Année 2016-2017 :
"Épigénétique et ADN égoïste"

20 Février, 2017

Cours III

L'impact des éléments transposables et de leurs reliques sur le développement.

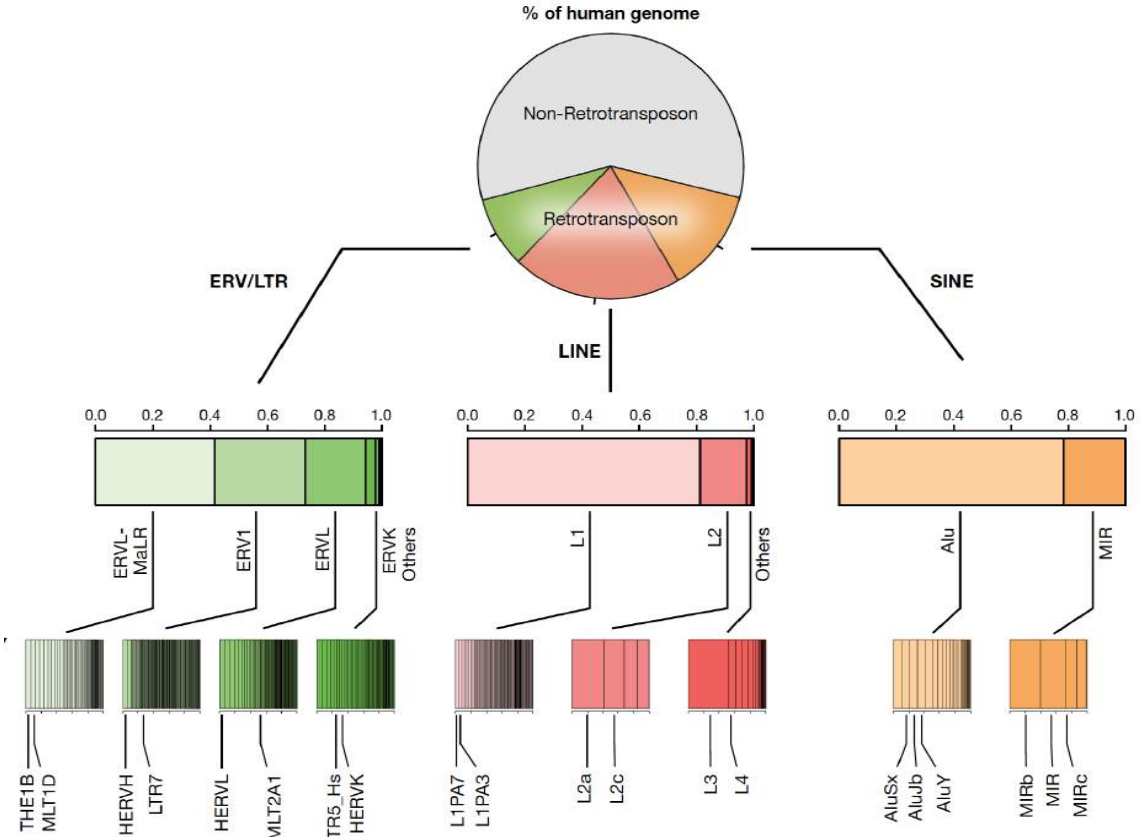
The impact of transposable elements and their relics on development

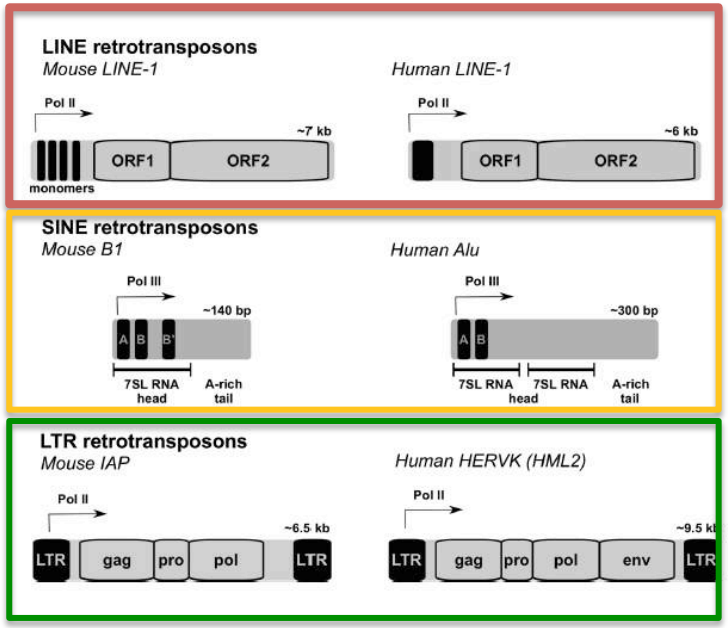
Epigenetic Control as a Defense but also a Resource for the Host and its Selfish Parasites

This week:
DNA-based targeting of TEs for silencing
Expression, control and potential roles of TEs during development

- RNA interference and Epigenetic silencing mechanisms co-evolved with TEs to protect the host genome but also provide opportunities for new functions
- Ongoing **arms race** between TE and Host => evolving attack + defense strategies
- Epigenetic mechanisms: opportunity for heritable and reprogrammable control
- RNA and DNA-targeting of epigenetic machinery: ancient RNAi strategies (piRNAs and protecing the germ line - last week)
- KRAB-Zinc finger proteins (this week)

Mammalian Retrotransposons





- ✧ Humans: a few (<10) "Hot" L1s account for most L1 and Alu retrotransposition
- ✧ Human ERVs are generally immobile except for HERVH/HERVK
- ✧ In mice several 100 young, potentially mobile LINEs
- ✧ In mice, ERVs (esp IAP and ETns) account for 10% of spontaneous mutations
- ✧ In both mice and humans, truncated/mutated TEs are ***still transcriptionally active:***
- ✧ **=>** material for regulatory landscapes of host genes…

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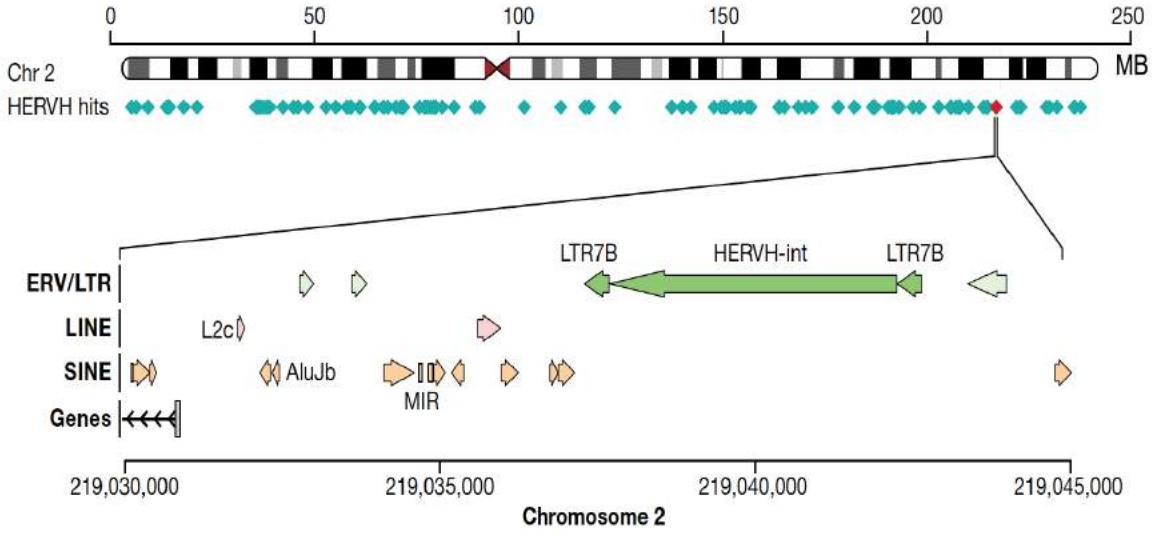

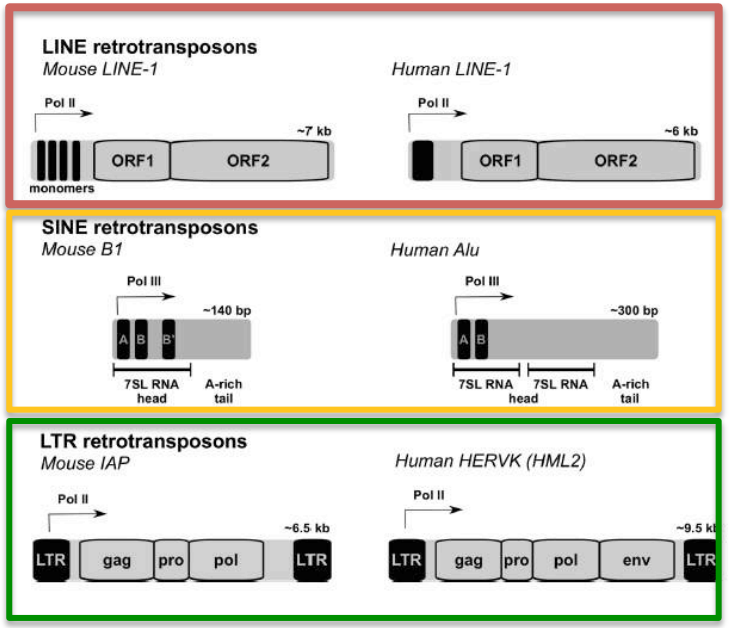

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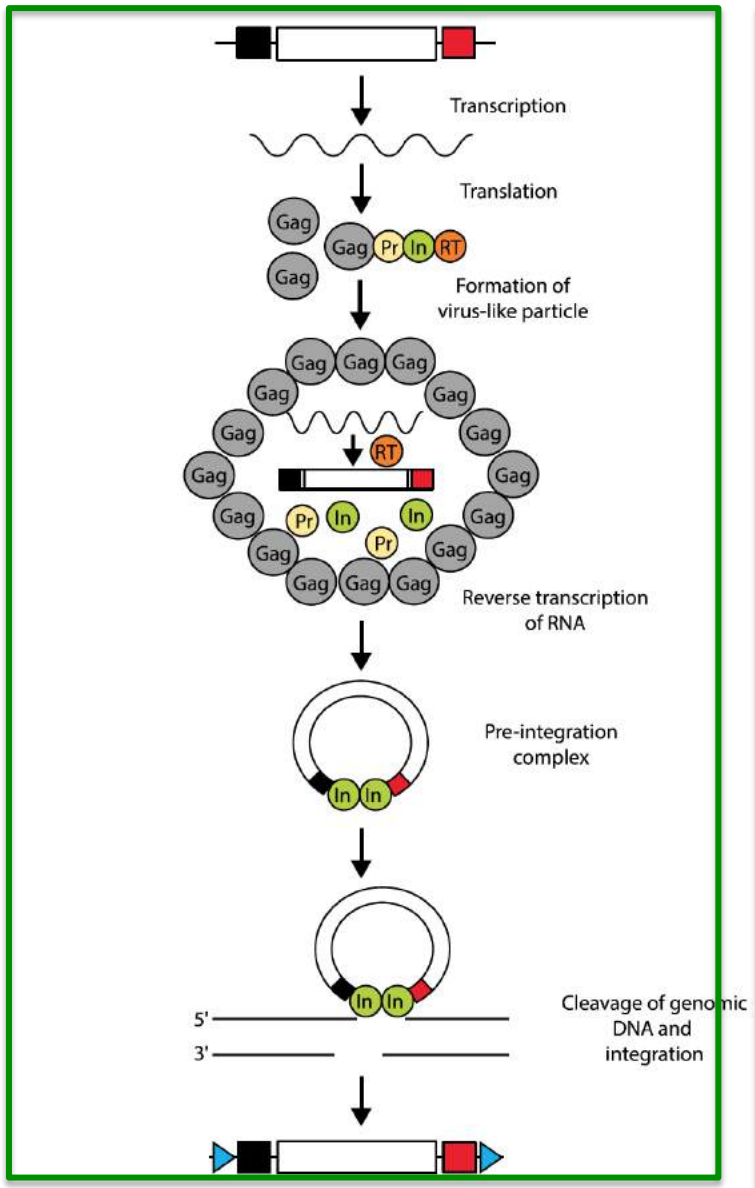
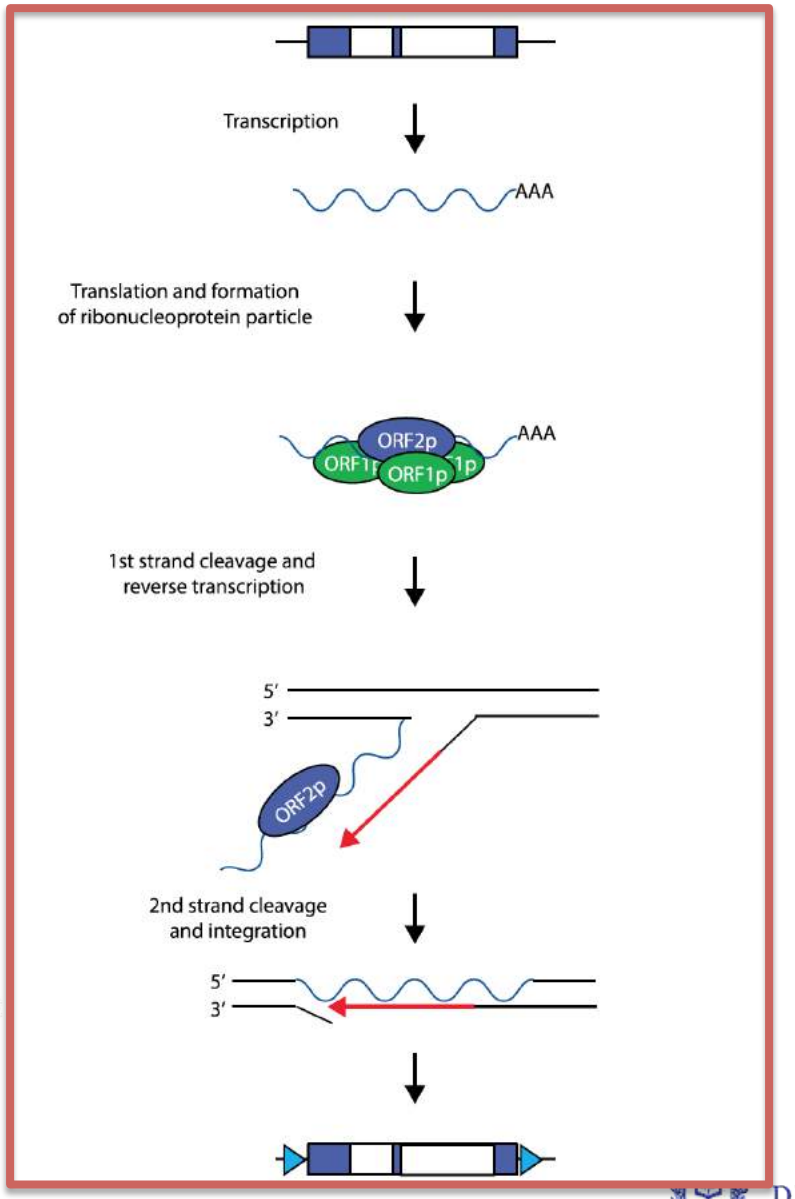
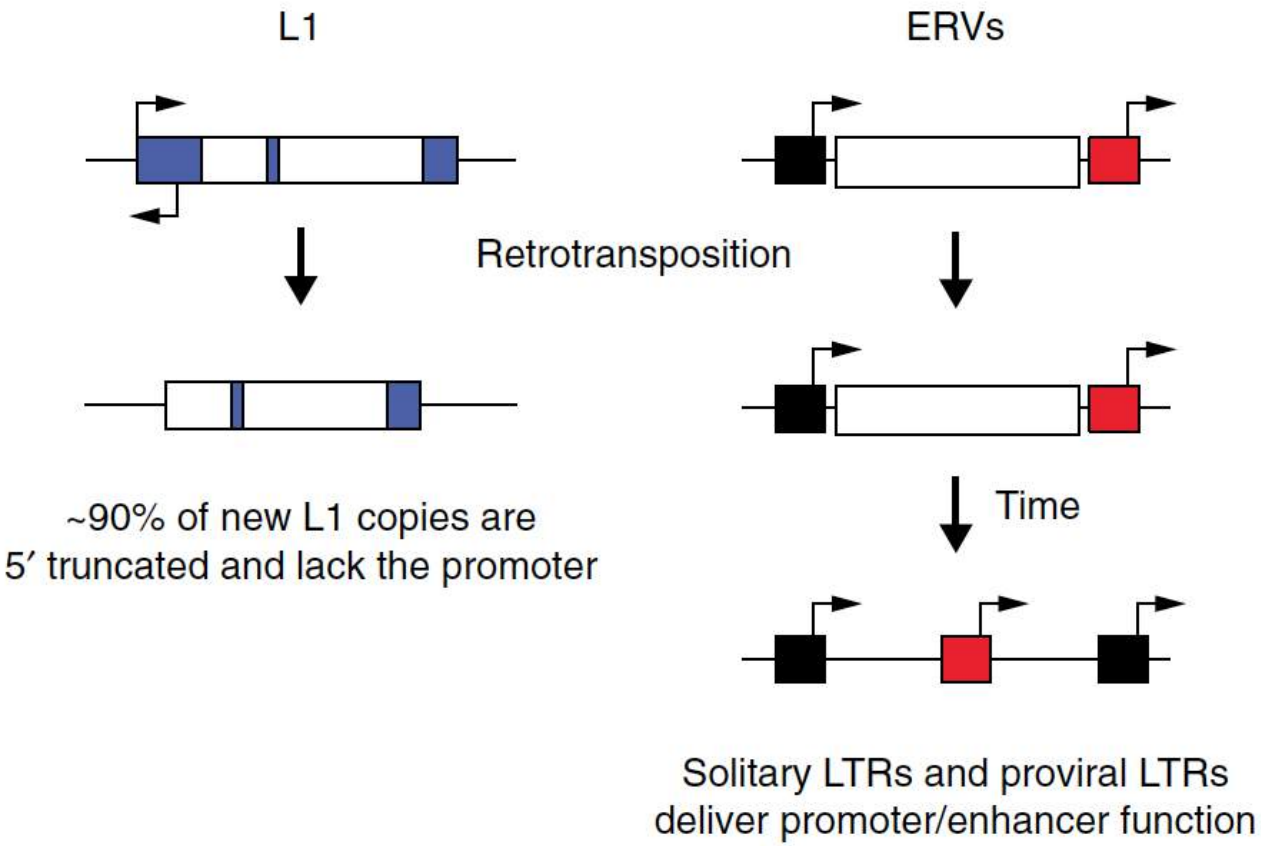
LTR and non-LTR Retrotransposons





From Gerdes et al, 2016

LTR and non-LTR Retrotransposons



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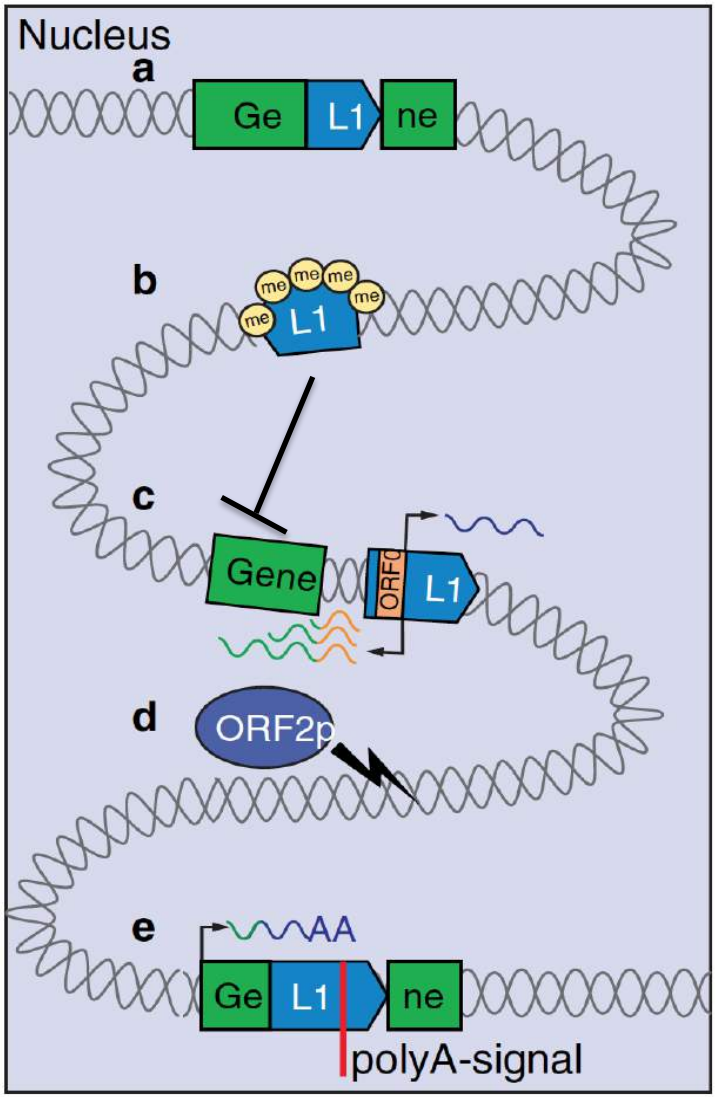
Impact of TE insertions on Gene Regulation



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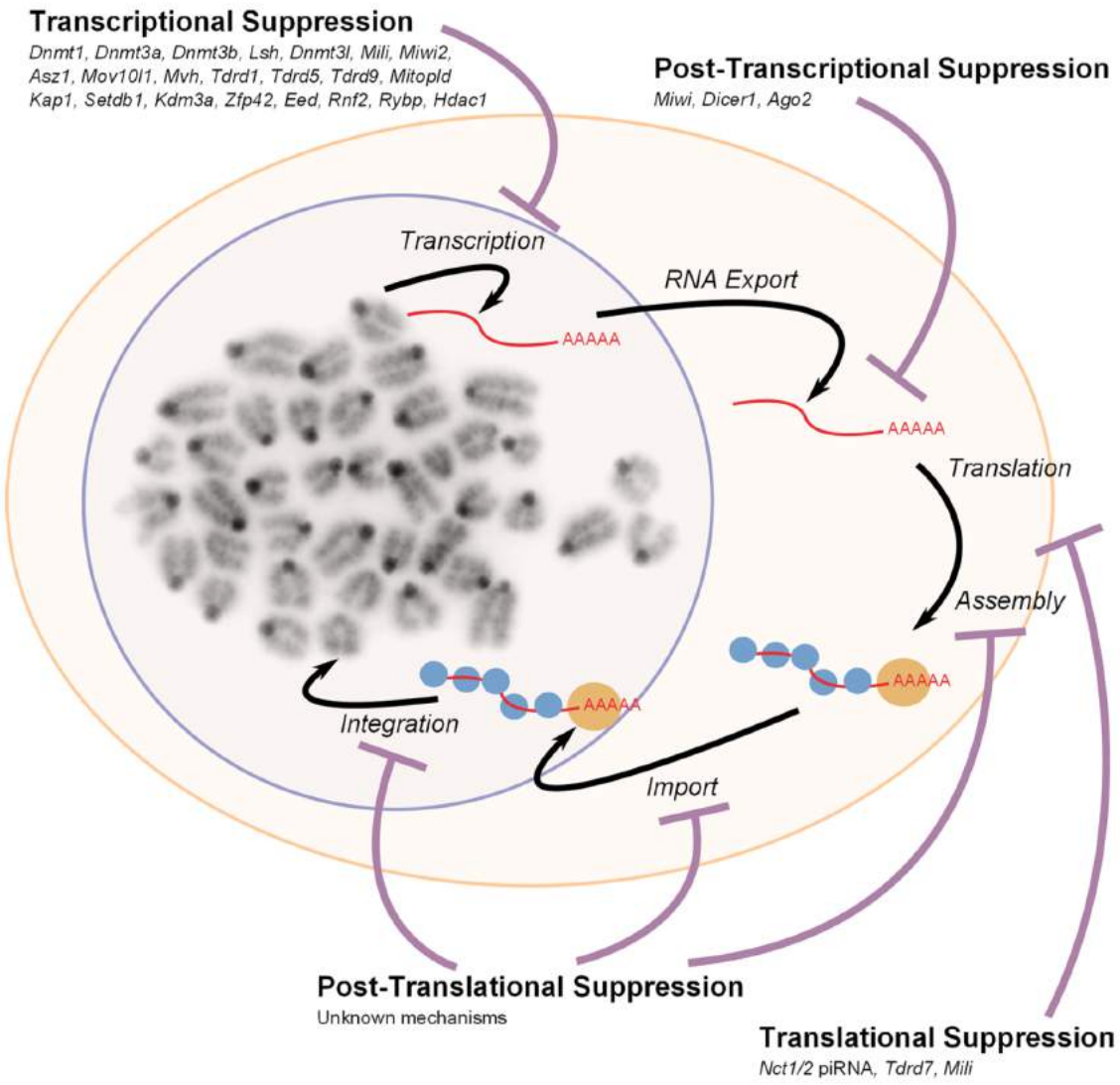
Retrotransposon Control Strategies?



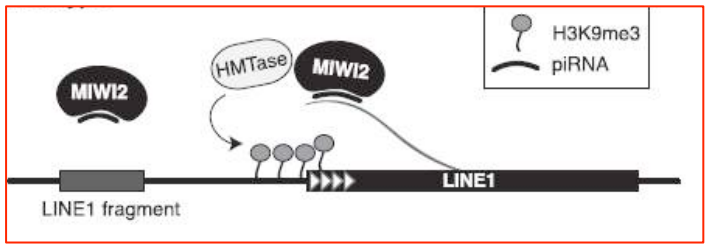
Targeting of TEs for repression

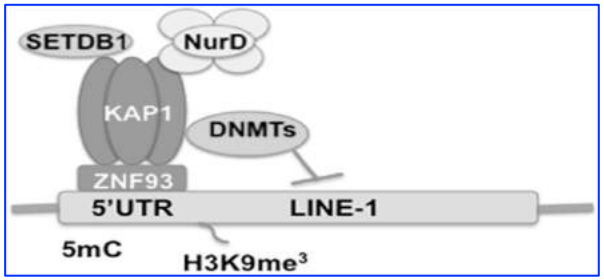
Both RNA and DNA based mechanisms of TE recognition exist:

- RNA interference is an almost universal feature of TE control

  Small RNAs derived from TEs can:

  - target TE mRNA for degradation and translational inhibition
  - target TE chromatin for heritable **epigenetic modifications** (H3K9me/HP1; DNA me)



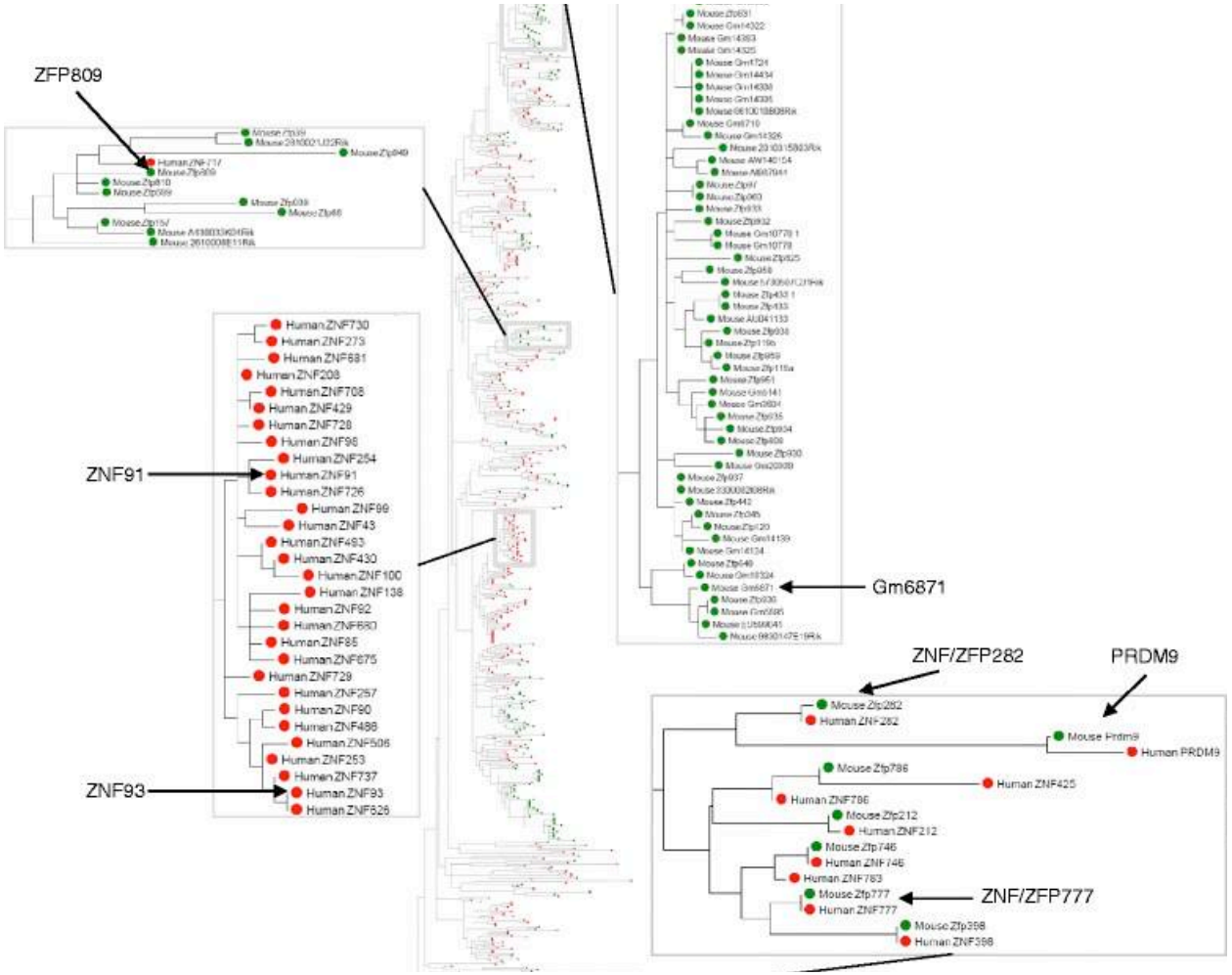


- DNA sequences of TEs can be recognized by repressor proteins (zinc finger proteins) that bind specifically and can recruit **heterochromatin-inducing** factors

- Different eukaryotes exploit different types and combinations of controls
  - control strategy also varies depending on cell type, or developmental stage
  - as well as on the nature and age of the TE
  - the older TE relics and their control are often co-opted for host gene regulation

Targeted TE silencing by the Krüppel-associated box zinc finger proteins (KRAB-ZFP) and KAP1/TRIM28

KRAB-ZFPs are largest family of gene regulating proteins in mammals



Castro-Diaz, 2014; Ecco et al, 2016; Karimi et al, 2011; Matsui et al, 2010; Rowe et al, 2010; Wolf and Godd 2009; Wolf et al, 2015

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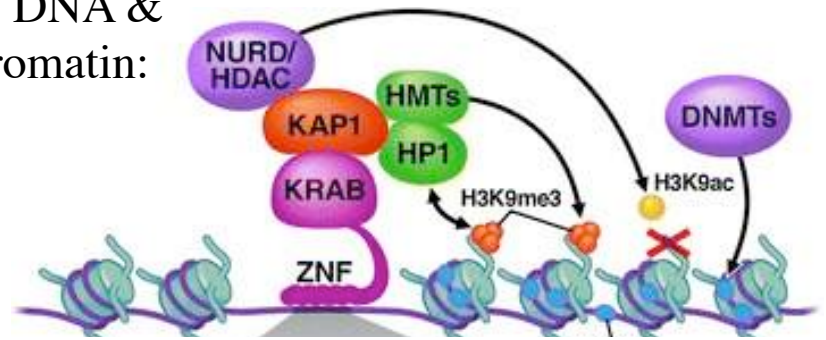
KRAB-ZFP binding to DNA & induction of heterochromatin:



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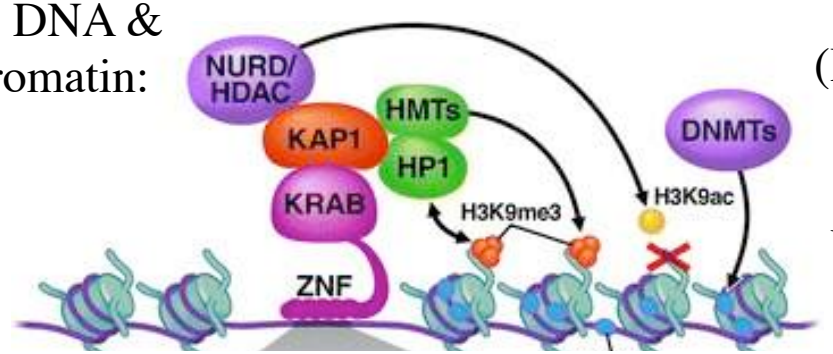
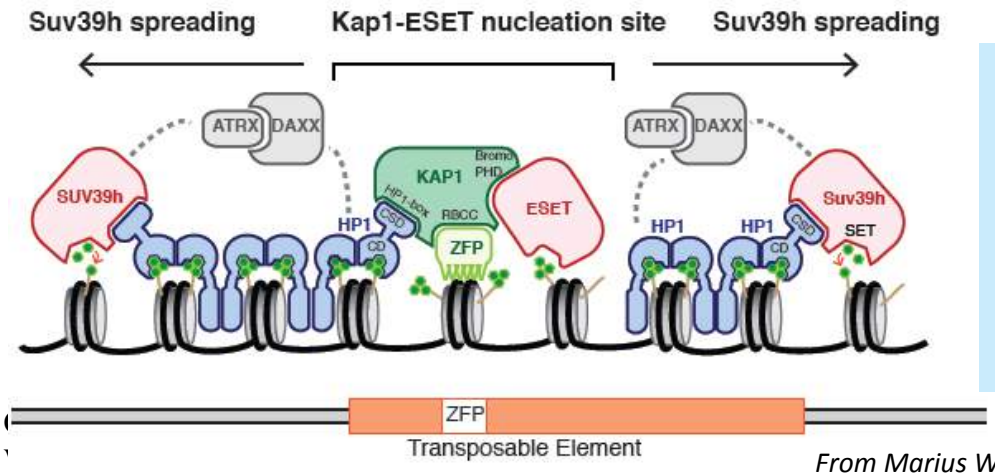
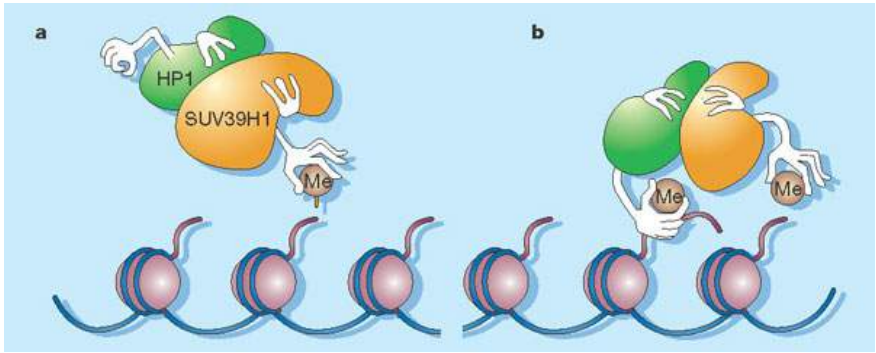


DNA methyltransferases (DNMTs) methylate genomic CpG sites, leading to heritable silencing – this is usually a downstream event

KAP1/TRIM28 is recruited through the **KRAB** domain
It interacts with the **NURD/HDAC** repressor complex which catalyzes removal of H3K9ac
It also interacts with histone methyltransferases (HMTs) (e.g. **SETDB1/ESET**) =>H3K9me3.
HP1γ interacts with both KAP1 and H3K9me3 -> and heterochromatin may **spread locally** via HP1 and SUV39H HMTase? (**see Cours 2015**)





From Marius Walter
Inspired by Bulut-Karsioglu et al., 2014 and Elsässer et al., 2015

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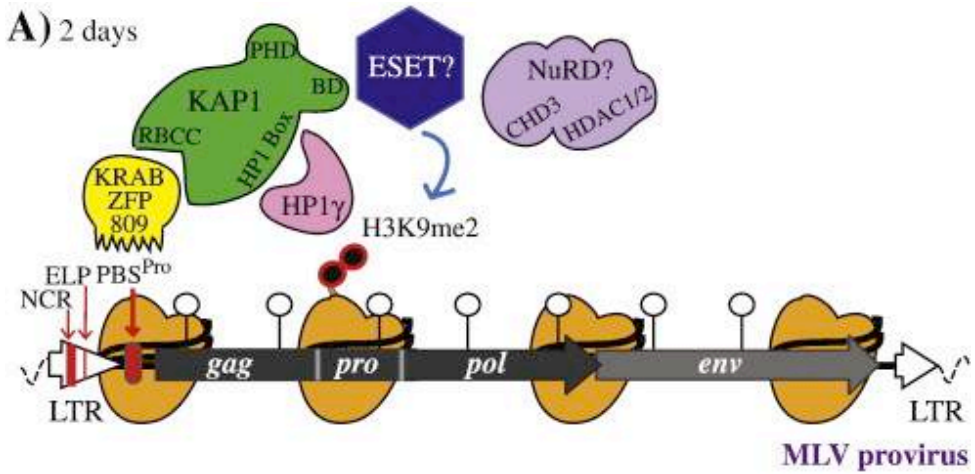


DNA methyltransferases (DNMTs) methylate genomic CpG sites, leading to heritable silencing – this is usually a downstream event

During differentiation, repression can be reinforced and/or replaced by DNA methylation

The KRAB zinc finger protein ZFP809 is required to initiate epigenetic silencing of endogenous retroviruses

Gernot Wolf,[1,2] Peng Yang,[1] Annette C. Füchtbauer,[2] Ernst-Martin Füchtbauer,[2] Andreia M. Silva,[2] Chungoo Park,[1,4] Warren Wu,[1] Anders L. Nielsen,[3] Finn S. Pedersen,[2] and Todd S. Macfarlan[1]

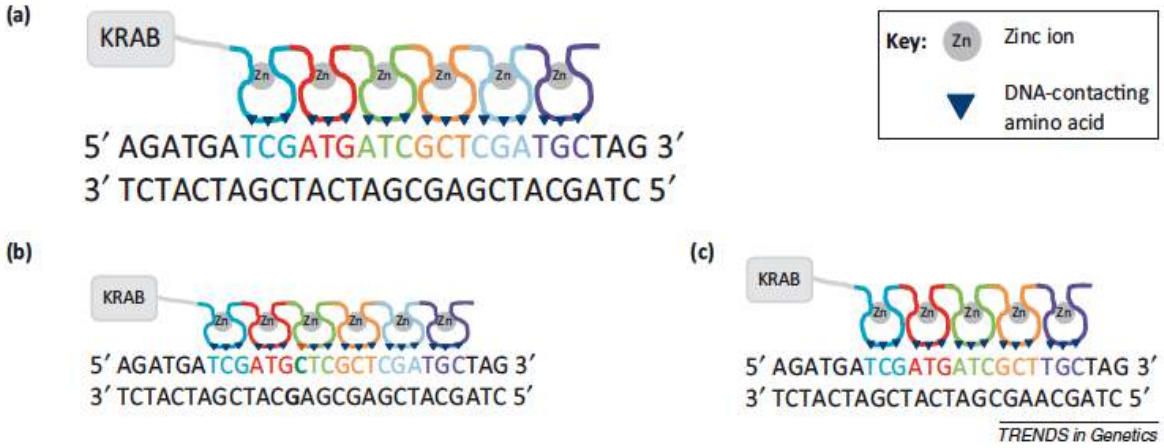
- ZFP809 knockout mice – see reactivation of ZFP809-targeted ERVs in somatic tissues.
- ERV reactivation accompanied by shift from repressive to active chromatin (H3K9me3 loss). DNA methylation only slightly affected.
- ZFP809 is required to *initiate* ERV silencing during embryonic development but becomes largely dispensable in somatic tissues (conditional KO/rescue)

Cas
Wol

Evolution of KRAB-ZFPs to repress specific TEs

DNA-binding specificity of ZFP809 is evolutionarily conserved in rodents and predates the endogenization of retroviruses now targeted by ZFP809 in Mus musculus.

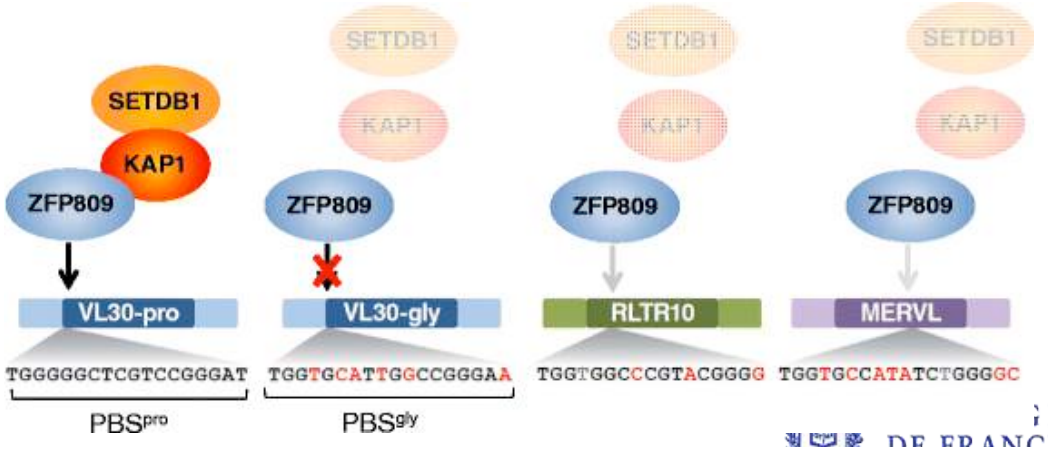
ZFP809 evolved to recognize foreign DNA and establish H3K9 methylation–based epigenetic silencing of ERVs.



Example of differential ZFP809 binding to various ERVs:

ZFP809 target sequences identified by ChIP-seq shown with differences from the canonical binding sequence highlighted in red.

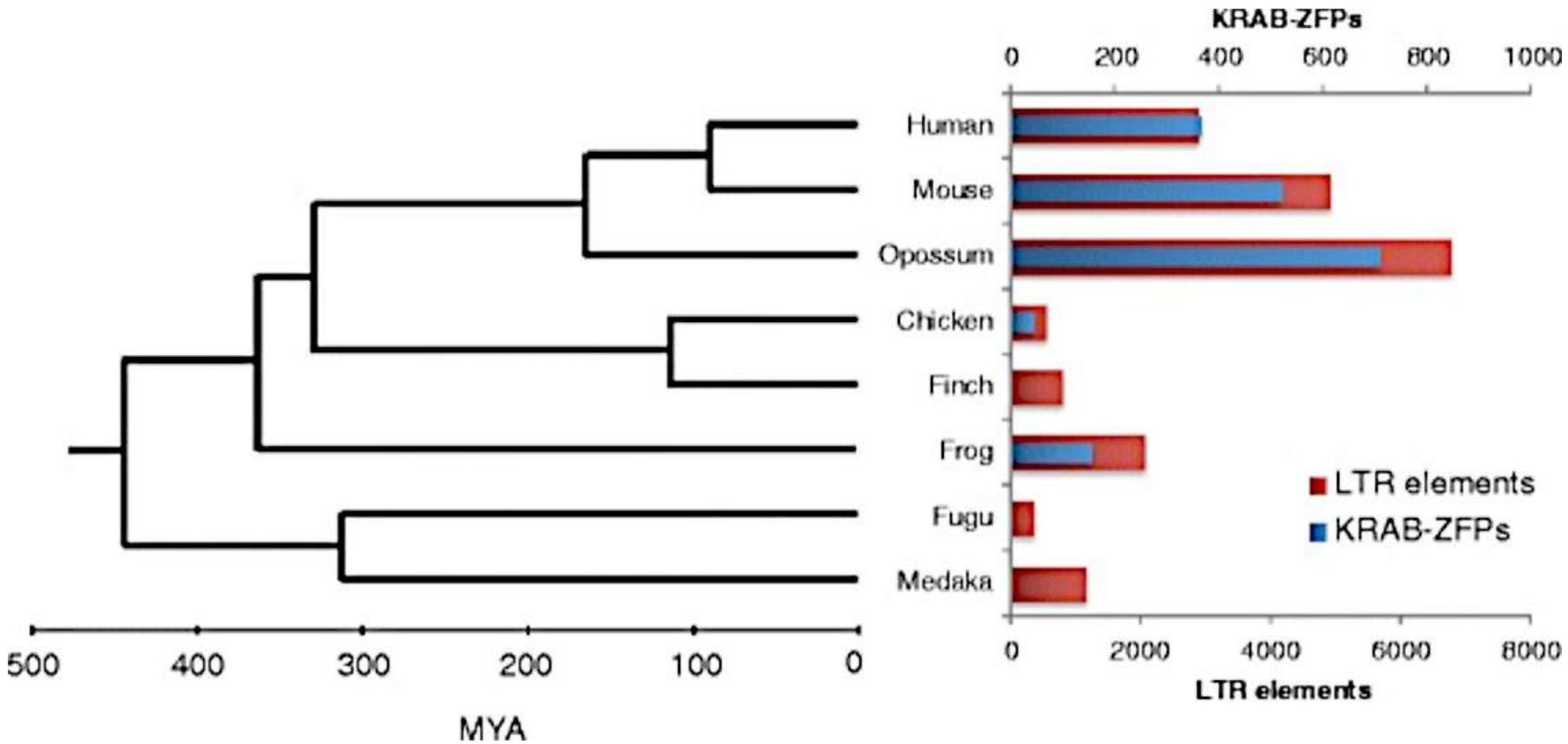
Weak ZFP809 binding does not lead to formation of KAP1/SETDB1 repressor complex



Nowick et al, 2013; Wolf et al, 2015

KRAB-ZFPs are evolving rapidly in mammals along with LTR-elements

Estimated number of LTR elements and KRABs in vertebrates:

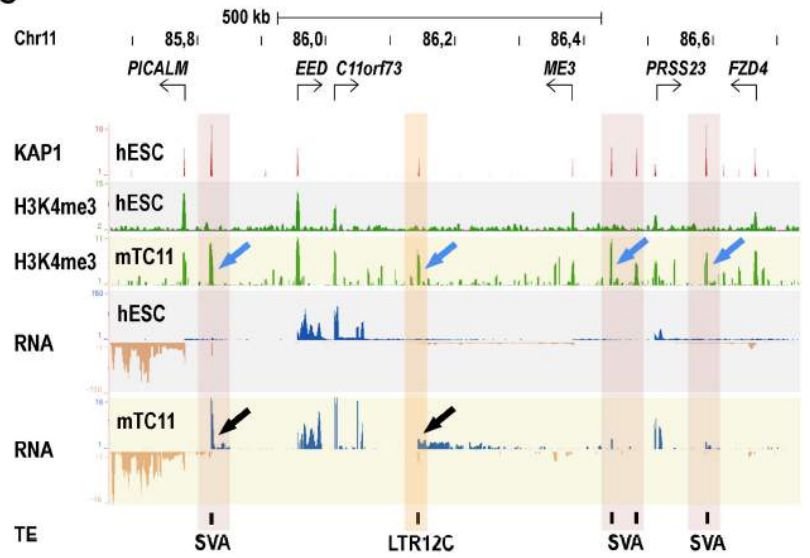


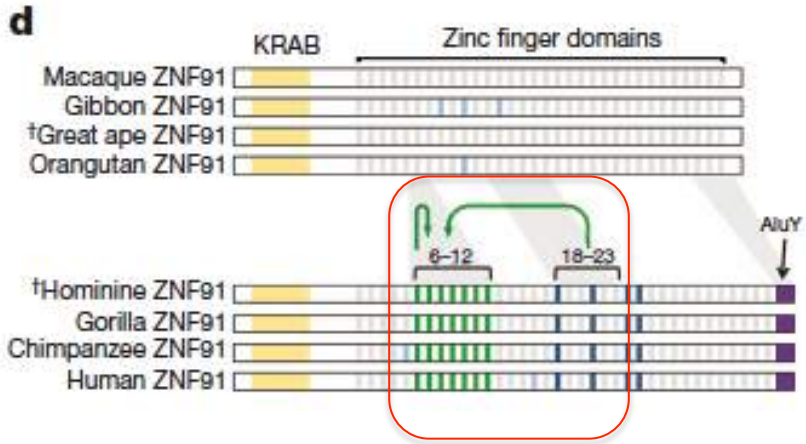
Evolutionary arms race between TEs and KRAB-ZFPs?

An evolutionary arms race between KRAB zinc-finger genes *ZNF91/93* and SVA/L1 retrotransposons

Frank M. J. Jacobs[1*†], David Greenberg[1,2*†], Ngan Nguyen[1,3], Maximilian Haeussler[1], Adam D. Ewing[1†], Sol Katz Benedict Paten[1], Sofie R. Salama[1,4] & David Haussler[1,4]

- Human chromosome 11 with its own TEs placed in a mouse ESCs (with their murine KRAB ZFP repertoire)
- Human TEs on Ch11 become reactivated and lose KAP1 binding (presumably due to lack of appropriate hKRAB-ZFP…)
- Screen for Primate KRAB-ZFPs that could now impose repression of human TEs (out of 170 primate-specific KZFPs, chose 14 most highly expressed in human ESCs
- Found **ZNF91** - most dramatically decreased SVA-driven luciferase activity in mESCs
- Changes in the Zn fingers of ZNF91 between 8–12 Myr ago *improved* the protein's ability to bind and repress SVA.





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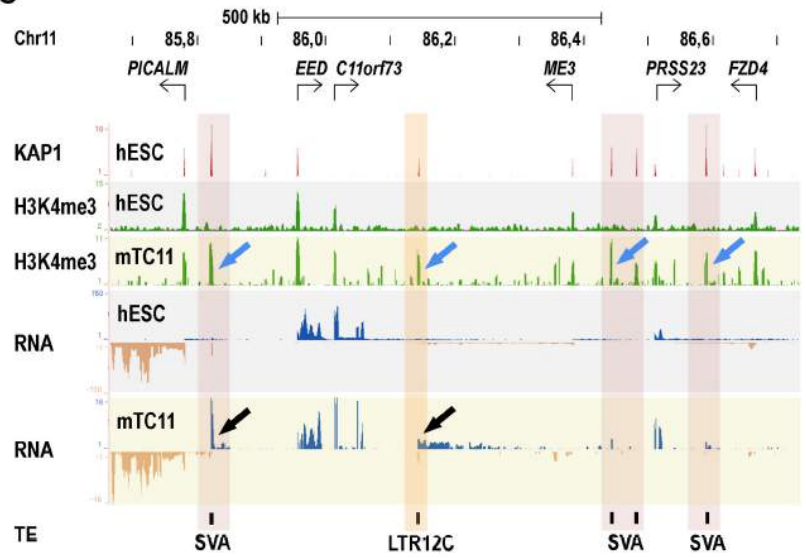
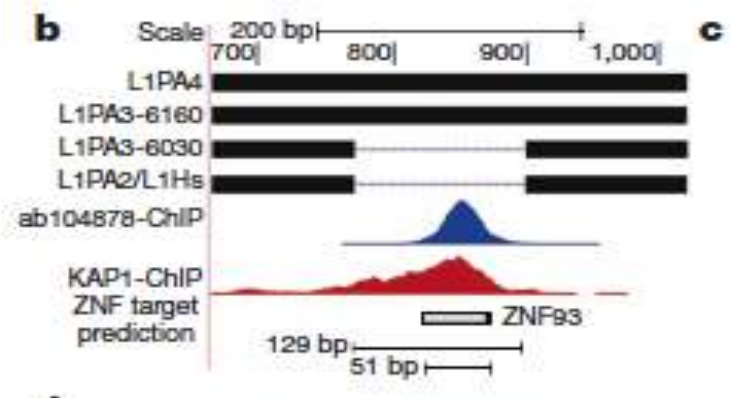
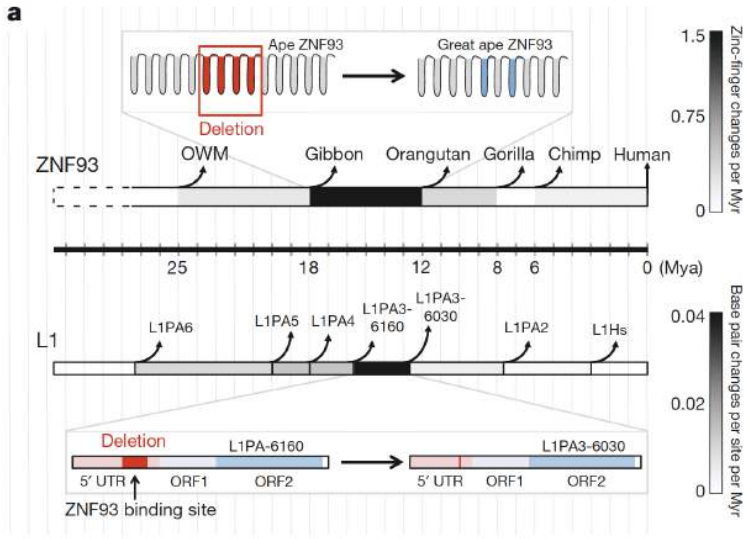
- Changes in the Zn fingers of ZNF91 between 8–12 Myr ago *improved* the protein's ability to bind and repress SVA.
- Another KRAB-ZFPs**, ZNF93** was identified as being able to repress a reporter with the 5'UTRof a KAP1-positive human **LINE L1PA4** element

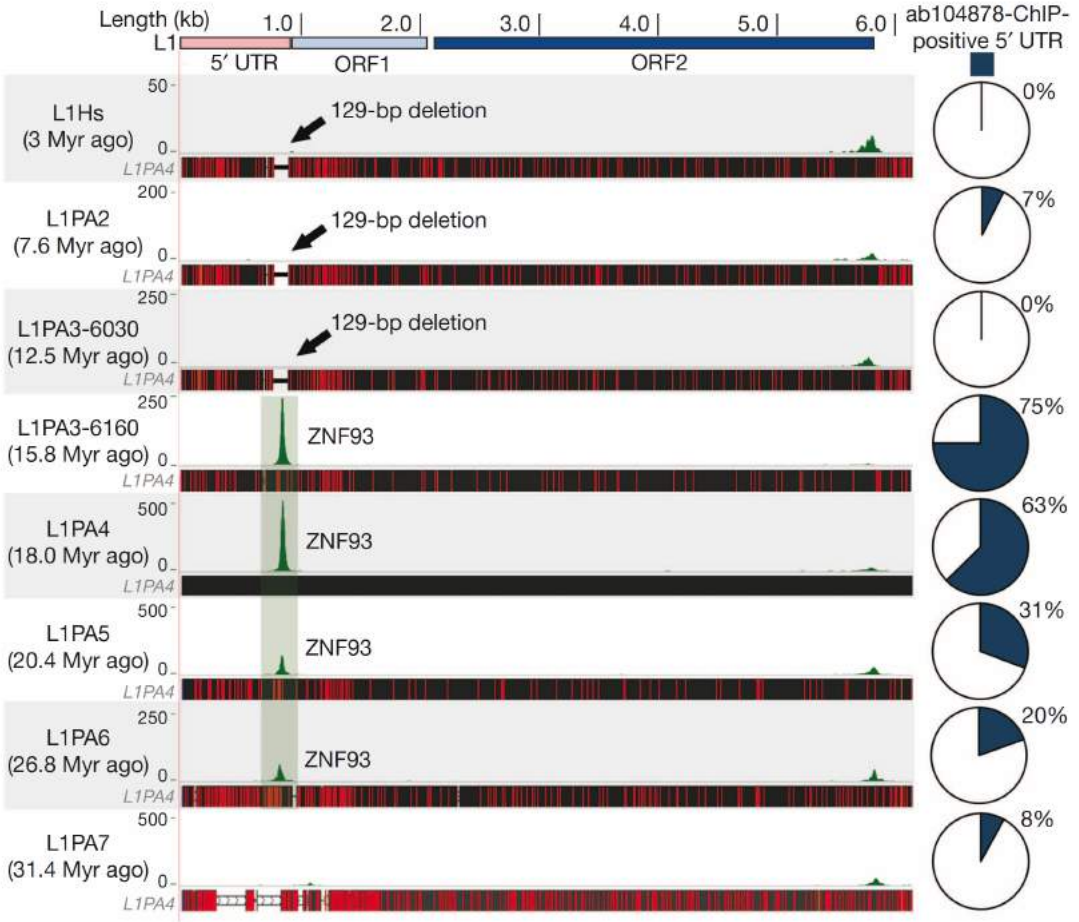




COLLÈGE DE FRANCE 1530

Evolutionary arms race between TEs and KRAB-ZFPs?

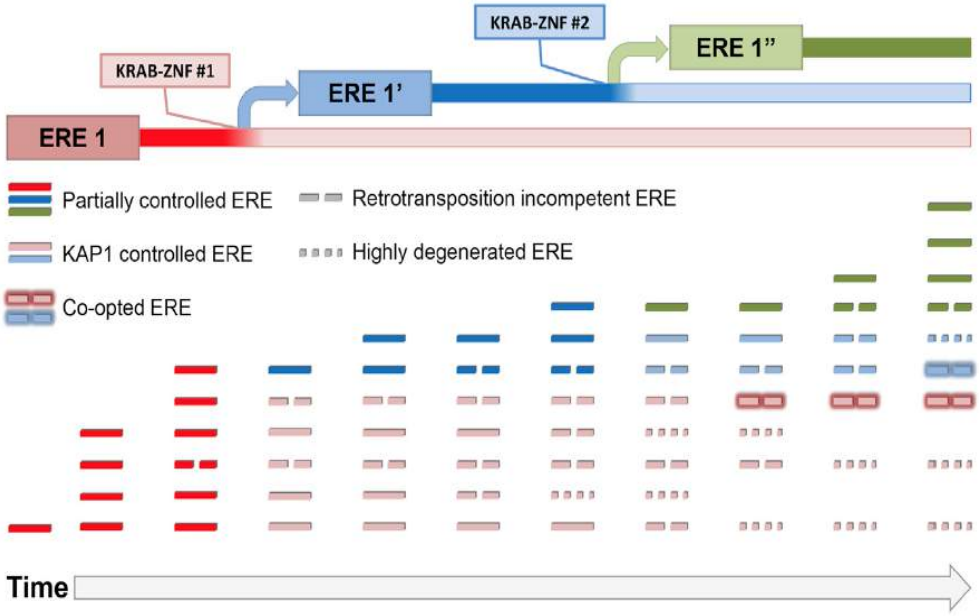


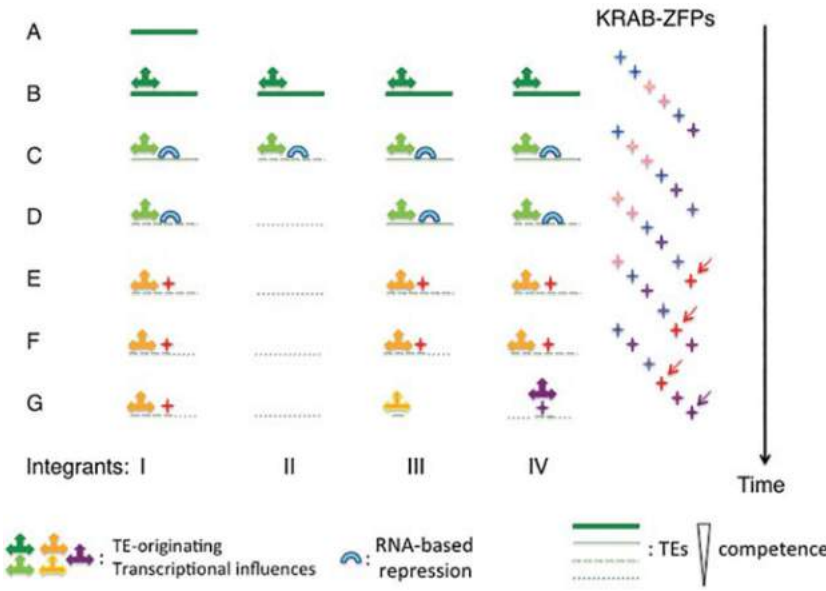


- Macaque ZNF93 does not have the ability to repress the 129-bp or 51-bp element of L1PA4 in the luciferase assay
- But Human ZNF93 cannot repress all LINEs of the L1PA3 lineage: eg L1 Hs Which deleted the ZNF93 binding site!

ZNF93 evolved in primates to repress the primate L1 lineage
But 12.5 million years ago, the L1PA3-subfamily of TEs escaped ZNF93's restriction through the removal of the ZNF93-binding site

Evolutionary arms race between TEs and KRAB-ZFPs?





- TEs are initially partially controlled by mechanisms such as RNAi
- => some retrotransposition can occur
- Over time, a KRAB-ZNF evolves that binds the TE, leading to its full repression.
- Rare pre-existing KRAB-ZNF-resistant TE mutants can then spread through the genome, whereas the previously dominating strain is inhibited.
- Old TEs progressively accumulate mutations, preventing transposition potential.
- Rare integrants undergo positive selection, can be co-opted and fixed, if beneficial to the host eg new promoters or enhancers rewiring transcriptional networks or new proteins (eg syncytin – placenta)

From Imbeault and Trono, 2014

Evolutionary arms race between TEs and KRAB-ZFPs?

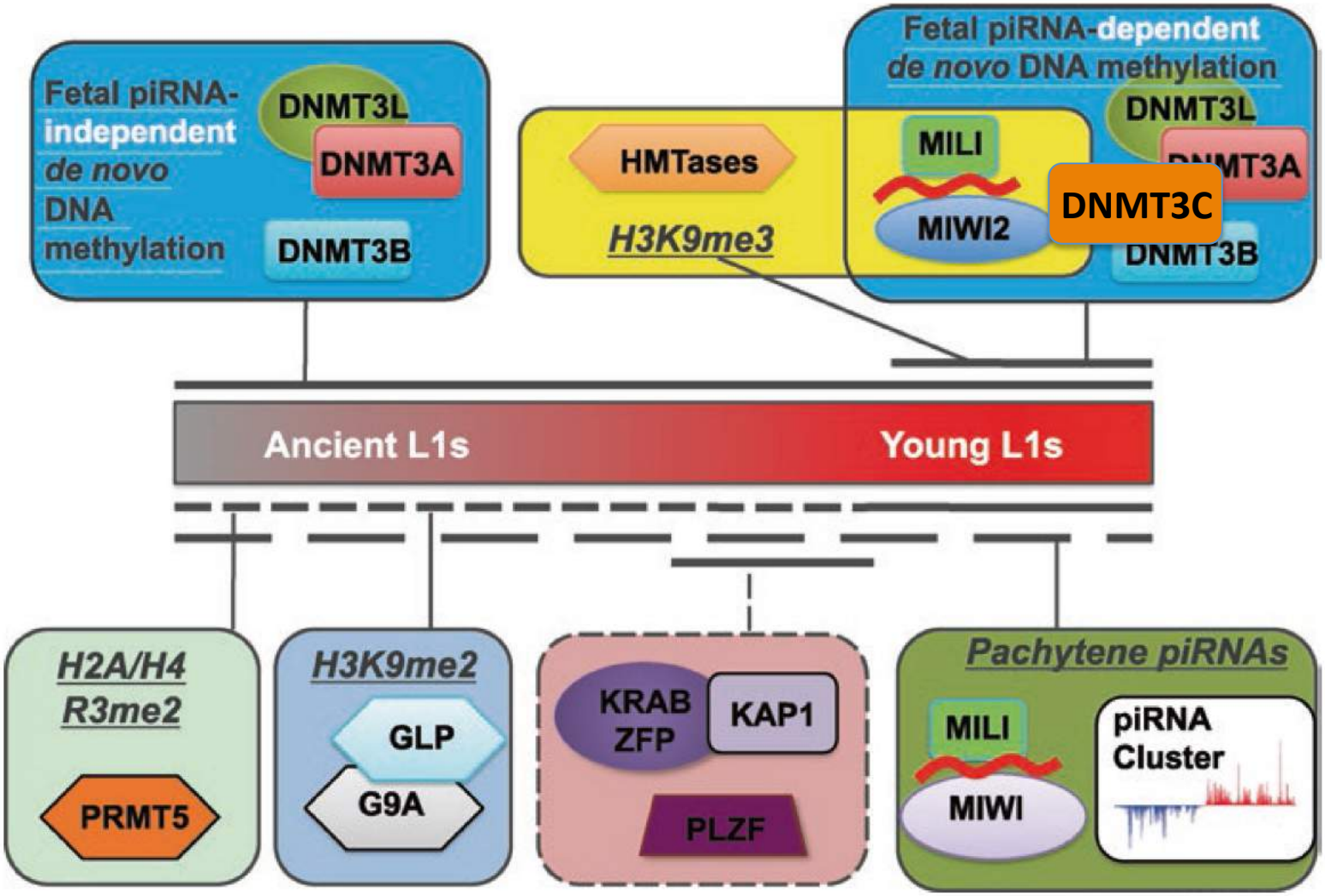
• KRAB-ZFPs can target repression of TEs in a sequence-specific manner, and some can target specific types of TEs (Castro-Diaz et al., 2014; Ecco et al., 2016; Wolf and Goff, 2009; Wolf et al., 2015).

• However some young and presumably active TEs **escape** KAP1-mediated silencing as KRAB-ZFPs have not yet evolved to target these sequences (Castro-Diaz et al., 2014; Jacobs et al., 2014).

• Other mechanisms (eg RNAi) target the silencing of young, and active TEs (Castro-Diaz et al., 2014; Jacobs et al., 2014).

• Tissue-specific expression of some KRAB-ZFPs may underlie tissue-specific host gene expression in somatic tissues through their effects on TEs (Ecco et al., 2016)
⇒ Primary role of KRAB-ZFPs is to control host programs and they are used to target TEs
⇒ Which in turn are exploited over evolution to regulate host genes…

Different strategies for silencing of ancient and young TEs



S.J. Newkirk and W. An, 2016

TE Transcriptional Control Strategies

TE expression requires:

- Permissive chromatin environment
- Transcription Factor availability
- No RNAi targeting

TE repression:

- RNA-targeted epigenetic repression
- DNA-targeted repressive factors
- Creates a repressive chromatin environment

• Relative importance of both targeting machinery (RNA or DNA based) and epigenetic mechanisms depends on the TE type and age, and the cell type (Crichton et al., 2014; Gerdes et al., 2016; Rowe and Trono, 2011; Schlesinger and Goff, 2015).

• Multiple histone modifications, including methylation at histones H3K4, H3K9, H2A/H4R3 and H3K27 as well as histone acetylation, have been implicated in TE transcriptional repression (Brunmeir et al., 2010; Di Giacomo et al., 2014; Karimi et al., 2011; Kim et al., 2014; Leeb et al., 2010; Macfarlan et al., 2011; Matsui et al., 2010; Reichmann et al., 2012).

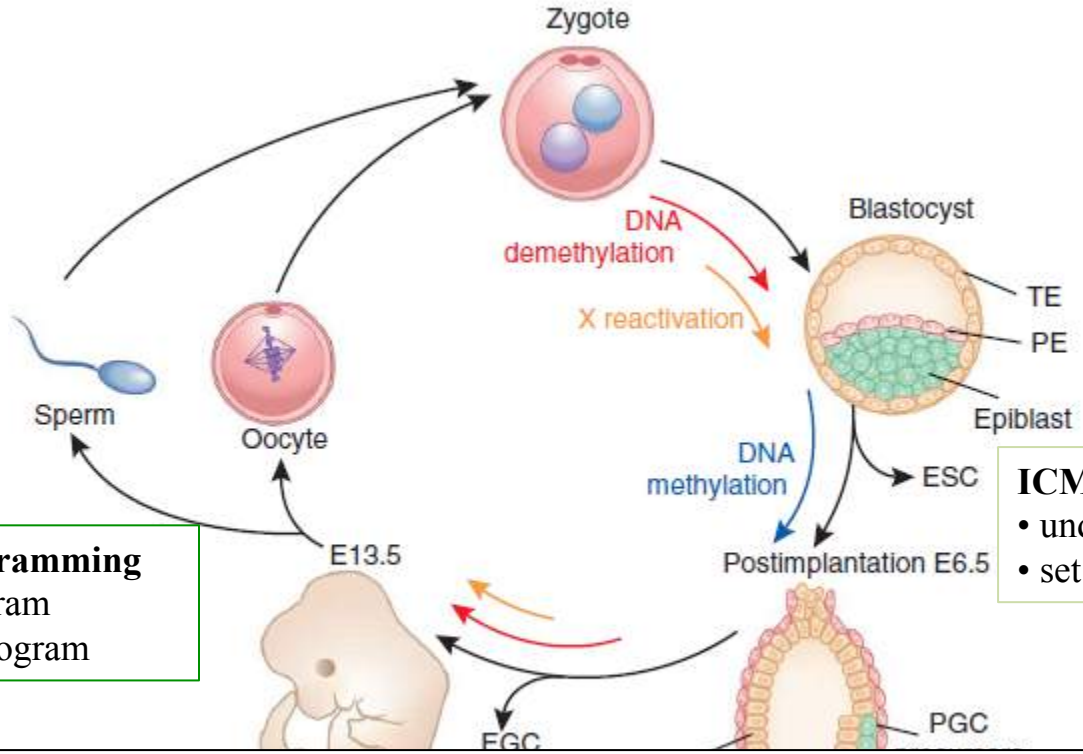
• The most common histone modification used to repress a large number of TEs is H3K9me3 (Karimi et al., 2011; Matsui et al., 2010; Rowe et al., 2010) deposited at TE sequences by the hHMTase SETDB1 via (KRAB-ZFPs) and associated co-repressor TRIM28/KAP1 (Castro-Diaz et al., 2014; Ecco et al., 2016; Karimi et al., 2011; Matsui et al., 2010; Rowe et al., 2010; Wolf and Goff, 2009; Wolf et al., 2015).

• DNA methylation plays a key role in repressing both mouse and human LINE-1 elements, and some mouse ERVs including IAP elements, in germ line and soma (Bourc'his and Bestor, 2004; Karimi et al., 2011; Walsh et al., 1998). Decreased DNA methylation during specific developmental time windows necessitates other silencing strategies.

Epigenetic Reprogramming in Development

- **Prepare for development (epigenesis)**
- **Preserve some epigenetic marks (parental imprints), erase others**

Zygotic Reprogramming
- undo gamete programs
- set up totipotency



Germ Line Reprogramming
- undo somatic program
- set up germ line program

ICM Reprogramming
- undo/prevent TE program
- set up pluripotency

In the developing germ line and in the early embryo, DNA Methylation and other chromatin marks are globally lost.
Most epigenetic marks are erased at each generation **(COURS 2014)** (except at young TEs)

How are TEs controlled during these critical periods?
In early embryos, mainly via DNA binding repressor proteins (KRAB-ZfP)
In the germ line piRNAs involved in re-establishing de novo silencing **(COURS II)**

Epigenetic Reprogramming in Development

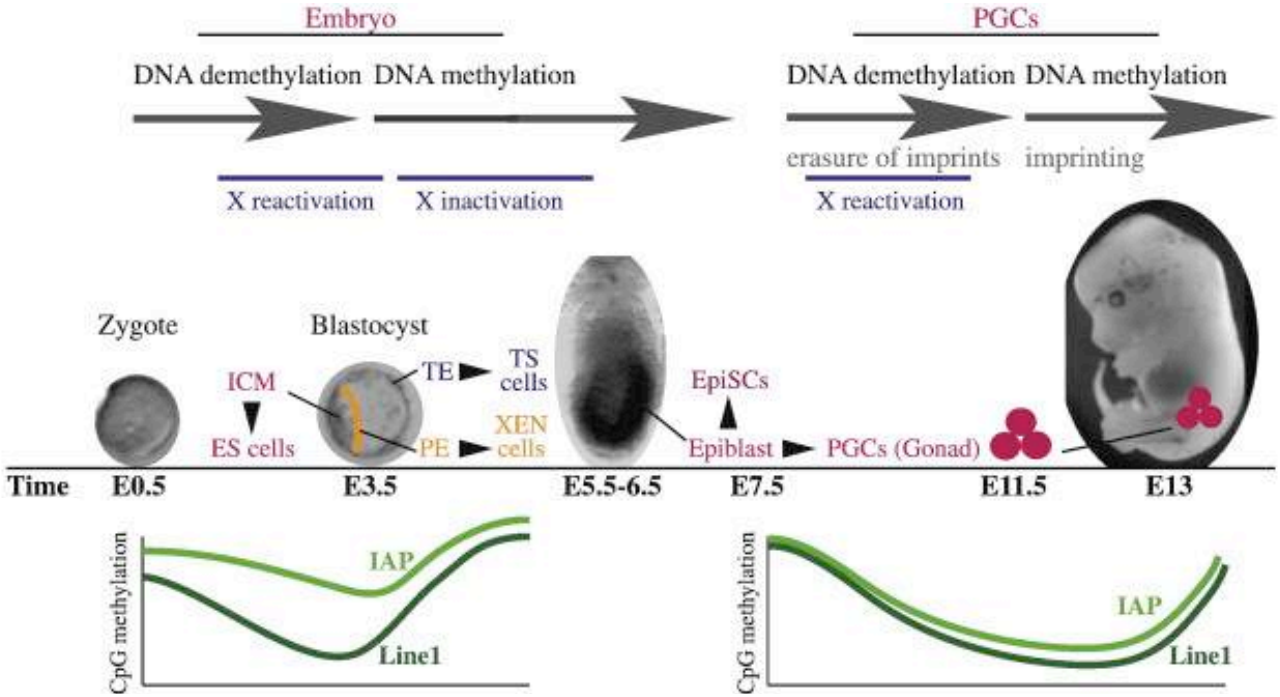
- For TEs to be successful they need to be *expressed and functional* in developing germ cells or in precursors to germ line (early embryo, pluripotent cells).

- For Host - *repression* of TE expression and mobility is particularly important to protect the host genome at these stages. However TE activity may also be **exploited** for gene regulation or new gene functions

- Dysregulated expression of TEs linked with defects in various developmental processes in mice:

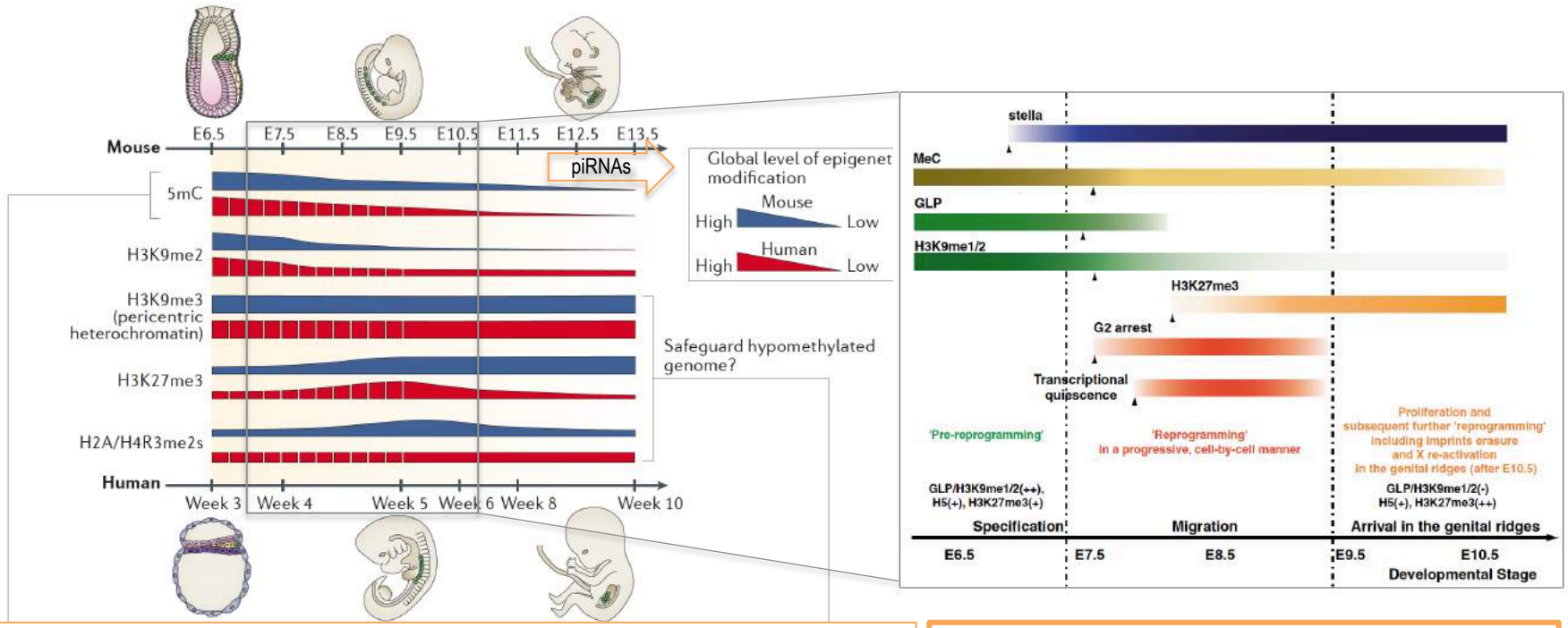
- aberrant proliferation of male germ cells (Galli et al., 2005)
- defects in oogenesis (Malki et al., 2014; Su et al., 2012)
- disruption of homologous chromosome synapsis during meiosis (Bourc'his, 2008: reviewed in Crichton et al., 2014; Öllinger et al., 2010)
- activation of the unfolded protein response during B lymphocyte differentiation (Pasquarella et al., 2016)
- inappropriate activation of innate immune responses (Herquel et al., 2013; Stetson et al., 2008)

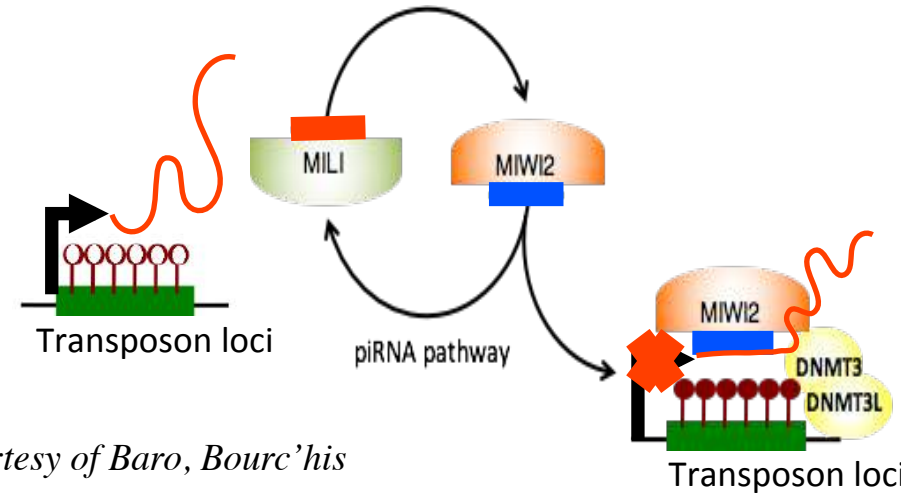
Developmental Dynamics of DNA methylation and Expression of TEs



Rowe and Trono 2011

Epigenetic Reprogramming in the Germ Line





Courtesy of Baro, Bourc'his

H3K27me3 and H2A/H4R3me2 seem to be globally enriched during period when both DNAme and H3K9me are lost – and before piRNA pathway?

Required for TE control?

(Ng et al, 2013, Liu et al, 2014)

Hackett et al, 2014
Seki et al, 2007

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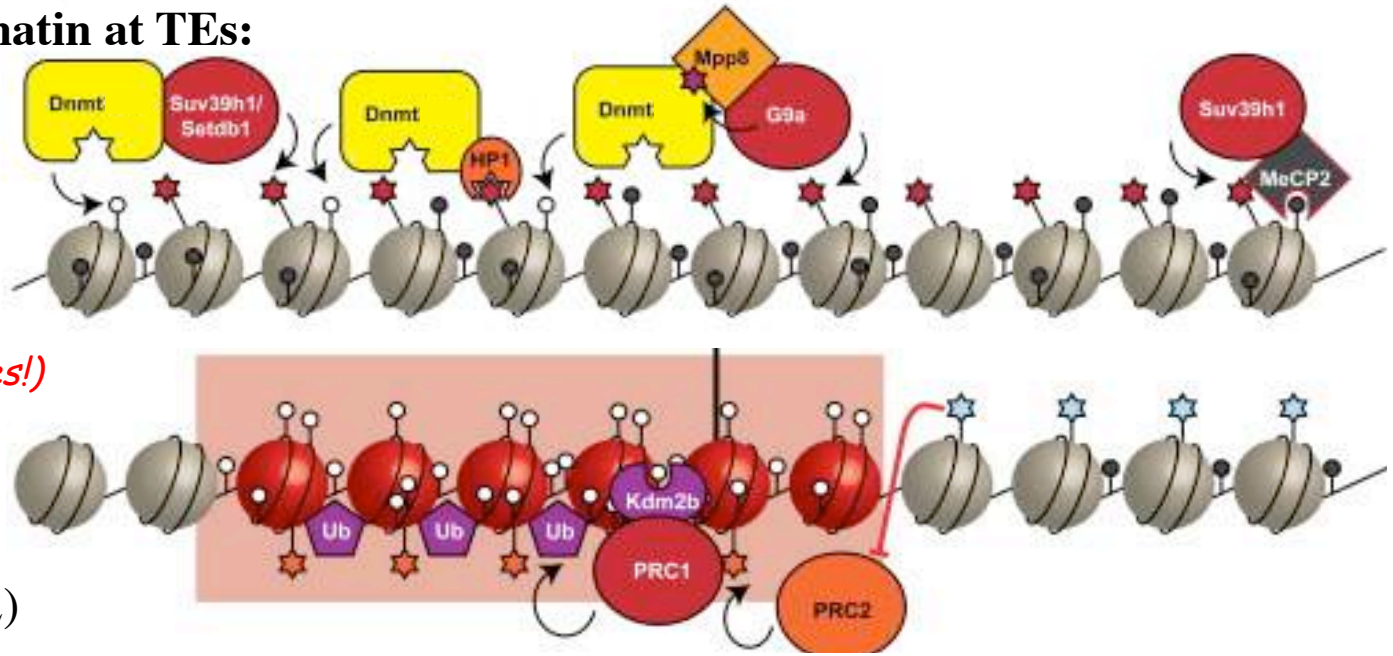
Epigenetic Reprogramming in the Germ Line

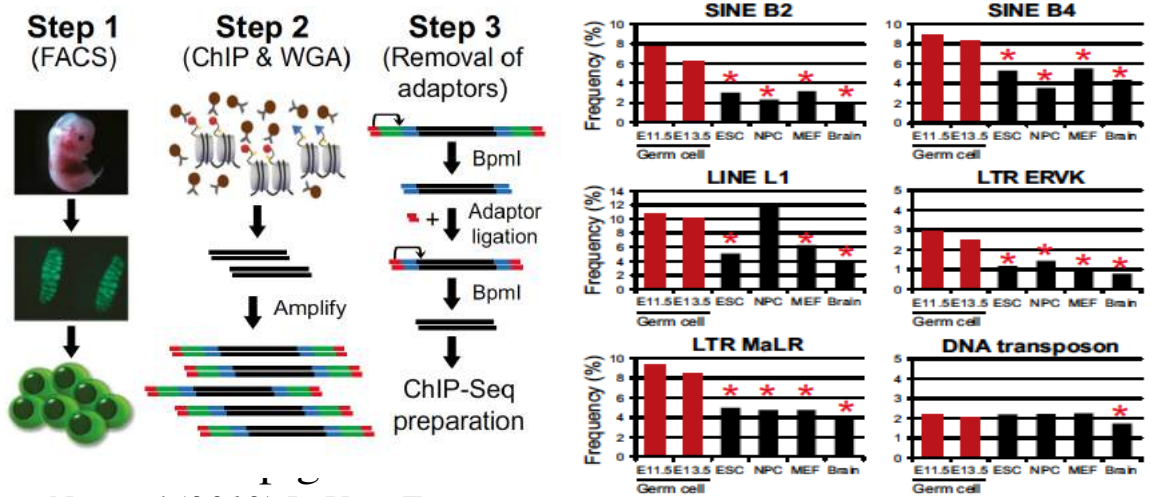
Repressive Chromatin at TEs:

H3K9me2/3 &
DNA methylation

(Hypothetical schemes!)

H3K27me3
(& H2A/H4R3me2)

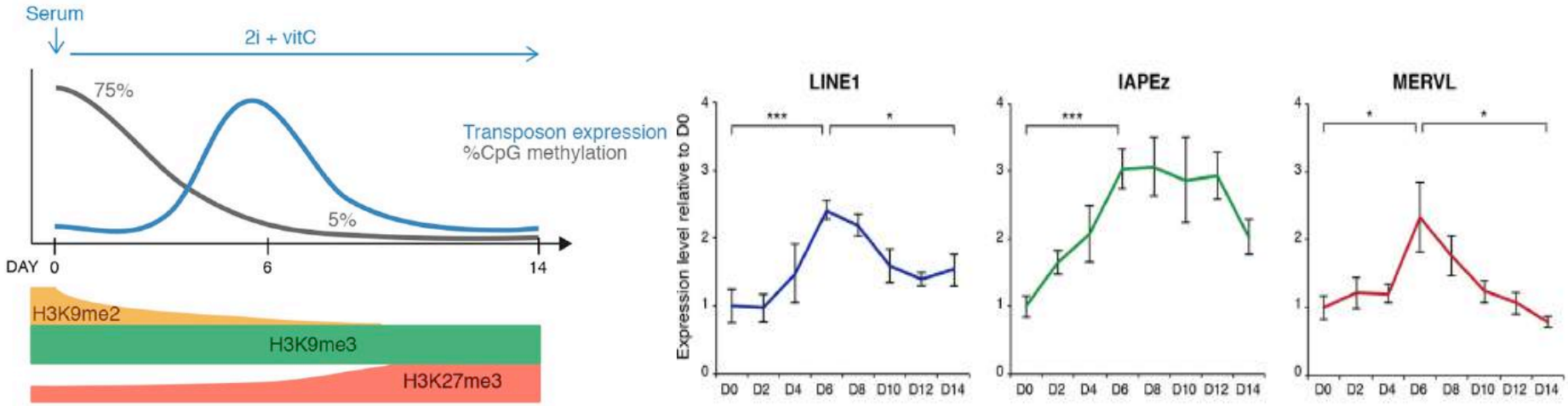




Ng et al (2013) In Vitro Epigenetic Priming of Human Pluripotent Stem Cells Reveals Germ Cell Molecular Signatures. Dev. Cell 24:324

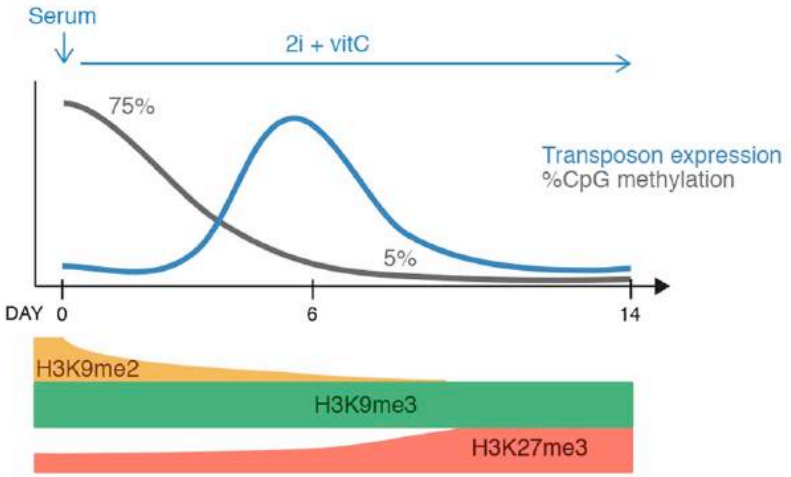
Or *in vitro* model for PGC formation

Epigenetic control of TEs in ESCs



- Mouse embryonic stem cells mimic the loss of DNA methylation that occurs during embryonic development – when culture in 2i + Vitamin C
- DNA methylation-independent mechanisms silence transposons in ESC: knocking-out the 3 active DNA methyltransferases (*Dnmt*-tKO) does not yield significant de-repression of transposons, except Intracisternal A Particle (IAP) elements (Karimi et al, 2011; Matsui et al, 2010)
- When DNA methylation is lost progressively, multiple families of transposons are reactivated at first but are later put back into a silent mode by alternative mechanisms.
- An epigenetic switch towards histone-based control is progressively implemented as DNA methylation disappears: see specific and overlapping roles of H3K9 and H3K27 trimethylation in controlling distinct transposon families upon DNA demethylation.

Epigenetic control of TEs in ESCs

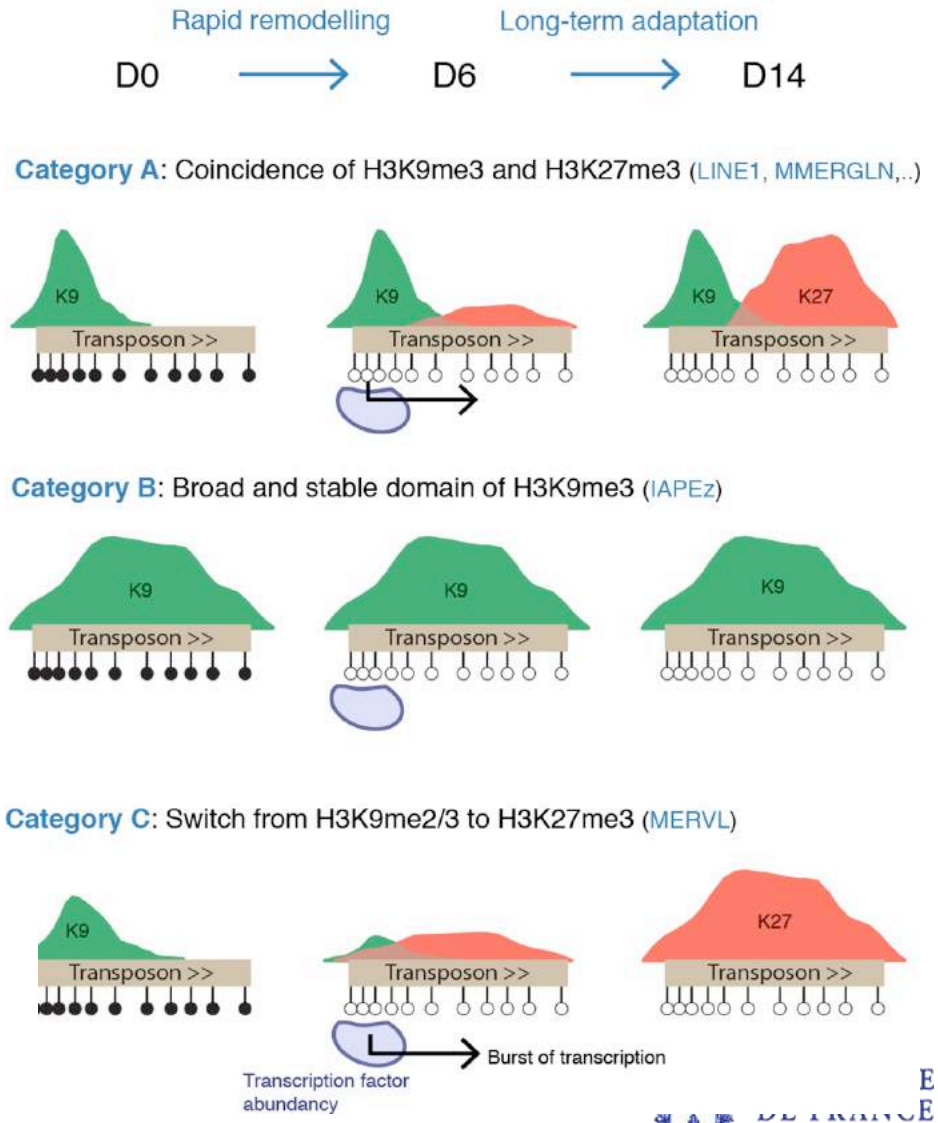


Multiple alternative strategies exist to repress TEs in the absence of DNA methylation.

Targeting strategies?

TF– Chromatin – RNA -?

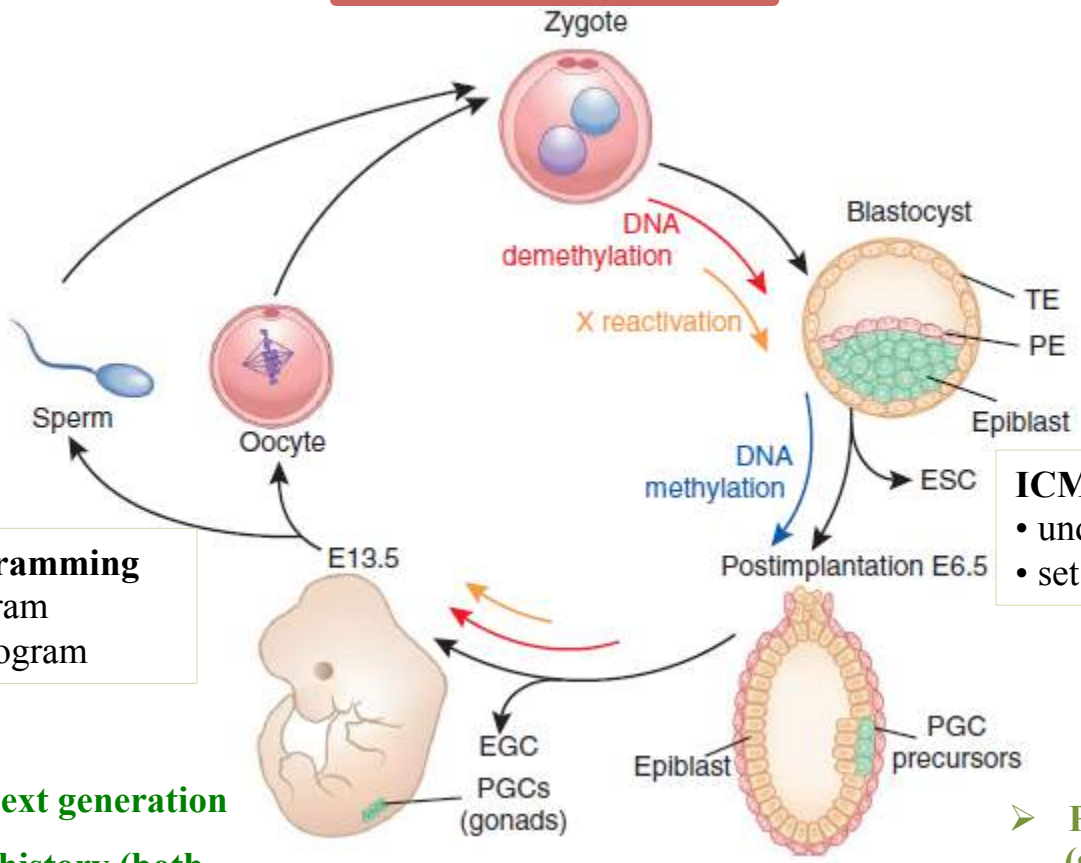
Stem cell-specific pathways probably also in action at the post-transcriptional level (RNAi, anti-viral pathways etc)



Epigenetic Reprogramming in Development

- **Prepare for development (epigenesis)**
- **Preserve some epigenetic marks (parental imprints), erase others**

Zygotic Reprogramming
- undo gamete programs
- set up totipotency



Germ Line Reprogramming
- undo somatic program
- set up germ line program

ICM Reprogramming
- undo/prevent TE program
- set up pluripotency

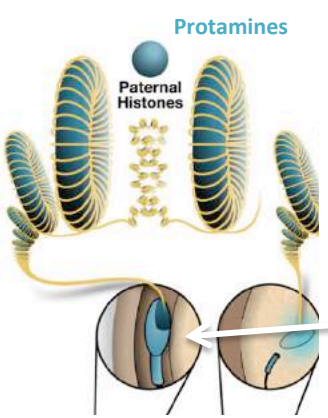
- **Prepare for the next generation**
- **Erase epigenetic history (both programmed and accidental)**

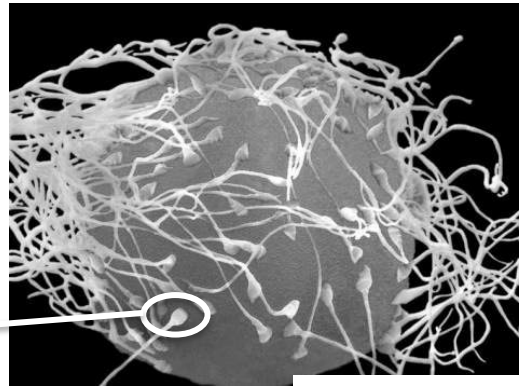
- **Prepare for the epiblast (soma and germ line)**

Adapted from Cantone and Fisher, 2013

Zygotic Reprogramming (COURS en 2014)

Two highly specialized cells, the egg and the sperm, fuse to form a totipotent cell, the zygote

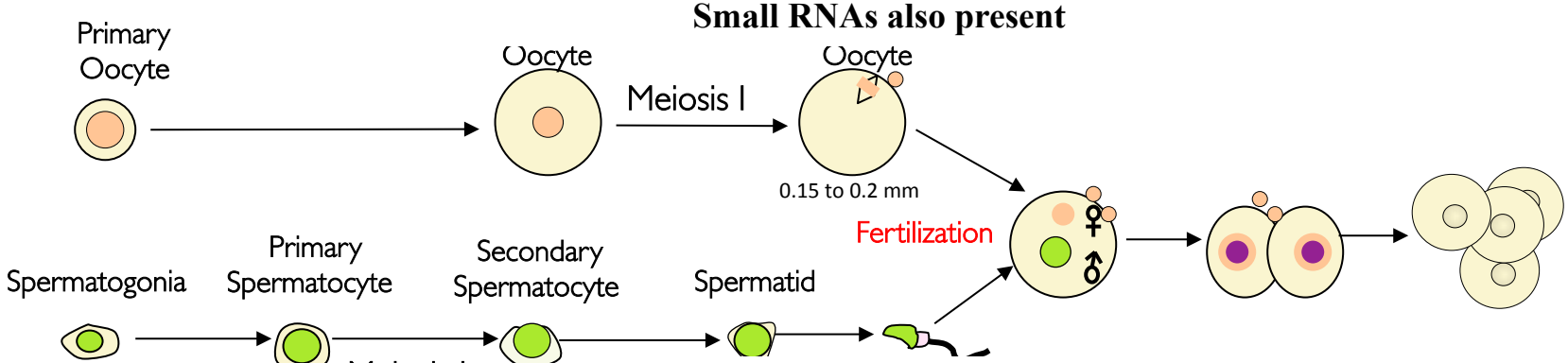




Huge maternal store of proteins, mRNA (to be translated later) to ensure early development, and to enable reprogramming upon fertilization.

LINE and IAP transcripts and proteins present

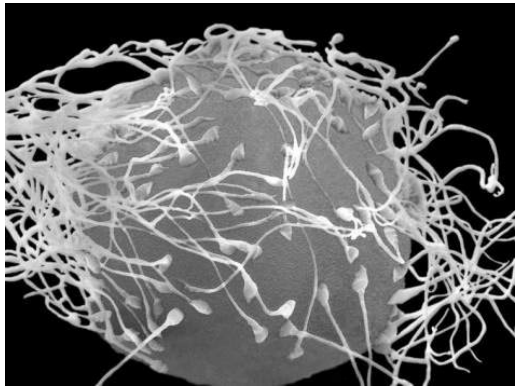
Small RNAs also present

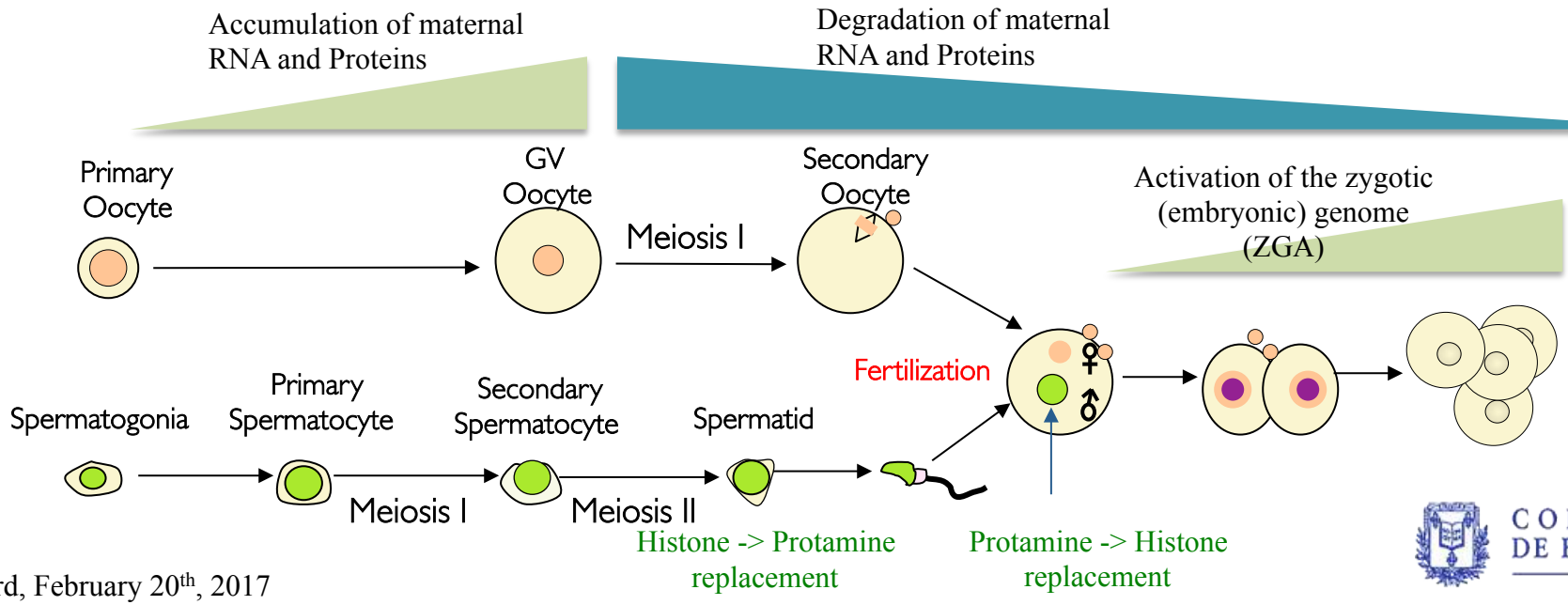


Highly packaged genome (protamines, few/ no histones) and a small amount of RNA

Zygotic Reprogramming (COURS en 2014)

Two highly specialized cells, the egg and the sperm, fuse to form a totipotent cell, the zygote



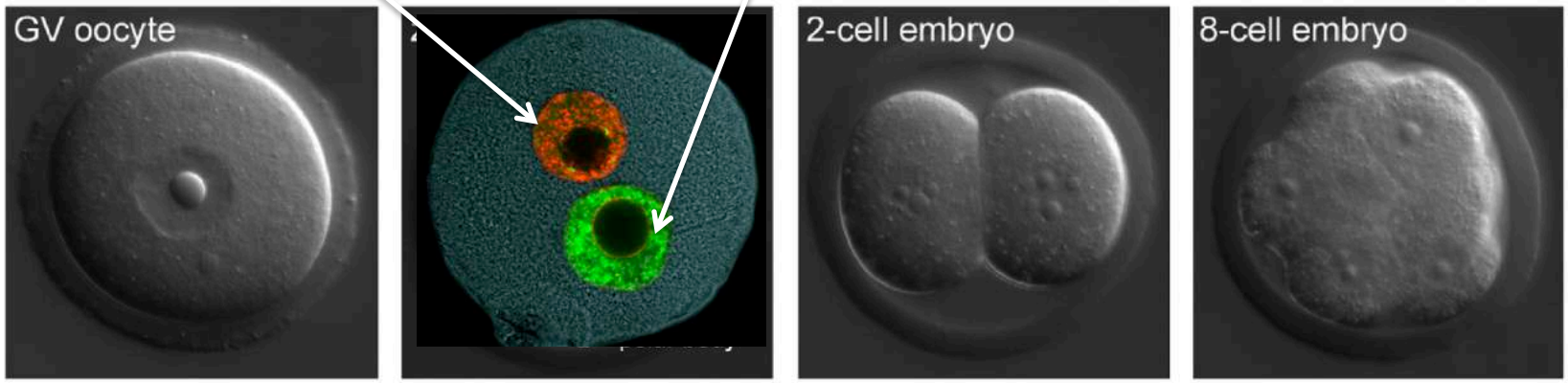


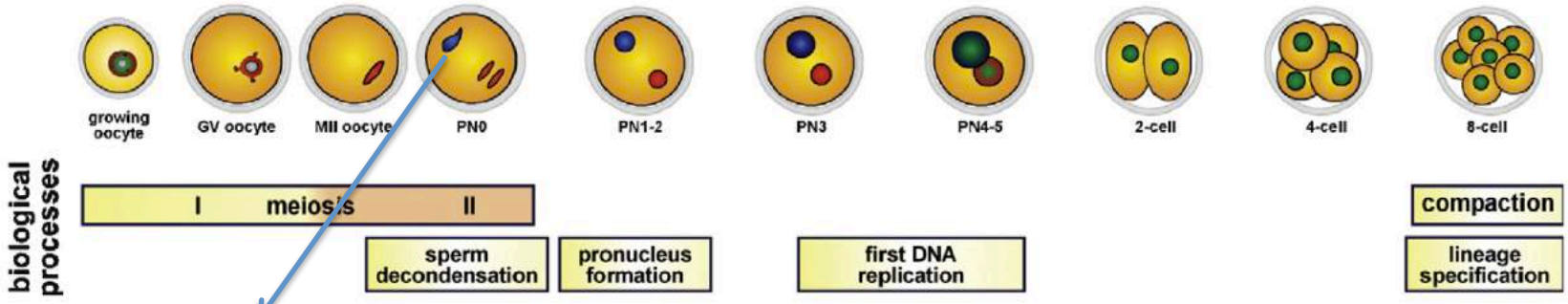
Fertilization triggers massive reorganization of the paternal and maternal epigenomes (prior to transcription)

Maternal interphase pronucleus

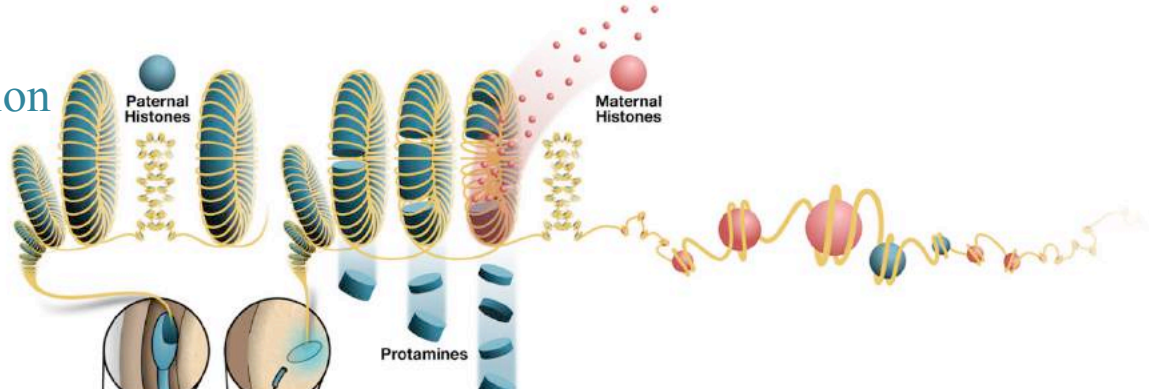
Paternal interphase pronucleus

The two parental pronuclei remain separate initially

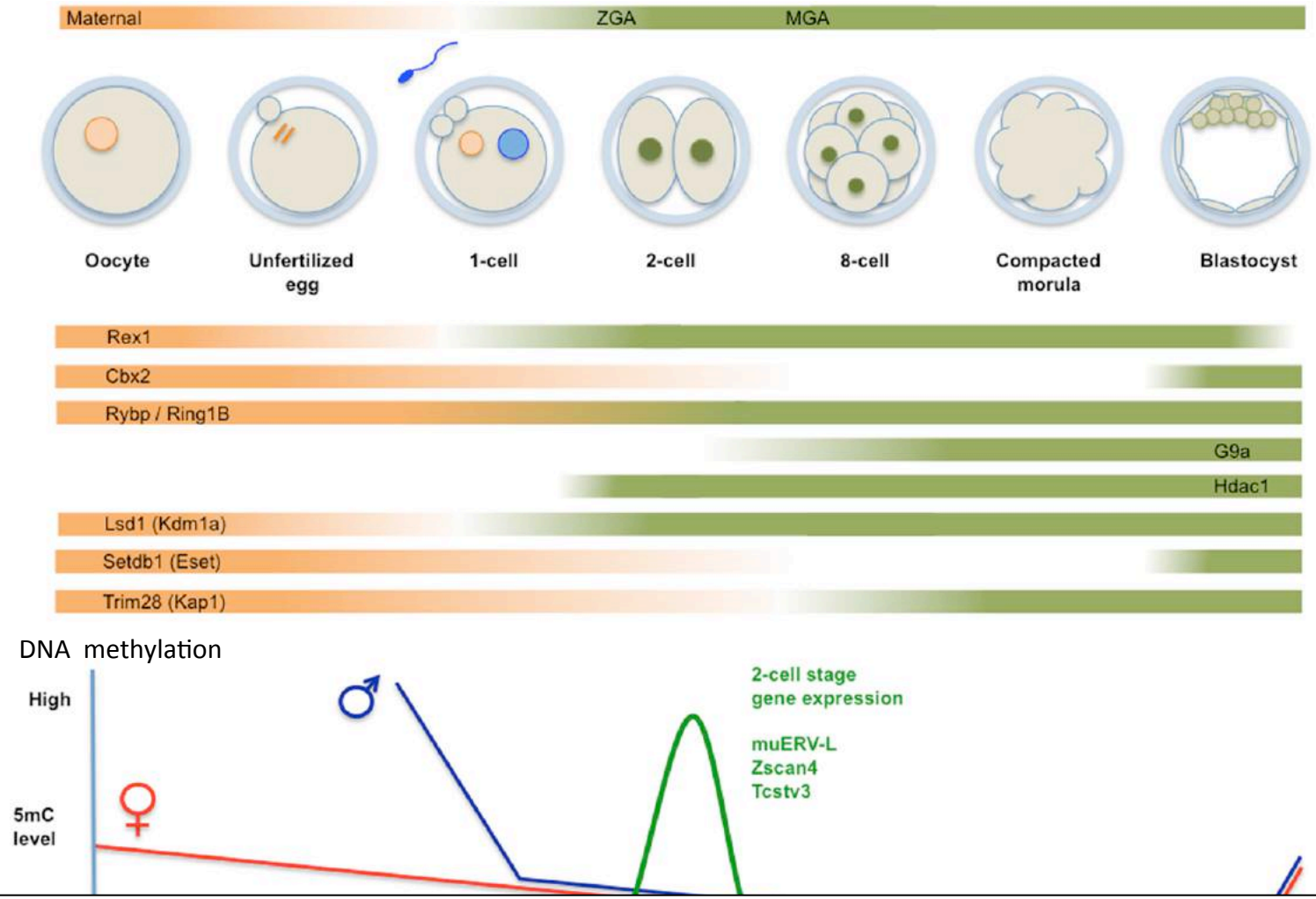




Protamine eviction, maternal histone incorporation



Epigenetic Dynamics during Early Embryogenesis

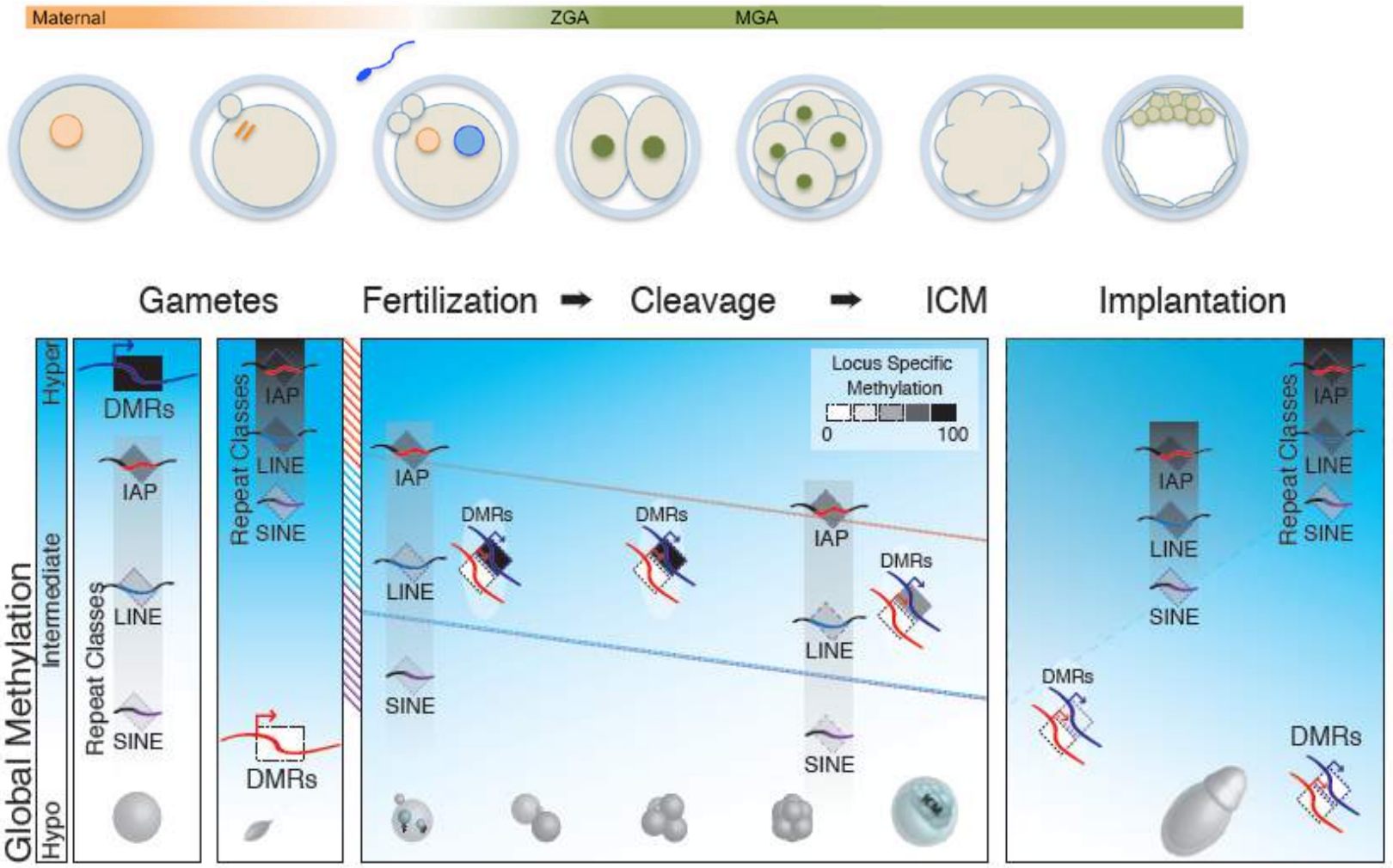


A few regions are protected from demethylation: Zfp57 and KAP1/Trim28 are required for the post-fertilization maintenance of maternal and paternal methylation imprints.

(Li et al, 2008; Messerschmidt et al, 2012)

Also true for some TEs? Not yet known…

Epigenetic Dynamics during Early Embryogenesis



DNA METHYLATION STATES OF **SPECIFIC** TEs POORLY CHARACTERISED

Challenging due to their repetitive nature

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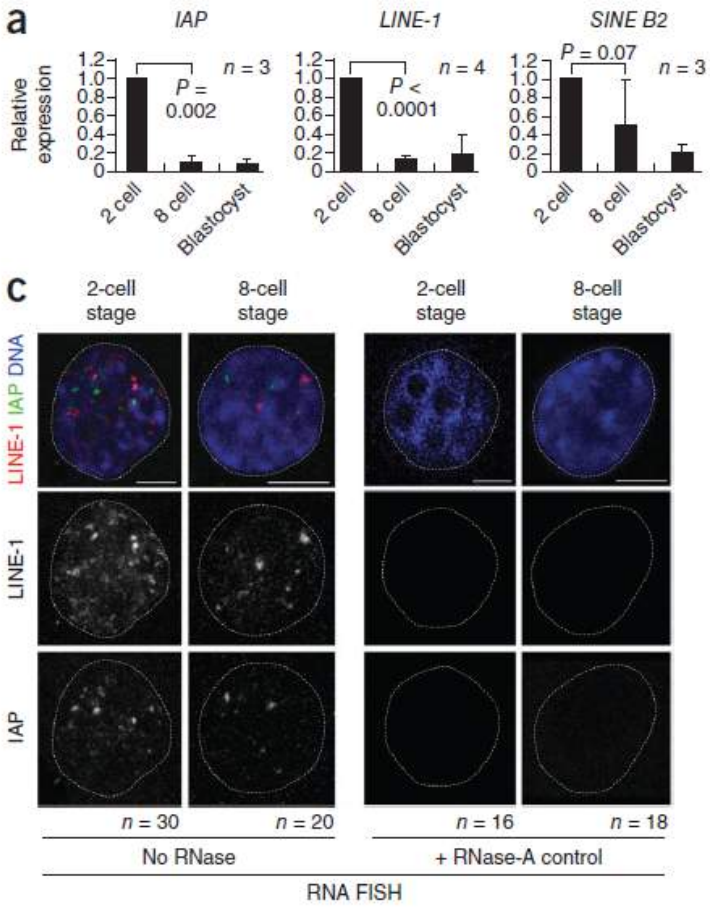
Expression of Repeat Elements after Fertilization?

In early mouse embryo: global DNA hypomethylation and no piRNA machinery mean that repeats can become expressed - very high LINE and ERV expression

- Highest TE transcripts are at 2-cell stage – eg MERVL activated at 2-cell stage then rapidly repressed
- Different TEs show very different dynamics



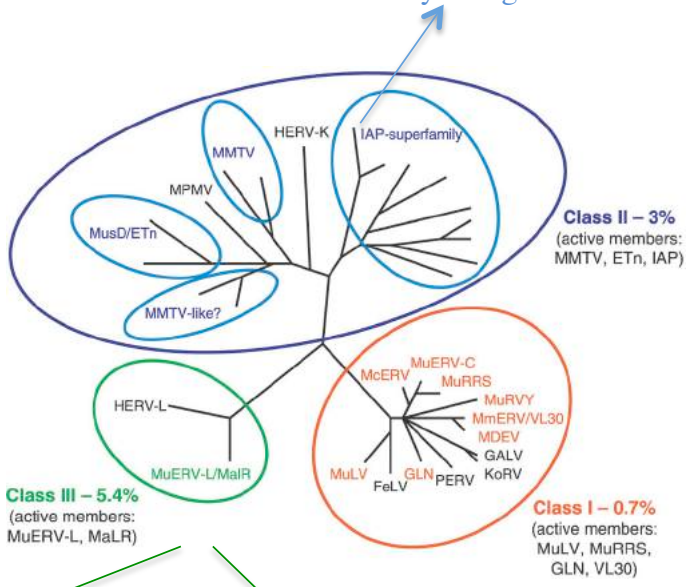
(Bachvarova, 1988; Efroni et al., 2008; Evsikov et al., 2004; Packer, Manova, & Bachvarova, 1993; Peaston et al., 2004).

Control of Repeat Elements after Fertilization?

In early mouse embryo: global DNA hypomethylation and no piRNA machinery mean that repeats can become expressed - very high LINE and ERV expression

- Highest TE transcripts are at 2-cell stage – eg MERVL activated at 2-cell stage then rapidly repressed
- Different TEs show very different dynamics

IAP RNAs: high levels in fully-grown oocytes, decrease in 1-cell embryos, increase again during development to the blastocyst stage



MT MaLR LTR retrotransposon transcripts are highly abundant in mouse oocytes but decrease in abundance as pre-implantation

Epsilon-type retroviral-like particles, encoded by MuERVL ERVL LTR retrotransposons, are *not* present in fully-grown oocytes, transiently increase in 2-cell embryos, then disappear as pre-implantation development proceeds

(Bachvarova, 1988; Efroni et al., 2008; Evsikov et al., 2004; Packer, Manova, & Bachvarova, 1993; Peaston et al., 2004).

Control of Repeat Elements after Fertilization?

In early mouse embryo: global DNA hypomethylation and no piRNA machinery mean that repeats can become expressed - very high LINE and ERV expression

Developmental Cell, Vol. 7, 597–606, October, 2004, Copyright ©2004 by Cell Press

Retrotransposons Regulate Host Genes in Mouse Oocytes and Preimplantation Embryos

- Highest TE transcripts are at 2-cell stage – eg MERVL activated at 2-cell stage then rapidly repressed
- Different TEs show very different dynamics
- A quarter of these TE sequences are at 5'ends of chimeric transcripts with exons from endogenous mouse loci.
- Chimeric transcripts only in oocytes and preimplantation embryos, originating from developmentally regulated LTR promoters spliced onto host genes. Some, but not all, chimeric transcripts encode novel protein

Exaptation? Mammalian hosts are co-opting retrotransposons to drive gene expression and other functions during these stages of development.
Or just a **consequence** of open chromatin and lack of adequate control?

Table 3. Intron/Exon Structure of Selected Chimeric Transcripts

Transposable element	Symbol	Schematic
MTA	*Stk3*	
	5730494M16Rik	
	Speer5-ps1	
	Zfp277	
	Spin	
	D7Ertd445e	
	Dncic2	
	D6Ertd365e	
	C230040D10Rik	
	Abcb1b	
	Fert2	
	Ski	
MTC	*Vdac2*	
	Nfil3	
	Dnajc11	
	Trp53bp1	
MTD	*D6Ertd527e*	
	AU017455	
	Rnf24	
RLTR10/MTE	*Pard3*	
MT-int (MTA)	*Calr3*	
MT-int (MTC)	*E330021D16Rik*	
MT2B	*2610005H11Rik*	

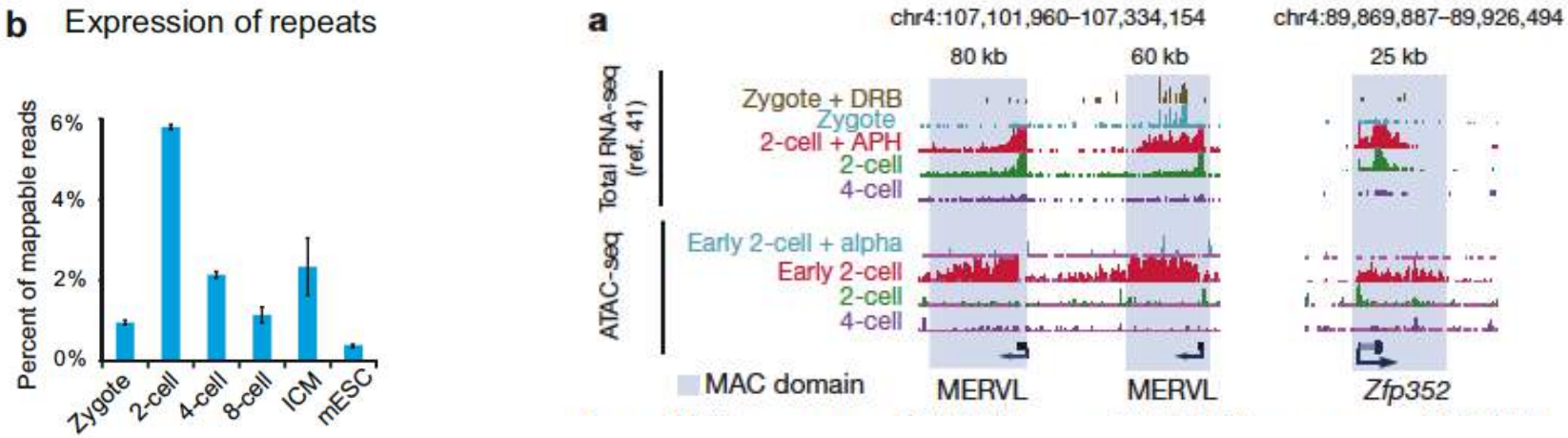
Examples of chimeric transcript structure determined by alignment of the transcript to the Ensembl annotated mouse genome ass release 13.30.1. The retrotransposon alternative first exon (red box) is shown in relation to the contiguous gene; white boxes – conver transcript exons omitted in chimeric transcript; black boxes, conventional transcript exons included in chimeric transcript.

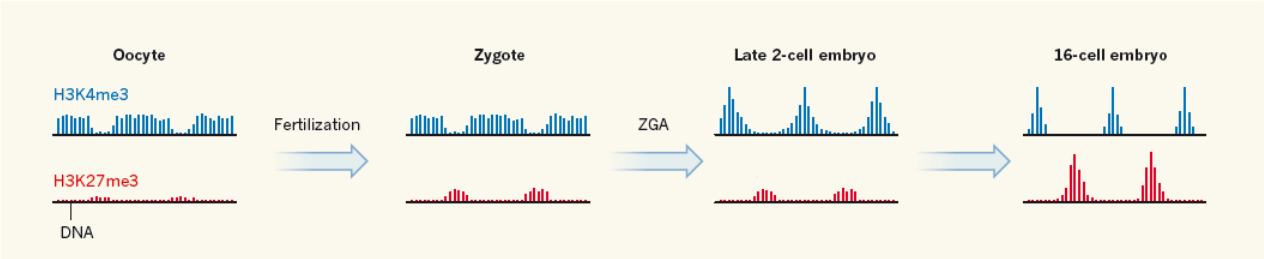
(Bachvarova, 1988; Efroni et al., 2008; Evsikov et al., 2004; Packer, Manova, & Bachvarova, 1993; Peaston et al., 2004).

Dynamic Transcription of Distinct TE classes during early Mouse Development

The landscape of accessible chromatin in mammalian preimplantation embryos

Jingyi Wu[1,2*], Bo Huang[3*], He Chen[4], Qiangzong Yin[1], Yang Liu[2,5], Yunlong Xiang[1], Bingjie Zhang[1], Bofeng Liu[1], Qiujun Wang[1], Weikun Xia[1], Wenzhi Li[6], Yuanyuan Li[1], Jing Ma[1], Xu Peng[7], Hui Zheng[1], Jia Ming[6], Wenhao Zhang[1], Jing Zhang[8], Geng Tian[9], Feng Xu[7,10], Zai Chang[8], Jie Na[6], Xuerui Yang[2,5] & Wei Xie[1,2]



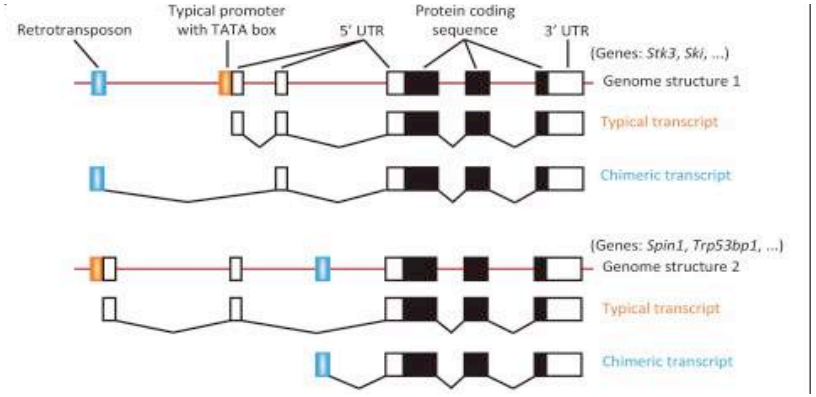


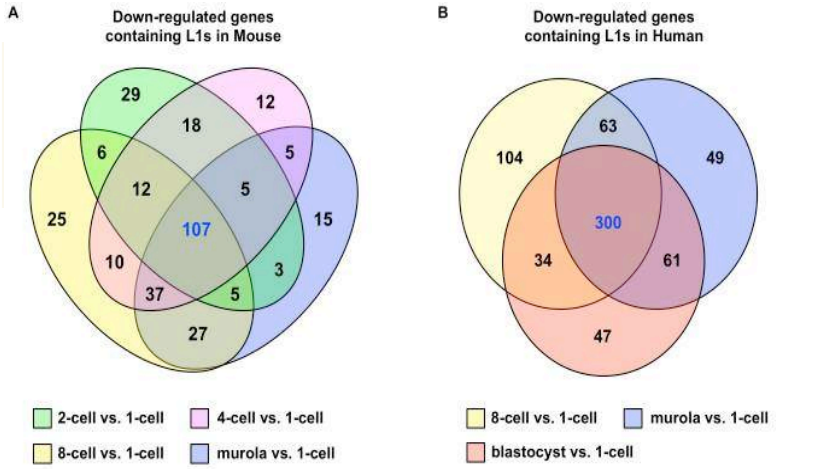
Unlike any somatic cells, see high chromatin accessibility both at promoters and more distant sites at repeats at the 2-cell stage.
Around MERVL see large, open domains become progressively restricted and as H3K27me3 domains start to appear

(Bac 04).

Dynamic Transcription of Distinct TE classes during early Mouse Development

- Are these expressed TEs just symptomatic of a loss in epigenetic control and presence of activators?
- Or might they play a role(s) in early development? (Peaston et al. 2004, Beraldi et al, 2006 and others)
- Provide strong alternative promoters to host genes? (Peaston et al 2004; Li et al 2014)
- Orchestrate the reorganisation of the early epigenome? (Wu, Huang et al, 2016 and others)
- Enable maintenance of high transcriptional activity to facilitate epigenomic reprogramming and EGA? (Hall et al, 2014)
- Influence developmental silencing of some genes with intragenic LINEs? (Ngamphiw et al, 2014)





C

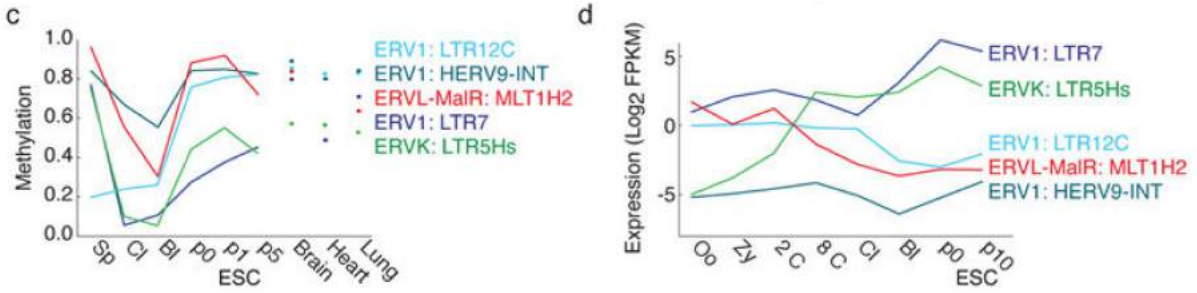
Orthologous down-regulated genes containing L1s

Kcnq1(16535) - **KCNQ1**(3784), **Rad51l1**(19363) - **RAD51B**(5890), **Rabgap1l**(29809) - **RABGAP1L**(9910), **Fut8**(53618) - **FUT8**(2530), **Pde3a**(54611) - **PDE3A**(5139), **Lmbr1**(56873) - **LMBR1**(64327), **Vav3**(57257) - **VAV3**(10451), **Rsrc1**(66880) - **RSRC1**(51319), **Ccdc132**(73288) - **CCDC132**(55610), **Tusc3**(80286) - **TUSC3**(7991), **Hivep1**(110521) - **HIVEP1**(3096), **Rims2**(116838) - **RIMS2**(9699), **Tox**(252838) - **TOX**(9760), **Cntn4**(269784) - **CNTN4**(152330)

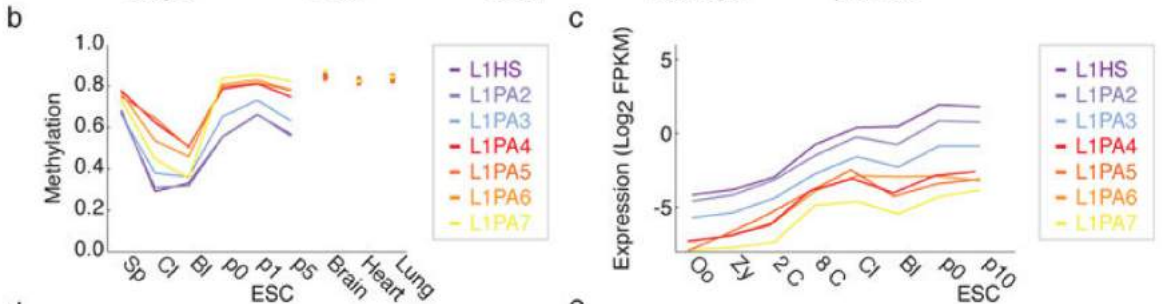
DNA Methylation and Expression Dynamics of TEs in Early Human Embryogenesis

- As in mice, human embryos show dynamic TE expression
- ERVs show dynamics loss and gain of DNA methylation
- Compared to ERVs, LINEs maintain higher methylation levels
- Only the primate-specific, still potentially mobile L1PA phylogeny is dynamically expressed
- Human-specific L1HS and its two closest ancestors, L1PA2 and L1PA3, are demethylated early, while older elements maintain higher embryonic methylation

ERVs
LTR subfamily dynamics are divided into early and late pre-implantation phases



LINEs
Emergent L1PA subfamilies escape DNA methylation-based repression during pre-implantation growth

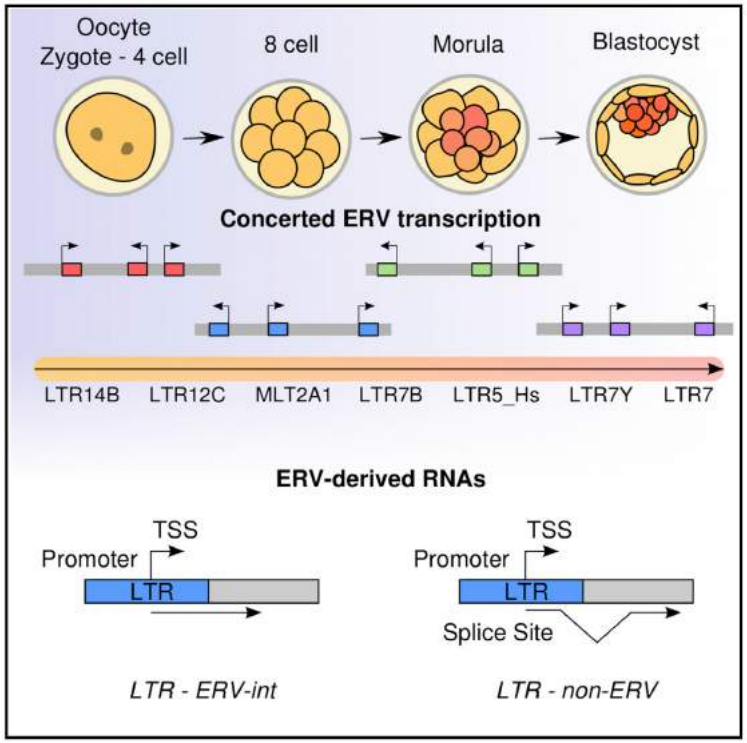


Smith et al (2014) DNA methylation dynamics of the human preimplantation embryo. Nature 511: 611-615

Dynamic Transcription of Distinct TE classes during early Human Development

Dynamic Transcription of Distinct Classes of Endogenous Retroviral Elements Marks Specific Populations of Early Human Embryonic Cells

Jonathan Göke,[1,*] Xinyi Lu,[2] Yun-Shen Chan,[2] Huck-Hui Ng,[2,3,4,5] Lam-Ha Ly,[1] Friedrich Sachs,[2,3] and Iwona Szczerbinska[2,3]

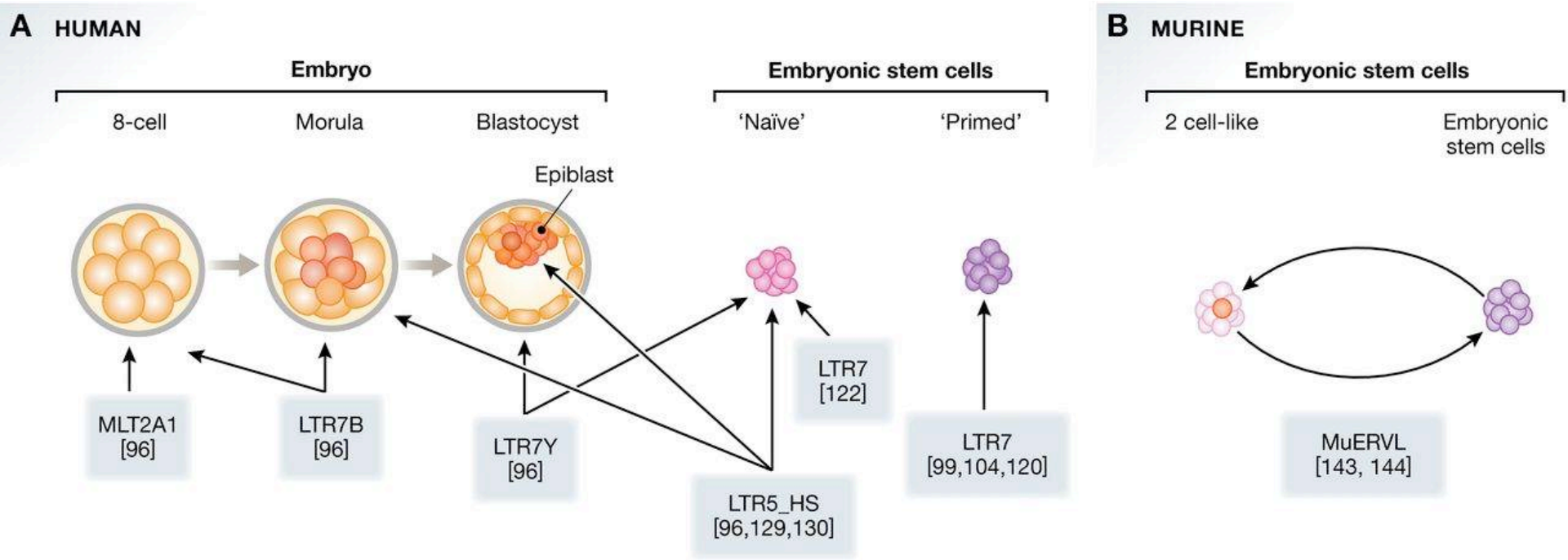


Specific families of ERVs are transcribed in human preimplantation embryos.

Transcribed ERVs are stage-specific and frequently spliced with non-ERV exons, generating a wide variety of co-expressed RNAs that demarcate the distinct cell populations in early human embryos

- ERVs are systematically transcribed in pre-implantation embryos
- Specific ERV families characterize different developmental stages
- Long terminal repeats regulate & initiate stage-specific transcription
- Preserved splice sites link stage-specific ERVs to the non-repetitive transcriptome

Specific ERVs mark the different cellular identities in early embryonic development



(A) Specific ERV families are expressed in the early human embryo, and in naïve and primed human embryonic stem cells (ESCs).

(B) In mouse, some ERVs are specifically activated in the two-cell stage. These ERVs are spontaneously expressed in cells which show features of two-cell-like totipotent cells.

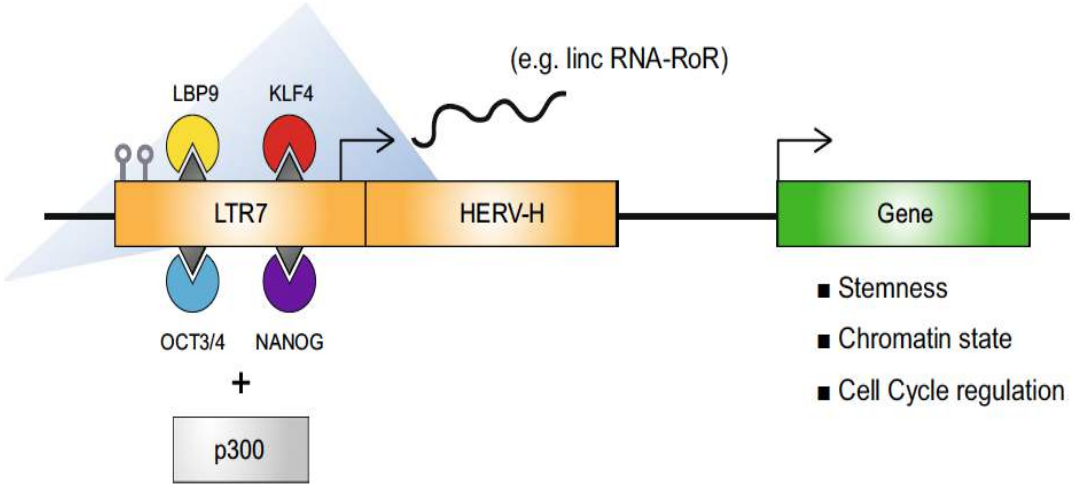
Santoni et al, 2012; Gifford et al, 2013; Lu et al, 2014; Macfarlan et al, 2012; Fort et al, 2014; Kapusta et al, 2013

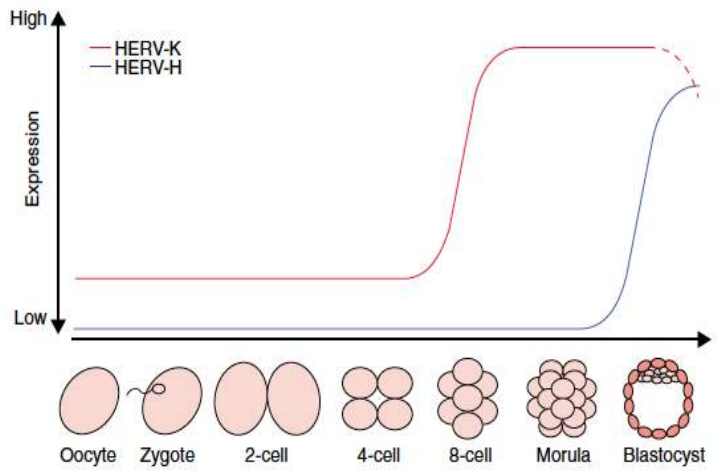
Some ERVs drive Non-coding RNAs: Role in Pluripotency?

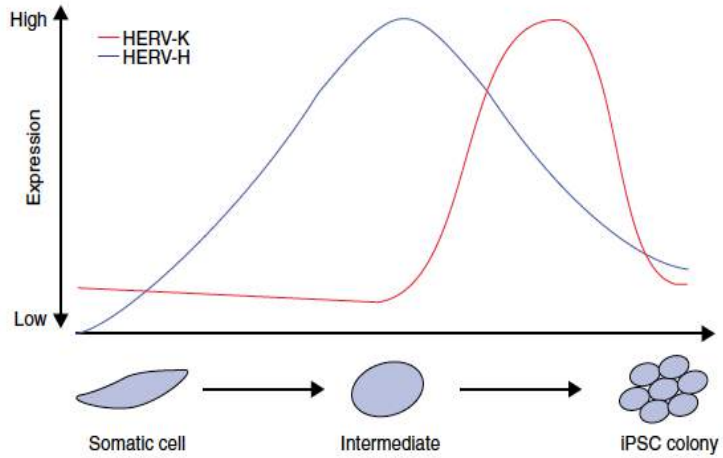
Retrotransposons may shape species-specific embryonic stem cell gene expression?

- HERV-H activity overlaps with pluripotent state:
- HERV-H expression may 'define' naïve stem cells.
- HERV-H may regulate stem cell gene expression??
- HERV-H recruits the TF, LBP9 which is essential for ground-state pluripotency…
- HERV-H must be silenced to guarantee successful cell differentiation
- Inappropriate expression of HERV-H and K ERVs could interfere with reprogramming to iPS?

More functional tests required!







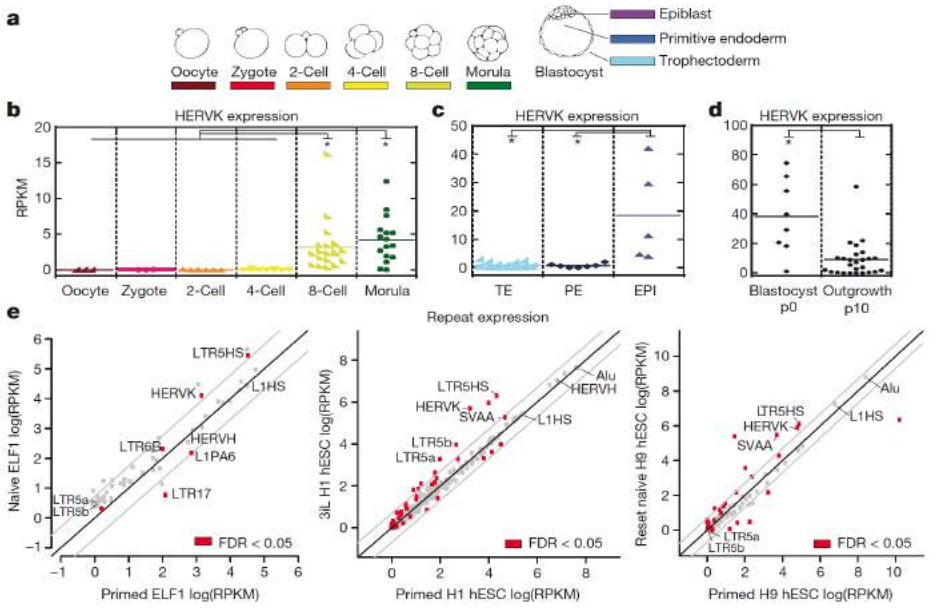
Robbez-Masson and Rowe *Retrovirology (2015) 12:45*

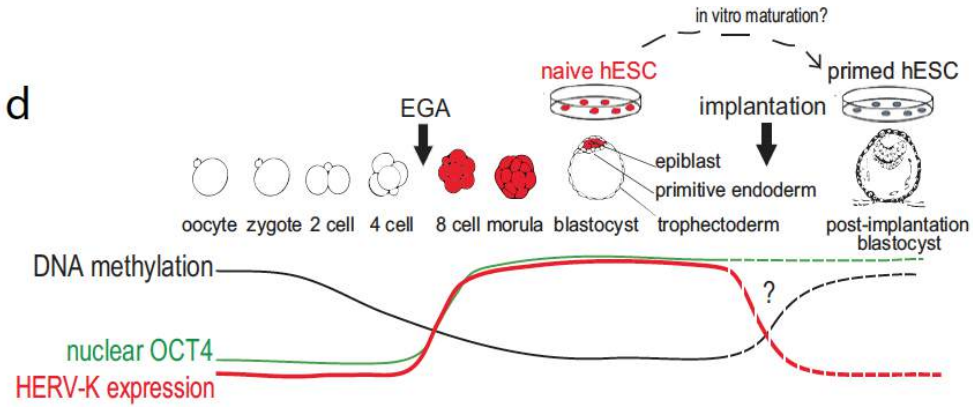
Endogenous Retroviral Expression in Human Pre-implantation Embryos

Intrinsic retroviral reactivation in human preimplantation embryos and pluripotent cells

Edward J. Grow[1], Ryan A. Flynn[2], Shawn L. Chavez[3,4,5], Nicholas L. Bayless[6], Mark Wossidlo[1,3,4], Daniel J. Wesche[3], Lance Martin[2], Carol B. Ware[7], Catherine A. Blish[8], Howard Y. Chang[2], Renee A. Reijo Pera[1,3,4,9] & Joanna Wysocka[3,10,11]

Endogenous retroviruses (ERVs) are remnants of ancient retroviral infections, and comprise nearly 8% of the human genome[1]. The most recently acquired human ERV is HERVK(HML-2), which repeatedly infected the primate lineage both before and after the divergence of the human and chimpanzee common ancestor[2,3]. Unlike most other human ERVs, HERVK retained multiple copies of intact open reading frames encoding retroviral proteins[4]. However, HERVK is transcriptionally silenced by the host, with the exception of in certain pathological contexts such as germ-cell tumours, melanoma or human immunodeficiency virus (HIV) infection[5–7]. Here we demonstrate that DNA hypomethylation at long terminal repeat elements representing the most recent genomic integrations, together with transactivation by OCT4 (also known as POU5F1), synergistically facilitate HERVK expression. Consequently, HERVK is transcribed during normal human embryogenesis, beginning with embryonic genome activation at the eight-cell stage, continuing through the emergence of epiblast cells in preimplantation blastocysts, and ceasing during human embryonic stem cell derivation from blastocyst outgrowths. Remarkably, we detected HERVK viral-like particles and Gag proteins in human blastocysts, indicating that early human development proceeds in the presence of retroviral products. We further show that overexpression of one such product, the HERVK accessory protein Rec, in a pluripotent cell line is sufficient to increase IFITM1 levels on the cell surface and inhibit viral infection, suggesting at least one mechanism through which HERVK can induce viral restriction pathways in early embryonic cells. Moreover, Rec directly binds a subset of cellular RNAs and modulates their ribosome occupancy, indicating that complex interactions between retroviral proteins and host factors can fine-tune pathways of early human development.





E. Heard, February 20th, 2017

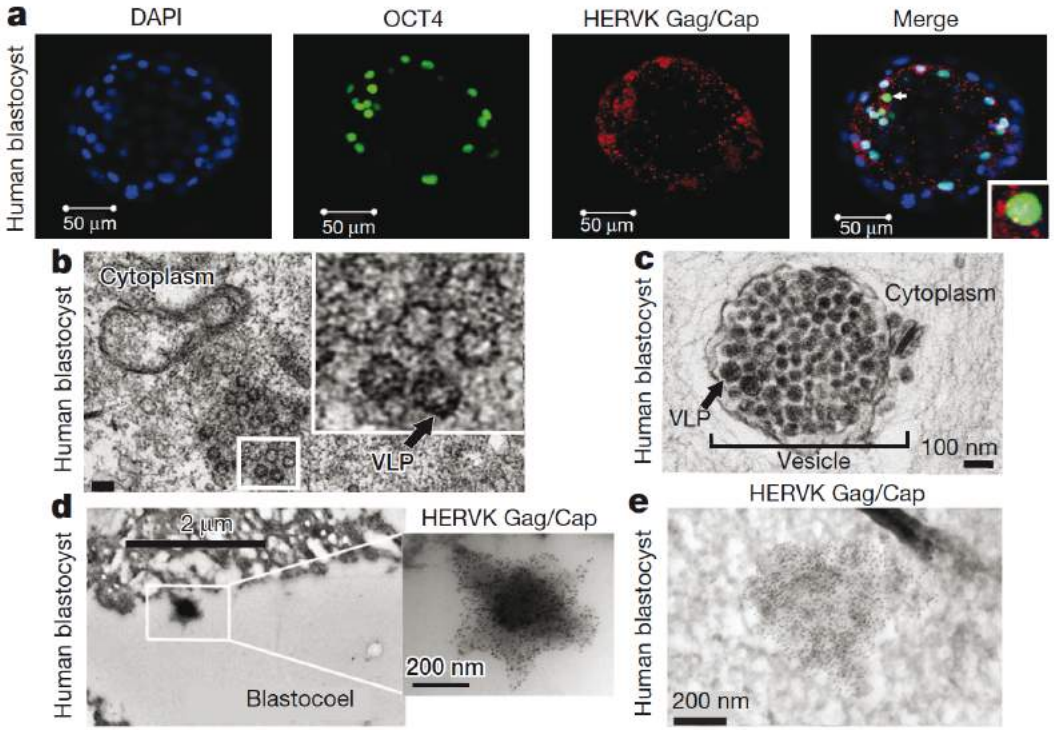
COLLÈGE DE FRANCE 1530

Endogenous Retroviral Expression in Human Pre-implantation Embryos

Intrinsic retroviral reactivation in human preimplantation embryos and pluripotent cells

Edward J. Grow[1], Ryan A. Flynn[2], Shawn L. Chavez[3,4,5], Nicholas L. Bayless[6], Mark Wossidlo[1,3,4], Daniel J. Wesche[3], Lance Martin[2], Carol B. Ware[7], Catherine A. Blish[8], Howard Y. Chang[2], Renee A. Reijo Pera[1,3,4,9] & Joanna Wysocka[3,10,11]

Part of a protective mechanism?
Induction of HERV-K particles in early embryos may induce host viral restriction pathways to protect from subsequent infection?



Virus-like particules in the cytoplasme

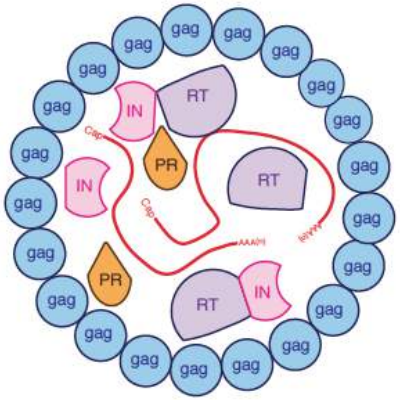
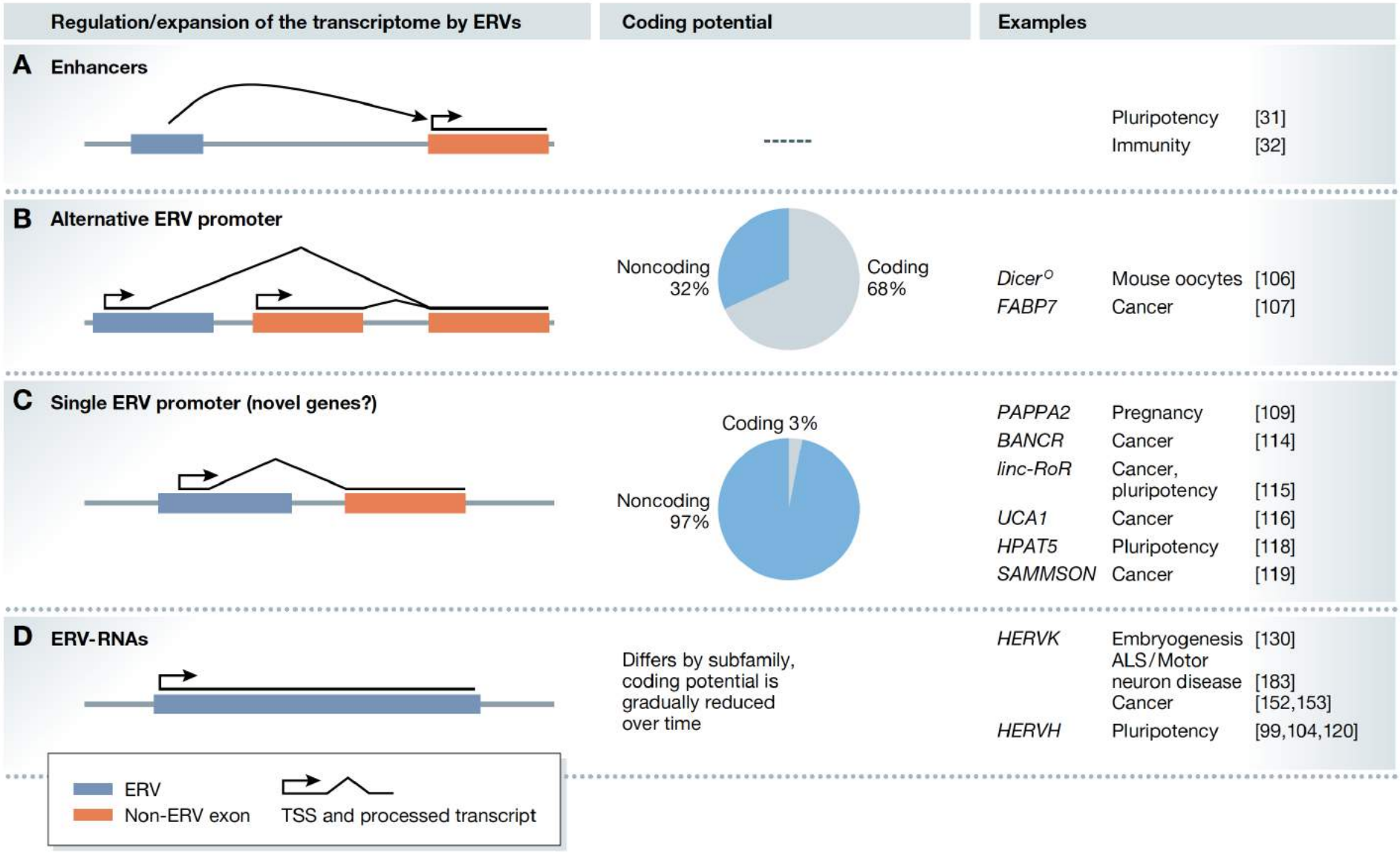


Figure 3 | Human blastocysts contain HERVK proteins and viral-like particles. a, Immunofluorescence of human blastocysts (days post-fertilization

TEs regulate and expand the transcriptome
for better or for worse



TEs regulate and expand the transcriptome during the very first stages of life

1. Distinct classes of TEs seem to be specifically expressed in mouse and human pre-implantation development

Exaptation?
Mammalian hosts are co-opting retrotransposons to drive gene expression and other functions during these stages of development.

Consequence
of open chromatin and lack of adequate control?

Santoni et al, 2012; Gifford et al, 2013; Lu et al, 2014; Macfarlan et al, 2012; Fort et al, 2014; Kapusta et al, 2013

TEs regulate and expand the transcriptome during the very first stages of life

1. Distinct classes of TEs seem to be specifically expressed in mouse and human pre-implantation development
2. It is not *entire subclasses* active at any given time but a specific subset of integrants – due to combined influence of trans-activators/repressors and local chromatin constraints – *raises question of Cause vs Consequence*
3. Some TEs (or their relics) may have been coopted for the purposes of gene regulation and orchestration of a number of processes during early embryonic development.
4. In mouse, large fraction of 2-cell stage activated genes are driven from the LTR of mouse-specific MERV-L
5. In human, ERV-derived mRNA transcripts and long non-coding RNAs found throughout pre-implantation development (2-cell to blastocyst) and in embryonic stem cells
6. In human – OCT4 factor binds LTR of HERVH: pluripotency of hESCs correlates with expression of some HERVH loci
7. Role for HERVH-lncRNAs and enhancer activity of HERHV LTR7 in maintenance of pluripotent state?

Santoni et al, 2012; Gifford et al, 2013; Lu et al, 2014; Macfarlan et al, 2012; Fort et al, 2014; Kapusta et al, 2013

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L'implication des éléments transposables dans les maladies : mutations et épimutations

The implication of transposable elements in disease: mutations and epimuations