CHAIRE ÉPIGÉNÉTIQUE ET MÉMOIRE CELLULAIRE

Année 2016-2017 :
"Épigénétique et ADN égoïste"

13 Février, 2017

Cours II

Le rôle de l'épigénétique dans la régulation des éléments transposables.

The role of epigenetics in the regulation of transposable elements

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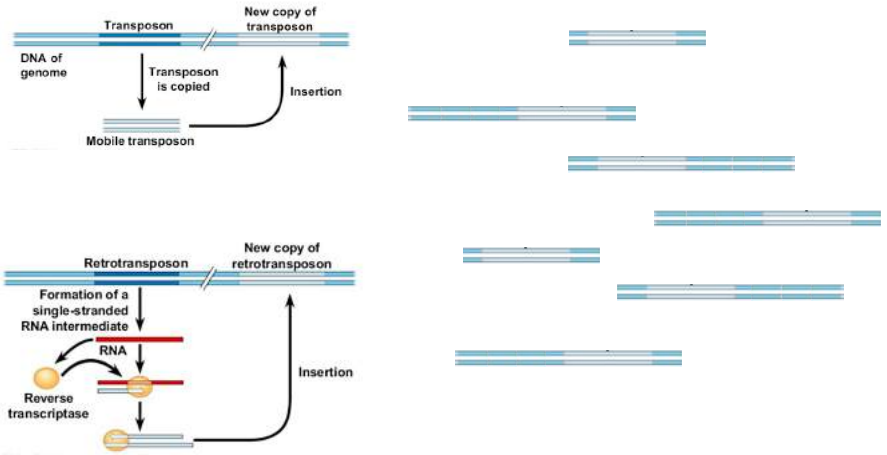
Transposable Elements

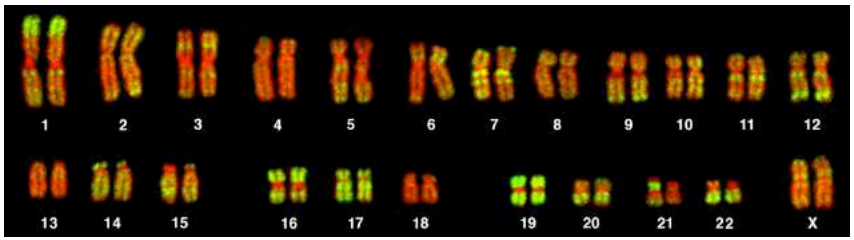
From Selfish DNA, Junk DNA…
To Genome architects, Genetic and Epigenetic tools

Transposable Elements

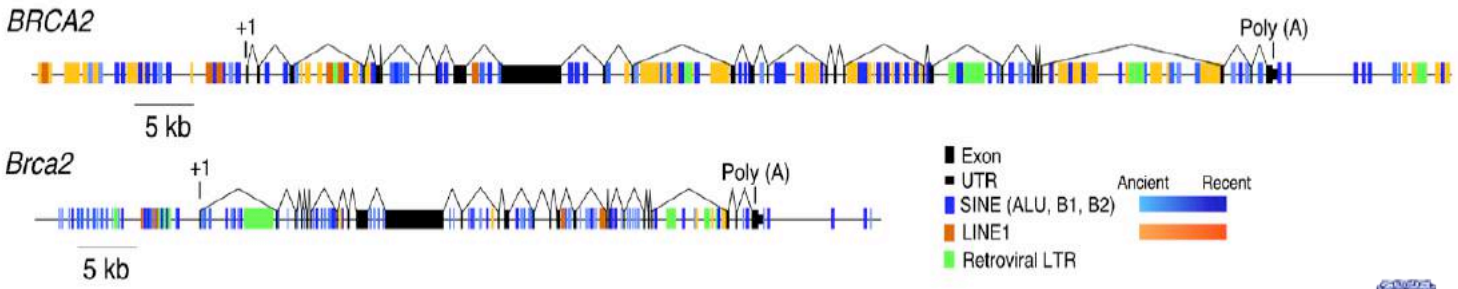
From Selfish DNA, Junk DNA…
To Genome architects, Genetic and Epigenetic tools

- ✧ Sequencing of genomes has revealed the massive presence of TEs and their relics
- ✧ Making up most of the 98% of "non-coding" genome – "junk" or "hidden treasures"?
- ✧ Latest census : **$4.5x10^6$** transposable elements in the human genome!





Bolzer et al, Plos Biol. 2005



Courtesy of Tim Bestor

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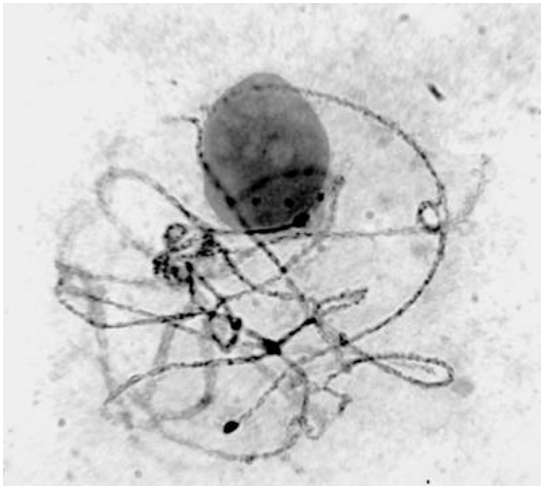
Transposable Elements

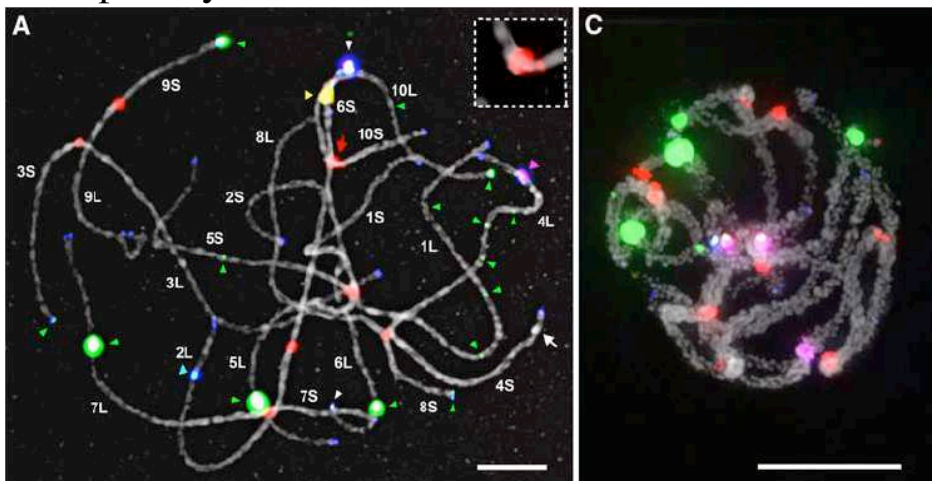
From Selfish DNA, Junk DNA…
To Genome architects, Genetic and Epigenetic tools

- ✧ The Maize genome, studied by McClintock is made up of 85% TEs!
- ✧ Transposable elements are most highly enriched in Heterochromatin – silent but with potential to be active



Barbara McClintock, 1951

TE repeat hybridisation on Maize chromosome 9

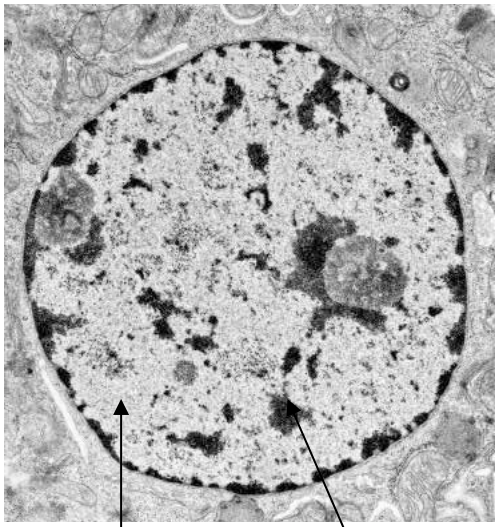


Wang et al, Plant Cell 2006

Heterochromatin and the Epigenetic Silencing of Genes and TEs

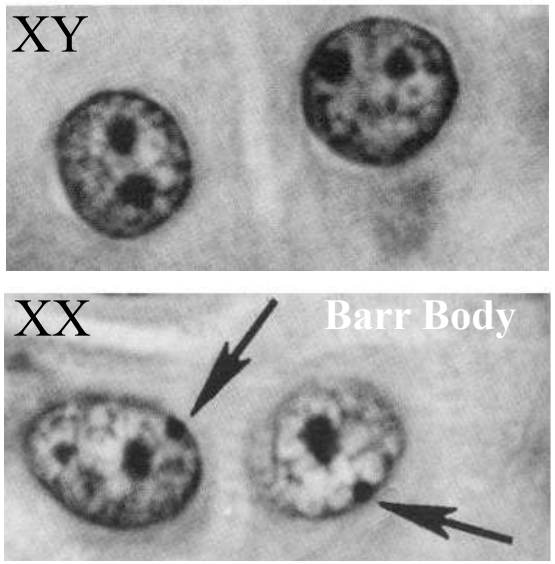
Heterochromatin and Euchromatin
Emile Heintz, 1929

Voir COURS 2015



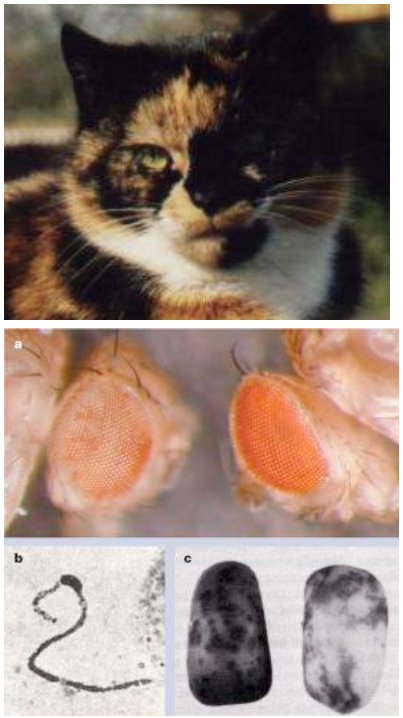
Euchromatin Heterochromatin

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



Bertram et Barr, 1949

The heterochromatic inactive X
M. Lyon, 1961



Barbara McClintock proposed that "*changes in quantity, quality or structural organization of heterochromatic elements may well alter the kind and/or degree of particular exchanges that occur, and in this way control the chromosome organization and the kind and the relative effectiveness of genic action*" (McClintock, 1950).

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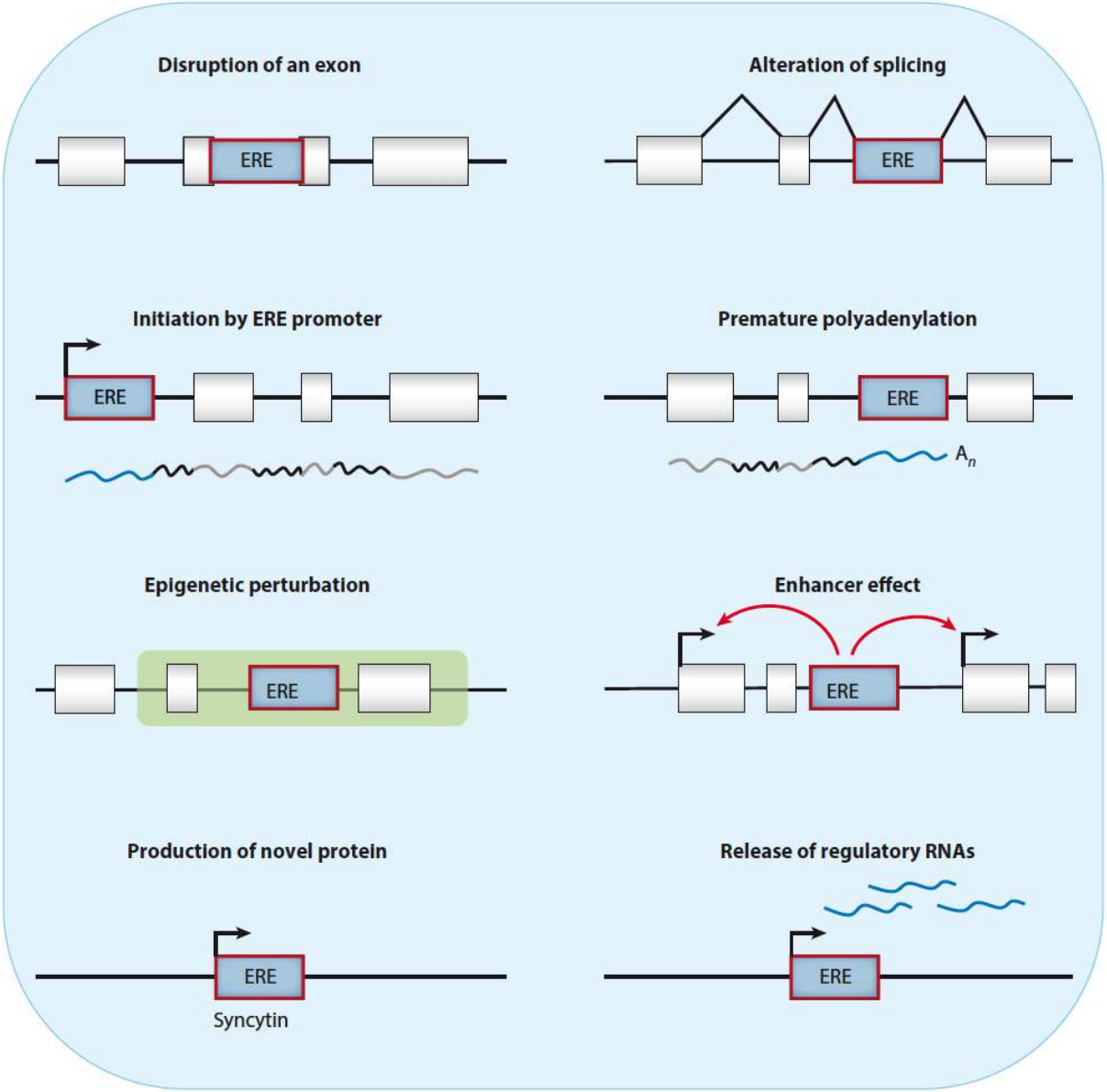
Transposable Elements (TEs) as Generators of Genetic Diversity and Modulators of Gene Expression

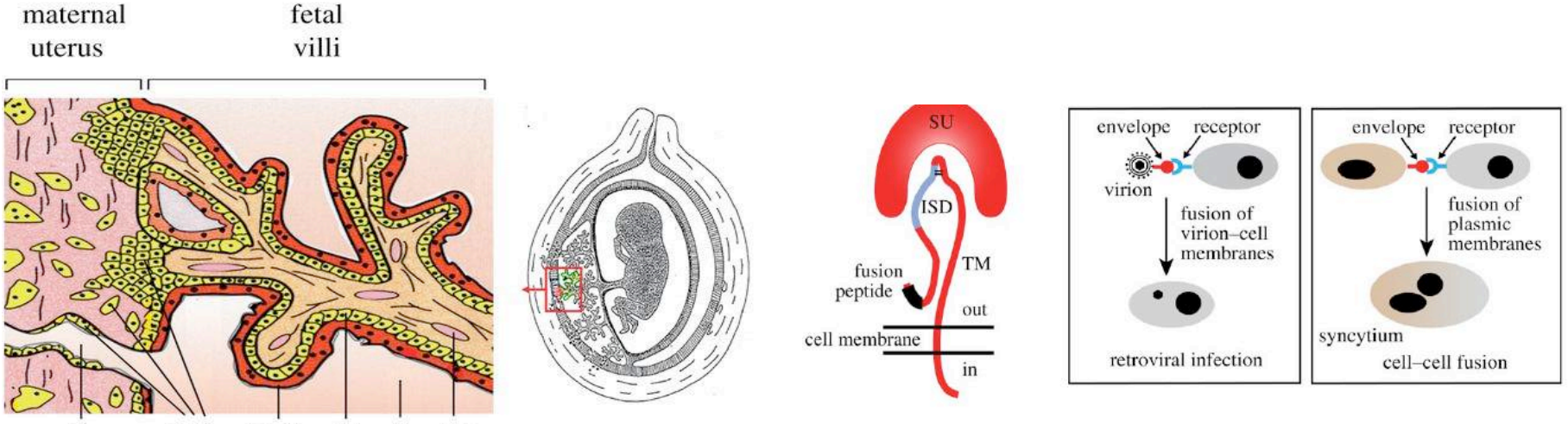
- TEs = powerful genetic force engaged in the evolution of higher species.
- Genomes are spattered with thousands of copies => leads t recombination events inducing deletions, duplications
- They can disrupt existing genes but also provide new protein-coding sequences
- They exert a wide range of transcriptional influence, either directly or via host mechanisms responsible for their control



Transposable elements as generators of genes for new (host) functions

- **Placentation**:

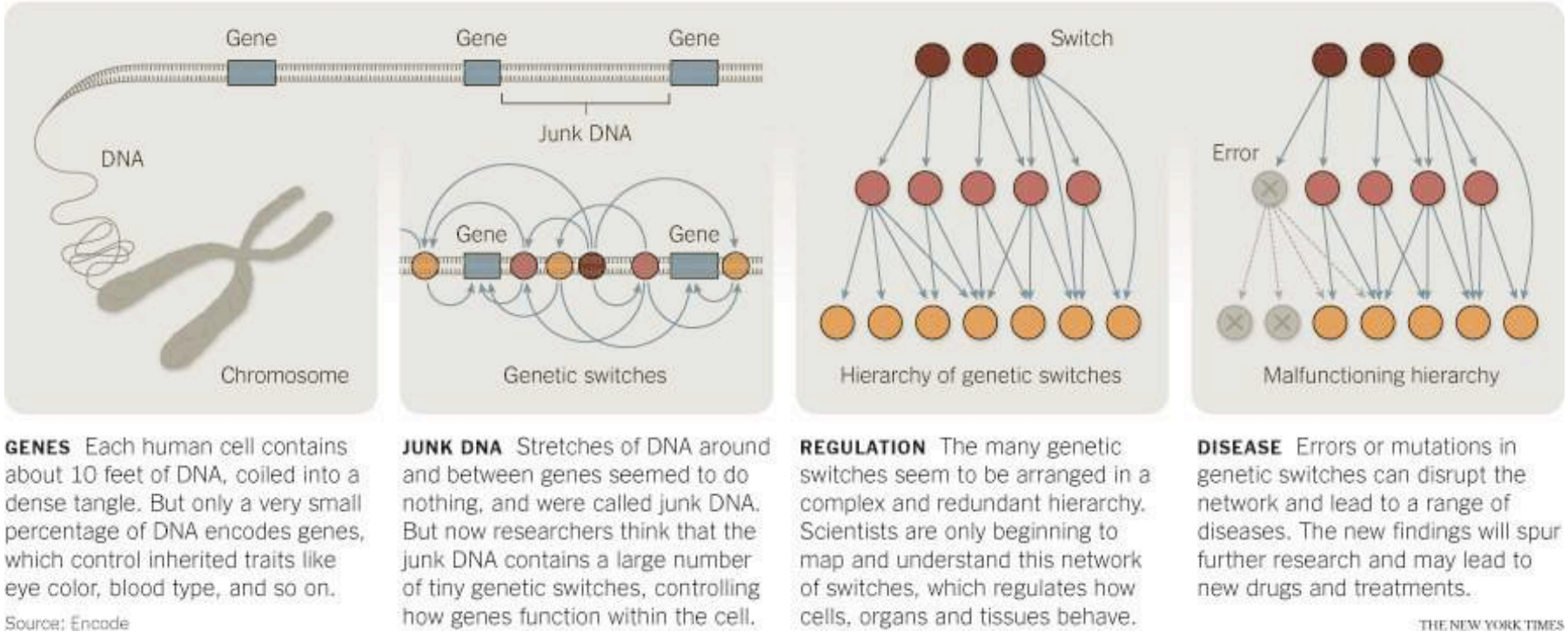
Syncytin - required to form the syncytiotrophoblast double layer – essential for maternal/fetal exchange. Exaptation of an *env* gene: emergence of mammalian ancestors with a placenta from egg-laying animals? New *env*-derived syncytin genes, each providing its host with a selective advantage (see Lavialle, Heidmann et al, 2012)



- **Immune System**:

Recombination-activating genes *RAG1* and *RAG2*, which are critical for V(D)J recombination and adaptive immune system development, originated from domestication of a member of the *Transib* family of DNA transposons, approx. 500 Mya (Kapitonov & Jurka 2005, Zhou et al. 2004).

Transposable elements as generators of phenotypic diversity through the modulation of gene expression (genetic and epigenetic)



Architects of gene regulatory landscapes – providing specific DNA binding sites for factors that control gene expression – enhancers, locus control regions (super-enhancers), insulators etc

TE fragments/relics provide "Controlling Elements"

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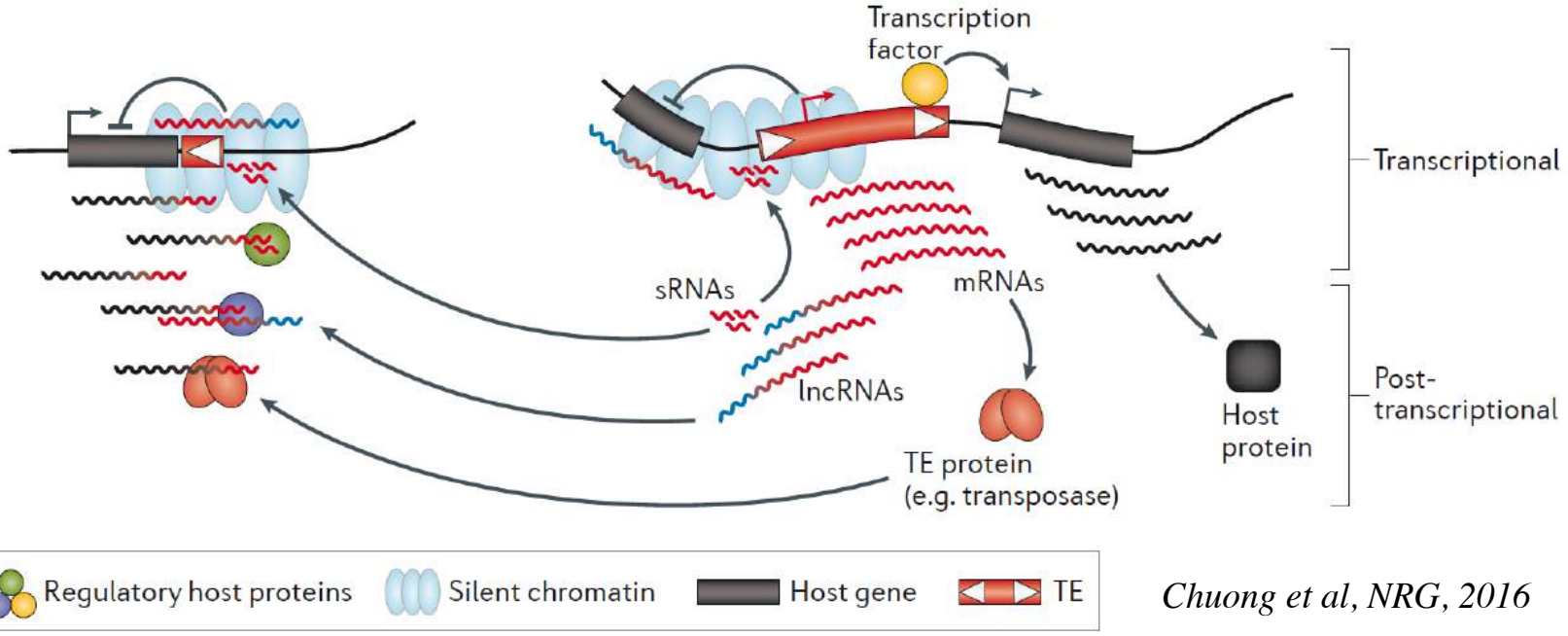
Transposable elements as generators of phenotypic diversity through the modulation of gene expression (genetic and epigenetic)

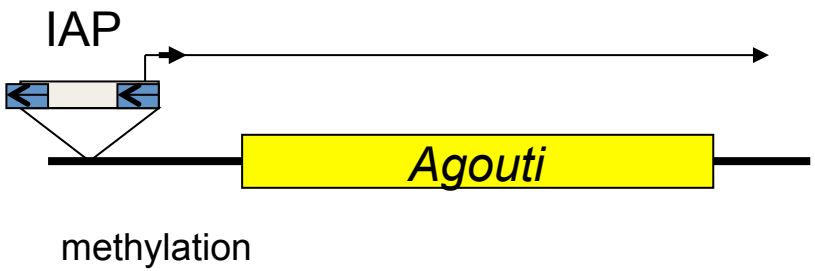
New alleles

New epialleles

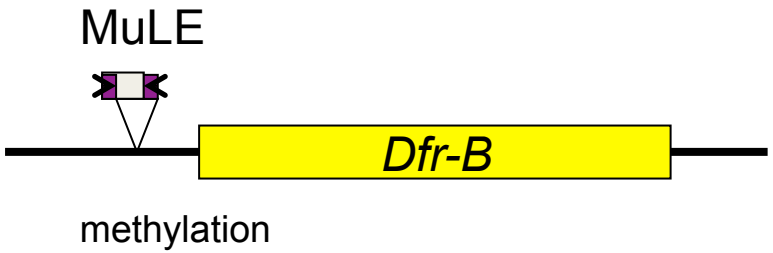


Chuong et al, NRG, 2016

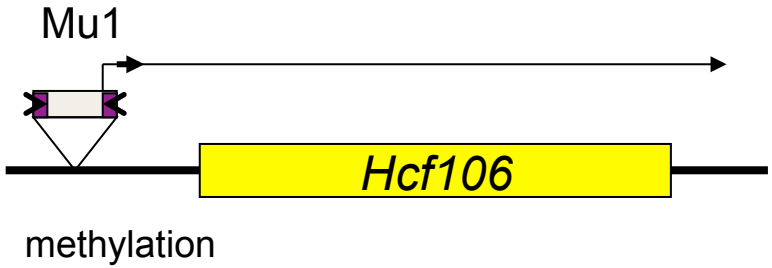
TEs can regulate genes via DNA methylation in plants and animals

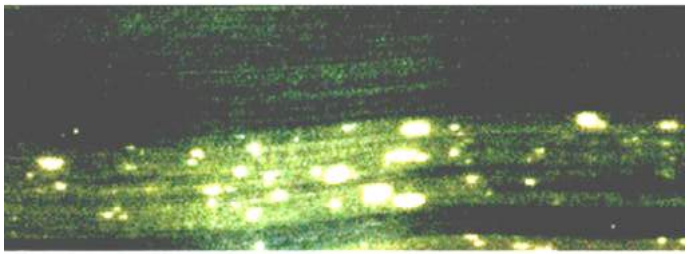


Morgan et al., 1999



Iida et al., 2004



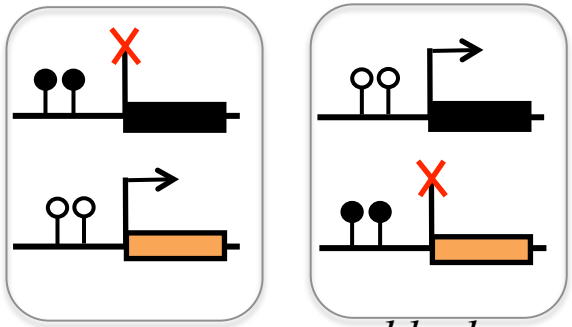


Martienssen et al., 1990

Courtesy of Rob Martienssen

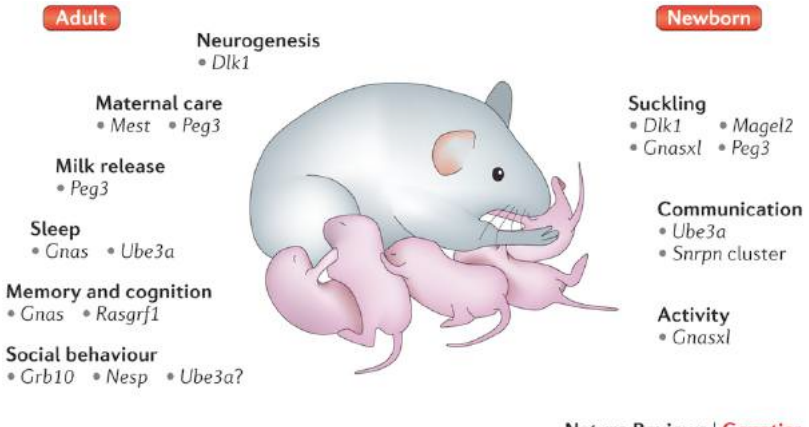
TEs are implicated in epigenetic phenomena

X-chromosome inactivation

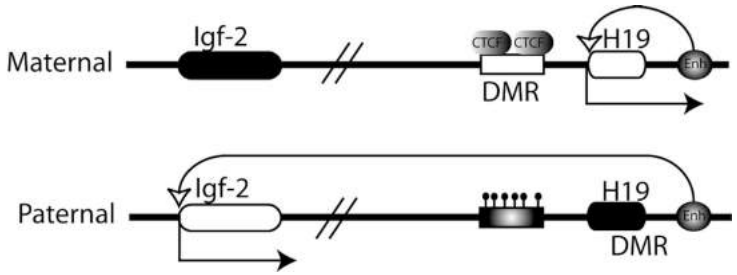


Imprinting

Imprinted Genes Control Embryonic & Neonatal Growth



Nature Reviews | Genetics



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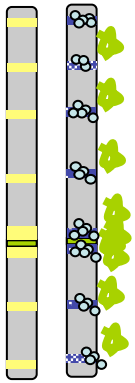
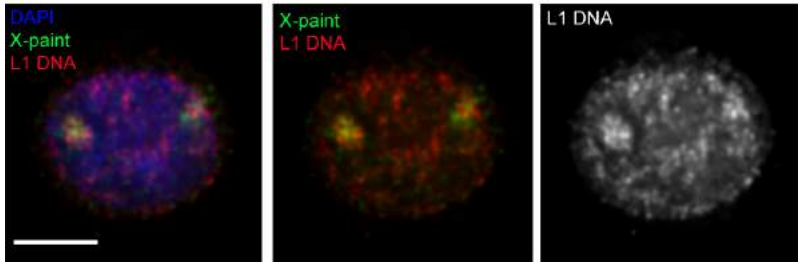
TEs are implicated in epigenetic phenomena

X-chromosome inactivation



Xa Xi

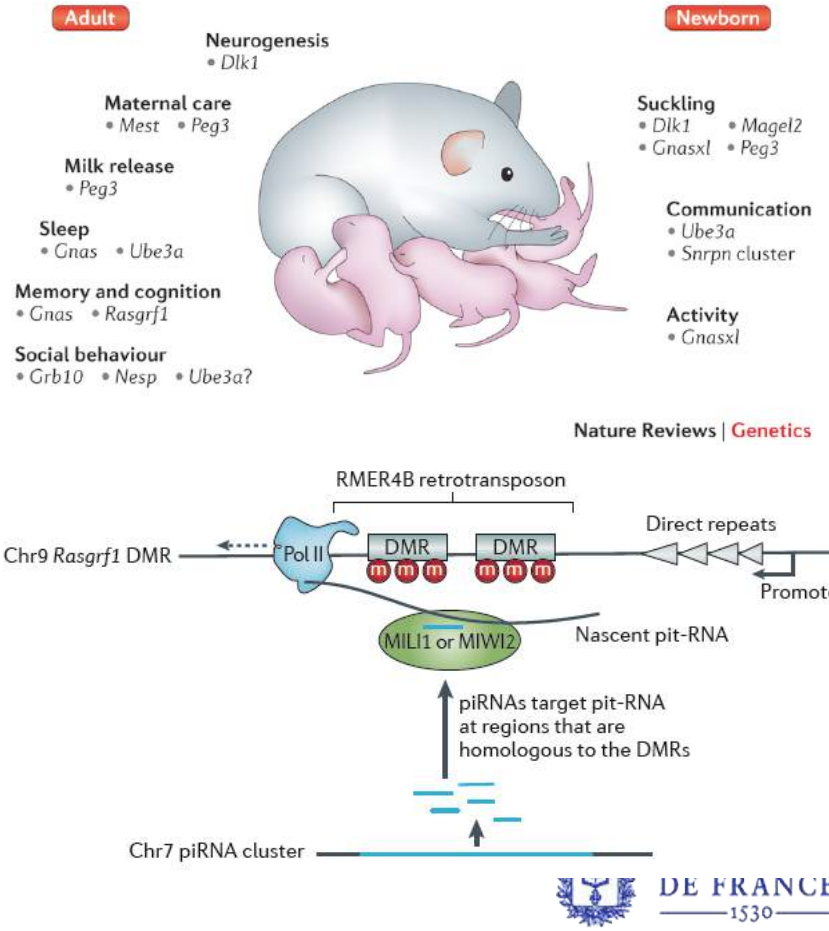


Chow et al, Cell 2010

The X chromosome is specifically enriched in LINE retroelements, which may facilitate X inactivation (Lyon LINE Hypothesis, 1998)

Imprinting

Imprinted Genes Control Embryonic & Neonatal Growth

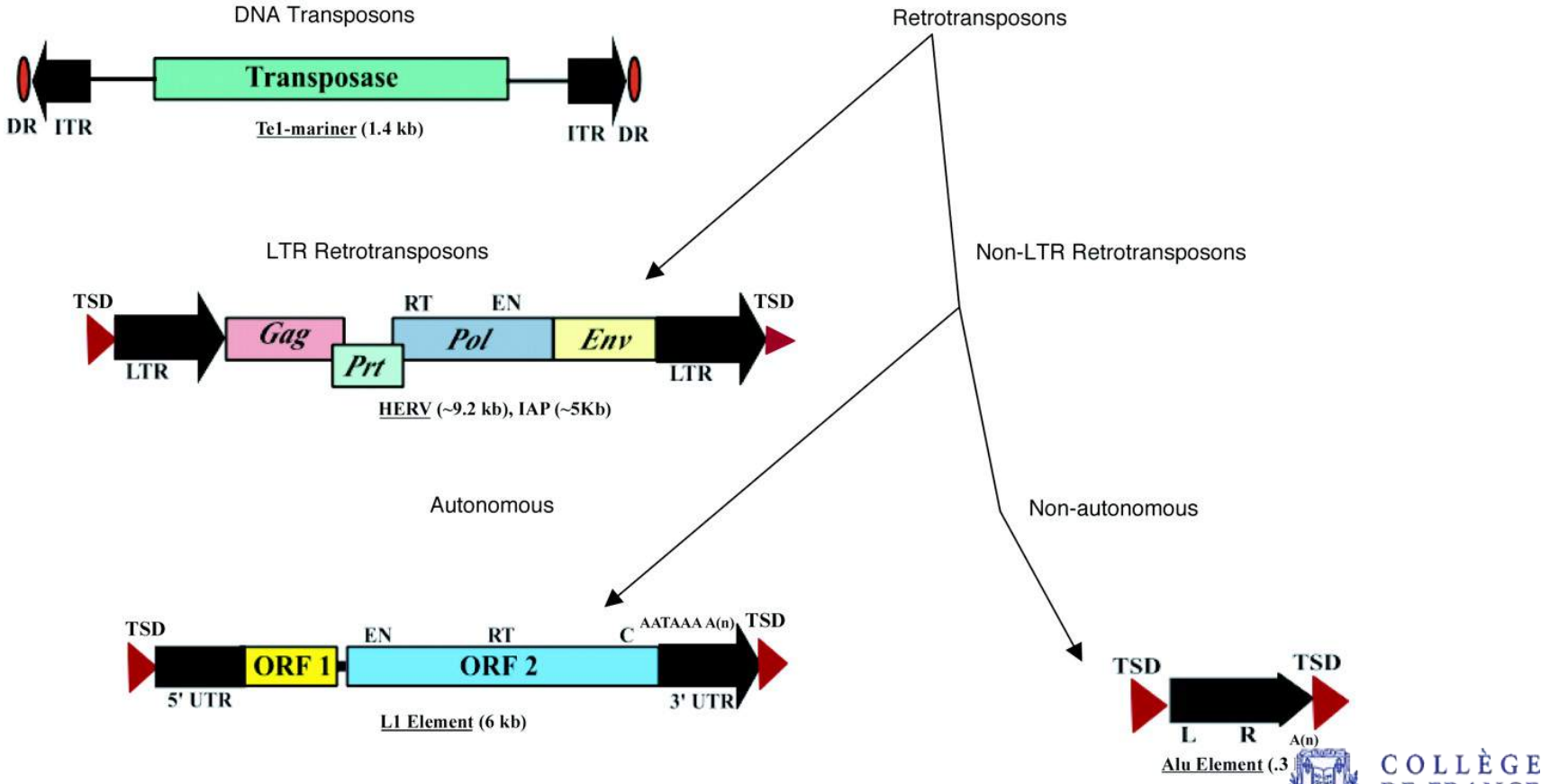


Epigenetic Mechanisms and Transposable Elements

- Epigenetic silencing may have evolved as a means to defend the genome from parasites such as TEs and viruses (Doerfler et al, 1991; Yoder et al, 1997).
- On the other hand, it may only be thanks to the existence of epigenetic mechanisms that TEs can persist in a host after they first appear (Slotkin and Martienssen, 2007; Fedoroff, 2012).
- Epigenetic changes allow for the dynamic balance between silencing and escape required to protect the host, but also enable TE (selfish) spread as well as selection for new functions within the host.
- Epigenetic silencing represents an opportunity for both heritable and reprogrammable expression.

Classes of Transposable Elements

Different distributions and dynamics in different organisms
Transposons vary in structure and manner of proliferation
These features are used to classify them
Each class contains **autonomous** elements that can self-proliferate,
and **non-autonomous** elements that cannot.



Haig H. Kazazian Jr. Science 2004;303:1626-1632

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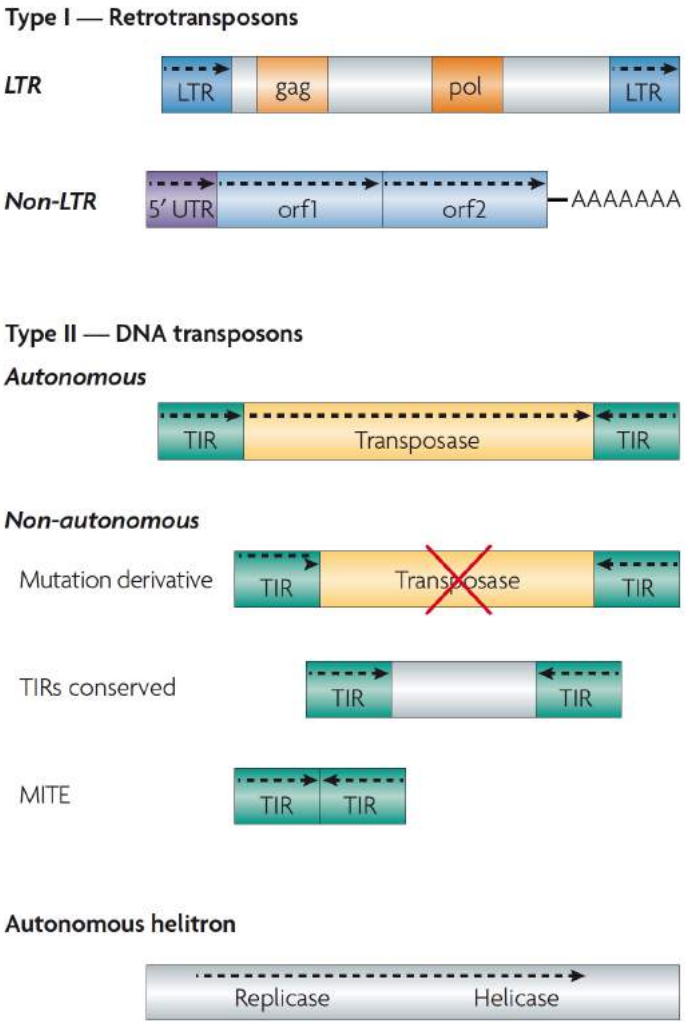
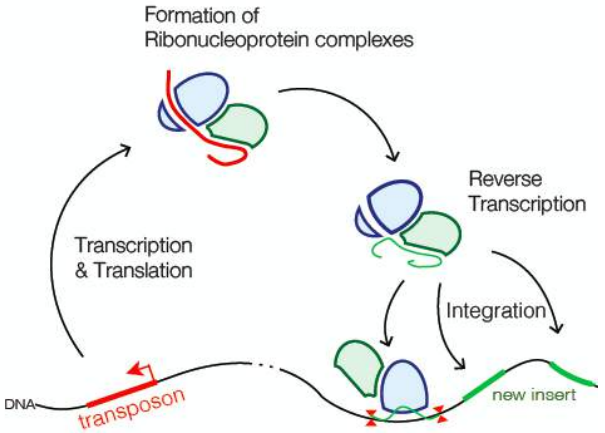
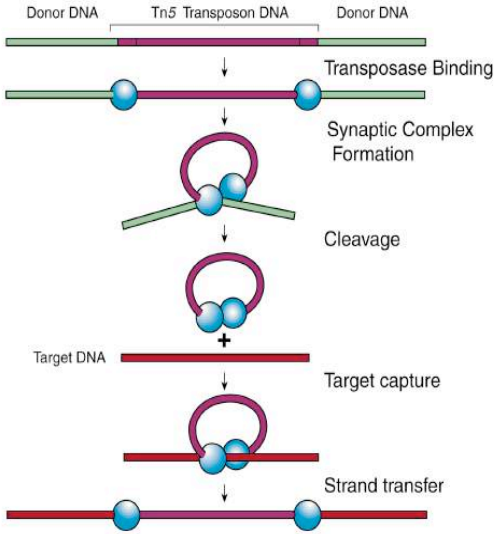
Transposable Elements:

Class I (copy-and-paste) and Class II (cut-and-paste)







Slotkin and Martienssen, 2007

Adapted from Davies et al, 2000 – Science

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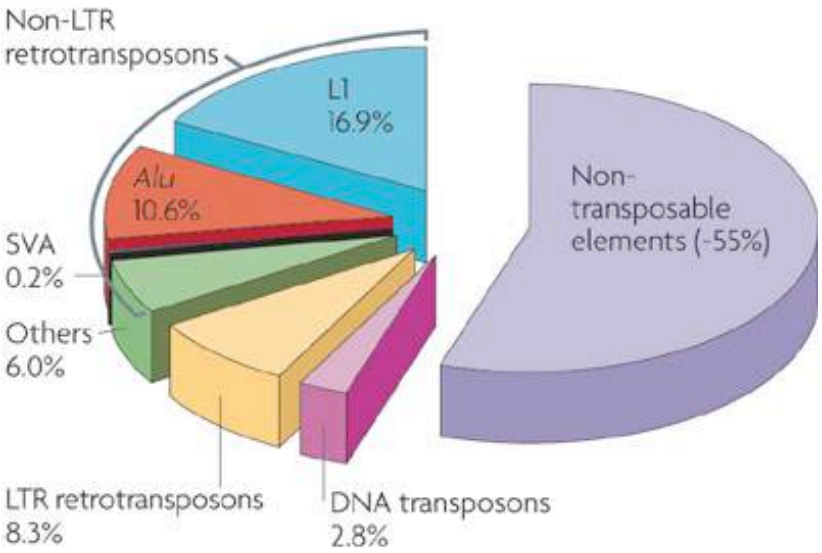
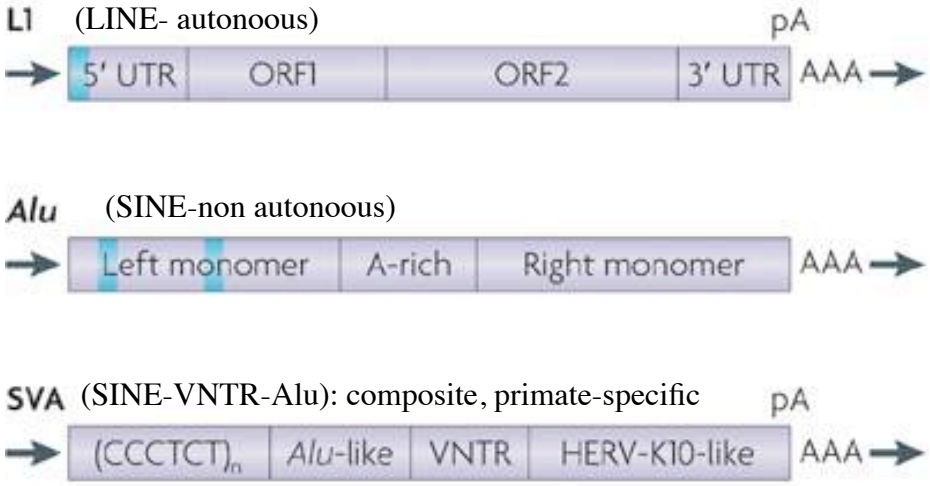
Human Retrotransposons



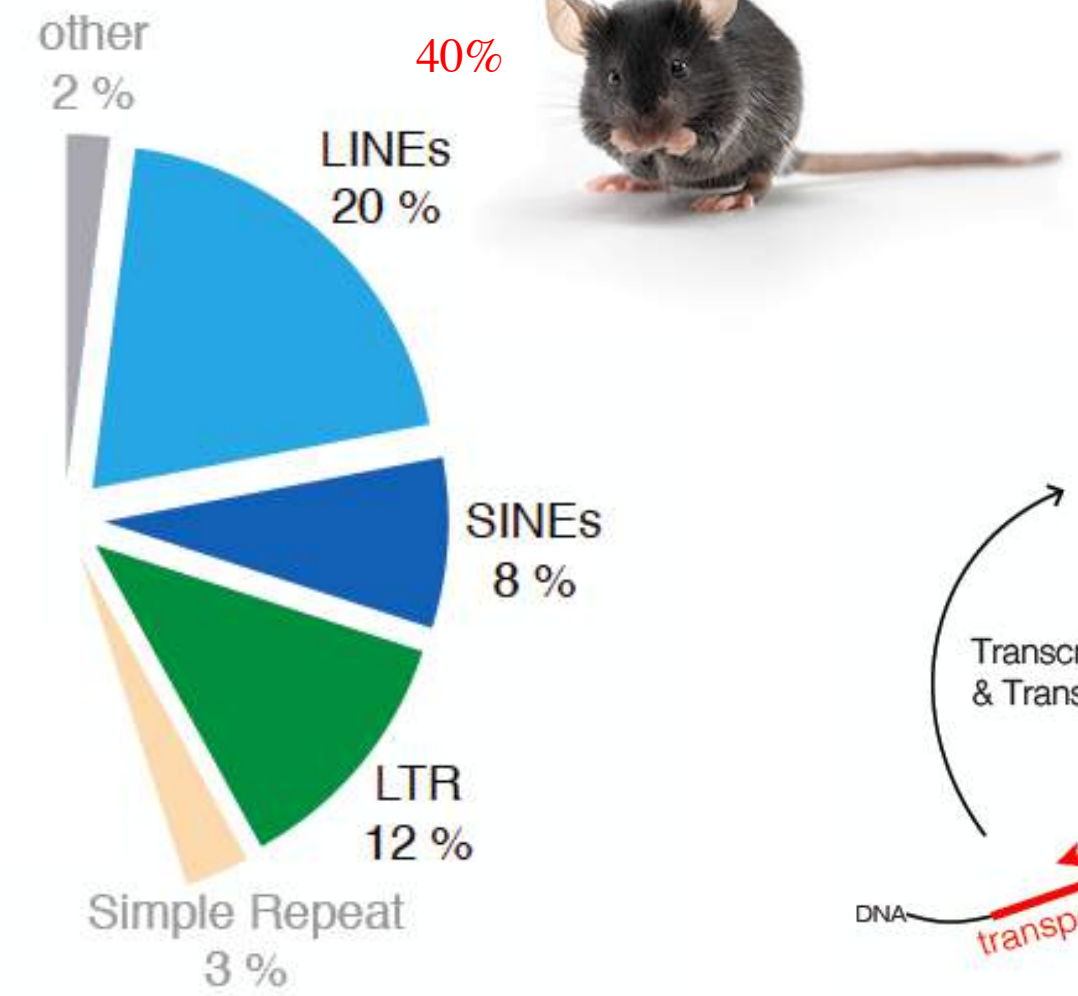


- ✧ The human genome contains few DNA transposons and none seem to be active
- ✧ But it contains millions of copies of retrotransposons
- ✧ 1.2 million copies of the (non-autonomous) Alu repeat in the human genome
- ✧ >100,000 L1 sequences exist in human genome, most inactive (mutated/truncated)
- ✧ Only the LINE-1 (L1) family, remains the primary source of retrotransposition
- ✧ A few (<10) "Hot" L1s account for most L1 and Alu retrotransposition in humans
- ✧ "Hot" L1s are extremely polymorphic and specific to a few individuals;
- ✧ They may participate in creating somatic variation during life span, and also in disease (**COURS III et IV)**
- ✧ LTR retrotransposons are present but only few are active in humans

E

Mouse Retrotransposons

Mice have many more active LINEs and ERVs than humans



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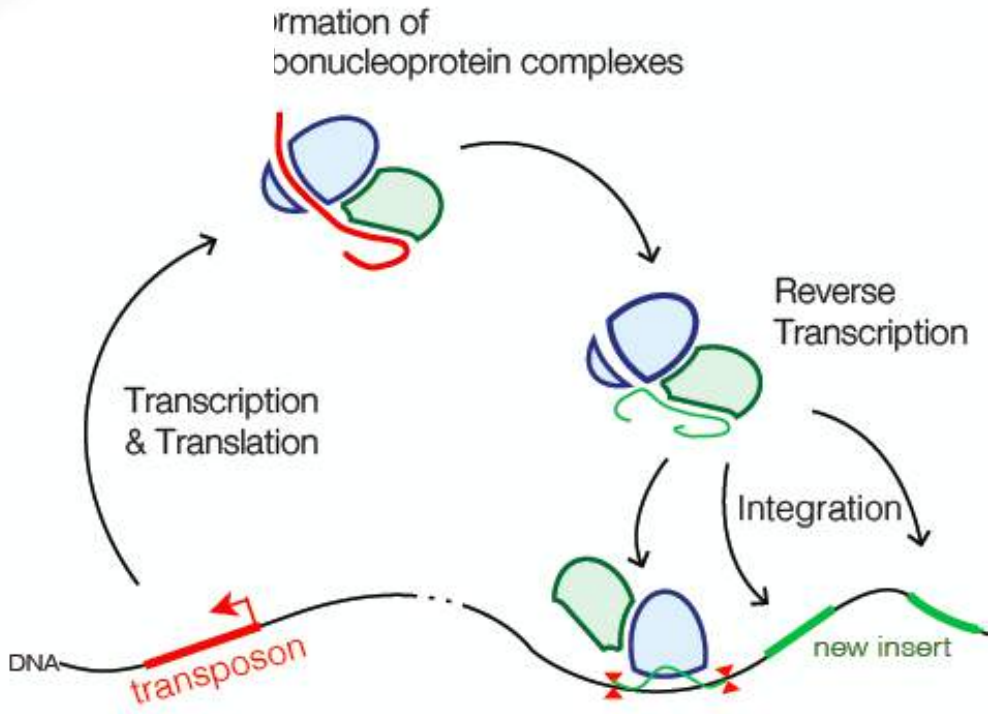
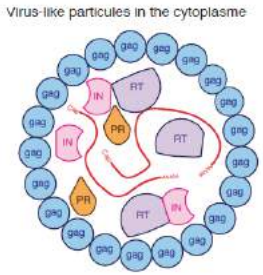
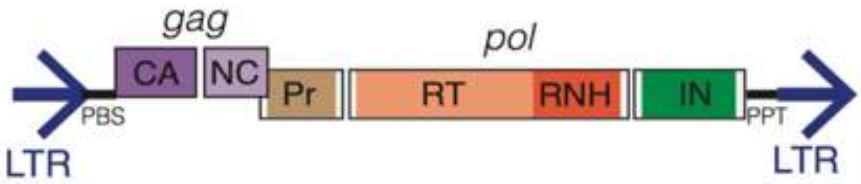


Marius Walter/Deborah Bourc'his

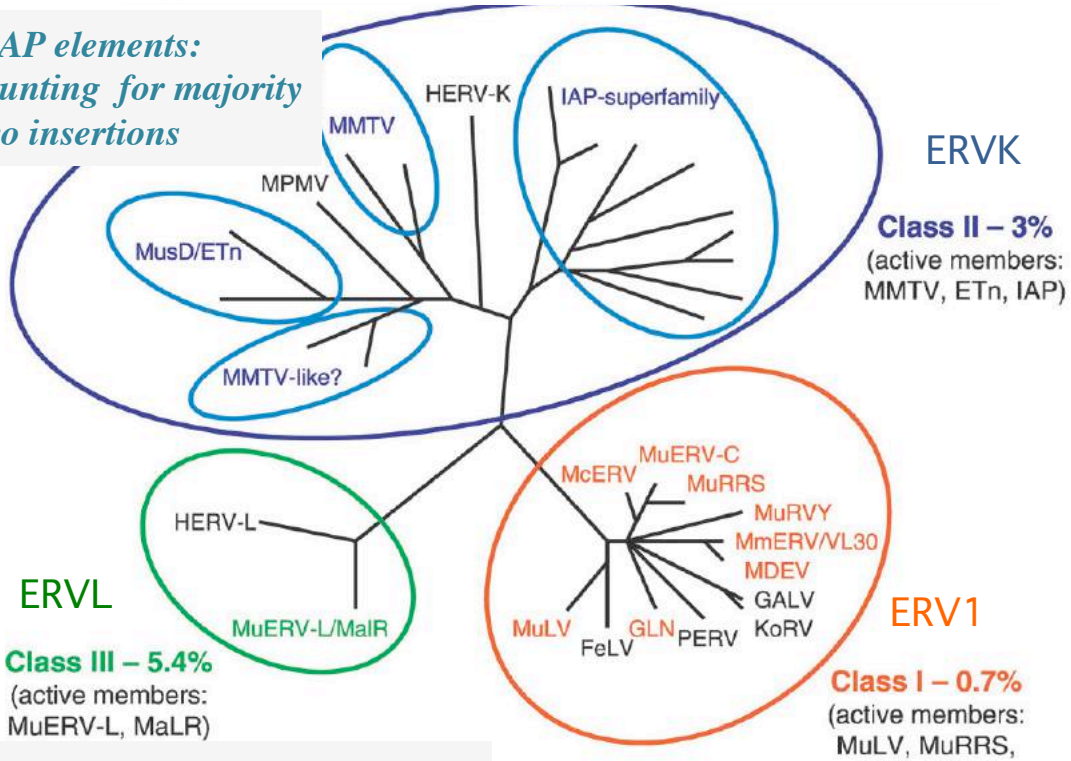
LTR elements: Endogenous retroviruses



LTR-retrotransposon (5-20 kb)



ETn and IAP elements: most active, accounting for majority of de novo insertions



Most ancient - coopted for roles in germ line and development (more next week)

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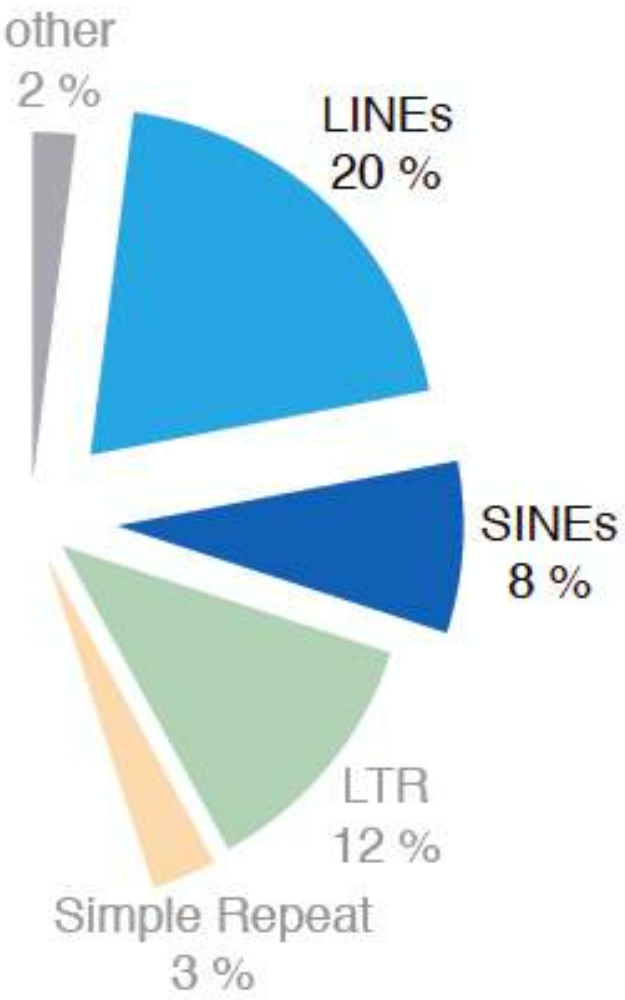
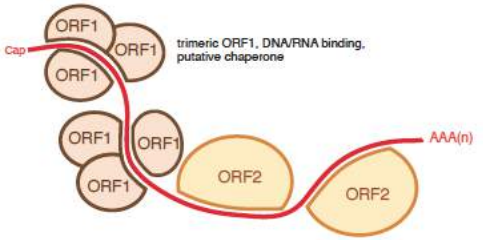
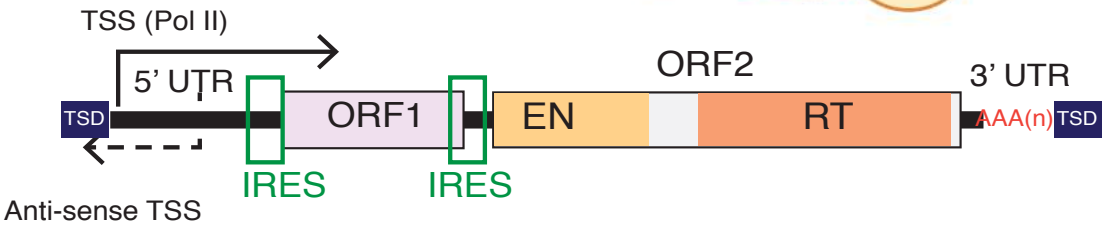
Non-LTR Elements

LINEs/SINEs- Long and Short Interspersed Elements





mouse LINE1 (~ 6 kb)



SINEs (~ 100-300 bp)

7SL RNA head

Alu - 280bp (human)

B1 - 135pb (mouse)

tRNA head

B2 - 175pb (mouse)

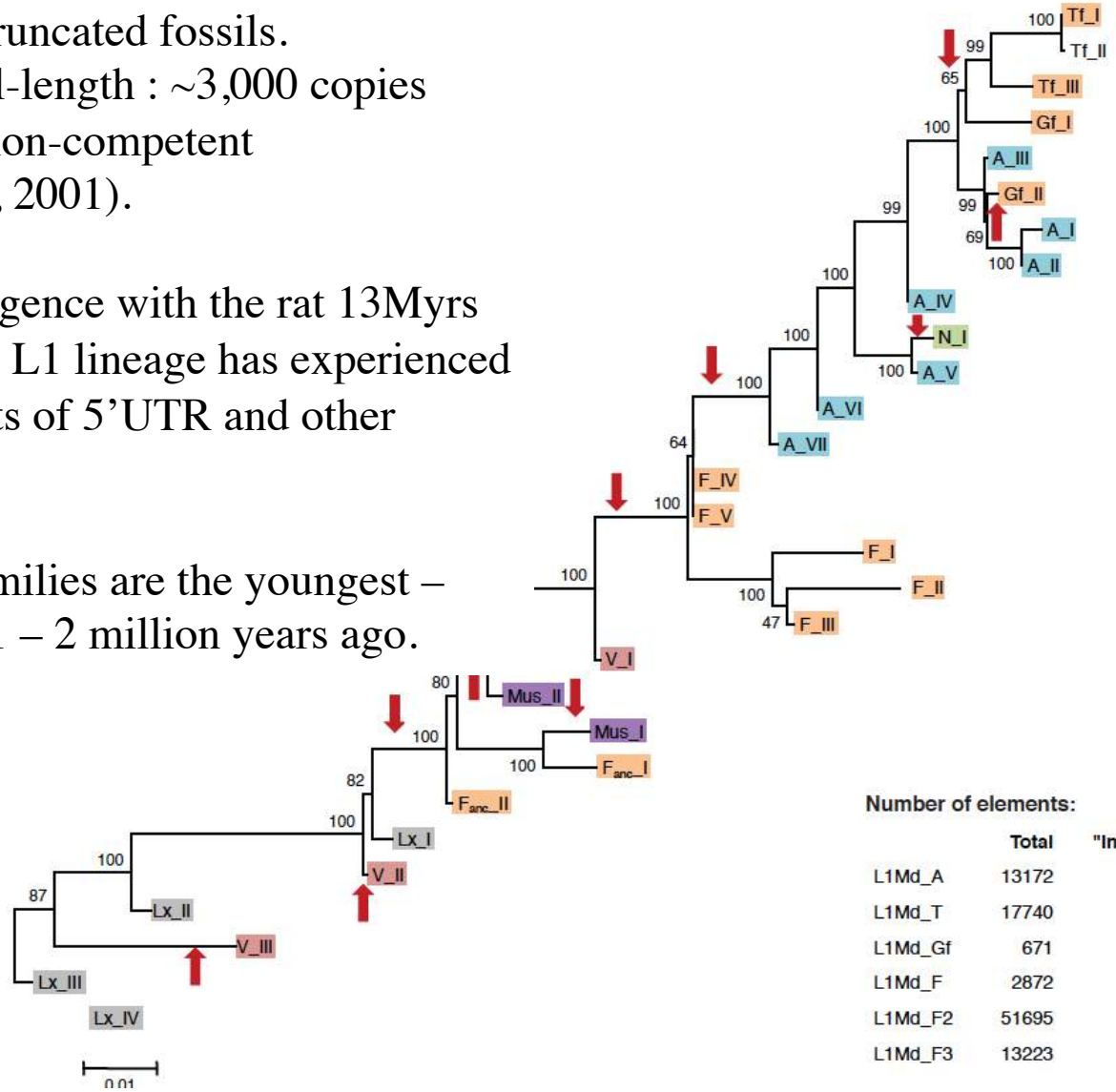
Marius Walter/Deborah Bourc'his

Evolution of Mouse LINE 1 Elements

Most L1s are truncated fossils.
20,000 L1s full-length : ~3,000 copies retrotransposition-competent (Goodier et al., 2001).

Since the divergence with the rat 13Myrs ago, the mouse L1 lineage has experienced 11 replacements of 5'UTR and other changes.

Most active families are the youngest – emerging just 1 – 2 million years ago.



Number of elements:

	Total	"Intact"
L1Md_A	13172	4424
L1Md_T	17740	6506
L1Md_Gf	671	344
L1Md_F	2872	705
L1Md_F2	51695	7316
L1Md_F3	13223	1458

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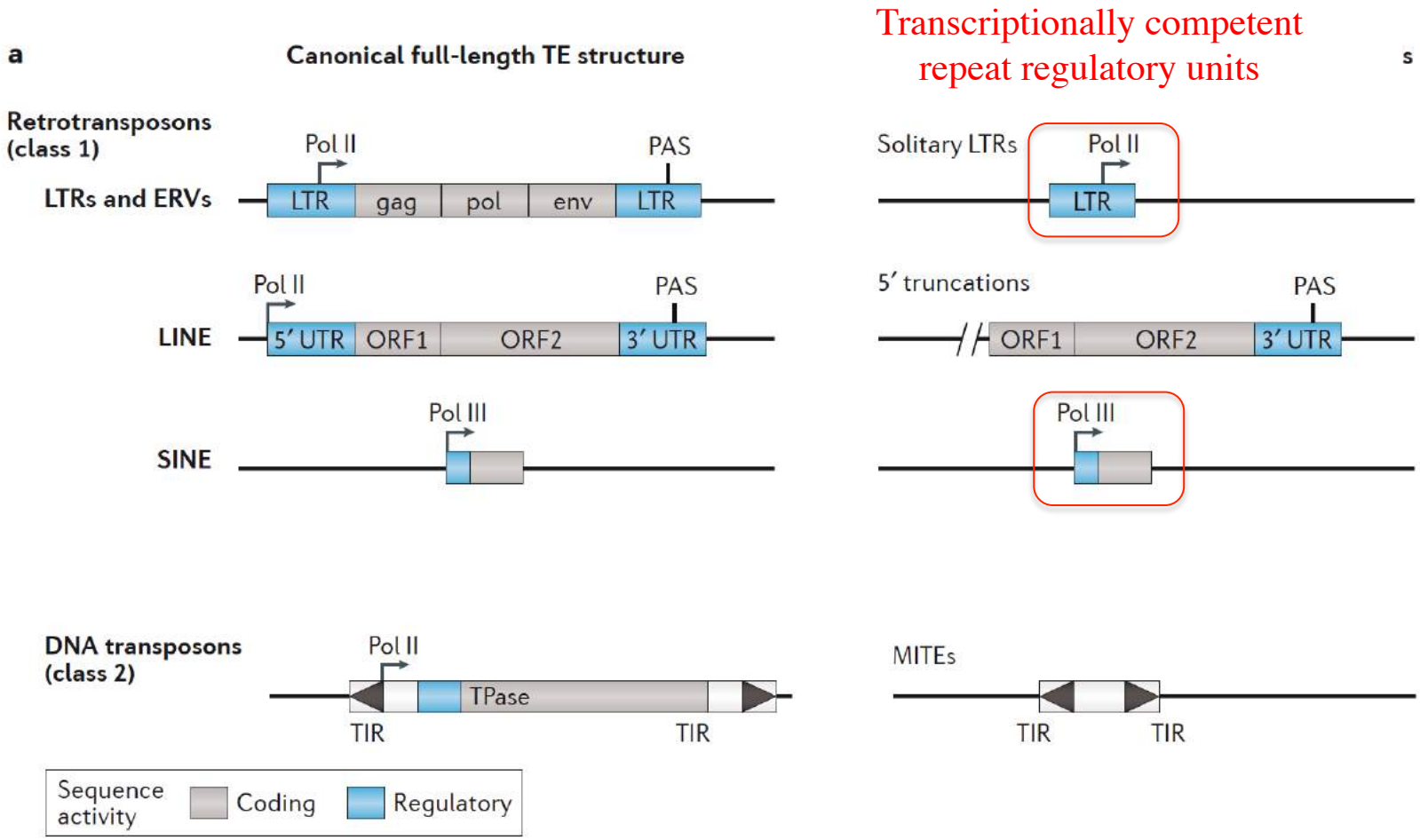
Transposable Elements



Most Transposons and Retrotransposons are truncated or mutated => cannot be mobilised, but are still *transcriptionally* competent…

Controlling TEs: an Evolutionary Arms Race

- TEs have been exploited through evolution to create new functions but their **unrestrained spread** would **rapidly lead to lethality**.
- Yet they persist in all eukaryotic genomes… How?

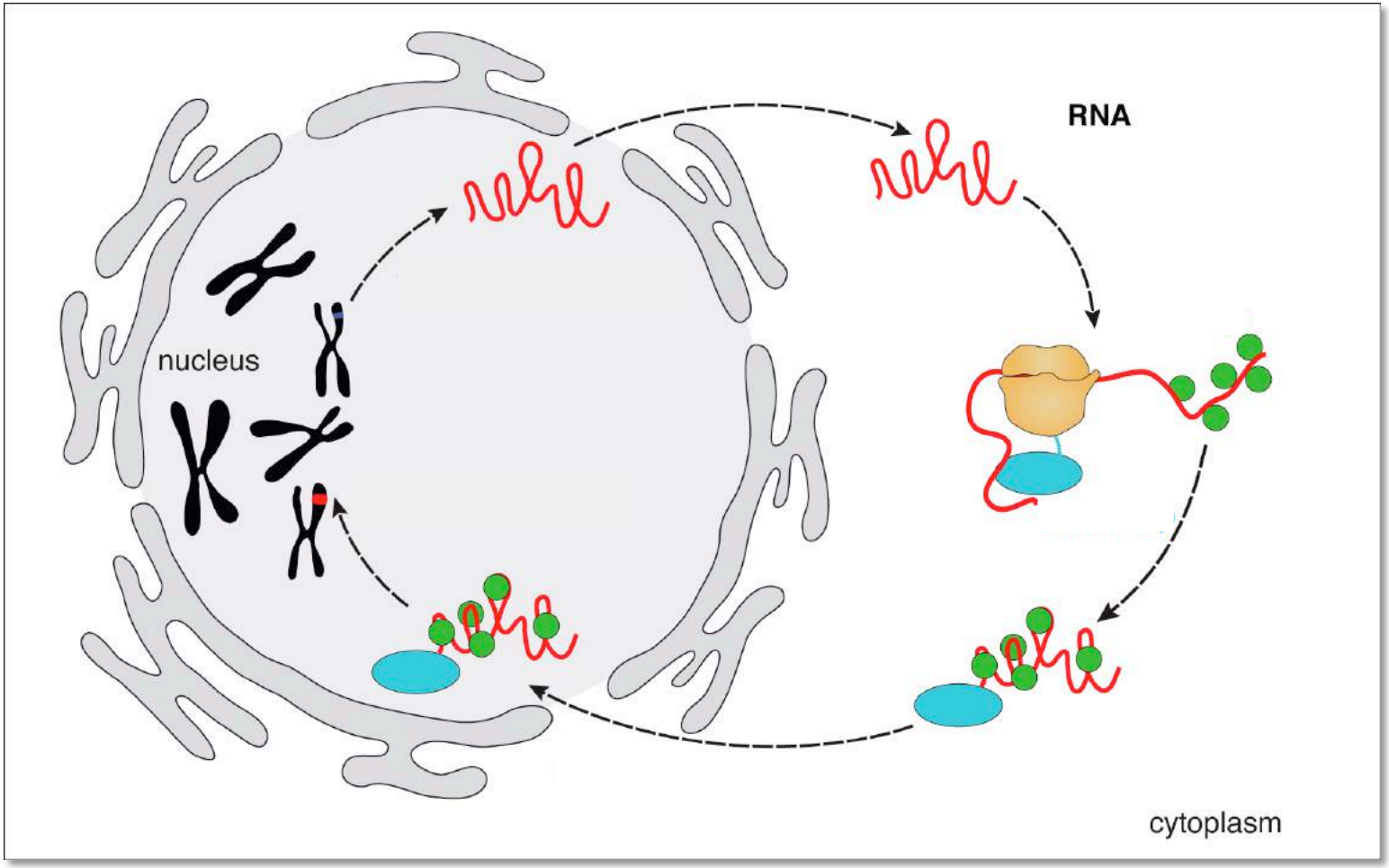
- Depending on where a new TE copy inserts into the genome, this can
  - **disrupt normal gene function** and lethality or disease => no propagation…
  - **have an advantageous effect** (rarely): new gene function, regulation etc
  - **have no effect**, simply adding to the overall size of the genome
  - **have no immediate effect** – because it becomes silenced (**cryptic**)
- To avoid the potentially deleterious effects of active TEs, the genome has evolved '**defense**' mechanisms to suppress their activity: ***post-transcriptional*, *transcriptional* and *epigenetic***

Controlling TEs: an Evolutionary Arms Race

- Over evolutionary time, eukaryotic genomes undergo repeated episodes of TE invasion, or reactivation (eg genomic "shocks" triggering re-expression of existing TEs).
- This drives the evolution of new repressive mechanisms, and so on….

"An evolutionary arms race has shaped the genomes of primates, including humans". (Image David Greenberg, UCSC). http://news.ucsc.edu/2014/09/jumping-genes.html

Retrotransposon (LINE1) life cycle



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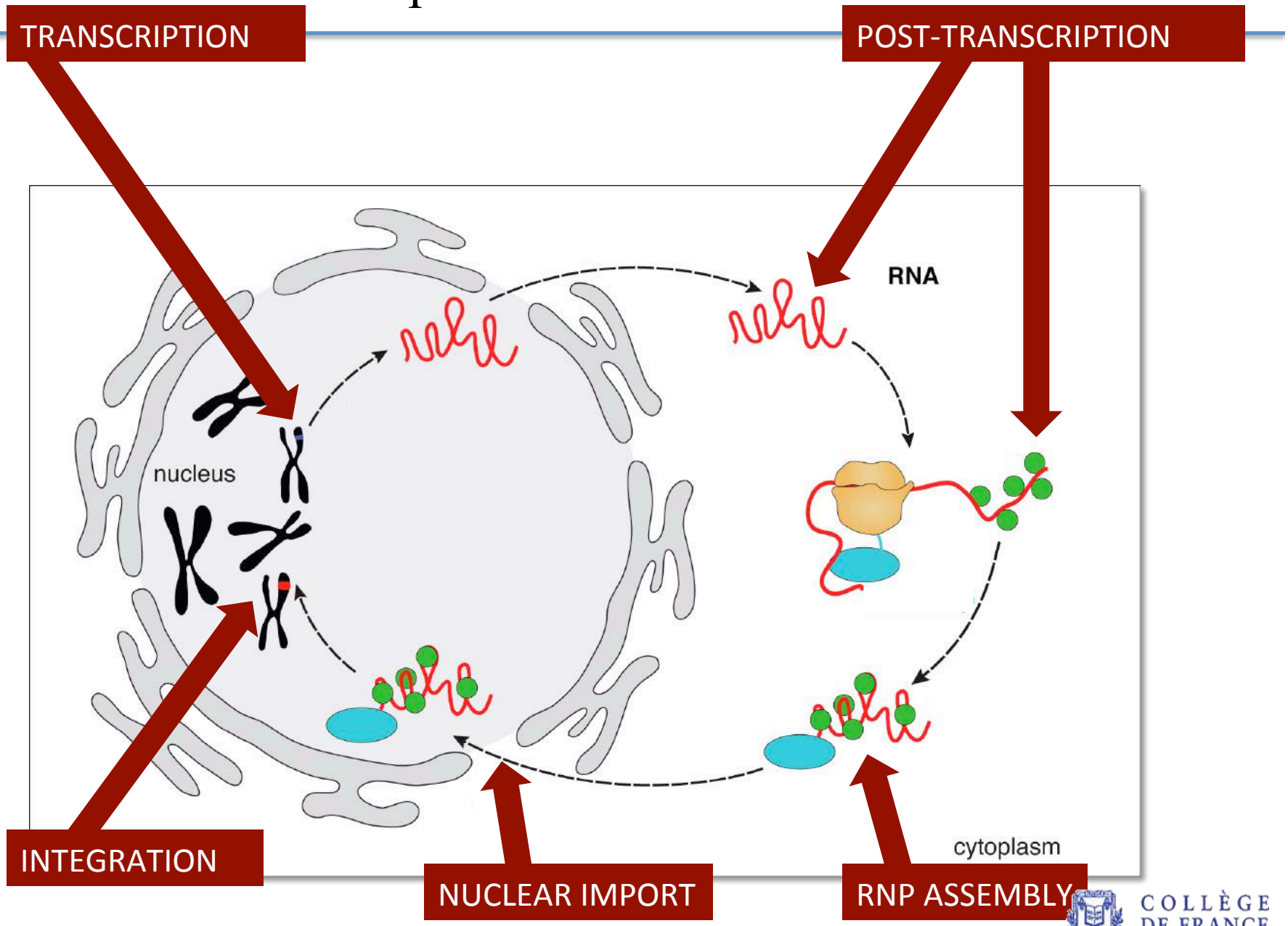
Multiple Levels of Control?



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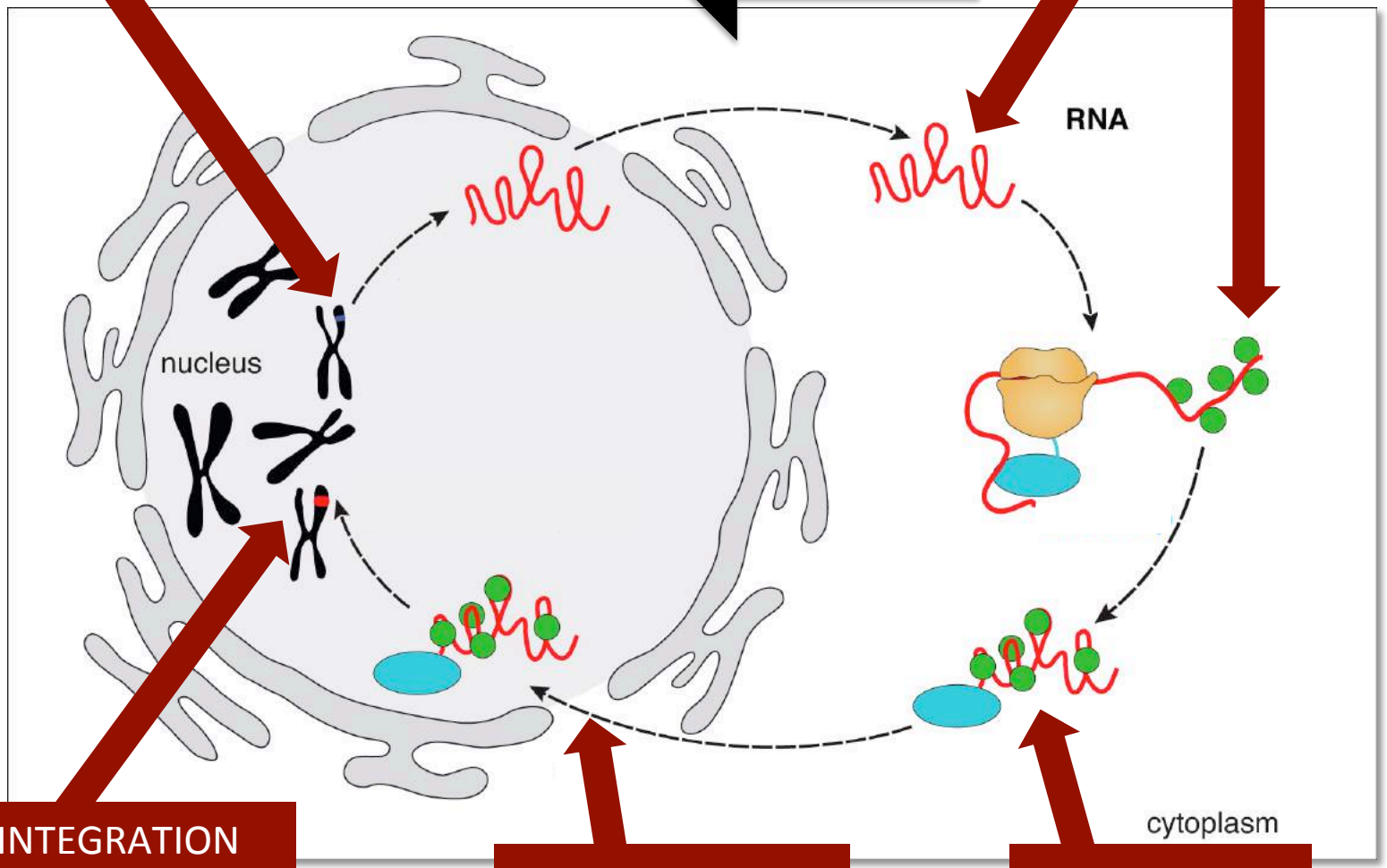
Multiple Levels of Control?



TRANSCRIPTION

DNA methylation: DNMT1, DNMT3C, DNMT3L

H3K9 methylation: SetB1, KAP1

POST-TRANSCRIPTION

RNA editing: APOBECs

RNA interference: PIWI/piRNA

INTEGRATION

Repair: ERCCX

NUCLEAR IMPORT

RNP ASSEMBLY

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Transcriptional and Epigenetic Control of TEs

- How is a TE recognized and targeted for silencing?
  RNA and DNA based mechanisms
- How is this silencing maintained?
  Epigenetic mechanisms
- When and how is it reversed?
  In the germ line, during development, in somatic cells

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

Craig Mello and **Andy Fire**
Nobel Prize, 2006
"for their discovery of RNA interference - gene silencing by double-stranded RNA"

Targeting of TEs for repression

Both RNA and DNA based mechanisms of TE recognition exist:

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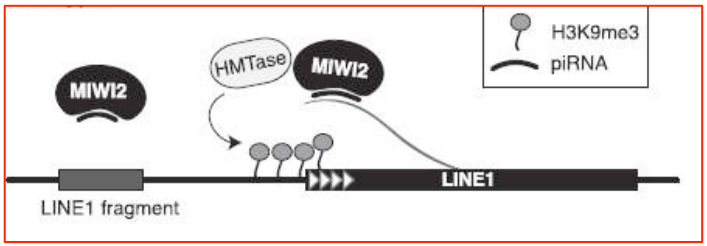
  - target TE mRNA for degradation and translational inhibition
  - target TE chromatin for heritable **epigenetic modification**



- RNA interference is the most evolutionary conserved strategy to combat TEs
- Key RNAi factors seem to be derived from last common eukaryotic ancestor
- RNA-Induced Silencing Complexes (RISC) promote mRNA decay or transcriptional silencing and epigenetic modifications (RITS complex)
- Variations of RNAi strategies for TE defense exist in all organisms (not yeast)
- Common feature: Target specificity through complementarity with the guiding sncRNA

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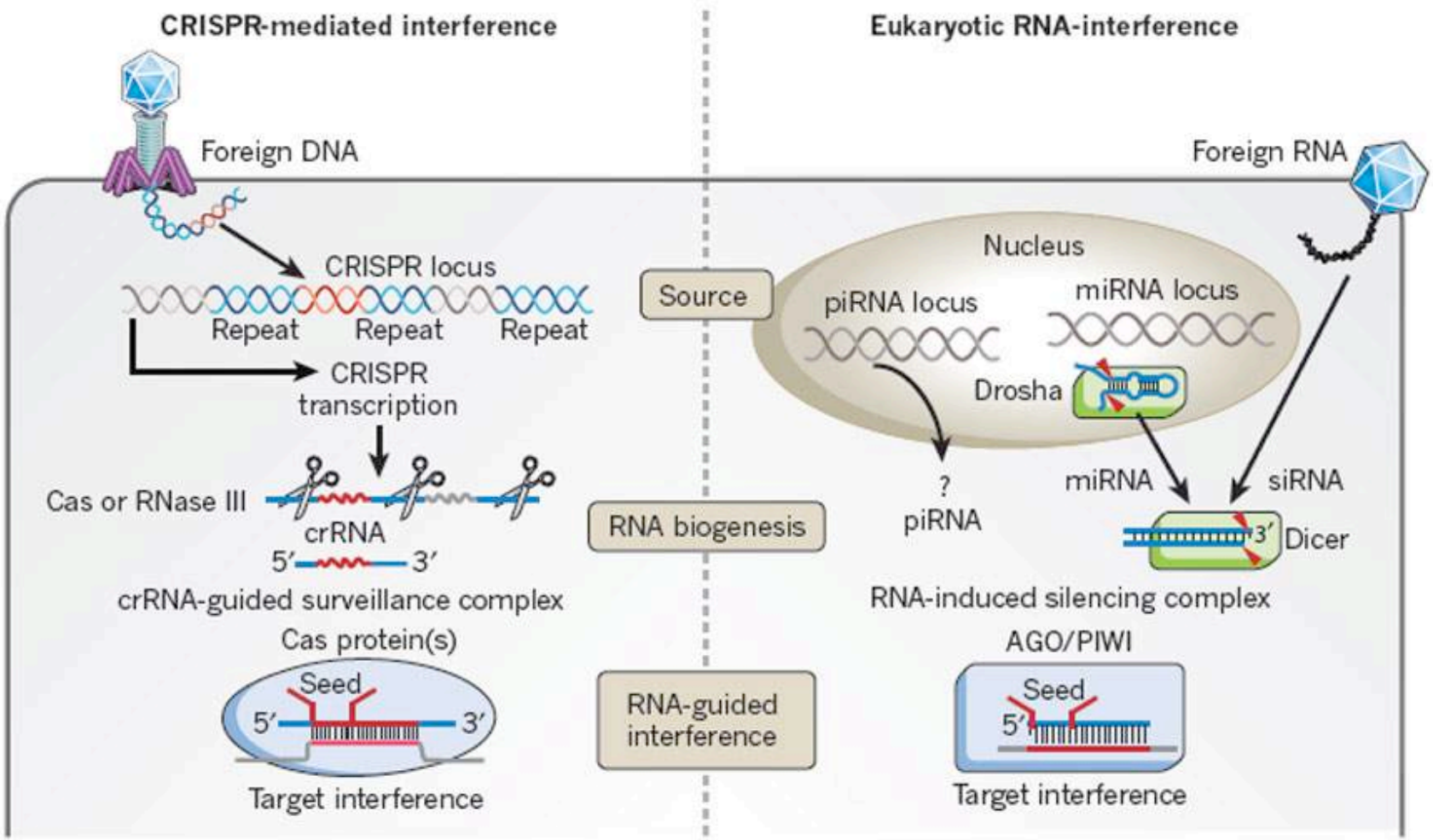
RNA-guided interference strategy: eukaryotic RNAi (RNA cleavage) and bacterial CRISPR-mediated interference (DNA cleavage)



Wiedenheft et al, NRG 2012

Targeting of TEs for repression

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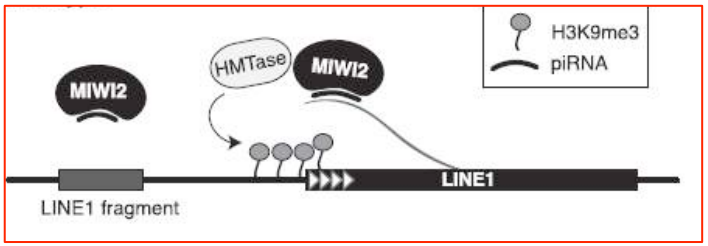

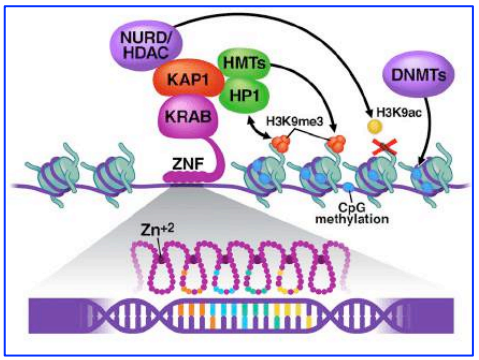

- DNA sequences of TEs can be recognized by repressor proteins (zinc finger proteins) that bind specifically and can recruit **heterochromatin-inducing** factors

(More next week – COURS III)

- Different eukaryotes exploit different types and combinations of controls – and these can also vary depending on cell type, or developmental stage.

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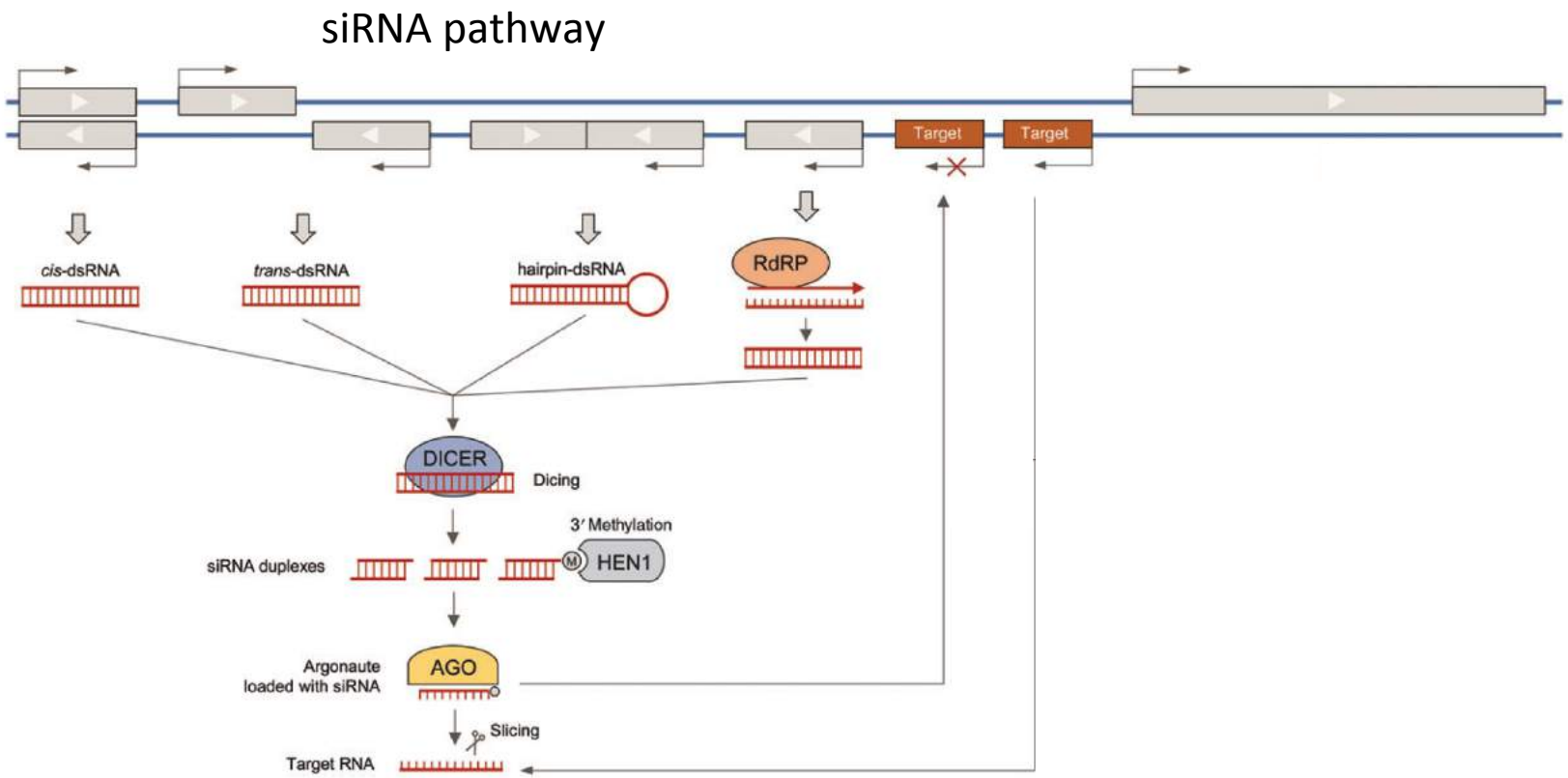
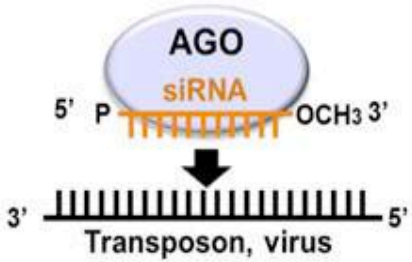
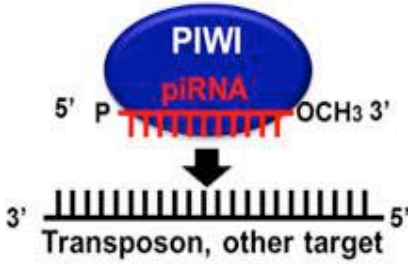
RNAi Pathways: RNA-induced silencing



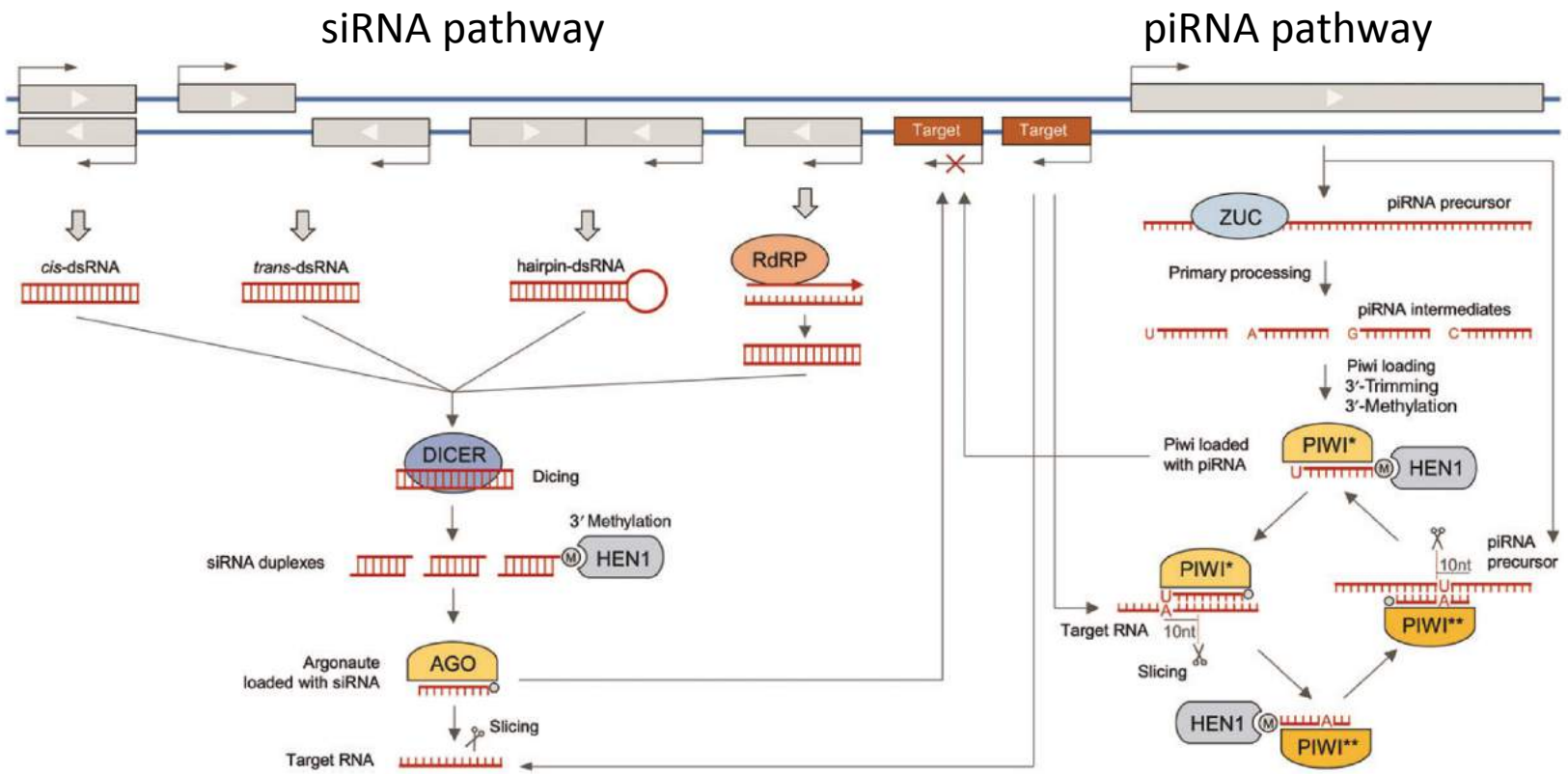
Gebert and Rosenkranz (2015) WIREs RNA
"RNA-based regulation of transposon expression"

RNAi Pathways: RNA-induced silencing

	AGO		PIWI
Expression	**All tissues**		**Germline and cancer**
Homologs of human	AGO1, AGO2, AGO3, AGO4		HIWI, HILI, PIWIL3, HIWI2
Mouse	AGO1, AGO2, AGO3, AGO4		MIWI, MILI, MIWI2
Drosophila	AGO1, AGO2		PIWI, AUB, AGO3
Bound small RNA	miRNA	siRNA	piRNA
Length (nt)	20–23	20–23	25–31
Precursor	Hairpin-structured RNA	dsRNA	ssRNA
Biogenesis	Drosha, Dicer	Dicer	Dicer-independent
Function	 Regulation of mRNA stability and translation	 Regulation of transposon, protection from viral infection	 Regulation of transposon, unknown function

- With their internally repetitive and rearranging structures, the expression of TEs commonly makes antisense RNAs, hairpin RNA, duplex RNA...
- Such double stranded RNA will be recognised & cleaved by Dicer generating small interfering siRNAs
- Small RNAs are loaded onto Argonaute proteins which will then find and cleave the target RNAs (PTGS), but can also enter the nucleus where they can cooperate with chromatin factors to induce transcriptional silencing (TGS)

RNAi Pathways: RNA-induced silencing

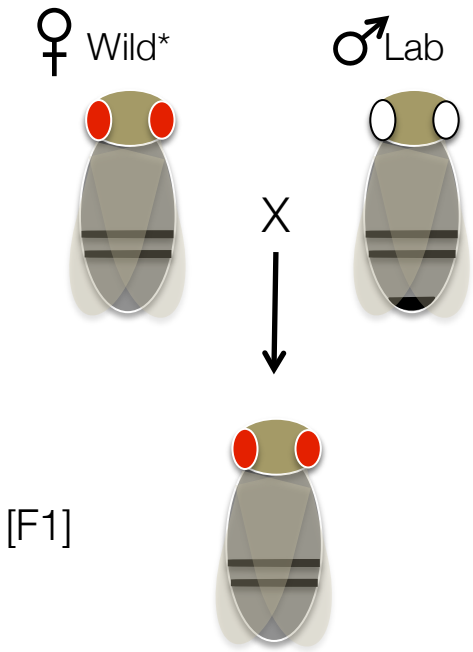


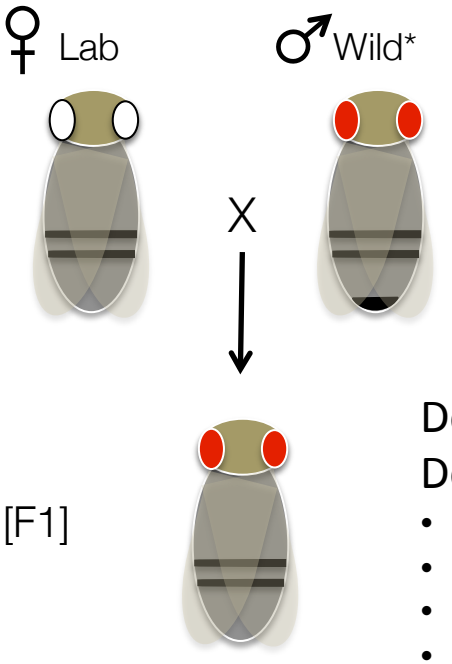
TEs that produce double stranded RNA (in cis or trans) will be detected by Dicer and cleaved to generate small interfering siRNAs

TE clusters generate single stranded RNA that is processed into small RNAs (piRNAs) and amplified, in order to establish TE repression in the germ line in animals, when epigenetic silencing is temporarily released

Drosophila Hybrid Dysgenesis

P-element induced hybrid dysgenesis affects germline function of F1 hybrid progeny in a non-reciprocal fashion





Defective Germline Development:

- Mutation Induction
- Chromosomal rearrangements
- DNA damage
- Loss of germ cells

Fertile

Sterile

Courtesy of Felipe Texeira

Schaefer et al, 1979 – Genetics

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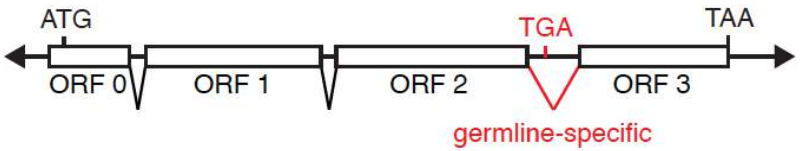
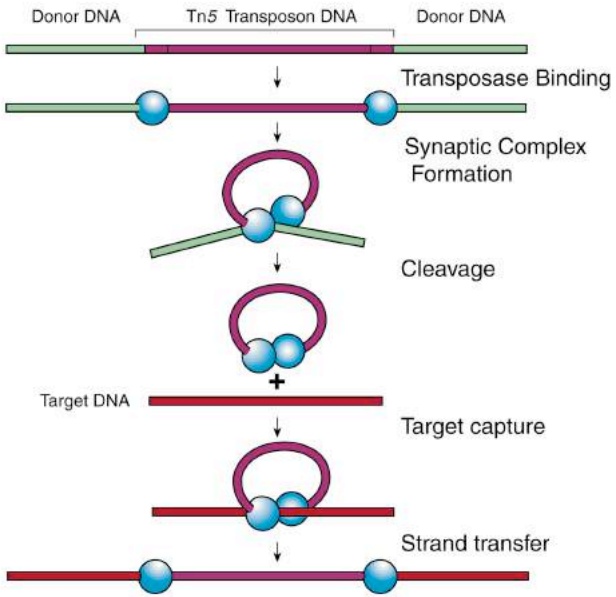
Hybrid dysgenesis is caused by P-element DNA transposon

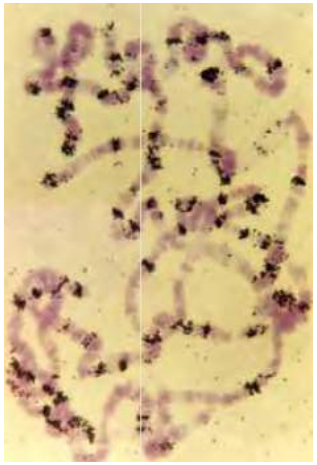
P-element





Adapted from Davies et al, 2000 – Science

P-strain
(after 1970s)



from S. Ronsseray

Wild*
(after 1970s)

Many P-elements

Lab
(before 1950s)

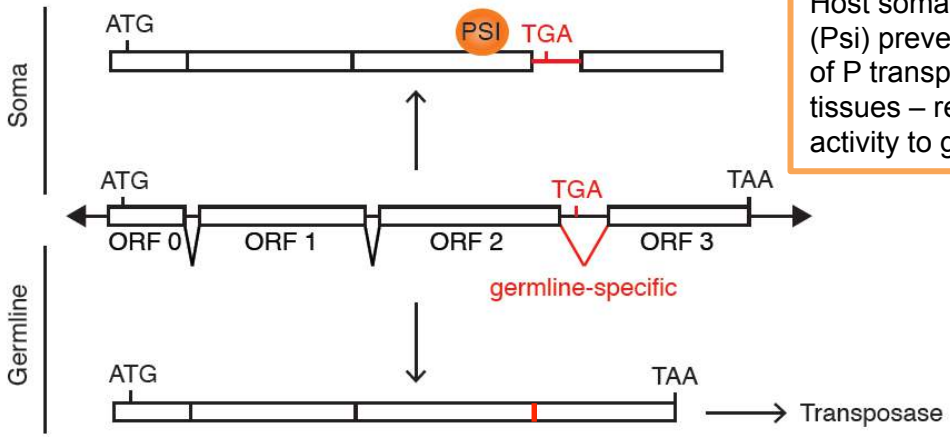
No P-elements

Wild*
Harwich strain

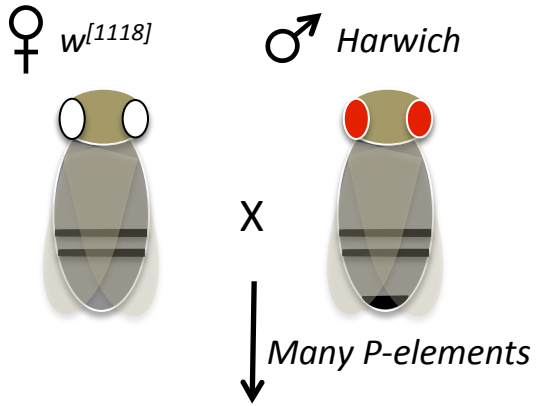
Felipe Texeira

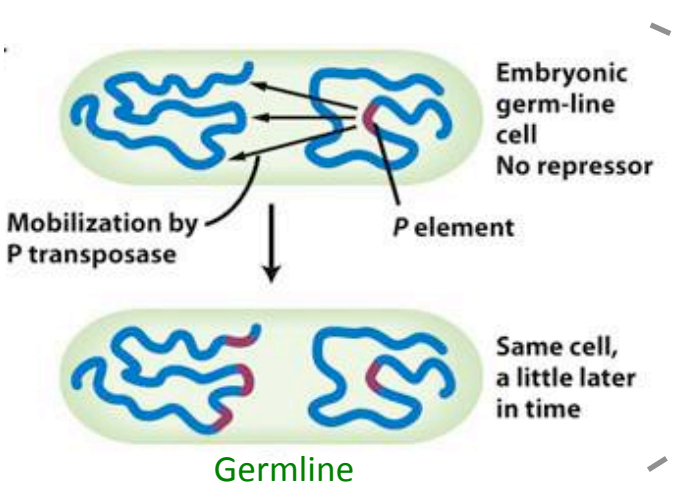
Rapid spread of P elements in all populations of D. melanogaster worldwide, in less than 50 years (Anxolabehere et al., 1988).

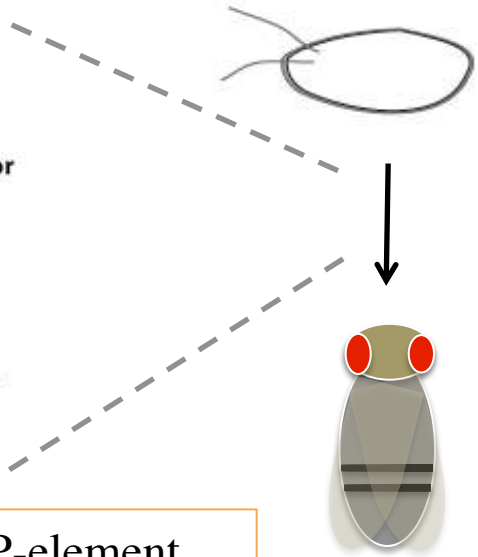
Germline defects are probably caused by genomic instability



Host somatic splicing factor (Psi) prevents the production of P transposase in somatic tissues – restricts P-element activity to germline.







Felipe Texeira

What is the nature of the "repressor" of P-element mobilisation, that is transmitted by females only?

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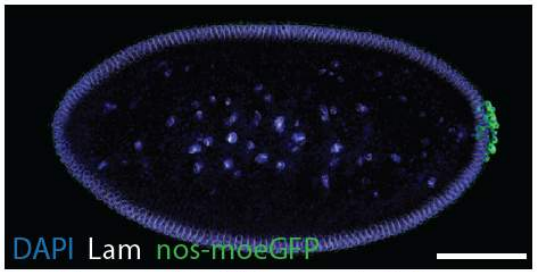
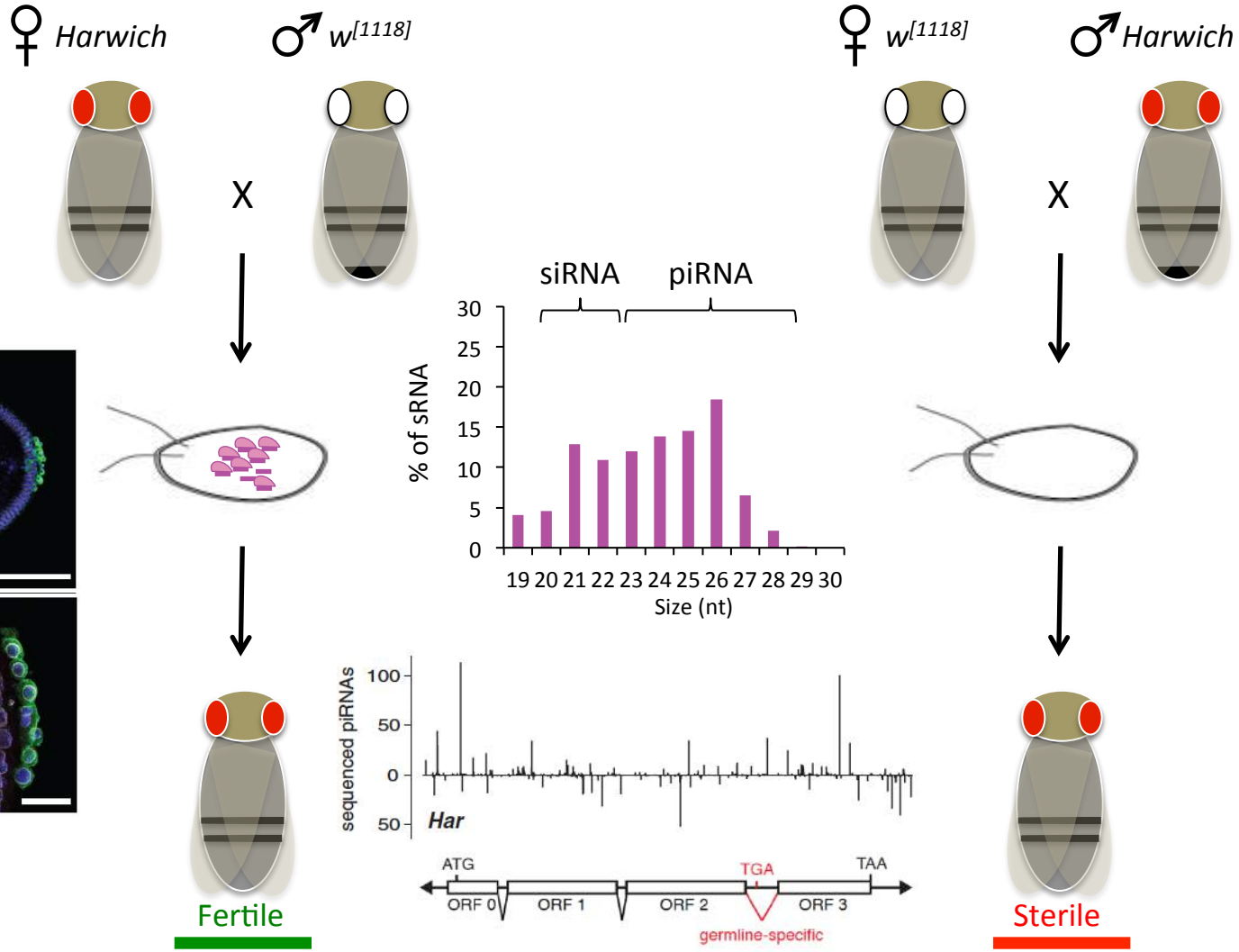
Epigenetically inherited small RNAs are associated with F1 germline protection



Felipe Texeira



E. Heard, February 13th, 2017

sRNA data from Brennecke, Malone et al, 2008 – Science

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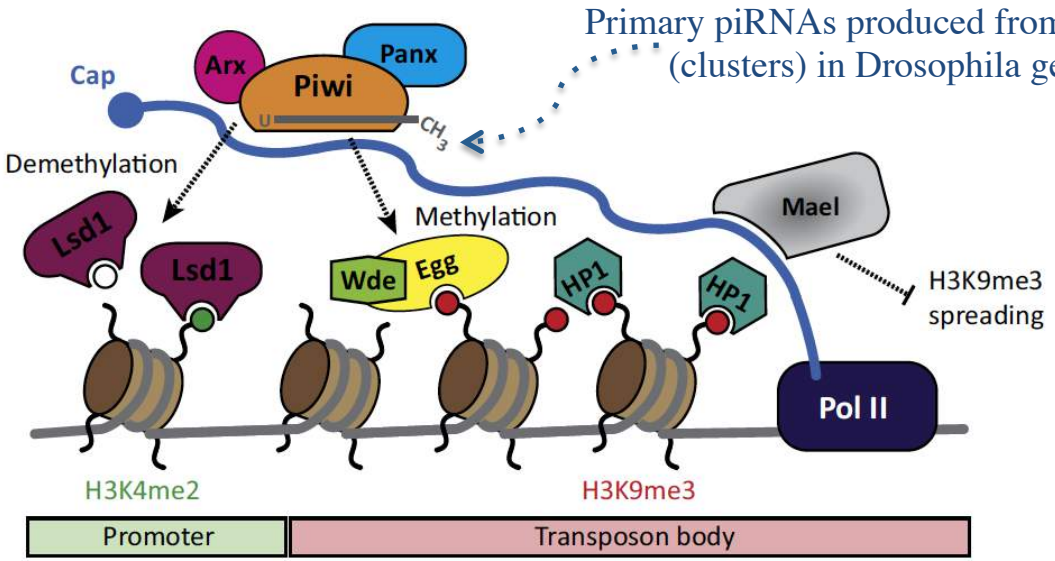
PIWI-interacting RNA (piRNA)-Mediated Transcriptional Silencing

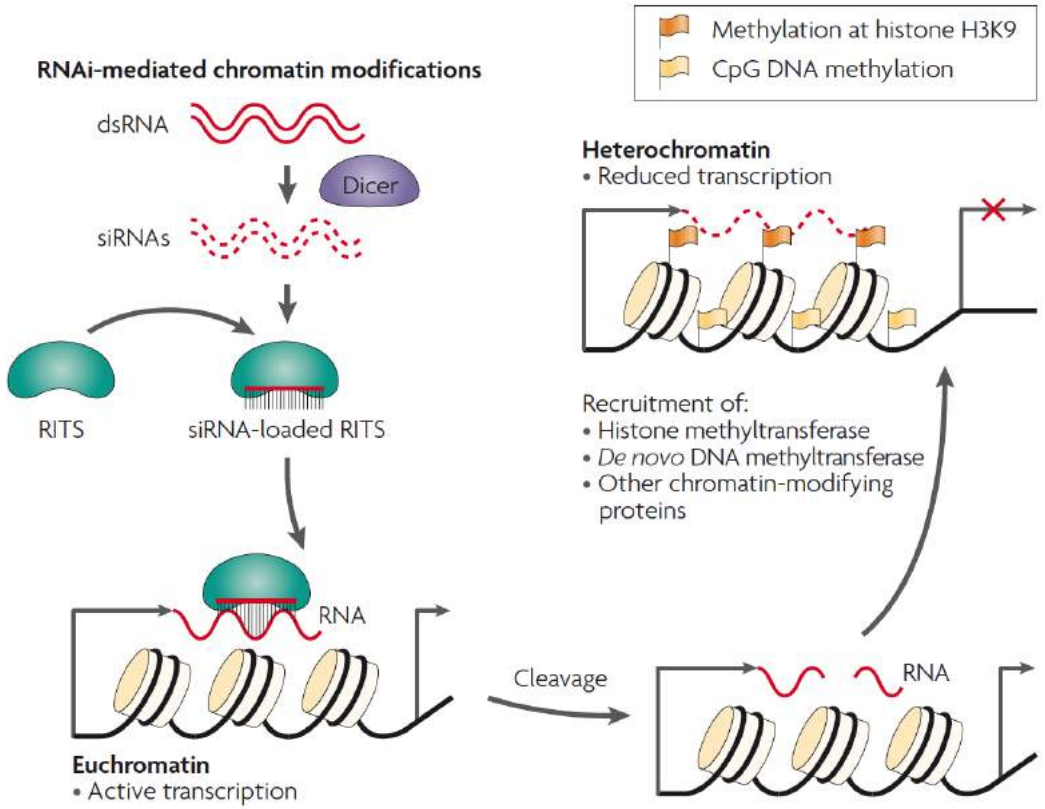
Drosophila ovary:



From Czech and Hannon, TIBs, 2016

1. piRNA-Piwi/Asterix (Arx) complexes scan for, and detect, nascent transposon transcription.
2. Upon target engagement, Piwi recruits the Panoramix (Panx) complex (Piwi, Arx, and Panx) & induces co-transcriptional repression by recruiting general silencing machinery factors.
3. Lysine-specific demethylase 1 (Lsd1) removes active histone marks (H3K4me2) from transposon promoter regions, enabling efficient TE suppression at *transcriptional* level.
4. => Transposon bodies receive repressive histone 3 lysine 9 trimethylation (H3K9me3) marks via HMTase Eggless (Egg) + cofactor Windei (Wde).
5. Subsequent recruitment of HP1 to H3K9me3 leads to heterochromatin formation.
6. Maelstrom (Mael), a putative single-stranded RNA-binding protein, is required for transcriptional silencing and blocks H3K9me3 spread.

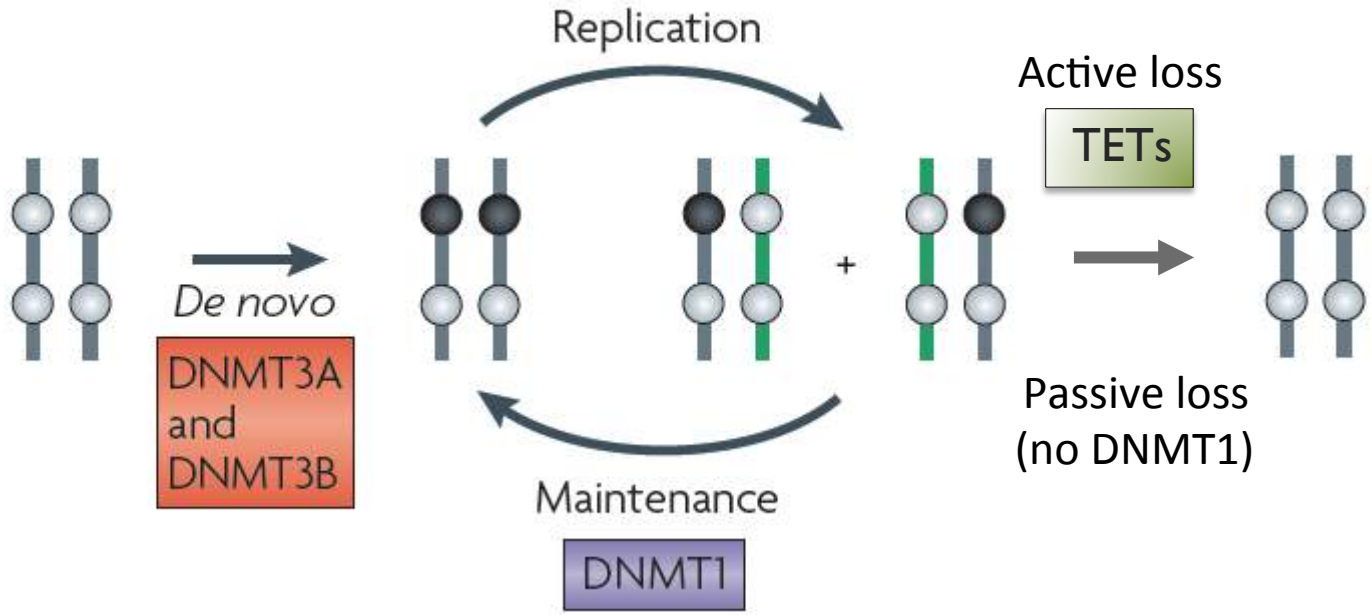
RNAi mediated Epigenetic Silencing of TEs



Different chromatin factors ensure heritable transposon silencing:
H3K9 methylation, DNA methylation...

Slotkin and Martienssen, NRG 2007

DNA Methyltransferases: Orchestrators of DNA Methylation



Jones P.A. *et. al.* 2009. *Nat Rev Genet.*

- **DNMT1 preferentially methylates hemimethylated DNA => maintenance MTAse**
- **DNMT3A/3B show equal preference for hemimethylated and unmethylated DNA**
- **DNMT3L stimulates DNMT3A/3B activity**
- **DNMT1 is present in all cells – DNMT3A/B are more context specific**

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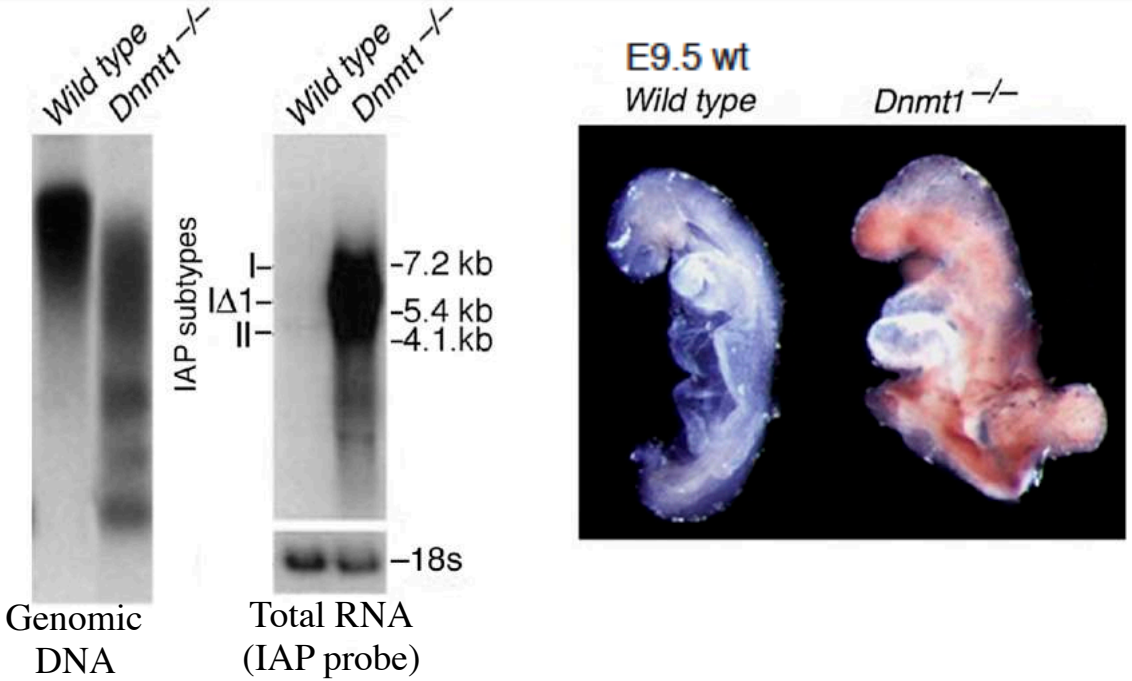
DNA Methylation is essential for TE silencing in Mammals



- Mutants in the maintenance DNA Mtase, Dnmt1show early lethality (around E9.5) and result in aberrant activation of the most aggressive TEs (IAPs) (Walsh et al, 1998)

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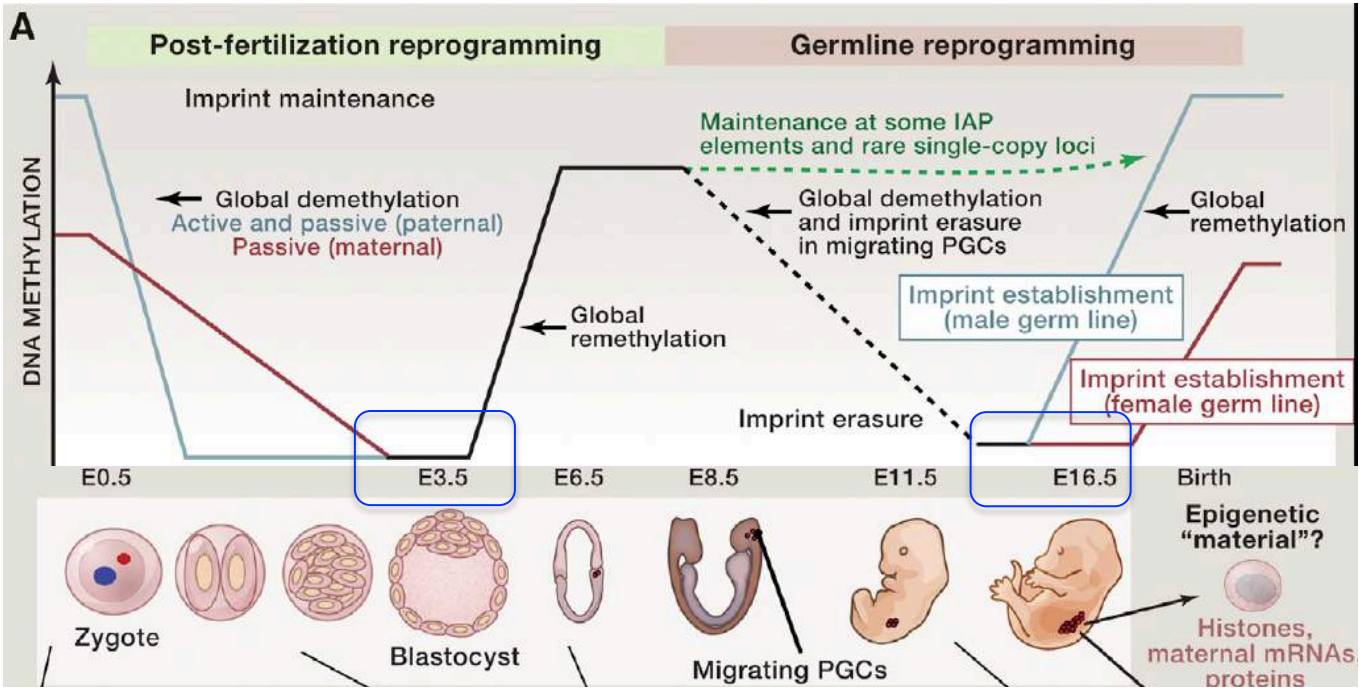
Epigenomes are globally reprogrammed in the mammalian germ line and during early development



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 a few single copy loci and young TEs)

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

CONTROLLING TEs : (I) Germ line (II) Soma

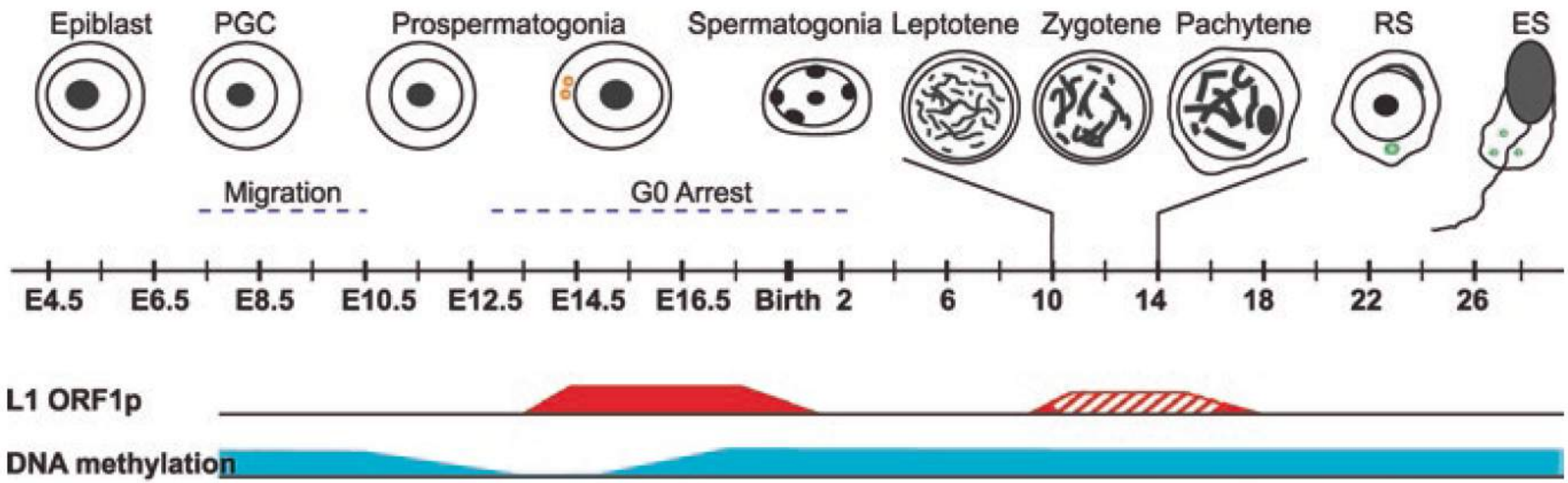
Critical times for “host” protection from TE activity: in germ line and early embryogenesis before germ line is laid down

TEs that are vertically transmitted must be active in germ line – and host defense systems must be applied then

Different organisms use different strategies – the male and female germ lines can even employ different strategies

Somatic protection is also required to protect the organism at least long enough for it to reproduce…

The piRNA pathway, DNA methylation and Histone modifications ensure transposon silencing in the mouse germ line



S.J. Newkirk and W. An, 2017

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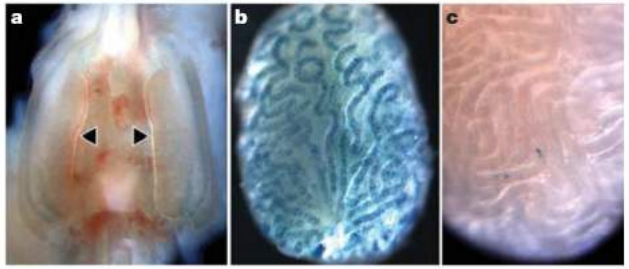
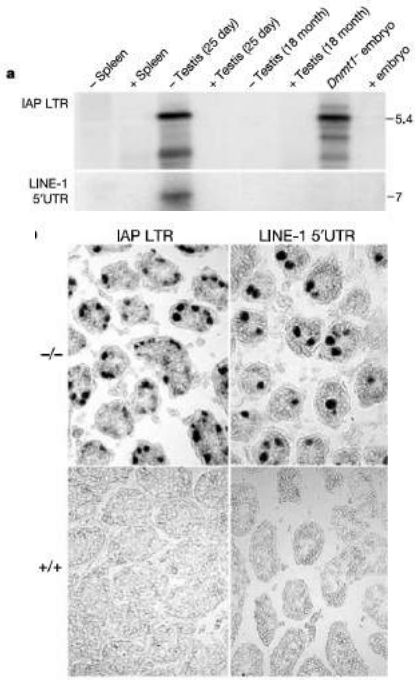
DNA Methylation is essential for TE silencing in the male germ line of mice

Meiotic catastrophe and retrotransposon reactivation in male germ cells lacking Dnmt3L

Déborah Bourc'his & Timothy H. Bestor

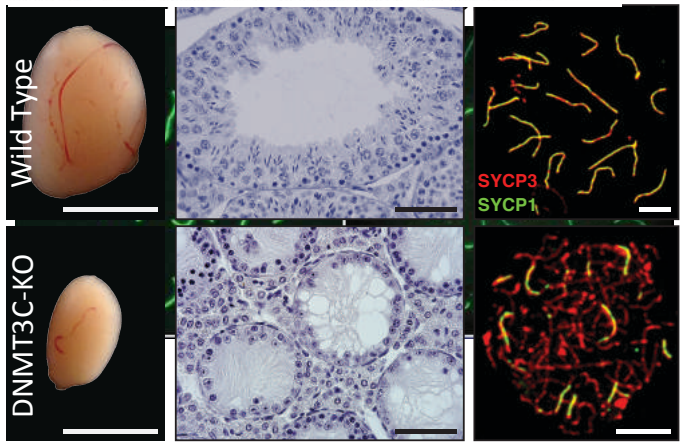


Bourc'his and Bestor, Nature 2008



The DNA methyltransferase DNMT3C protects male germ cells from transposon activity

Joan Barau,[1] Aurélie Teissandier,[1,2] Natasha Zamudio,[1*] Stéphanie Roy,[3] Valérie Nalesso,[4,5,6] Yann Hérault,[4,5,6] Florian Guillou,[3] Déborah Bourc'his[1†]



Barau et al, Science 2017

- Mutants in Dnmt3L (cofactor of *de novo* MTases Dnmt3A/3B) show arrest in spermatogenesis due to aberrant activation of repeat elements (Bourc'his and Bestor, 2008)
- Recently a novel, male germ line-specific *de novo* DNMT, Dnmt3C was discovered in the mouse. Its knock out gave a similar arrest during spermatogenesis (Barau et al, 2017). DNMT3C is a duplication of DNMT3B and it targets evolutionarily young transposons.

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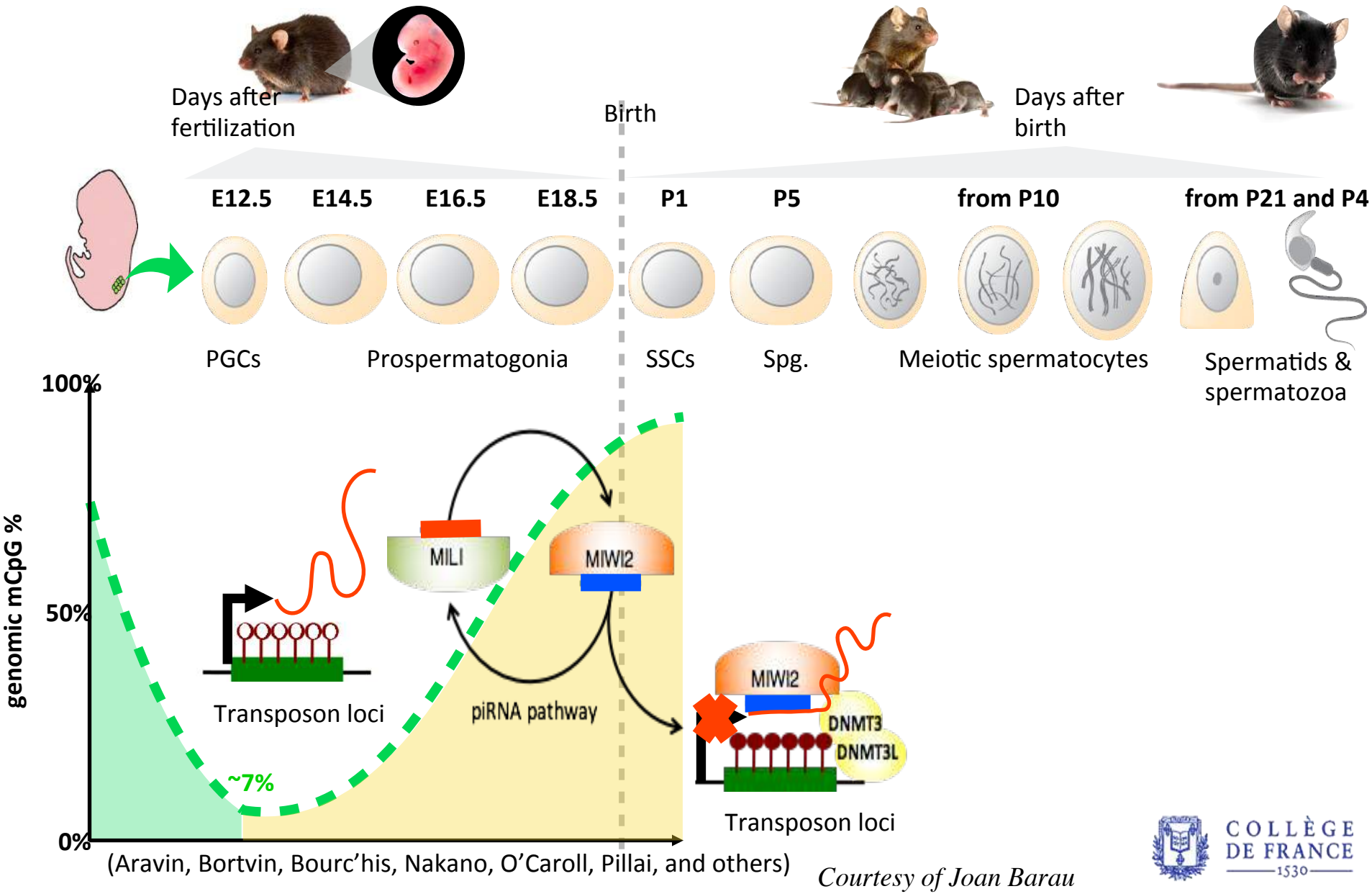
The piRNA pathway and DNA methylation collaborate to ensure transposon silencing in the mouse germ line



(Aravin, Bortvin, Bourc'his, Nakano, O'Caroll, Pillai, and others)

Courtesy of Joan Barau

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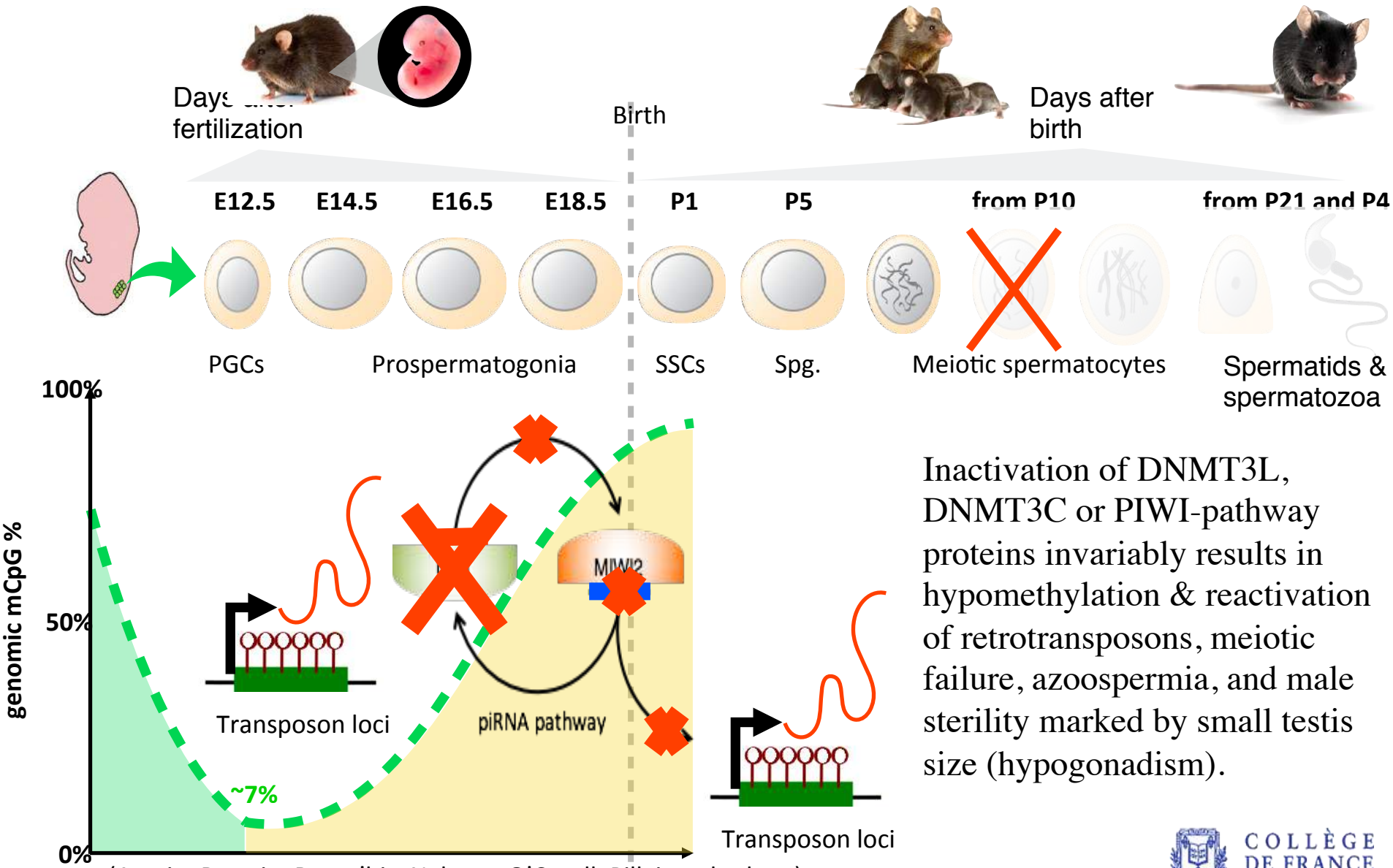
The piRNA pathway and DNA methylation collaborate to ensure transposon silencing in the mouse germ line



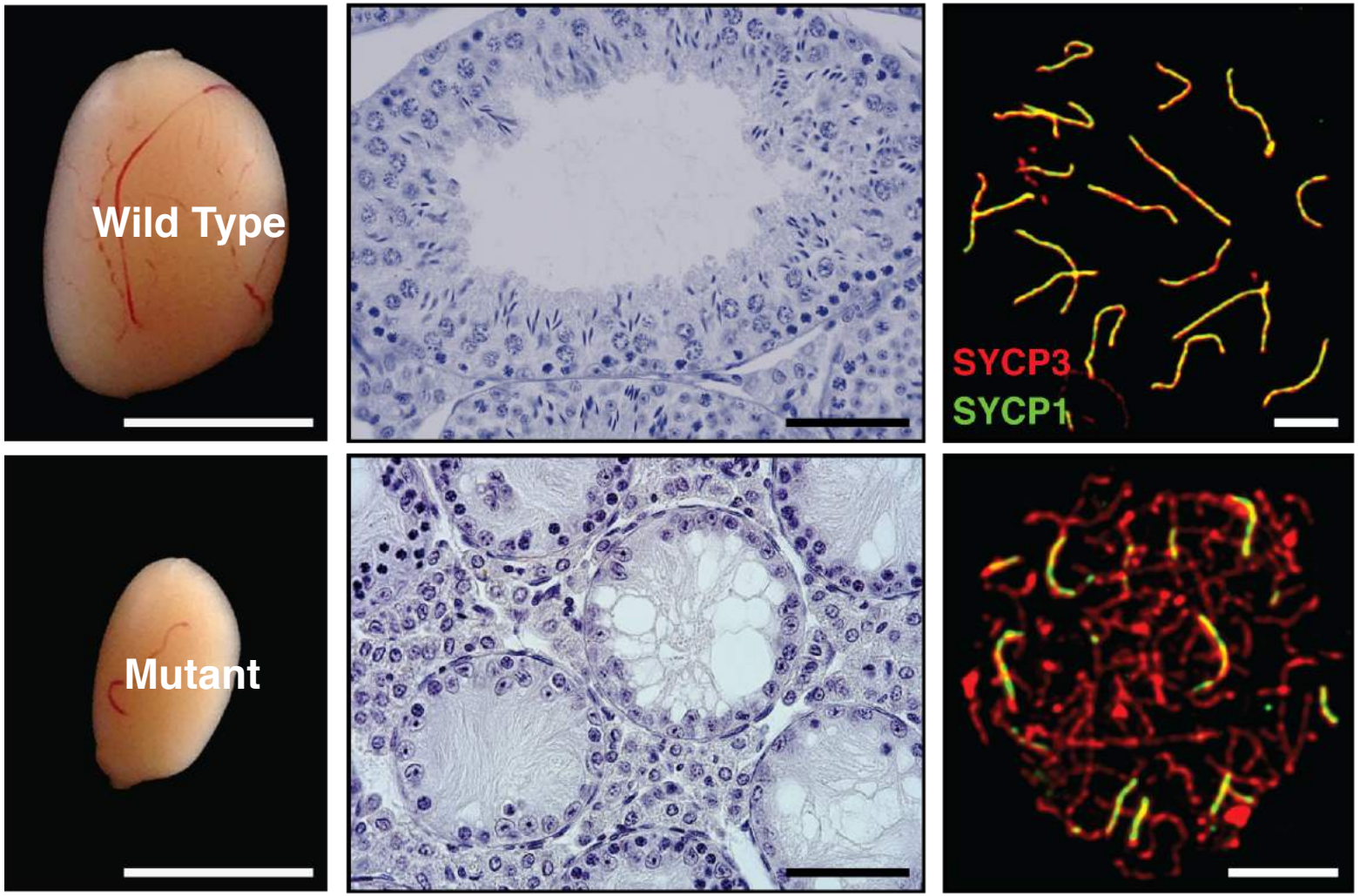
(Aravin, Bortvin, Bourc'his, Nakano, O'Caroll, Pillai, and others)

Inactivation of DNMT3L, DNMT3C or PIWI-pathway proteins invariably results in hypomethylation & reactivation of retrotransposons, meiotic failure, azoospermia, and male sterility marked by small testis size (hypogonadism).

Courtesy of Joan Barau

COLLÈGE DE FRANCE 1530

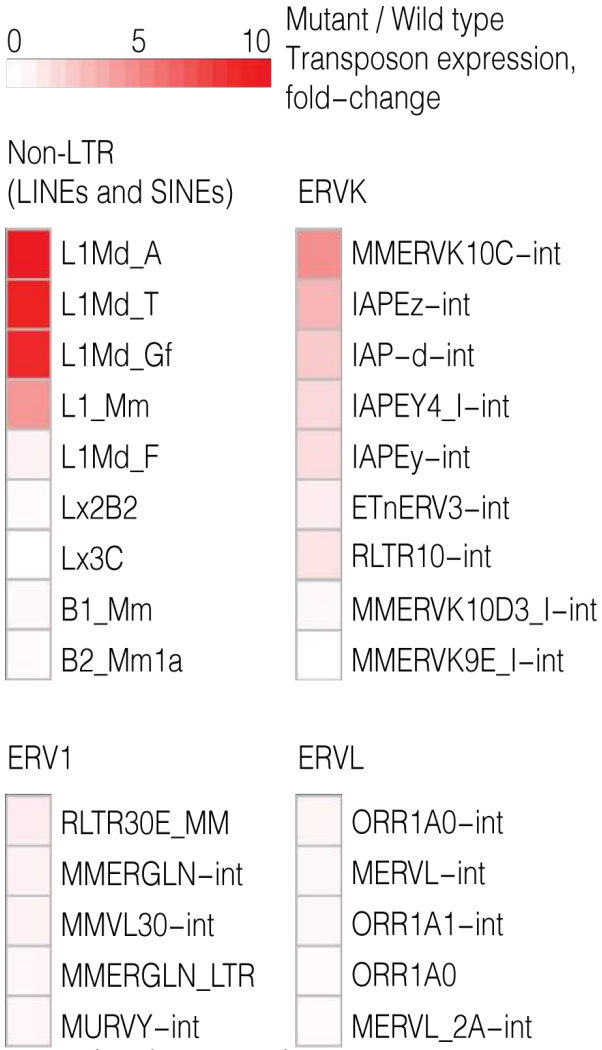
Dnmt3L, Dnmt3C and Piwi mutants all give similar developmental arrest of spermatogenesis and infertility

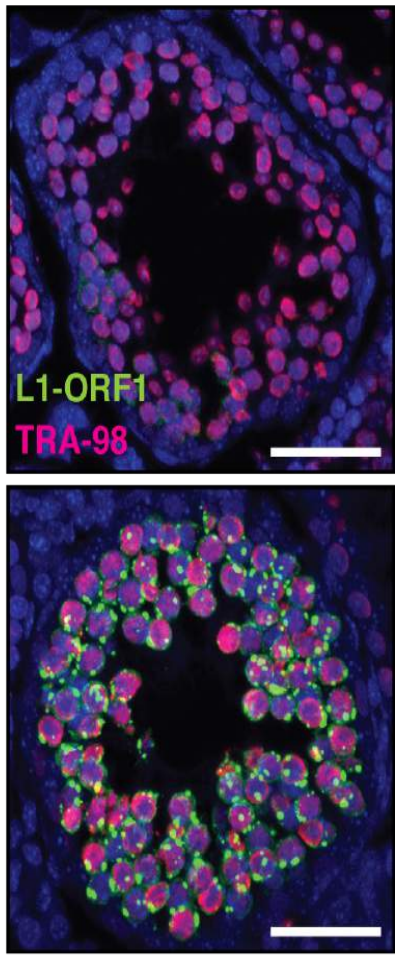


Courtesy of Joan Barau/Deborah Bourc'his

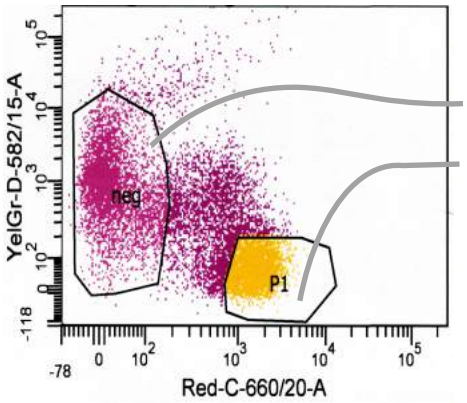
Dnmt3C and Dnmt3L KO mice fail to repress transposons in the male germ line

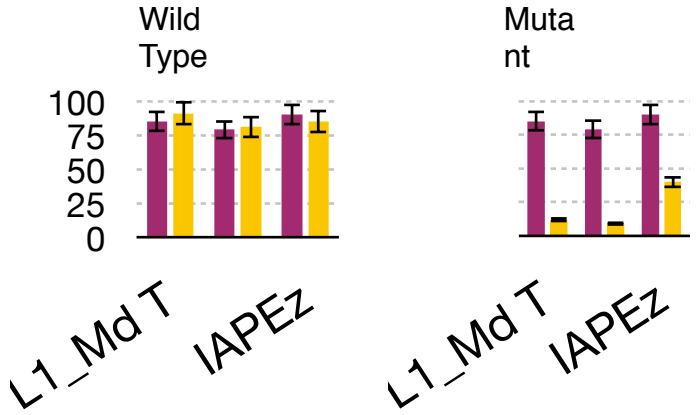
Transposon reactivation





Transposon hypomethylation



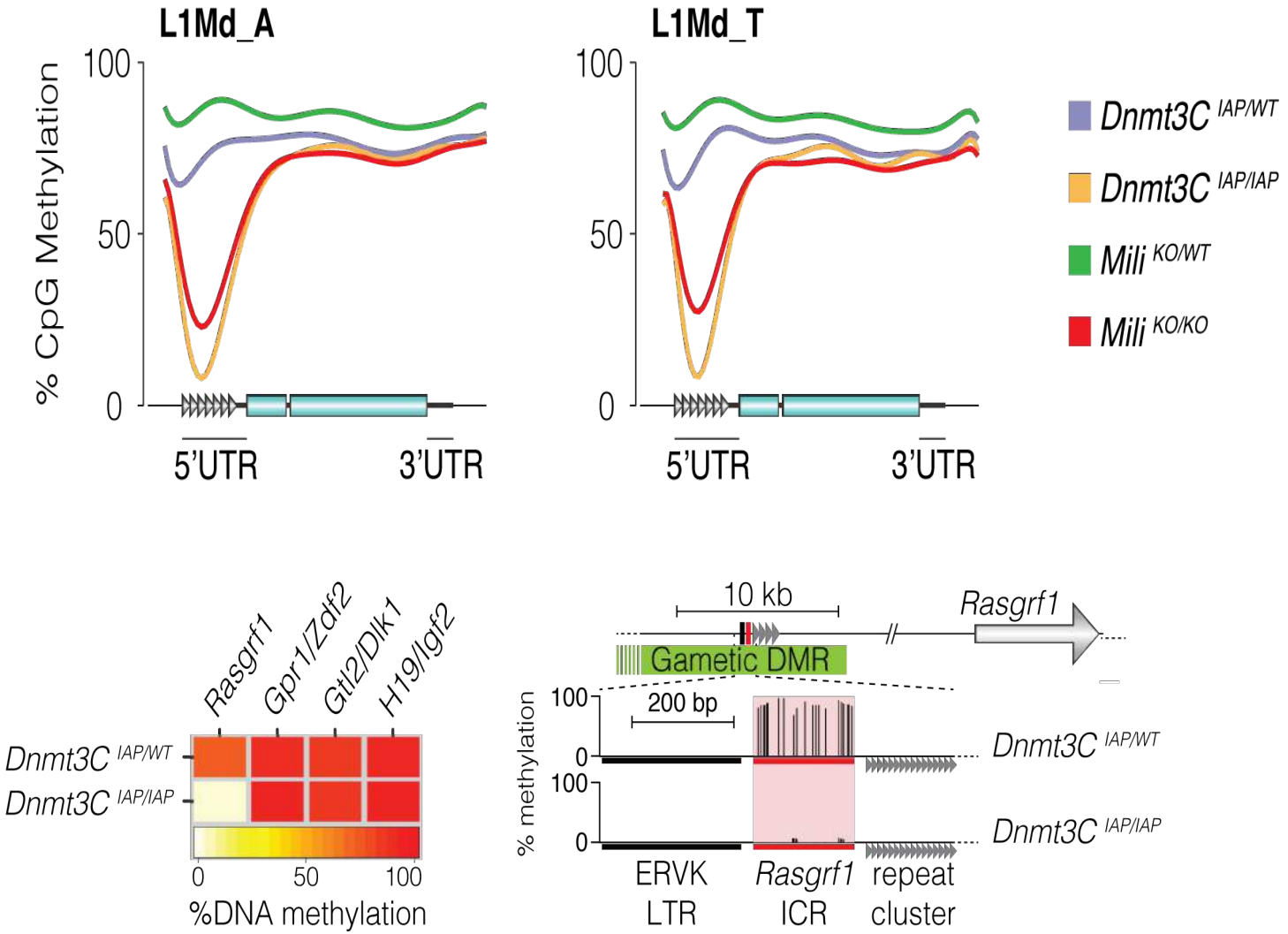


Barau et al, Science 2017

Courtesy of Joan Barau

COLLÈGE DE FRANCE 1530

A methylation defect at evolutionarily young, active LINEs reminiscent of small (pi)RNA directed DNA methylation



Barau et al, Science 2017 *Courtesy of Joan Barau/Deborah Bourc'his*

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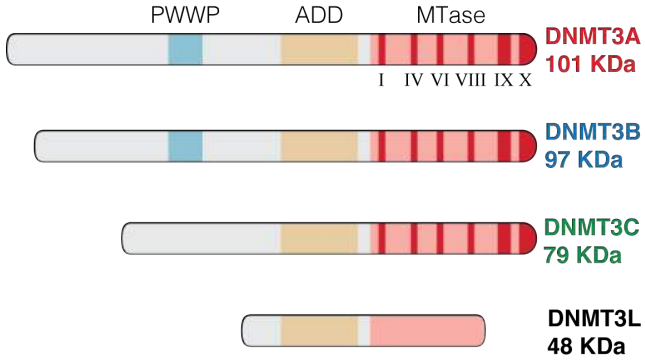
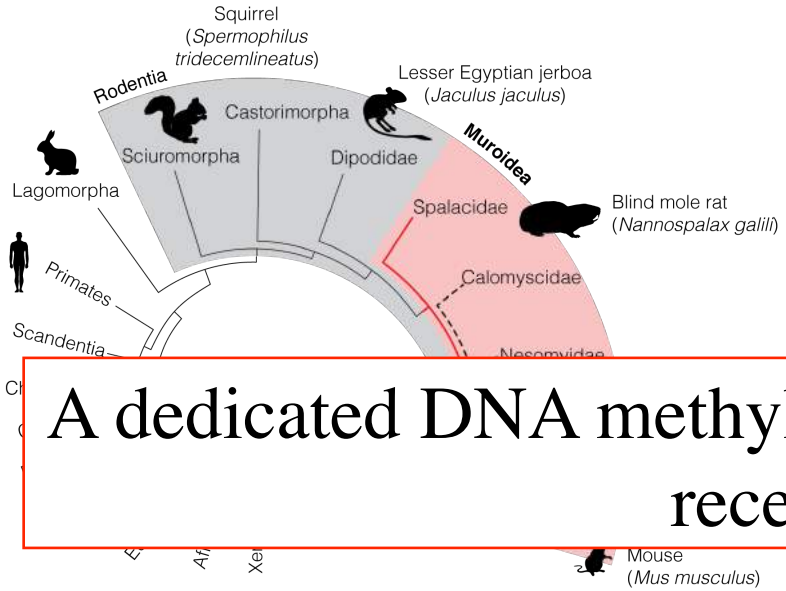
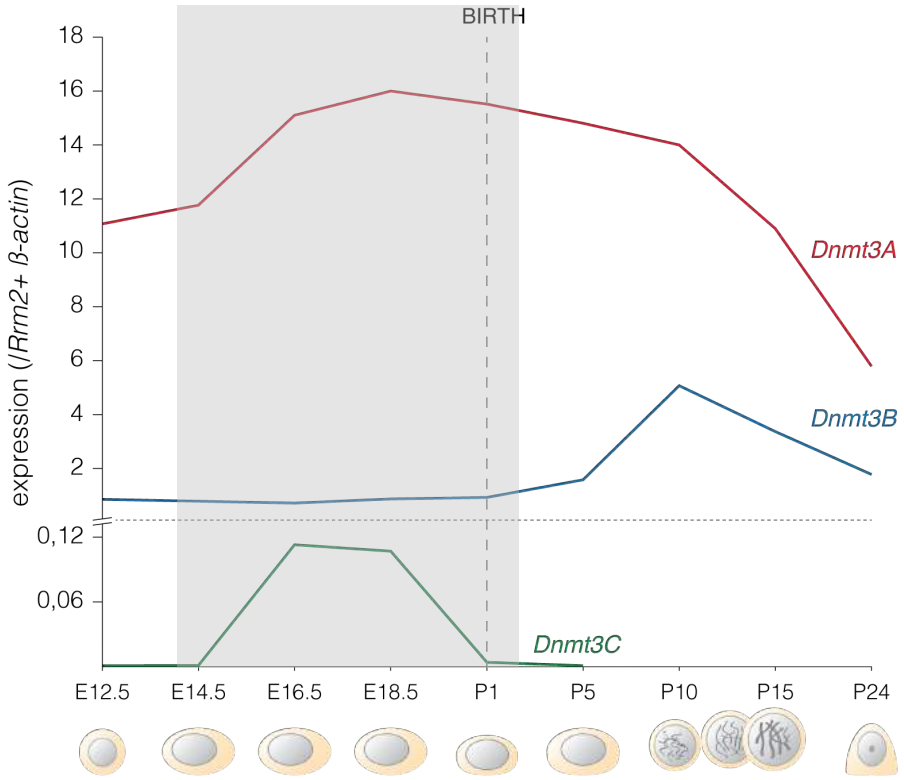
Dnmt3C appears to have evolved specifically in some rodents







A dedicated DNA methylation activity to protect against recent LINEs?

Barau et al, Science 2017

Courtesy of Joan Barau/Deborah Bourc'his

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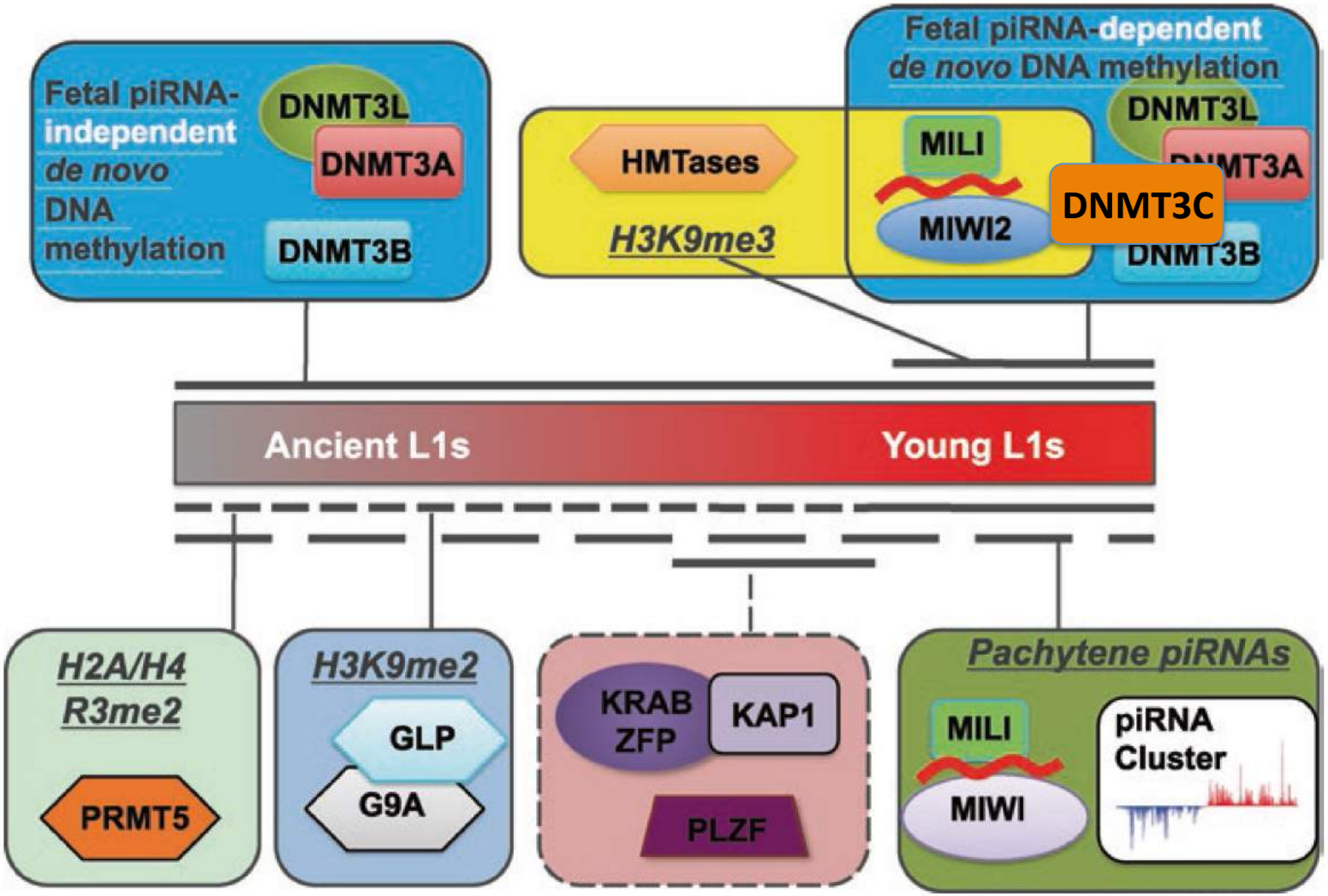
Different strategies for silencing of ancient and young LINEs in mouse germ line



S.J. Newkirk and W. An, 2016

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

- **RNA interference** and **Epigenetic** silencing mechanisms **have co-evolved** with TEs: they provide a means to protect the genome from aberrant expression and mobility – but with opportunities enabling vertical transfer of TEs and appearance of new host functions.
- Ongoing **arms race** between TE and Host provides a powerful means for evolution of epigenetic mechanisms which can modulate control, to the benefit of both TE and Host.
- Epigenetic silencing represents an opportunity for both heritable and reprogrammable expression => metastable alleles, sensitivity to environment, fuel for adaptation…
- DNA-targeting of silencing machinery and the remarkable KRAB-Zinc finger proteins involved – both as regulators of gene expression and as arms against TEs: **NEXT WEEK!**

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CHAIRE ÉPIGÉNÉTIQUE ET MÉMOIRE CELLULAIRE

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.

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