CHAIRE ÉPIGÉNÉTIQUE ET MÉMOIRE CELLULAIRE

Année 2013-2014 :

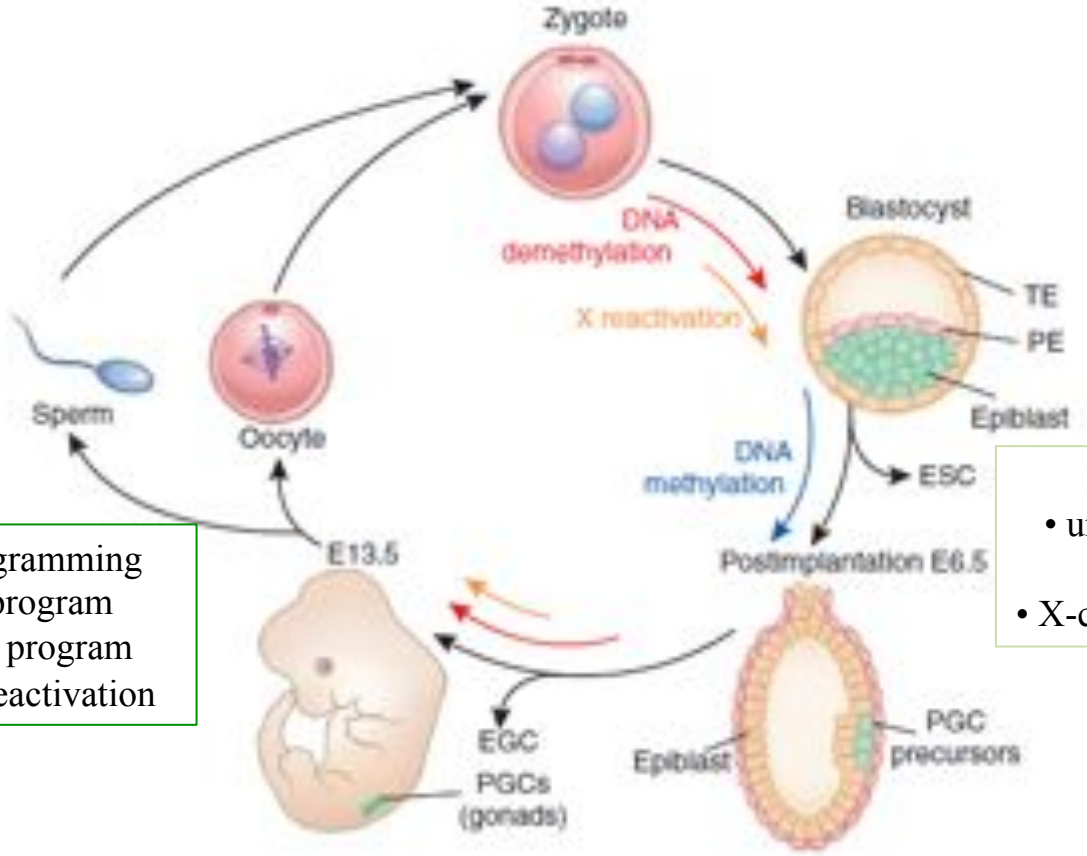
“**Reprogrammations développementales, induites et pathologiques** ”

<u>Cours IV</u>

« Mécanismes moléculaires au cours de la reprogrammation »

Developmental and Experimental Reprogramming

Zygotic Reprogramming
• undo gamete programs
• set up totipotency

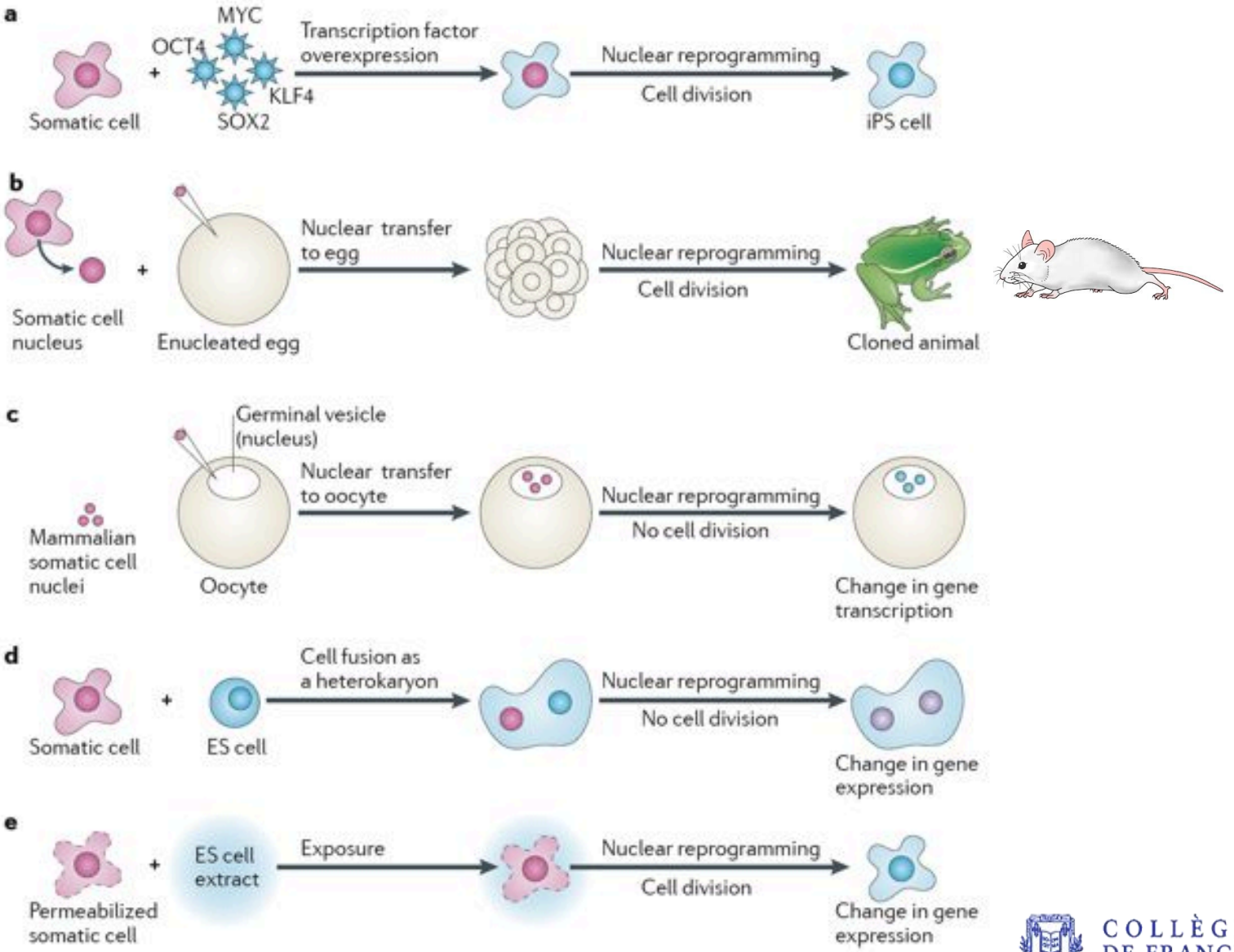


Germ Line Reprogramming
• undo somatic program
• set up germ line program
• X-chromosome reactivation

ICM Reprogramming
• undo/prevent TE program
• set up pluripotency
• X-chromosome reactivation

Adapted from Cantone and Fisher; 2013

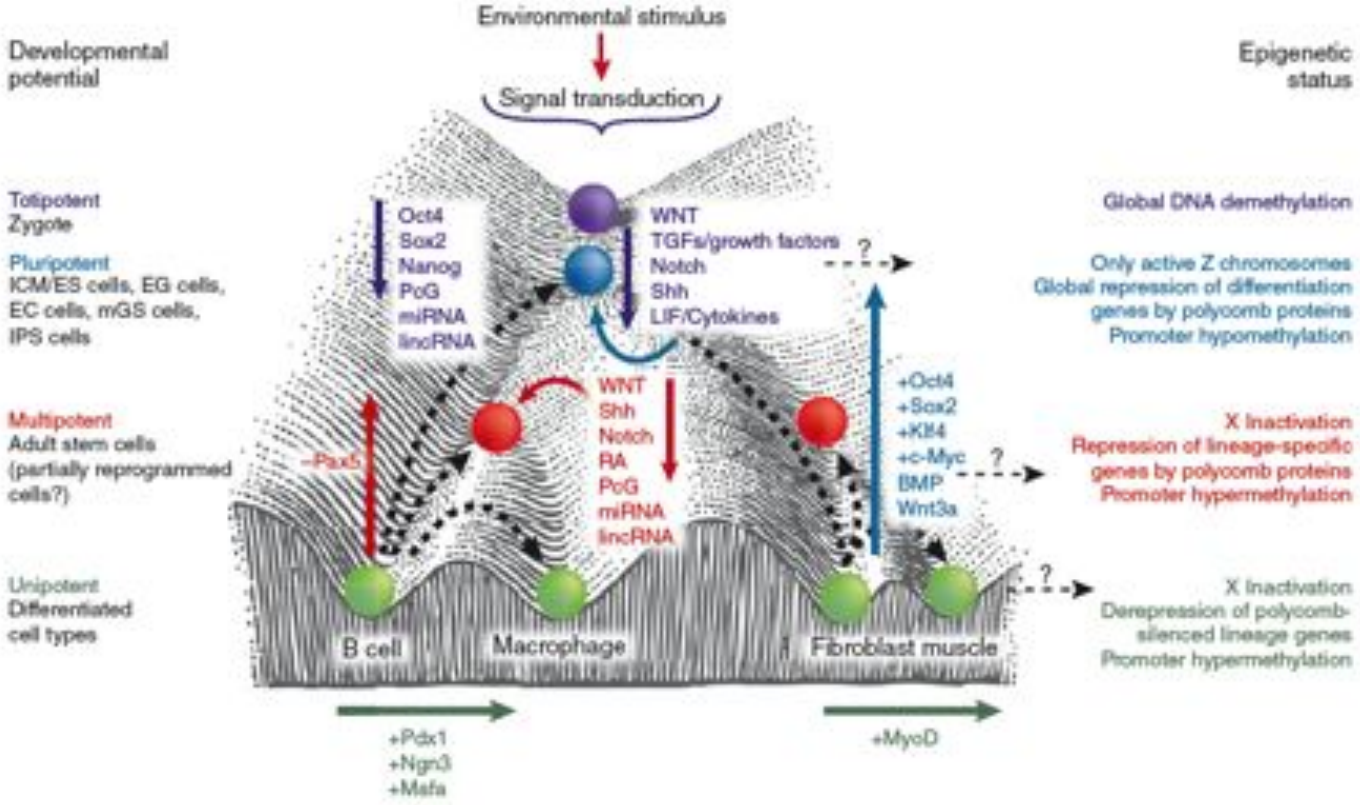
Developmental and Experimental Reprogramming



Jullien et al, 2011

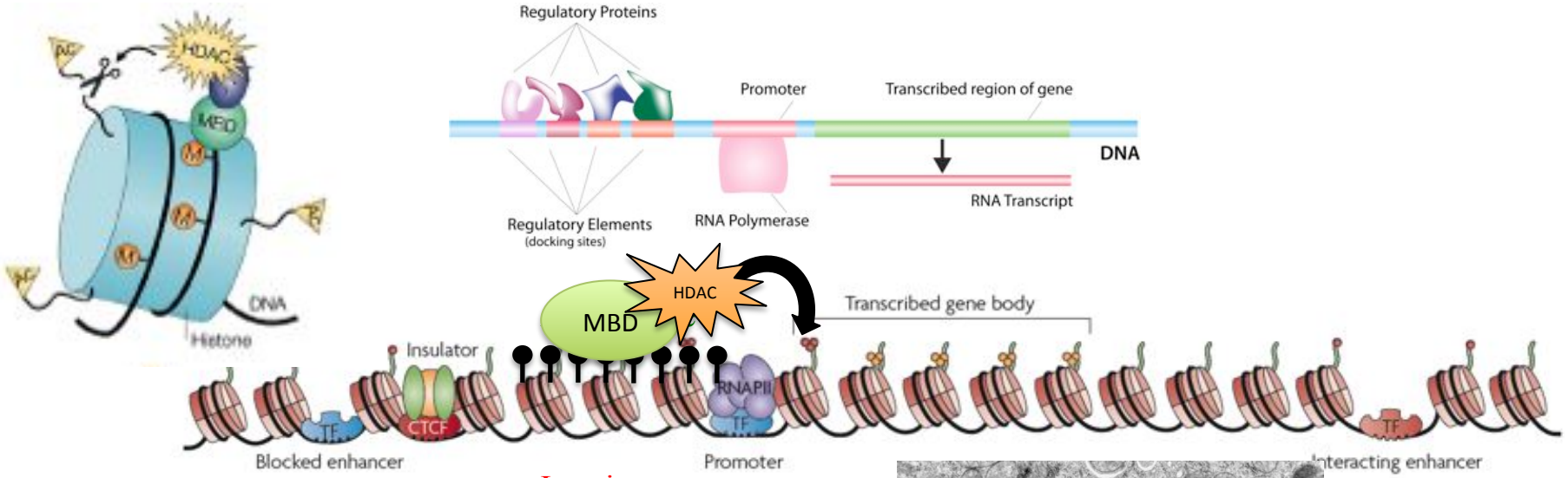
Developmental and Experimental Reprogramming

What are the mechanisms?
What are the signals and what are the barriers?



Adapted from Plath and Hochedlinger, 2009

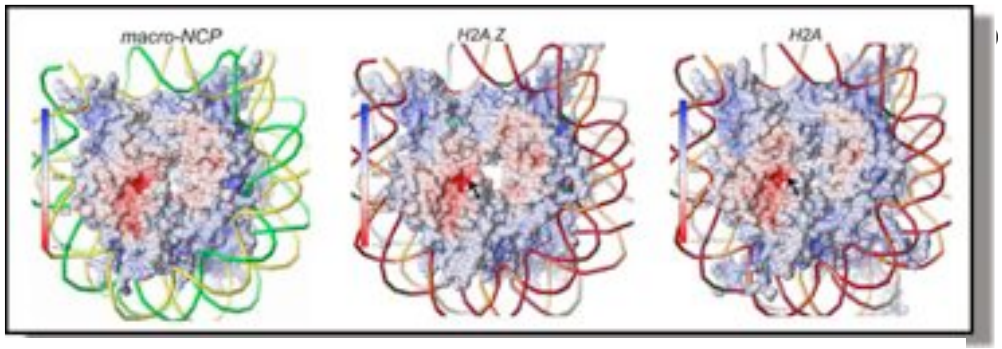
Gene regulation in the context of chromatin



Inactive gene

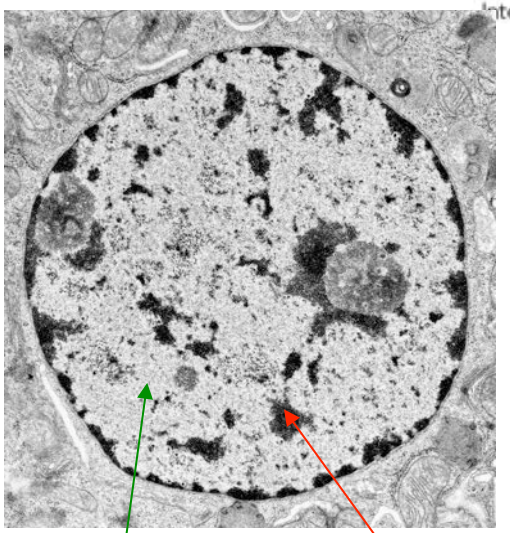
Repressive chromatin

DNA methylation



Multiple histone variants

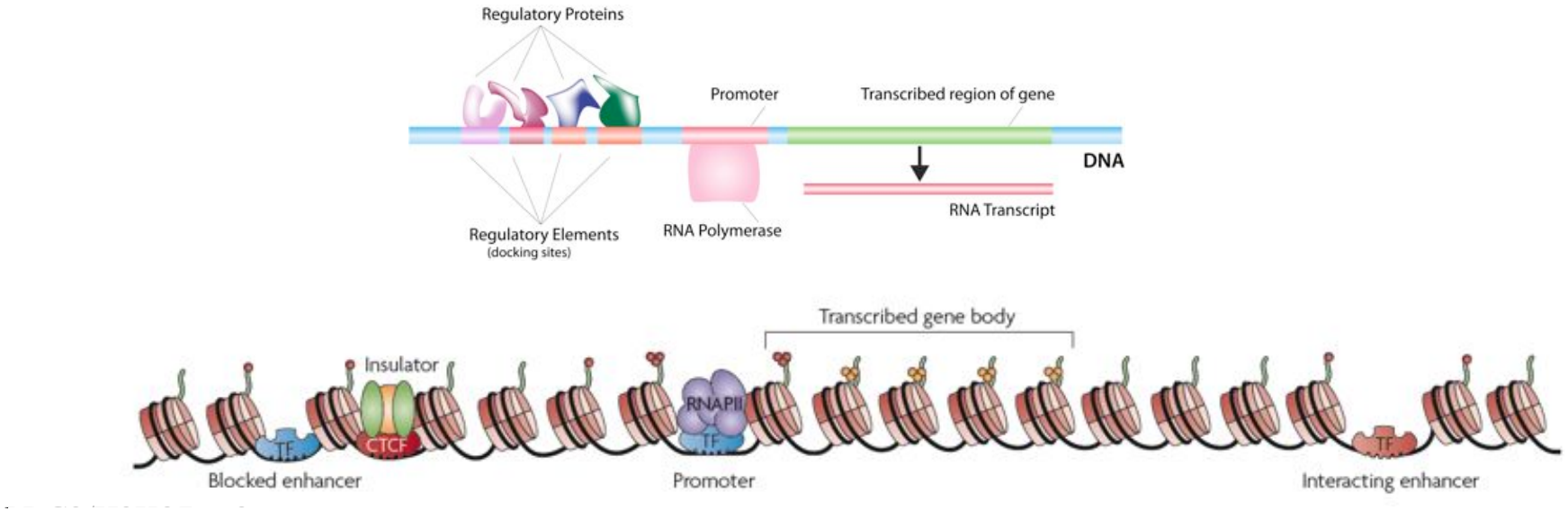
=> different structures, timing, distributions



Euchromatin Heterochromatin

http://medcell.med.yale.edu/histology/

Gene regulation in the context of chromatin

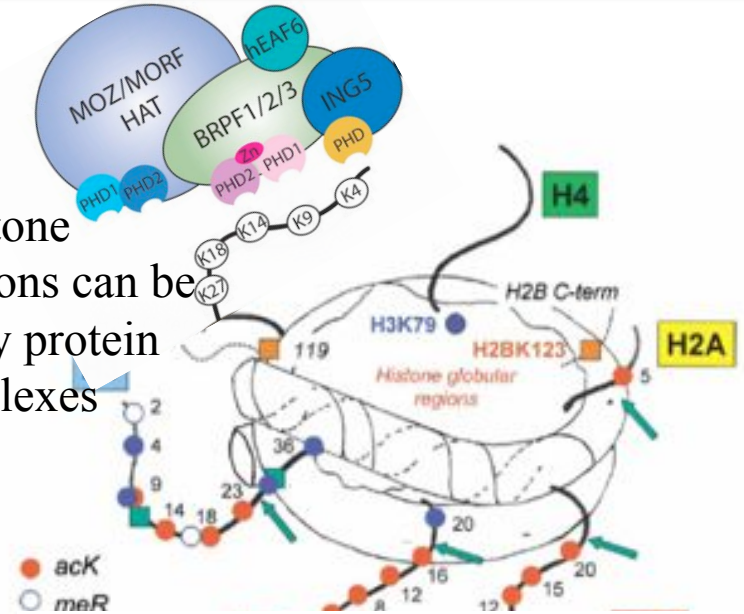


Chromatin

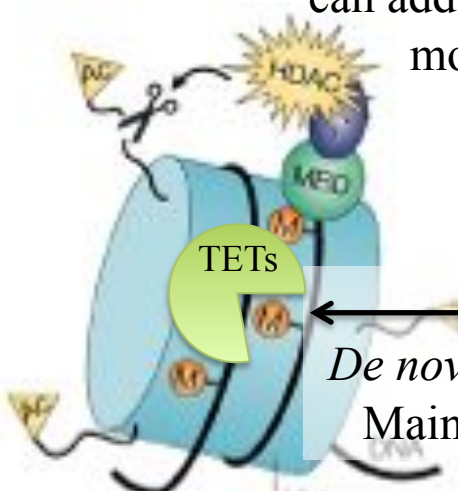
- is a *facilitator* of DNA-based processes such as transcription
  - can enable *propagation* of active or inactive states
- can act as a *epigenetic barrier* against changing gene activity states and thus *preserve cell identity*

Reversing chromatin states, overcoming epigenetic barriers?

Histone modifications can be bound by protein complexes



Histone modifying enzymes can add or remove these modifications



Dnmts

De novo: Dnmt3a,3b, 3L

Maintenance: Dnmt1

Passive mechanisms

During cell division / DNA replication

Remove chromatin factors and/or partners involved in self-perpetuation

eg Dnmt1/5meC; HP1/H3K9me3/Suv39; PRC2/H3K27me3;

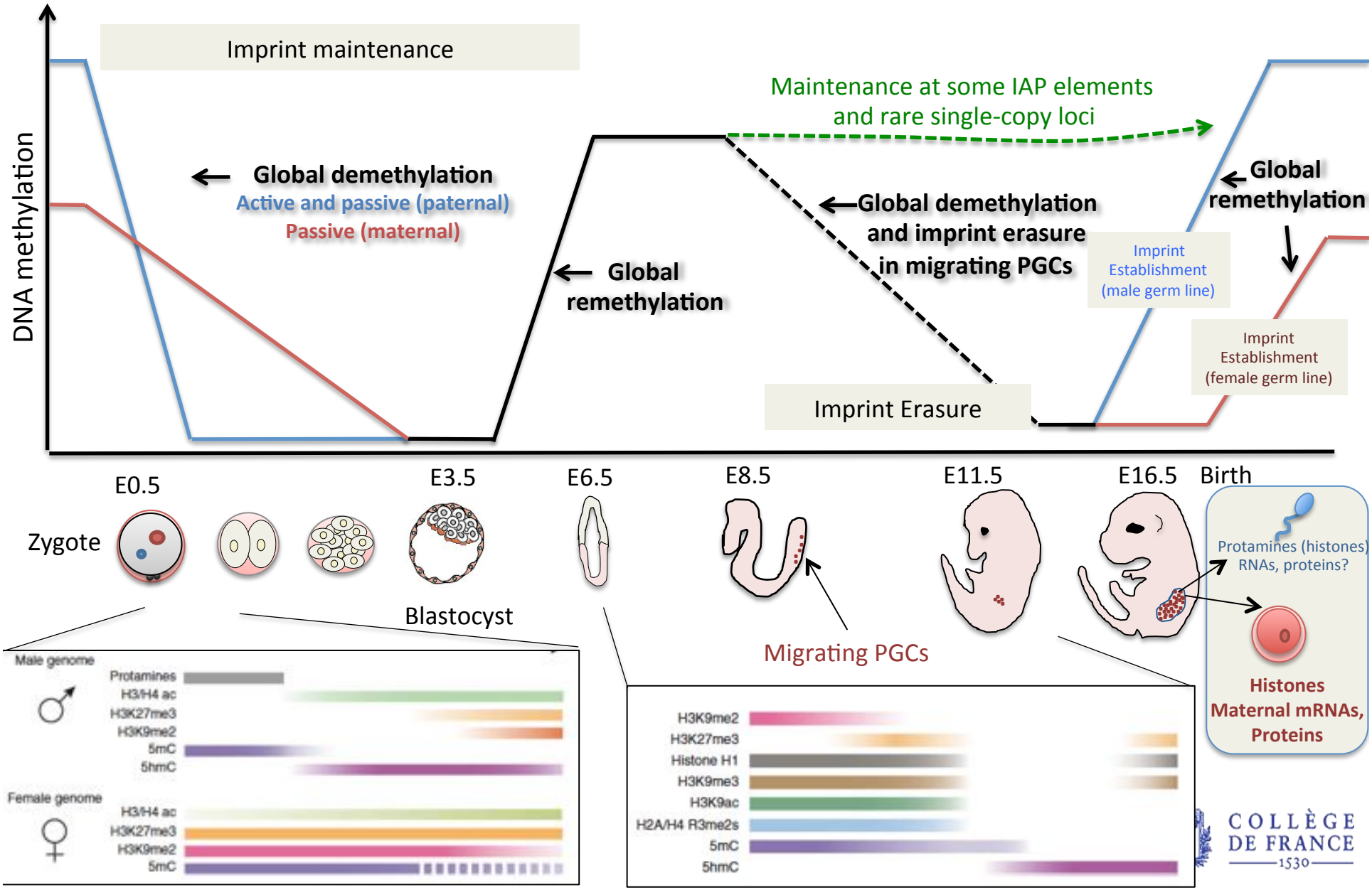
G9a/Glp /H3K9me2; Uhrf1/2

Active mechanisms

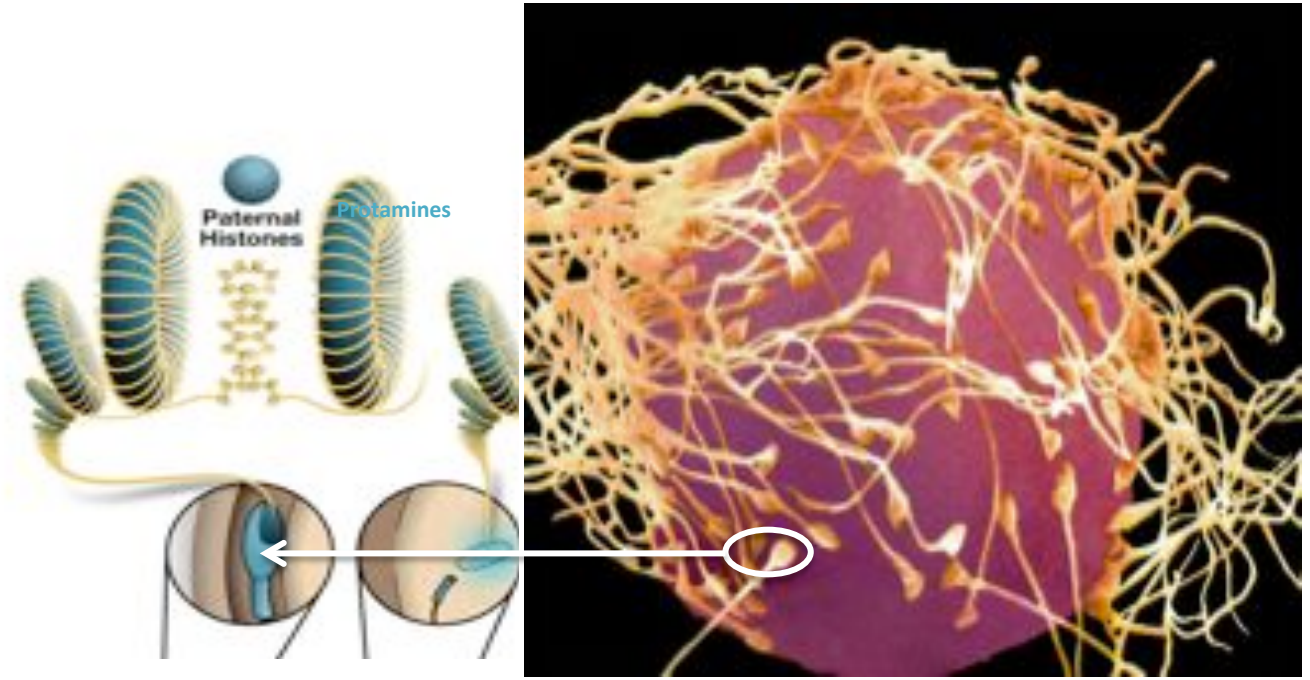
Chromatin remodelling, Histone exchange

Expression of factors that reverse chromatin states (eg TET enzymes: 5meC->5hmeC, histone demethylases or deacetylases)

Epigenetic reprogramming in mammals



Epigenetic Dynamics following Fertilization



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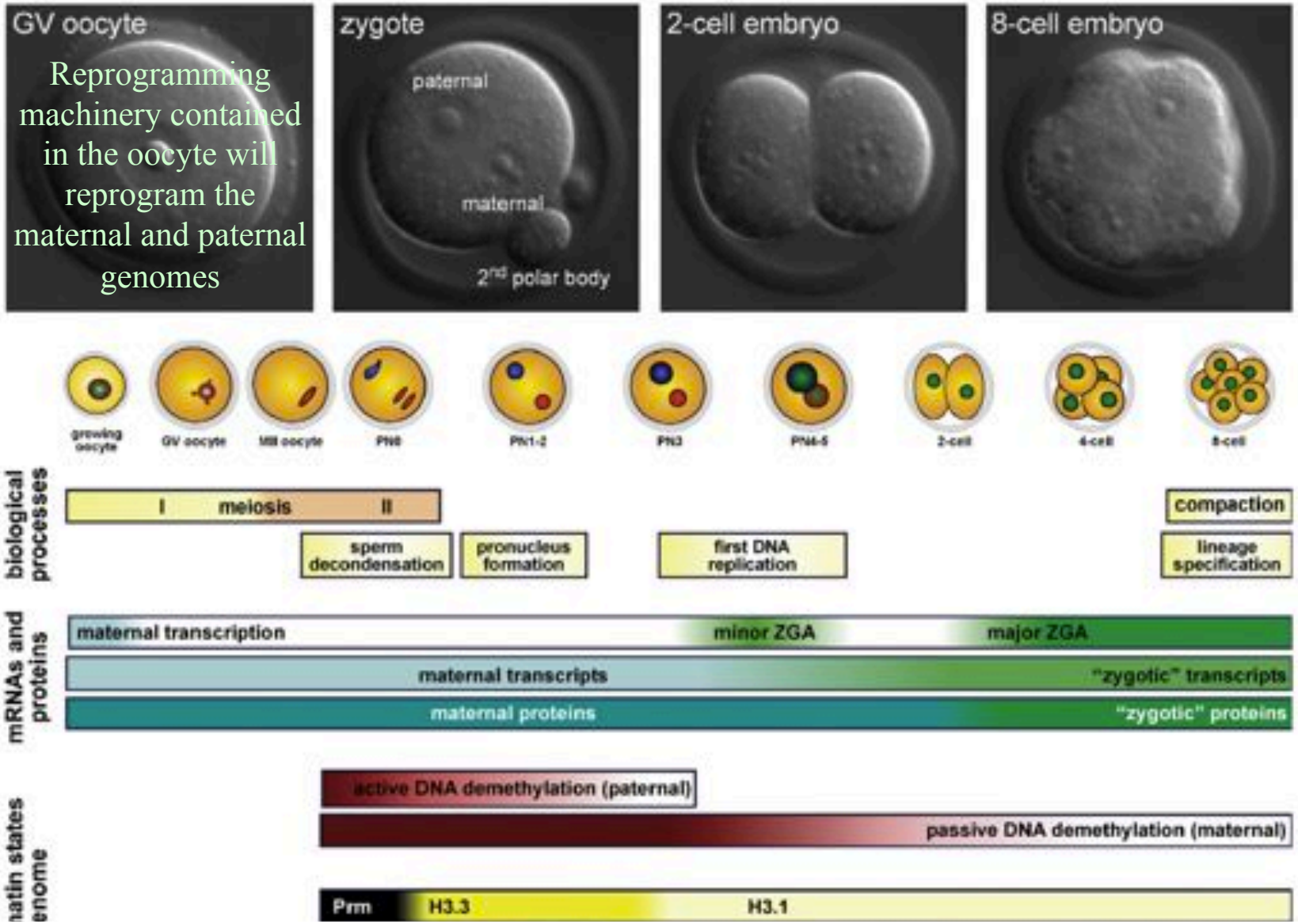
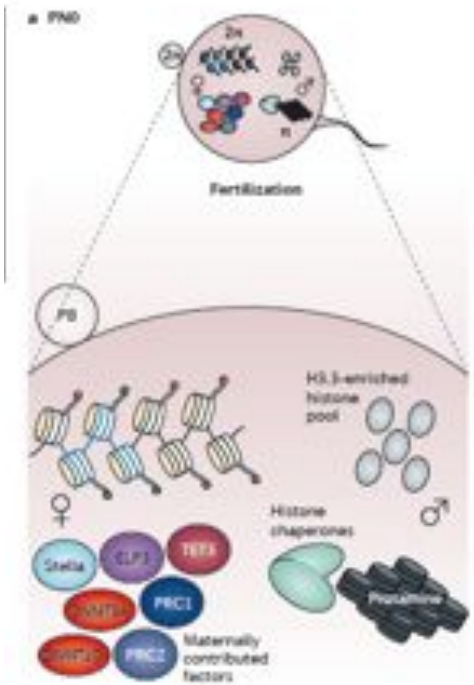


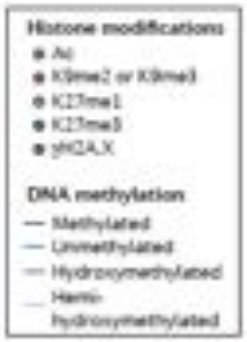
Epigenetic Dynamics in the Zygote



Reprogramming requirements in the zygote:

• Protamine histone exchange requires maternal chromatin remodellers, histones and chaperones:

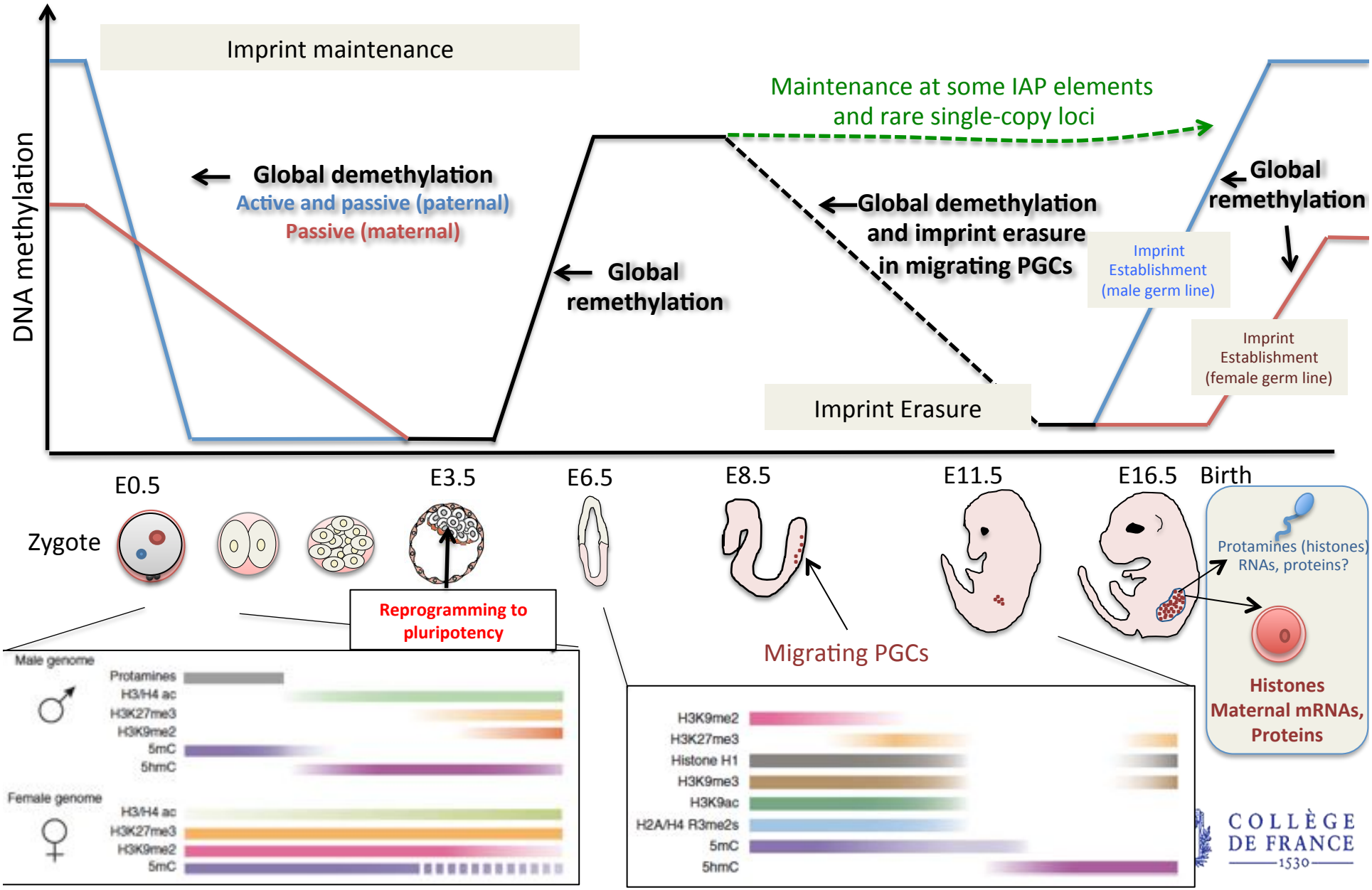
- histone variant H3.3 (van der Heijden et al., 2005)
- histone H3K4me3 (Torres-Padilla et al., 2006)
- DNA demethylation (Mayer et al., 2000).



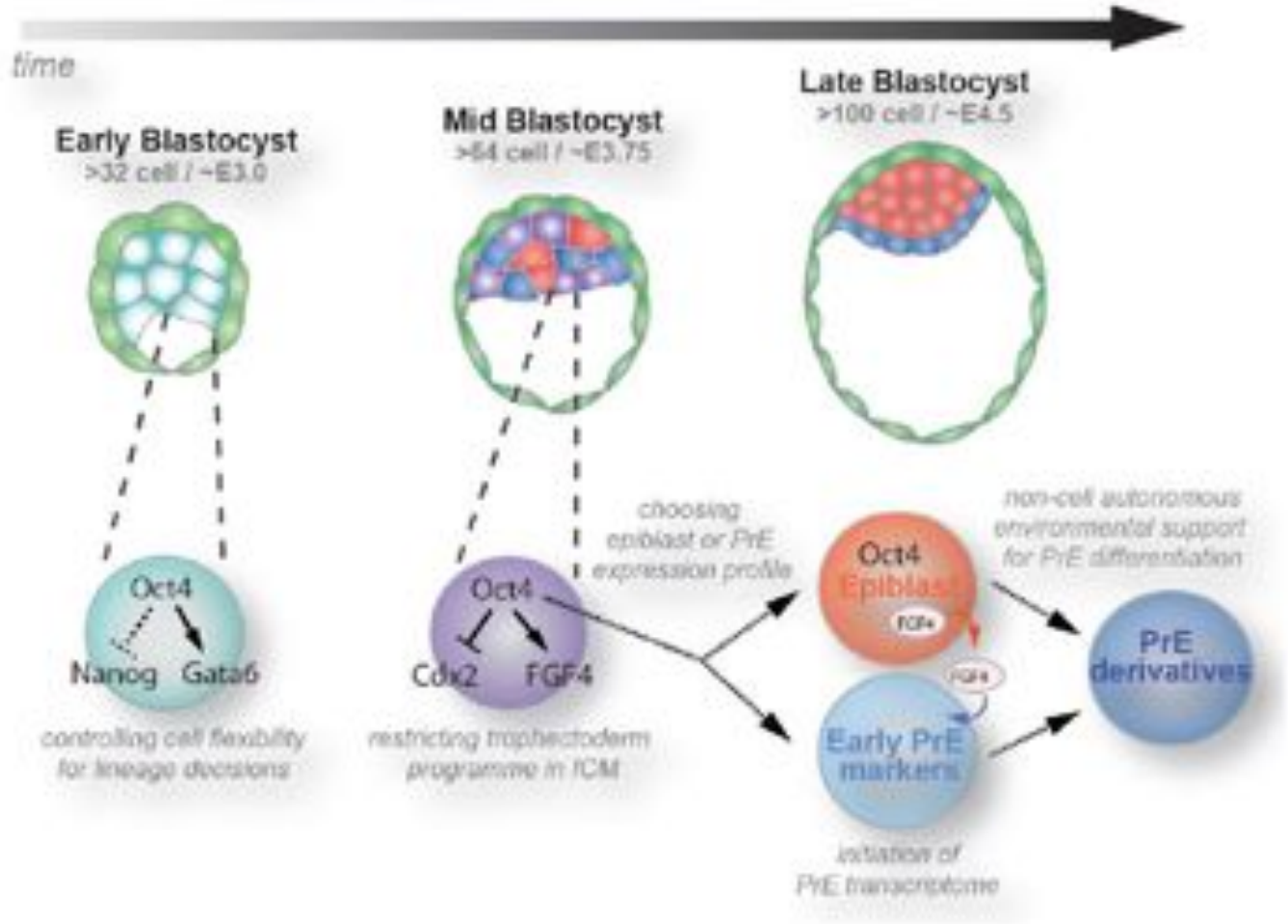
im28 are required for the postfertilization maintenance of maternal and paternal methylation imprints.
Li et al, 2008; Messerschmidt et al, 2012.

Adapted from Smith and Meissner, 2013

Epigenetic reprogramming in mammals



Pluripotency factors are required to determine early cell fate and for reprogramming in the ICM

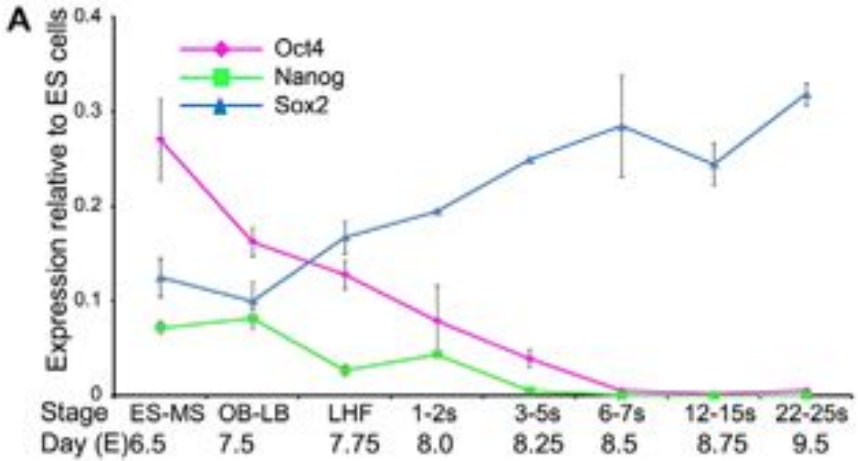


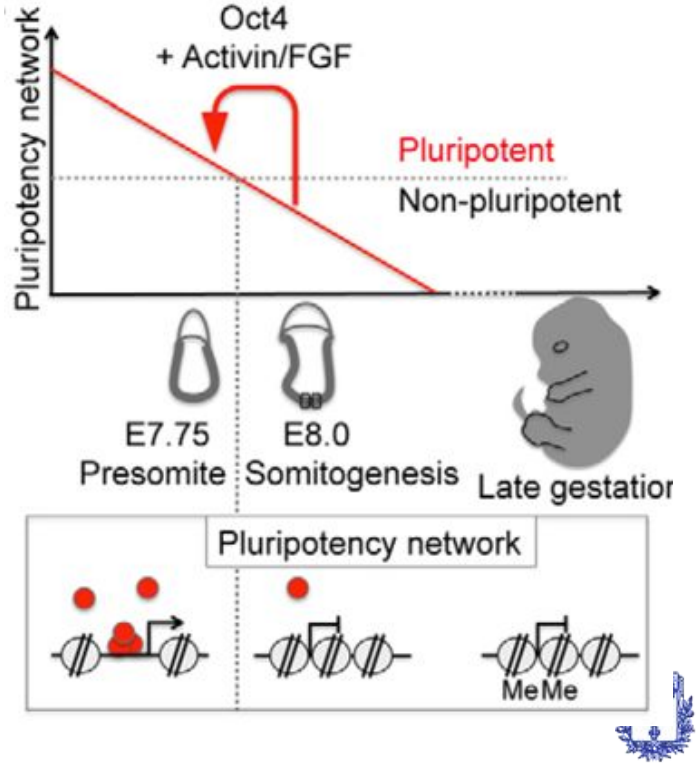
Le Bin et al, 2014

Pluripotency is rapidly lost in the post-implantation Embryo

- Levels of pluripotency gene regulatory network activity decline during post-implantation development, reaching a threshold at the onset of somitogenesis (E8.0) where levels become too low to sustain pluripotency.
- Decreased accessibility of regulatory elements invasion may extinguish pluripotency.
- Ectopic expression of Oct4 in co-operation with activin/FGF, can revive the pluripotent state initially, but DNA methylation rapidly stabilizes the non-pluripotent state.

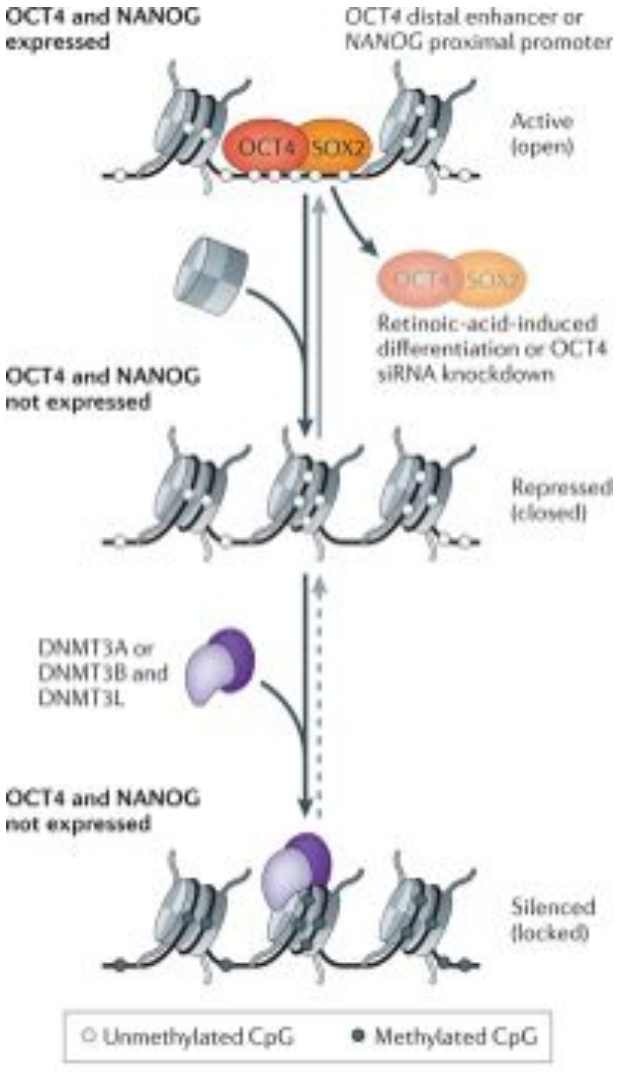
Osorno et al (2012) Development 139, 2288-2298





Pluripotency is rapidly lost in the post-implantation Embryo

Potential Scenario



Active promoters and enhancers have nucleosome-depleted regions (NDRs) that are often occupied by transcription factors and chromatin remodellers.

Loss of factor binding during differentiation — leads to increased nucleosome occupancy of the regulatory region, providing a substrate for *de novo* DNA methylation.

Except in the emerging germ line (Primordial Germ Cells) where Oct4 continues to be expressed

DNA methylation subsequently provides added stability to the silent state and is likely to be a mechanism for more accurate epigenetic inheritance during cell division.

Jones, P. (2012) Functions of DNA methylation: islands, start sites, gene bodies and beyond *Nature Rev. Genetics* 13, 484-493

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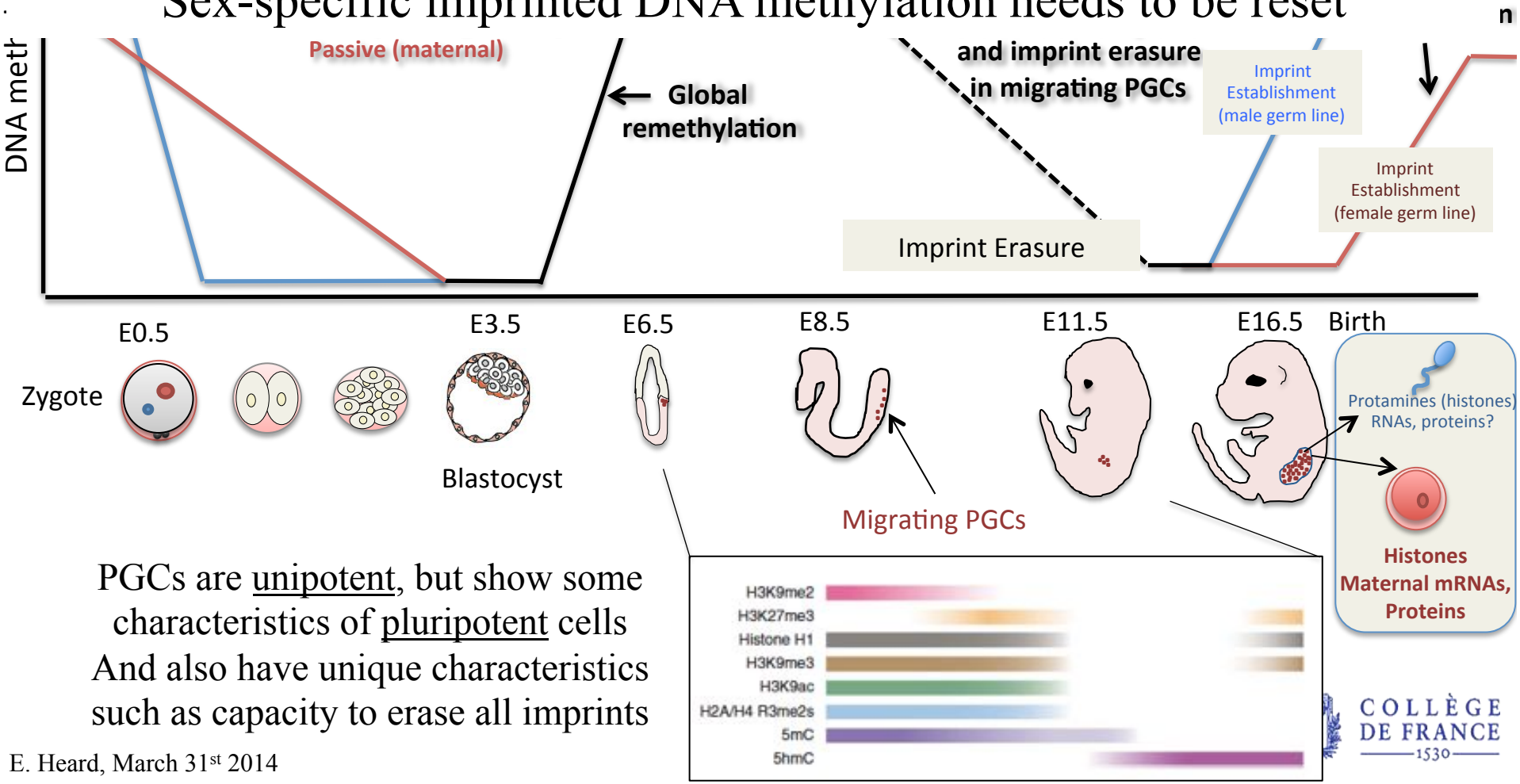
Epigenetic reprogramming in the germ line

Genome-wide reprogramming is essential to prevent transmission of inappropriate information to the next generation:

Epimutations accumulated during the organism's life must be erased

Sex-specific imprinted DNA methylation needs to be reset



PGCs are <u>unipotent</u>, but show some characteristics of <u>pluripotent</u> cells

And also have unique characteristics such as capacity to erase all imprints

PGC specification: Initiating the genetic program for epigenetic reprogramming

Epigenetic Barrier

E4.5

Inner Cell Mass

Pluripotent

Primed Epiblast

Future PGCs are initially primed for somatic fate - though are still expressing Oct4

E5.75……

Competence

Inactive X chromosome
DNA methylation re-established
Repression of some pluripotency genes
Onset of somatic cell differentiation

Somatic cells

Specification

BMP signaling from extra-embryonic ectoderm induces *Blimp1* (*Blimp1* KO -> somatic program)

Blimp1/Prdm1: *transcriptional repressor required for specification (to suppress somatic program, induce PGC program)*

Tcfap2c/Ap2g *also required for specification*

Prdm14: *required for reaquisition of pluripotency*

E7.5

Primordial Germ Cells

Repression of somatic program

Initiation of epigenetic changes

Re-expression of pluripotency genes

Xi-reactivation

DNA demethylation

[Imprints erased]

→Epigenetic ground state

Courtesy of A. Surani

E. Heard, March 31st 2014

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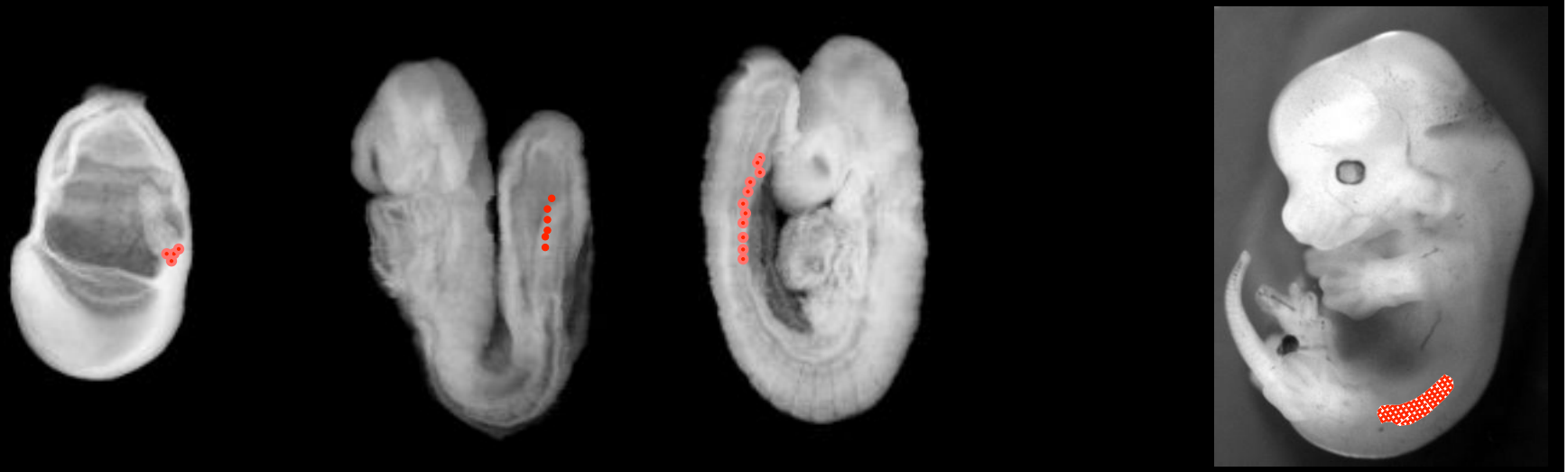
Reprogramming of PGCs upon entry into the genital ridge



E7.5 → E8.5 → E9.5 → E11.5 → E12.5

5mC = 12%

Popp et al, 2010

PGC Specification

Epigenetic Reprogramming (Step 1)

Loss of H3K9me2 (by down regulation of Glp/G9a HMTase) in preparation for later DNA demethylation?

Increase in H3K27me3 to compensate for K9 loss eg pluripotency genes?

Migration

Reprogramming (Step2)

DNA Demethylation

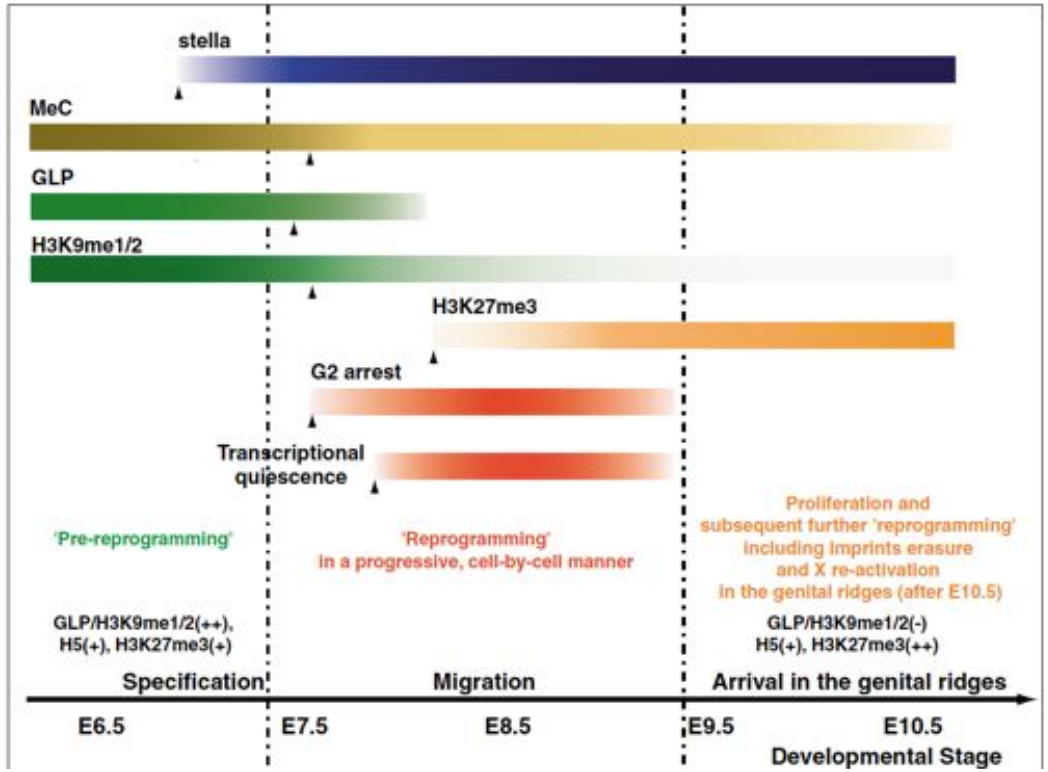
Loss of H3K27me3

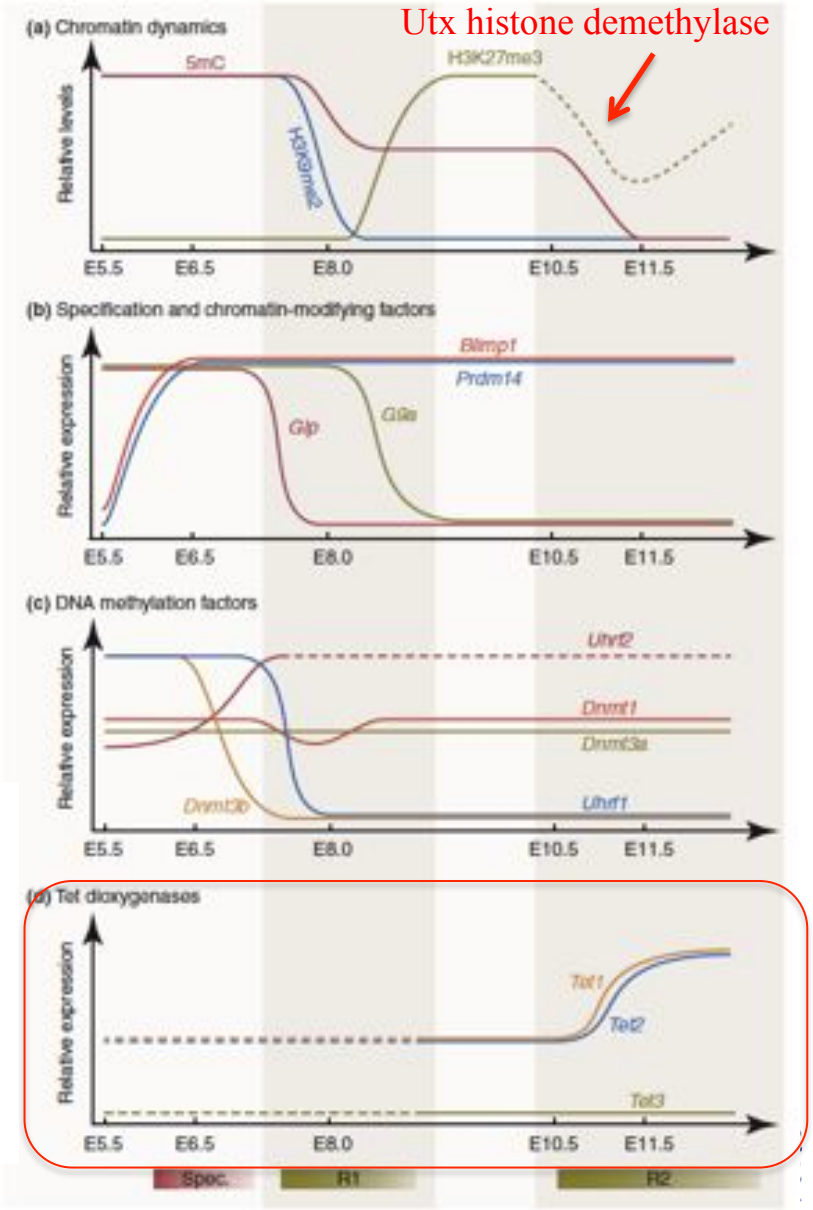
X-Reactivation

Imprints Erasure

Courtesy of A. Surani

Epigenetic Reprogramming in the Germ Line



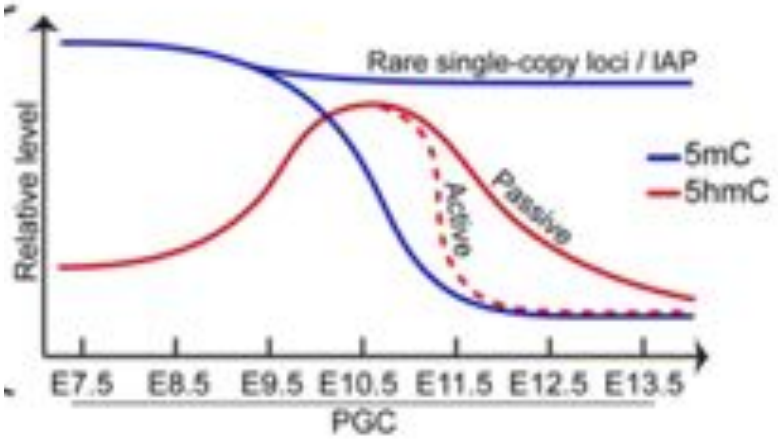


- By E10.5: DNA demethylation of some imprinted loci, transposons (eg LINE L1 and IAP), subset of germline-specific genes that are involved in genome defense against active transposons (Tex19.1 and Piwil2). Initiation of Xi reactivation.
- By E13.5 DNA demethylation of gene bodies, intergenic regions, imprinted domains and repeats previously protected from erasure in the zygote complete. Only exceptions: some IAPs and LTR-ERV1 repeats and a few hundred other single copy loci. Xi reactivation is also complete.

Germline DNA Demethylation Dynamics

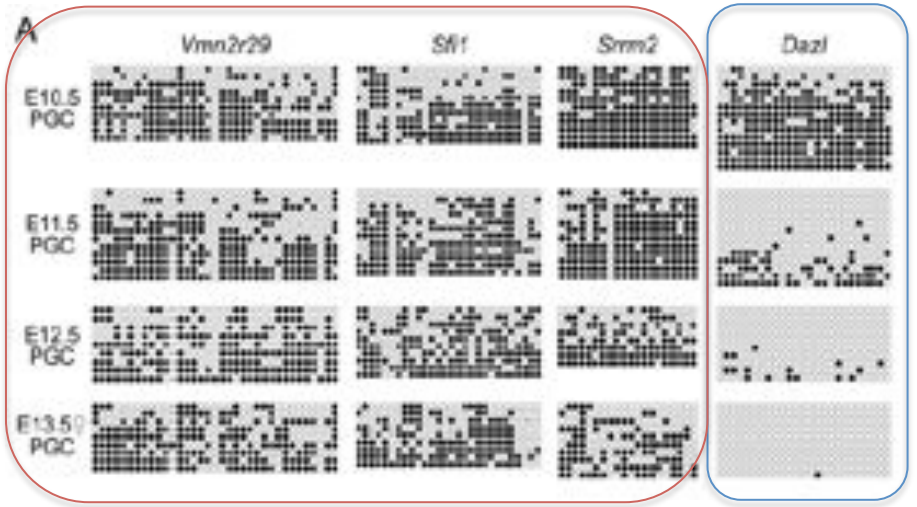
Whole genome bisulphite sequencing from E6.5 to E16.5

Hackett et al, Science, 2012
Seisenberger et al, Mol. Cell, 2012



Loci that escape systematic DNA demethylation in the mouse germ line

Dazl: a typical demethylated locus



- Erasure of CpG methylation (5mC) in PGCs occurs via conversion to 5-hydroxymethylcytosine (5hmC), driven by high levels of TET1 and TET2.
- Global conversion to 5hmC initiates in PGCs at embryonic day (E) 9.5-E10.5 and accounts for imprint erasure.
- Mechanistically, 5hmC enrichment is followed by gradual loss at a rate consistent with replication-coupled dilution.
- Conversion to 5hmC is an important component of parallel redundant systems that drive reprogramming in PGCs.

- 4730 loci escape demethylation (>40% 5mC) in PGCs: predominately repeat associated – in particular IAPTR1 (most active and dangerous element =>may need to be silenced even during germ line reprogramming)
- 233 single-copy loci with >40% 5mC, positional context or chromatin structure may contribute to their escape from reprogramming.

From Hackett et al, 2012

Resetting the Epigenome in the Germ Line

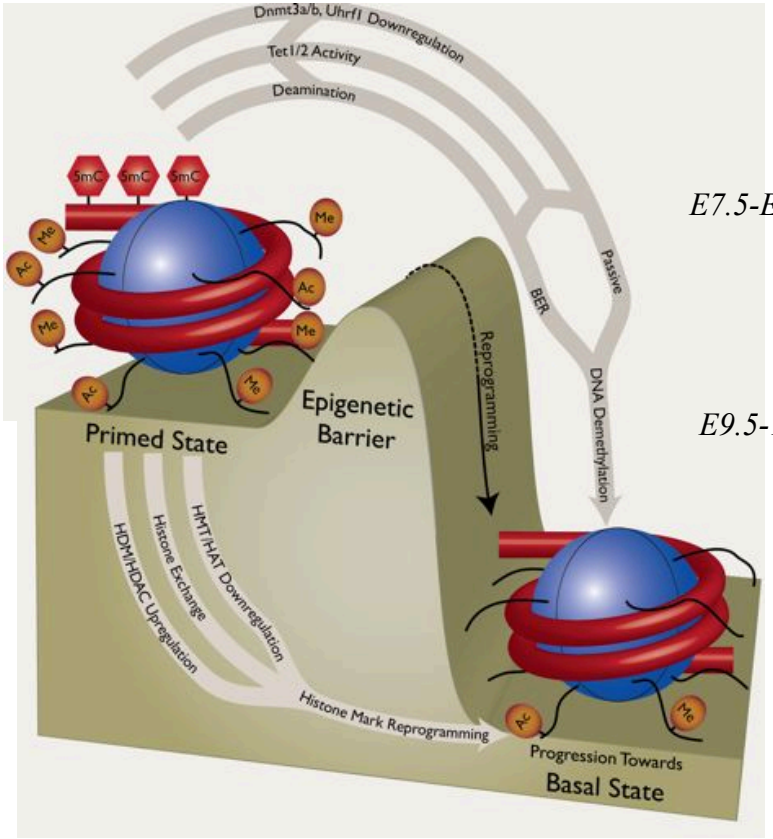
Parallel strategies presumably confer robustness to reprogramming in the germline so that genetic and epigenetic information can be faithfully conveyed to the next generation.

E6.0 *Blimp1, Prdm14, Ap2g*

Primed Epiblast state

H3K9me2 / 5mC / Xi

Parallel *redundant* mechanisms
(NB *Tet1, 2* loss have no effect on fertility or viability)



E7.5-E8.5

- *Erasure of H3K9me2, Glp repression and Kdm3a upregulation*
- *Down regulation of DNA methylation*

E9.5-10.5

- *Tet1 and Te2 Expression Conversion of 5mC->5hmC*
- *Higher order nuclear changes & base excision repair*

E11.5-E12.5

Oct4, Sox2, Nanog & Prdm14
XaXa
Demethylation
Imprints erased

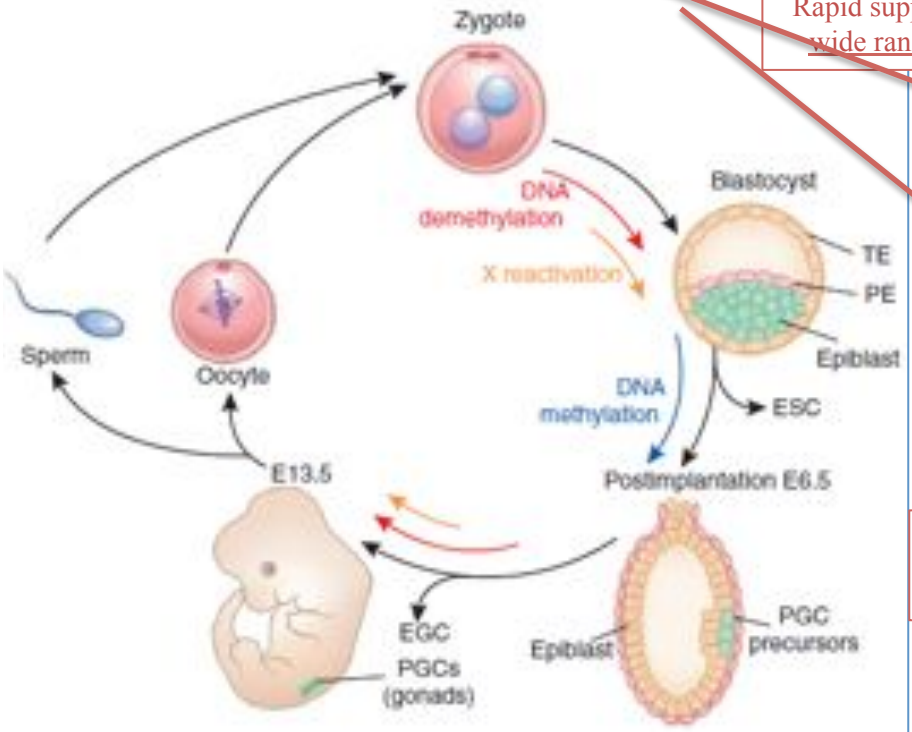
Hackett, Zylicz and Surani, 2012
Hackett, Sengupta, Zylicz……

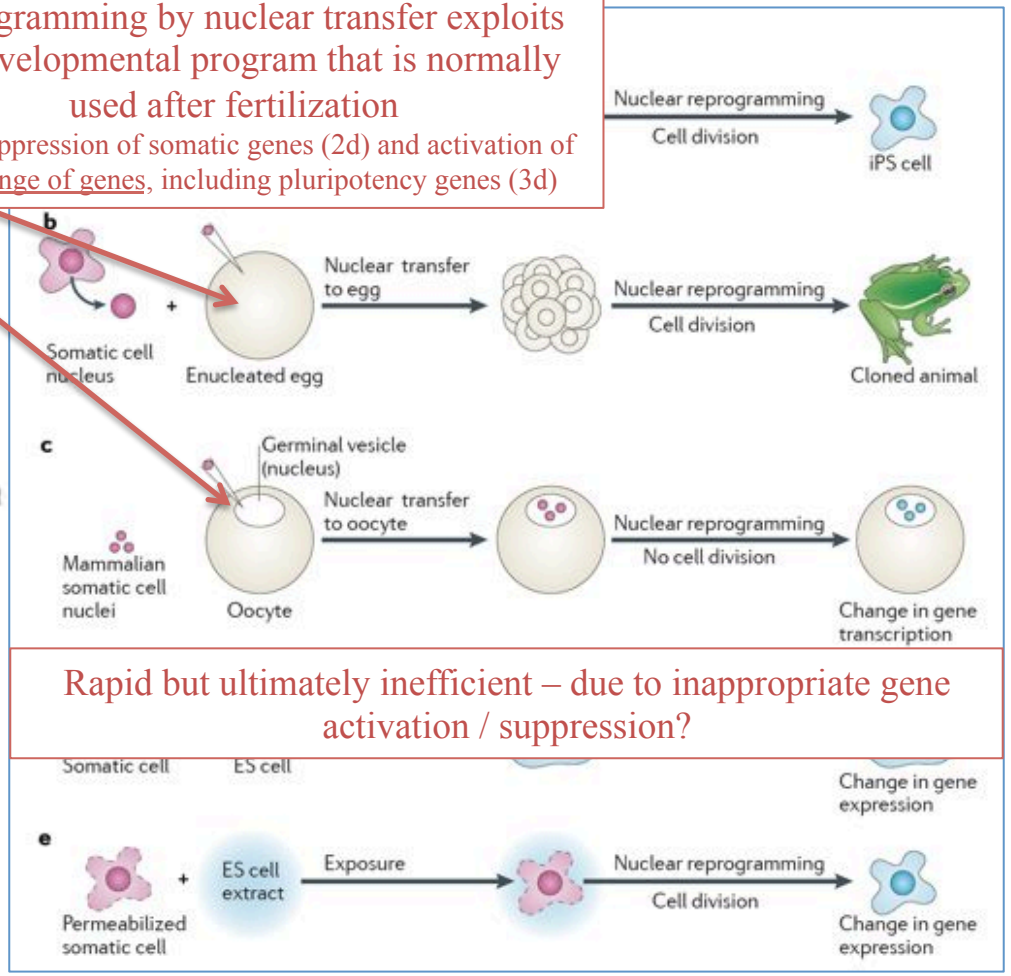
Developmental and Experimental Reprogramming

Zygotic Reprogramming => totipotency

Reprogramming by nuclear transfer exploits the developmental program that is normally used after fertilization

Rapid suppression of somatic genes (2d) and activation of wide range of genes, including pluripotency genes (3d)





Rapid but ultimately inefficient – due to inappropriate gene activation / suppression?

Germ Line Reprogramming => unipotency

Adapted from Cantone and Fisher; 2013; Jullien et al, 2011

Developmental and Experimental Reprogramming

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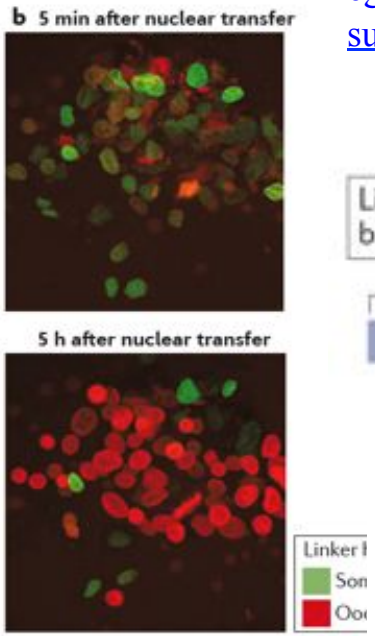
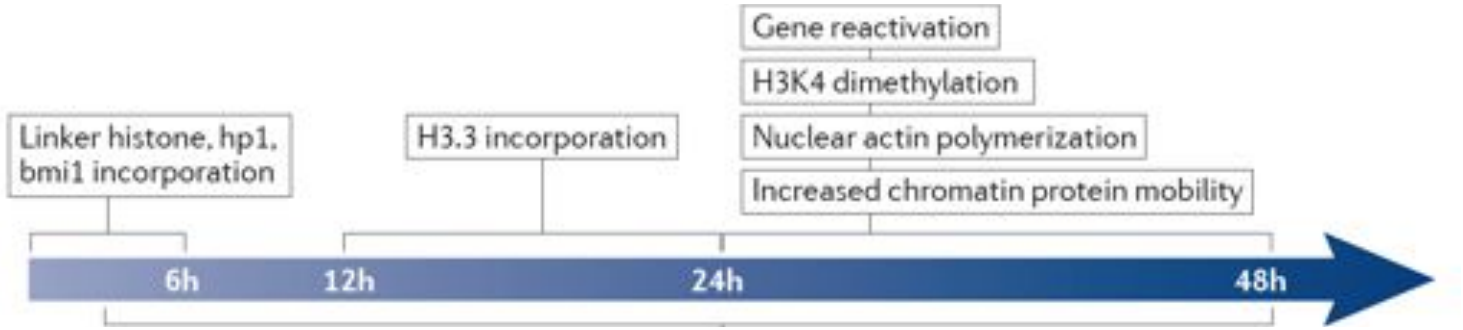
Somatic nuclear components (chromatin, transcriptional machinery) are displaced by egg/oocyte components (eg linker histones, core histone variants, TBP…), OR are supplemented by egg/oocyte components eg….)





Demethylation of the *Oct4* (*Pou5f1*) promoter occurs independently of DNA replication. (Simonsson, S. & Gurdon, J. DNA demethylation is necessary for the epigenetic reprogramming of somatic cell nuclei. Nat. Cell Biol. 6, 984–990 (2004).)

The BER and Tet3-mediated 5hmC pathways are implicated Wossidlo, M. et al. 5-Hydroxymethylcytosine in the mammalian zygote is linked with epigenetic reprogramming. Nat. Commun. 2, 241 (2011).

Adapted from Cantone and Fisher; 2013; Jullien et al, 2011

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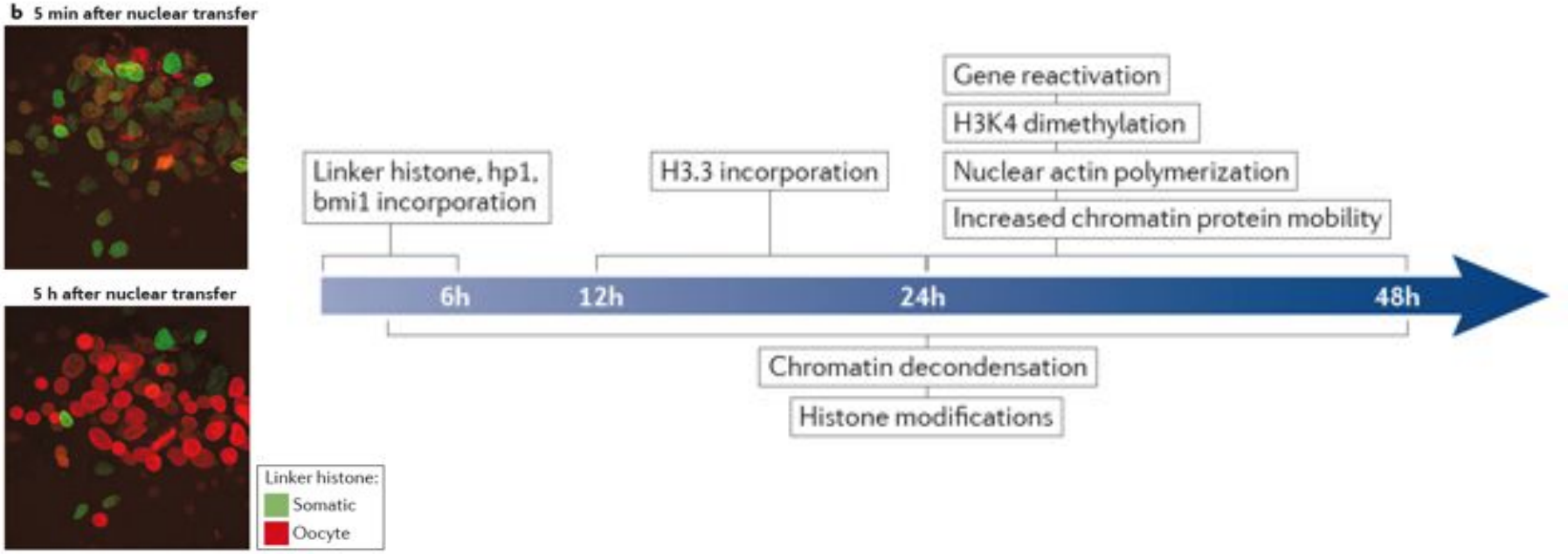

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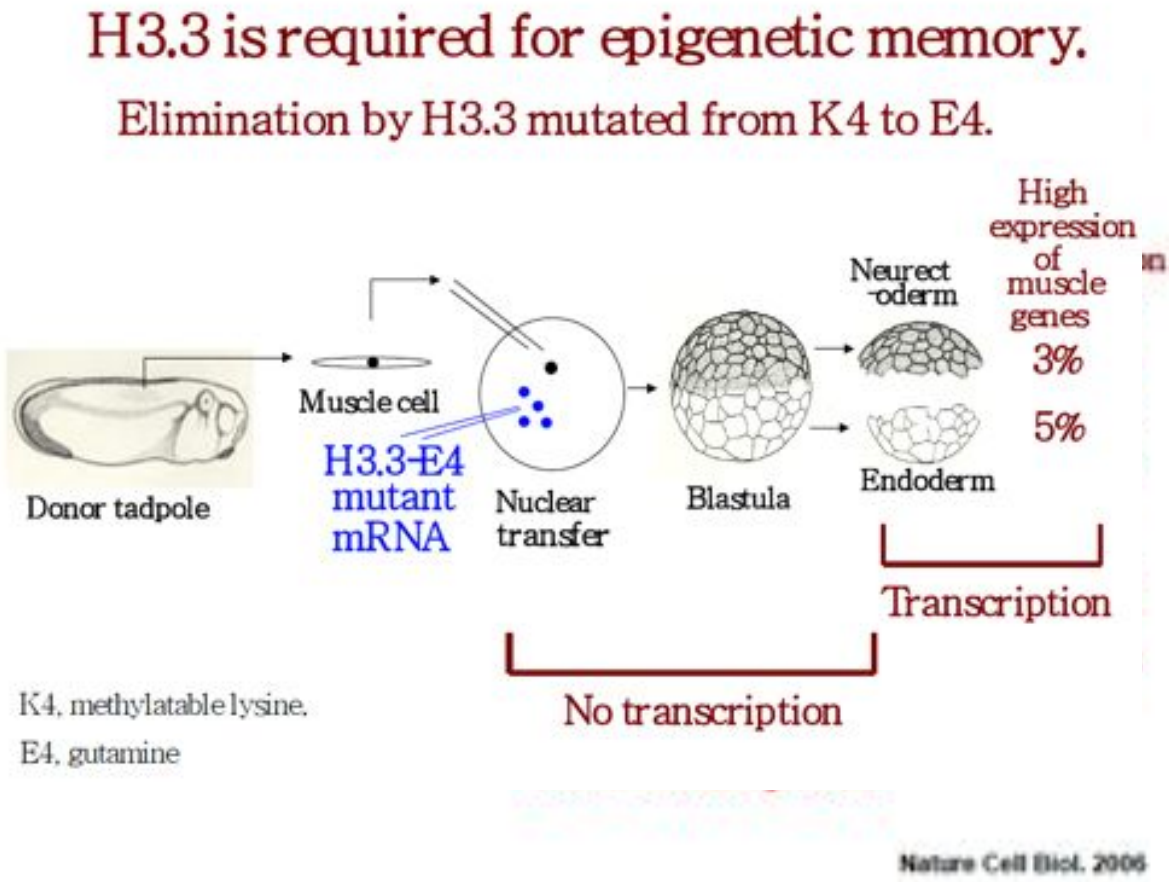
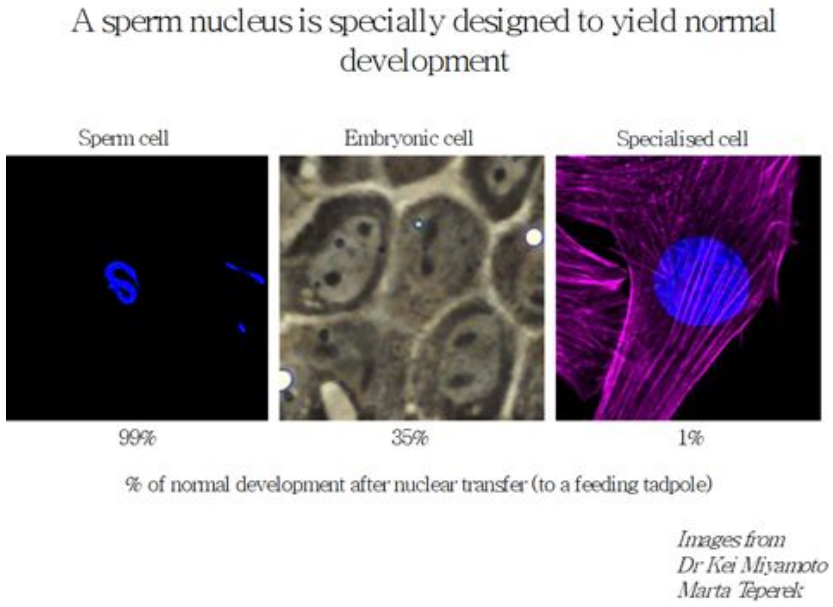
Developmental and Experimental Reprogramming



John Gurdon and colleagues

Developmental and Experimental Reprogramming

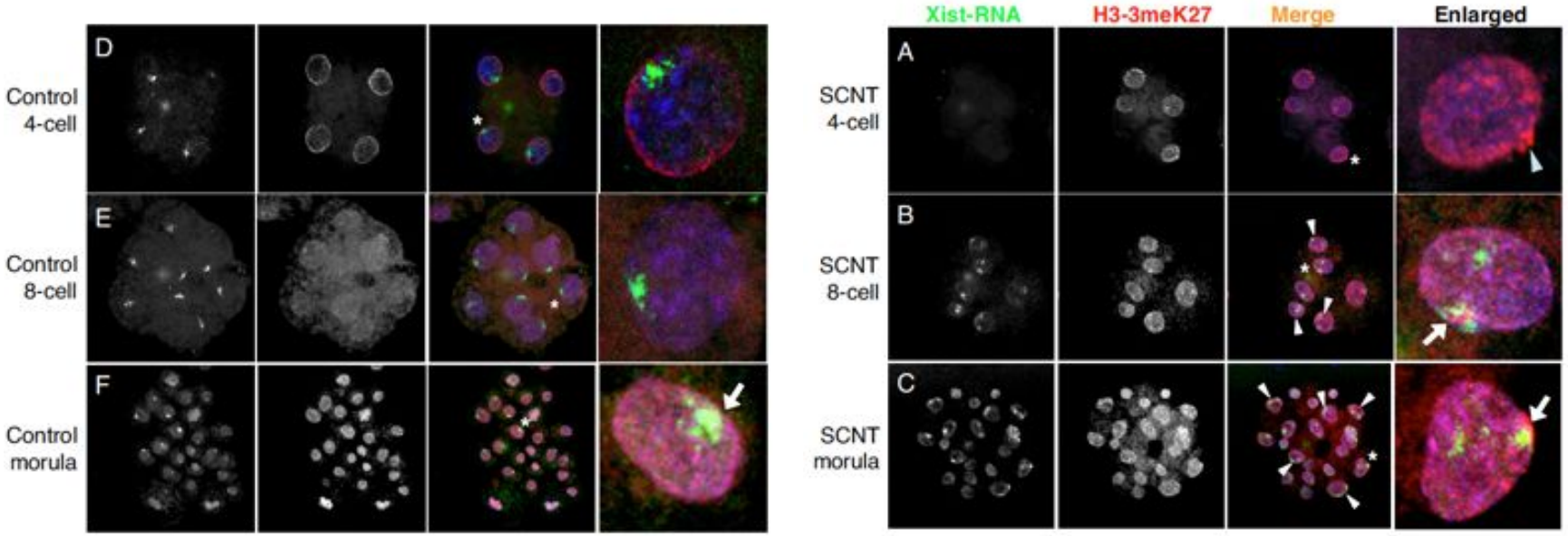
Somatic chromatin is NOT the template that the maternal reprogramming machinery is designed to work on….



Inefficient silencing, inefficient reactivation, as well as "mis-activation" can all occur, depending on gene and cell type of origin. Example of the X chromosome….

Aberrant X inactivation during SCNT

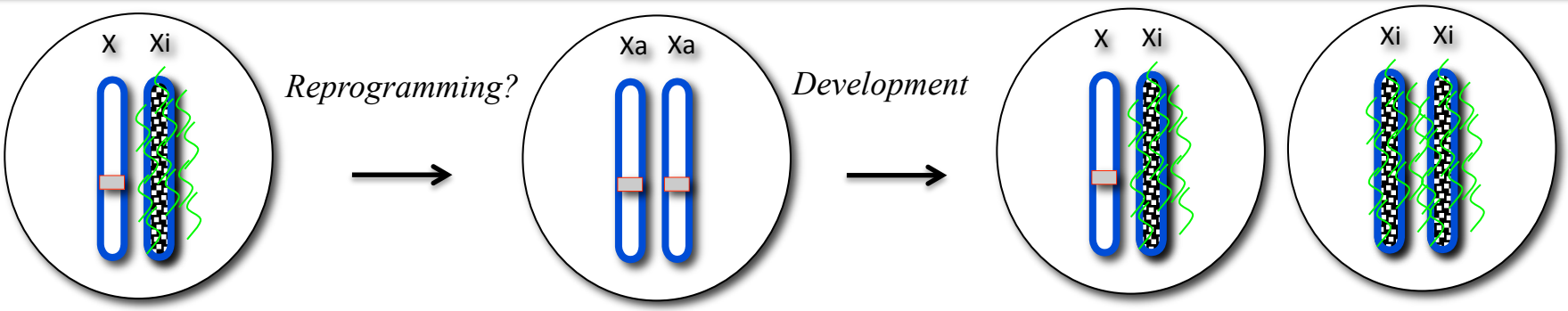
Aberrant reactivation of *Xist* from the active X in a somatic cells results in up-regulation of the single X in male and both X chromosomes female nuclei used in SCNT experiments



Bao et al, 2004. "Initiation of epigenetic reprogramming of the X chromosome in somatic nuclei transplanted to mouse oocyte"

- The inactive X is rapidly reactivated following SCNT and initiates inactivation again at the 4-cell stage
- The active X shows aberrant *Xist* expression and initiates aberrant XCI… leading to cellular pertubations due to X-chromosome functional nullisomy?

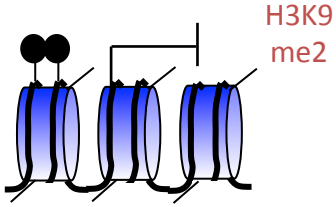
Aberrant X inactivation during SCNT

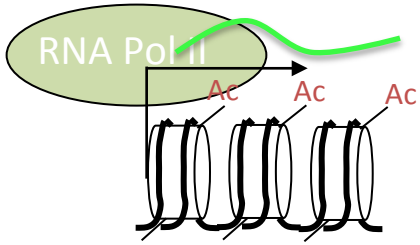


Controlling locus of X inactivation:

Inactive *Xist* gene on Xa

Xist OFF





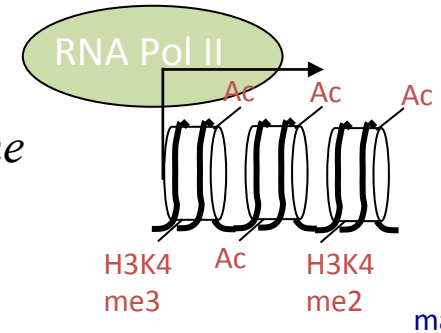
Active Xist gene on Xi

Xist ON

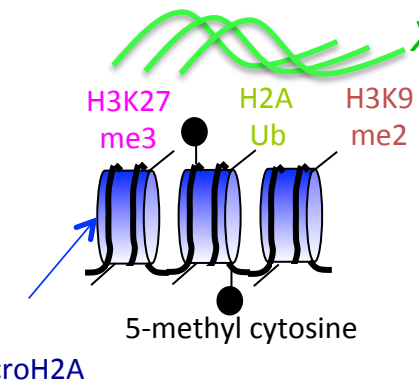
Target genes of X inactivation:

Active X chromosome

X-linked gene ON

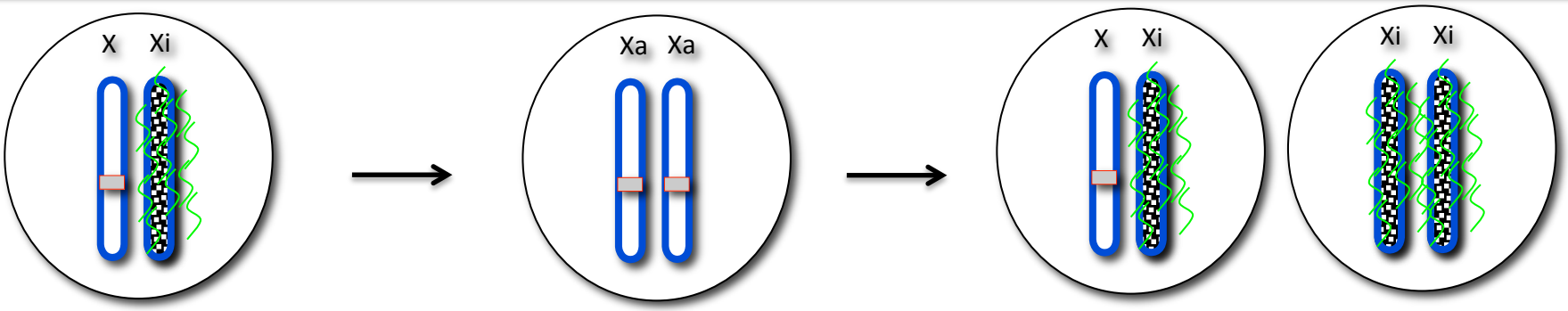


Inactive X chromosome



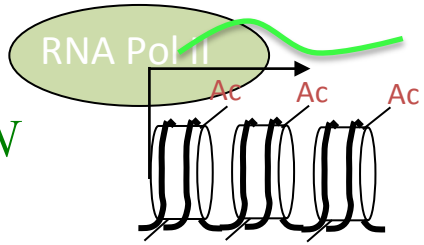
X-linked gene OFF

Aberrant X inactivation during SCNT



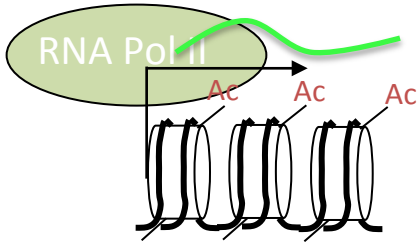
Controlling locus of X inactivation:

Xist OFF- ON



Xist reactivated on previously active X

Active Xist gene on Xi

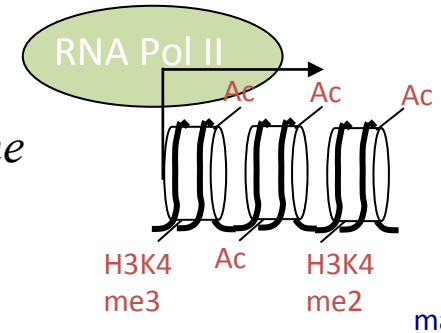


Xist ON

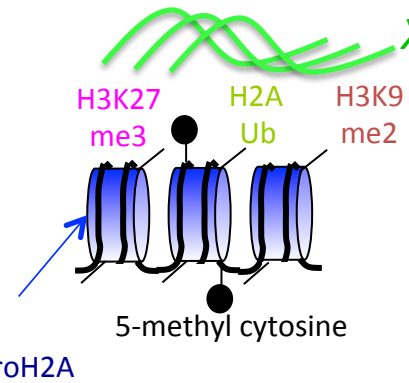
Target genes of X inactivation:

Active X chromosome

X-linked gene
ON

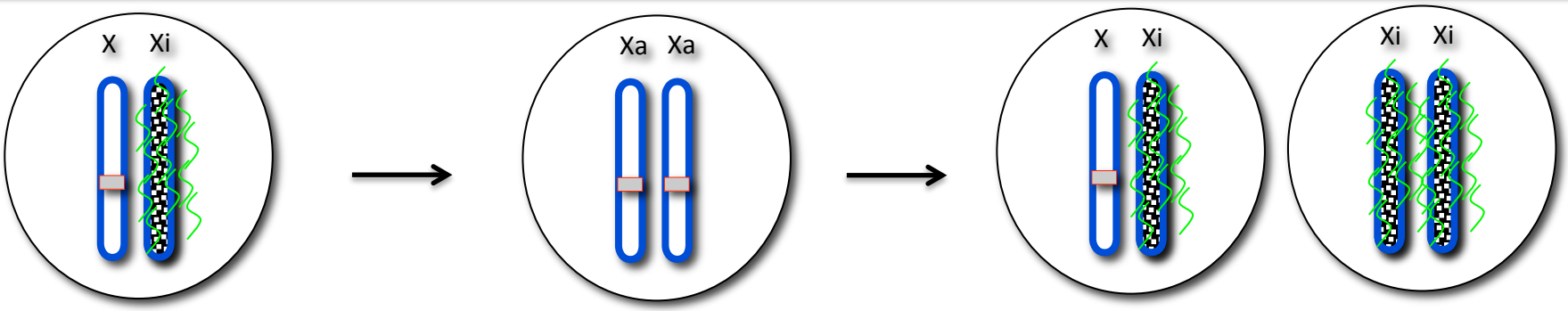


Inactive X chromosome



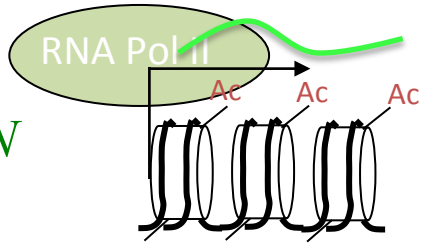
X-linked gene
OFF

Aberrant X inactivation during SCNT



Controlling locus of X inactivation:

Xist OFF- ON



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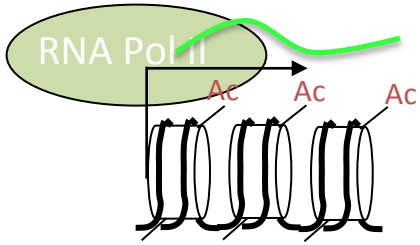
Active Xist gene on Xi

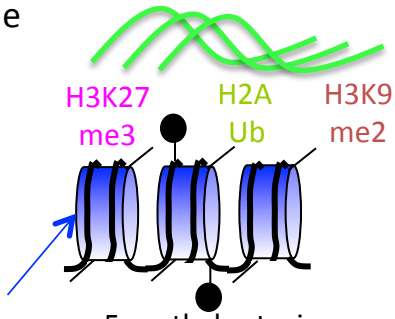


Xist ON

Target genes of X inactivation:

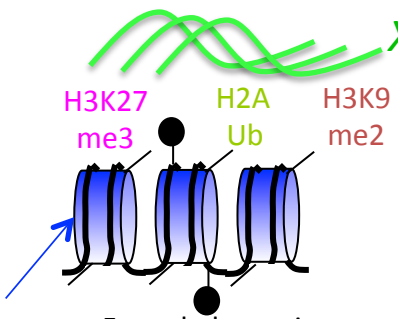
Active X chromosome

X-linked gene
ON -OFF



Inactive X chromosome

Xist RNA

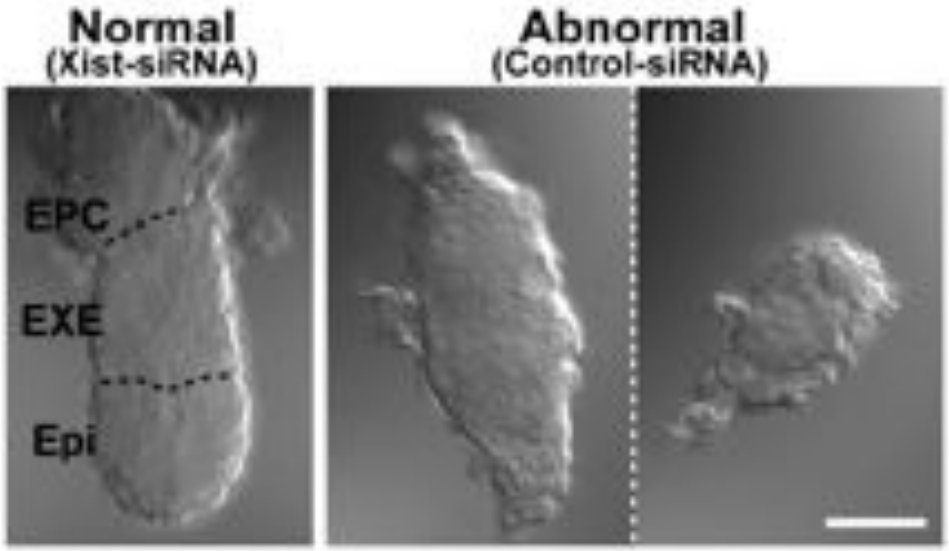


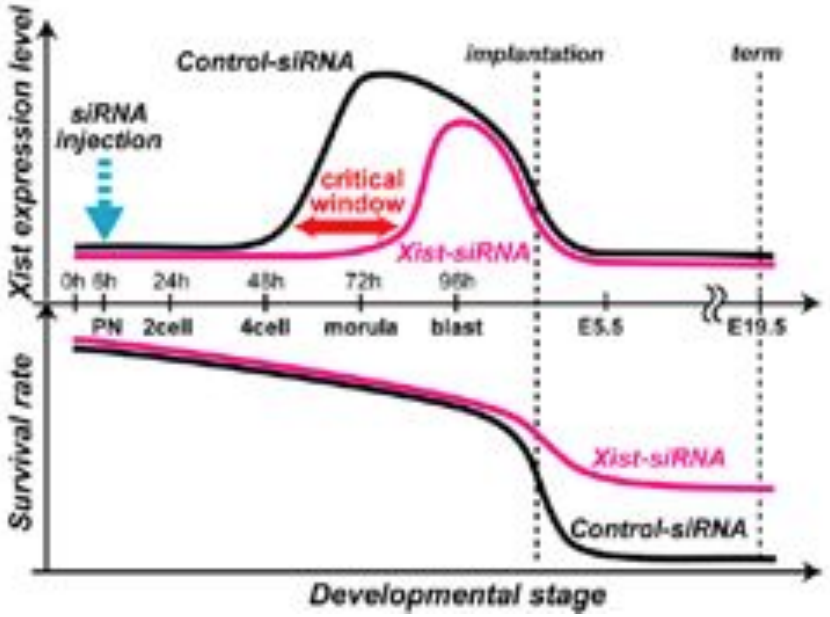
X-linked gene
OFF

Aberrant X inactivation during SCNT

Rescue of this phenotype when Xist RNA is *DEPLETED* in the donor nucleus

- Dramatically increased birth rates of male and female clones and decreased post-natal defects
- birth rate was further improved to about 20% by combining Xist-siRNA with 50nM trichostatin A (TSA) treatment (Matoba et al, PNAS, 2011)





SCNT leads to aberrant epigenetic memory AND aberrant activation of some genes (eg Xist) which can result in further aberrant events (eg X inactivation)

E.

SUMMARY
REPROGRAMMING MECHANISMS DURING SCNT

- Reprogramming events occur **rapidly** during SCNT
- High frequency of **aberrant gene regulatory events**

=>*due to maternal factors acting on somatic chromatin (not sperm)*

- **Pluripotency factors** are **not** involved initially – but become reactivated during SCNT (eg *Oct4*, *Sox2*)
- **Histone variants** seem to play a key role in promoting (H3.3) or preventing (mH2A) gene activity during SCNT reprogramming

May "interfere" with normal developmental program during SCNT

The behaviour of the X demonstrates that:

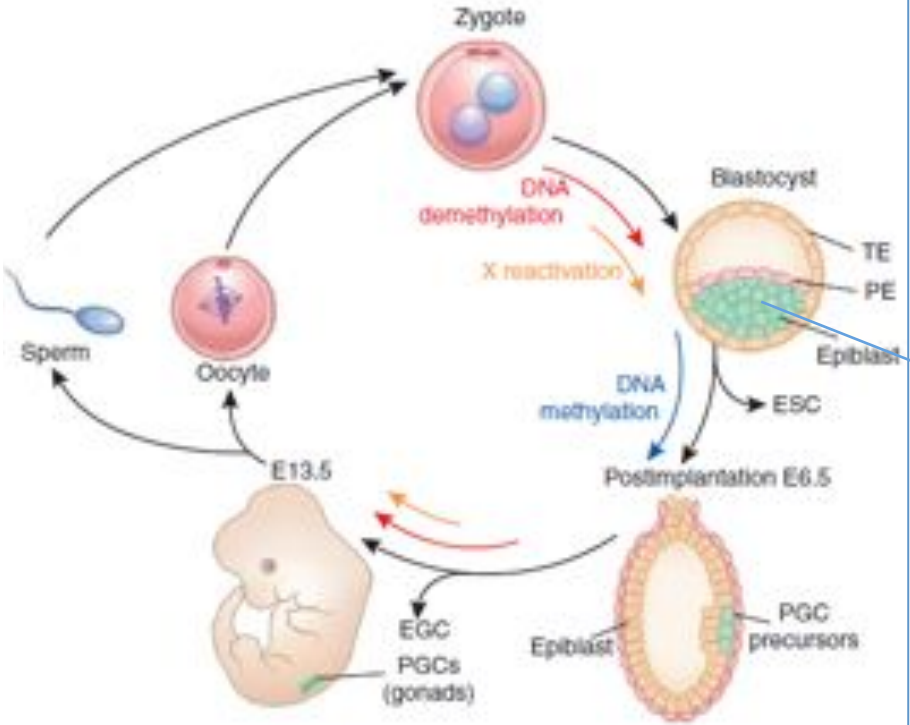
(i) The treatment of chromatin in the zygote is <u>not</u> equivalent to that in pluripotent cells (ICM or ES or iPS)

(ii) Zygotic reprogramming leaves a somatic memory on the Xi and aberrantly activates the *Xist* gene on the Xa

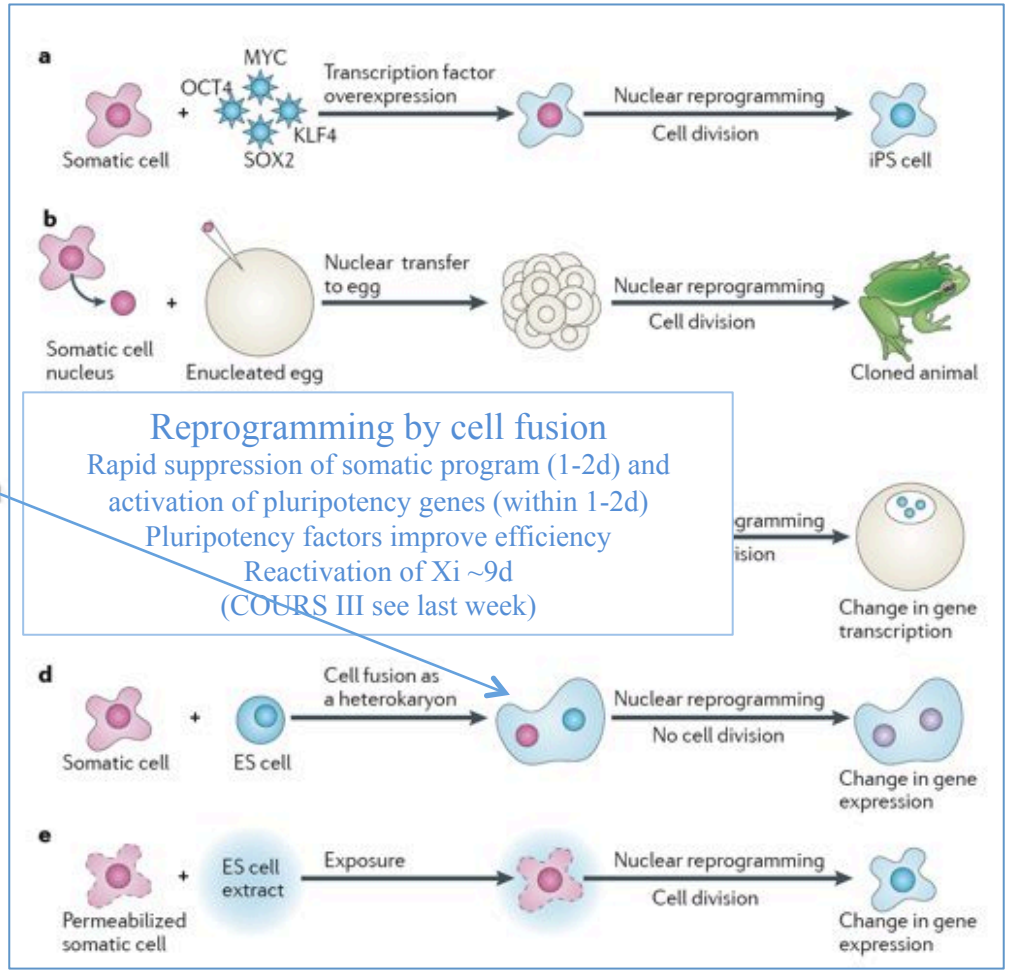
(iii) Memory on the Xi is erased in the ICM/ES/iPS (see Eggan et al, 2005; Okamoto et al, 2004)

Developmental and Experimental Reprogramming

Zygotic
Reprogramming
=> totipotency



Germ Line
Reprogramming
=> unipotency

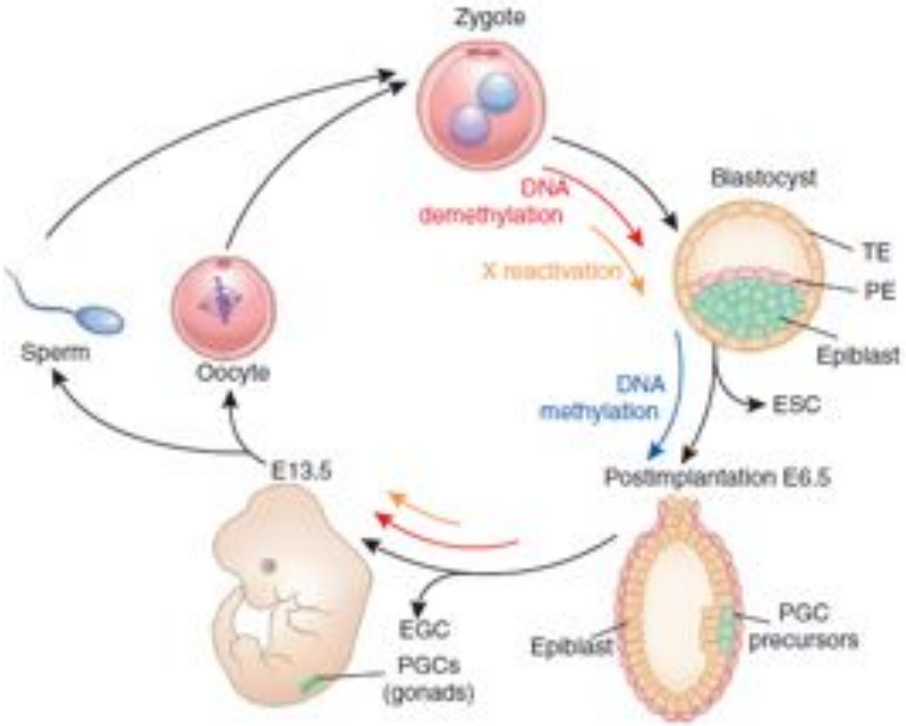


Reprogramming by cell fusion
Rapid suppression of somatic program (1-2d) and activation of pluripotency genes (within 1-2d)
Pluripotency factors improve efficiency
Reactivation of Xi ~9d
(COURS III see last week)

Adapted from Cantone and Fisher; 2013; Jullien et al, 2011

Developmental and Experimental Reprogramming

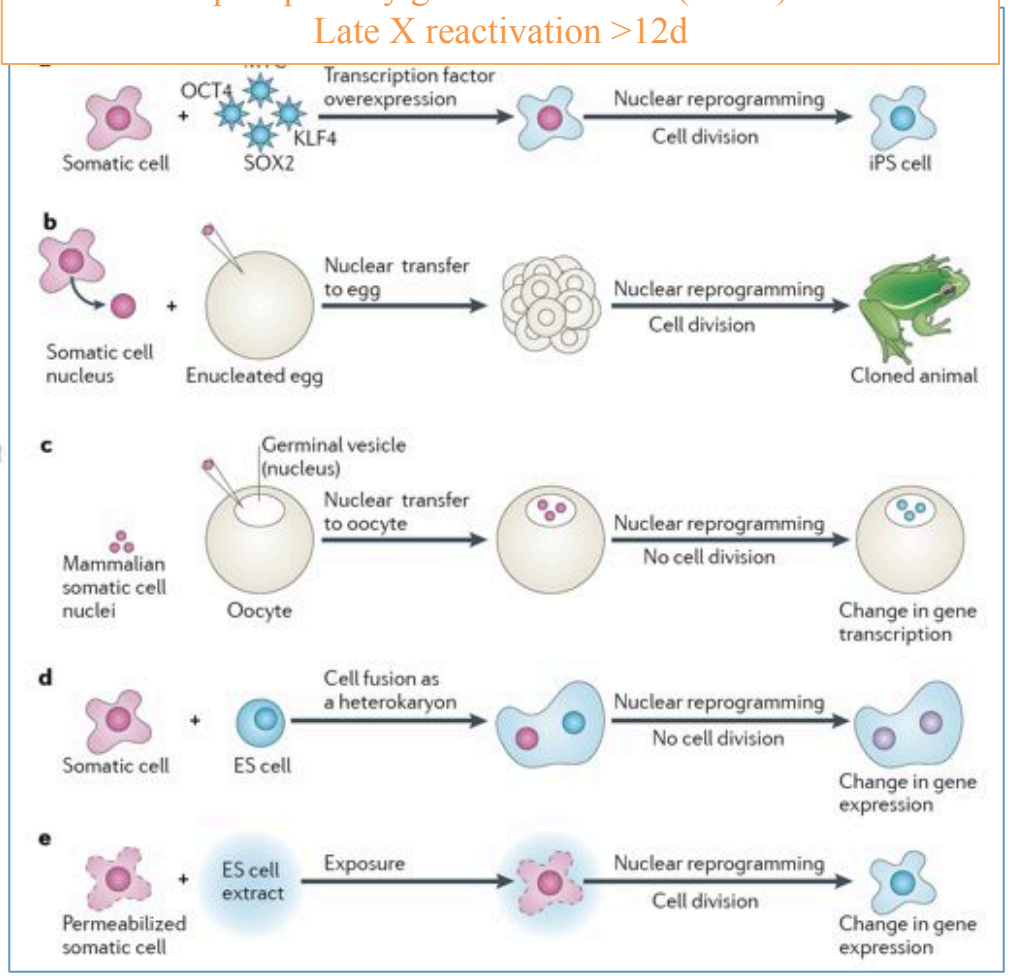
Zygotic
Reprogramming
=> totipotency



Germ Line
Reprogramming
=> unipotency

Reprogramming by OKSM?

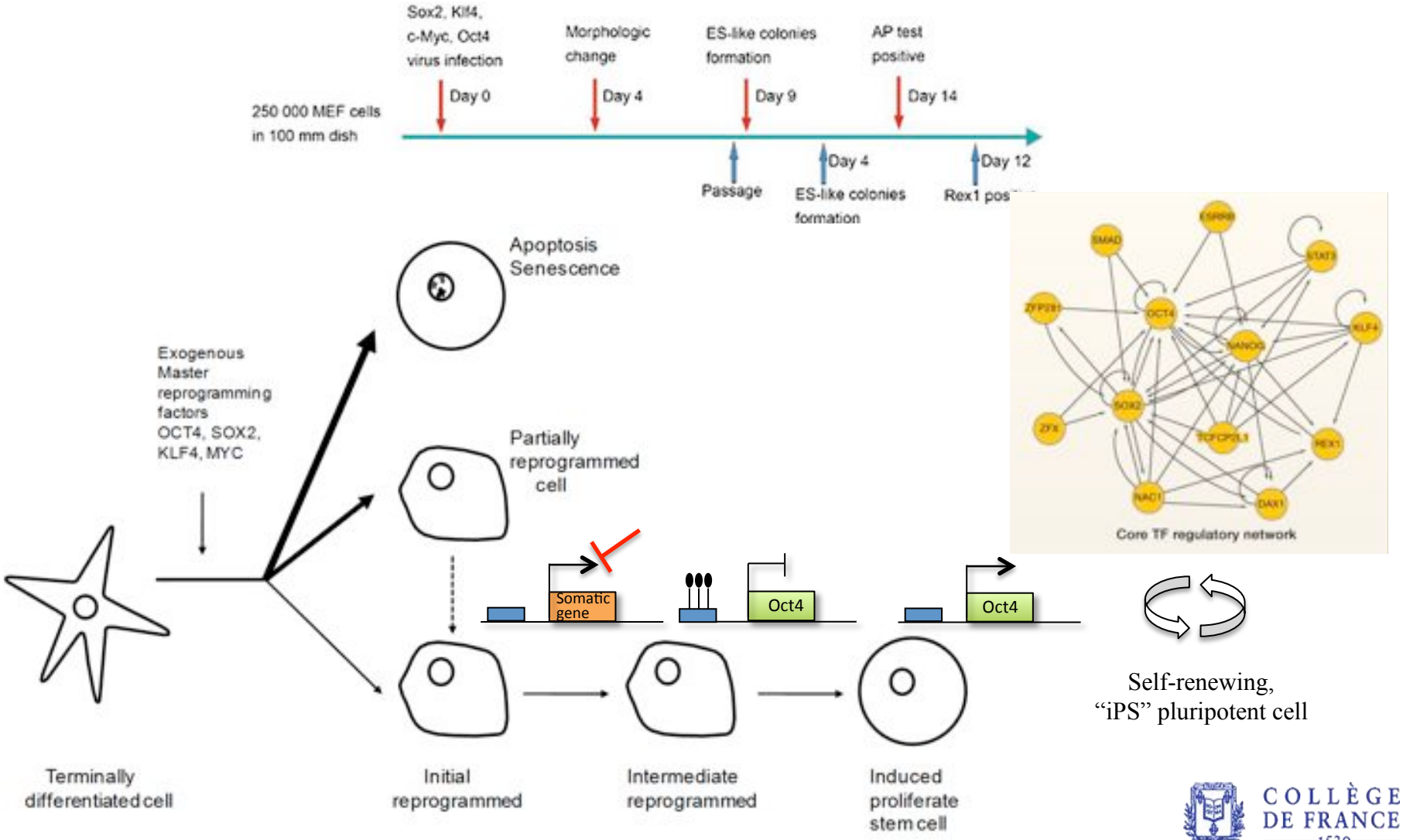
Rapid silencing of some somatic cells (1-2d) but much later pluripotency gene reactivation (8-12d)
Late X reactivation >12d



Adapted from Cantone and Fisher; 2013; Jullien et al, 2011

Mechanisms of induced pluripotency by TFs

Acquisition of pluripotent state is slow (2-3 weeks) and inefficient (0.1-3%)
⇒ TFs must need to overcome various epigenetic roadblocks or barriers…?



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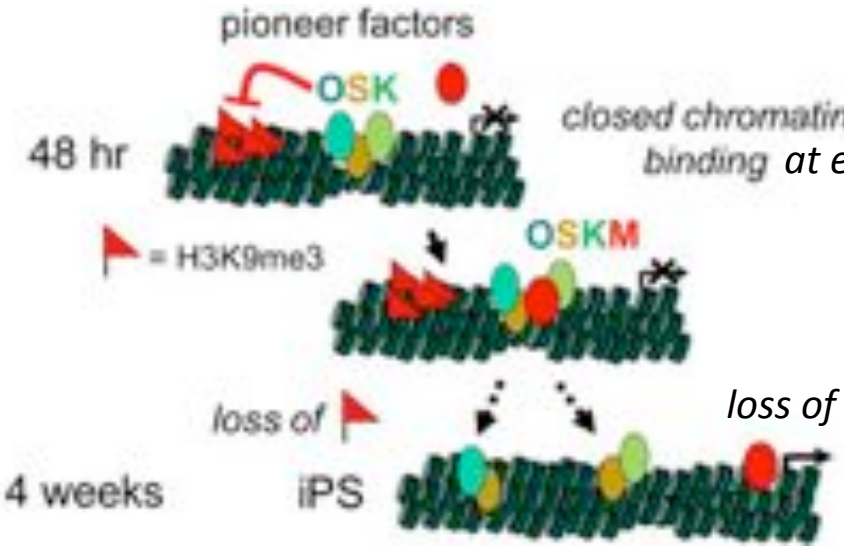
Mechanisms of induced pluripotency by TF

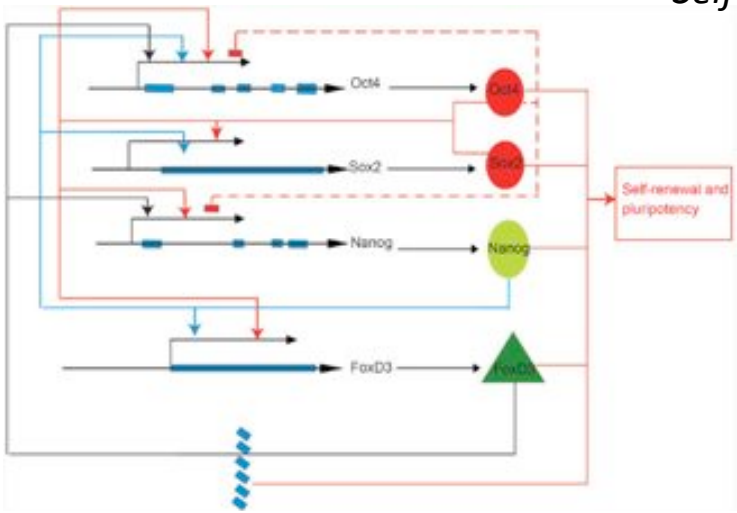
Acquisition of pluripotent state is slow (2-3 weeks) and inefficient (0.1-3%)
⇒ TFs must need to overcome various epigenetic roadblocks or barriers…?

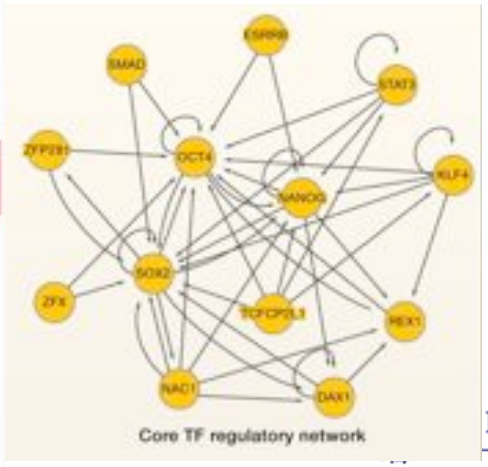


at enhancers => poising them for later activation?

loss of DNA methylation

Independence from exogenous OKSM
Self-sustaining feedback loop



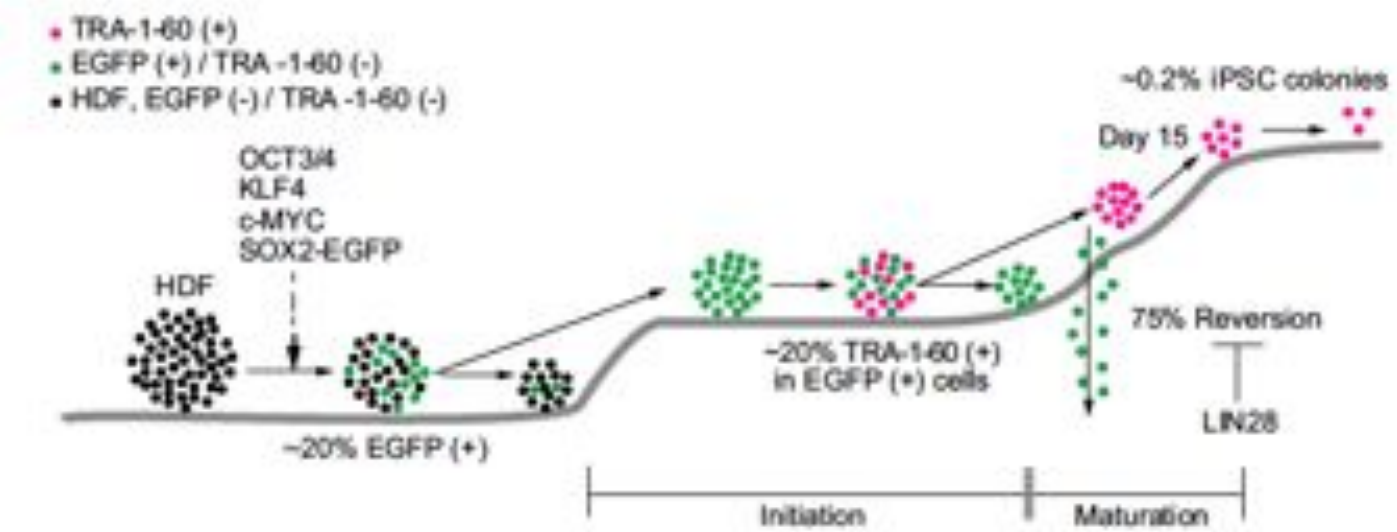


Induced Pluripotency is slow and inefficient

Acquisition of pluripotent state is slow (2-3 weeks) and inefficient (0.1-3%)
⇒ TFs need to overcome certain roadblocks or barriers…?

Maturation, not initiation, is the major roadblock during reprogramming toward pluripotency from human fibroblasts

Koji Tanabe[a], Michiko Nakamura[a], Megumi Narita[a], Kazutoshi Takahashi[a], and Shinya Yamanaka[a,b,1]



Mechanisms of induced pluripotency by TF

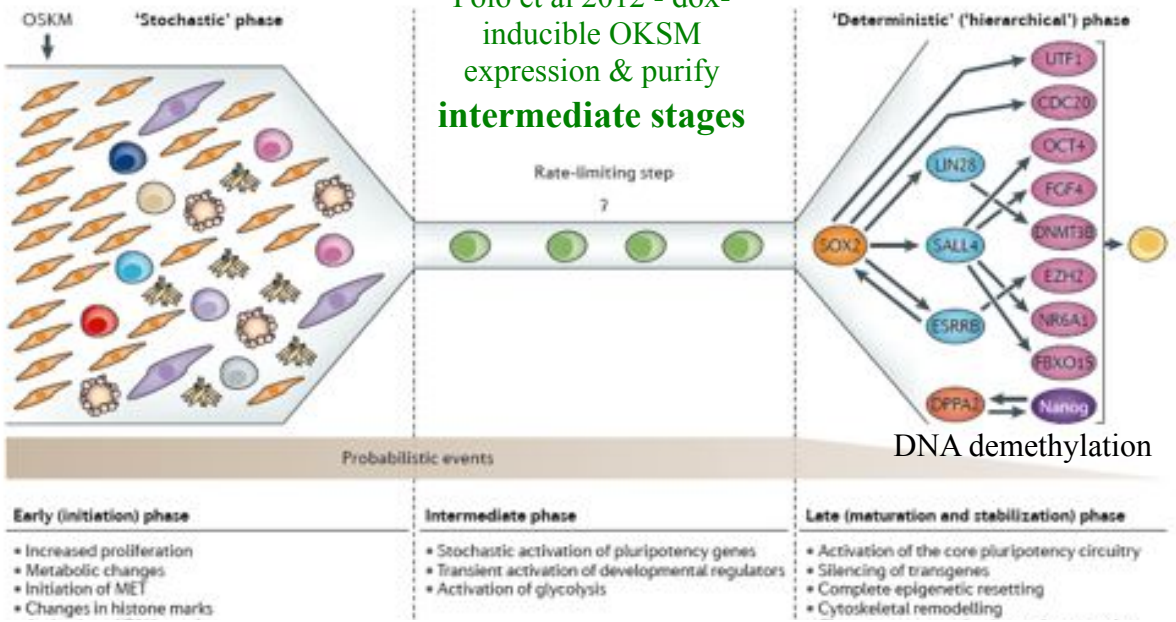
Acquisition of pluripotent state is slow (2-3 weeks) and inefficient (0.1-3%)
⇒ TFs must need to overcome various epigenetic roadblocks or barriers…?

Somatic cell population

Cell division is required for reprogramming

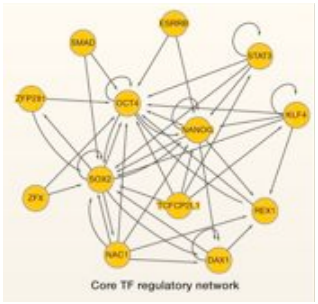
All cells can be reprogrammed but a subset of cells with "ultrafast" cell cycle may be priviledged

Polo et al 2012 - dox-inducible OKSM expression & purify **intermediate stages**



DNA demethylation

Self-renewing, "iPS" pluripotent cell

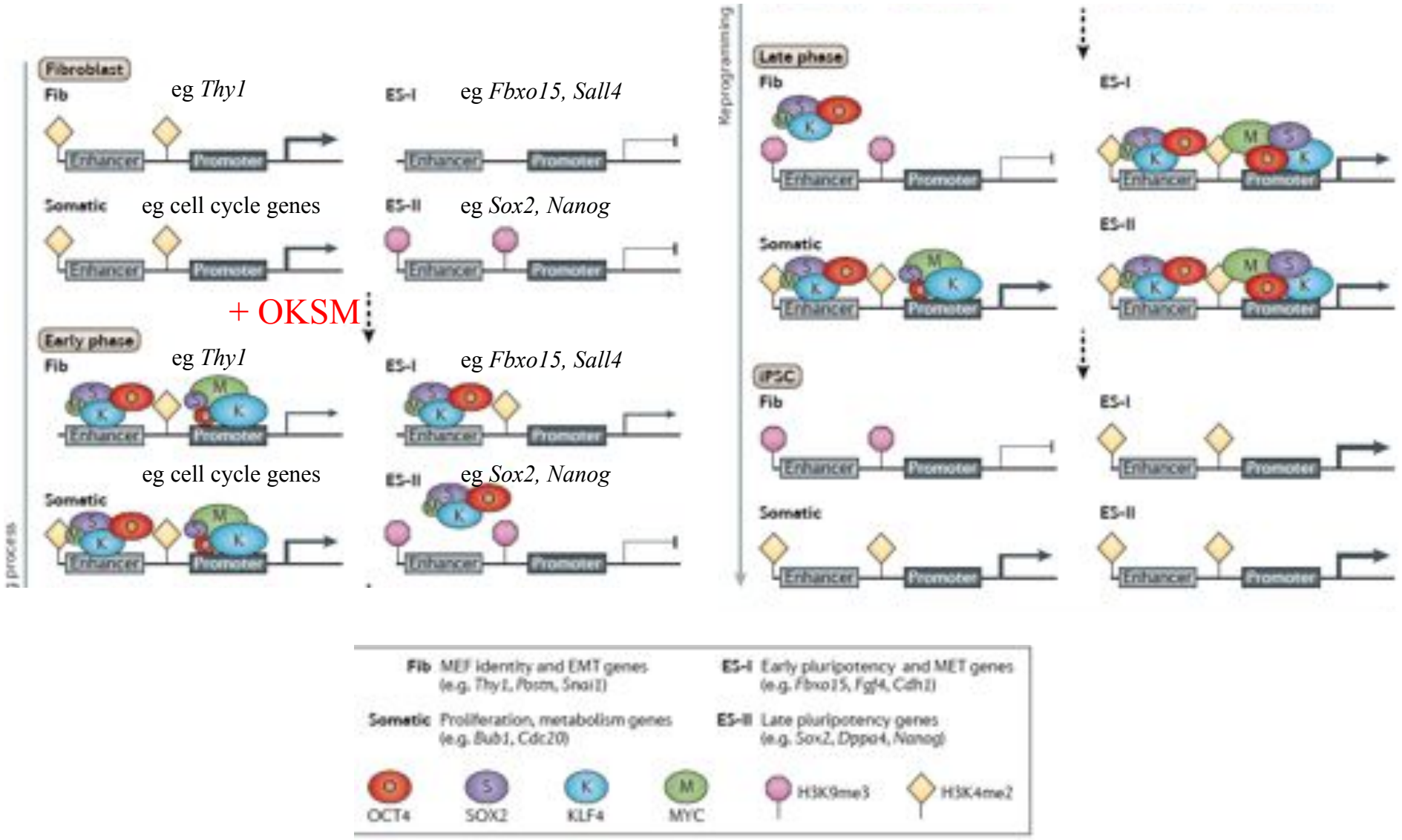


1. Early cell cycle changes and transitions to an epithelial state
2. Cells that continue to express somatic genes in the population are refractory to subsequent events
3. Later hierarchical activation of the pluripotency network; independence from exogenous TFs

Changing cell identity is accompanied by several epigenetic changes, with genome-wide resetting of DNA methylation status and X-chromosome reactivation being amonst the last events
(Buganim et al., 2012; Golipour et al., 2012; Polo et al., 2012).

Bugganim, Faddah and Jaenisch, NRG, 2013

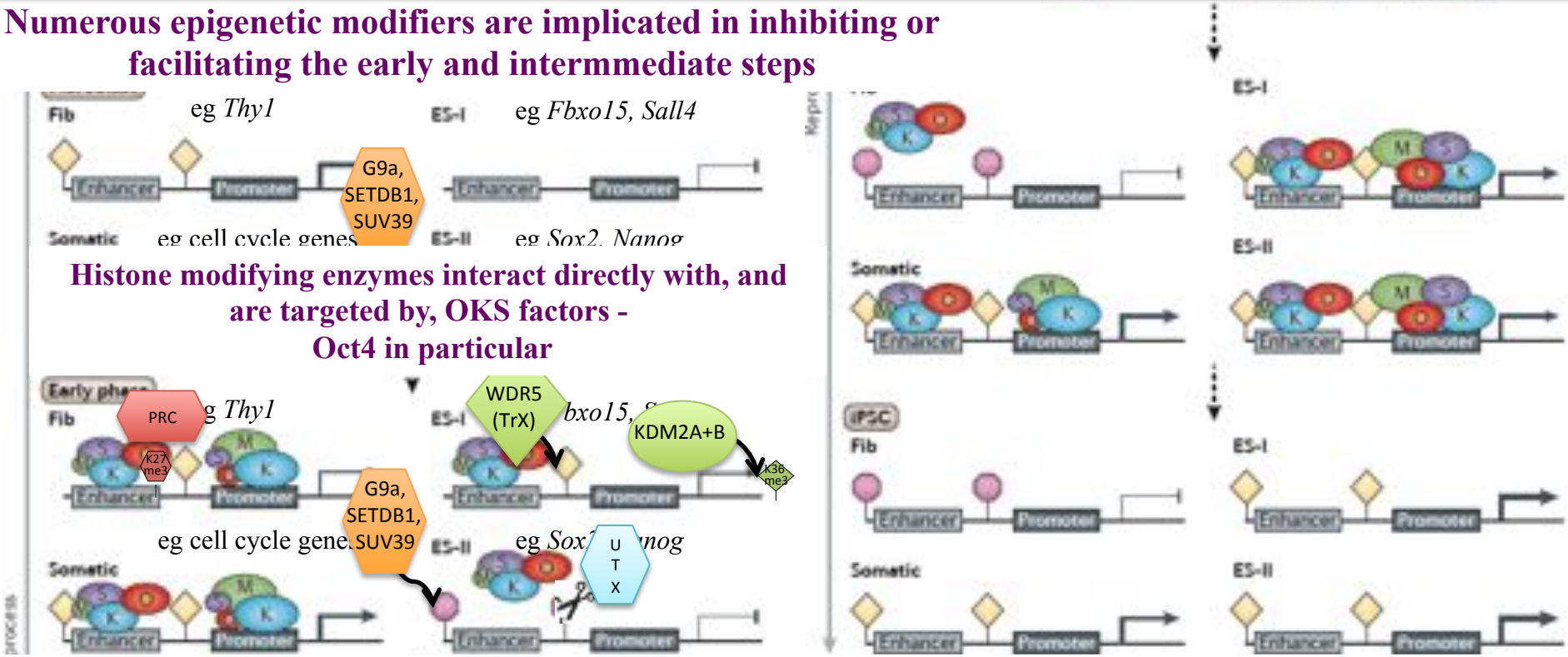
Mechanisms of induced pluripotency by TF



Bugganim, Faddah and Jaenisch, NRG, 2013

Mechanisms of induced pluripotency by TF

Numerous epigenetic modifiers are implicated in inhibiting or facilitating the early and intermmediate steps

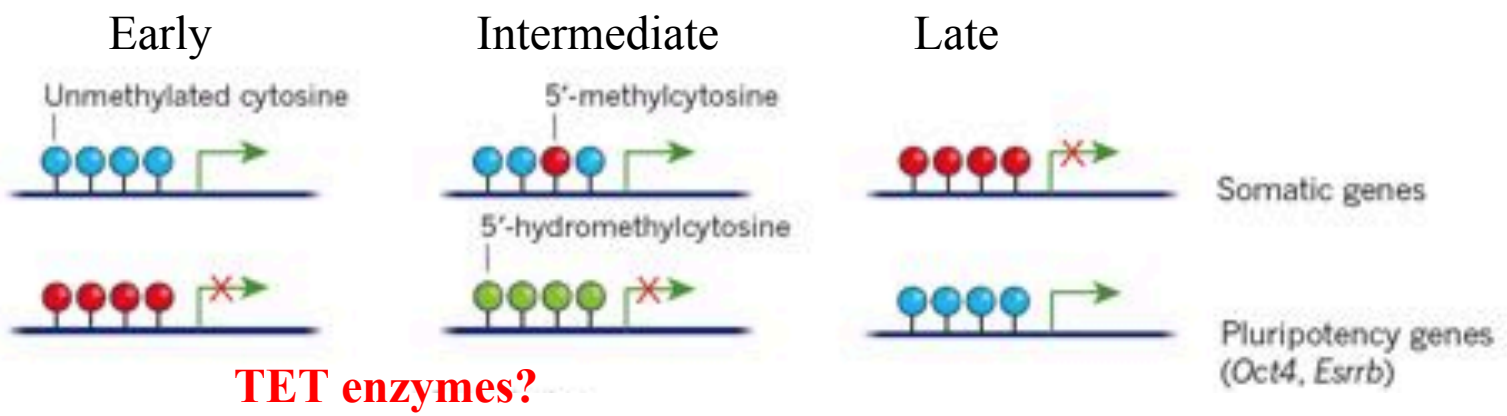


Drugs that interfere with histone modifying enzymes facilitate reprogramming process:
Eg Valproic acid, TSA, SAHA (HDAC inhibitors)
BIX 01294 (G9a H3K9me HMT inhibitor)
Parnate (LSD1 H3K9me/K4me demethylase)

Chemical modulators of signalling pathways can also improve reprogramming:
Eg GSK3 inhibitor (activates Wnt signaling) and MEK inhibitors ("2i" medium)

See Zhang, Li, Laurent and Ding, 2012 for review

DNA Methylation changes during iPS induction

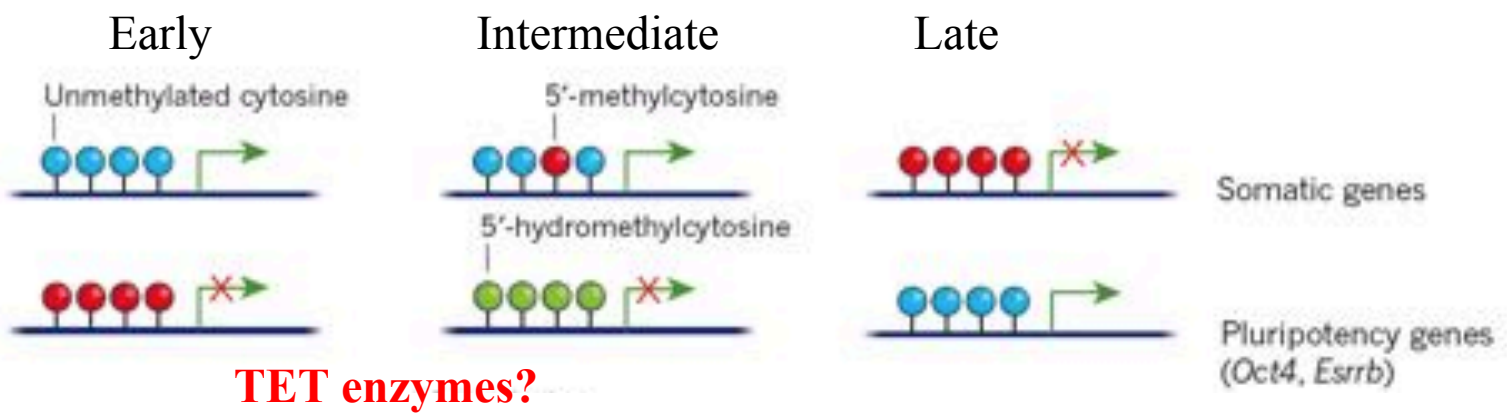


Dnmt3a and Dnmt3b KO have no effect on reprogramming (Pawlak and Jaenisch, 2011).
⇒ silencing of lineage-specific genes is mainly via H3K27 or H3K9 methylation?

Endogenous pluripotency genes are initially methylated – how are they demethylated?

- Decreased Dnmt1 levels facilitate reprogramming => some passive loss
- TET 1 + 2 enzymes interact directly with Nanog
- TET1/2/Nanog over-expression facilitates iPS
- TET1 overexpression can substitute for Oct4 in iPS reprogramming => TET1 probably required for activation of endogenous pluripotency genes…but still not clear How!

DNA Methylation changes during iPS induction



Active or passive DNA demethylation? (as for germ line, not entirely clear…)

Tet2 is required during early phases of iPS reprogramming for remodeling the chromatin at the promoters of key pluripotency genes in a demethylation-independent manner. Doege, C.A. *et al.* Early-stage epigenetic modification during somatic cell reprogramming by Parp1 and Tet2. *Nature* **488**, 652–655 (2012).

Tet1 also increases iPS reprogramming efficiency – probably by accelerating Oct4 transcriptional activation. Tet1 interacts with both Oct4 and Nanog, and a complex including Oct4, Nanog, and Tet1 may exist. Tet1 can replace Oct4 in the rOKSM reprogramming cocktail. Costa et al. (2013) and Gao et al. (2013).

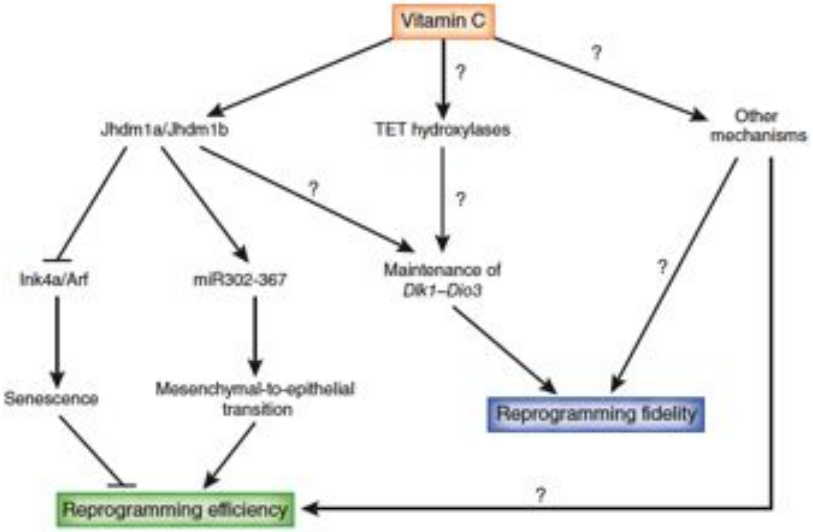
Costa et al. and Gao et al. show that Tet1 is an important component of the reprogramming process (although ESCs with a double knockout of Tet1 and Tet2 maintain pluripotency and can form viable, fertile offspring) (Dawlaty et al., 2013).

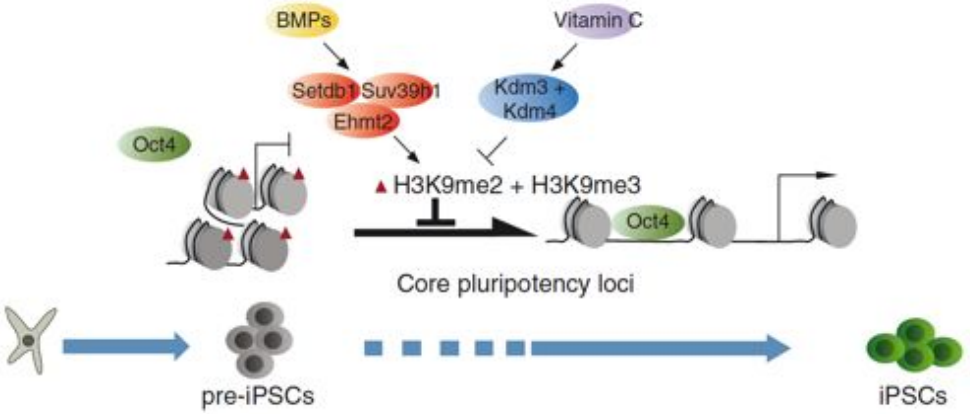
TET Enzymes, Vitamin C and iPS

Vitamin C enhances the efficiency of iPS cell generations (Esteban et al, 2010).

Vitamin C also improves the quality of reprogramming, allowing the generation of all-iPSC mice from both mouse fibroblasts and B lymphocytes (Stadtfeld et al, 2012).

Vitamin C – maintains normal maternal expression of some imprinted genes (eg Dlk1-Dio3), participates in overcoming the H3K9me epigenetic barrier, modulates Tet1 action (positive or negative) in reprogramming (Chen et al, 2013)…





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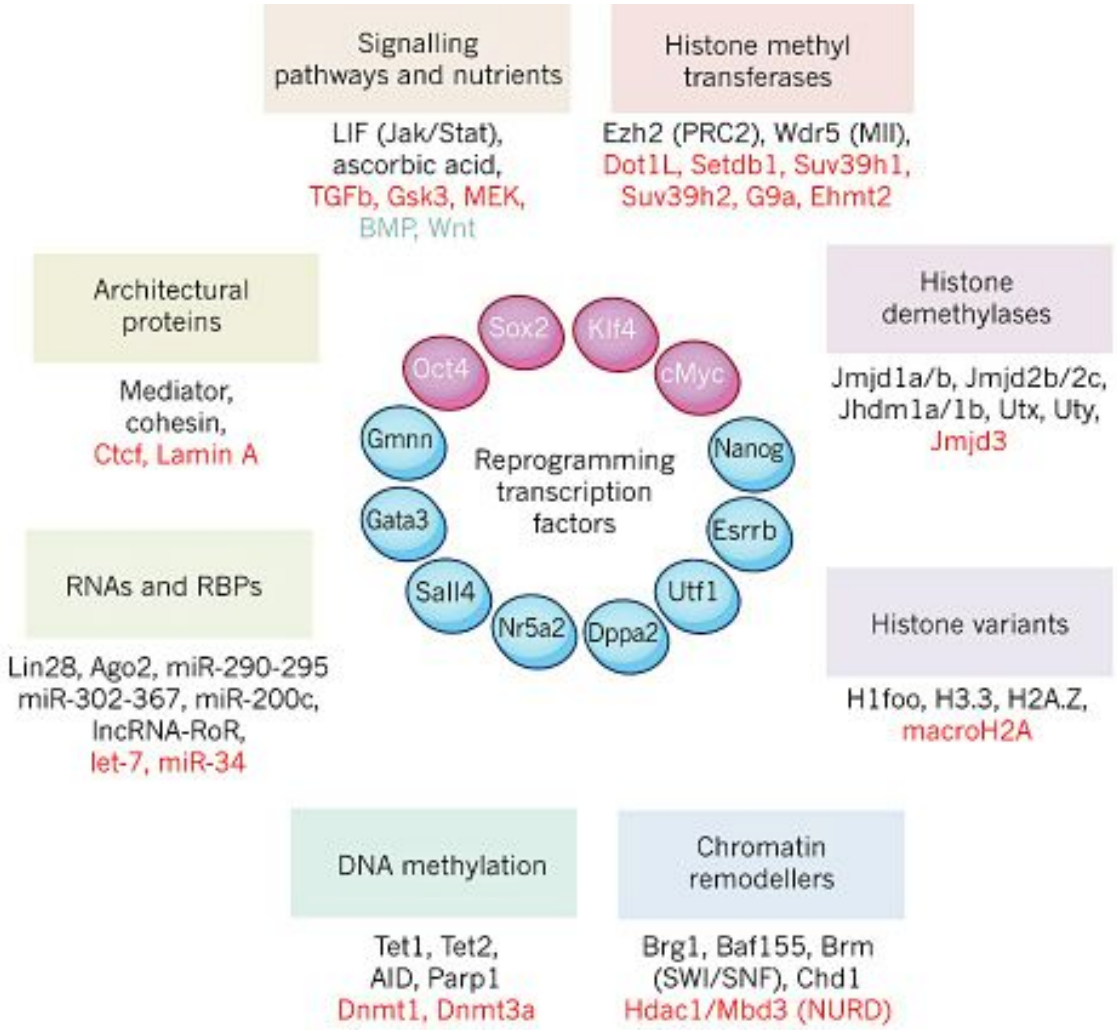
Factors involved in the Induction of Pluripotency

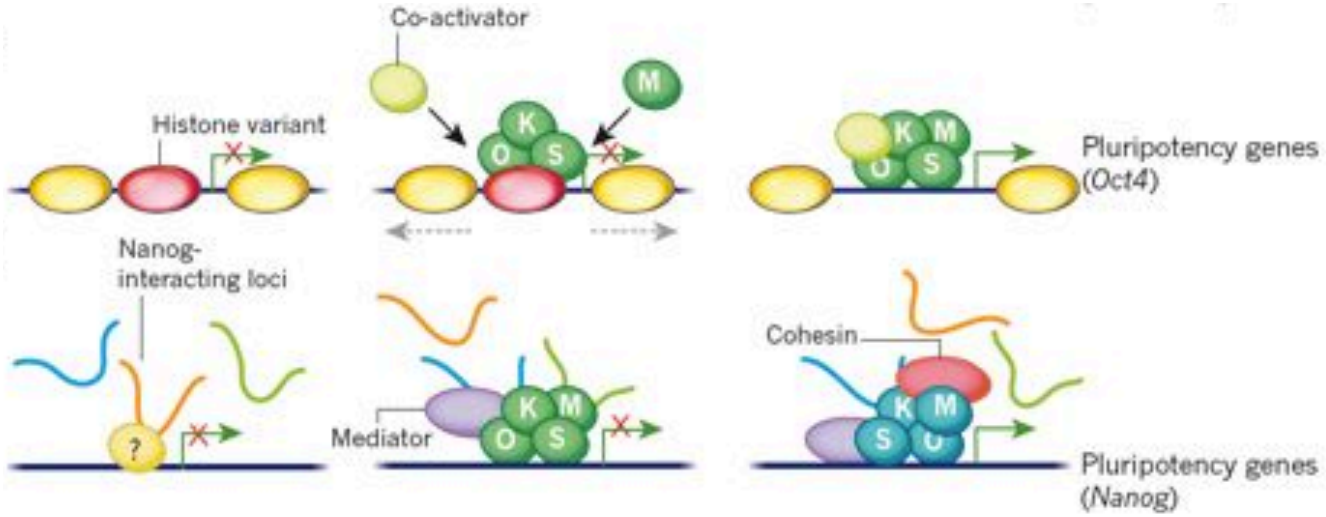


From Apostolou and Hochedlinger, Nature 2013

Nuclear and chromosome reorganisation during iPS

Klf4 Organizes Long-Range Chromosomal Interactions with the *Oct4* Locus in Reprogramming and Pluripotency

Zong Wei,[1,2] Fan Gao,[1,2,3,4] Sewoon Kim,[1,2] Hongzhen Yang,[1,2] Jungmook Lyu,[5] Woojin An,[2] Kai Wang,[3,4] and Wange Lu[1,2,*]



Nuclear and chromosomal organisation may also participate in the establishment of pluripotency

However cause and effect may be difficult to distinguish!

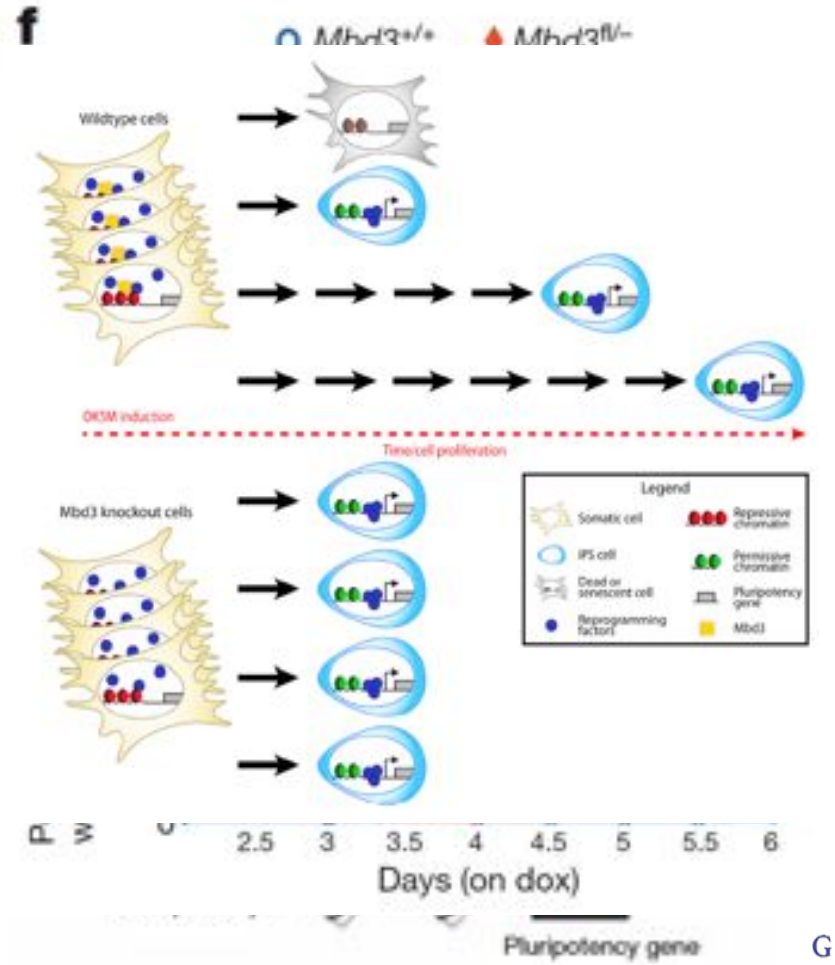
Overcoming Epigenetic Barriers in Reprogramming?

Removing Reprogramming Roadblocks: Mbd3 Depletion Allows Deterministic iPSC Generation

Justin Brumbaugh[1,2] and Konrad Hochedlinger[1,2,3,*]

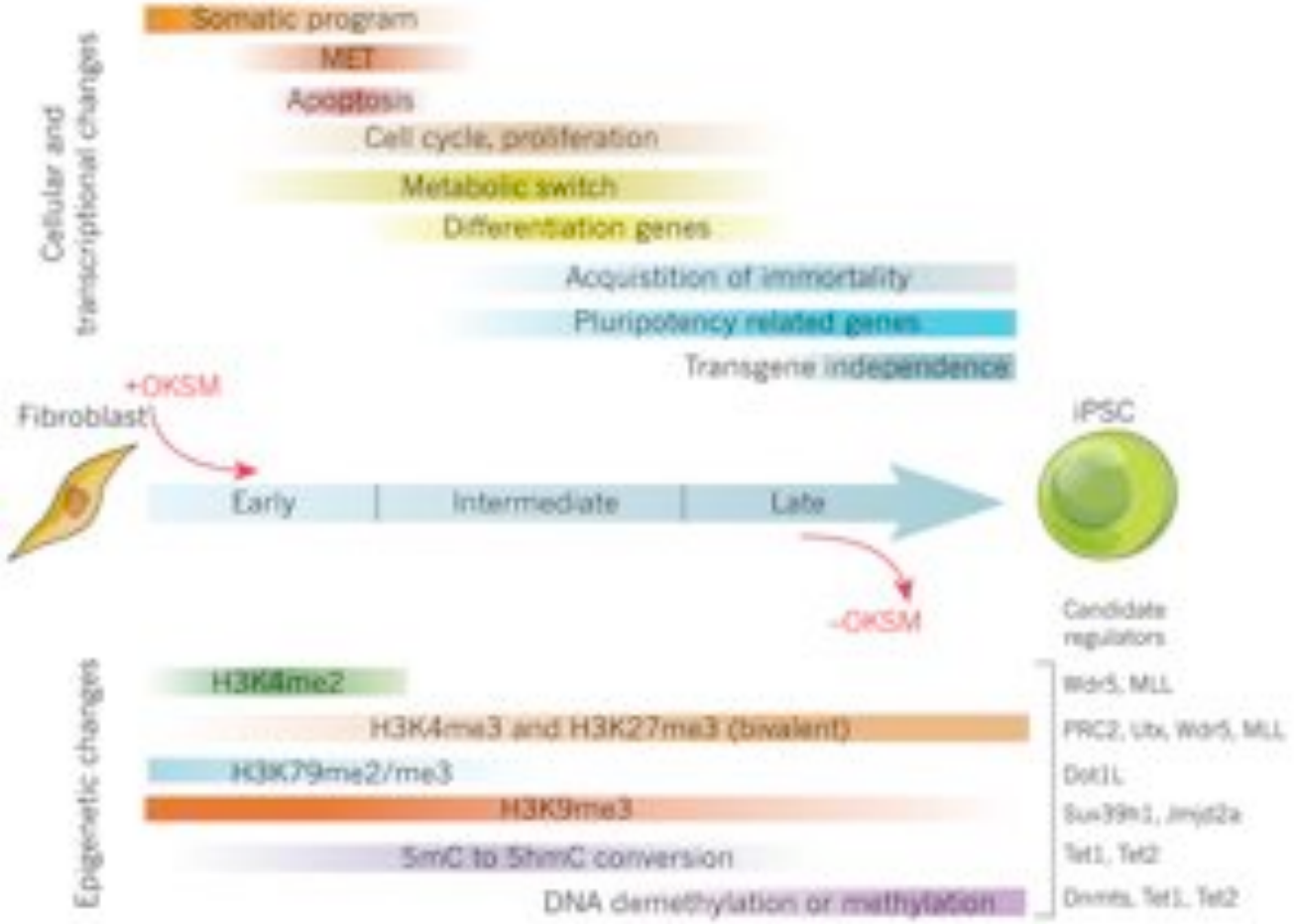
Reis et al (2014) "Deterministic direct reprograming of somatic cells to pluripotency". *Nature* 502, 65-70.

- Mbd3 depletion in fibroblast leads to rapid, 100% iPS efficiency!
- Mbd3 is NOT present at OKS target genes *prior* to exogenous OKSM.
- Mbd3/NURD become recruited (directly?) after OKSM induction – and act as *repressors* of pluripotency genes, counteracting reactivation.
- OKS and the positive iPS propelling factos Utx and Wdr5 were both essential even in Mnd3 -/- cells.
- => "Gas and Brakes" model: OKSM interact with multiple partners that both *promote* (Utx, Wdr5) and *prevent* (Mbd3, NuRD) pluripotency gene activation. Absence of Mbd3 allows uninterrupted progression of iPS – with no "intermediate" phase – but possible higher risk of uncontorlled proliferation?



Summary on Mechanisms of induced Pluripotency

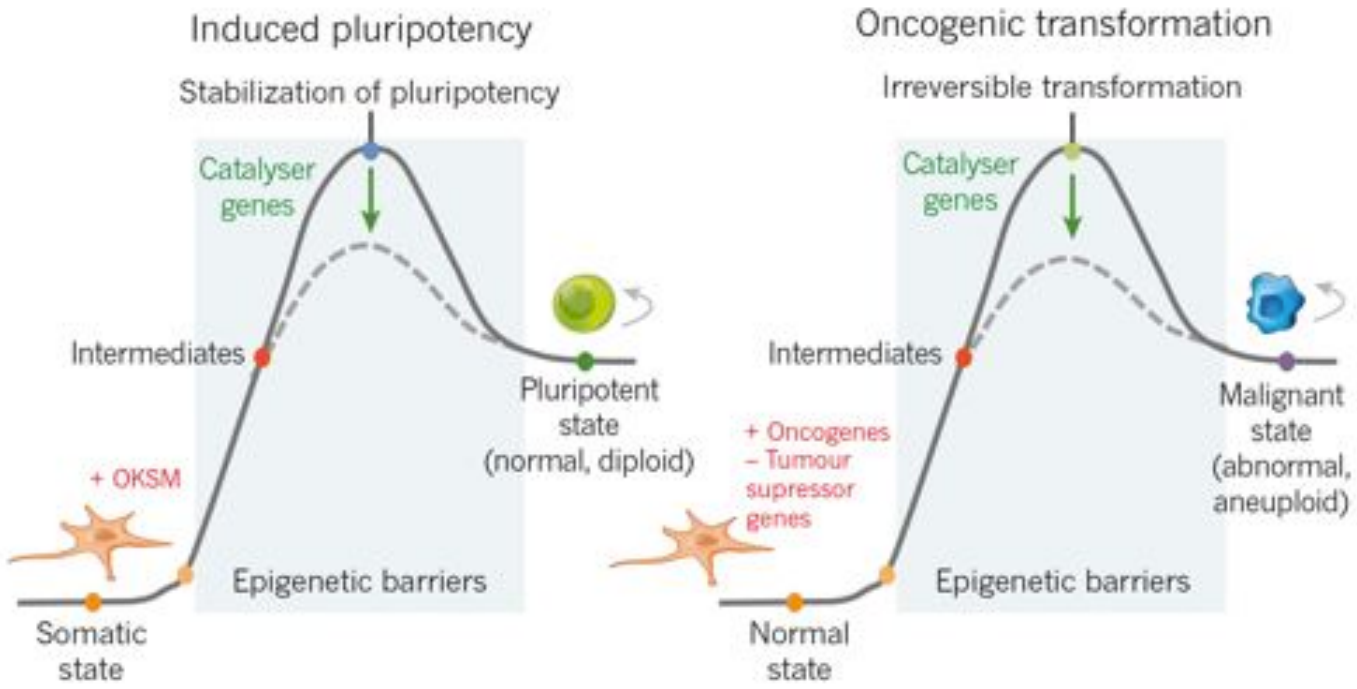
Some similarities to developmental reprogramming –
shutting down of somatic gene expression; activation of pluripotent or totipotent program
but also major differences – in *precision*, *timing* and *efficiency*



Apostolou and Hochedlinger, NRG, 2015

Similar Epigenetic Barriers in Reprogramming and Cancer?

Apostolou and Hochedlinger, NRG, 2015



- Similar epigenetic barriers are faced by **nascent iPS** cells and **pre-malignant** cells
- Same epigenetic regulators, Utx, mH2A, Jhdm1b, PRC2, Tet2, Dnmts are involved in iPS and tumorigenesis
- Progentior and stem cells may be more prone to tumorigensis and reprogramming than differenitated cells – due to a more "permissive" epigenetic environment (eg much lower H3K9 methylation and DNA methylation)?

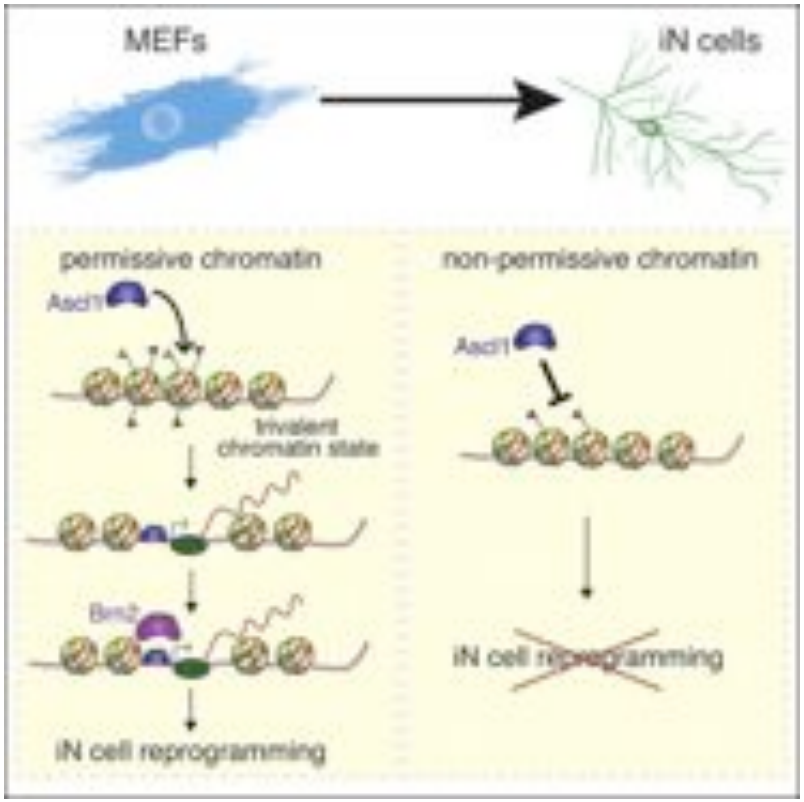
⇒ Must check iPSCs stringently for **mutation** or **epimutation** prior to any therapeutic use!!

⇒ ***Or* direct programming** as an alternative to induced pluripotency and redifferentiation…

E.

Direct cell conversion mechanisms

Hierarchical mechanism for direct conversion of *fibroblasts* into induced *neuronal* cells by the transcription factors Ascl1, Brn2, and Myt1l.



- Ascl1 acts as "on-target" pioneer factor - immediately occupies cognate sites in fibroblasts.
- Brn2 and Myt1l do not access fibroblast chromatin productively on their own -rely on Ascl1
- Unique trivalent chromatin signature (H3K4me1, H3K27acetyl, and H3K9me3) in the host cells predicts the permissiveness for Ascl1 pioneering activity among different cell types.

⇒ a precise match between pioneer factors and the chromatin context at key target genes determines transdifferentiation to neurons and likely other cell types.

CONCLUSION

"The idea that the differentiated state of a cell may not be terminal but rather, stable, underscores the importance of identifying environmental factors that maintain the stable differentiation cell state, an aspect that has not been widely explored and that might offer novel solutions for producing fully functional mature cell types from human pluripotent stem cells."

Sanchez- Alvarez and Yamanaka, Cell 40th Anniversary issue
"Rethinking Differentiation: Stem Cells, Regeneration, and Plasticity"