CHAIRE ÉPIGÉNÉTIQUE ET MÉMOIRE CELLULAIRE

Année 2021
“Mémoire cellulaire ”

15 mars, 2021

Cours 3
Suite et fin: *Stabilité et plasticité au cours du développement*

Maintien de l’identité cellulaire dans les cellules non-prolifératives
Maintaining cellular identity in non-dividing cells

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CHAIRE ÉPIGÉNÉTIQUE ET MÉMOIRE CELLULAIRE

COURS 1 (lundi 1er mars 10h-12h)
Introduction

COURS 2 (lundi 8 mars 10h-12h)
Stabilité et plasticité au cours du développement
Stability and plasticity during embryonic development

COURS 3 (lundi 15 mars 10h-12h)
Maintien de l'identité cellulaire dans les cellules non-prolifératives
Maintaining cellular identity in non-dividing cells

COURS 4 (lundi 22 mars 10h-12h)
Stabilité génétique et épigénétique au cours du vieillissement
Genetic and epigenetic stability during ageing

COURS 5 (lundi 29 juin 10h-12h)
Perte d'idéntité cellulaire au cours de la reprogrammation et dans des pathologies
Losing cellular identity during reprogramming and in disease

COURS II - CONTINUED

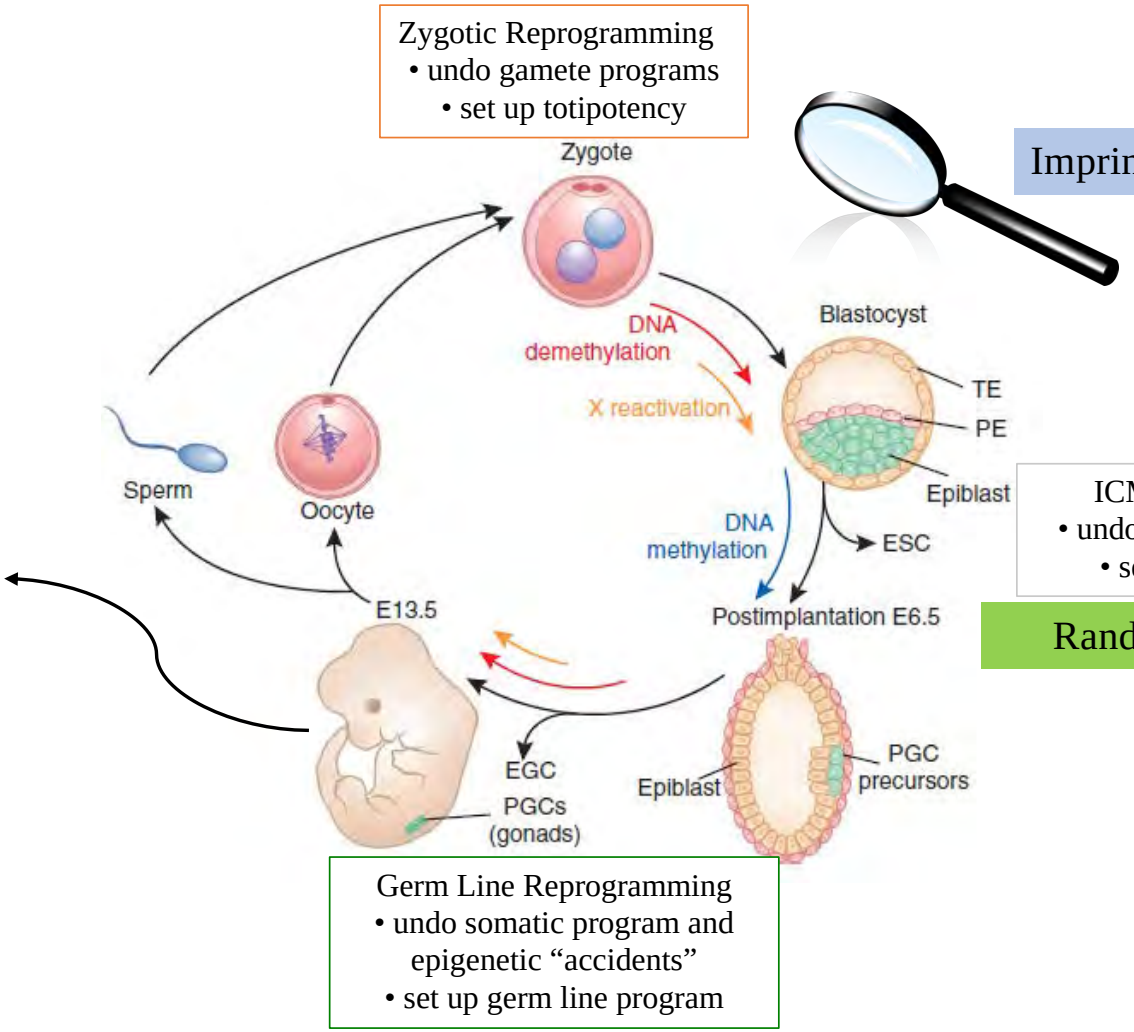
1. Cellular memory during embryogenesis: stability and plasticity
2. Tracing cell identity and cell fate during embryogenesis
3. Establishing cellular memory during development
4. Epigenetic dynamics during early mouse development
5. Strategies that enable cellular memory: the epigenetic machineries
6. Lessons from X-chromosome inactivation: stability and plasticity in development

THIS WEEK:

- more about establishing memory from embryo to soma
- transient allelic effects during early development

- Next week - COURS III+IV –

Stability and Plasticity during Mammalian Pre-Implantation Development



Imprinted X inactivation

ICM Reprogramming
• undo/prevent TE program
• set up Pluripotency

Random X inactivation

E. Heard, 8 mars, 2021

Adapted from Cantone and Fisher, 2013

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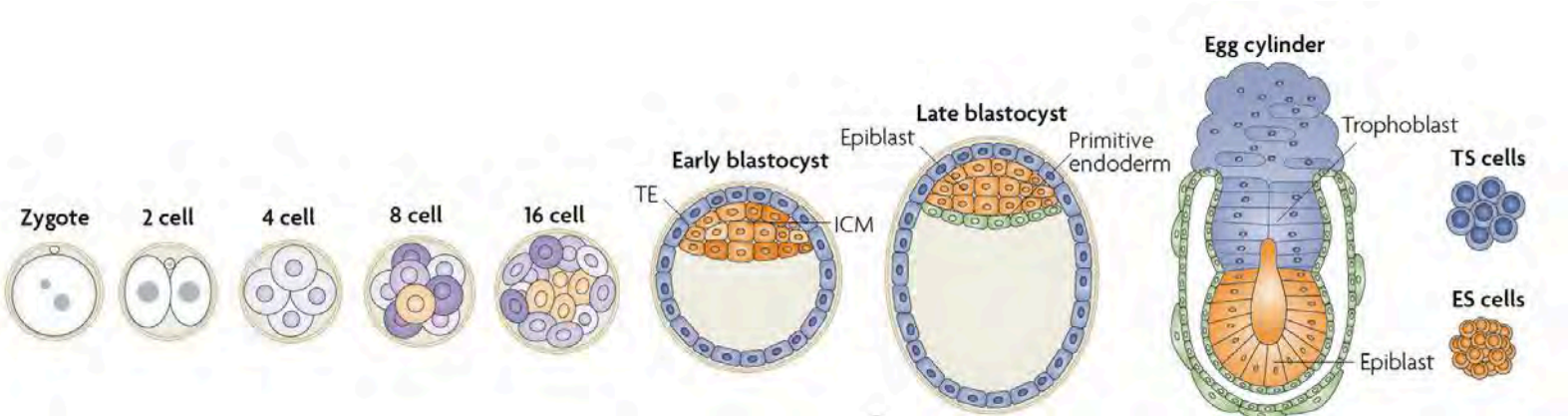
Establishing and maintaining early lineage decisions during Mouse development



• Progressive restriction of cellular plasticity from 4-cell stage

• Positional cues start to play a role at ~8-16 cell morula stage:

-**inner cells** tend to form inner cell mass (epiblast = soma + germ line; primitive endoderm)

-**outer cells** tend to form trophectoderm TE (extra-embryonic tissues)

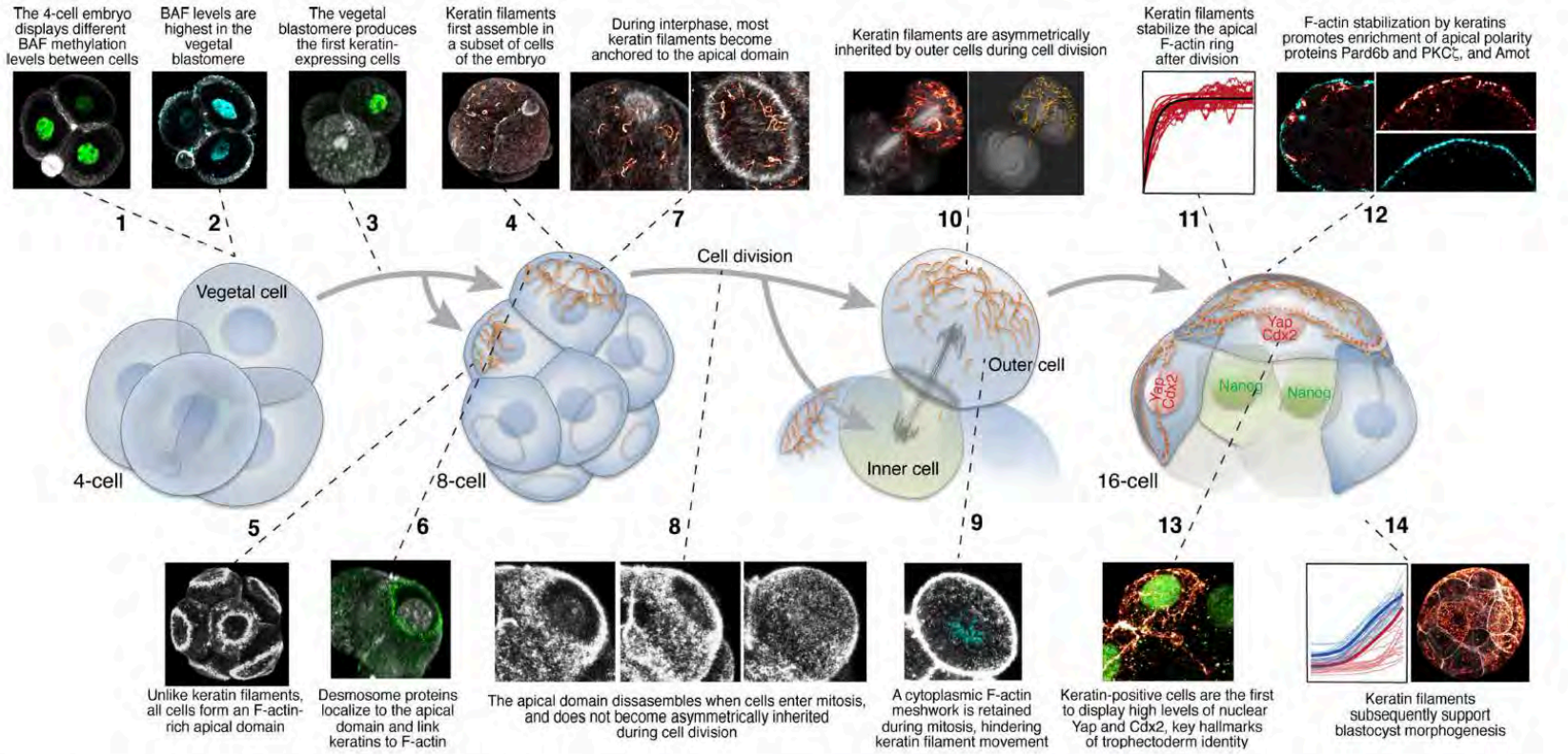
• Key transcription factors are essential to determine cell fate and establish cell lineages of the early embryo

• Chromatin factors (eg histone modifiers CARM1, SETDB1, PRC2, G9a) provide permissive (or non-permissive) environment for cell fate, and/or predispose a cell towards a particular lineage.

• Chromatin marks and DNA methylation also progressively lock in active and inactive states

Early decisions directing cell fate

Keratins are asymmetrically inherited fate determinants in the mammalian embryo
They specify the first Trophectoderm cells by inducing CDX2 via YAP
BAF-mediated heterogeneity at the 4-cell stage leads to cell-cell variation in keratin expression
Early cell-to-cell variability is transmitted through divisions to influence lineage fate.

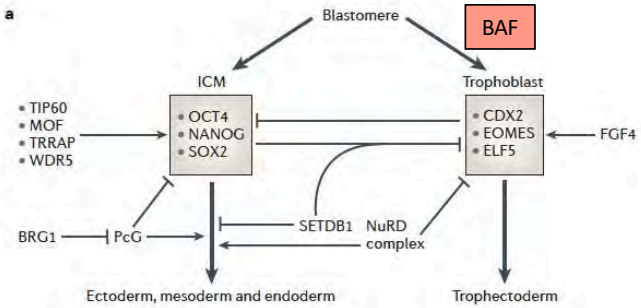


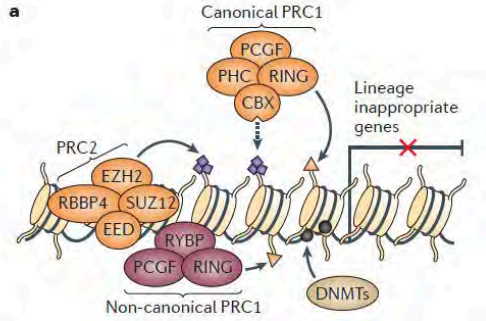
Chromatin: enabling developmental transitions and memorising activity states?

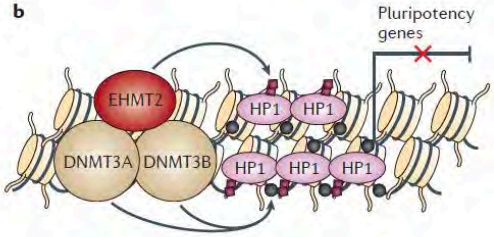
Chromatin modifiers and remodellers: regulators of cellular differentiation

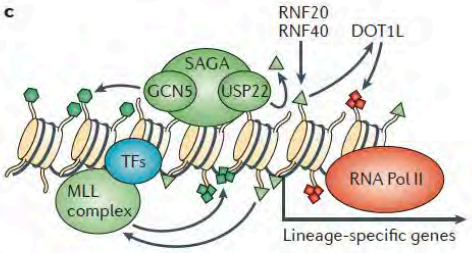
Taiping Chen[1–3] and Sharon Y. R. Dent[1–3]

Abstract | Cellular differentiation is, by definition, epigenetic. Genome-wide profiling of pluripotent cells and differentiated cells suggests global chromatin remodelling during differentiation, which results in a progressive transition from a fairly open chromatin configuration to a more compact state. Genetic studies in mouse models show major roles for a variety of histone modifiers and chromatin remodellers in key developmental transitions, such as the segregation of embryonic and extra-embryonic lineages in blastocyst stage embryos, the formation of the three germ layers during gastrulation and the differentiation of adult stem cells. Furthermore, rather than merely stabilizing the gene expression changes that are driven by developmental transcription factors, there is emerging evidence that chromatin regulators have multifaceted roles in cell fate decisions.









Chen and Dent, Nature Reviews Genetics, 2014

E. Heard, 8 mars, 2021

Developmental phenotypes due to mutation of chromatin modifiers : can have one or more roles in development

	Modifier	Function	Mutant Phenotype	Maternally Inherited	ES Cell Derivation	Reference
	Histone Modifications					
H3K9me pathways	Glp/Ehmt1	HMTase	Severe growth retardation and lethality at E9.5; reduction of H3K9me1 and H3K9me2 in embryos	ND	yes	Tachibana et al. (2005)
	G9a/Ehmt2	HMTase	Loss of H3K9 methylation in euchromatin; developmental and growth arrest at E8.5	yes	yes	Tachibana et al. (2002)
	Eset/ SETDB1	HMTase	Peri-implantation lethality (between E3.5 and E5.5); defects in ICM outgrowth	yes	no	Dodge et al. (2004)
	Suv39h1 Suv39h2	HMTase	Double knockout shows loss of H3K9 methylation in heterochromatin; polyploidy in MEF cells; chromosome pairing defects during spermatogenesis; male sterility and death of some double-mutant embryos at E14.5	ND	yes	Peters et al. (2001)
H3K27me pathways	Ezh2/ Enx-1	HMTase PRC2 complex	Growth defect of the primitive ectoderm; peri-implantation lethality	yes	no	O'Carroll et al. (2001)
	Eed	PRC2/3 complex	Defective gastrulation; failure to maintain inactive X in trophoblast cells	yes	yes	Shumacher et al. (1996)
	Suz12	PRC2/3 complex	Early postimplantation lethality; gastrulation defects	yes	ND	Pasini et al. (2004)
	YY1	PRC2/3 interaction	Defects in epiblast cell growth/survival; peri-implantation lethality	yes	no	Donohoe et al. (1999)
	Ring1b/ Rnf2	Ubiquitin ligase PRC1 complex	Gastrulation defects; lethality by E9.5	yes	ES viable	Voncken et al. (2003)
	DNA Methylation					
DNA methylation pathways	Dnmt1	DNA MTase	Genome-wide demethylation; developmental arrest at E8.5	yes	yes	Li et al. (1992)
	Dnmt3a	DNA MTase	Malfunction of gut; spermatogenesis defects; postnatal lethality (~4 weeks of age)	yes	yes	Okano et al. (1999)
	Dnmt3b	DNA MTase	Demethylation of minor satellite DNA; mild neural tube defects; embryonic lethality at E14.5–E18.5	yes	yes	Okano et al. (1999)

Full repression of repeats and certain genes

Bivalent states: ready for signal to activate or silence

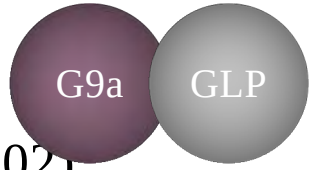
Primed: setting up chromatin state for later gene expression

Fully active: gene expression

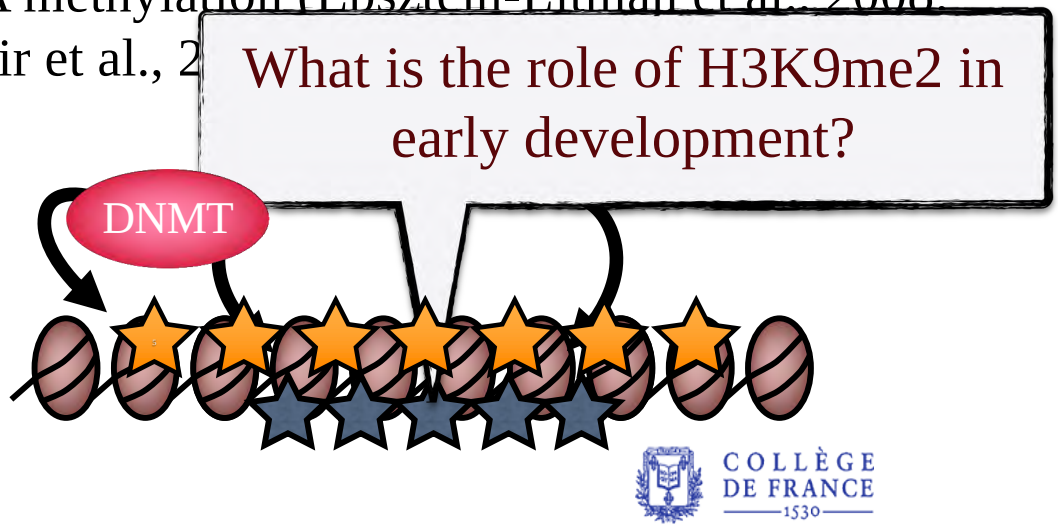
What is the role of G9a during development

Jan Zylicz
Novo Nordisk Foundation
Center for Stem Cell Biology,
University of Copenhagen

G9a helps establish silenced domains



- G9aKO lethal at ~E9.5, but why? (Tachibana et al., 2002)
- G9a is an H3K9 methyltransferase (Tachibana et al., 2001)
- In vitro H3K9me2 domains extend into developmentally regulated genes
- H3K9me2 inhibits reprogramming of somatic cells
- G9a targets de novo DNA methylation (Epsztein-Litman et al., 2008; Myant et al., 2010; Auclair et al., 2



Azim Surani's Lab

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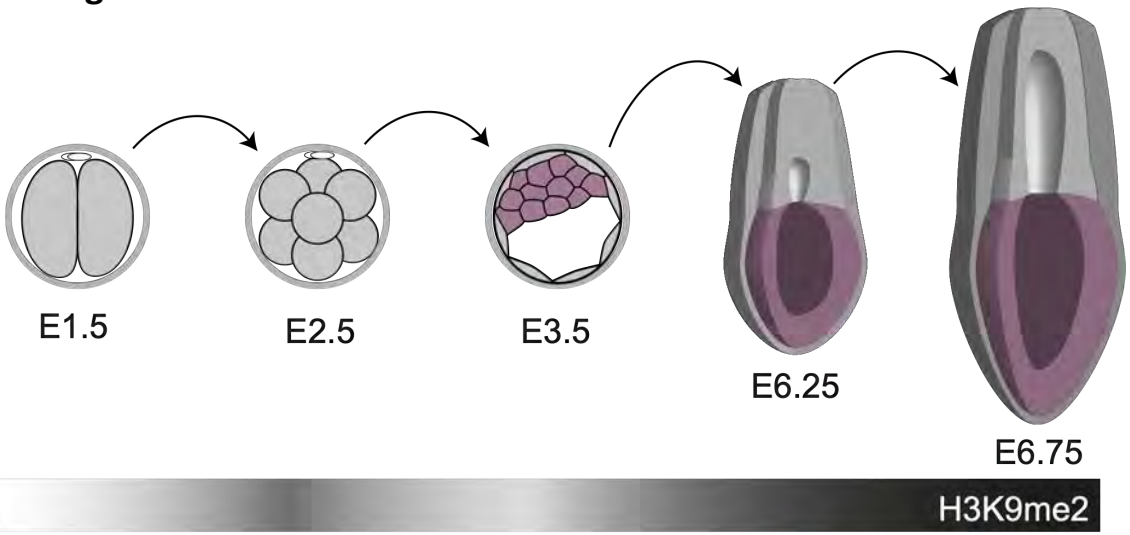
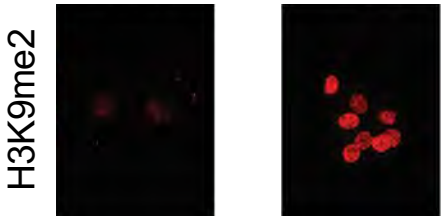
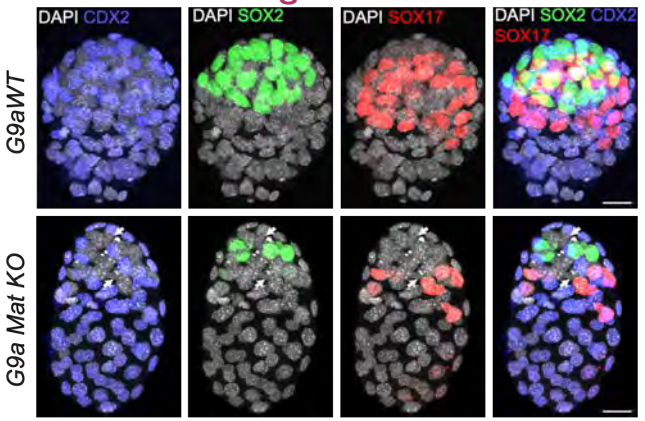
Maternal G9a regulates lineage segregation

Maternal G9a represses a subset of genes induced at 4 cell stage.

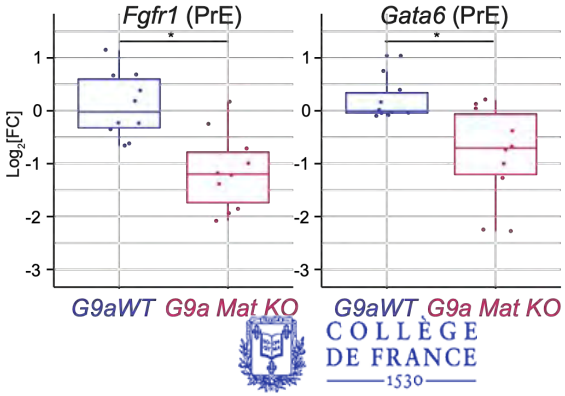




Lineage marker IF



Single Morula RNAseq



Zylicz* & Borensztein* et al., 2018 eLife

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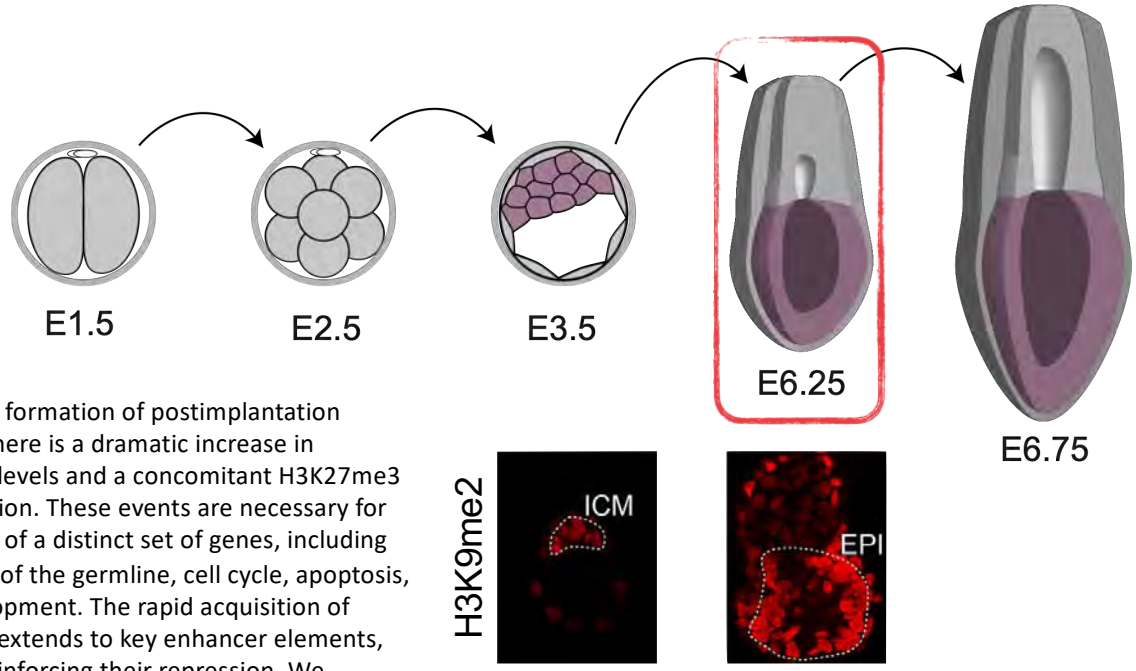
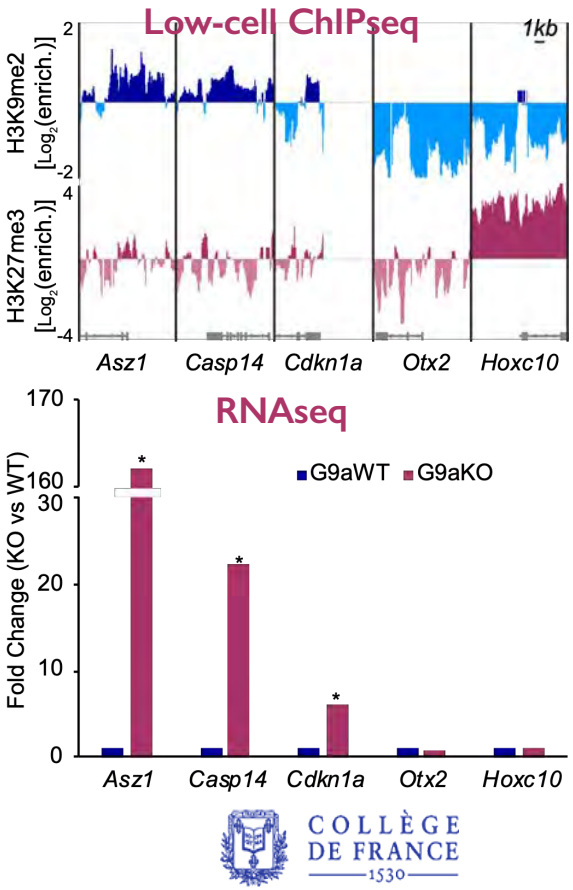
G9a represses late germline and proliferation genes



H3K9me2 accumulates at implantation

During the formation of postimplantation epiblast, there is a dramatic increase in H3K9me2 levels and a concomitant H3K27me3 redistribution. These events are necessary for repression of a distinct set of genes, including regulators of the germline, cell cycle, apoptosis, and development. The rapid acquisition of H3K9me2 extends to key enhancer elements, thereby reinforcing their repression. We propose that such epigenetic programming of epiblast primes a specific gene regulatory network, which is a necessary prerequisite for embryogenesis.



Zylicz et al., 2015 eLife

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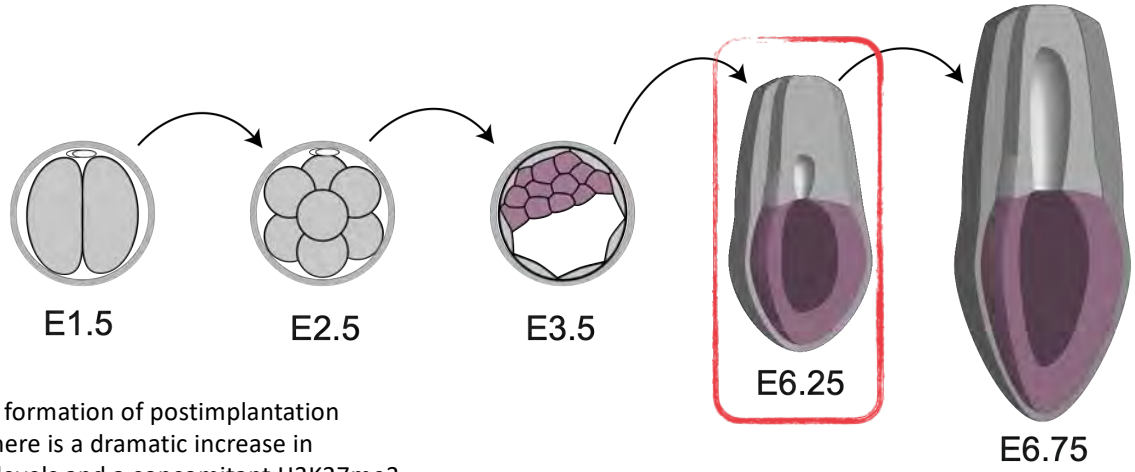

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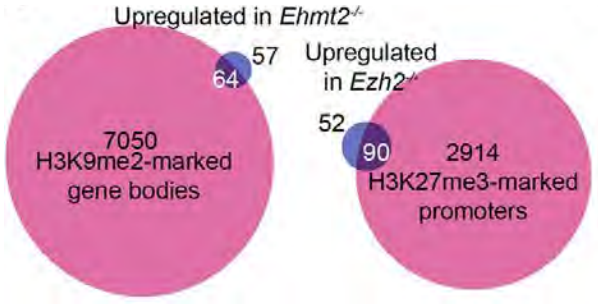
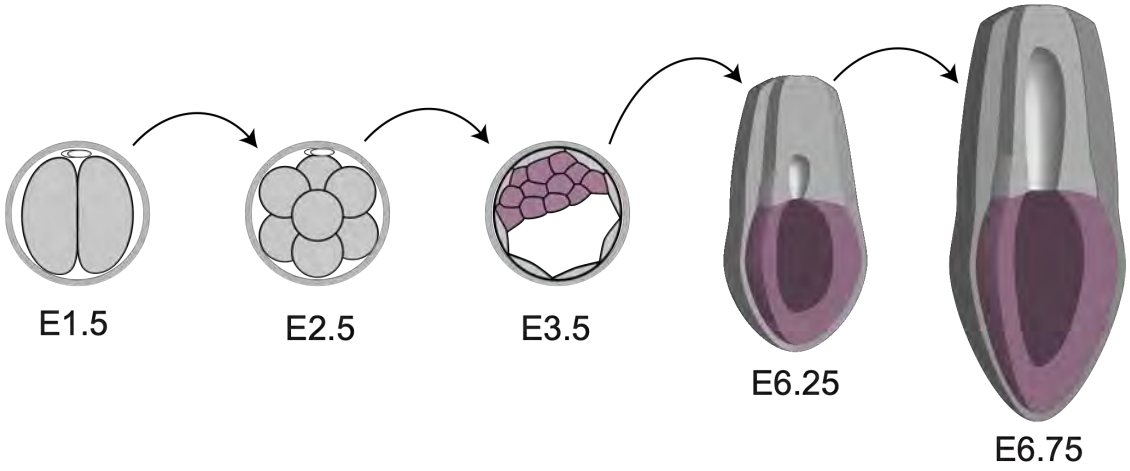
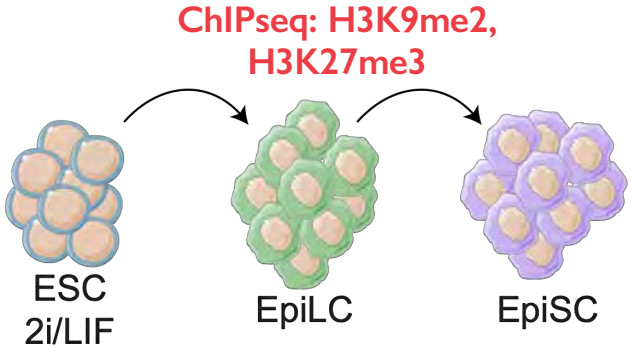
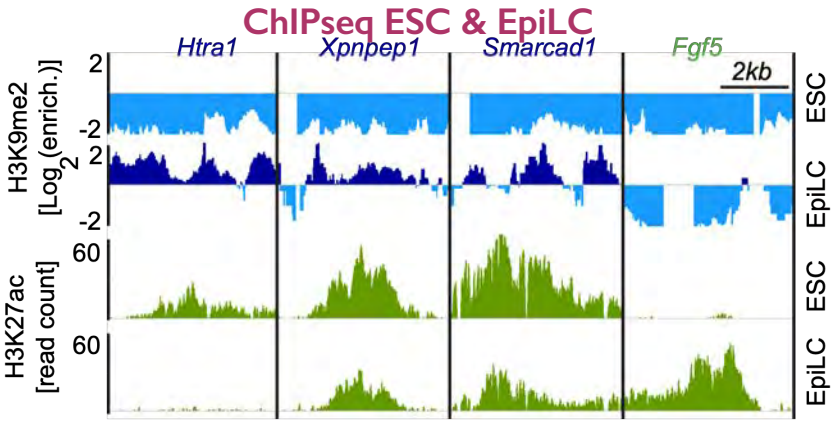


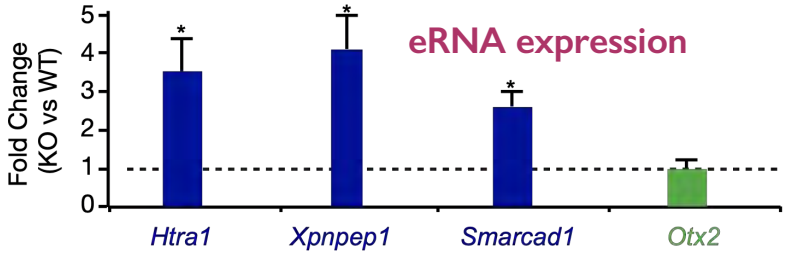
Zylicz et al., 2015 eLife

G9a rapidly represses enhancers during differentiation









Zylicz et al., 2015 eLife

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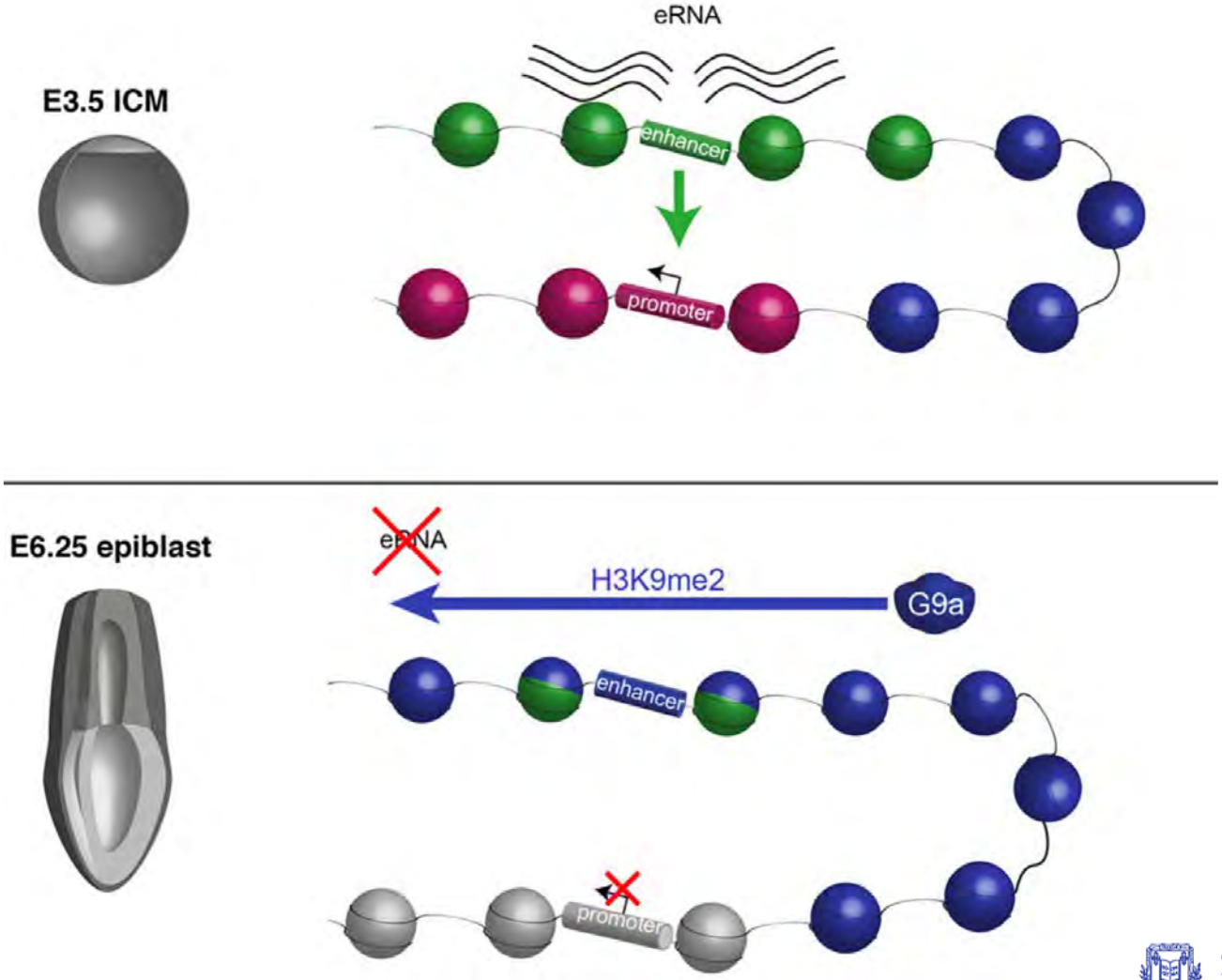
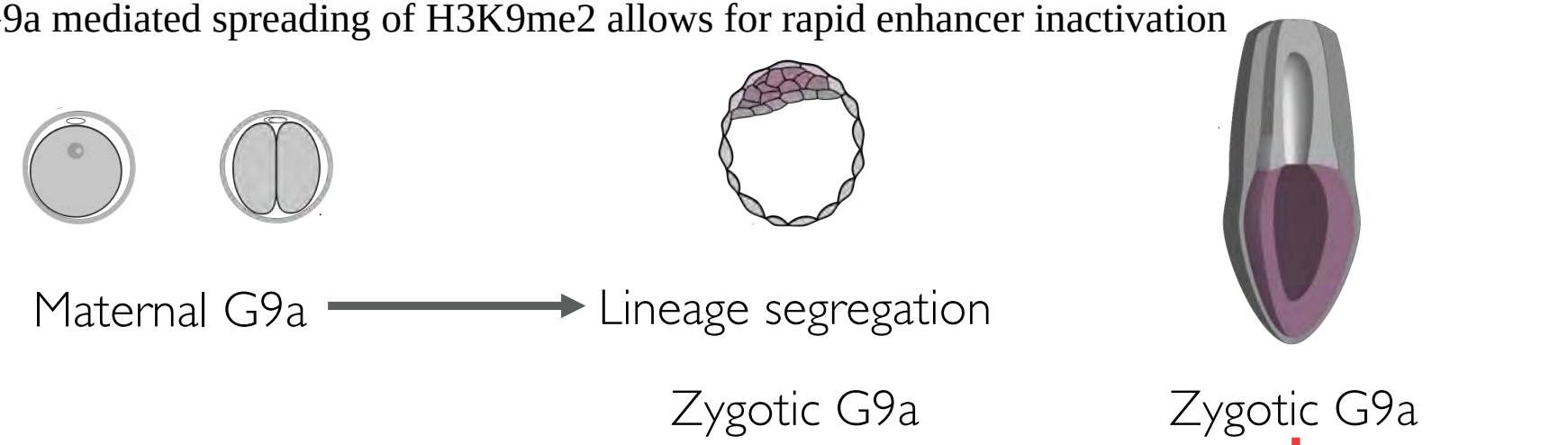


Zylicz et al., 2015 eLife

G9a plays at least two roles in early development

- Maternal G9a pre-sets lineage segregation: several maternally-contributed HMTs pre-set the embryo for later development
- Repressive marks accumulate globally but silence specific transcriptional programmes
- G9a mediated spreading of H3K9me2 allows for rapid enhancer inactivation

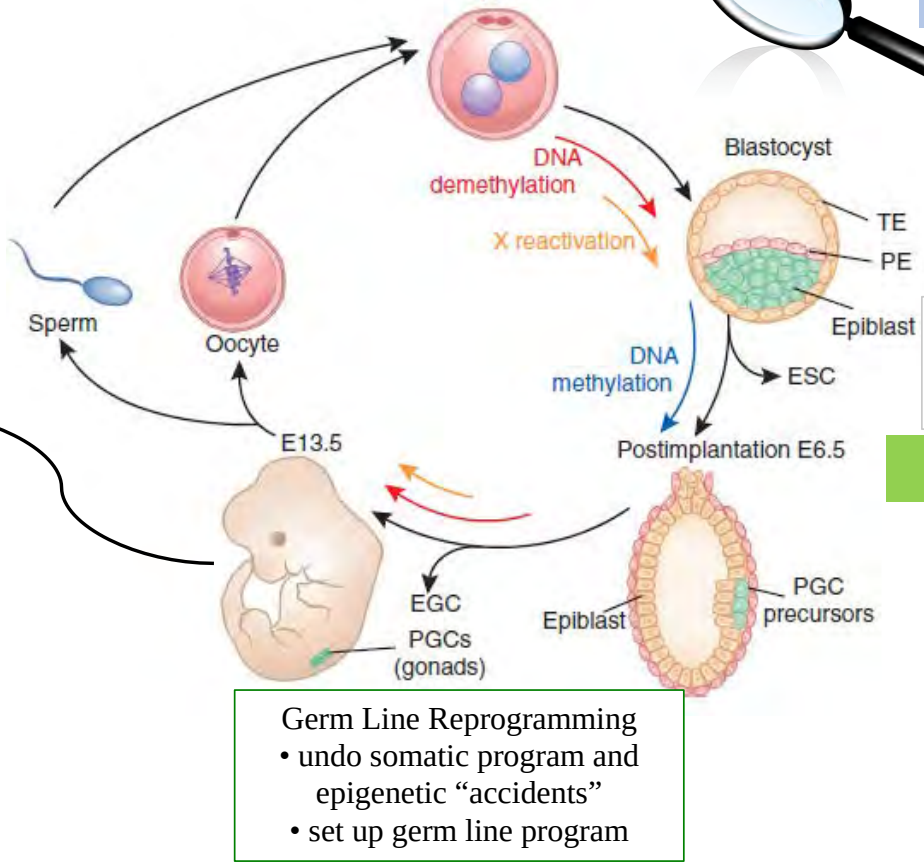


Zylicz* & Borensztein* et al., 2018 eLife

3D Chromatin Structure and Memory of the Parental Epigenomes

Zygotic Reprogramming
• undo gamete programs
• set up totipotency

Imprinted X inactivation



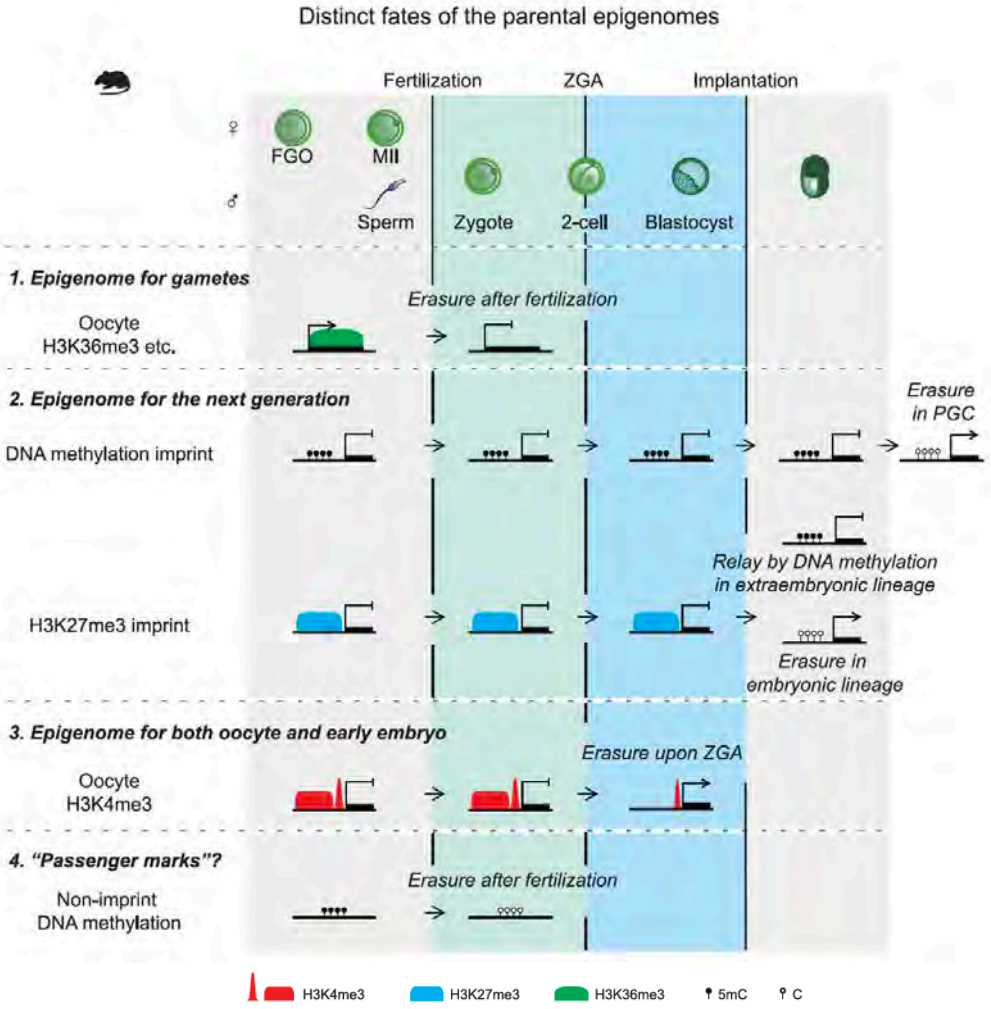
ICM Reprogramming
• undo/prevent TE program
• set up Pluripotency

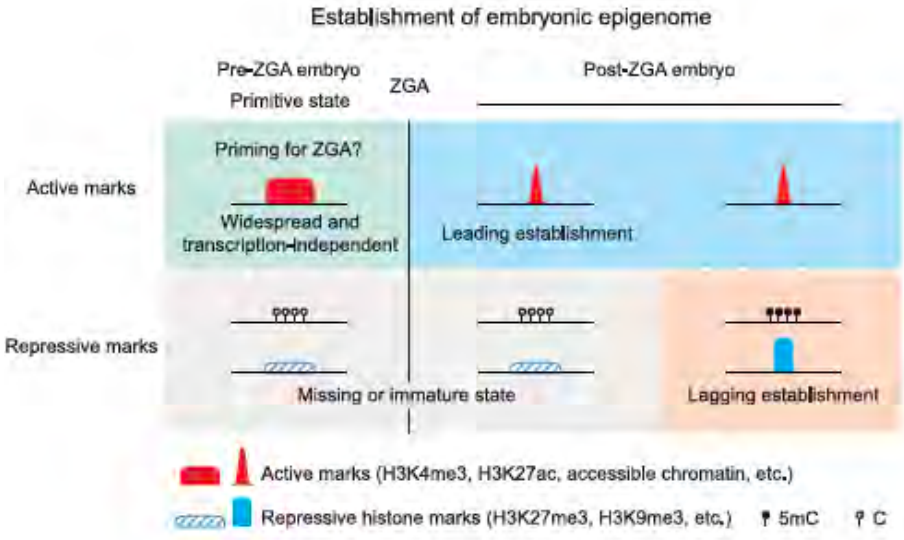
Random X inactivation

Germ Line Reprogramming
• undo somatic program and epigenetic "accidents"
• set up germ line program

Adapted from Cantone and Fisher, 2013

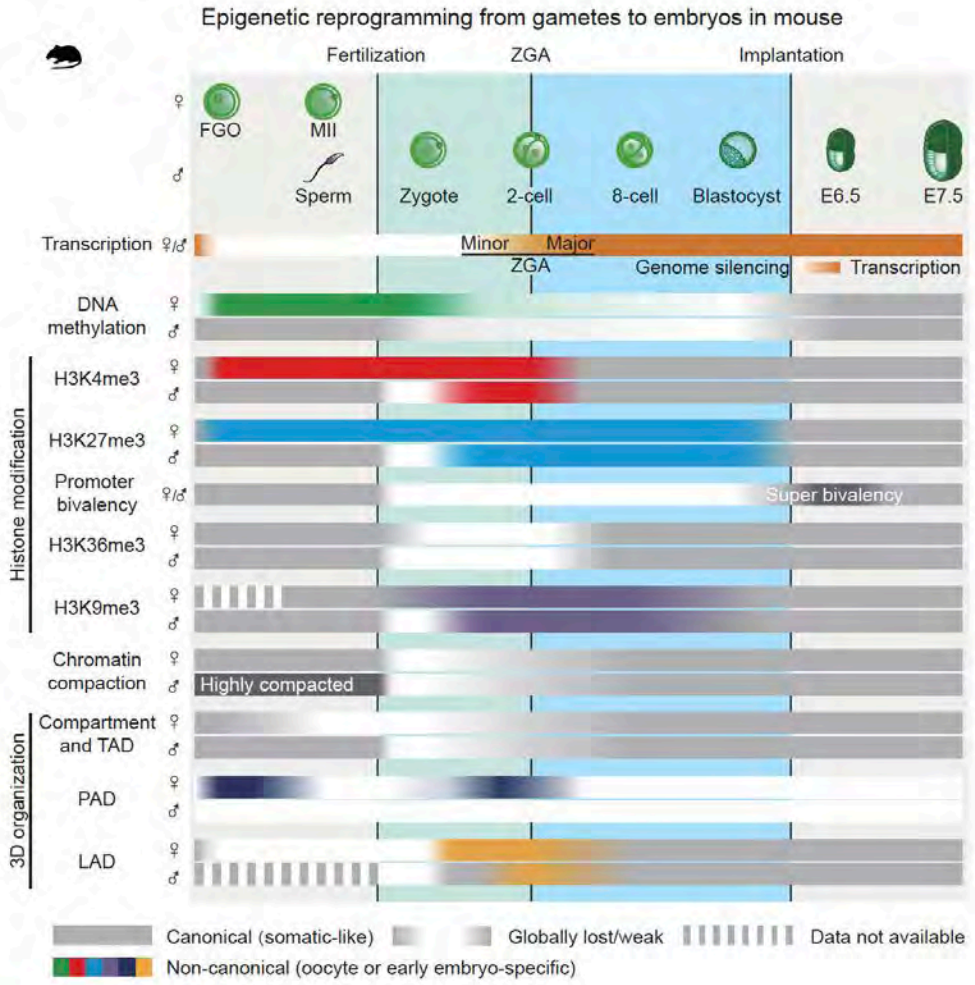
The Fates of the Parental and Embryonic Epigenomes

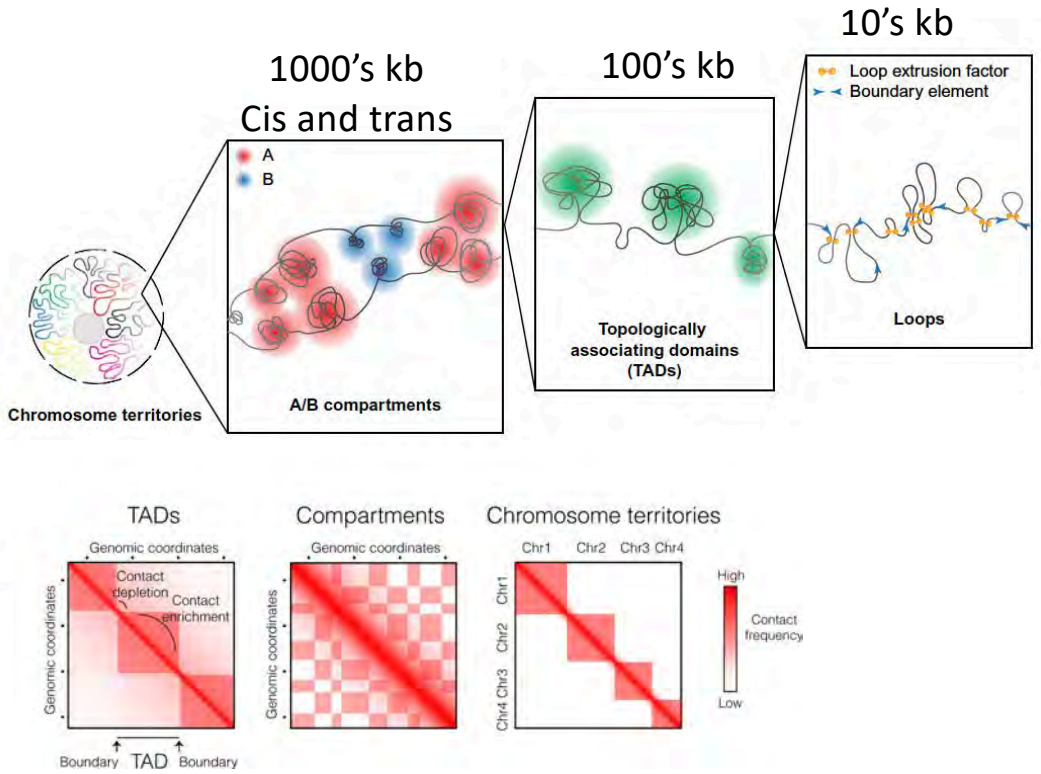




Xia and Xie, Stem Cell Reports 2020

Epigenetic Reprogramming from Gametes to Embryos





FGO, full-grown oocyte;
LAD, lamina-associated domain;
PAD, Polycomb associating domain;
TAD, topological associating domain;
ZGA, zygotic genome activation.

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Xia and Xie, Stem Cell Reports 2020

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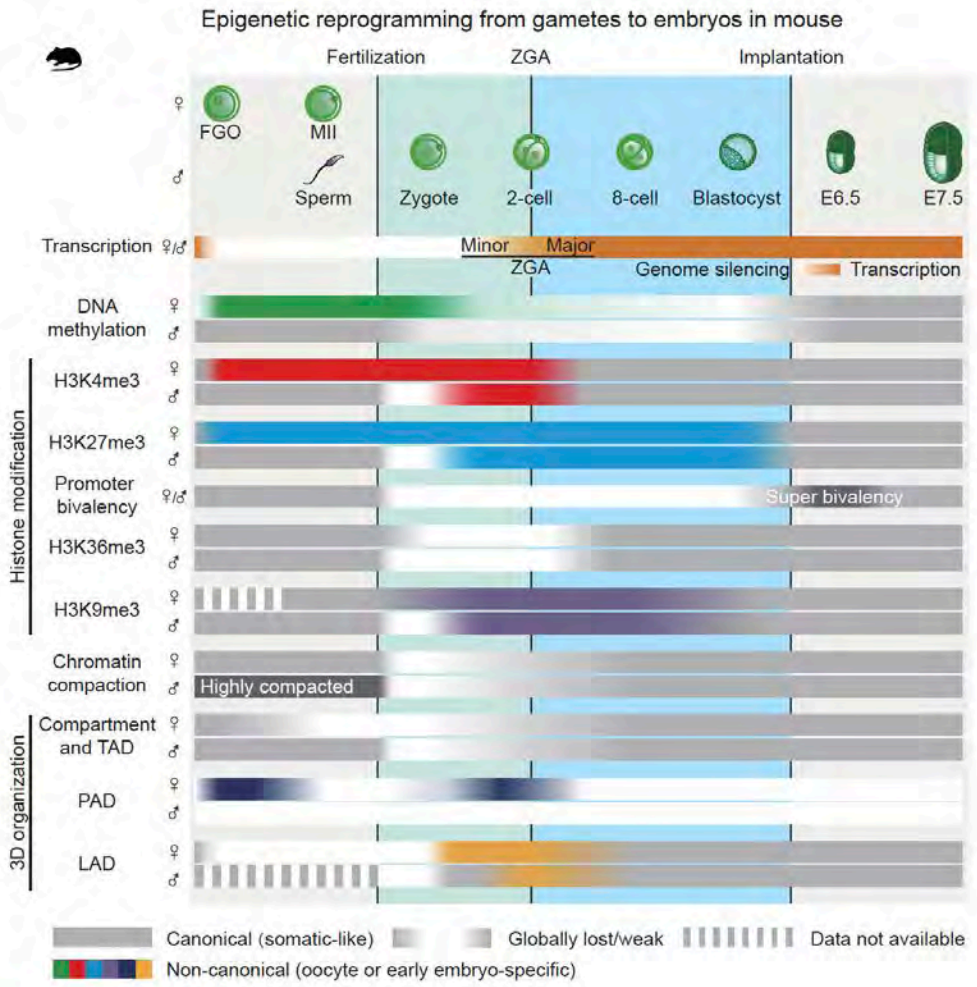
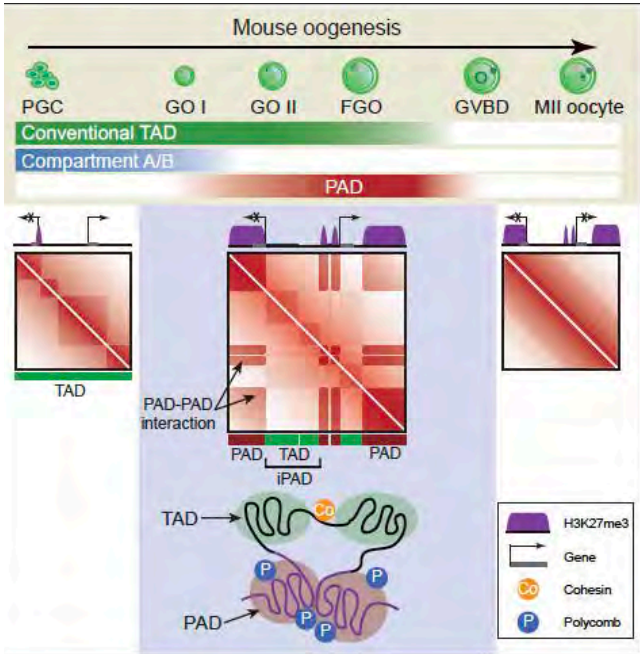
Epigenetic Reprogramming from Gametes to Embryos



E. Heard, 15 mars, 2021

Xia and Xie, Stem Cell Reports 2020

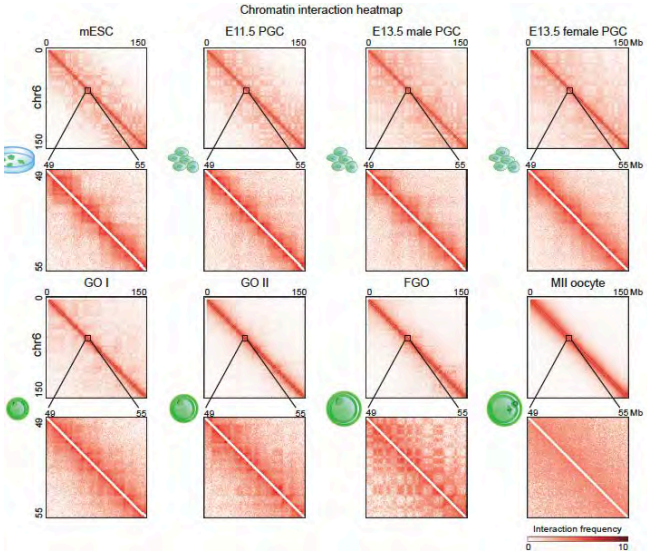
Polycomb Associated Domains (PADs)



Du et al., 2020, Molecular Cell 77, 825–839

- Hi-C analysis of meiotic chromatin architecture during mouse oocyte development
- Late-stage mouse oocytes show unique H3K27me3-marked Polycomb-associating domains
- PADs disassemble upon meiotic resumption but briefly reappear in early embryos
- PADs are regulated by Polycomb proteins and independent of cohesin

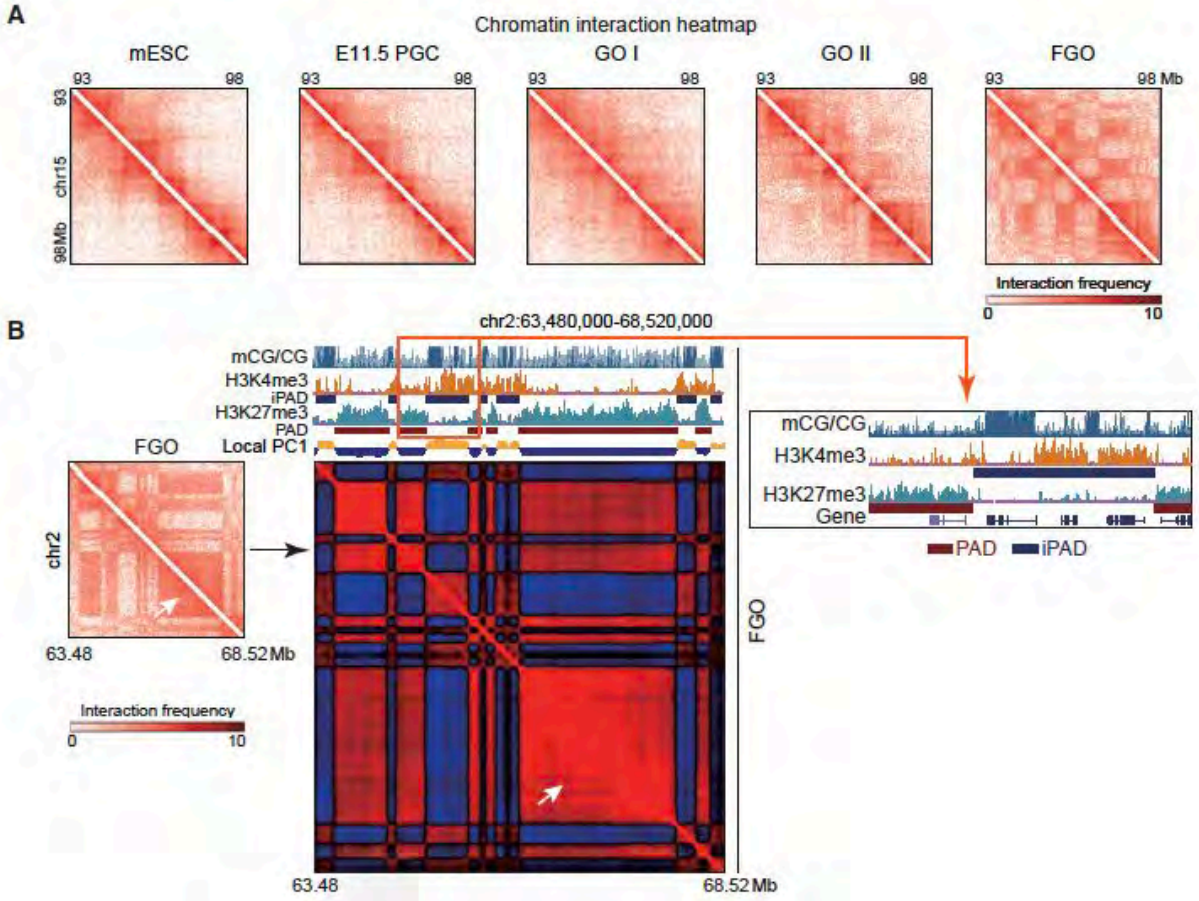
Appearance of Polycomb Associated Domains (PADs) during Oogenesis



Globally, the interaction heatmaps show that PGCs have relatively similar patterns compared to mESCs both for compartments and TADs. similarly to E11.5 PGCs and E13.5 PGCs

Oocytes at late stages, including GOs II, FGOs, and MII oocytes, show distinct chromatin organization. The most evident feature is the depletion of distal interactions and plaid patterns (compartments) across the chromosomes At local levels, their chromatin organizations are also distinct from those in mESCs and PGCs.

GOs II and FGOs appear to have a local compartment-like structure while MII oocytes entirely lack defined compartments but instead show a uniform chromatin interaction profile as reported before (Du et al., 2017; Ke et al., 2017).



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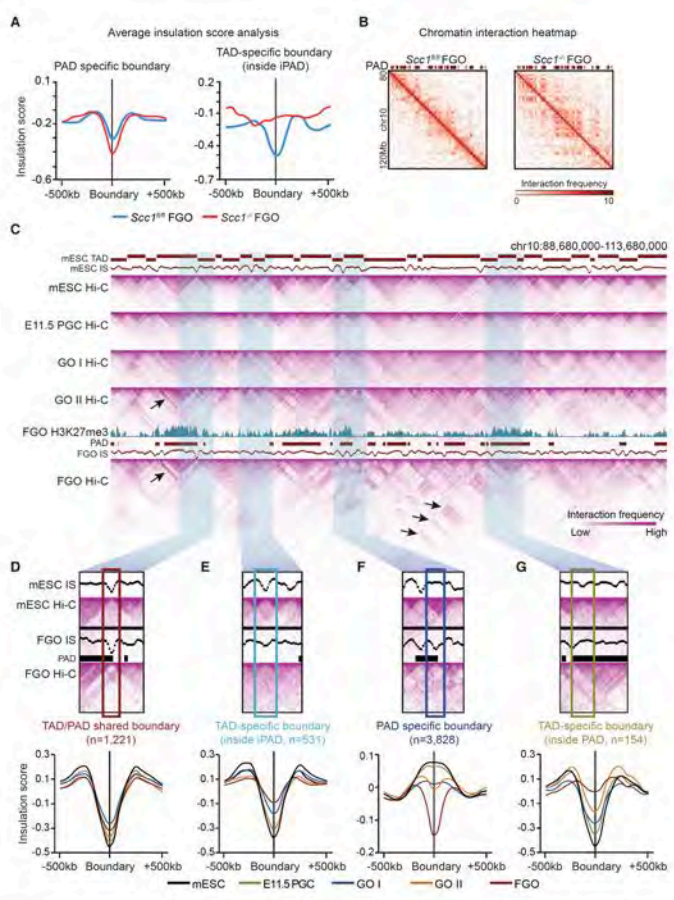
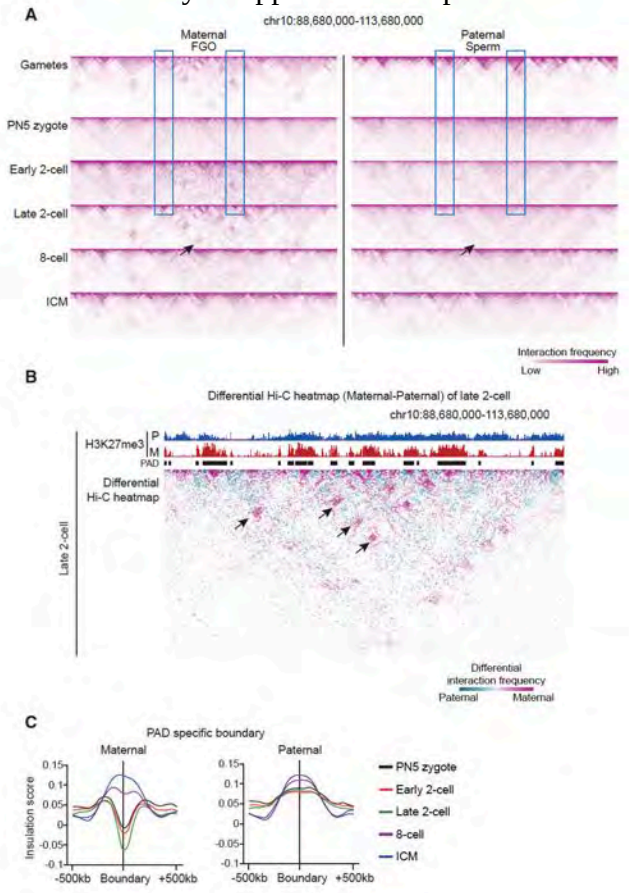
PADs are established during oogenesis, disappear in MII oocytes but reappear in early embryos and disappear around 8-cell stage

Dynamics of TADs and PADs during Oocyte Development



PADs Are Disassembled after Exiting from Diplotene Arrest but Briefly Reappear in Preimplantation Embryos



Similar to TADs and chromatin compartments (Du et al., 2017; Ke et al., 2017), PADs are also not found in MII Oocytes.

Zheng et al., 2016 showed that distal H3K27me3 in mouse oocytes is inherited after fertilization and persists until blastocyst.

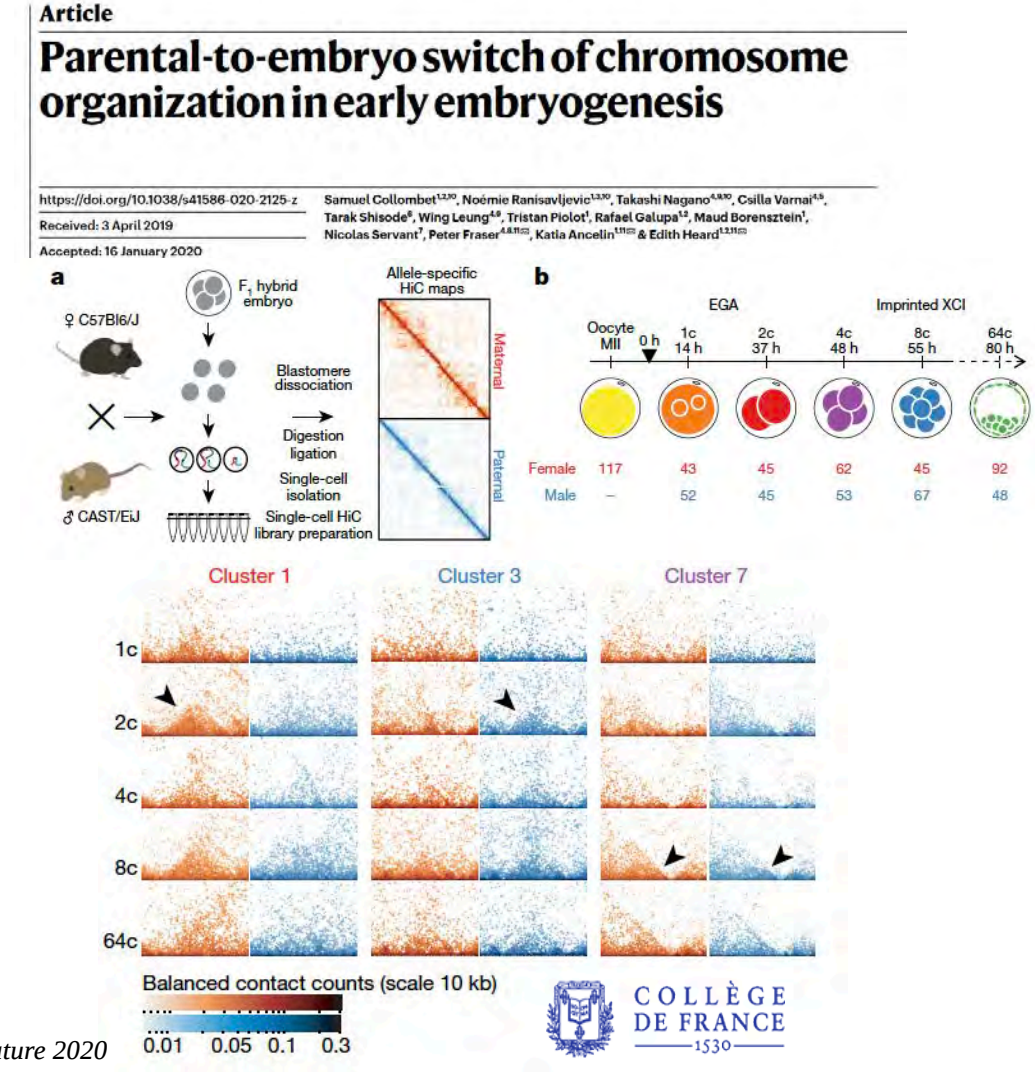
PADs are also present in early embryos after fertilization. PADs and their compartmental interactions appear to emerge in early 2-cell embryos and become further evident in late 2-cell embryos. PADs begin to fade away in the 8-cell embryos and become undetectable in inner cell mass (ICM).

Du et al., 2020, Molecular Cell 77, 825–839

What is the role of parental 3D chromosome memory carried over from gametes to embryos?

Noemie Ranisavljevic,
Samuel Collombet
Katia Ancelin
Edith Heard, Institut Curie

Takashi Nagano, Csilla Varnai,
Peter Fraser, Babraham Institute, UK



Collombet, Ranisavljevic, Nagano et al, Nature 2020

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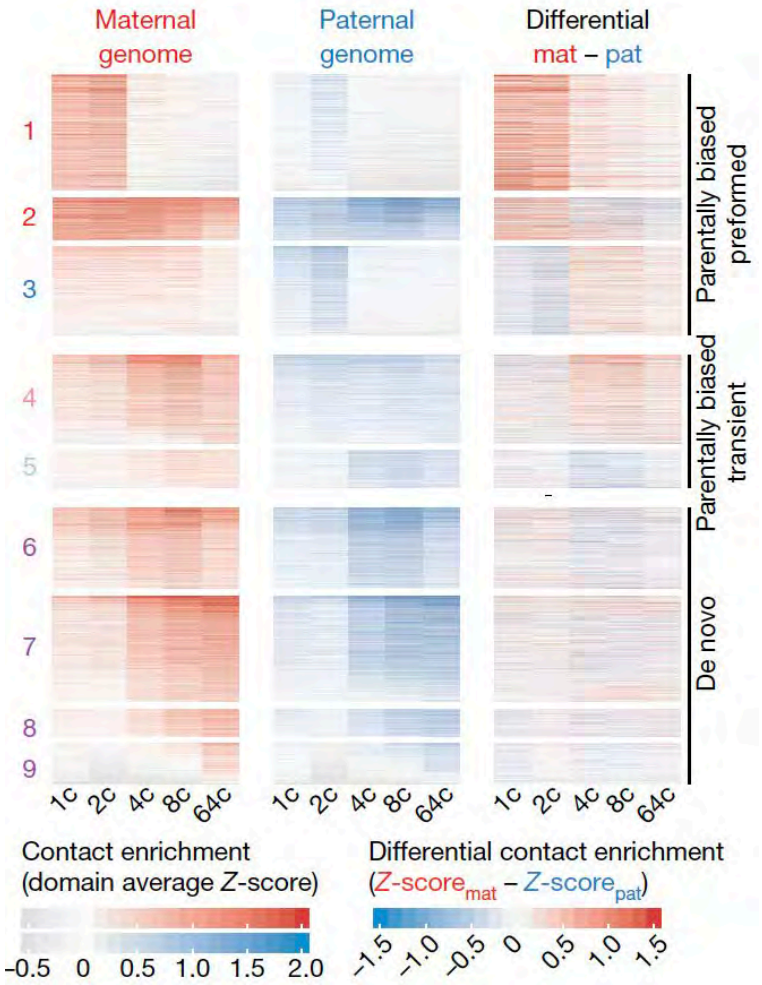
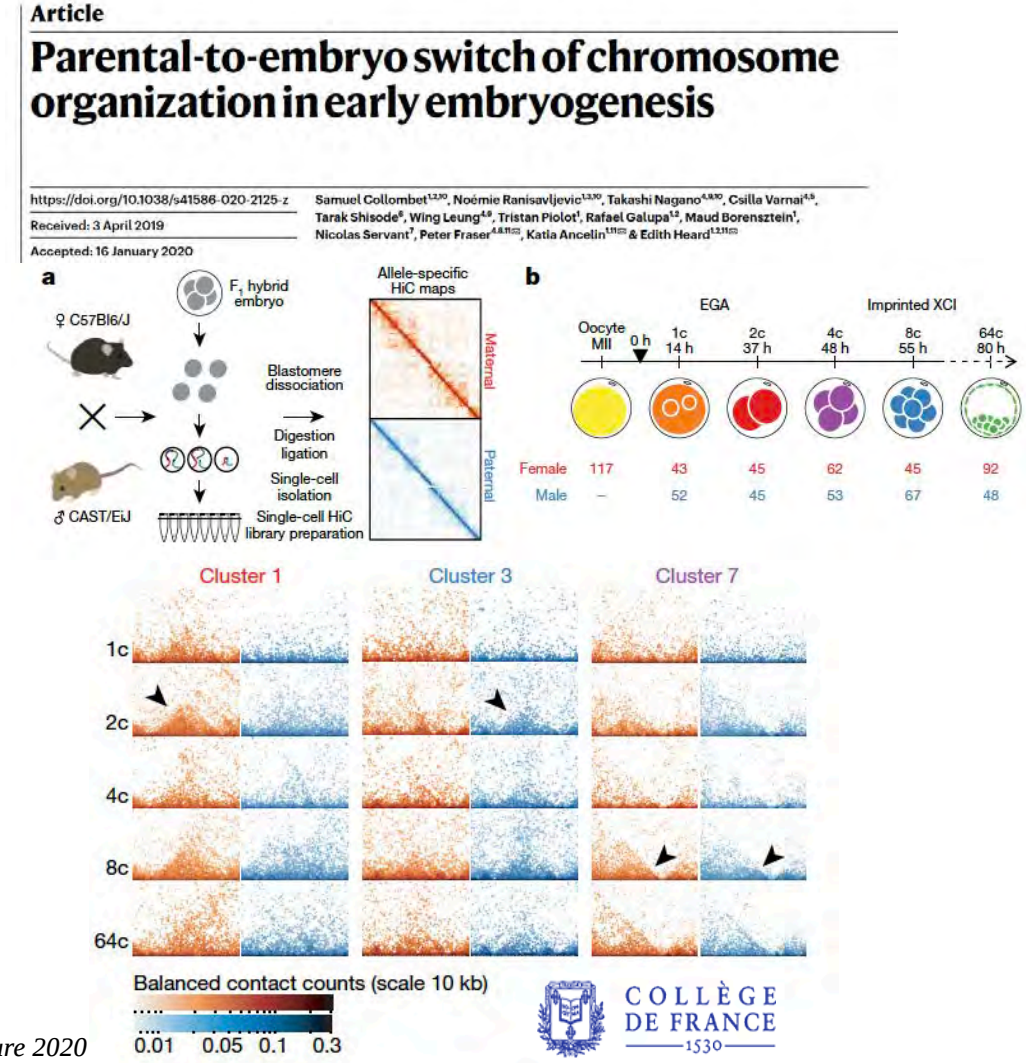
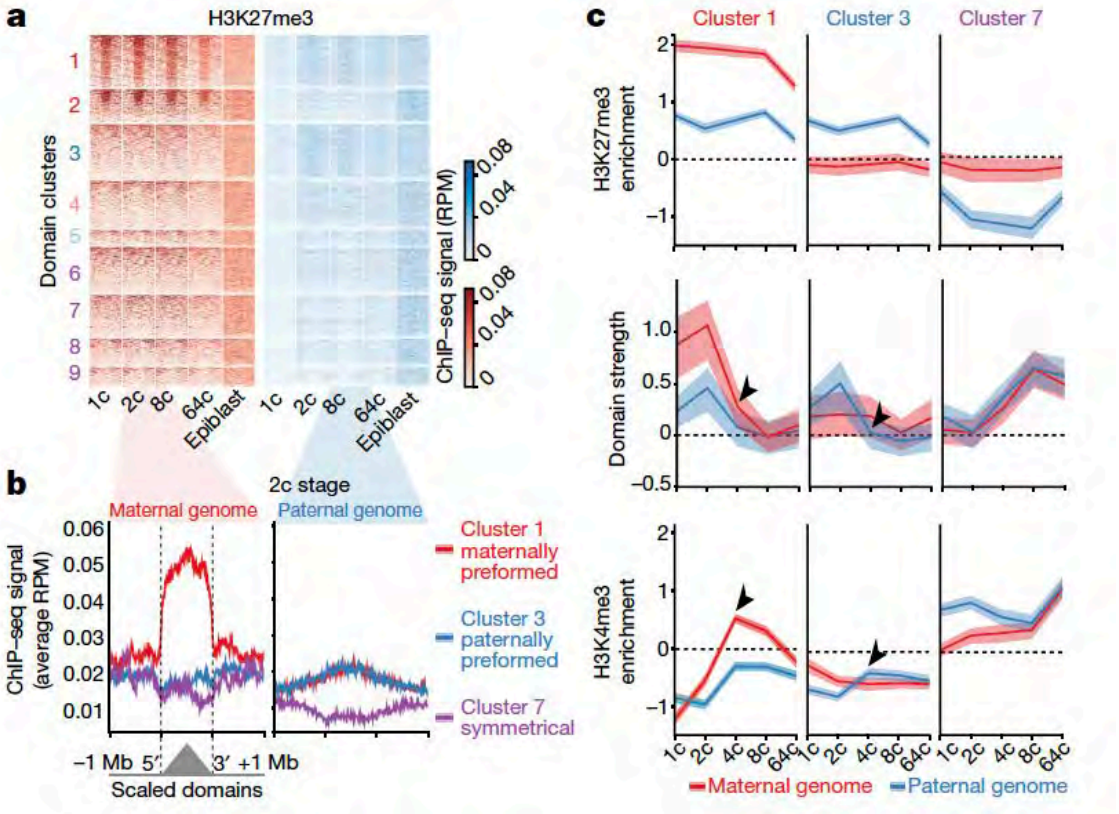


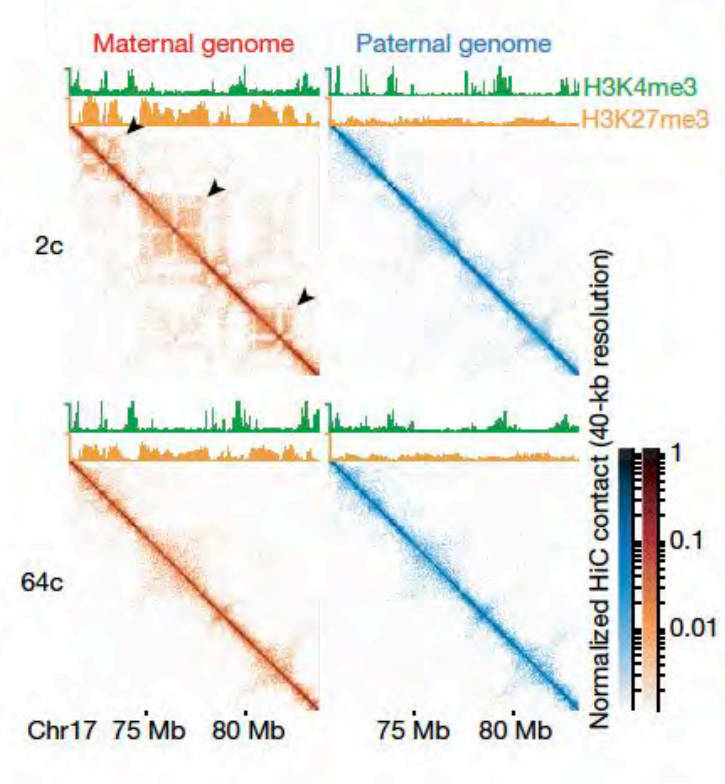


E. Heard, 15 mars, 2021

Collombet, Ranisavljevic, Nagano et al, Nature 2020

What is the role of parental 3D chromosome memory carried over from gametes to embryos?

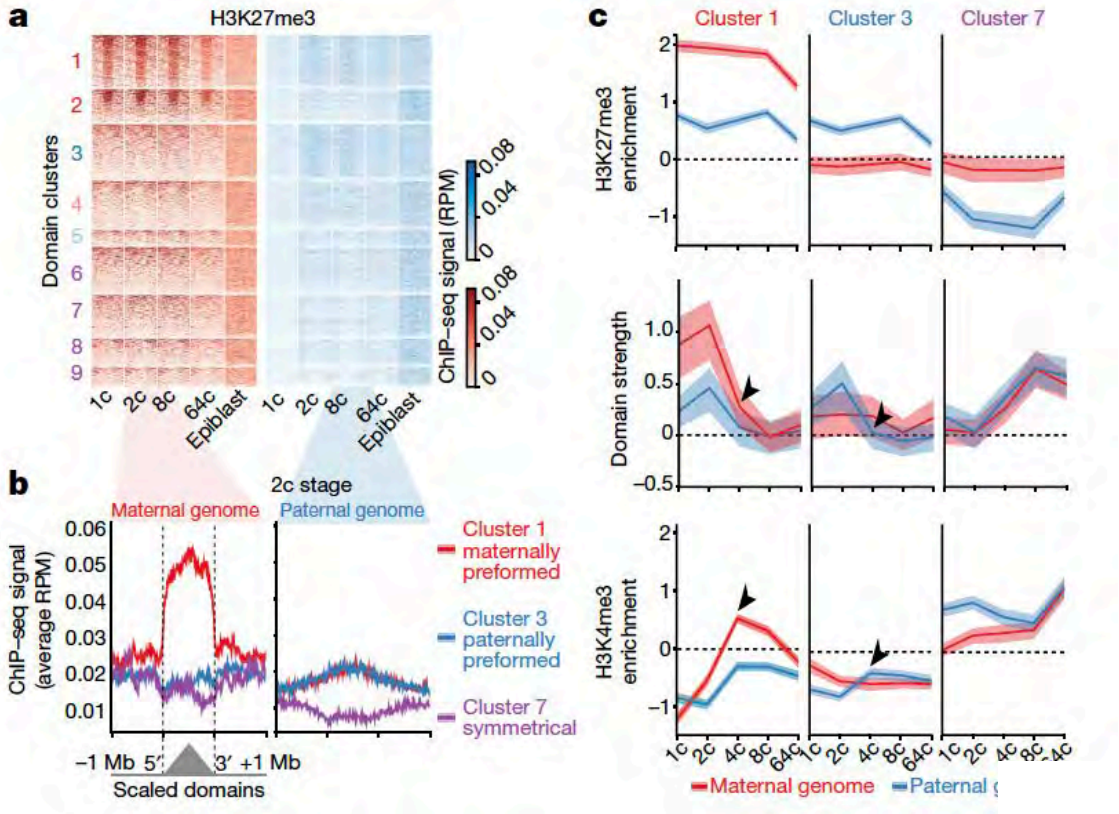


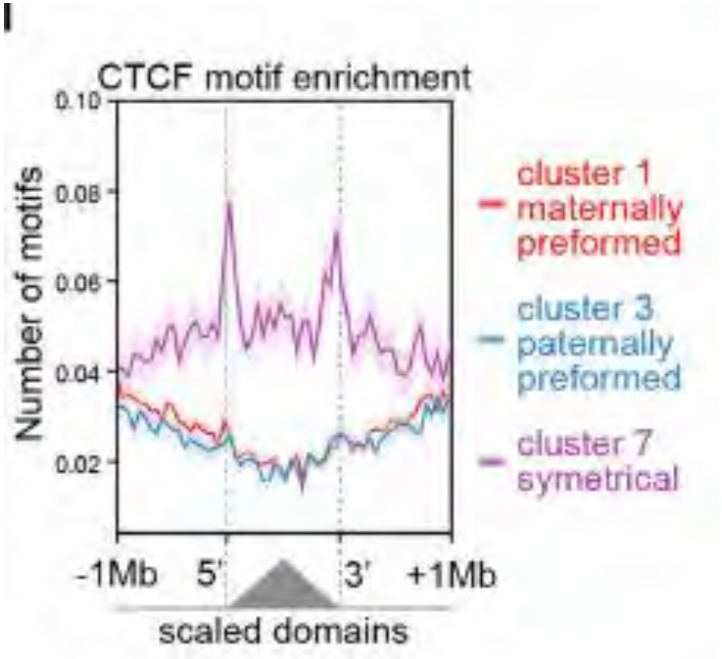


Both maternal and paternal early domains are enriched in H3K27me3 => PADs?

Collombet, Ranisavljevic, Nagano et al, Nature 2020

What is the role of parental 3D chromosome memory carried over from gametes to embryos?





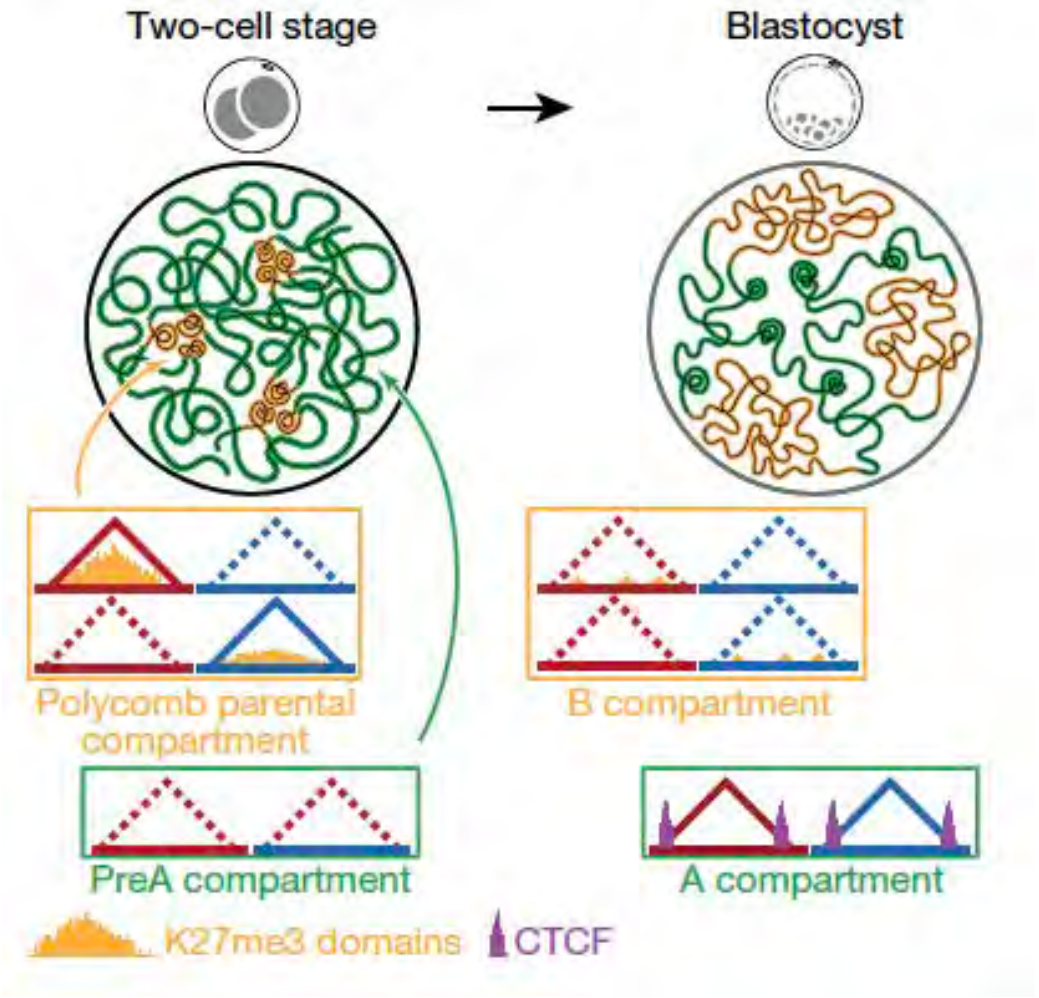
Both maternal and paternal early domains are enriched in H3K27me3 => PADs?

Only symmetrical domains that appear *de novo,* later are CTCF-flanked => TADs?

E. Heard, 15 mars, 2021

Collombet, Ranisavljevic, Nagano et al, Nature 2020

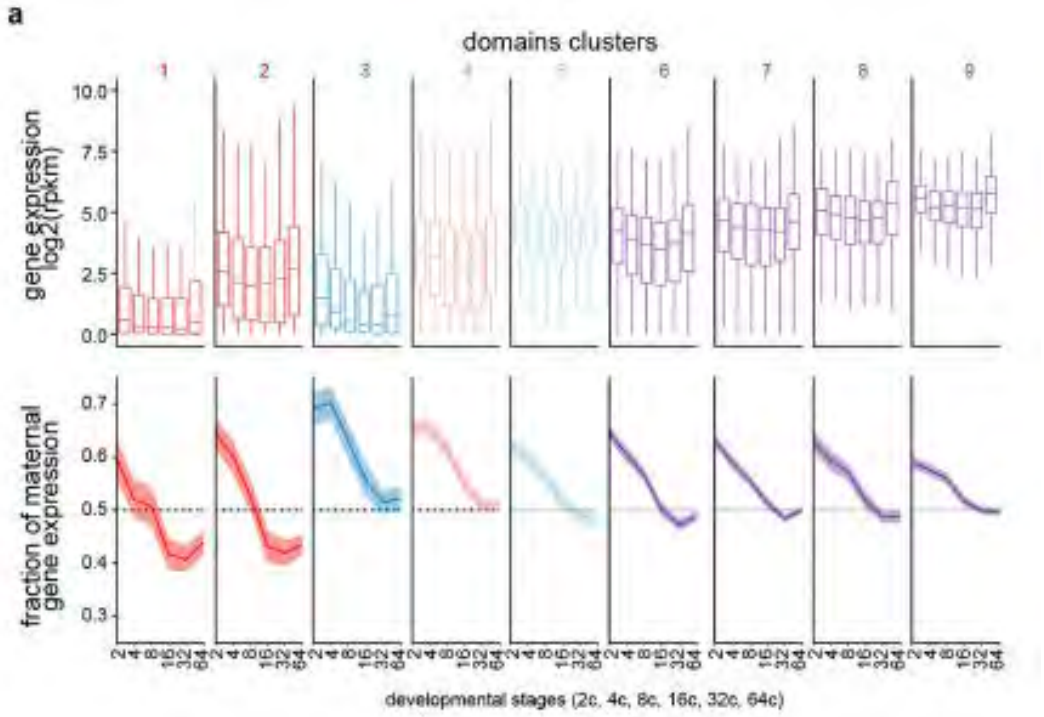
What is the role of parental 3D chromosome memory carried over from gametes to embryos?



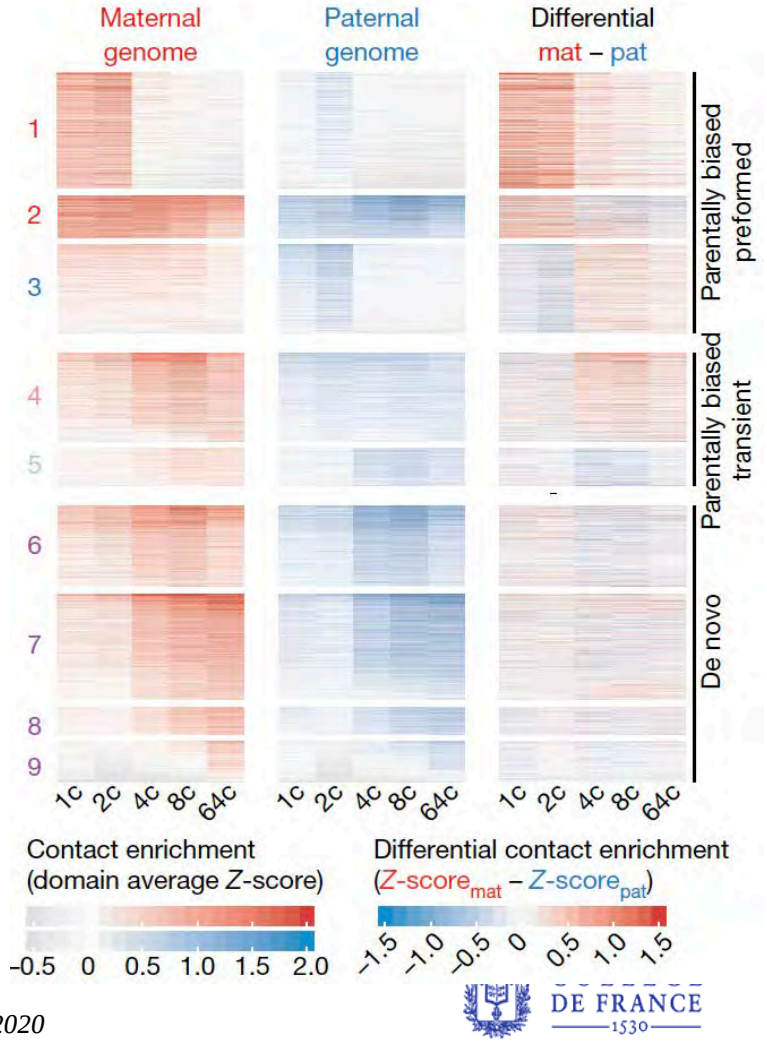
Collombet, Ranisavljevic, Nagano et al, Nature 2020

What is the role of parental 3D chromosome memory carried over from gametes to embryos?

Transient allelic asymmetry in gene expression



Maternal or paternal domains are associated with less paternal or maternal expression respectively



ure 2020

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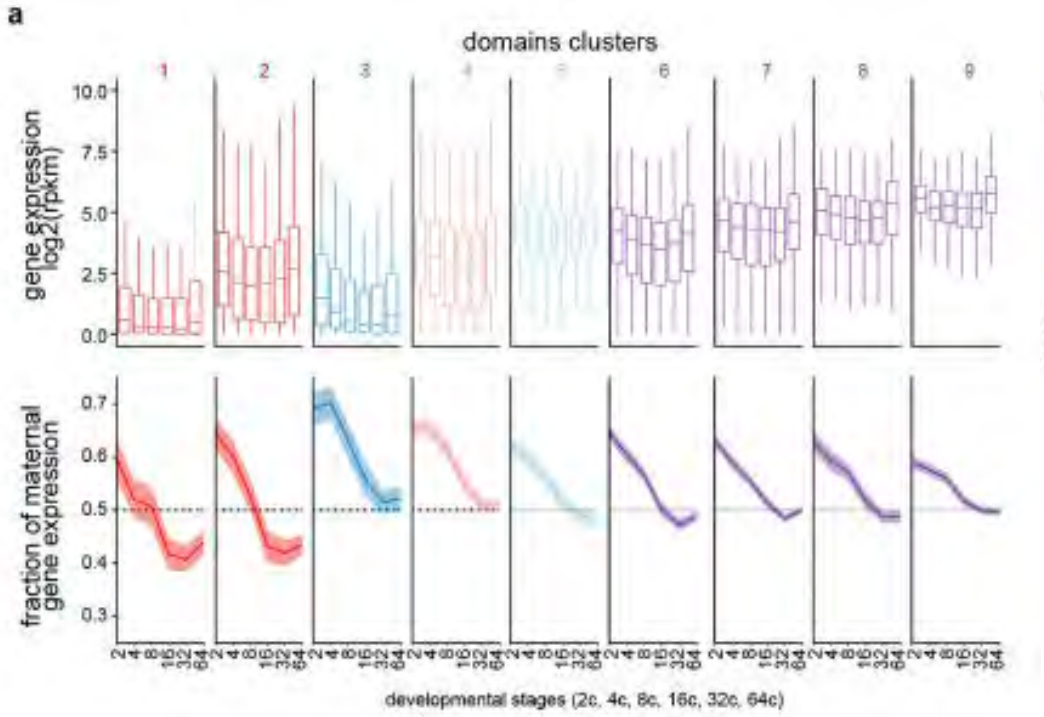
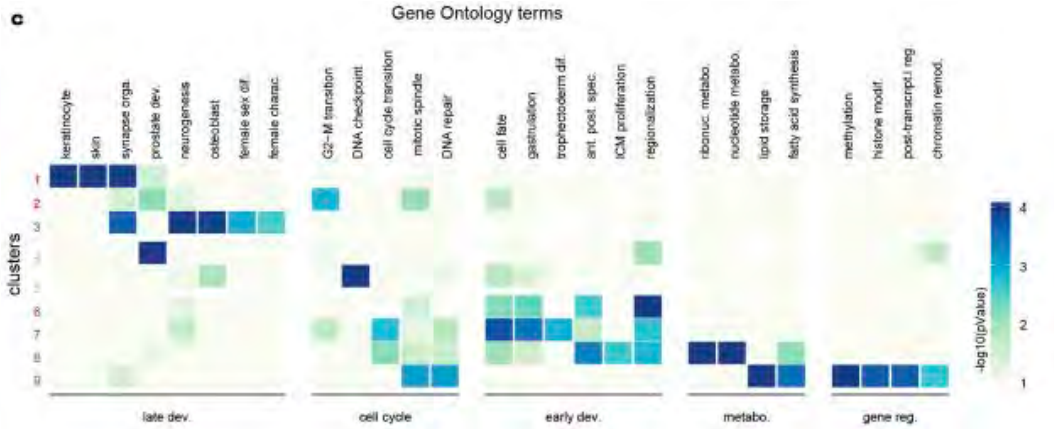
What is the role of parental 3D chromosome memory carried over from gametes to embryos?

Transient allelic asymmetry in gene expression

Allelic repression of tissue-specific genes





Maternal or paternal domains are associated with less paternal or maternal expression respectively

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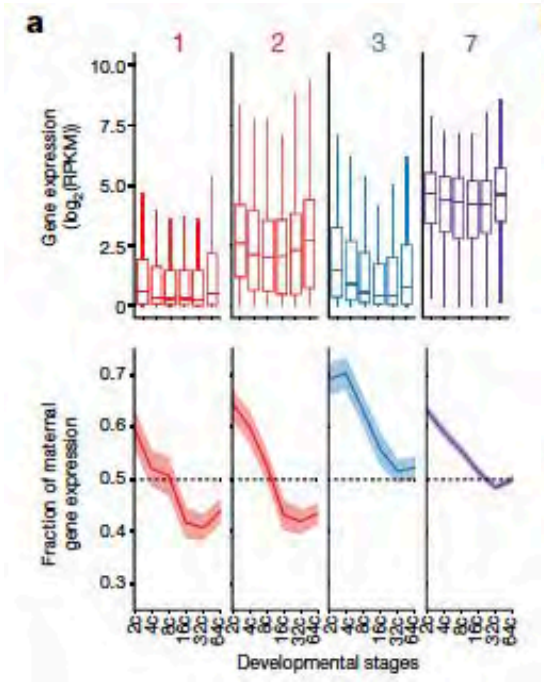
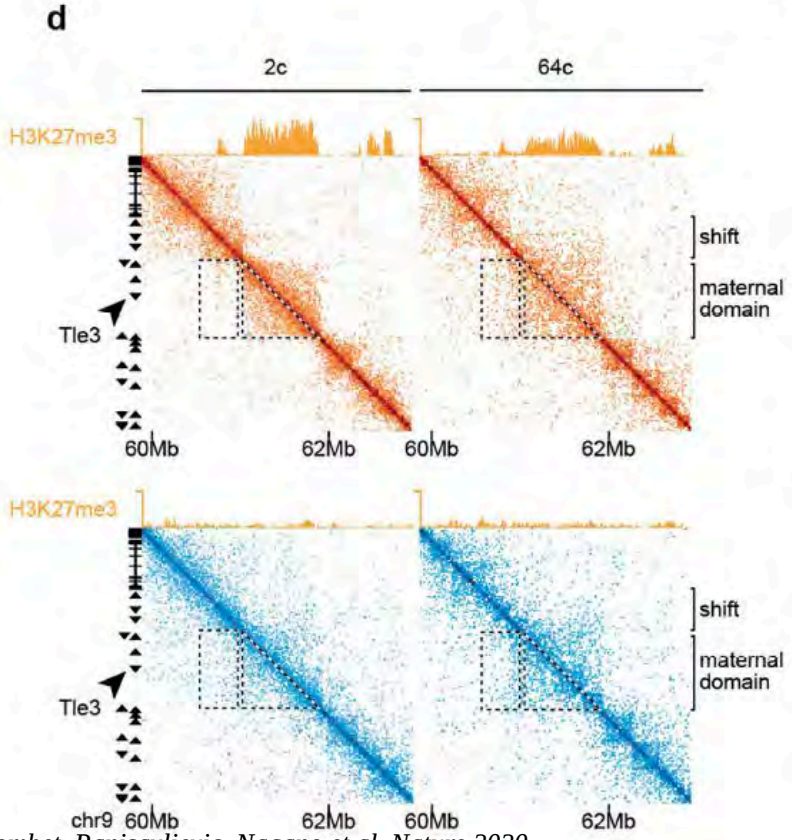
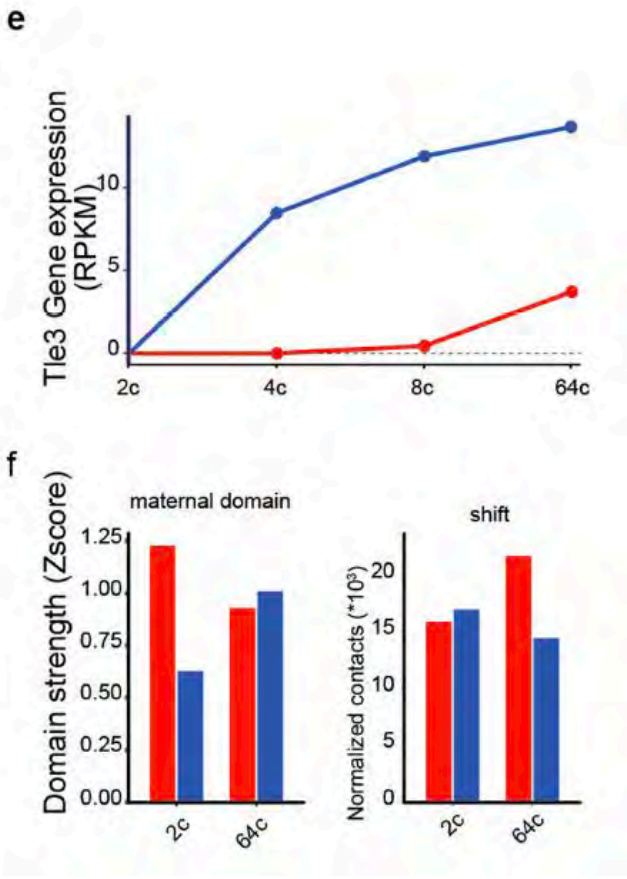
What is the role of parental 3D chromosome memory carried over from gametes to embryos?

Transient allelic asymmetry in gene expression

Transient imprinting







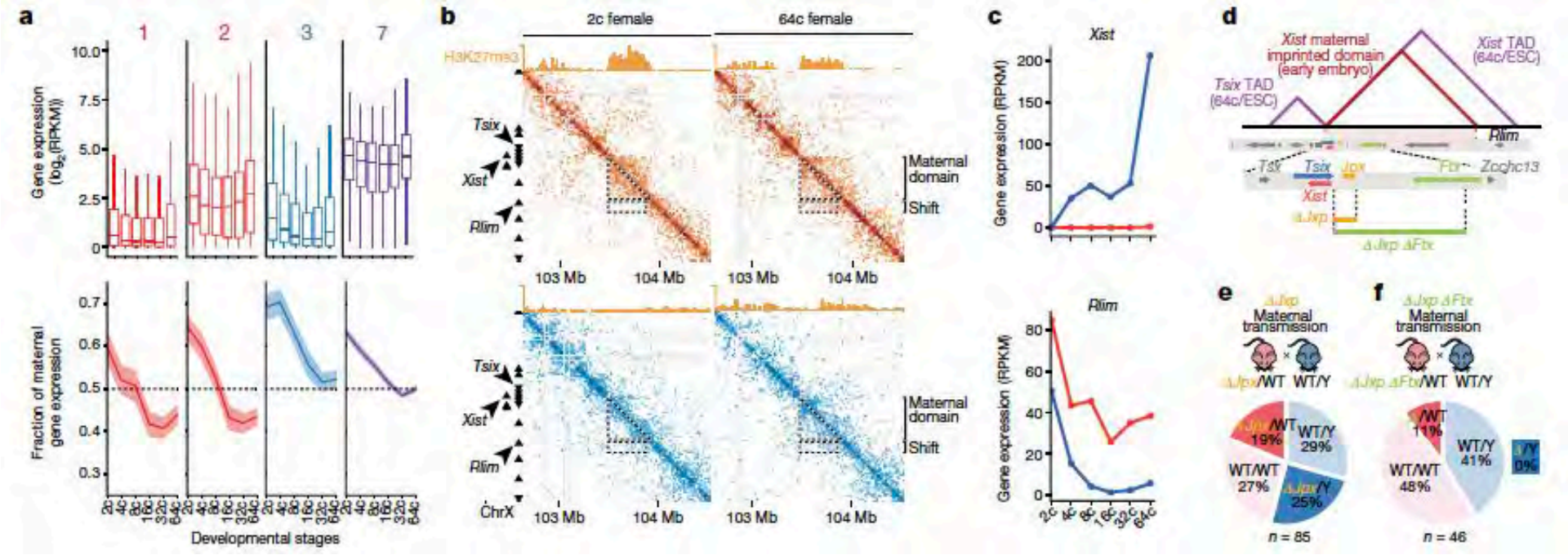
E. Heard, 15 mars, 2021

Collombet, Ranisavljevic, Nagano et al, Nature 2020

What is the role of parental 3D chromosome memory carried over from gametes to embryos?

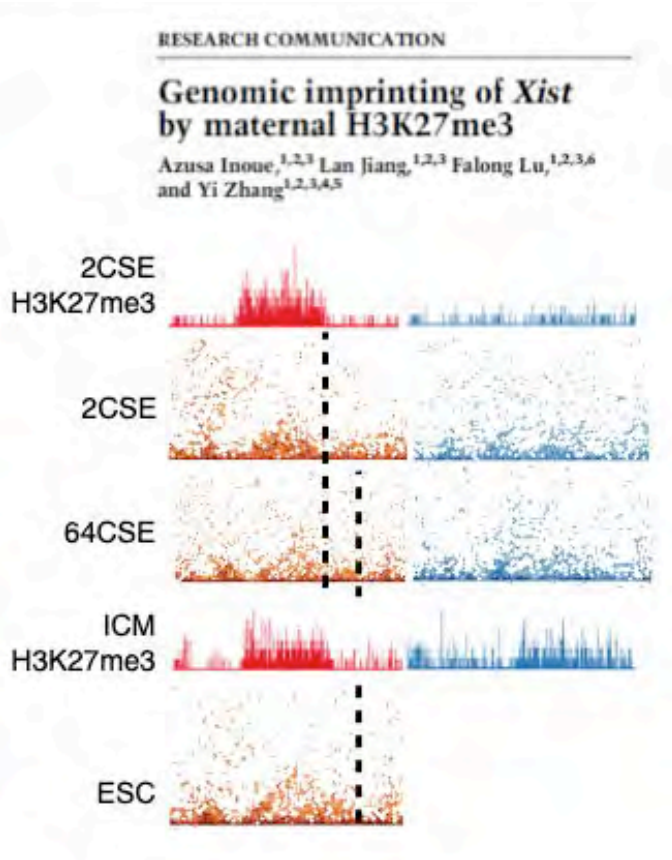
Transient allelic asymmetry in gene expression

Transient imprinting: the *Xist* gene



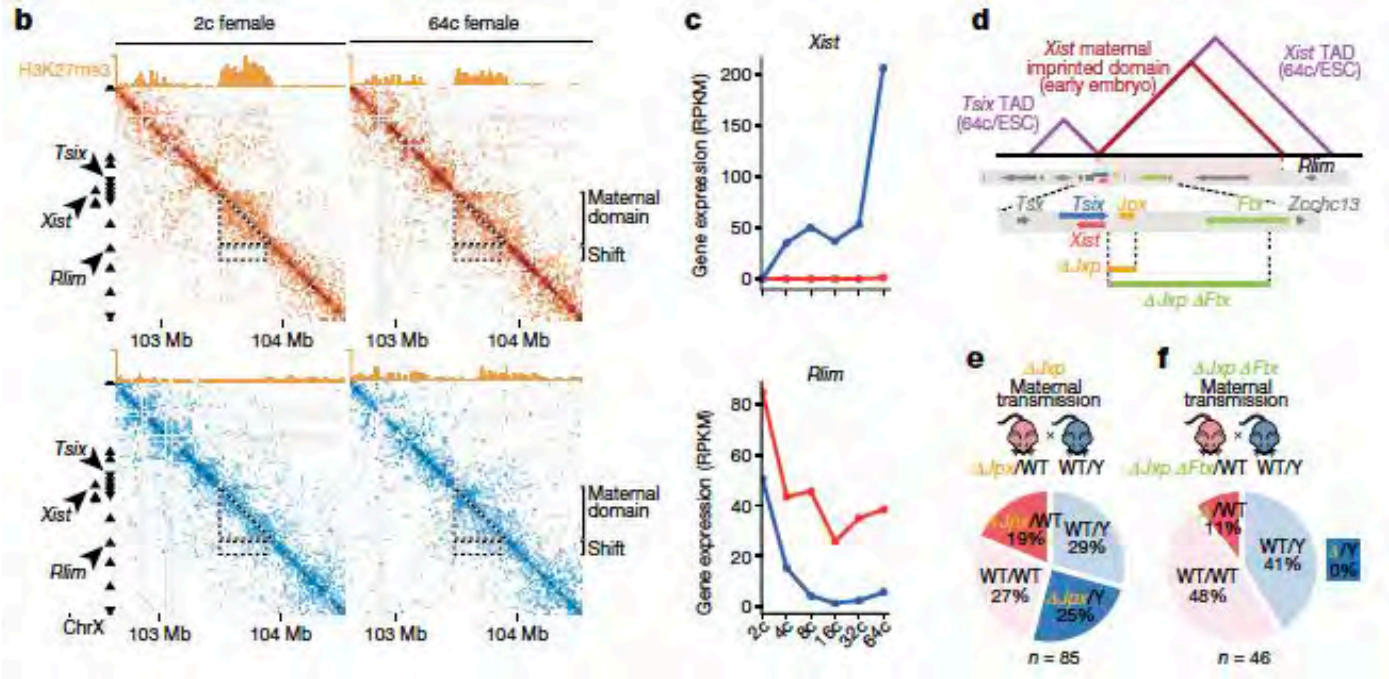
Collombet, Ranisavljevic, Nagano et al, Nature 2020

What is the role of parental 3D chromosome memory carried over from gametes to embryos?



Transient imprinting: the *Xist* gene

Deletion of the region spanning this domain leads to early lethality



E. Heard, 15 mars, 2021

Collombet, Ranisavljevic, Nagano et al, Nature 2020

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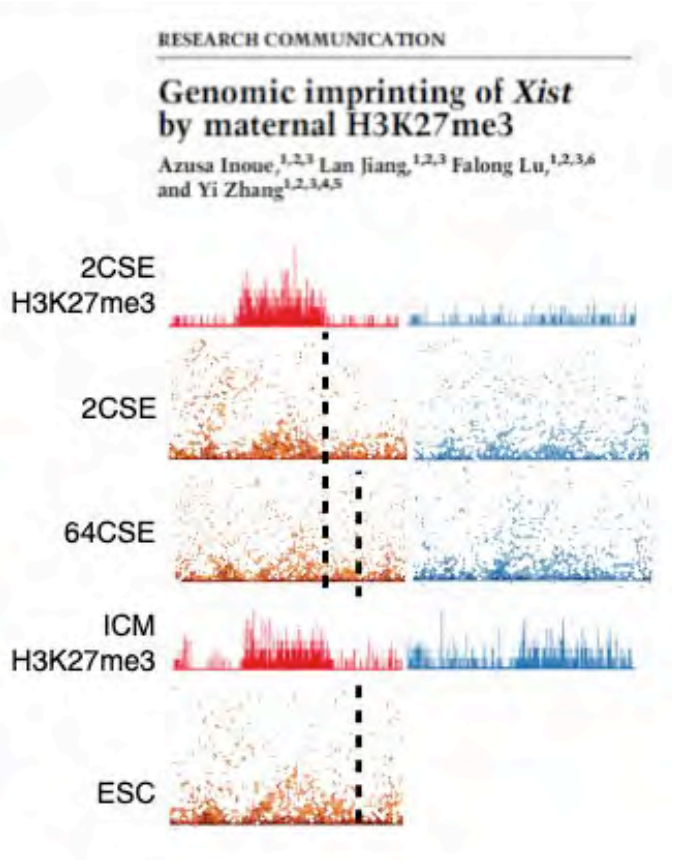
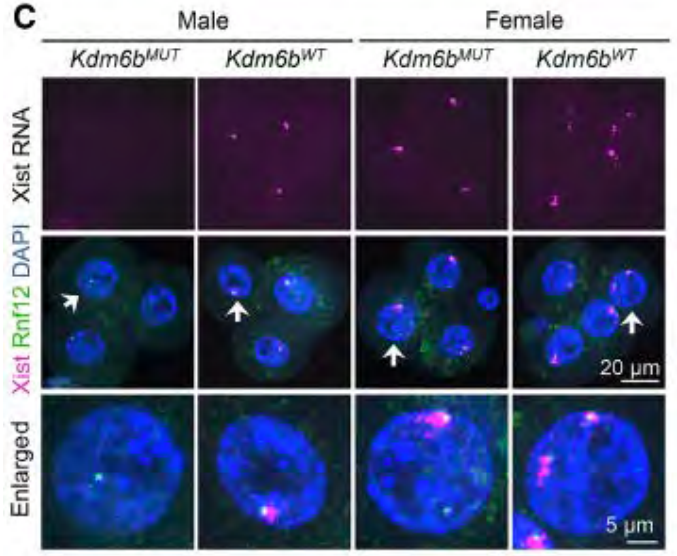
What is the role of parental 3D chromosome memory carried over from gametes to embryos?



Removal of H3K27me3 by injecting a demethylase result in aberrant up-regulation of Xist



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3D chromosome structure is associated with Polycomb domains and leads to transient “non-canonical” imprinting of genes including *Xist*

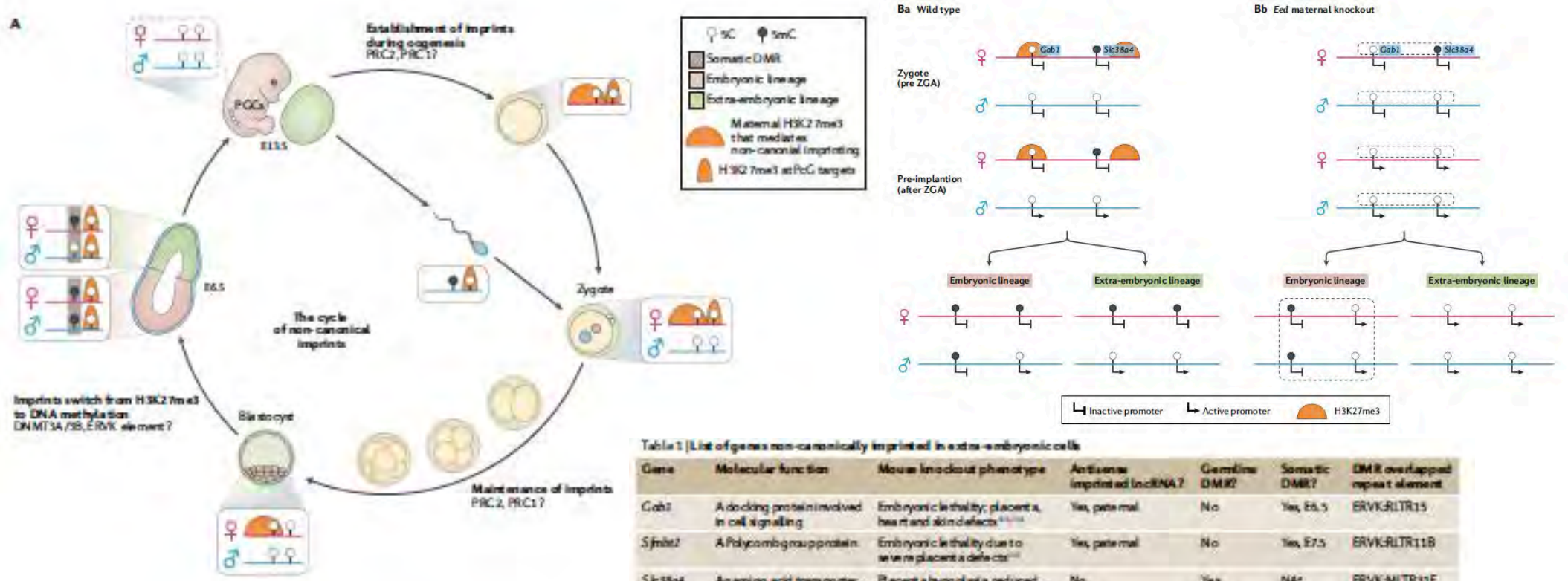


Table 1 | List of genes non-canonically imprinted in extra-embryonic cells

Gene	Molecular function	Mouse knockout phenotype	Antisense imprinted lncRNA?	Germline DMR?	Somatic DMR?	DMR overlapped repeat element
Gab1	A docking protein involved in cell signalling	Embryonic lethality; placenta, heart and skin defects	Yes, paternal	No	Yes, E6.5	ERVK-9-LTR15
Sfmbt2	A Polycomb group protein	Embryonic lethality due to severe placenta defects	Yes, paternal	No	Yes, E7.5	ERVK-9-LTR11B
Slc38a4	An amino acid transporter	Placenta hypoplasia, reduced fetal weight, 20% survival rate	No	Yes	NA*	ERVK-9-LTR31F
Phf17	A cofactor involved in histone acetylation	NA	No	No	Yes, E6.5	ERVK-9-LTR20C and RLTR31B
Smoc1	A matricellular protein involved in cell signalling	Perinatal lethality	Yes, paternal	No	Yes, E6.5	ERVK-9-LTR11B
Platr20	A lncRNA with unknown function	NA	Yes, paternal	No	Yes, E6.5	ERVK-9-LTR15
Gm12855	A lncRNA with unknown function	NA	No	No	Yes, E6.5	ERVK-9-LTR31A

E. Heard, 15 mars, 2021

X-chromosome inactivation as a model system for cellular memory and epigenetic dynamics

Zygotic Reprogramming

Imprinted X inactivation

ICM Reprogramming
• undo/prevent TE program
• set up Pluripotency

Random X inactivation

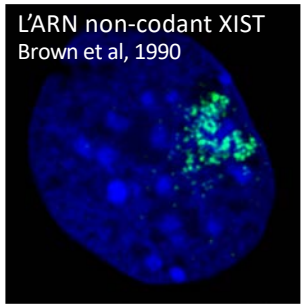
Germ Line Reprogramming

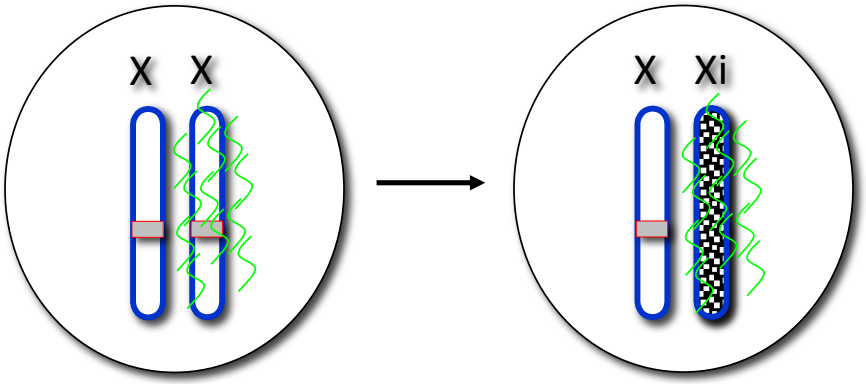
Adapted from Cantone and Fisher, 2013

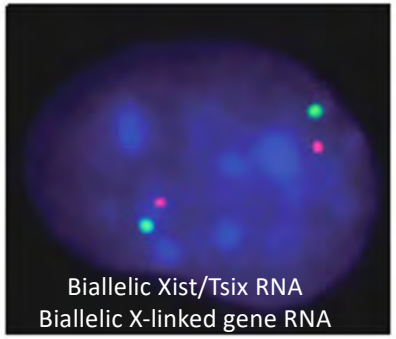
Initiating and maintaining X-Chromosome Inactivation

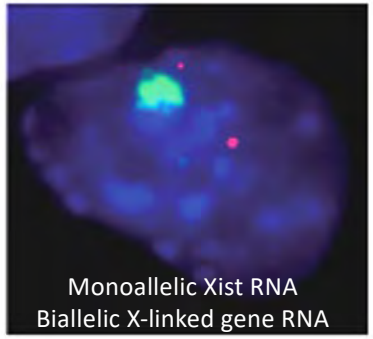
Initiation :
A non-coding RNA silences genes

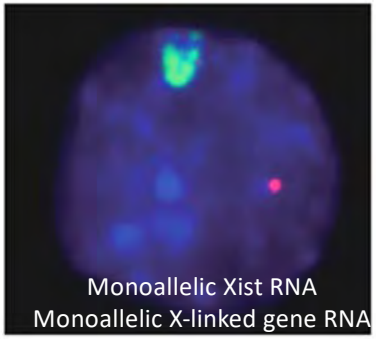
Maintenance:
Chromatin marks, Asynchronous replication,
Nuclear organisation











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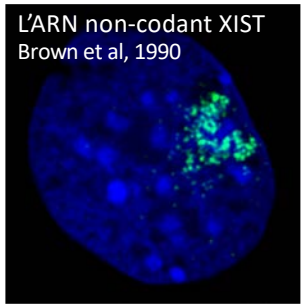
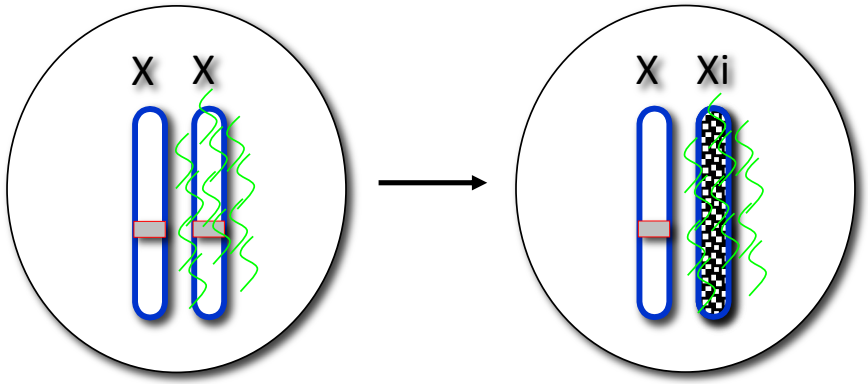
Initiating and maintaining X-Chromosome Inactivation

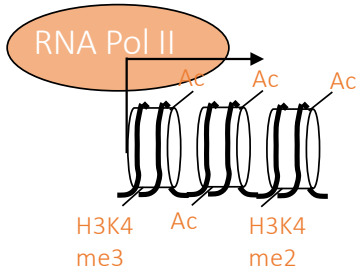
Initiation :
A non-coding RNA silences genes

Maintenance:
Chromatin marks, Asynchronous replication, Nuclear organisation

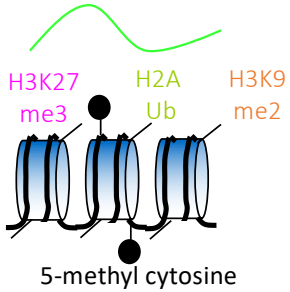




Active X chromosome



Inactive X chromosome



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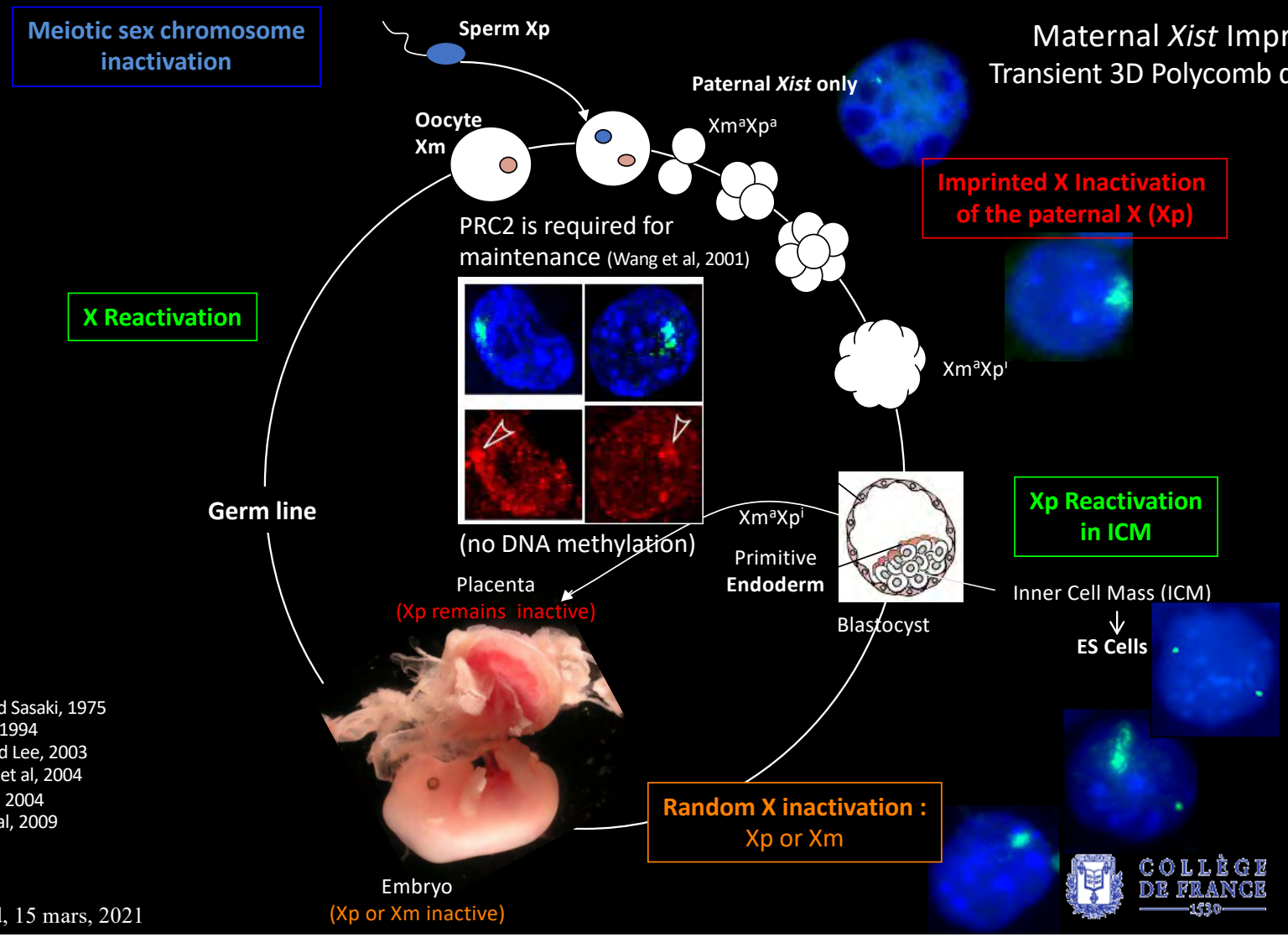
In vivo Dynamics of murine X inactivation



Takagi and Sasaki, 1975
Kay et al, 1994
Huyhn and Lee, 2003
Okamoto et al, 2004
Mak et al, 2004
Patrat et al, 2009

E. Heard, 15 mars, 2021

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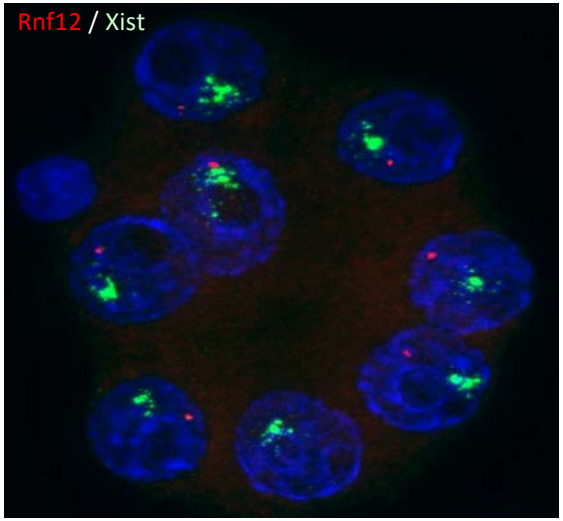
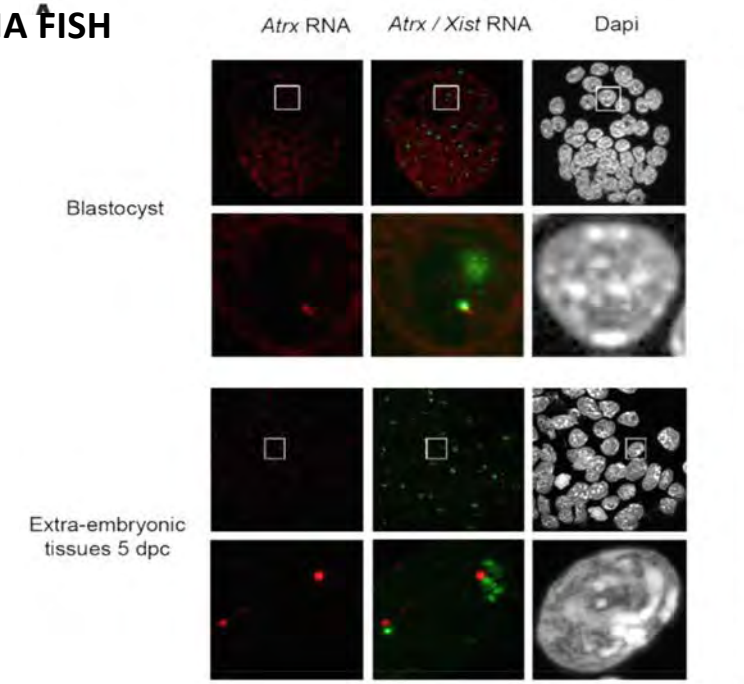
Timing and extent of XCI during murine pre-implantation development

Nascent transcript RNA FISH

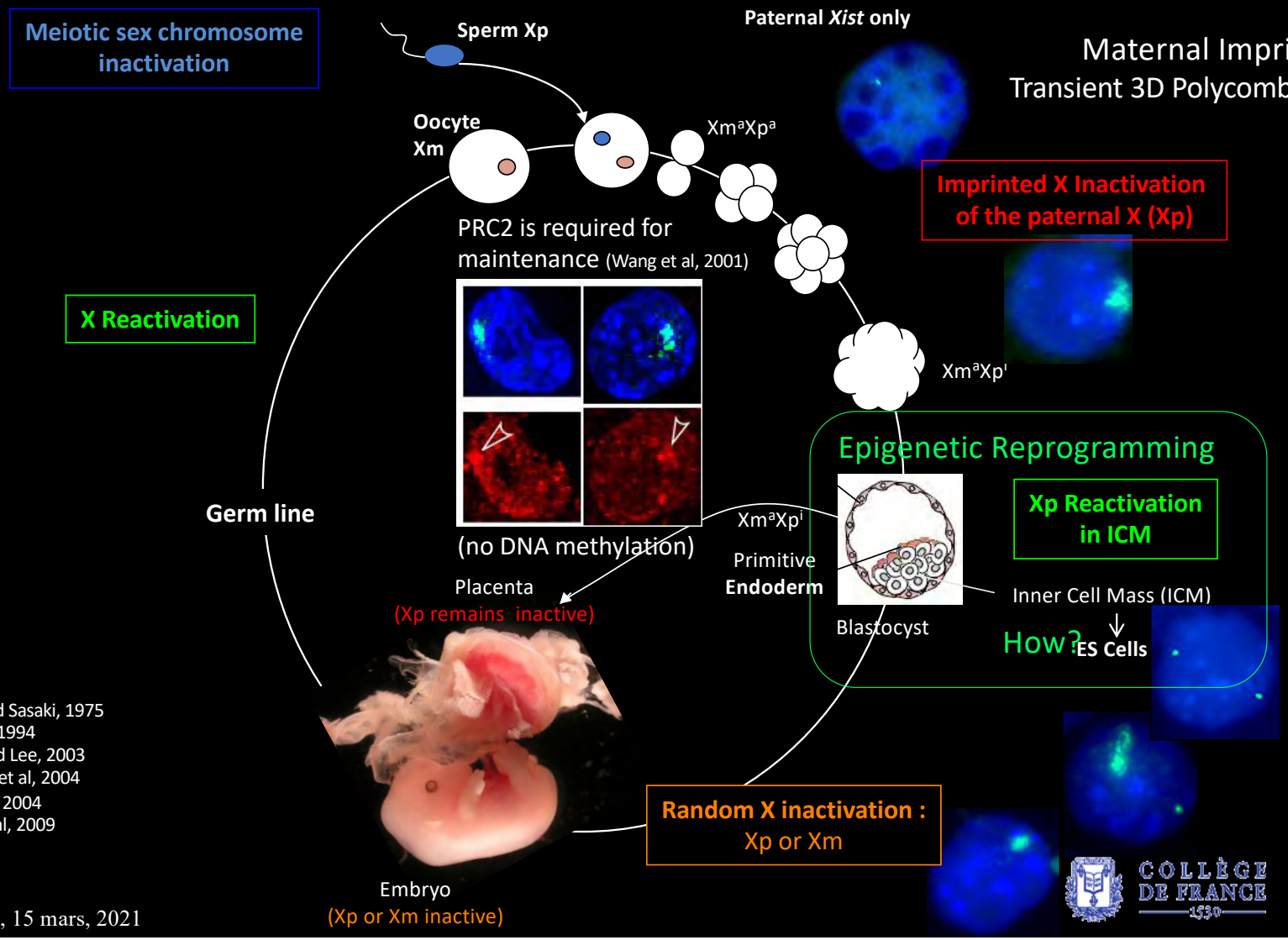




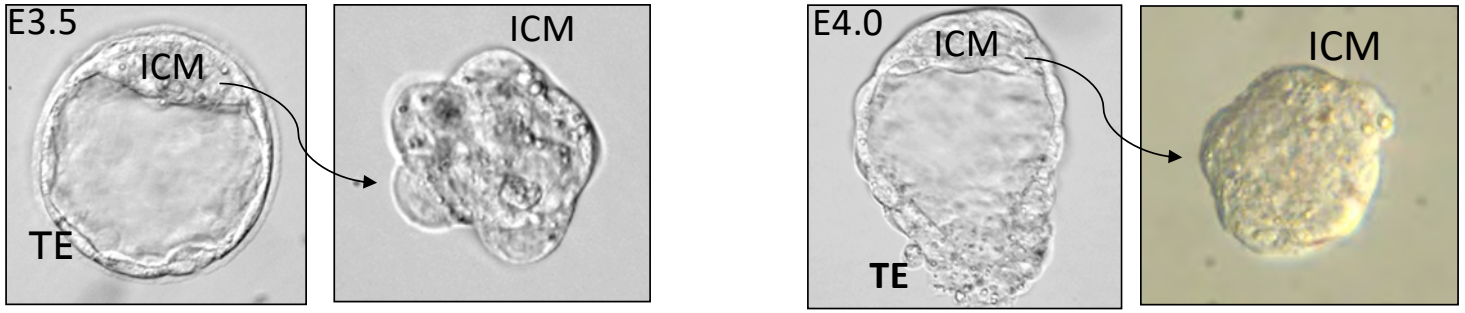
- Different genes show very different kinetics of XCI – rapid or very slow silencing
- Some genes show escape from the outset (eg *Utx, Jarid1c*)
- Others are inactivated and then reactivated in specific lineages (eg *Atrx*)
- Global reactivation happens in the inner cell mass but not the trophectoderm

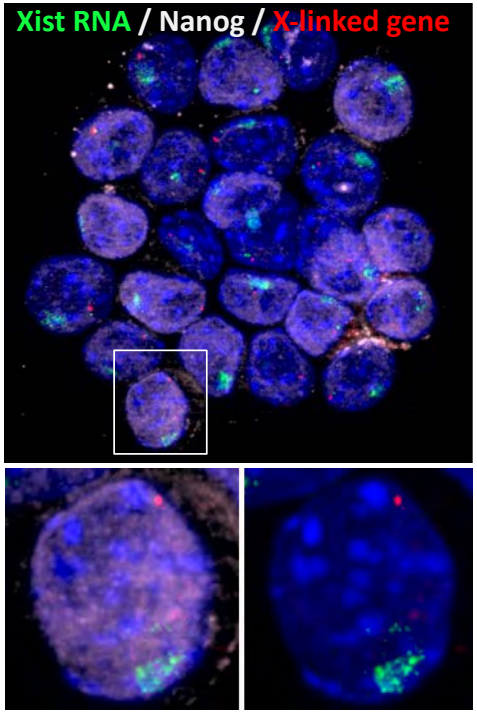
Patrat et al, *PNAS* 2009

In vivo Dynamics of murine X inactivation



Reactivation of the paternal X is linked to pluripotency

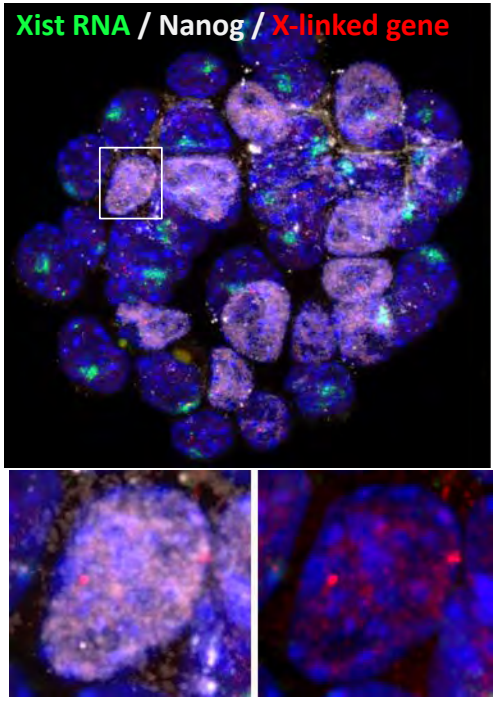




Ikuhiro
Okamoto

Increased
↑ Nanog, Oct4, Sox2
lead to *Xist* repression
& loss of PRC1/2
enrichment

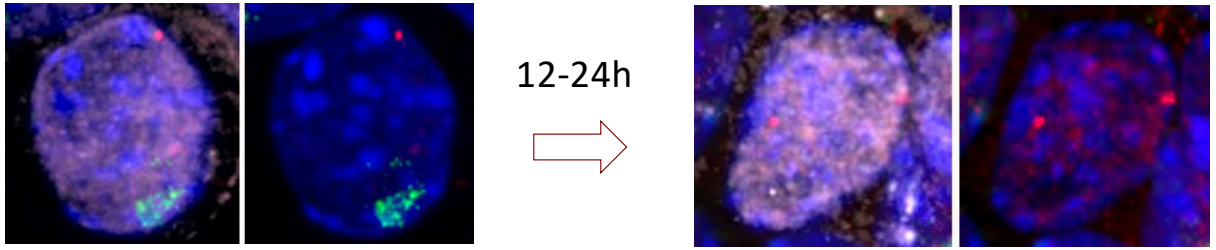
Okamoto et al, 2004
Mak et al, 2004
Navarro et al, 2007
Silva et al, 2009



Kinetics of Xp gene reactivation?

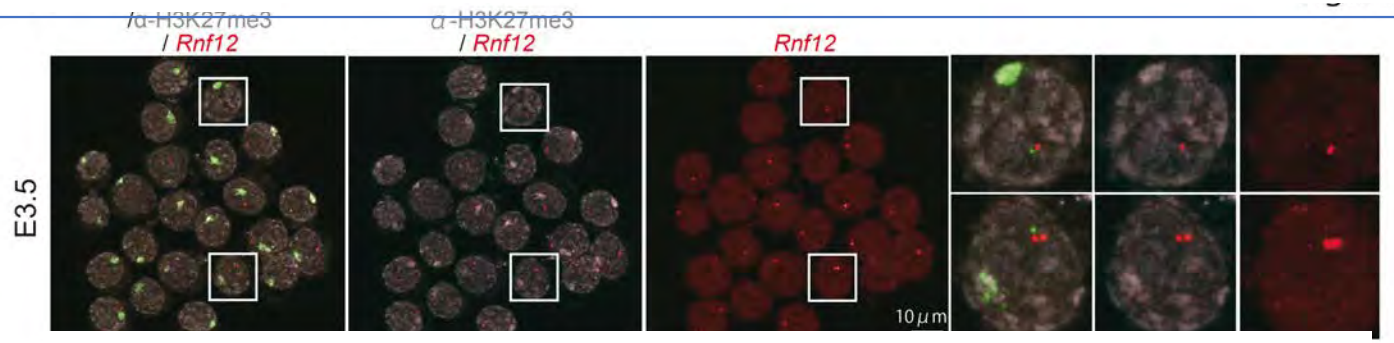
Borensztein, Okamoto et al, 2017

How is the Xi reactivated in the inner cell mass?

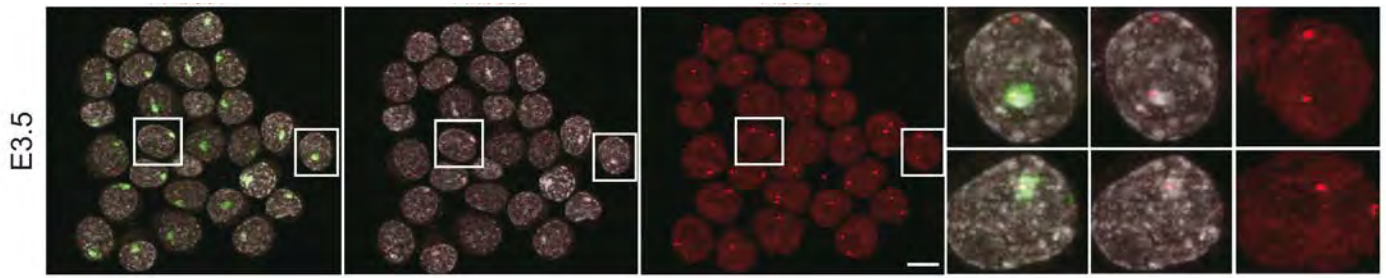


- *Xist* is down-regulated by up-regulation of pluripotency factors
- How and when are paternal X-linked genes re-expressed?
- How is the chromatin landscape of the paternal X reset?
- Do X-linked escapees have any role?

X-linked gene reactivation timing varies substantially in the ICM

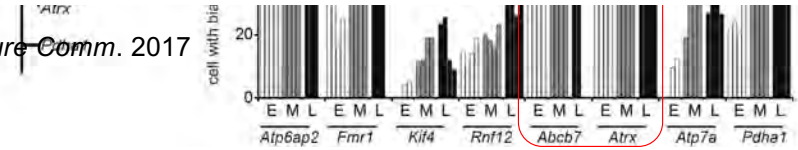


Some genes only reactivate after Xist and H3K27me3 loss



Some genes reactivate *even prior* to Xist and H3K27me3 loss

Perform single cell RNA seq in E3.5, E4.0 ICMs
to assess chromosome-wide timing of X reactivation



Borensztein, Okamoto et al, *Nature Comm*. 2017

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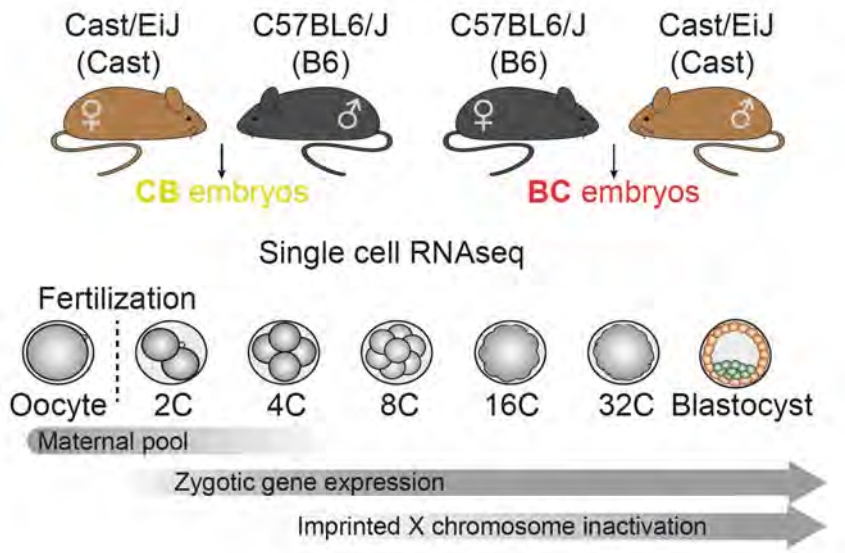
Single cell allelic profiling of X-chromosome inactivation and reactivation in mouse embryos

Single cell RNA-seq analysis



M. Borensztein

Inter-species crosses
=> F1 embryos
19 Millions SNPs; 1 SNP/100bp
1 SNP/650bp for the X
(Frazer *et al*, Nature, 2007)

Borensztein *et al*. Xist-dependent imprinted X inactivation and the early developmental consequences of its failure. *Nature Structural & Molecular Biology* **24:**226-233 (2017)

Borensztein, Okamoto et al . Contribution of epigenetic landscapes and transcription factors to X-chromosome reactivation in the inner cell mass. *Nature Communications* 8:1297 (2017)

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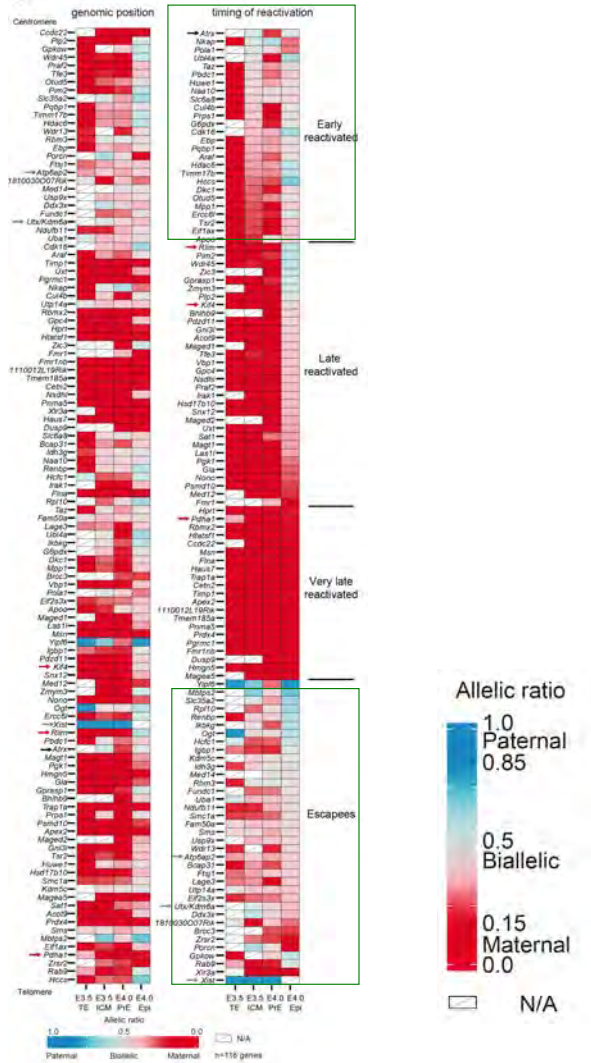
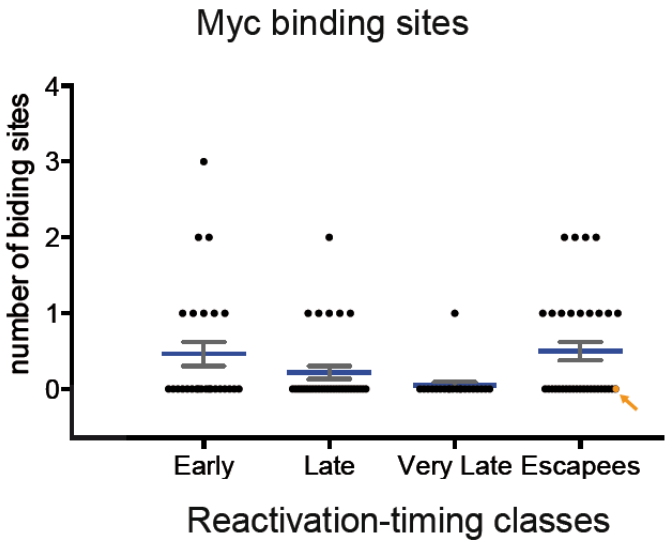
Different genes show very different kinetics of X-reactivations



Early-reactivated genes:
Association with Myc transcription factors



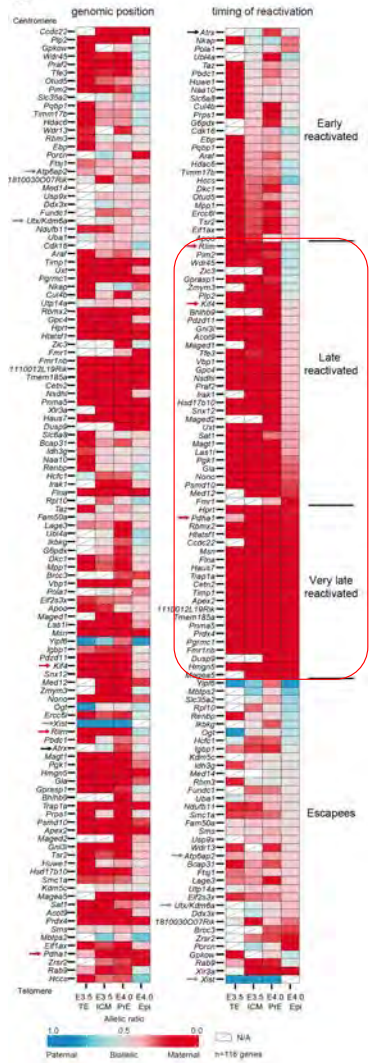
Some genes appear to have little "memory"
& are rapidly re-expressed via TFs such as Myc

(NB Pluripotency factor motifs are not specifically enriched at <u>early</u> reactivated genes)

COLLÈGE DE FRANCE 1530

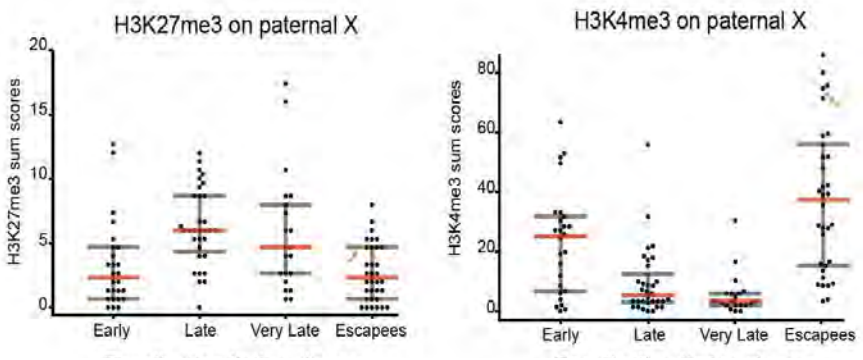
E. Heard, 15 mars, 2021

Different genes show very different kinetics of X-reactivation

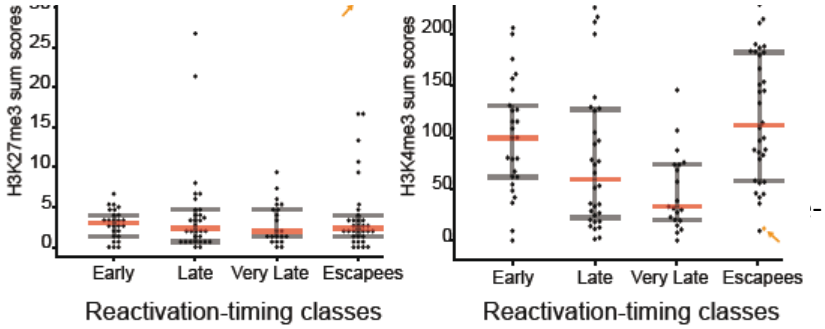


E. Heard, 15 mars, 2021

Epigenetic "memory" at some regions of the Xp?



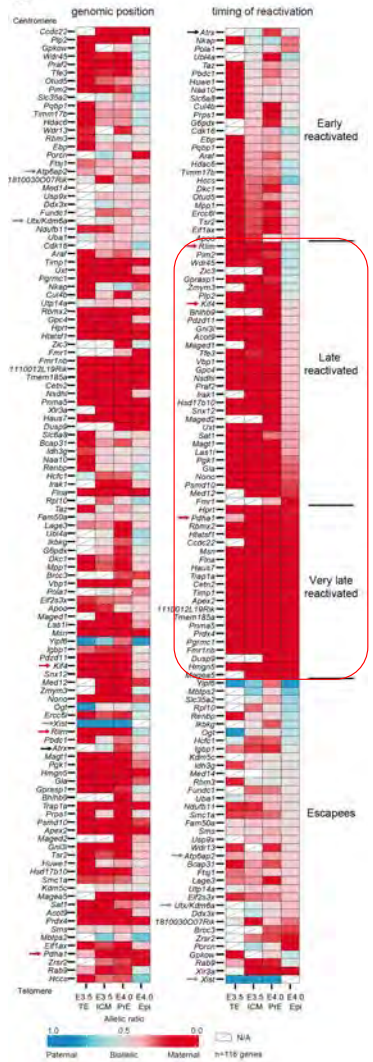
Late-reactivated genes are significantly enriched in H3K27me3 on the paternal X



Zheng, H. *et al.* Resetting Epigenetic Memory by Reprogramming of Histone Modifications in Mamma*ls. Mol. Cell* **63,** 1066–1079 (2016).

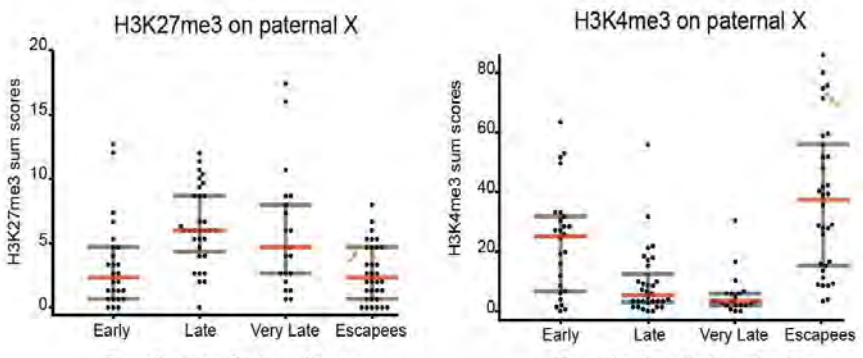
COLLÈGE DE FRANCE 1530

Different genes show very different kinetics of X-reactivation



E. Heard, 15 mars, 2021

Differences in epigenetic "memory" at different regions of the X?



How is this repressive epigenetic memory removed from late-reactivated genes?

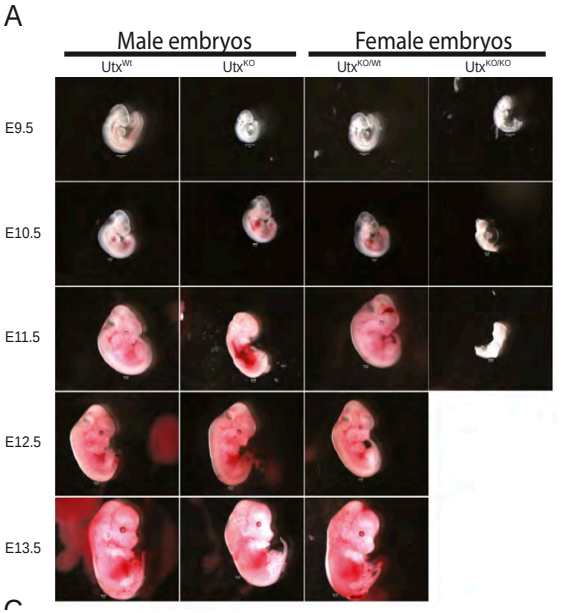
Is it lost passively (cell division) or is it actively erased (eg histone demethylase)?

COLLÈGE DE FRANCE 1530

Might Utx/Kdm6a H3K27me3 demethylase participate in loss of H3K27me3 during X reactivation in the ICM?

Kdm6a /Utx:

- H3K27 demethylase
- Ubiquitously expressed in embryos & somatic tissues
- Escapes X-chromosome inactivation
- Gender-specific tumor suppressor in T-cell acute lymphoblastic leukemia
- Sex-specific earlier lethality observed in *UTX* deleted mice (Jaenisch, Magnuson and Hanna labs)



Welstead *et al*, PNAS, 2012

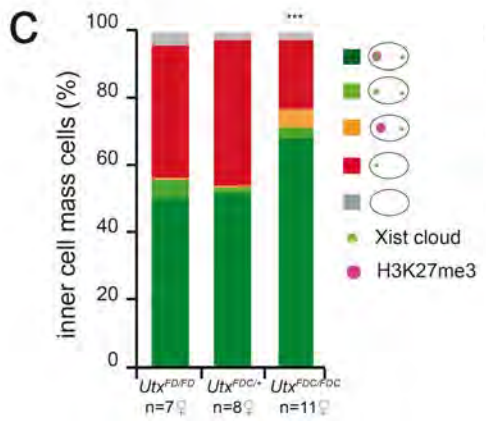
Yoo *et al*, 2012

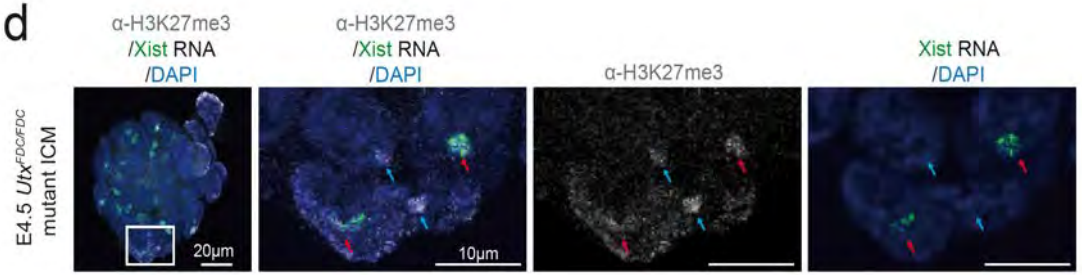
Might UTX participate in removal of H3K27me3 from the Xi?

COLLÈGE DE FRANCE 1530

Utx/Kdm6a facilitates loss of epigenetic silencing at some loci during Xp reactivation in the ICM

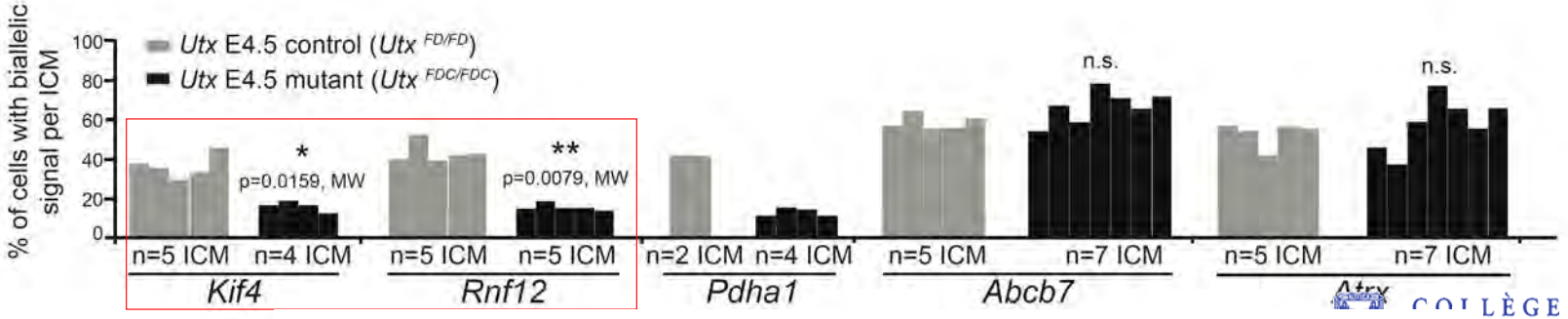
Collaboration with Konstantinos Anastassiadis (Biotec, Dresden)





In Utx mutant E4.5 female blastocysts:

- H3K27me3 remains aberrantly enriched on the Xp
- Only late-reactivated genes are affected by absence of *Utx*
- Observe <u>even slower reactivation</u> of X-linked genes that have an epigenetic memory associated with H3K27me3



Borensztein, Okamoto et al (2017) "Contribution of epigenetic landscapes and transcription factors to X-chromosome reactivation in the inner cell mass". Nature Communications 8:1297

CONCLUSIONS

- The memory of maternal germ line 3D Polycomb domain enables transient imprinting and prevents aberrant up-regulation of Xist on the maternal X in both male and female embryos
- X-chromosome dosage compensation is essential
- Some X-linked genes such as *Atrx* may be specifically required at higher doses in certain lineages, for the Xi?
- Diverse kinetics of XCI and *reactivation* in the ICM
  (Both DNA sequence and chromatin dependent)
- Some genes are reactivated very rapidly via TFs such as Myc
- Others reactivate more slowly and retain epigenetic memory (Polycomb)
  (Why? Now identify PcG retention features…)
- *Utx* – an H3K27 demethylase and an X-linked escapee - facilitates erasure of Polycomb memory on the inactive X in the ICM

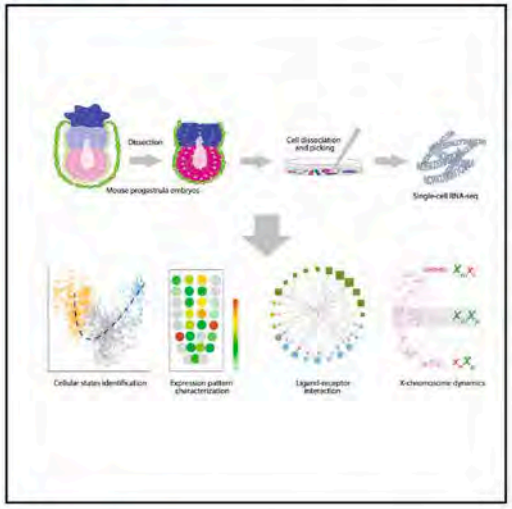
Single cell RNA seq reveals X-chromosome dynamics

Article

Cell Reports

Single-Cell RNA-Seq Reveals Cellular Heterogeneity of Pluripotency Transition and X Chromosome Dynamics during Early Mouse Development

Graphical Abstract



Authors

Shangli Cheng, Yu Pei, Liqun He, ..., Patrick P.L. Tam, Naihe Jing, Qiaolin Deng

Correspondence

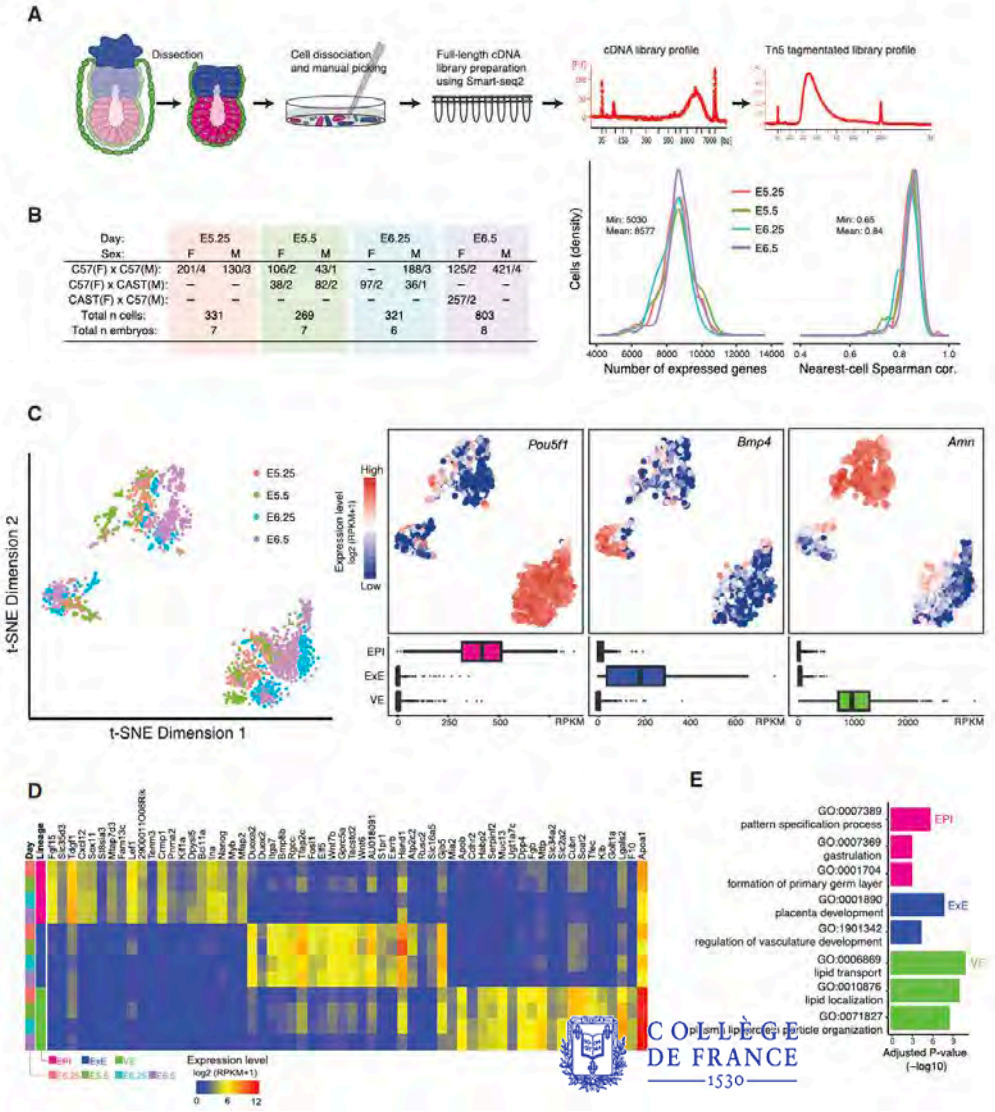
qiaolin.deng@ki.se

In Brief

Cheng et al. present a molecular roadmap at single-cell and allelic resolution that highlights the developmental process of epiblast cells transiting through pluripotency states and acquiring the primitive streak propensity ahead of gastrulation. In the epiblast of female embryos, the paternal X chromosome is reactivated before the completion of imprinted inactivation.

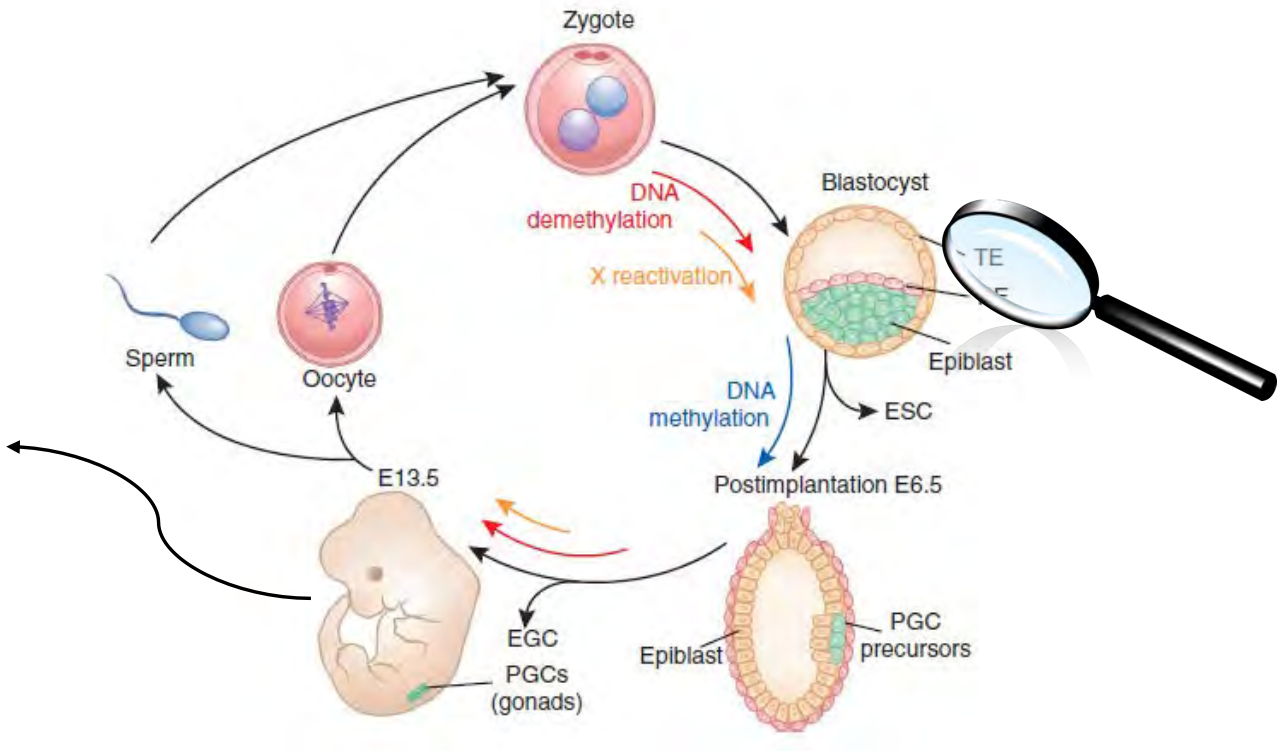
Highlights

- A comprehensive scRNA-seq roadmap of early mouse development before gastrulation
- Three cellular states of the epiblast cells transit the pluripotency continuum
- X-reactivation in the epiblast initiates before completion of imprinted X-inactivation
- Faster X-inactivation in visceral endoderm than in the extraembryonic ectoderm



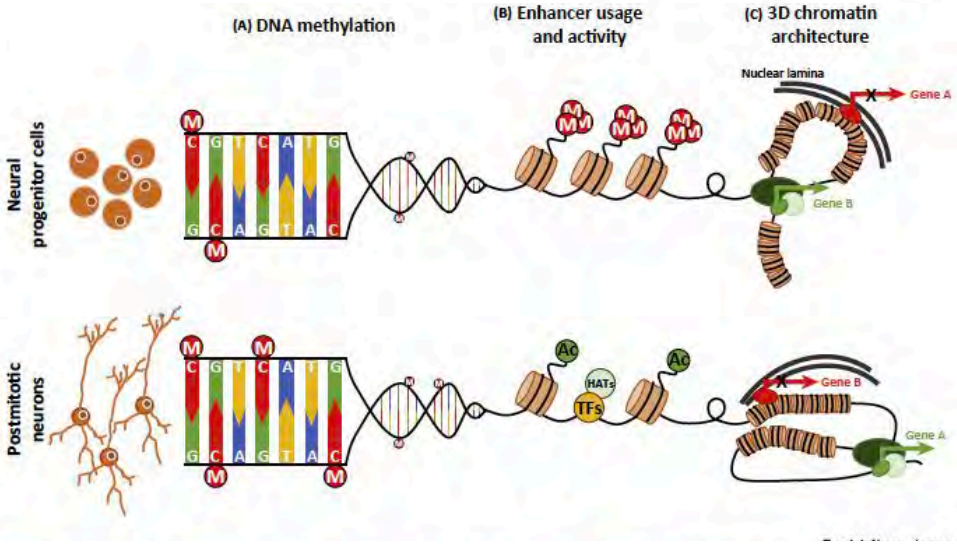
E. Heard, 15 mars, 2021

Establishing memory in the soma:
repressing pluripotency and priming / poising for later gene activity



Adapted from Cantone and Fisher, 2013

Setting up cellular memory states for later action



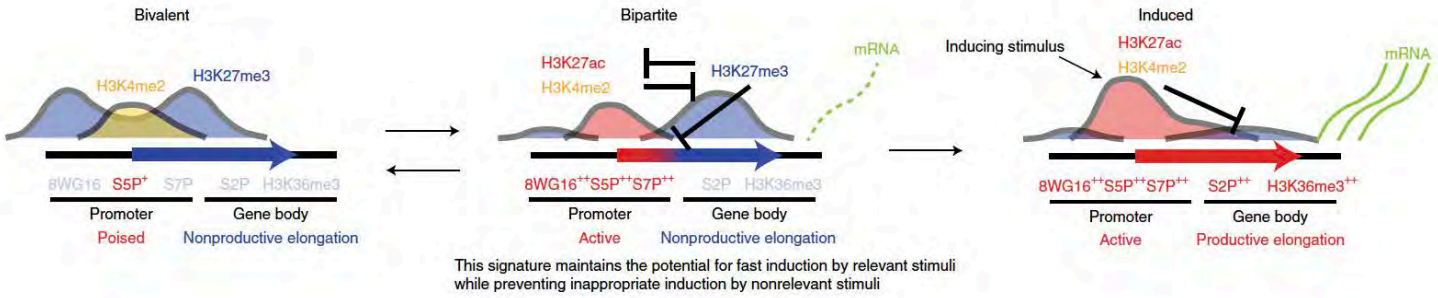
Chromosome architecture

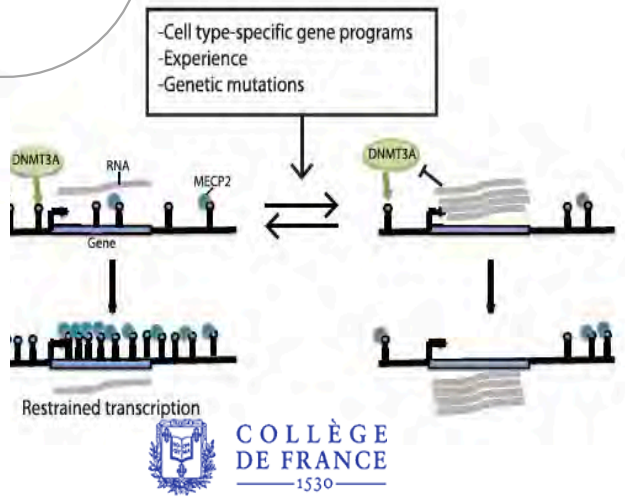
Chromatin

DNA methylation

Bookmarking factors

Pioneer factors





COLLÈGE DE FRANCE 1530

E. Heard, 15 mars, 2021

Safeguarding lineage-specific expression potential at bivalent promoters via Dppa2/4

***Dppa2* and *Dppa4* counteract de novo methylation to establish a permissive epigenome for development**

Kristjan H. Gretarsson and Jamie A. Hackett

Epigenetic priming by Dppa2 and 4 in pluripotency facilitates multi-lineage commitment

Mélanie A. Eckersley-Maslin, Aled Parry, Marloes Blotenburg, Christel Krueger, Yoko Ito, Valar Nila Roamio Franklin, Masashi Narita, Clive S. D'Santos and Wolf Reik

STEM CELL DIFFERENTIATION

Dppa2 and Dppa4 safeguard bivalent chromatin in order to establish a pluripotent epigenome

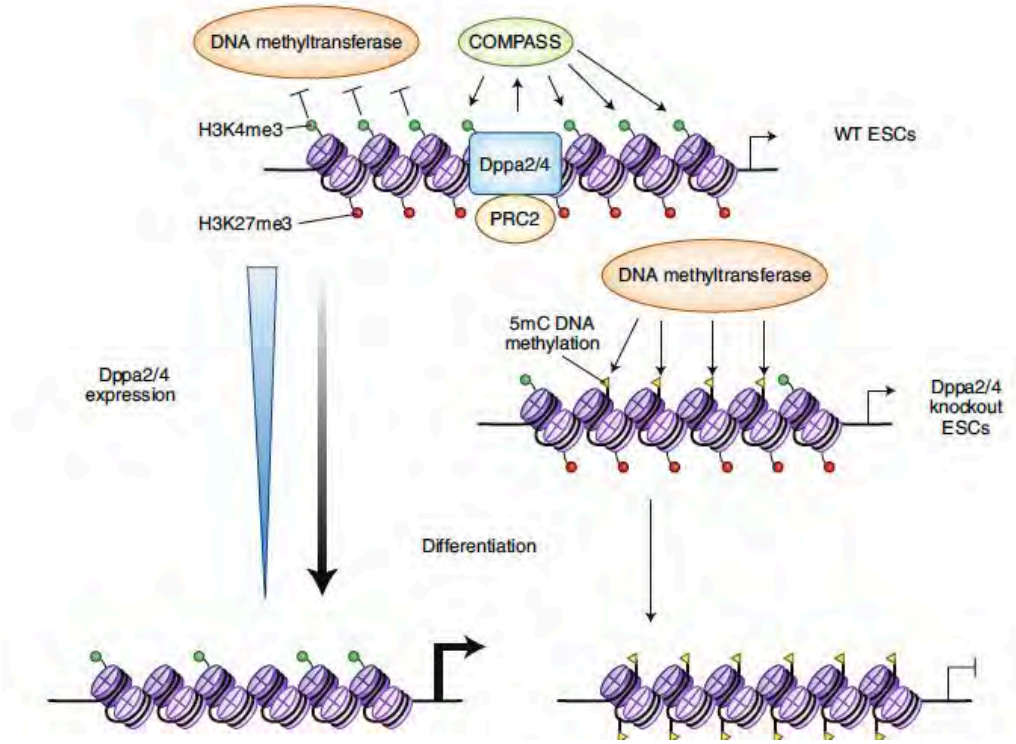
Bivalent chromatin domains contain opposing histone modifications that assist cell lineage specification. Two studies report a role for Dppa2 and Dppa4 in the establishment of bivalency and the prevention of de novo DNA methylation at development-related genes in mouse embryonic stem cells.

Early mammalian development entails genome-wide epigenome remodeling, including DNA methylation erasure and reacquisition, which facilitates developmental competence.

Dppa2 and Dppa4 are essential safeguards of focal epigenetic states. In absence of Dppa2 and Dppa4, developmental genes and young LINE1 elements, specifically bound by DPPA2, lose H3K4me3 and gain ectopic de novo DNA methylation in pluripotent cells.

Without Dppa2/4, lineage-associated genes acquire a repressive epigenetic memory, which renders them incompetent for activation during future lineage specification.

Dppa2/4 sculpt the pluripotent epigenome by facilitating H3K4me3 and bivalency to counteract de novo methylation



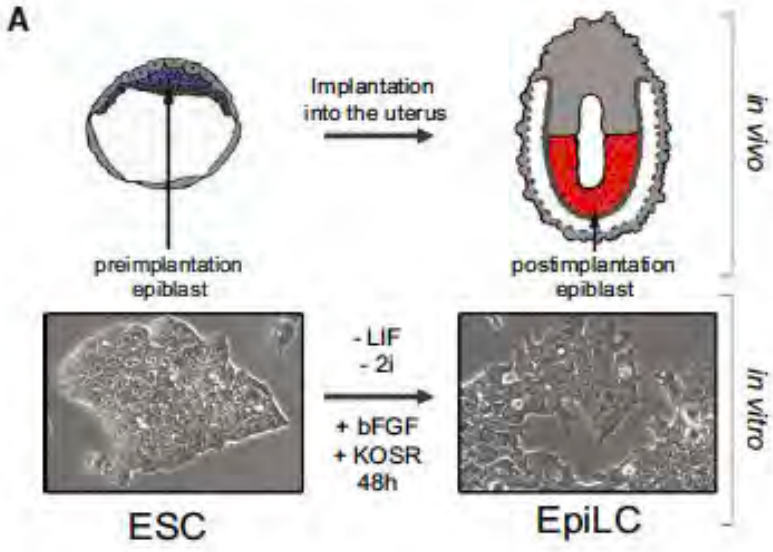
COLLÈGE DE FRANCE 1530

Priming cells for lineage specification by rewiring gene regulatory networks via specific TFs and alternative enhancers

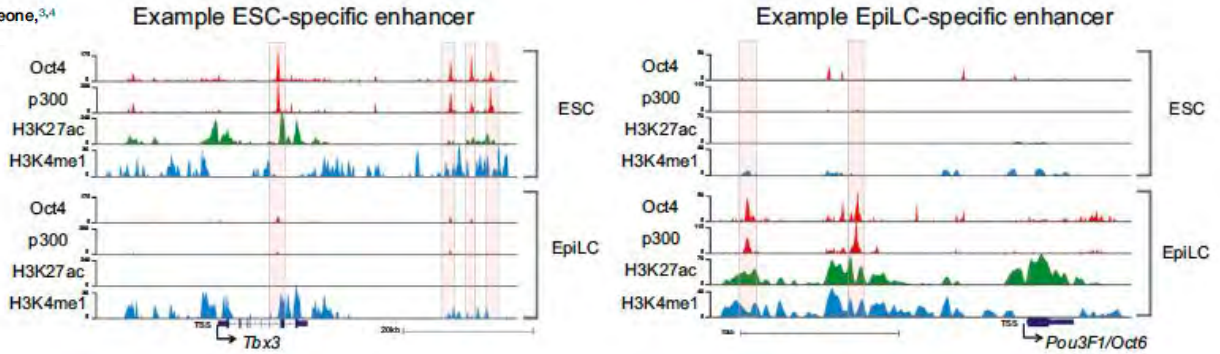
Reorganization of Enhancer Patterns in Transition from Naive to Primed Pluripotency

Christa Buecker,[1] Rajini Srinivasan,[1] Zhixiang Wu,[2] Eliezer Calo,[1] Dario Acampora,[3,4] Tiago Faial,[1] Antonio Simeone,[3,4] Minjia Tan,[2] Tomasz Swigut,[1,*] and Joanna Wysocka[1,5,*]

Cell Stem Cell 2014



Extensive epigenetic reprogramming occurs during the transition from naïve ESCs to formative EpiLCs.



- Naive and primed pluripotency is characterized by distinct signaling requirements, transcriptomes, and developmental properties,
- Both cellular states share key transcriptional regulators: Oct4, Sox2, and Nanog.
- Transition between the two pluripotent states is associated with widespread Oct4 relocalization, and global rearrangement of enhancer chromatin landscapes. Candidate mediators of primed state-specific Oct4 binding, including Otx2 and Zic2/3.
- Even when differentiation cues are blocked, premature Otx2 overexpression is sufficient to exit the naive state, induce transcription of a substantial subset of primed pluripotency-associated genes, and redirect Oct4 to previously inaccessible enhancer sites.
- However, the ability of Otx2 to engage new enhancer regions is determined by its levels, cis-encoded properties of the sites, and the signaling environment.
- **Capacity of transcription factors such as Otx2 and Oct4 to pioneer new enhancer sites is highly context dependent.**

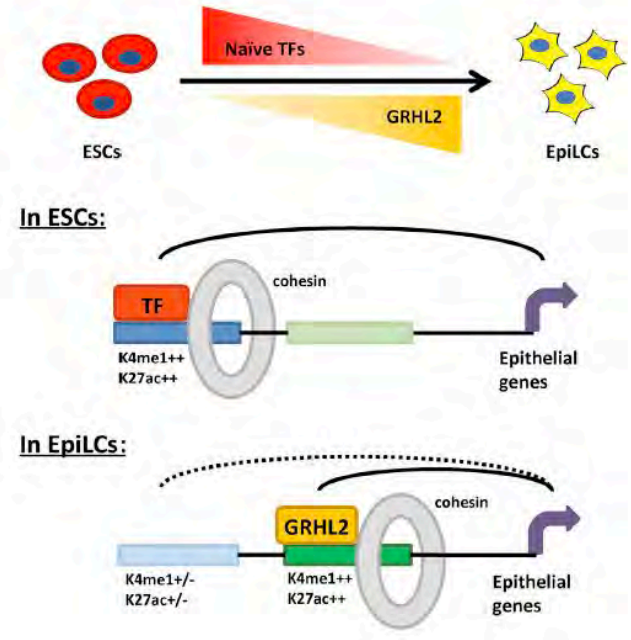
Priming cells for lineage specification by rewiring gene regulatory networks via specific TFs and alternative enhancers

Article

Cell Stem Cell

GRHL2-Dependent Enhancer Switching Maintains a Pluripotent Stem Cell Transcriptional Subnetwork after Exit from Naive Pluripotency

Chen et al, Cell Stem Cell 2018



- GRHL2 binds and activates enhancers during the transition from ESCs to EpiLCs
- GRHL2 maintains rather than activates target gene expression in EpiLCs
- GRHL2 target genes are regulated by distinct ESC-specific enhancers in ESCs
- GRHL2 loss results in an epithelial to mesenchymal-like transition in EpiLCs

Extensive epigenetic reprogramming occurs during the transition from naïve ESCs to formative EpiLCs. The transcription factor GRHL2 rewires a subset of enhancers during the transition without altering cognate gene expression. By doing so, GRHL2 subdivides the naïve pluripotency network prior to lineage diversification.

The enhancer landscape of pluripotent stem cells undergoes extensive reorganization during early mammalian development. The functions andmechanisms behind such reorganization, however, are unclear. Here, we show that the transcription factor GRHL2 is necessary and sufficient to activate an epithelial subset of enhancers as naive embryonic stem cells (ESCs) transition into formative epiblastlike cells (EpiLCs). Surprisingly, many GRHL2 target genes do not change in expression during the ESCEpiLC transition. Instead, enhancers regulating these genes in ESCs diminish in activity in EpiLCs while GRHL2-dependent alternative enhancers become activated to maintain transcription. GRHL2 therefore assumes control over a subset of the naive network via enhancer switching to maintain expression of epithelial genes upon exit from naive pluripotency. These data evoke a model where the naive pluripotency network becomes partitioned into smaller, independent networks regulated by EpiLC-specific transcription factors, thereby priming cells for lineage specification

E. Heard, 15 mars, 2021

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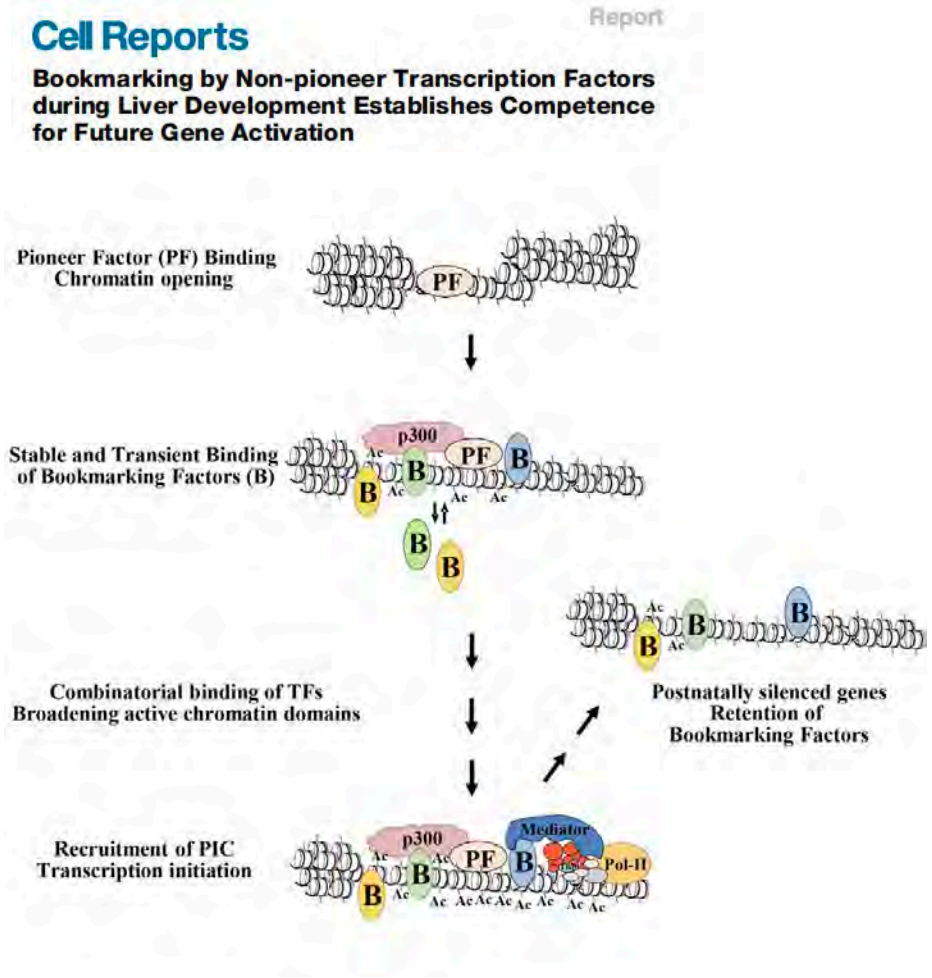
Stable gene expression patterns during liver development result from combinatorial activity of multiple transcription factors



HNF4a and C/EBPa are prominent hepatic transcription factors required for the activation of most hepatic genes, but they lack pioneer factor features, such as high-affinity binding to compacted nucleosomes and an ability to open condensed chromatin

- During liver development, master transcription
- factors bind to their targets in a temporally stable or dynamic manner.
- Early and persistent binding is necessary, but not sufficient, for gene activation.
- Stable gene expression patterns are the result of combinatorial activity of multiple transcription factors, which mark regulatory regions long before activation and promote progressive broadening of active chromatin domains.
- Both temporally stable and dynamic, short-lived binding events contribute to the developmental maturation of active promoter configurations.

Karagianni et al, Cell Reports 2020

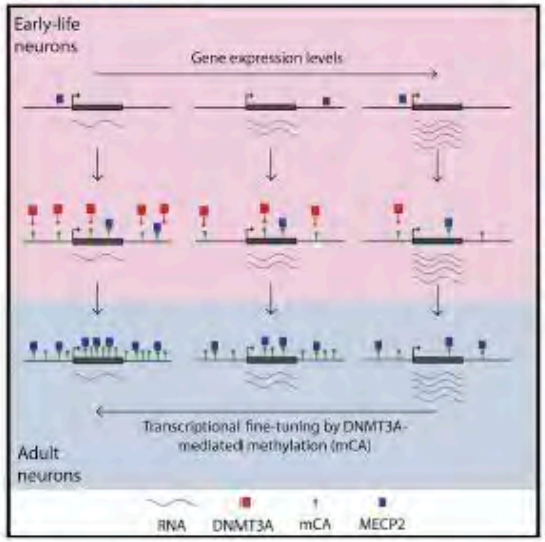
Deposition of mCA marks by DNMT3A within specific brain genes during early postnatal life is important for their regulation throughout life

Article

Cell

Early-Life Gene Expression in Neurons Modulates Lasting Epigenetic States

Stroud et al, 2017

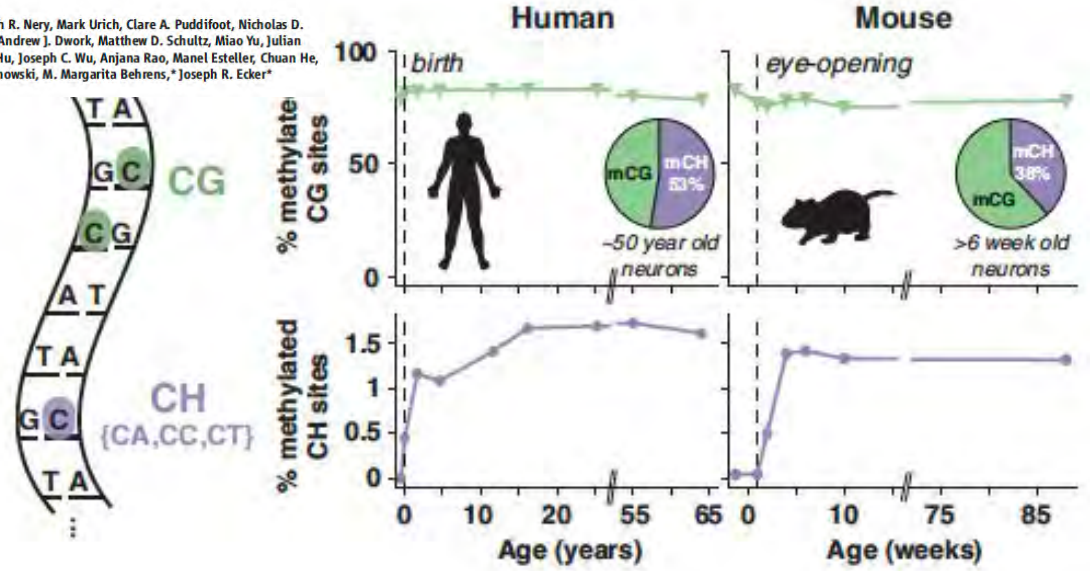


Highlights

- In the brain, DNMT3A binds the genome during early life to specify CA methylation
- DNMT3A preferentially binds across transcribed regions of lowly expressed genes
- DNMT3A binding across genes is modulated by the transcription states of genes
- mCA recruits MECP2 and fine-tunes gene expression in the adult brain

Global Epigenomic Reconfiguration During Mammalian Brain Development

Ryan Lister,* Eran A. Mukamel, Joseph R. Nery, Mark Urich, Clare A. Puddifoot, Nicholas D. Johnson, Jacinta Lucero, Yun Huang, Andrew J. Dwork, Matthew D. Schultz, Miao Yu, Julian Tonti-Filippini, Holger Heyn, Shijun Hu, Joseph C. Wu, Anjana Rao, Manel Esteller, Chuan He, Fatemeh G. Haghighi, Terrence J. Sejnowski, M. Margarita Behrens,* Joseph R. Ecker*



- Extensive methylome reconfiguration occurs during development from fetal to young adult.
- In this period, coincident with synaptogenesis, highly conserved non-CG methylation (mCH) accumulates in neurons, but not glia, to become dominant form of methylation in human neuronal genome.
- Multiple scales of brain cell DNA methylation:
- intragenic methylation patterns in neurons that distinguish genes with cell type–specific activity.
- novel mCH signature that identifies genes escaping X-chromosome inactivation in neurons.

COLLEGE DE FRANCE 1530

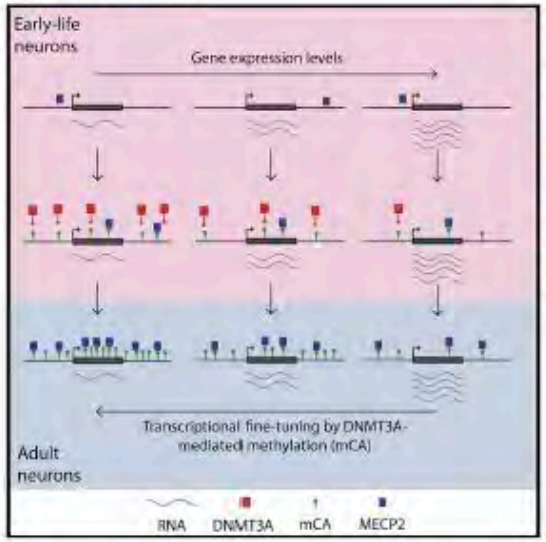
Deposition of mCA marks by DNMT3A within specific brain genes during early postnatal life is important for their regulation throughout life

Article

Cell

Early-Life Gene Expression in Neurons Modulates Lasting Epigenetic States

Stroud et al, 2017



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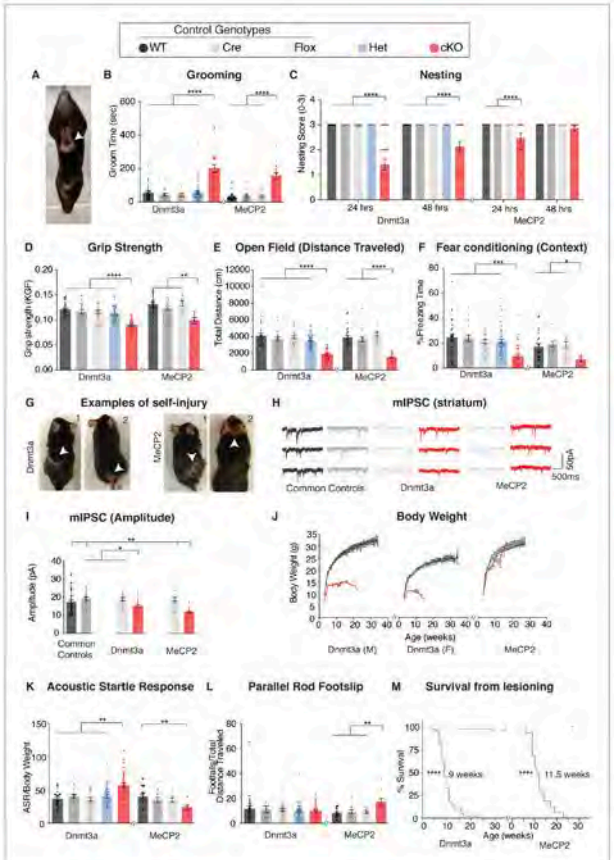
Losing Dnmt3a dependent methylation in inhibitory neurons impairs neural function by a mechanism impacting Rett syndrome

Lavery et al, Elife 2020

Loss of Dnmt3a or MeCP2 in inhibitory neurons produces overlapping but not identical behavioral phenotypes

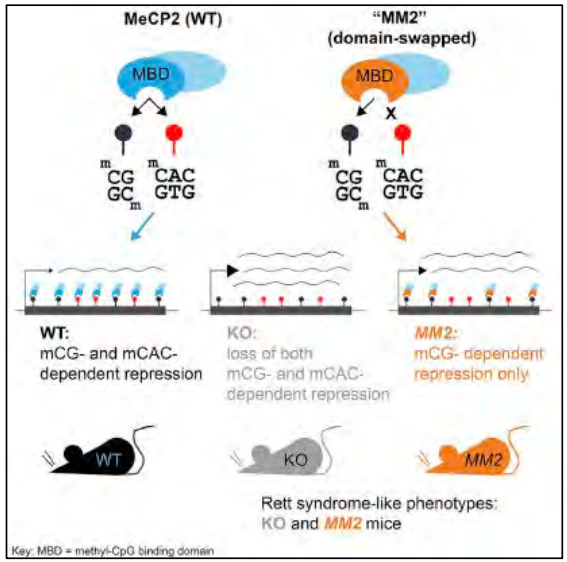


Article

Molecular Cell

Neuronal non-CG methylation is an essential target for MeCP2 function

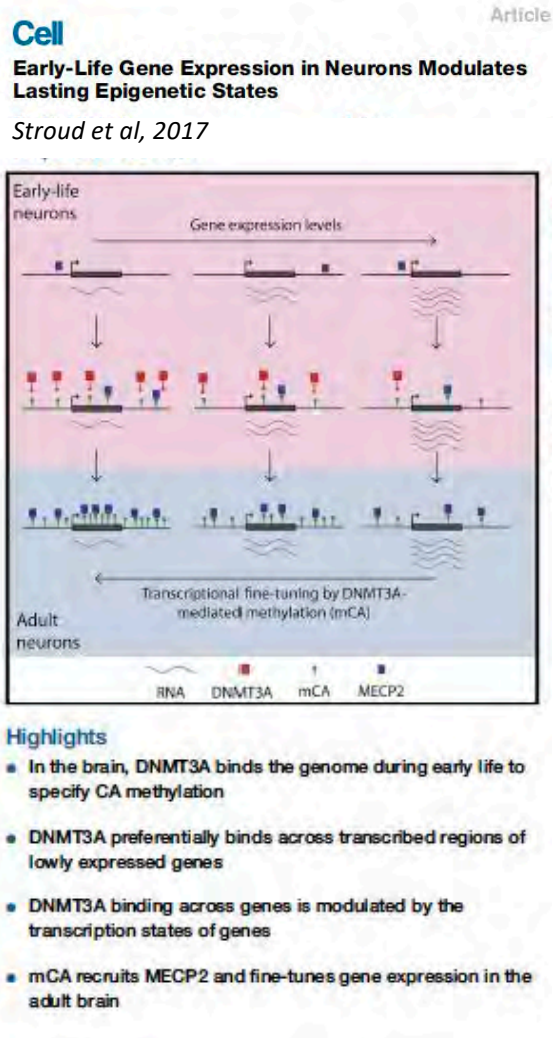
Tillotson et al, Mol Cell 2020



- MeCP2 has dual-binding specificity for mCG and mCAC motifs
- Chimeric protein MM2 contains a similar DNA binding domain that only recognizes mCG
- Knockin mice expressing MM2 display Rett-syndrome-like phenotypes
- Genes dysregulated in both *MM2* and *Mecp2* null mice may contribute to Rett syndrome

COLLEGE DE FRANCE 1530

Deposition of mCA marks by DNMT3A within specific brain genes during early postnatal life is important for their regulation throughout life



- In early life, DNMT3A binds broadly across the genome (less at promoters and enhancers)
- DNMT3A is recruited *transiently* during the early postnatal period, at lowly expressed gene bodies where it leads to mCA (and some mCpG) methylation
- Once a gene has been specified to be lowly expressed, the gene likely binds DNMT3A within its transcribed region and becomes methylated at CA sequences.
- mCA recruits MECP2 – and allows for fine tuning of transcription in specific neuronal subtypes
- Mutations in MECP2 cause RTT, and this is due to a loss of binding of MECP2 to mCA sequences across the neuronal genome
- mCA contributes to the fine-tuning of genes, including those with critical neuronal functions, in a neuronal subtype-specific manner at least in part by differentially recruiting MECP2 to neuronal gene bodies.
- Once bound to mCA, MECP2 appears to restrain gene transcription to a level of expression that is directly correlated with the number of mCA marks and MECP2 binding sites per gene, thus preferentially regulating some of the longest genes in the genome
- Genes that are misregulated in both DNMT3A and MECP2 mutants (316) show overlap – and the most severely dysregulated genes show highest levels of mCA => new candidate genes for Rett syndrome (*eg CNTN4 – neuronal membrane glycoprotein; AUTS2 is a transcriptional activator with non-canonical PRC1*)

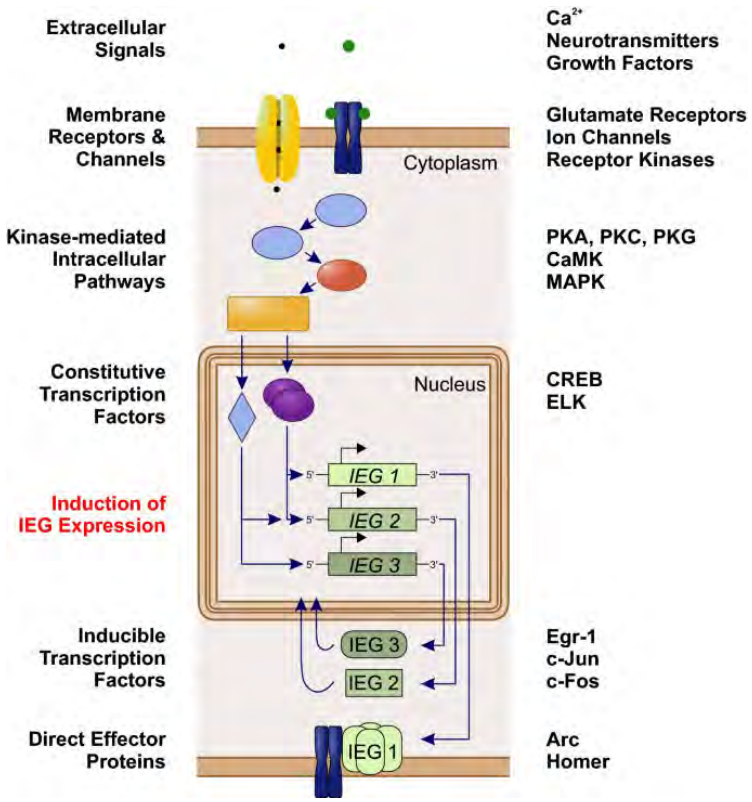
E. Heard, 15 mars, 2021

Bipartite Polycomb signature regulates stimulus-response transcription during development

- During development, cells are exposed to a variety of distinct environmental signals to which they may need to rapidly respond in a spatiotemporally regulated manner, in order to keep their differentiation schedule.
- Stimulus-response genes are essential for rapid cellular responses to extracellular signals.
- Among them, immediate early genes (IEGs) are induced in multiple cell types within minutes in a stimulus-dependent manner, often encoding transcription factors (for example, Fos and Egr1), which in turn regulate the expression of downstream late-response genes (LRGs) through activation of enhancers.
- Before induction, IEGs share key regulatory properties, which poise them for rapid stimulus dependent activation.
- In general, these include accessible promoters and enhancers bound by serum response factor, nuclear factor-κB, cyclic AMP response element-binding protein (CREB) and/or activator protein-1 transcription factors, which are posttranslationally modified upon stimulus response, as well as transcriptionally permissive histone modifications (H3K4me2/3) and paused RNA polymerase II (RNAPII).
- These IEGs are both general (ie induced in most cell types in response to different stimuli) and some are cell-type specific (responding to specific signals in different cell types).

- **How is spatiotemporal regulation and specificity of the IEG transcriptional response achieved in developing cells?**

- **How is untimely induction of IEGs in response to spurious signals is prevented?**

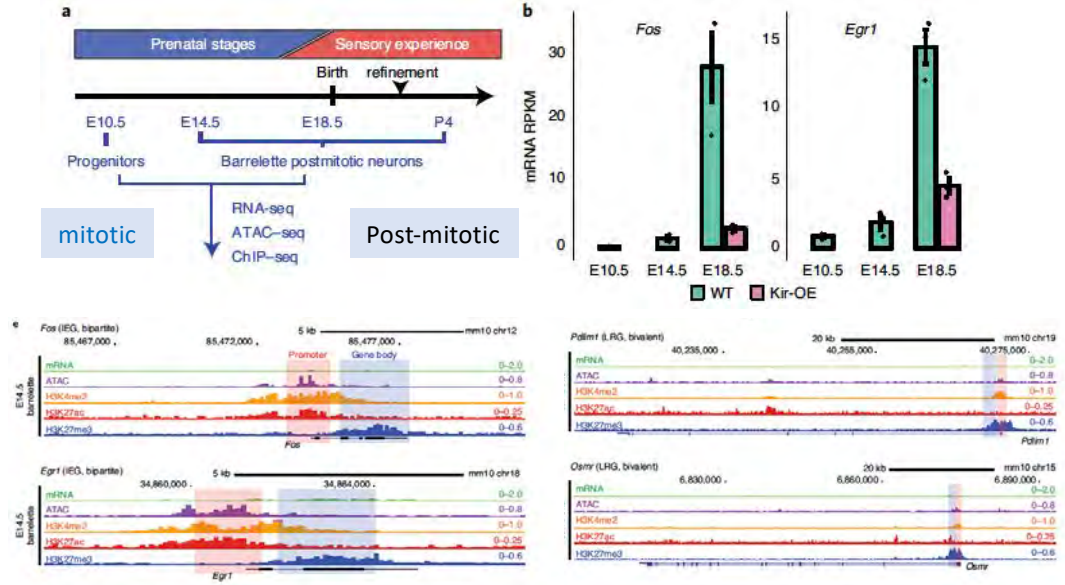


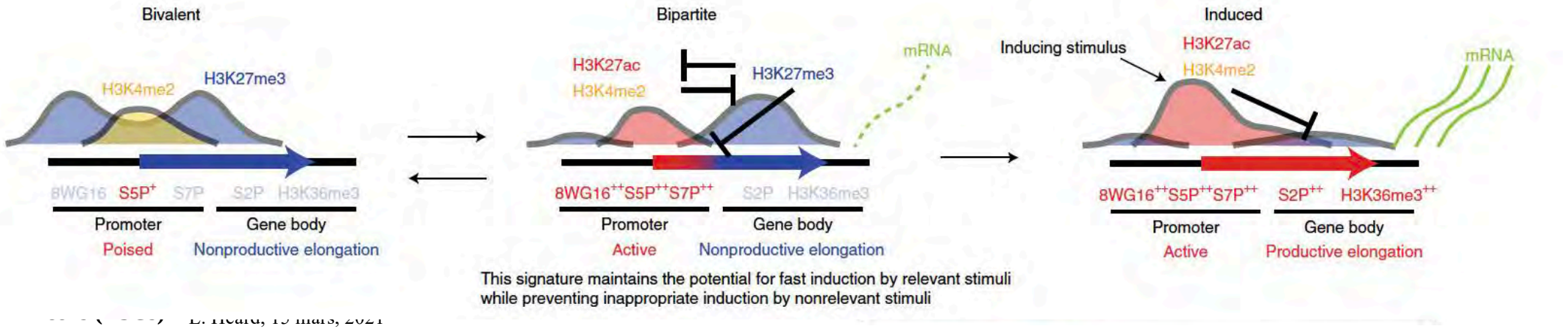
Kitazawa et al, Nature Genetics 2021

Bipartite Polycomb signature regulates stimulus-response transcription during development

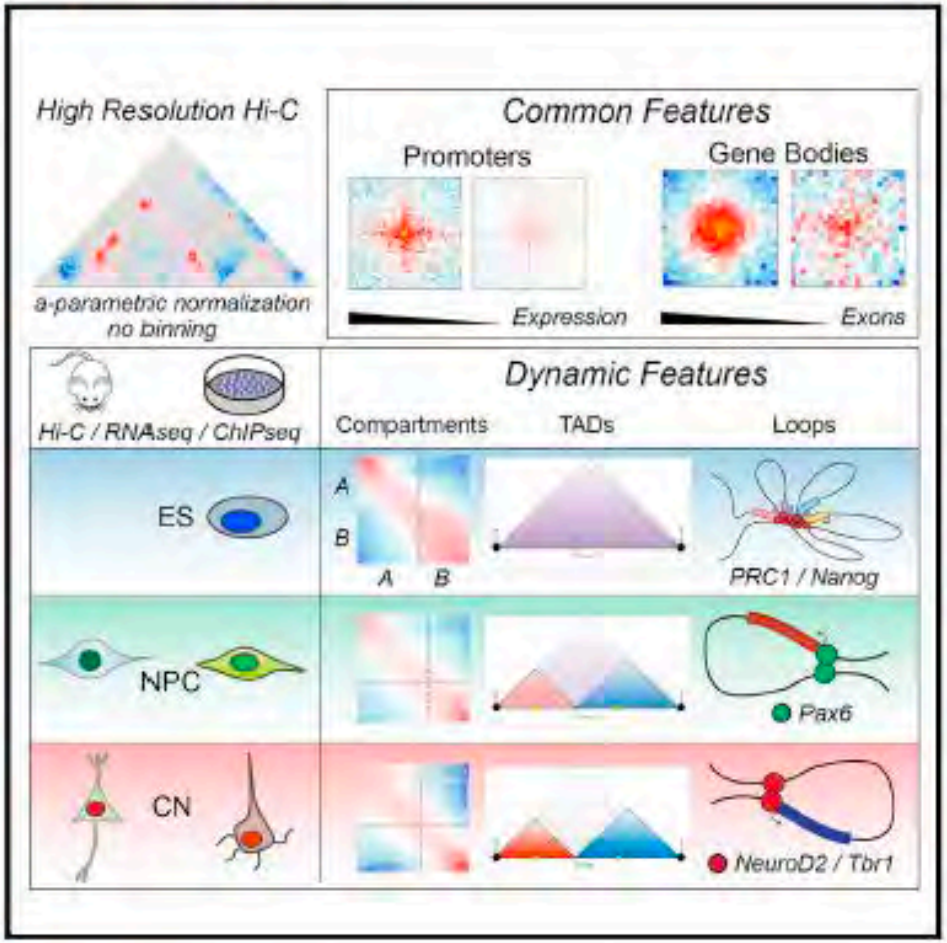
During development, response to environmental signals requires rapid, stimulus-dependent, transcriptional responses through induction of Immediate Early Genes (IEGs), encoding TFs – which in turn regulate activation of specific Late Response Genes (LRGs), driving cell-type-specific differentiation schedules.

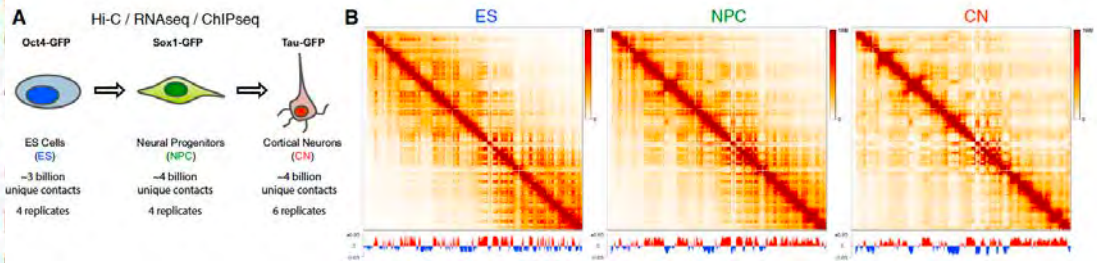
- A unique H3K27ac/H3K27me3 bipartite chromatin signature, modulates the rapidity and amplitude of the transcriptional response of inducible IEGs to distinct stimuli during development.
- Polycomb (Pc)-dependent H3K27me3 on gene bodies inhibits the productive elongation of RNAPII on bipartite genes (shown by PRC2 Ezh1/2 KO and Utx targeting)
- Polycomb marks the body of IEG genes and may act as a buffer against untimely high-level expression.
- Strong stimuli allow for the rapid removal of Pc marking of gene bodies and fast transcriptional induction (*active removal, not passive*)





3D organization to memorise gene regulatory landscapes?





- Ultra-deep Hi-C during mouse neural differentiation, both *in vitro* and *in vivo*
- Transcription is correlated with, but not sufficient for, local chromatin insulation
- Polycomb network is disrupted, while novel contacts between neural TF sites appear
- Dynamic contacts among exon-rich gene bodies, enhancer-promoters, and TF sites

Boyev et al, Cell 2018

SUMMARY

Cellular Memory: stability and plasticity during development

Orchestrating epigenesis

- Focus only on transcripton factor (TF) networks, signalling, and chromatin
- Most epigenetic factors (chromatin associated) play multiple different roles throughout development
- Establishing the earliest cell fate decision through transcriptional noise and chromatin remodeling
- Early parental asymmetries in gene expression due to chromatin and 3D organization – transient imprints and X inactivation
- Reactivation of the inactive X is driven by active processes: transcription factors and histone demethylase?
- Repressing and activating or priming genes as lineages are established
- Establishing the memory of somatic cell lineages through pioneer and bookmarking transcription factors as well as chromatin