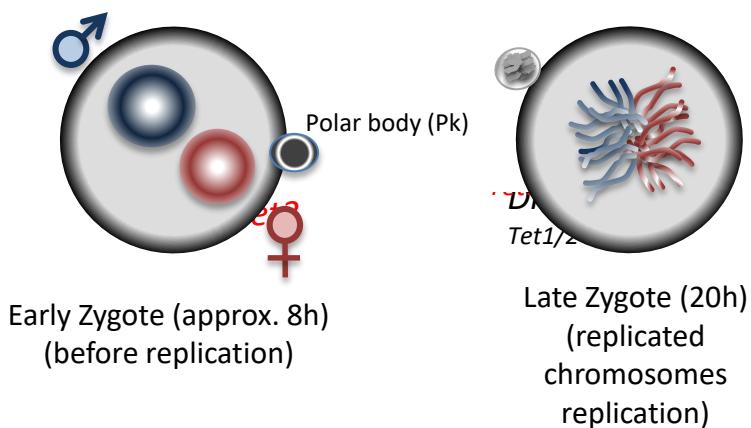
>200 embryonic and adult somatic cell types  
To form the tissues/organs of an individuum



# Genome wide DNA-methylation changes during development



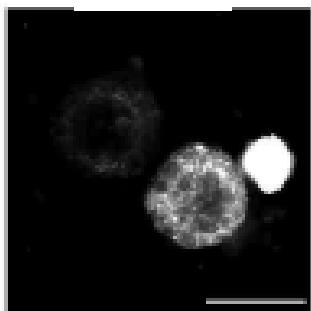
Georg Thieme Verlag, Stuttgart · New York · 2015  
Alfred Nordheim et al.: Molekulare Genetik, 10. Auflage



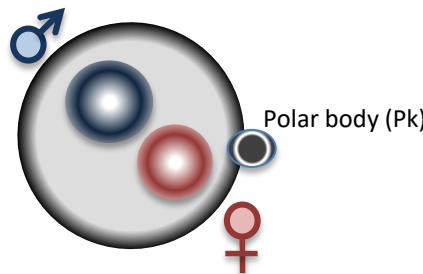
JW, Modified from Seisenberger et al  
Philos Trans R Soc Lond B Biol Sci.  
2013 January 5; 368(1609).

# DNA-demethylation in the first cell (zygote)

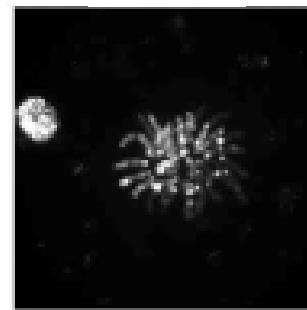
5mC



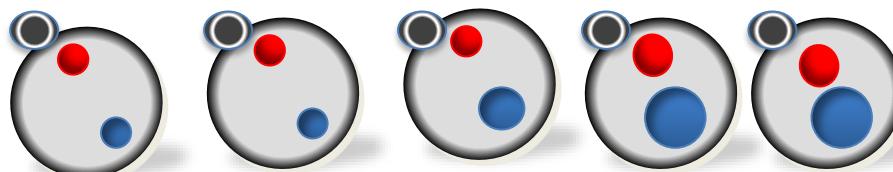
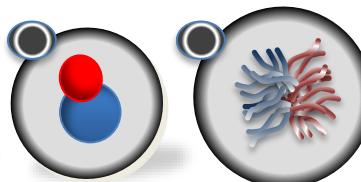
Early Zygote (G1 phase)  
(before DNA replication)



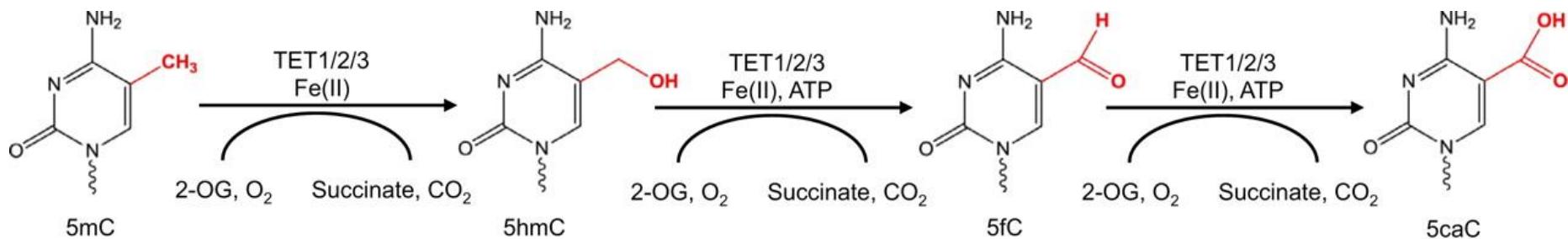
5mC



Late replicated zygote (20h)  
G2-M phase  
(condensed metaphase  
chromosomes)



# Oxidation of 5mC by Tet enzymes

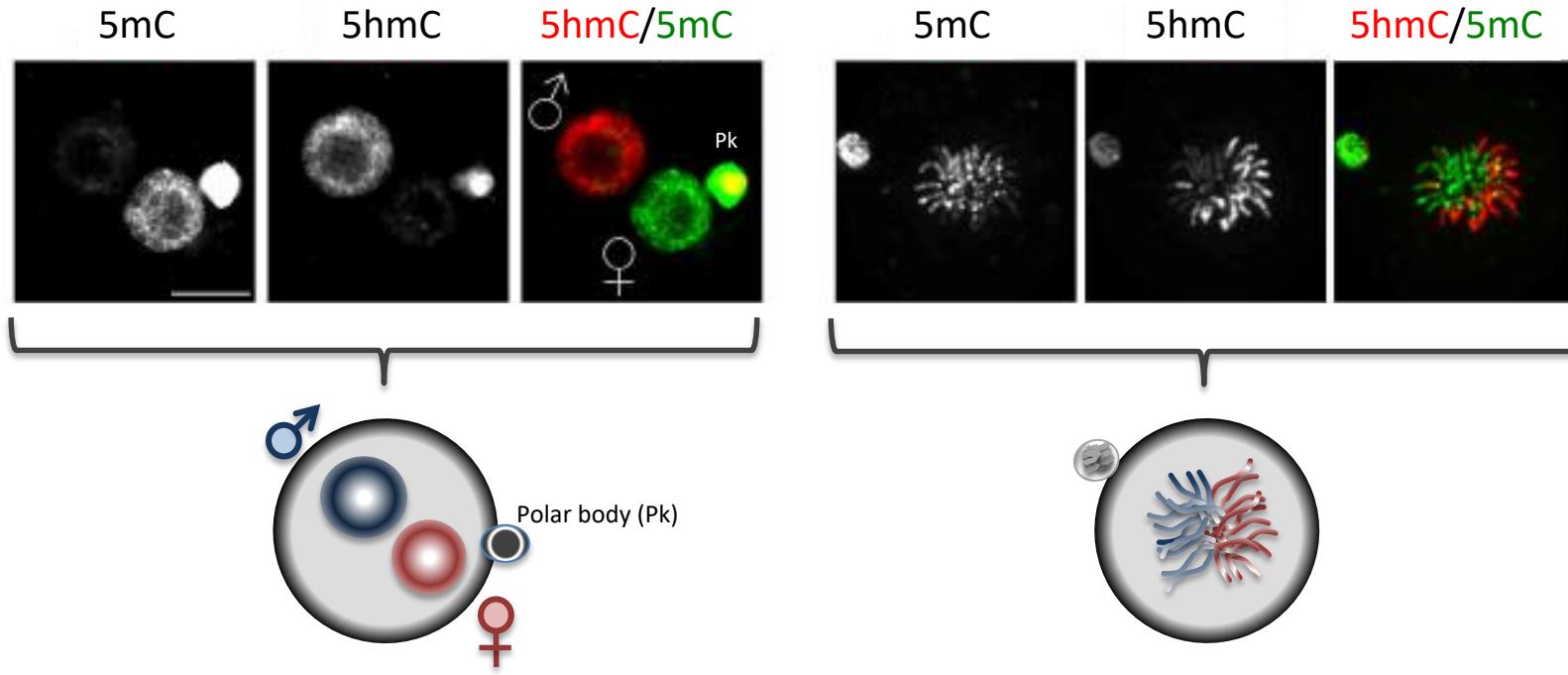


## Oxidation of 5mC

--> 5mC can be oxidized into 5hmC, 5fC and 5caC

--> TET (= Ten Eleven Translocation-) Oxygenases catalyse the oxidation using  $\alpha$ -Ketoglutarate as a co-factor

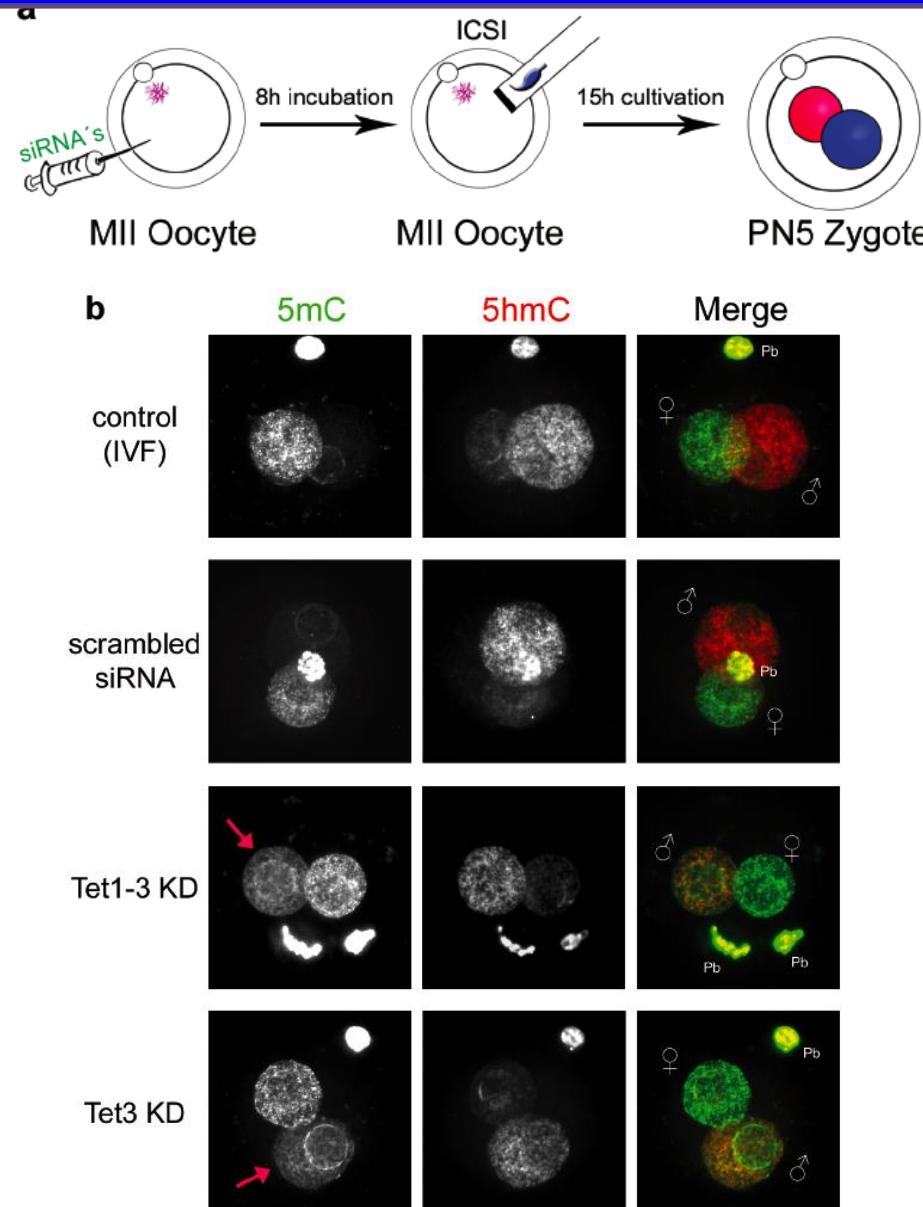
# DNA-demethylation in the first cell (zygote)



Paternal and maternal chromosomes show  
asymmetric 5mC and 5hmC methylation

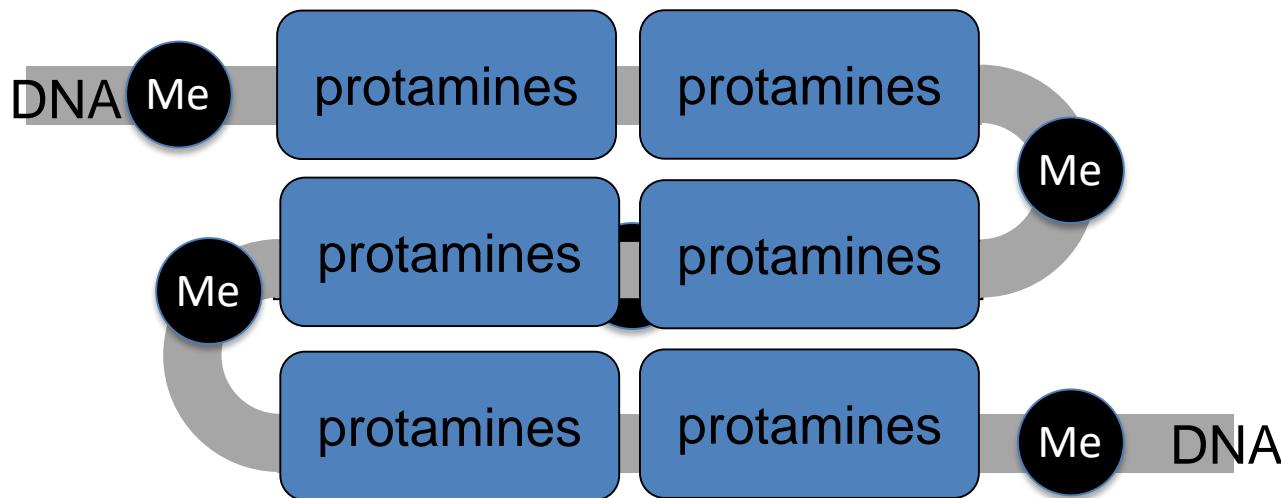
In the paternal pronucleus 5mC is converted to 5hmC but not (less) in the maternal pronucleus

# Knockdown of TET3 affects the 5hmC conversion in the paternal pronucleus



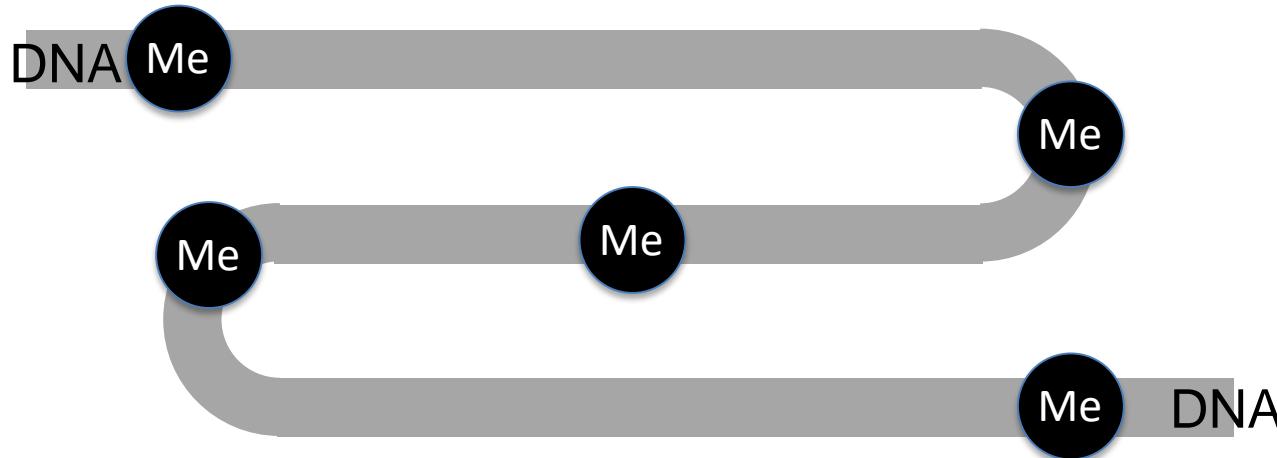
DNA-methylation changes and histone modifications are occurring side by side

# Epigenetic reprogramming in the zygote: 1. replacement of protamines in the paternal chromosomes



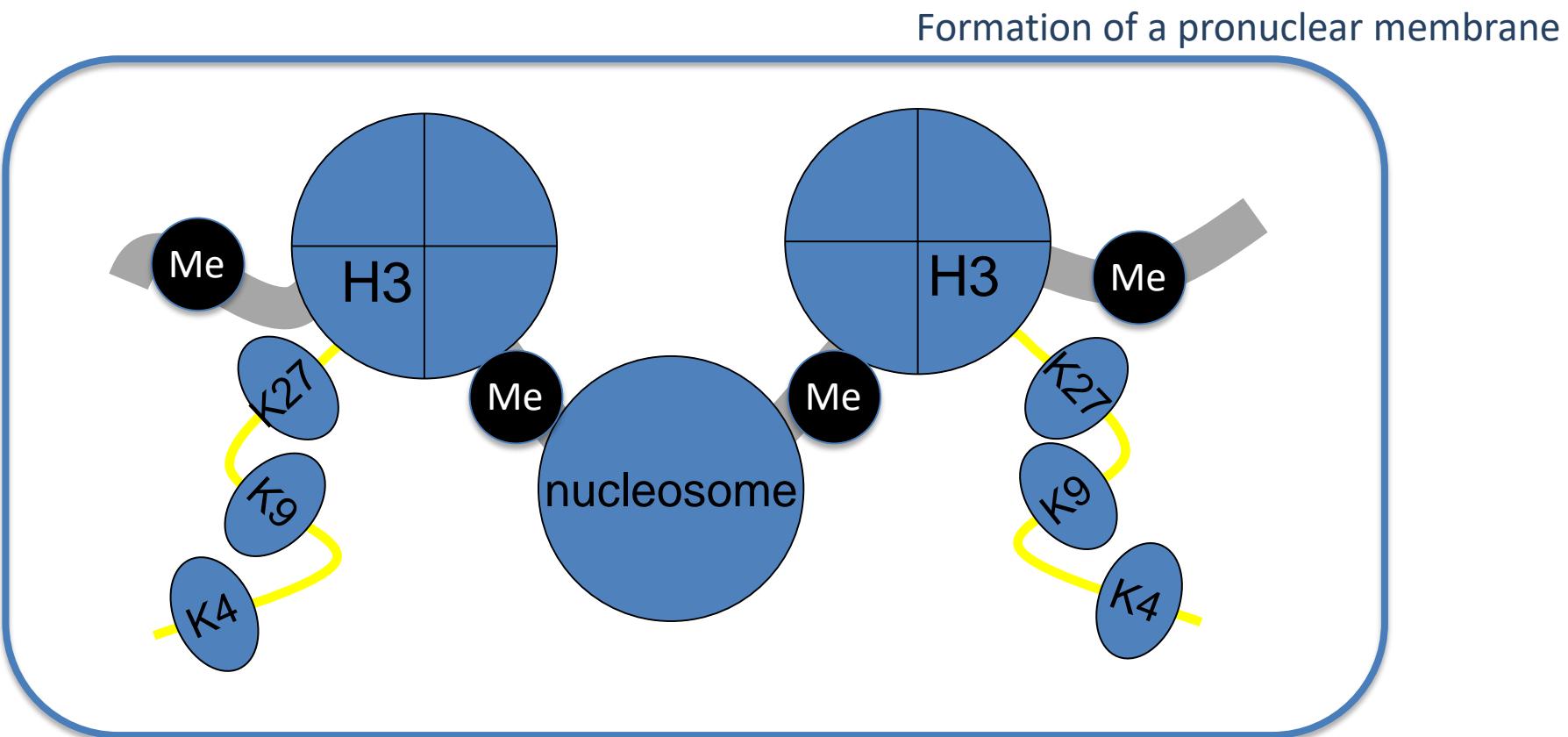
The incoming sperm chromosomes are packaged in protamines (= histone like proteins).

# Epigenetic reprogramming in the zygote: 1. replacement of protamines in the paternal chromosomes



Sperm membrane breaks down and protamines (= histone like proteins)  
are evicted

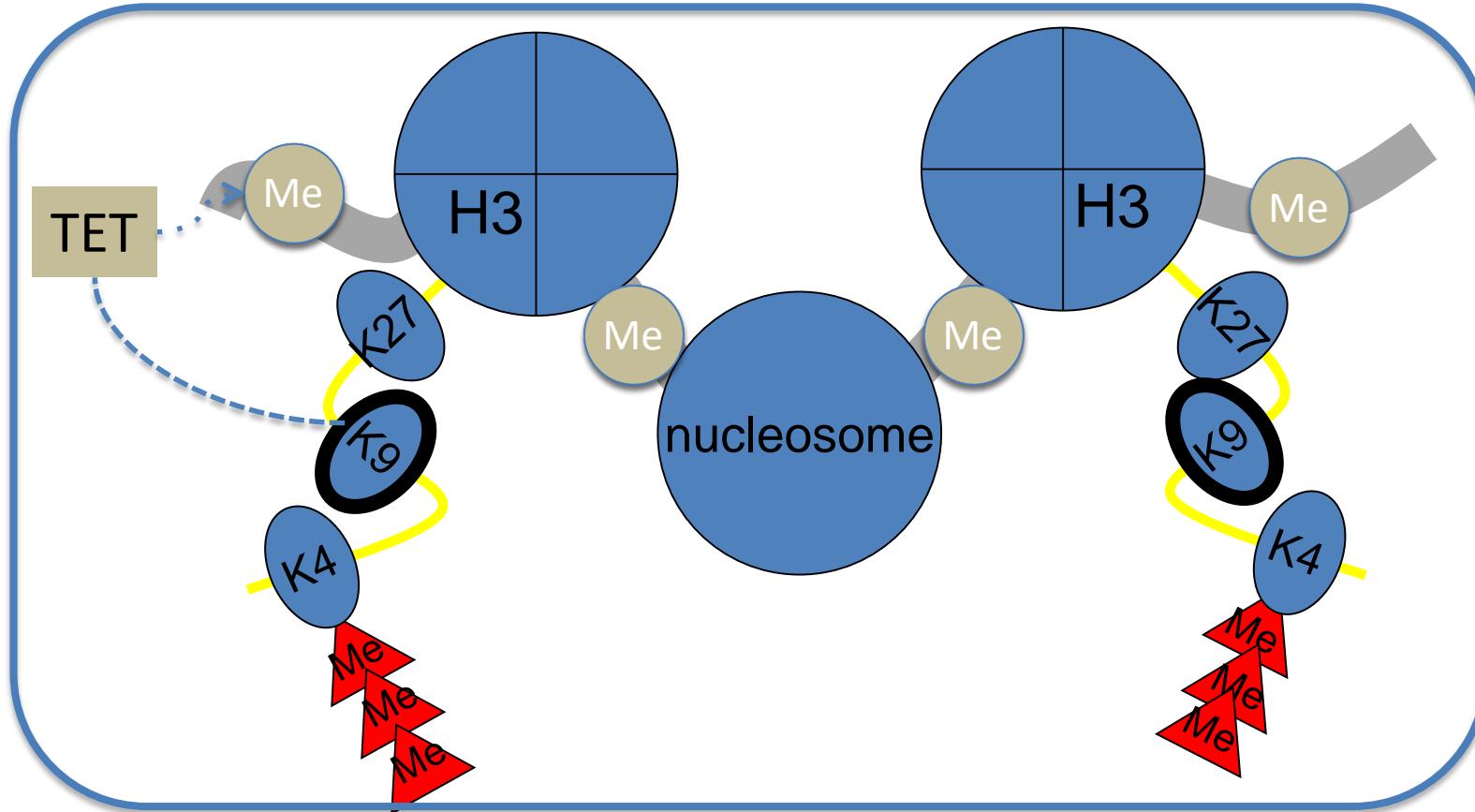
# DNA-demethylation in zygote: formation of a new paternal pronucleus with „normal“ chromatin



Paternal chromosomes are repackaged by histones coming from the egg cytoplasm.

Histones are initially only partially acetylated on H4 (not shown) but not methylated at lysines of the histone H3 tail

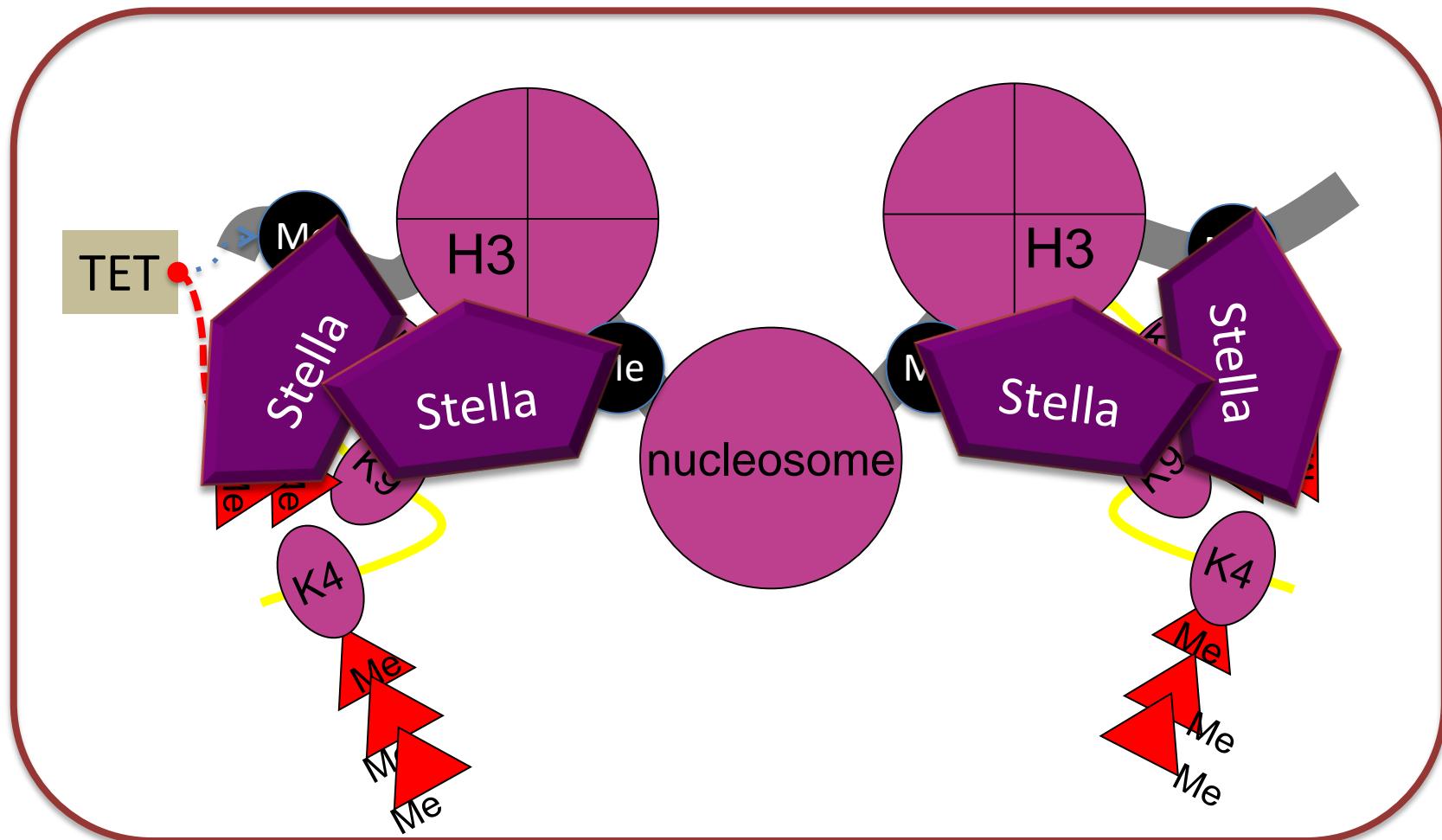
# DNA-demethylation in zygote phase II: paternal pronucleus



In G1 the paternal chromatin acquires gradual H3K4 methylation but not H3K9. The absence of H3K9me2 allows TET to recognize DNA methylation and convert 5mC to 5hmC

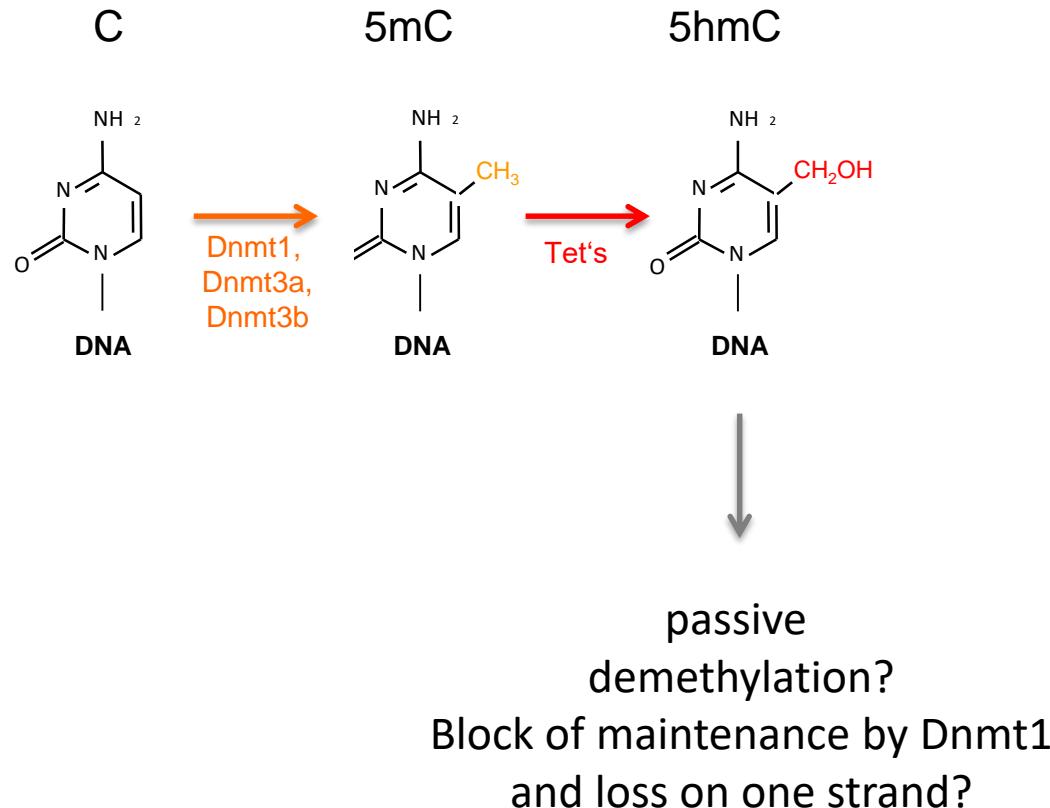


# DNA-demethylation in zygote : Epigenetics of the maternal pronucleus



The maternal chromosomes have a higher „level“ of histone modifications. DNA-methylation (5mC) is not converted into 5hmC. The presence of H3K9me3 prevents the oxidation by TET3 by recruiting the Stella protein

# What is the consequence of 5hmC oxidadation in the zygote?

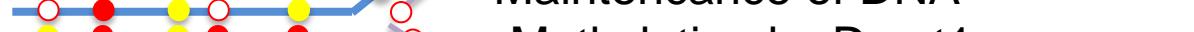


# Possible 5hmC influence epigenetic inheritance

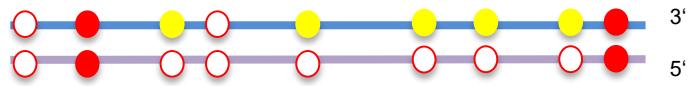
Before Replication



↓ TET



After Replication



Maintenace of DNA  
-Methylation by Dnmt1

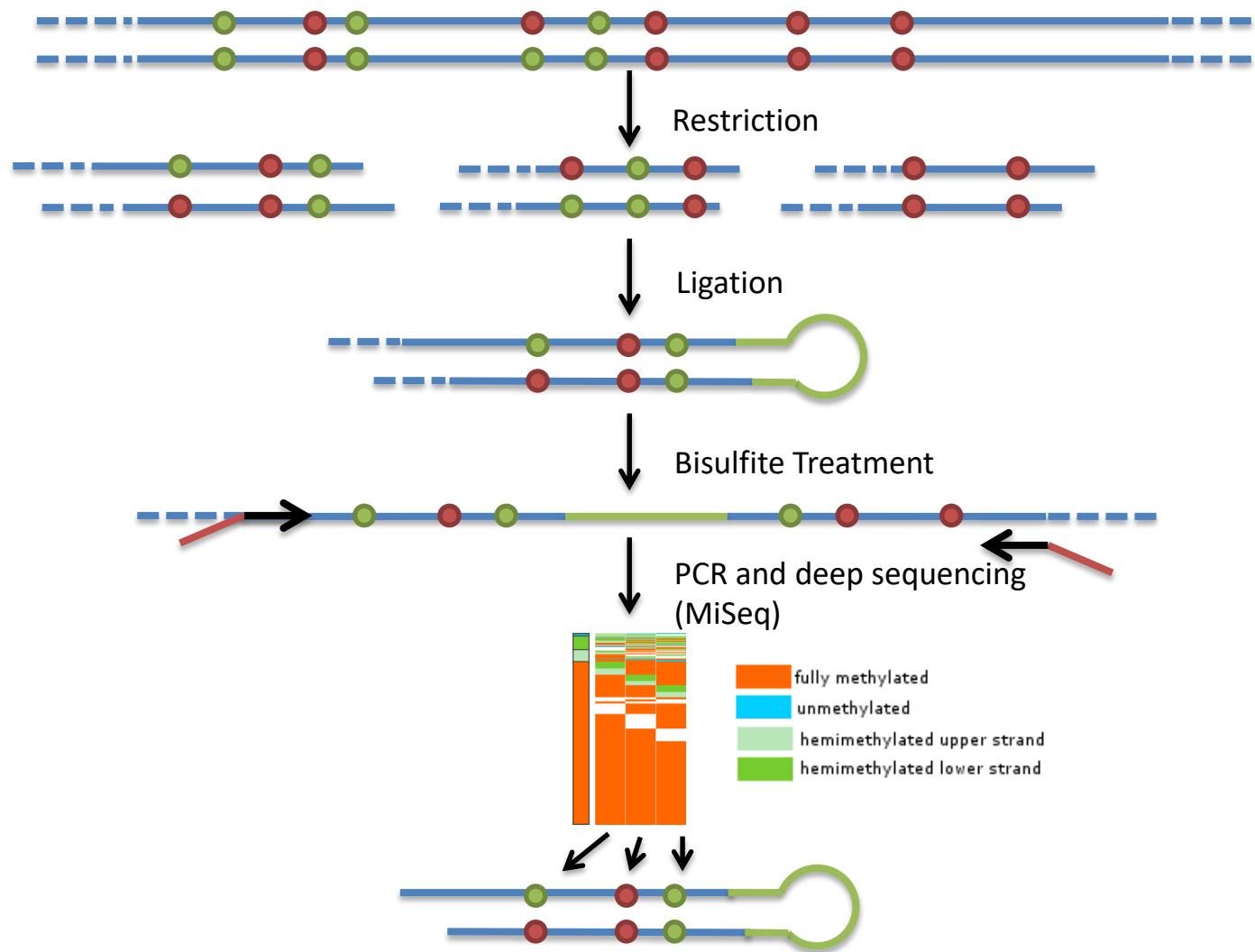


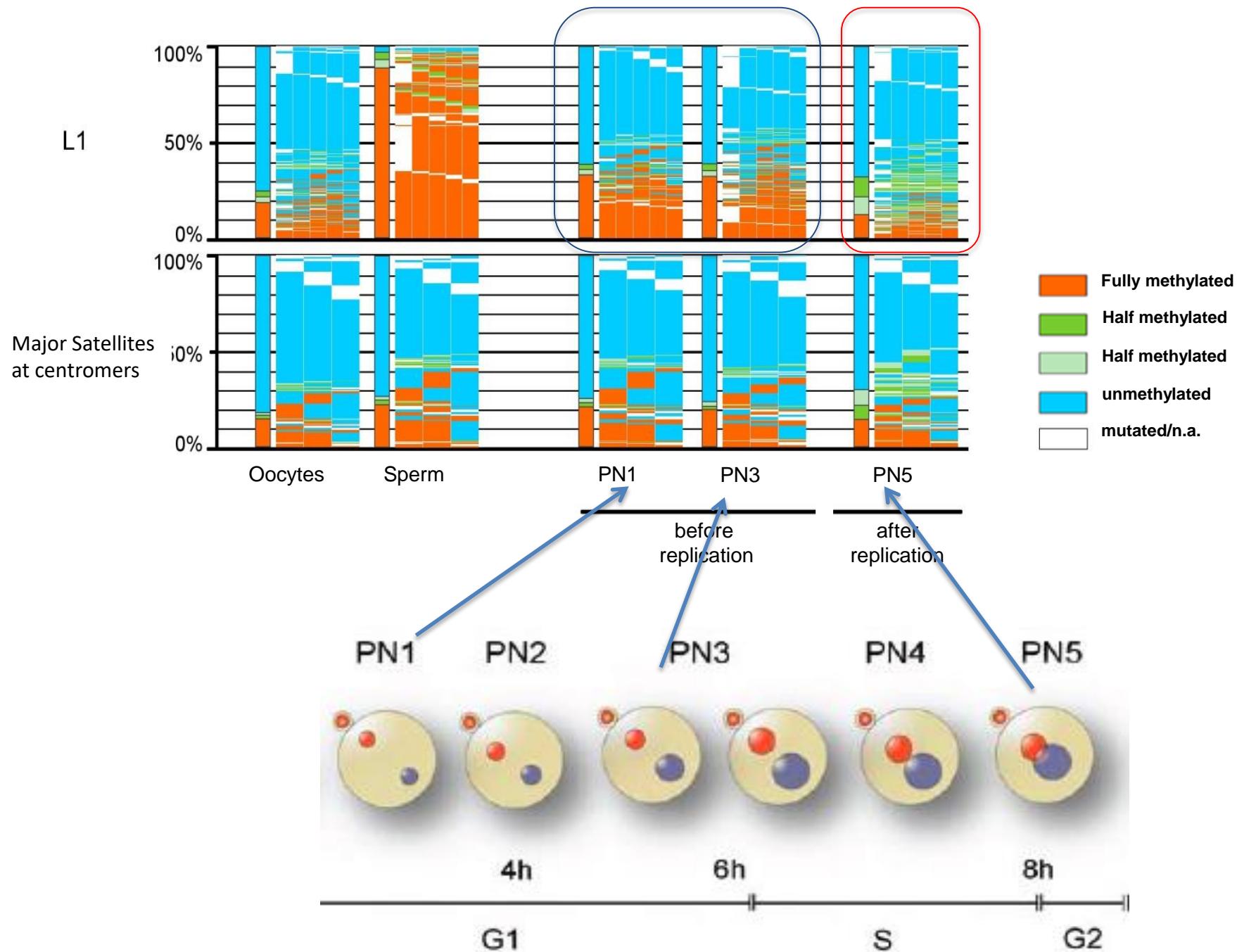
- Methylated CpG
- Hydroxymethylated CpG
- Unmethylated CpG



Dnmt1

# Hairpin-Bisulfite-Sequencing (HBS) resolves the status of DNA-methylation on both DNA-strands

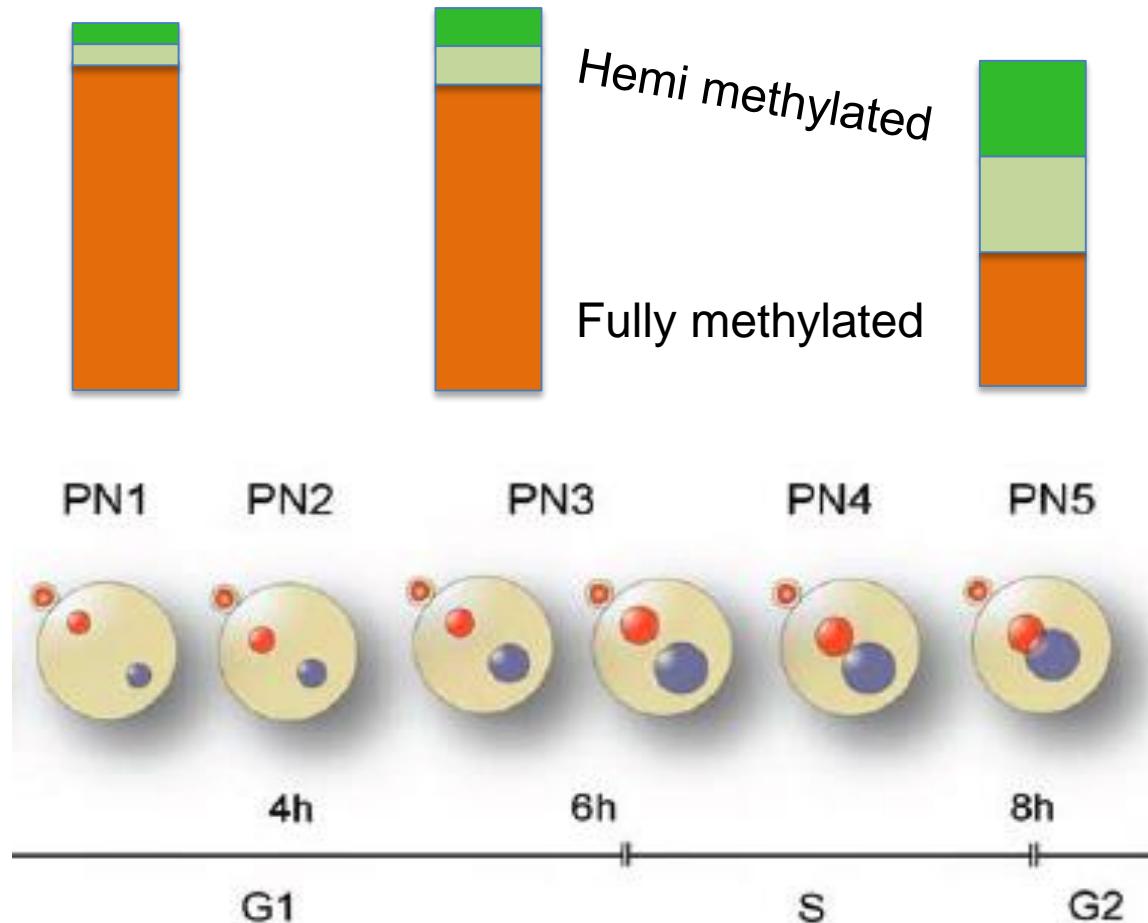




# DNA- Methylation is reduced after DNA replication

Hemi (=half) -methylated CpGs accumulate (only one DNA strand is methylated)

Conclusion: Maintenance methylation is blocked



# Oxidative modifications, active and passive demethylation – our current view

Before Replication



TET  
oxygenase

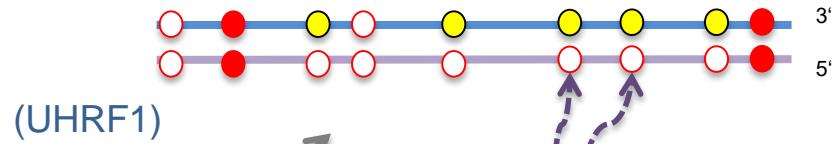
active  
Demethylation (repair,  
decarboxylation)



→



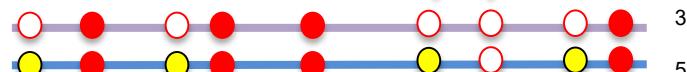
After Replication



(UHRF1)  
Dnmt1

Passive  
demethylation

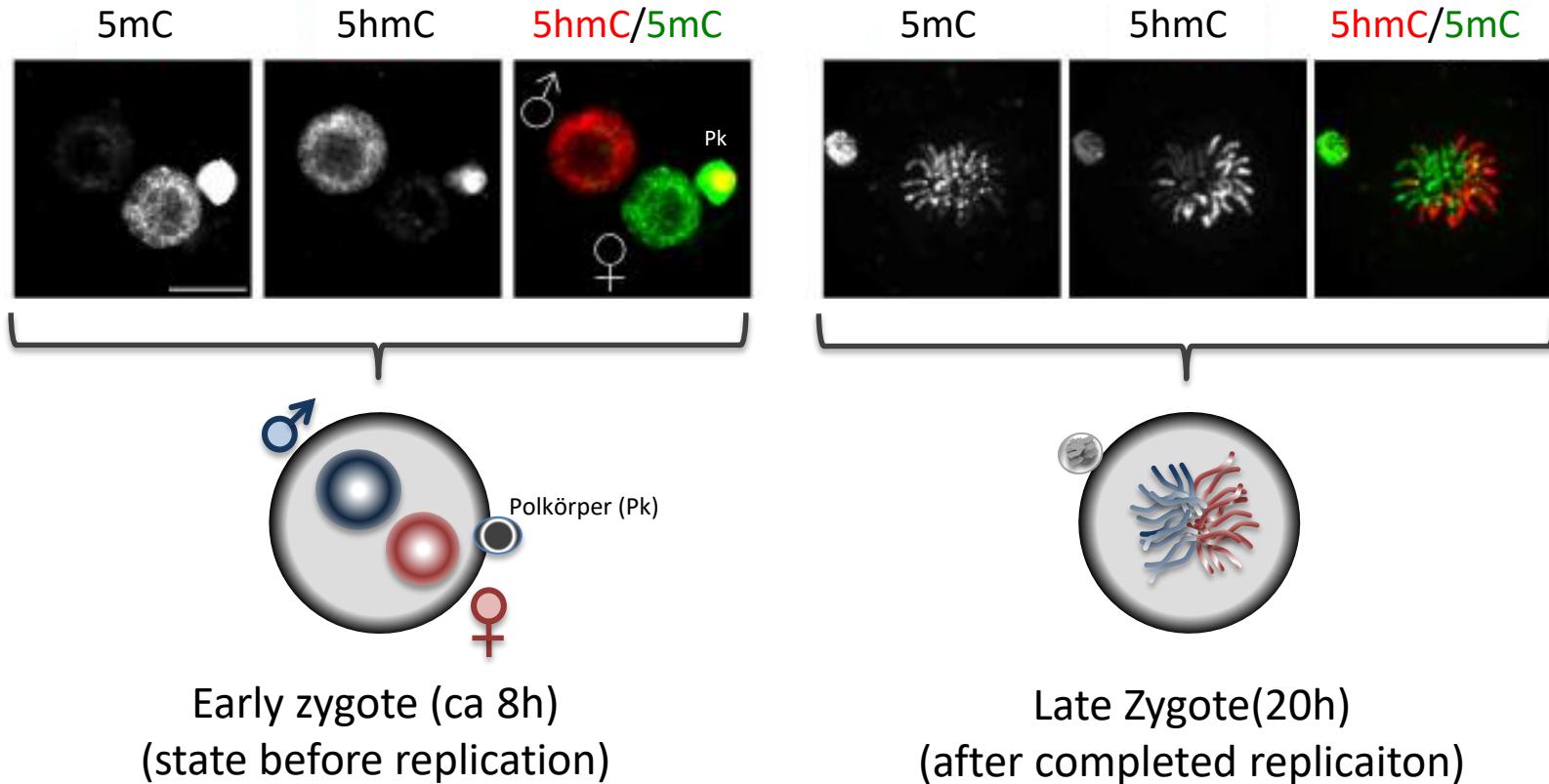
Dnmt3A



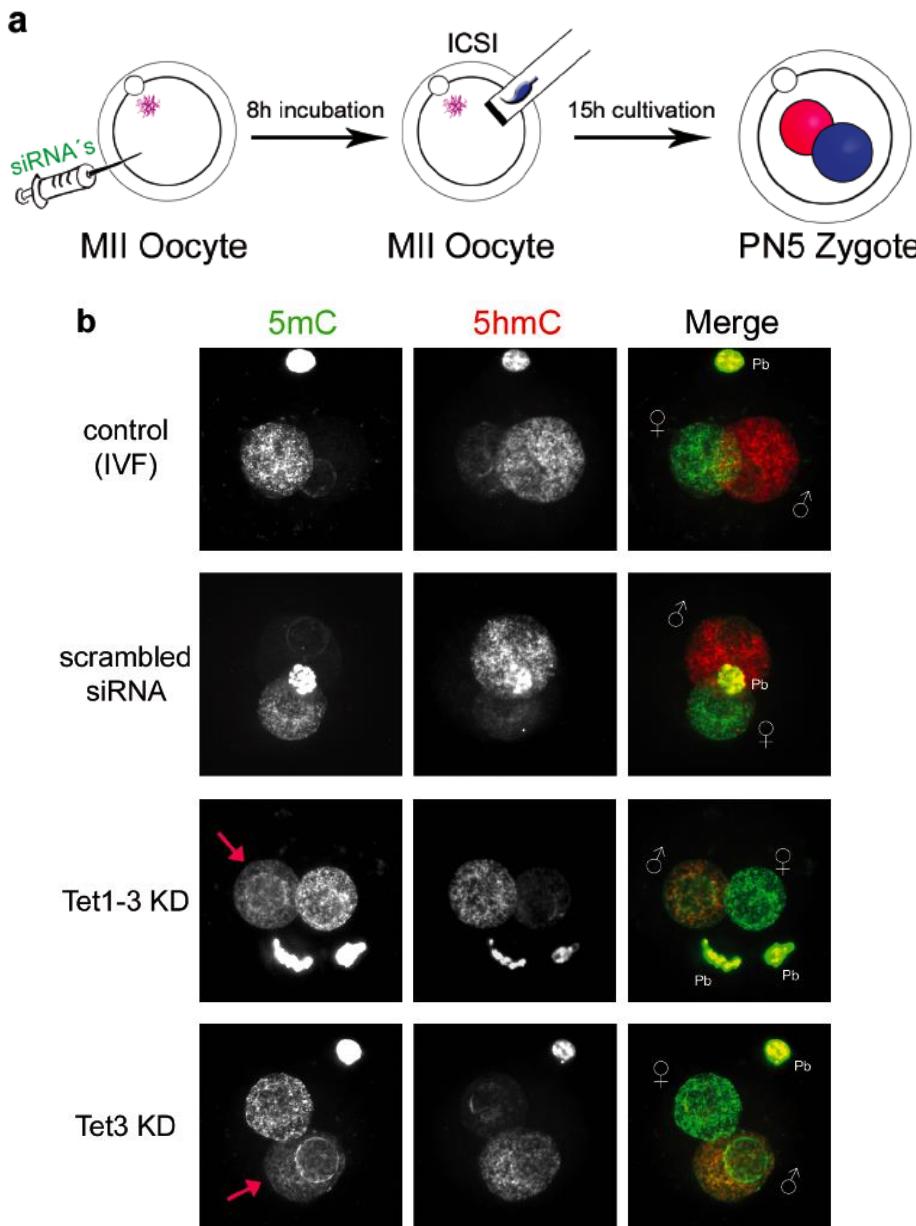
Dnmt3B

- 5mG
- 5hmC
- 5fC/5caC
- Unmethylated C
- Newly methylated CpG

# 5mC in the paternal genome but not in the maternal genome is converted into 5hmC

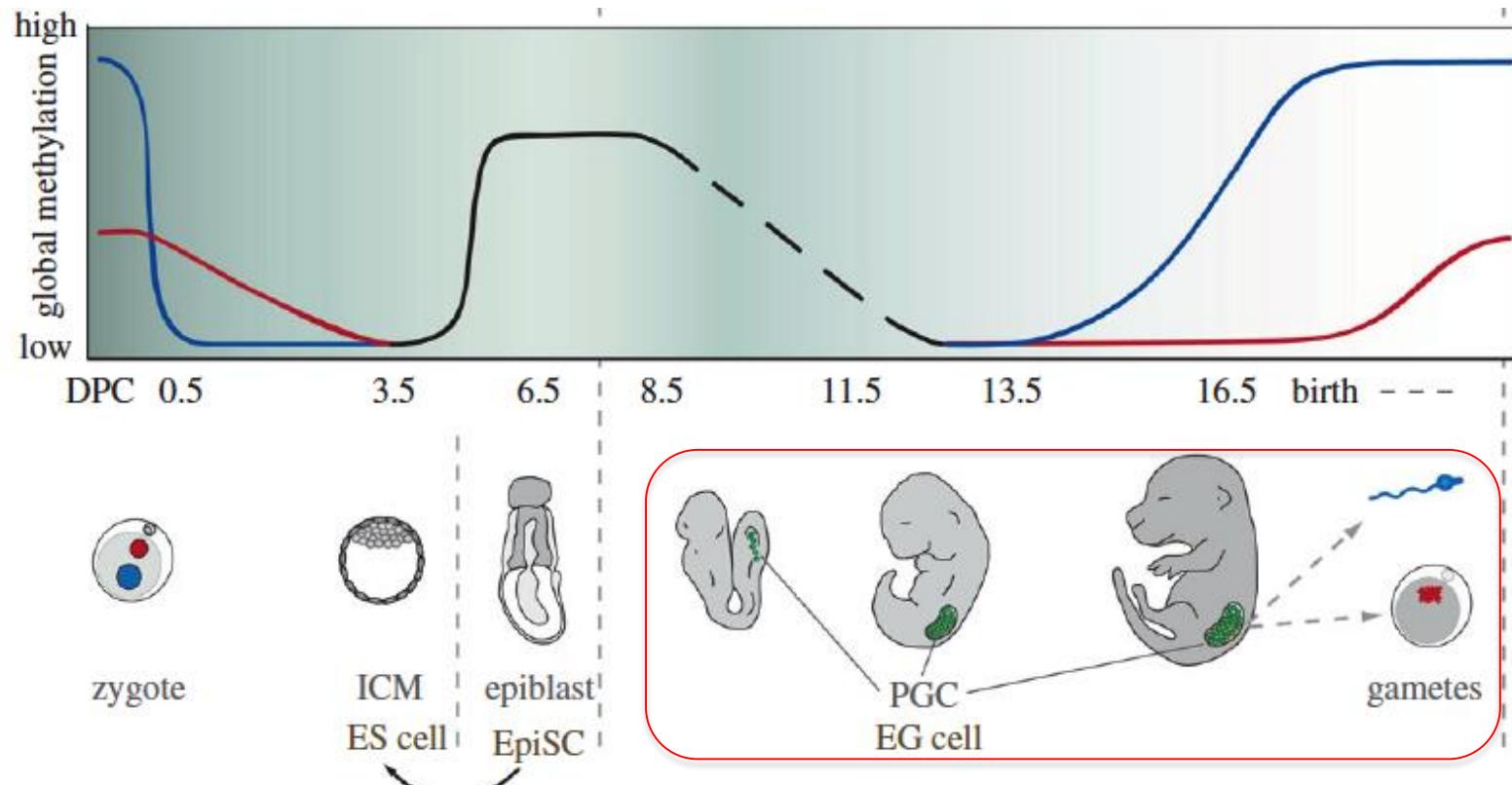


# Conversion of 5mC to 5hmC in the zygote is Tet3 dependent



# Reprogramming of genome wide methylation and imprints in the early germ line

# Epigenetic reprogramming in the germ cells

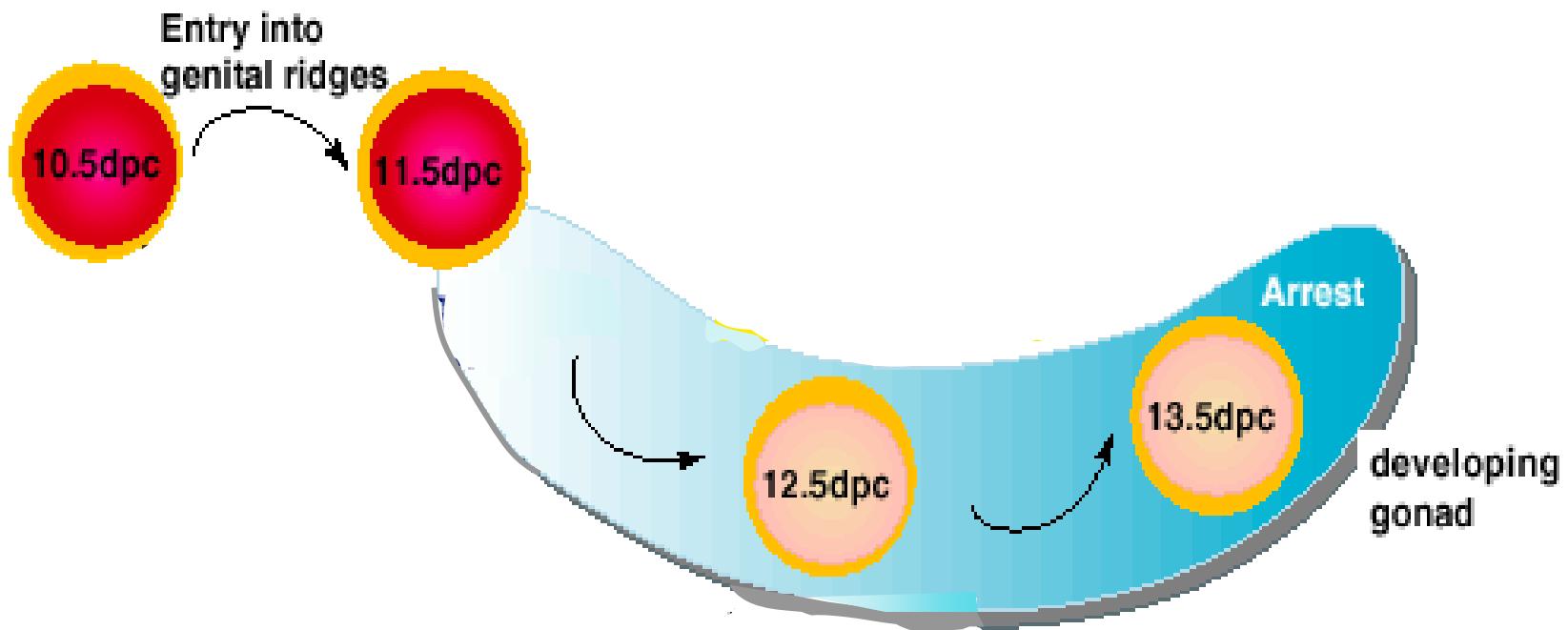


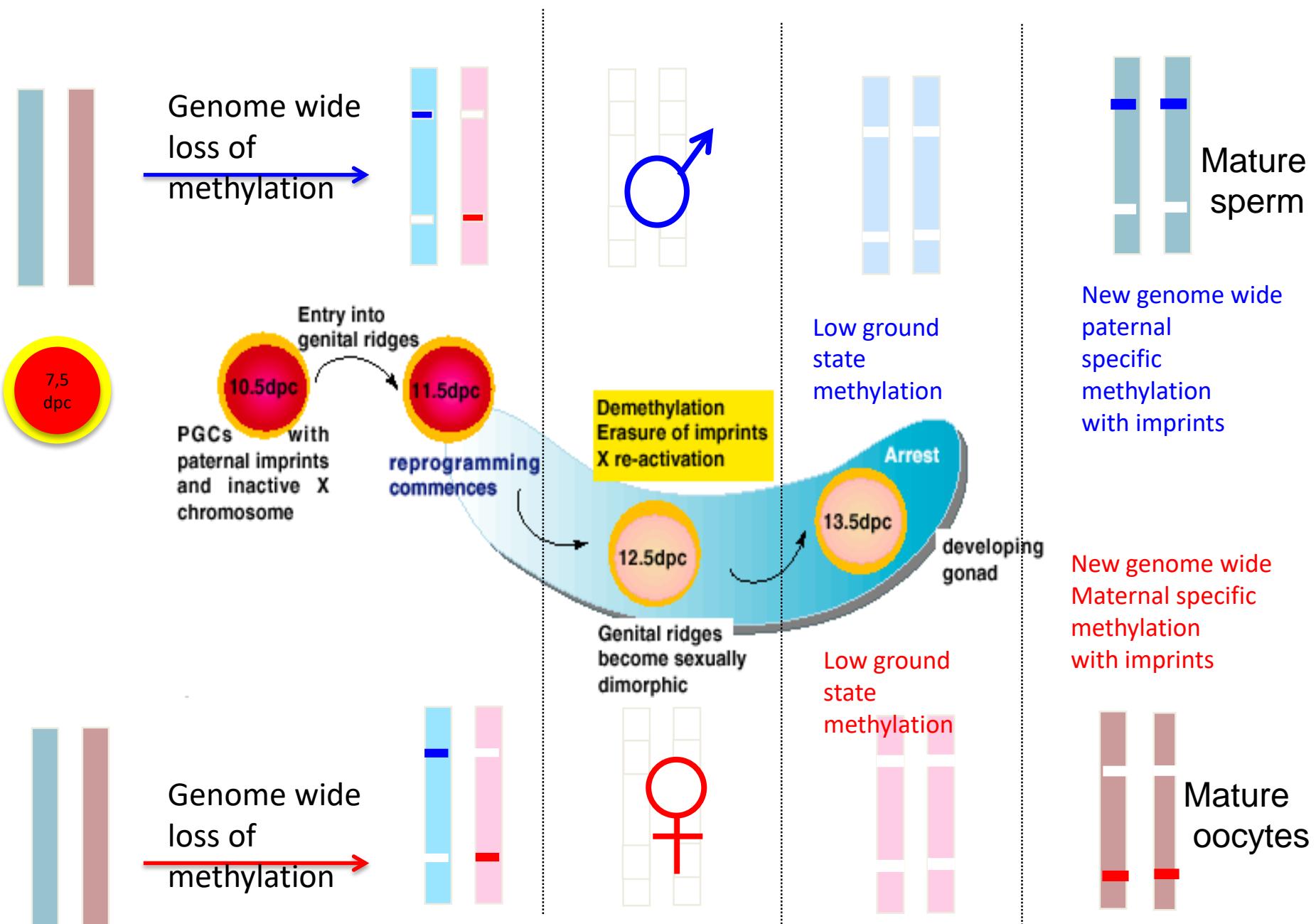
Seisenberger et al  
Philos Trans R Soc Lond B Biol Sci.  
2013 January 5; 368(1609).

# Epigenetic reprogramming in the germ cells

- Very early germ cells i.e. „primordial germ cells“ (PGCs) have a normal somatic epigenome.
- During maturation (and migration) of PGCs their „somatic“ epigenome is reprogrammed and set to a low „stem cell like“ ground state epigenome.
- Along with this resetting „genomic imprints“ are erased.
- During later germ cell maturation (generation of sperms or oocytes) new sex specific epigenetic programs are established including genomic imprints.

The reprogramming of PGCs is finished when PGCs migrate through the genital ridge and enter the Gonaden-Anlagen





# Epigenetic reprogramming in the germ cells

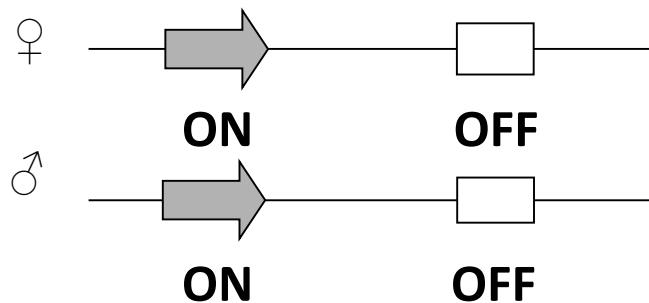
- Genome wide methylation is very much decreased in developing PGCs – this includes also the methylation on the inactive X-chromosome.
- Genomic imprints and the methylation at the XIST promoter (female only) are completely demethylated at late stages of early migrating germ cells
- The resetting of imprints is the prerequisite for a later establishment of new sex specific imprints in the developing germ cells (sperm and egg)
- The resetting of X-chromosomal methylation (at CpG islands) and at the XIST promoter is the prerequisite to reactivate both X-chromosomes in females

# Genomic Imprinting

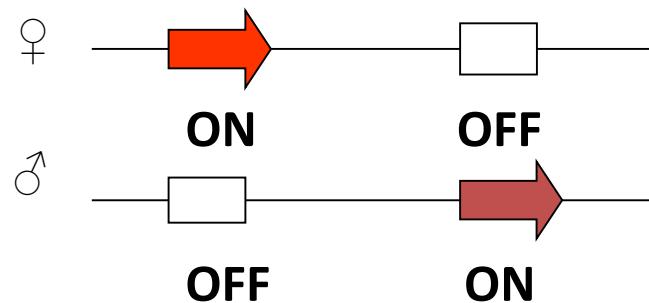
About 100 genes are expressed in a parental manner, i.e. only from either the paternal or the maternal chromosome

This imprinting is caused by an epigenetic marking (DNA-methylation) established in the germ line. The marking is sex specific.

Most Genes are expressed from both chromosomes



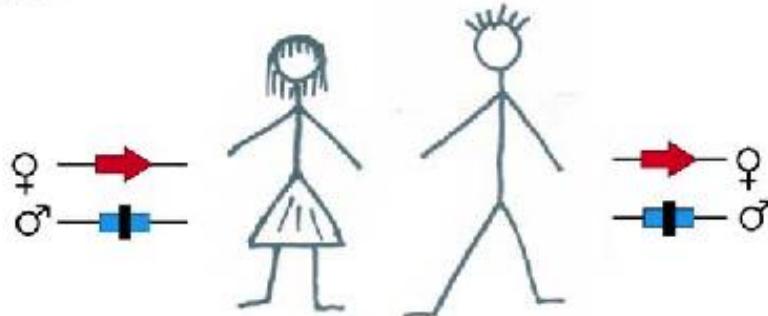
Imprinted genes are expressed only from one of the parental chromosomes



This imprinted expression causes a haploid gene dose of imprinted genes! The haploid gene dose is necessary to balance the expression of imprinted genes. The „off“ state is caused by epigenetic marks (DNA-methylation)

## Parents

The life cycle of genomic imprints



DNA-methylation imprint

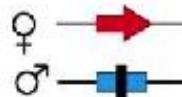
## Immature germ cells



## Mature germ cells

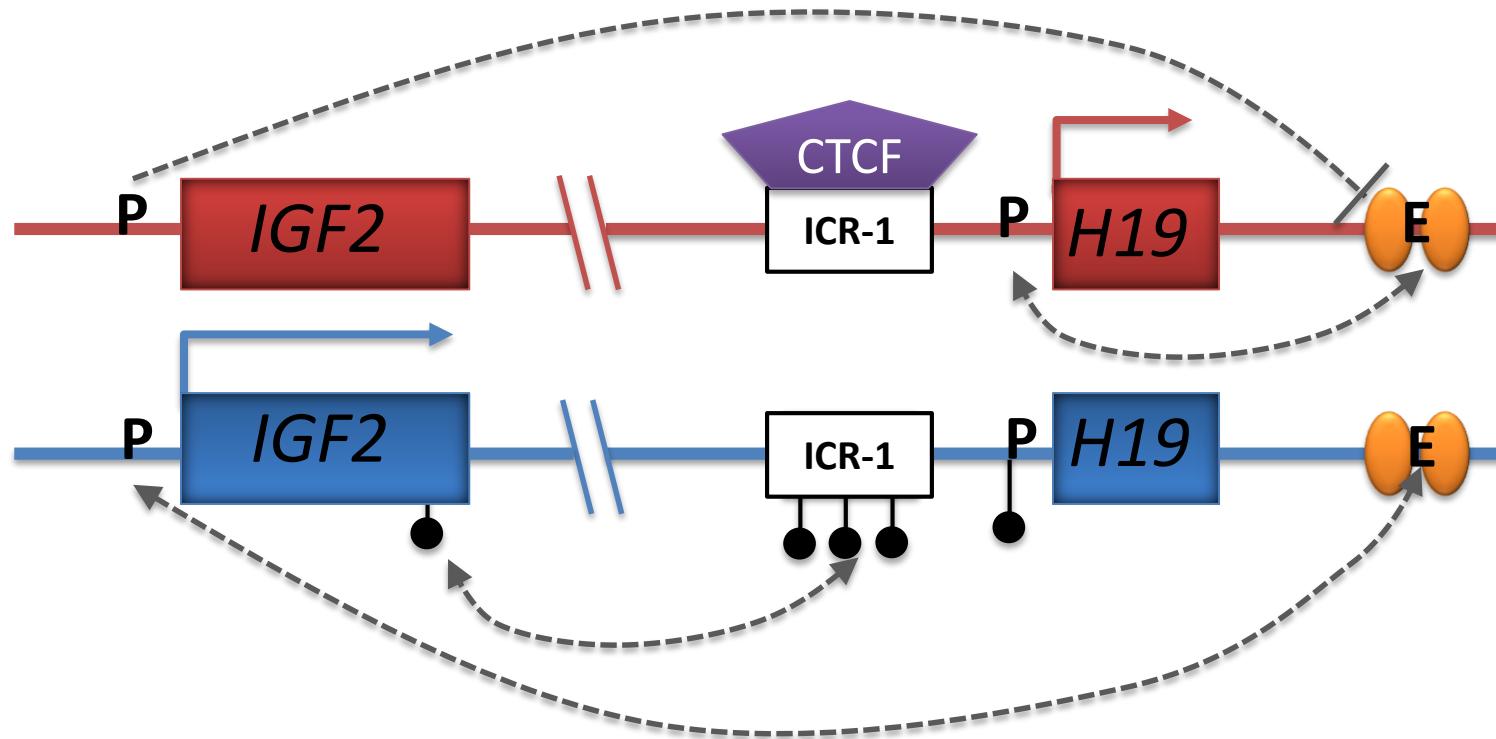


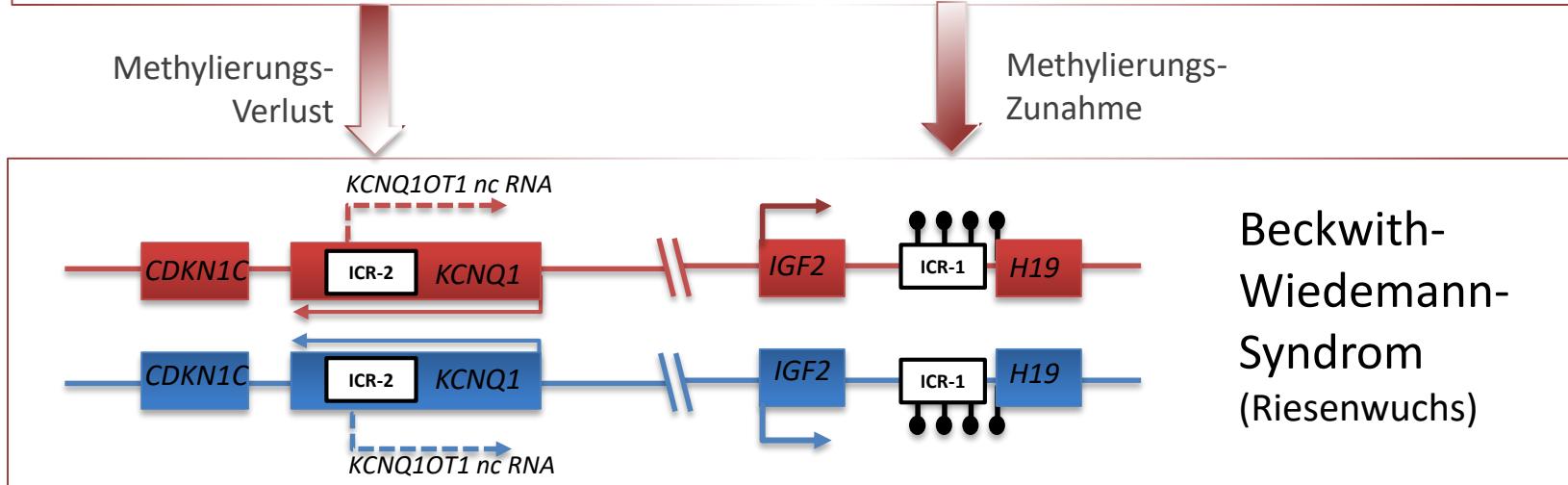
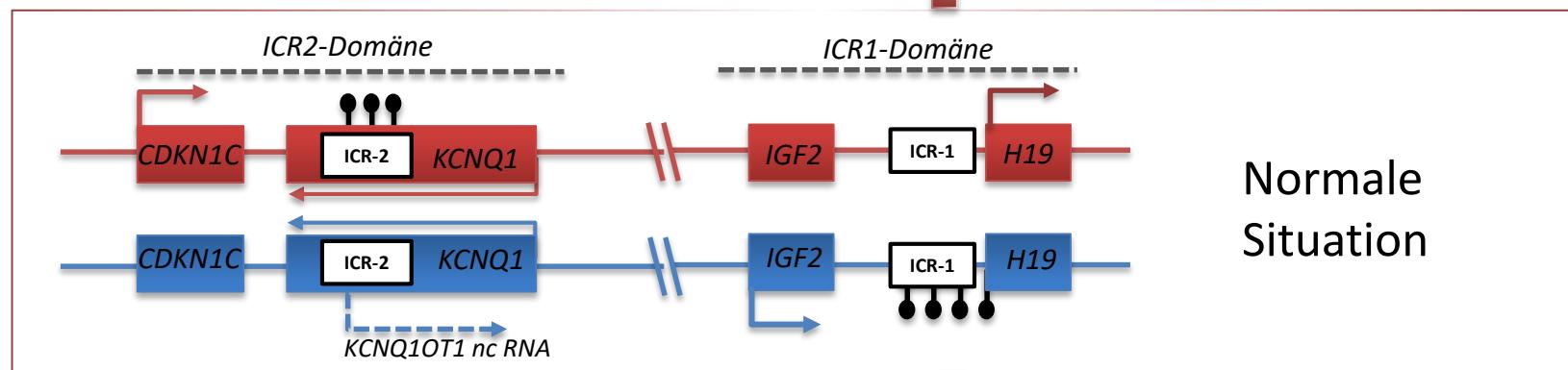
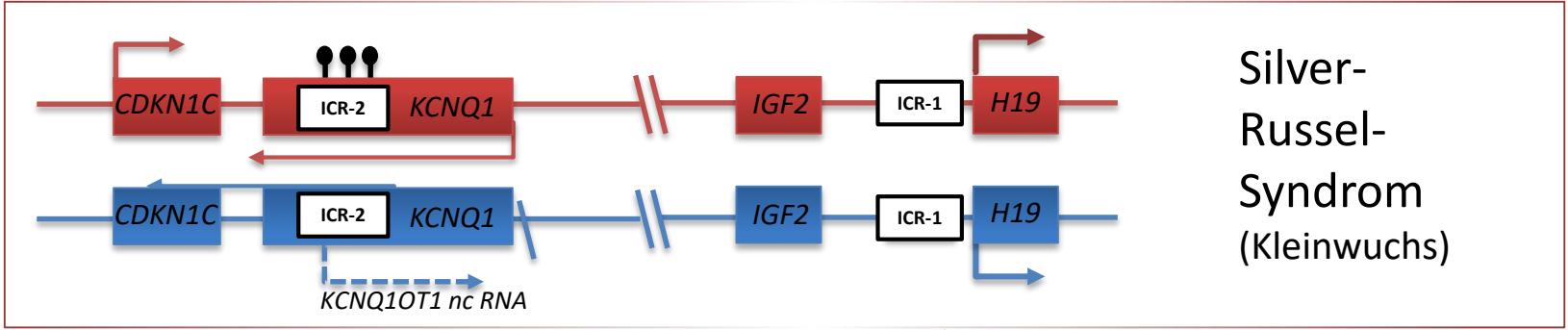
## Offspring



The graph shows as an example the fate of a paternal imprint

# Genomic Imprinting: Example of imprinted gene regulation





# Genomic Imprinting

Genomic imprints are important to balance the gene dosis of certain developmental genes.

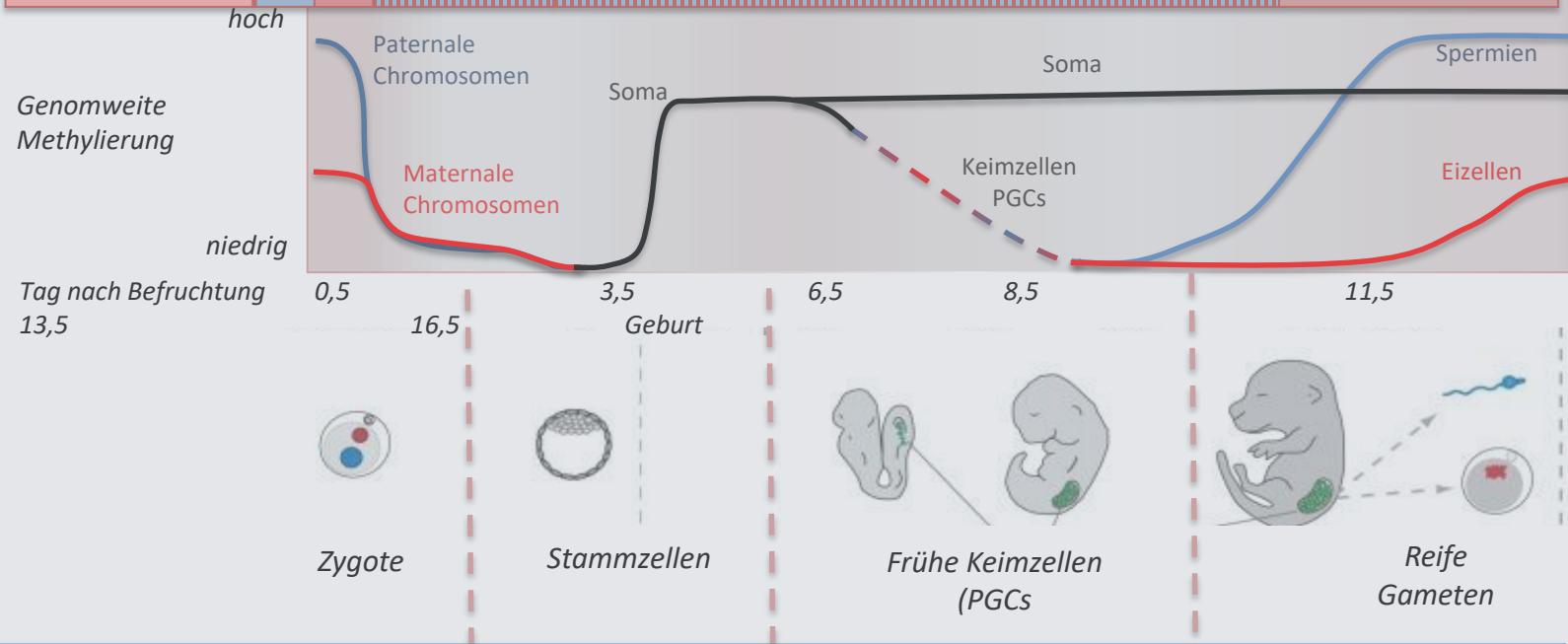
A loss or gain of imprints on the „wrong“ chromosome results in an unbalanced state and causes developmental diseases (Beckwith-Wiedemann Syndrome and Prader Willi Syndrome).

DNA-methylation imprints „survive“ the genome demethylation in the early embryo and are maintained throughout development – they are only erased in germ cells (of the next generation).

X-chromosomes were reset (demethylated) in the germ line and stayed unmethylated during early embryogenesis (i.e. both –chromosomes are active in early females!!)

X-chromosomes become epigenetically modified in females at the time of implantation i.e. during day 6-7 of embryonic development in all somatic cells (also the early PGCs)

Histonmodifikationen	Zygote	Früher Embryo	Soma	Frühe Keimzellen	Späte Keimzellen (Eizellen)	
H3K4me3	+	+	++	+	++	++
H3K27me3	-	+	++	+	+/-	+
H3K9me3	-	+	+	++	-	++
H3Ac	+	+	++	++	++	++



## DNA-Methyltransferasen

Dnmt3a	+	+	+/-	++
Dnmt3b	+	+	+/-	-
Dnmt3l	+	+	-	++
Dnmt1	++	++	-	-
Tet1-3	+++		++	?

# X-chromosome inactivation

Mammalian females have two X-chromosomes males only one.

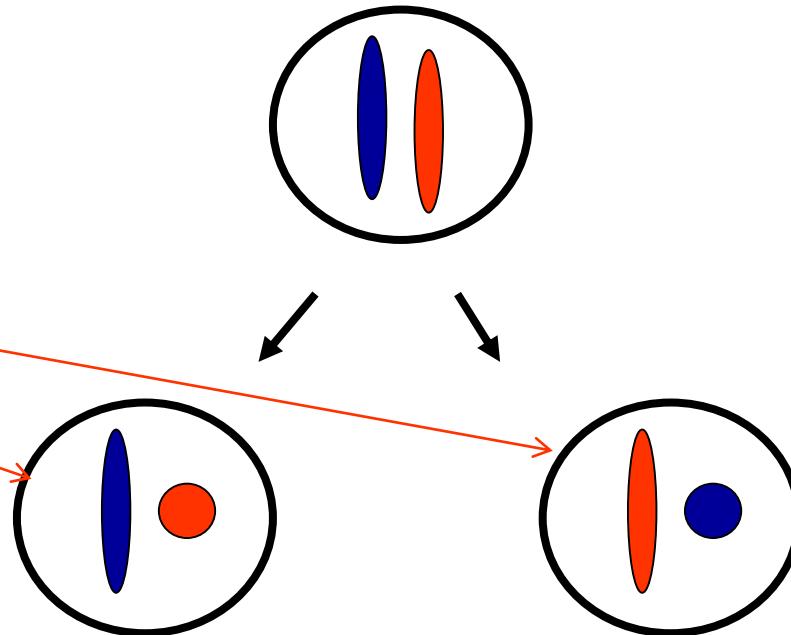
One of the female X-chromosomes is epigenetically silenced (Barr body) – i.e. almost all genes of this chromosome are transcriptionally silenced by repressive histone marks and DNA-methylation

This „inactivation“ depends on the XIST non coding RNA. Xist is the only gene transcribed from the inactive X-chromosome.

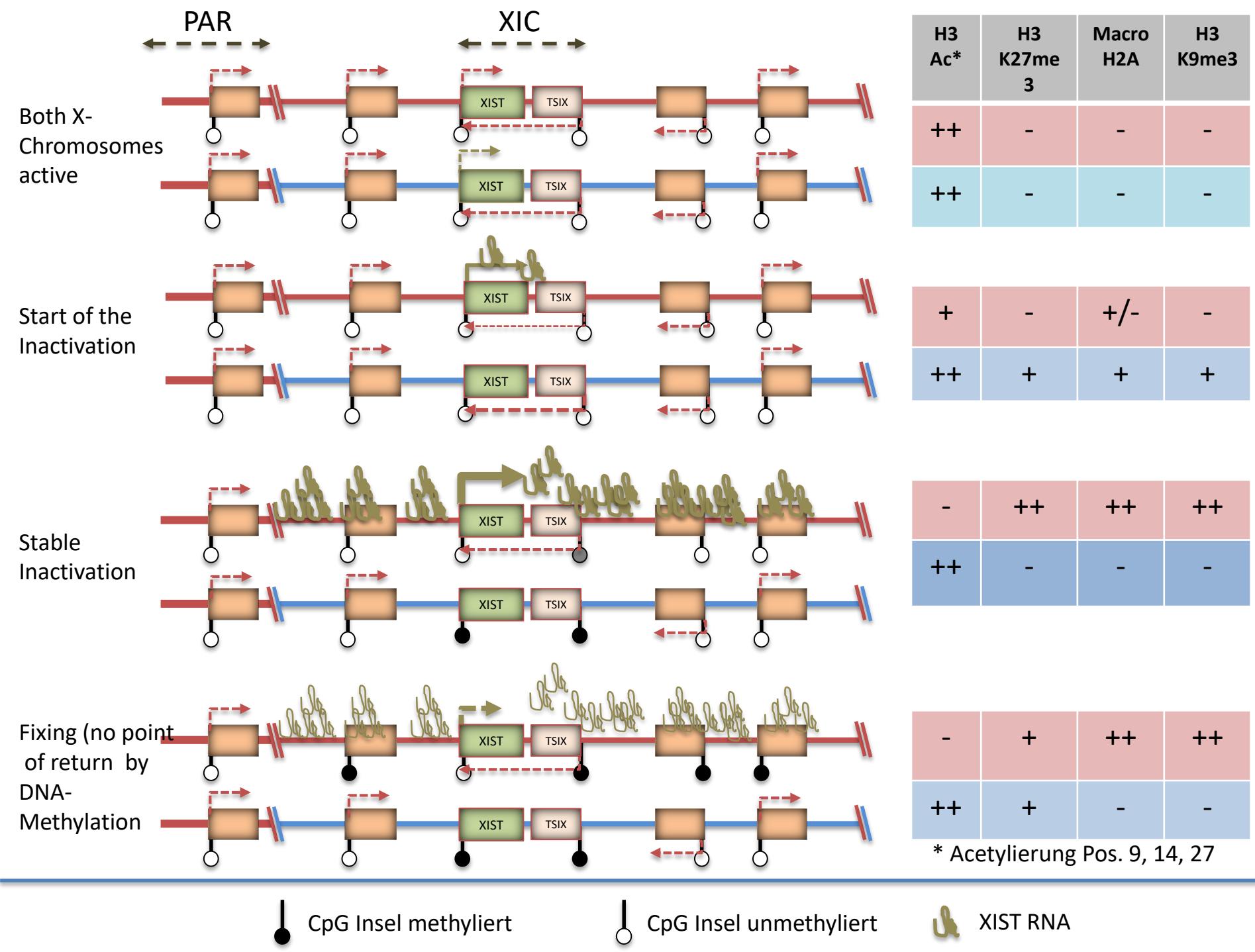
The inactivation starts at around d6-7 of embryonic development in all somatic cells.

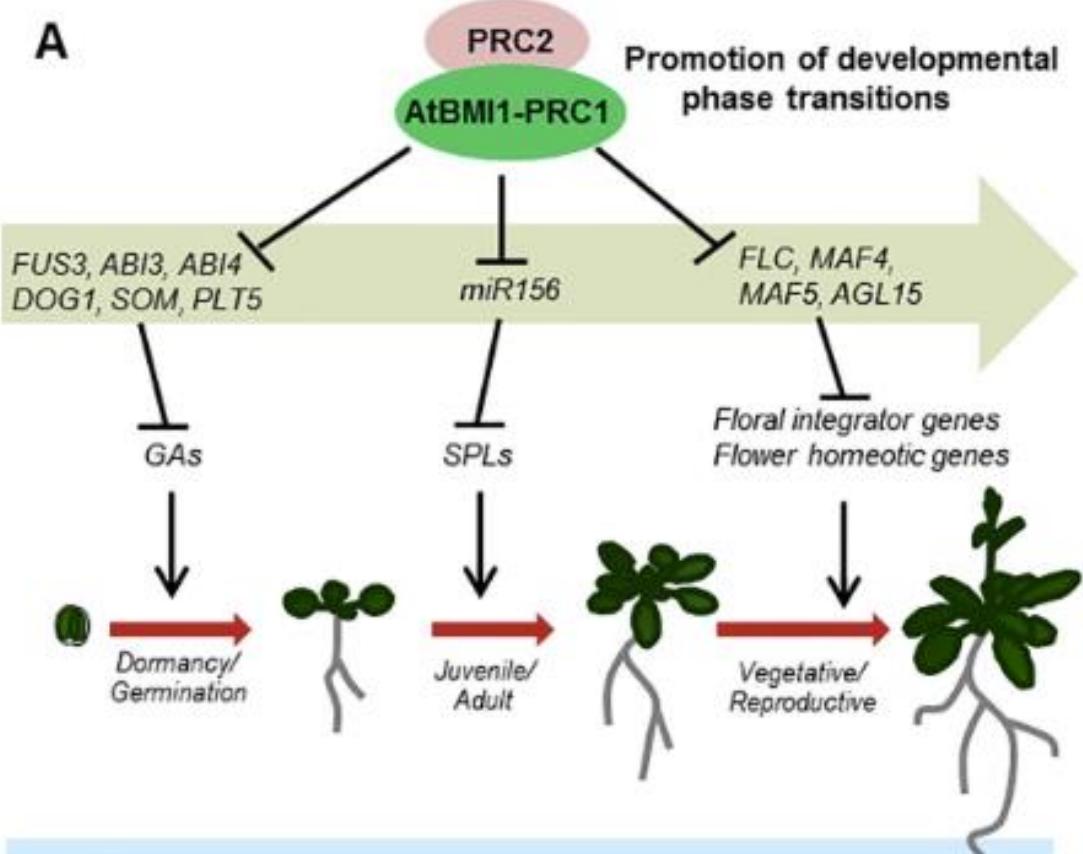
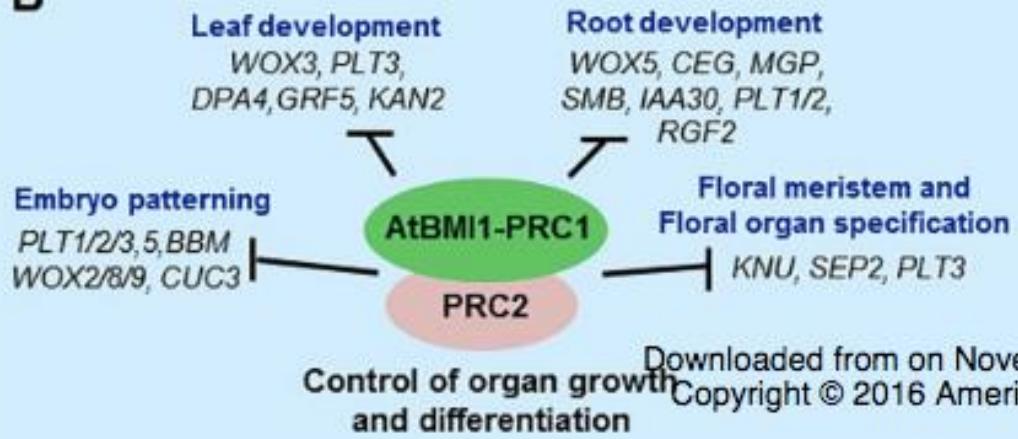
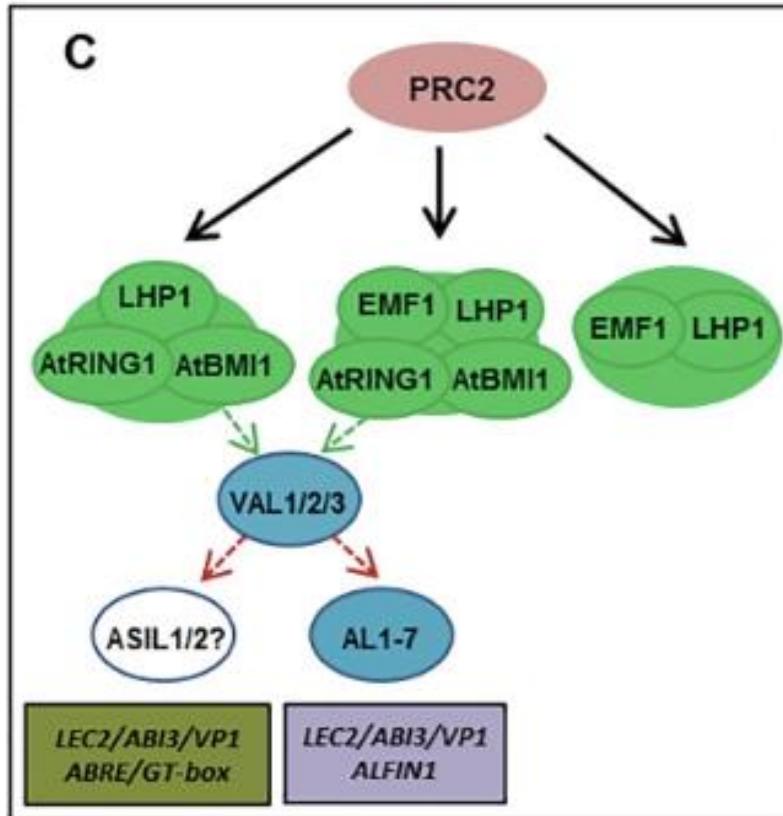
Once one X-chromosomes became silenced – there is a random choice which of them is chosen - the inactive status is inherited to all progenitor cells of this cell. This causes and X-chromosome „mosaicism“ in females.

## X-Chromosomen Inaktivierung: Zufällige Auswahl (choice) und Beibehaltung (maintenance)



Während der Entwicklung wird im Embryo festgelegt, welches X ( $X_m$  oder  $X_p$ ) in bestimmten Zellen aktiv ist und welches X-chromosome epigenetisch inaktiviert wird. In „Nachkommen“ dieser epiplastisch programmierten Zellen (= Sektoren des Fells) bleibt dieser Zustand epigenetisch stabil. Da die beiden X-Chromosomen der Calico Katzen unterschiedliche Fellfarballele (Genvarianten) tragen entsteht so eine gescheckte Fellfarbe!!!



**A****B****C**

# Epigenetic control of cell fate

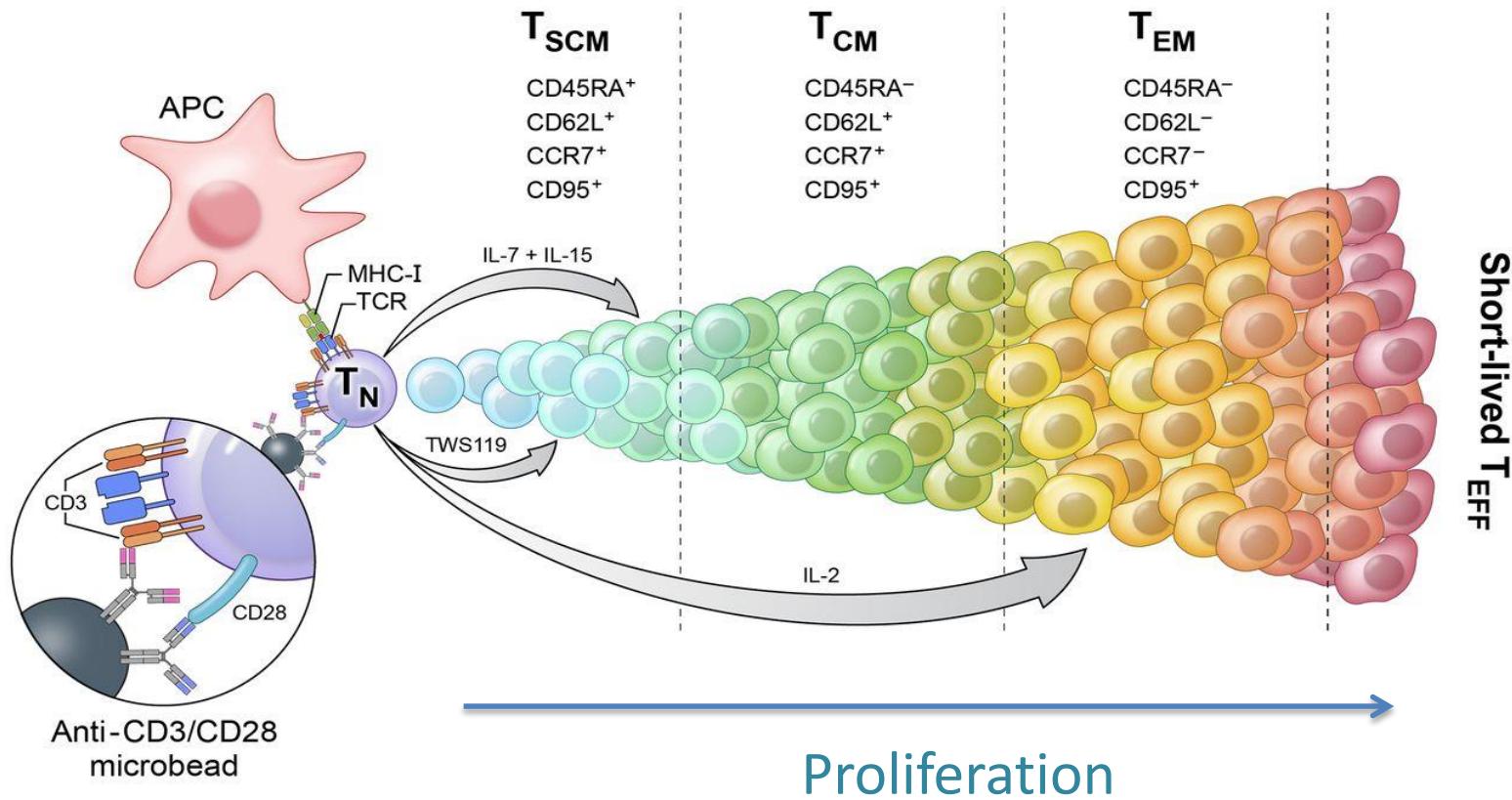
In some cell types of the body developmental changes are strictly controlled by epigenetic switches (changes in DNA-methylation or H3K27me3).

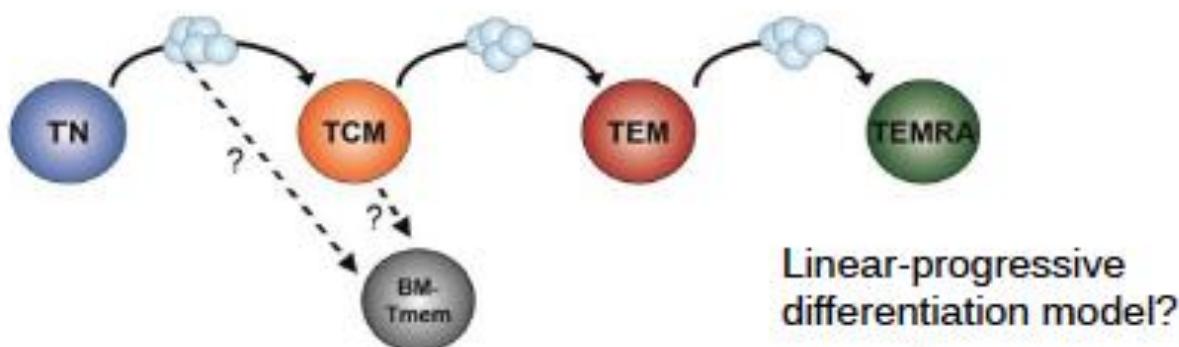
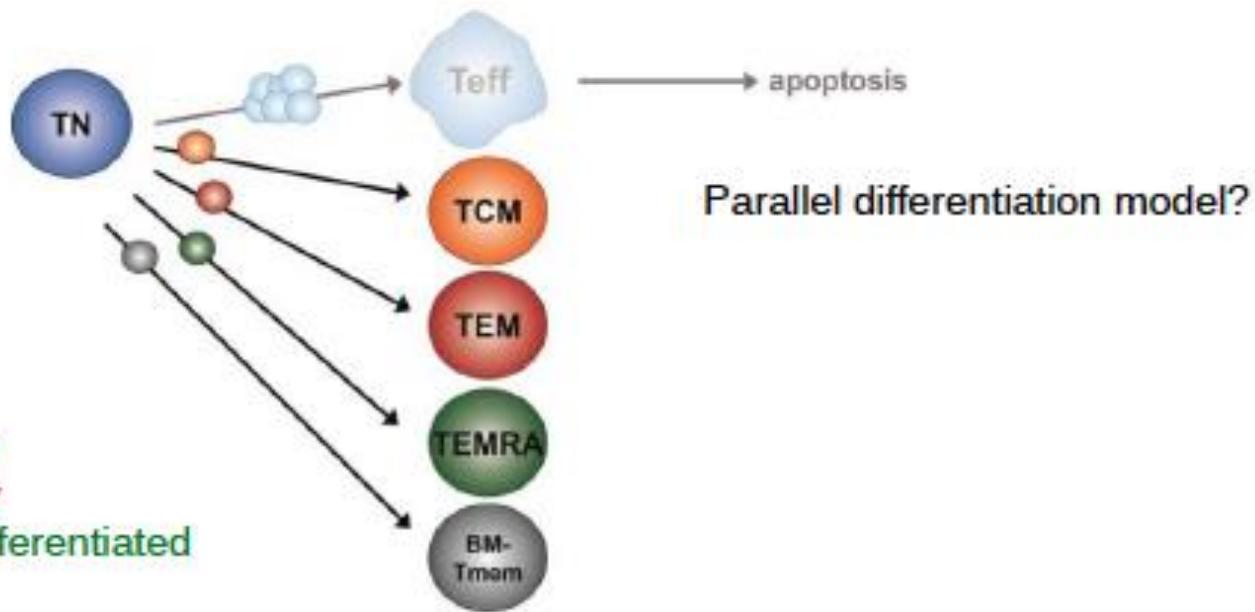
One such switch controls the activity of a key control genes FoxP3 which controls the fate of regulatory T-cells of the lymphoid lineage.

Upon differentiation of naive T-cells into mature regulatory T cells the DNA-methylation of an important regulatory element is specifically removed – the element can be bound by transcription factors.

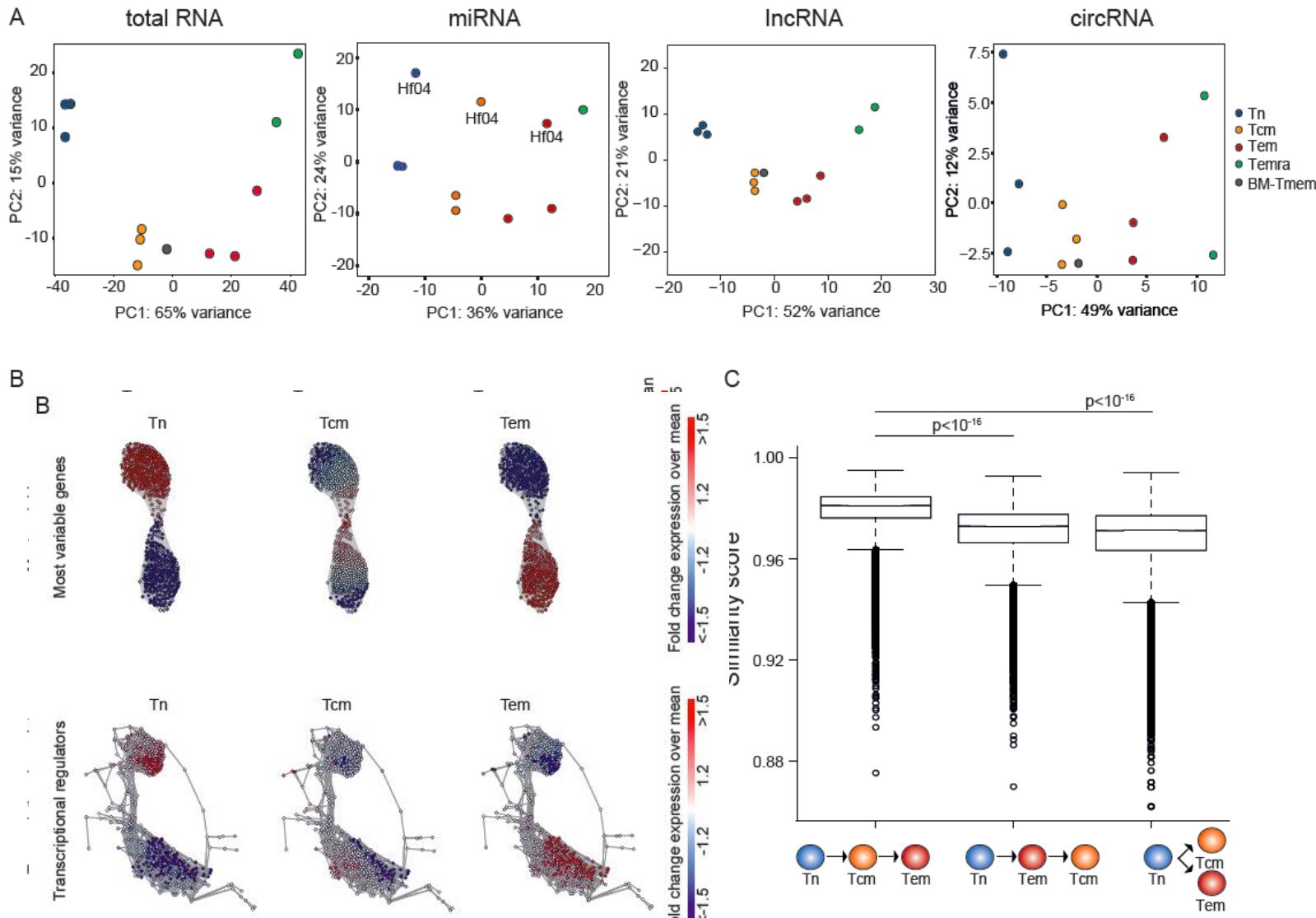
The DNA demethylation occurs by an active mechanism resembling processes of demethylation found at imprints and at the XIST promoter methylation in primordial germ cells.

# T-cell development

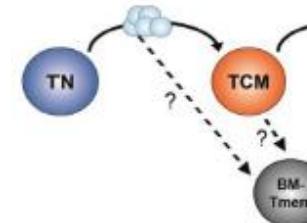
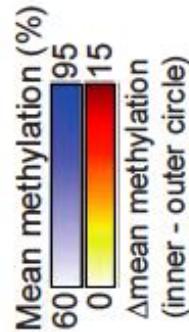
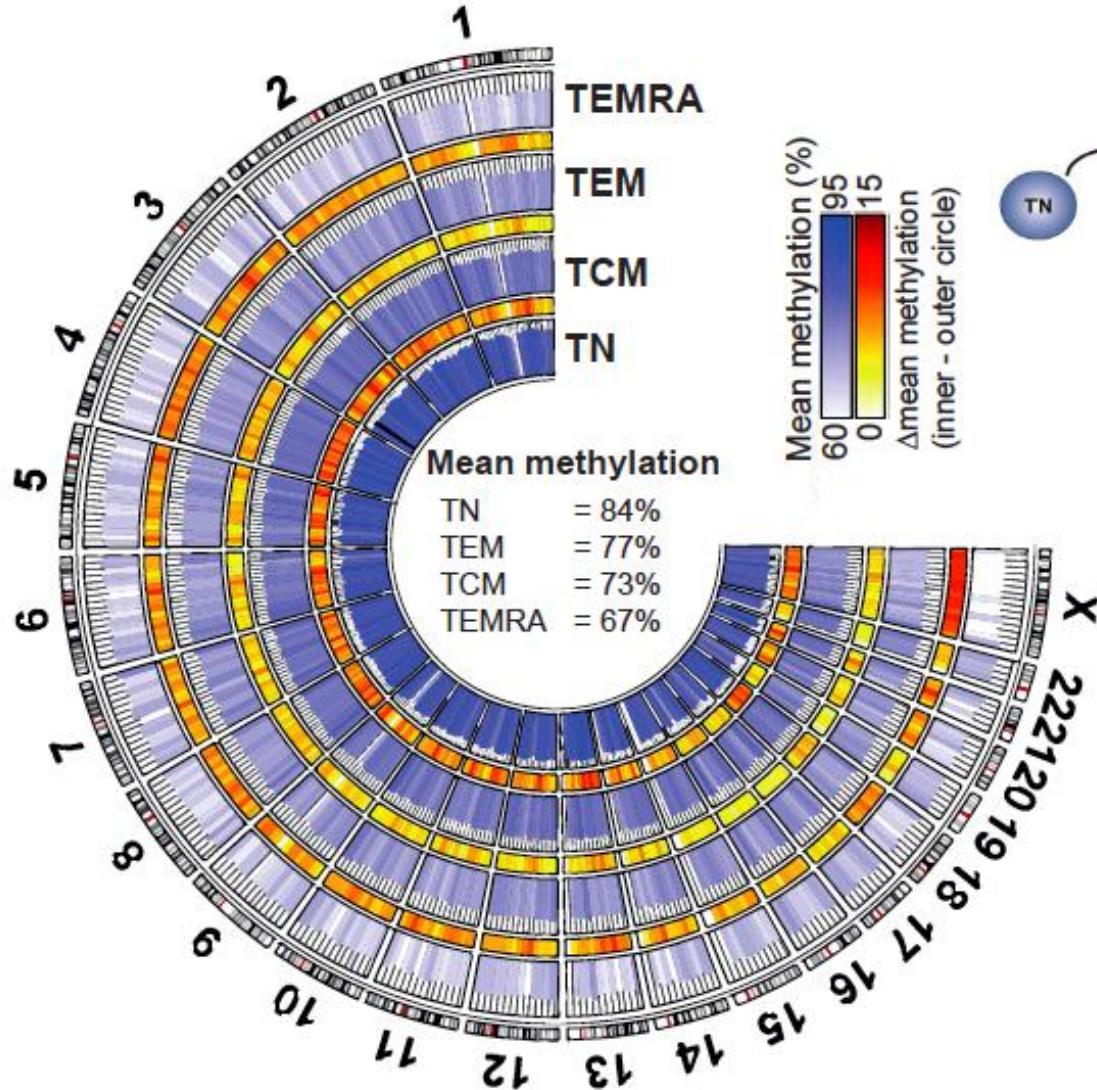




# RNA-expression and TF regulome/network predict a linear differentiation model



# Genome wide progressive loss of DNA methylation support a linear relationship of memory T-cells

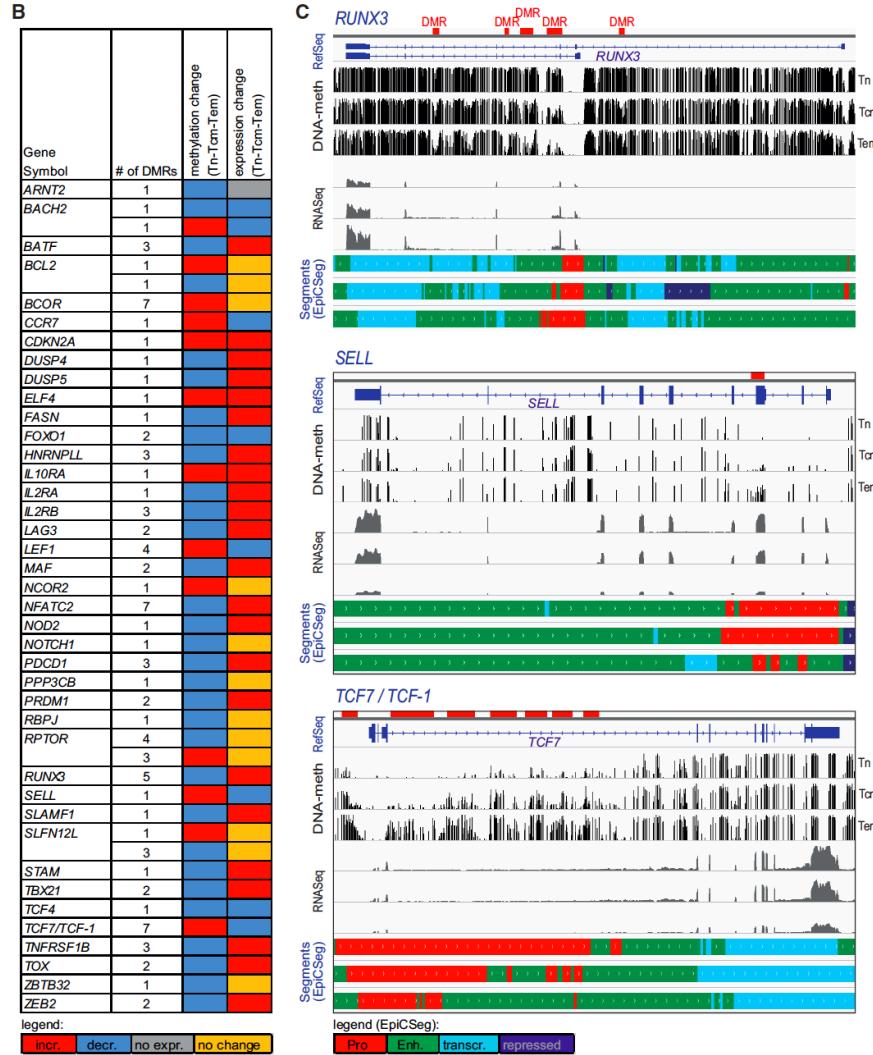


? ? ?

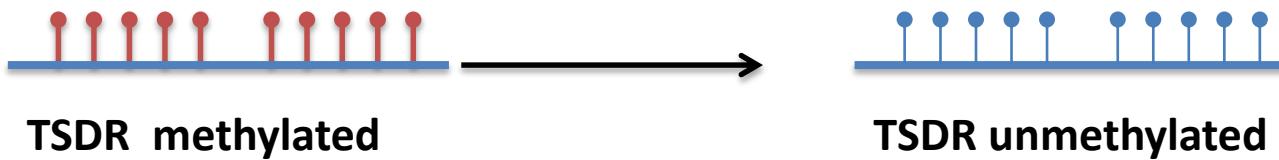
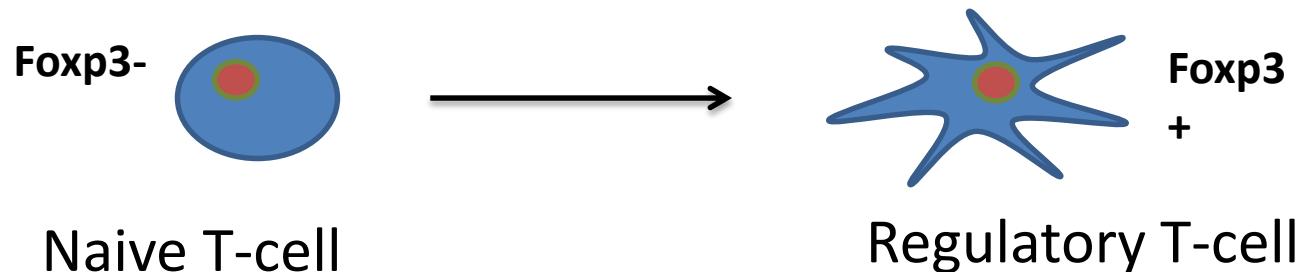
TN → TCM → TEM → TEMRA

Linear-progressive differentiation model?

# Key transcription factors controlling memory T-cells show DNA-methylation changes upon differentiation

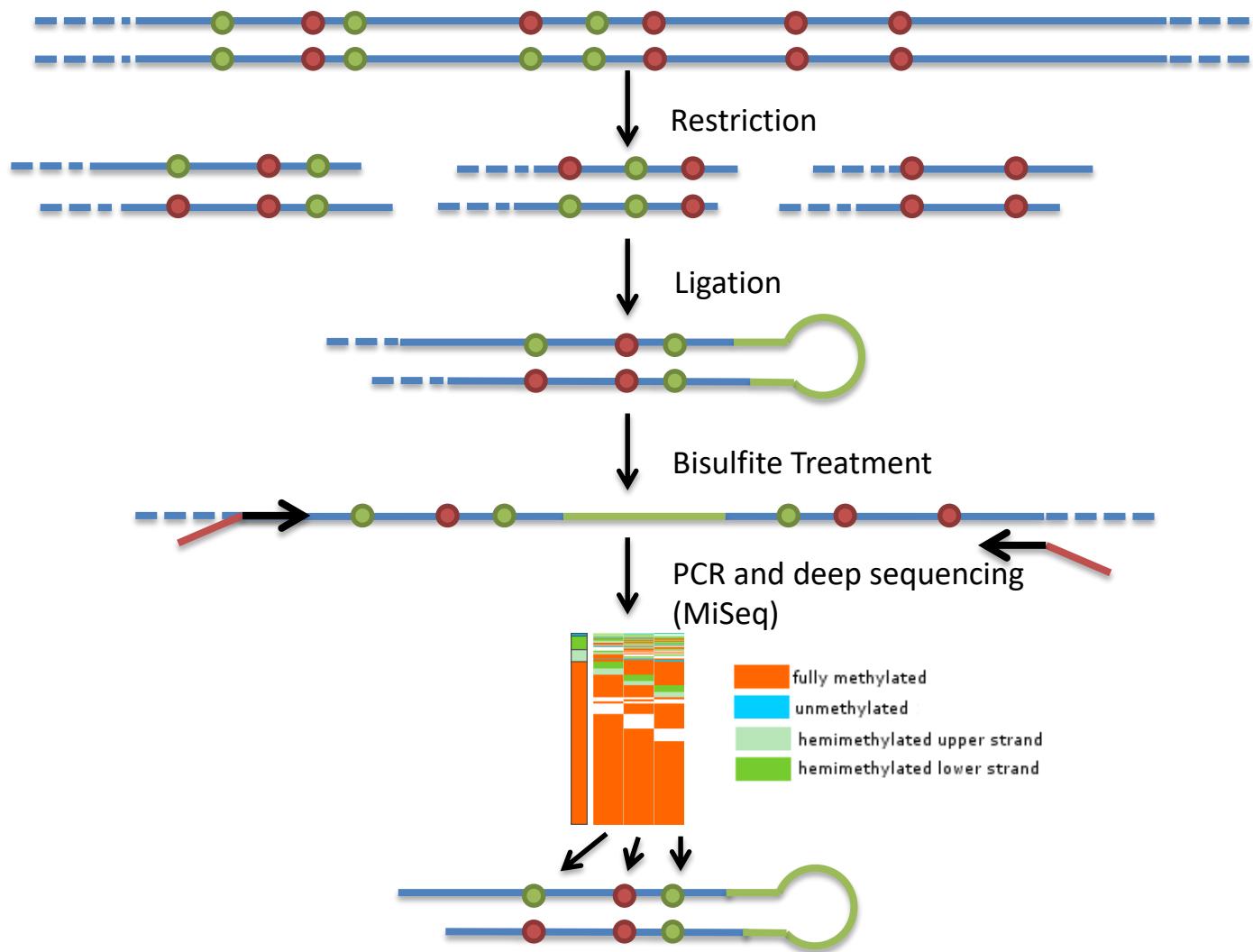


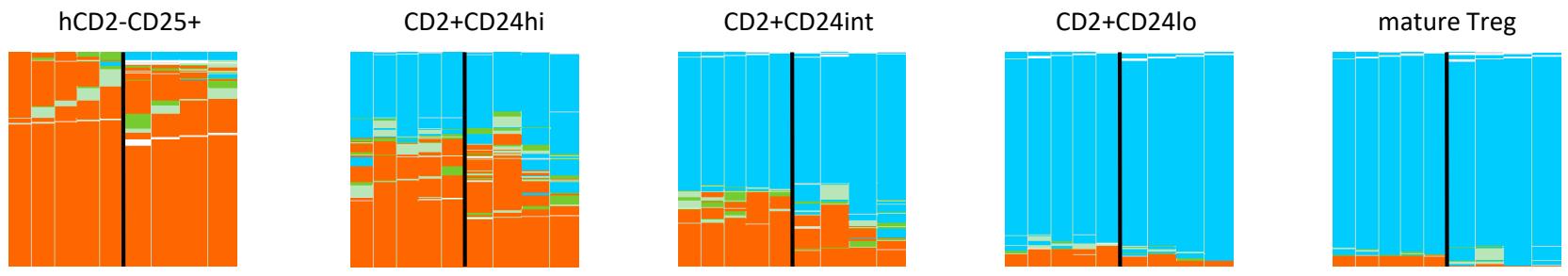
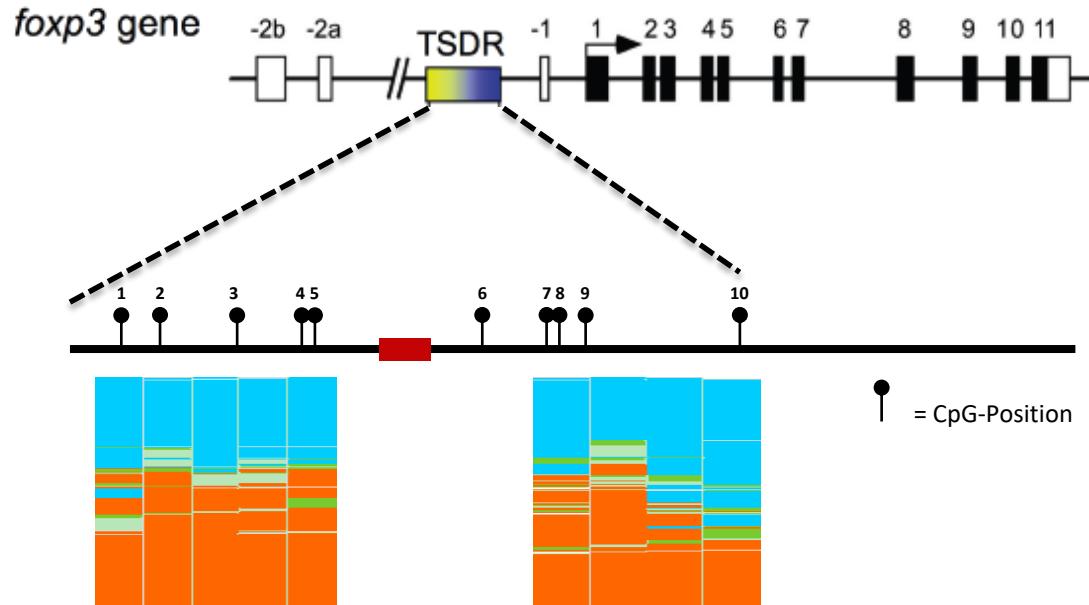
# FoxP3 expression control in regulatory T-cells (Tregs)



No cell division !!!

# Hairpin-Bisulfite-Sequencing to resolve the status of DNA-methylation on both DNA-strands



**A**

CD4<sup>+</sup> naive T-cells  
No FoxP3 expression



Maturing Tregs  
No cell division

- fully methylated
- unmethylated
- hemimethylated upper strand
- hemimethylated lower strand