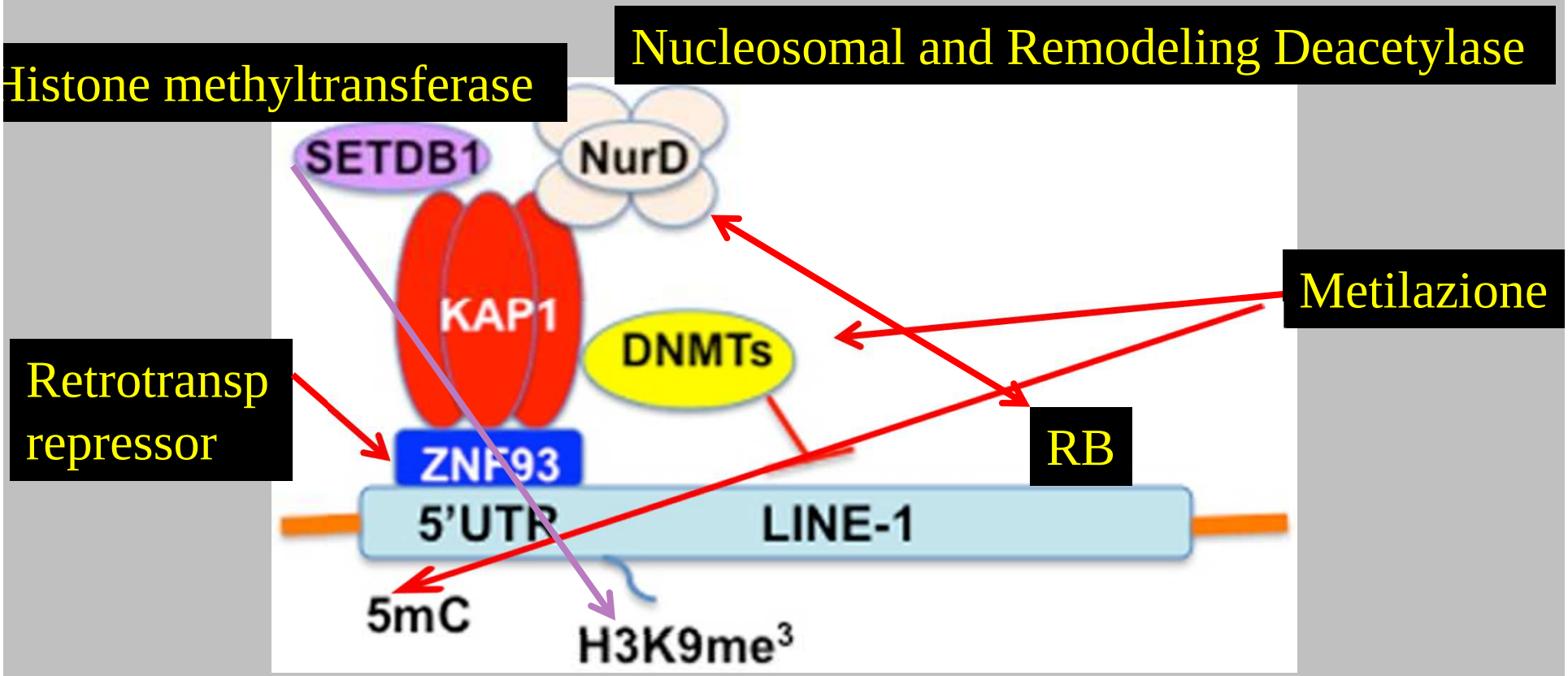
Come inibire l’espressione e trasposizione del Trasposone

Negative Control of the transcriptional activity of L1 in ES cells



KAP1 serves as a scaffold for heterochromatin complexes

Come inibire l’espressione e trasposizione del Trasposone

Riconoscimento delle sequenze L1

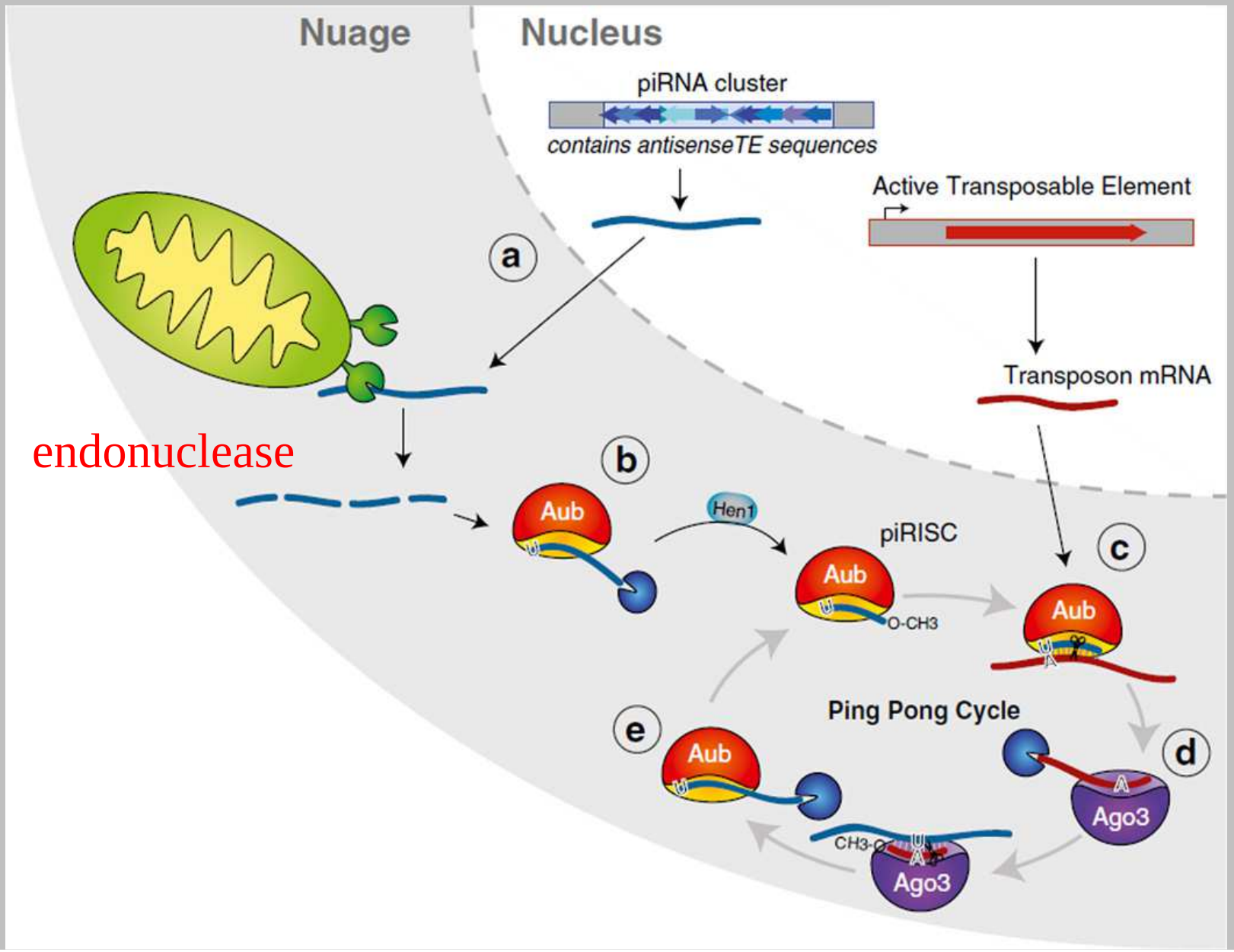
Le GUIDE - piRNA

piRNA biogenesis in the germline: From transcription of piRNA genomic sources to piRNA maturation.

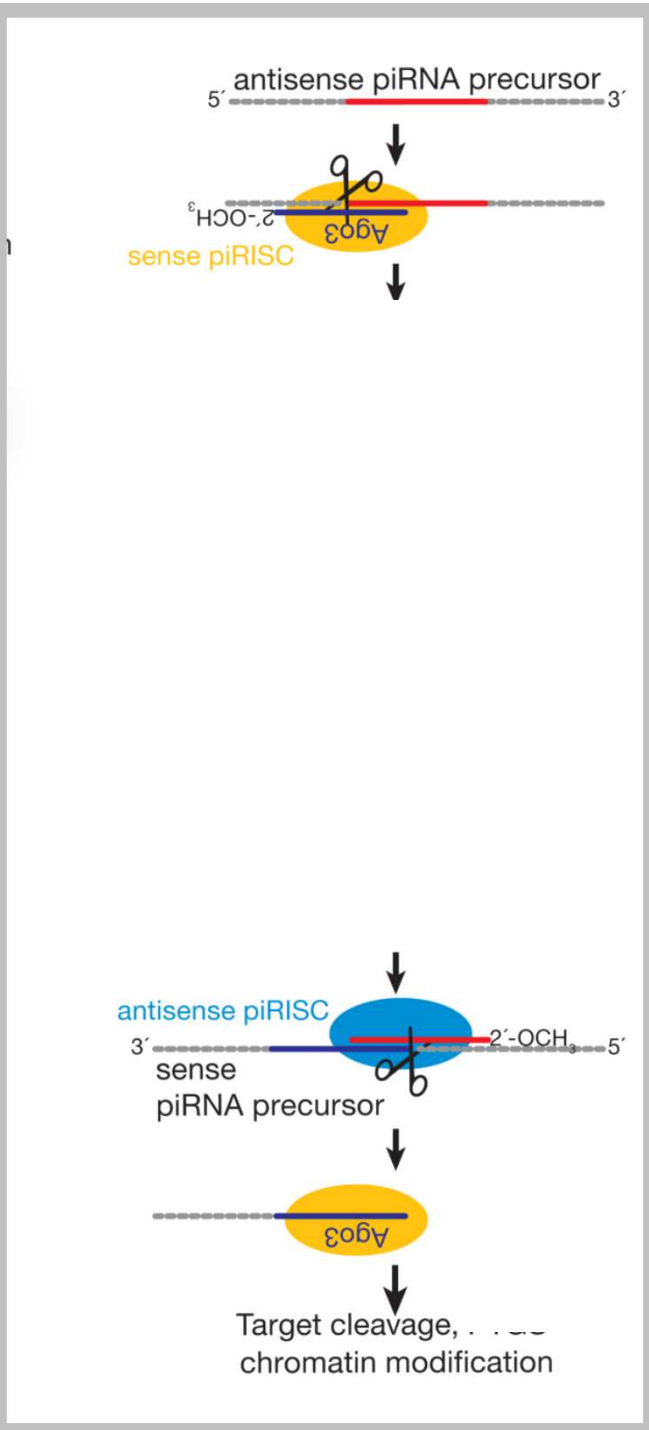
Hirakata et al Biochim Biophys Acta. 2015 Sep 5. review

- PIWI-interacting RNAs (piRNAs) are small non-coding RNAs enriched in animal gonads where they repress transposons to maintain genome integrity.
- Highly tissue-specific and adaptable nature of piRNA generation, as well as diversity of piRNA sequences.
- Focus on intracellular events from transcription of piRNA sources to piRNA maturation

Biogenesis of piRNAs



Pi RNA Pathway

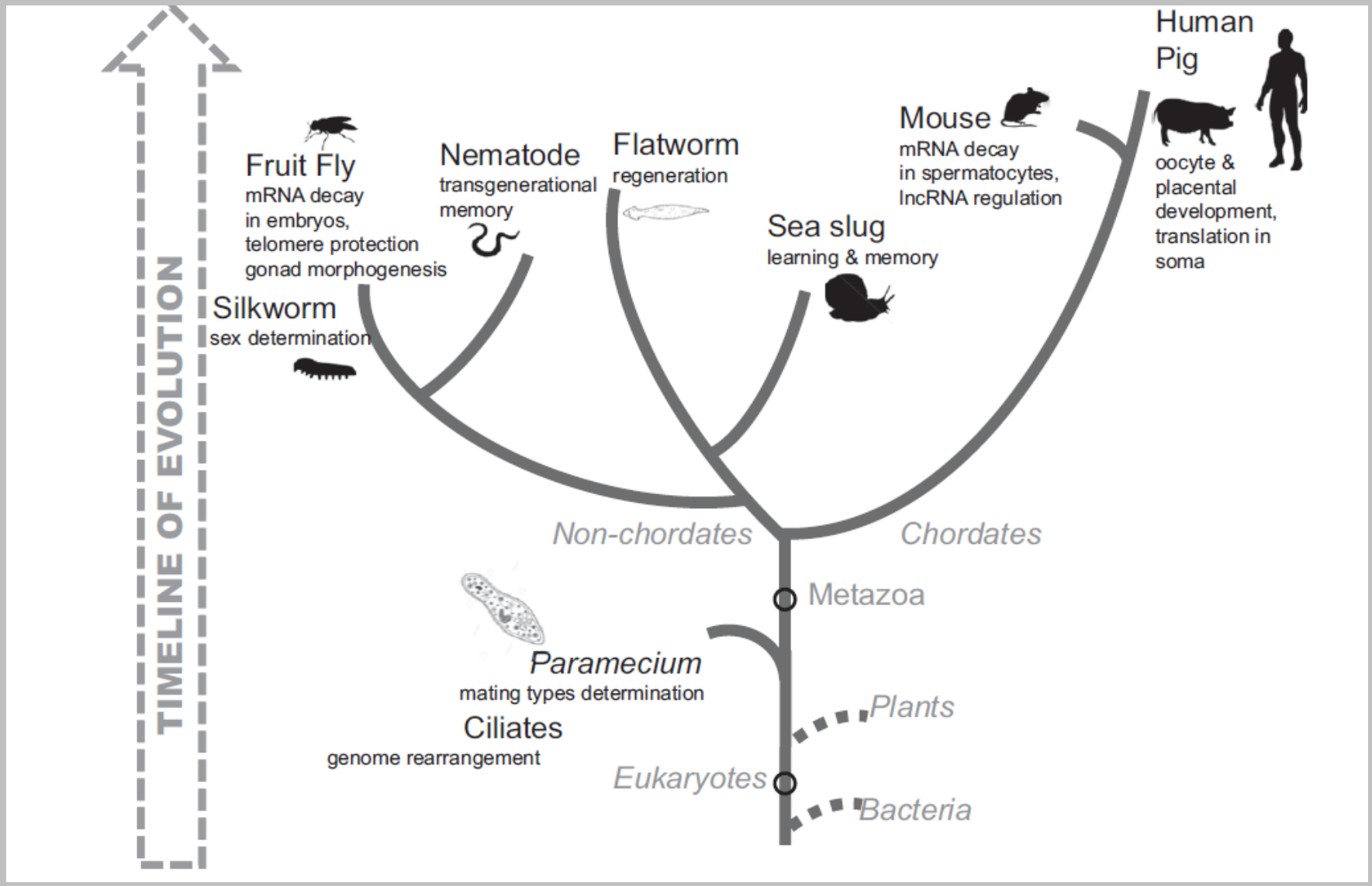


EVOLUZIONE RECENTE

remarkable differences can be observed even in **closely related species** reflecting the astonishing plasticity and diversity of these pathways.

Animal germ lines have evolved a dedicated class of 24- to 30-nucleotide (nt)-long small RNAs called Piwi-interacting RNAs (piRNAs)

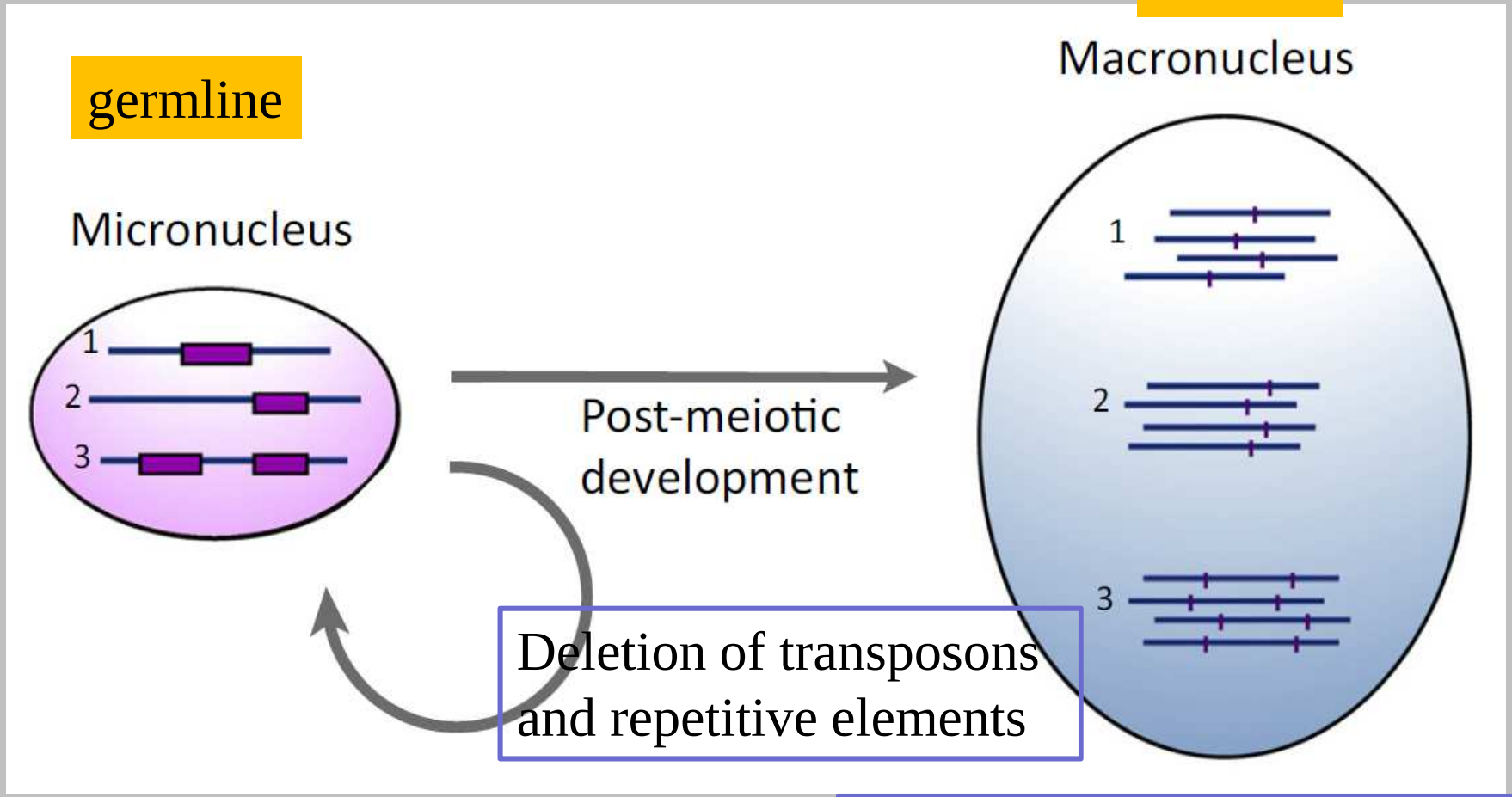
Diverse roles of piRNAs in different animals



February 2017 The FASEB Journal SARKAR ET AL

Ciliates and Transposons

Paramecium, Tetrahymena



Genome amplification (800X)!!

EVOLUZIONE RECENTE

remarkable differences can be observed even in **closely related species** reflecting the astonishing plasticity and diversity of these pathways.

Animal germ lines have evolved a dedicated class of 24- to 30-nucleotide (nt)-long small RNAs called Piwi-interacting RNAs (piRNAs)

-Piwi-like proteins- a subclass of Argonaute proteins- bind a class of small noncoding RNAs - piwi-interacting RNAs (piRNAs).

Come inibire l’espressione e trasposizione del Trasposone

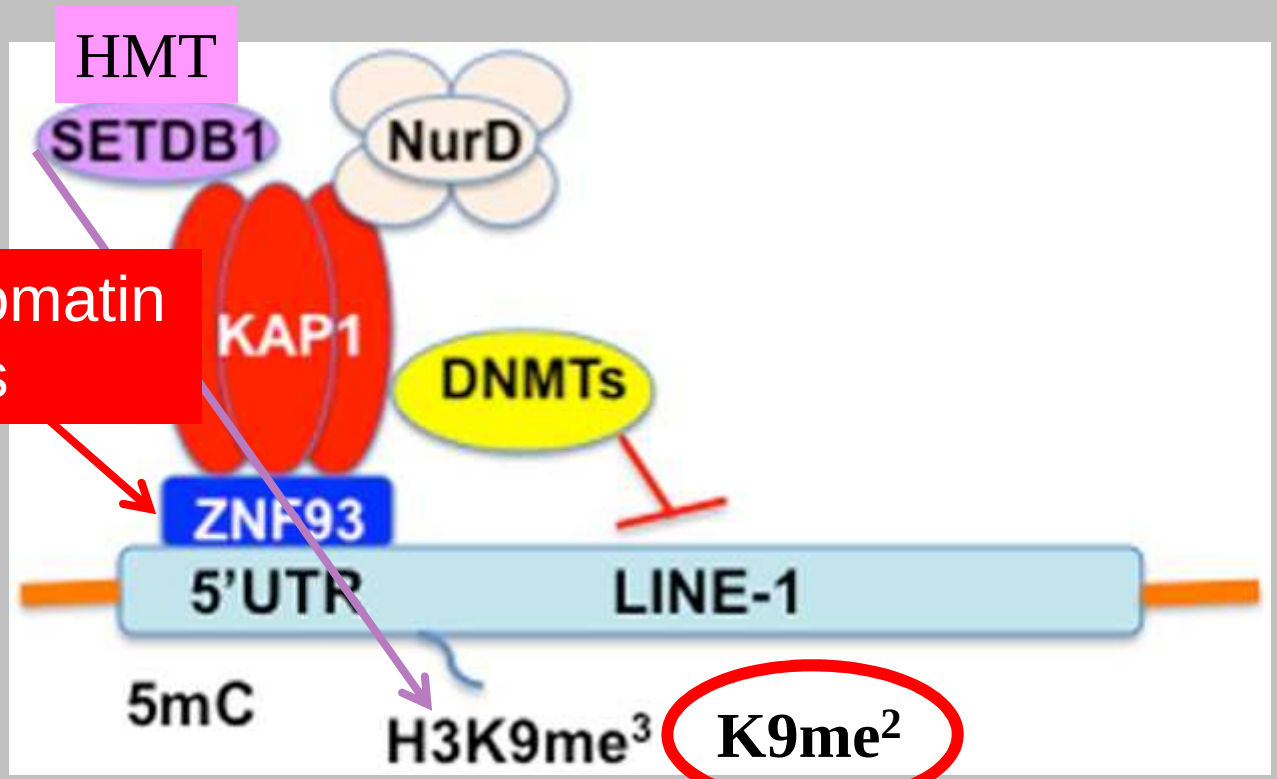
In mice, the piRNA pathway is mainly active in the male germ line

three Piwi proteins

MILI	Chromatin
MIWI	transcriptional silencing in embryonic germ cells by
MIWI2	DNA methylation marks on target transposon loci

Nuclear

Negative Control of the transcriptional activity of L1 in ES cells

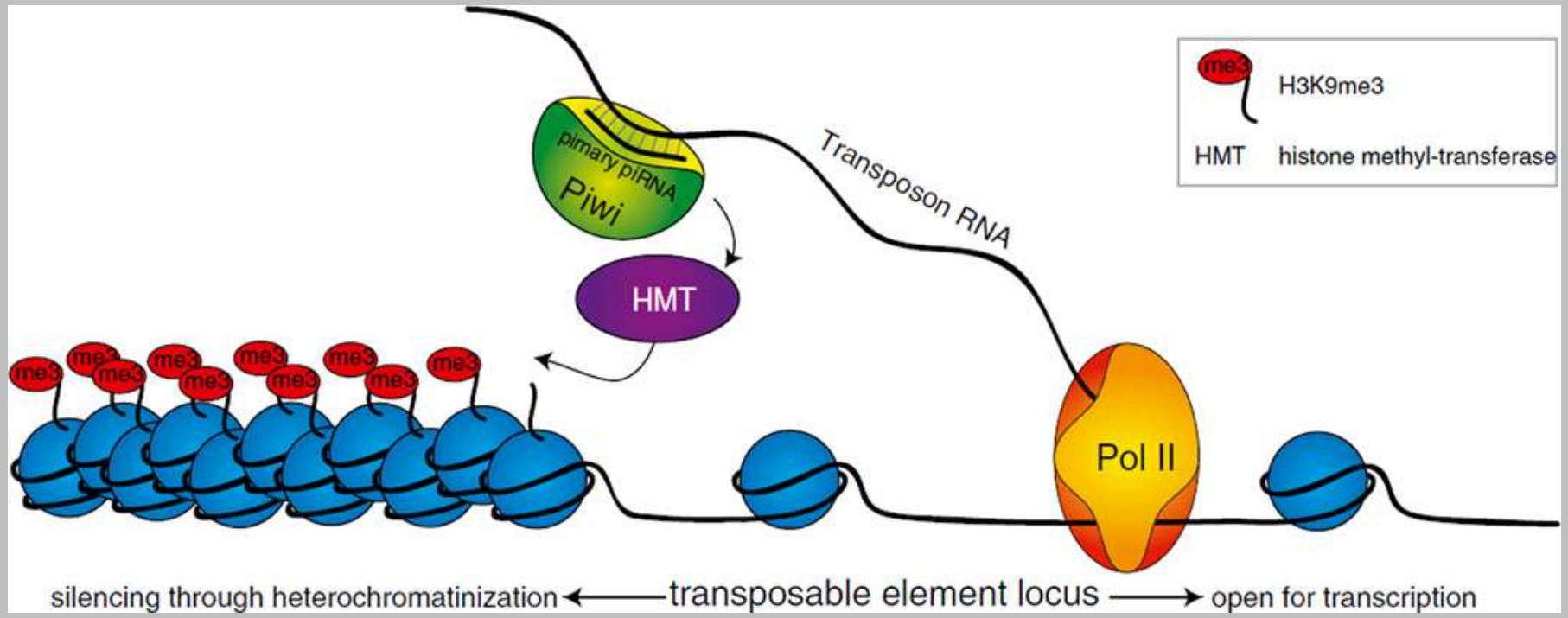


heterochromatin complexes

Riconoscimento

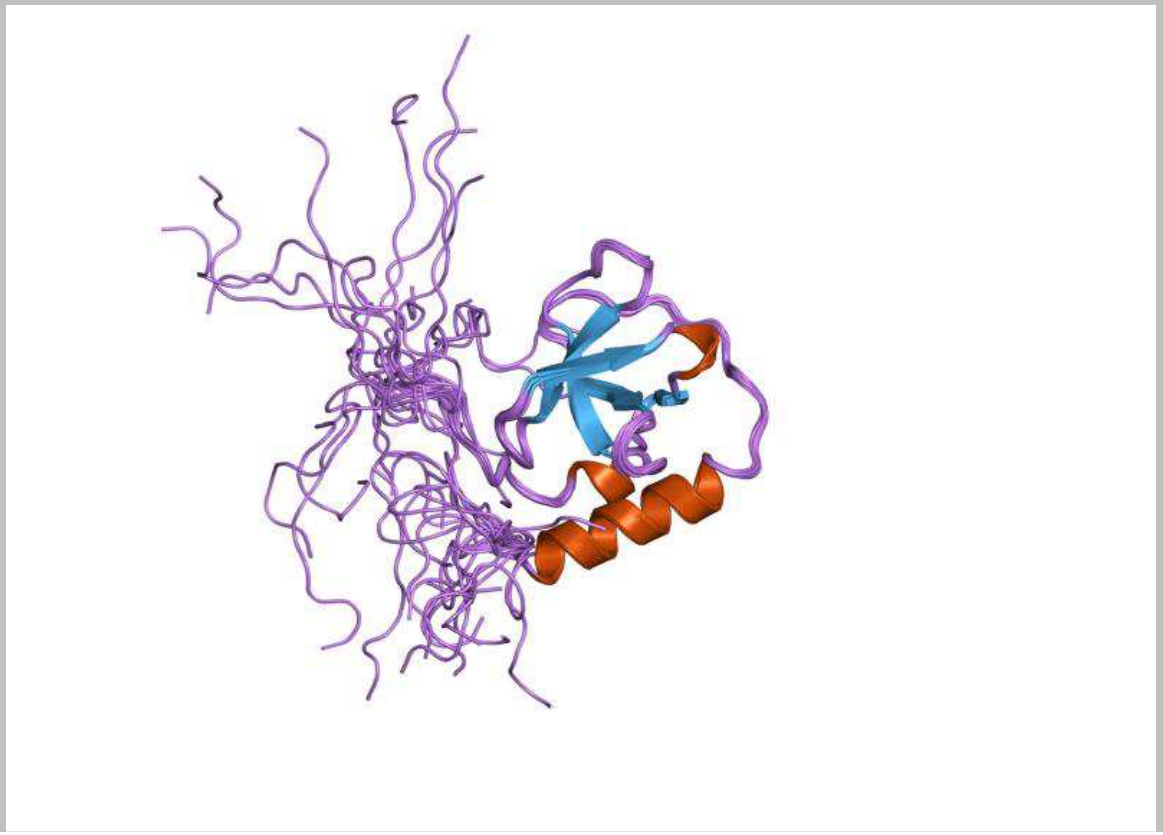
HMT istone metiltransferasi

nuclear function of the piRNA pathway



Tudor domain and proteins

The tudor domain TDRD is a conserved protein structural motif of 50 amino acids found in the Tudor (Drosophila).
a strongly bent anti-parallel β-sheet of five β-strands with a barrel-like fold recognizes symmetrically dimethylated arginineThe H3 dimethylated K9 modification cosuppresses L1 expression



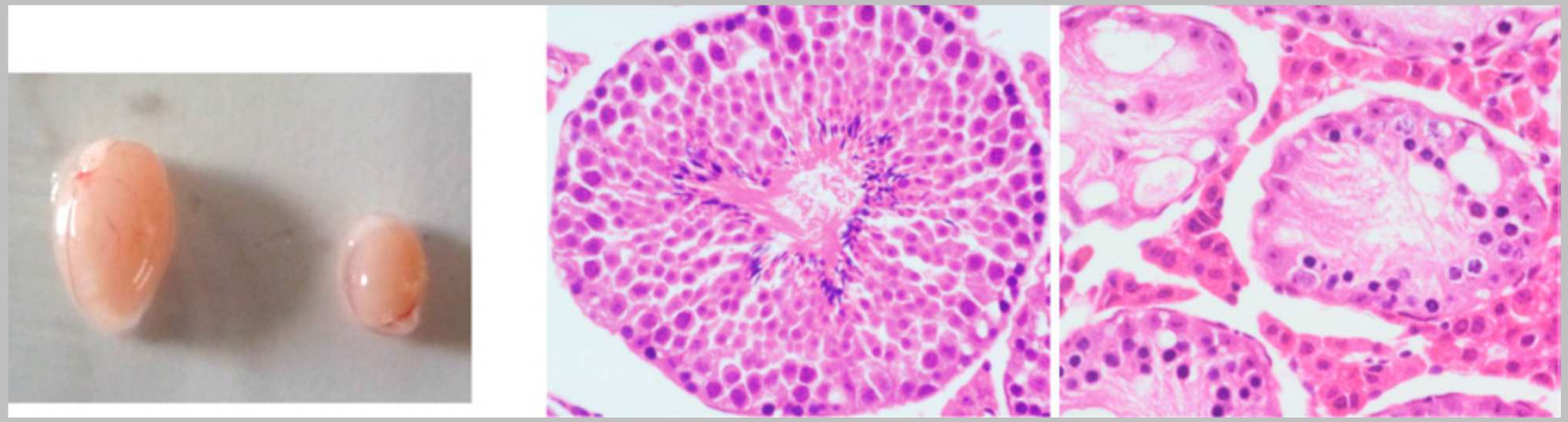
TDRD12 (Tudor Domain) is detected in complexes containing

Piwi protein **MILI**,
piRNAs,
TDRD1 (piRNA biogenesis)

Male mice carrying either a nonsense point mutation or a targeted deletion in the Tdrd12 locus are **infertile** and **derepress retrotransposons.**

TDRD12 is essential for production of piRNAs that enter
Piwi protein MIWI2.

Tdrd12 mutant male mice are infertile and display derepression of retrotransposons



Atrophied testes of homozygous
(−/−) Tdrd12 mutants

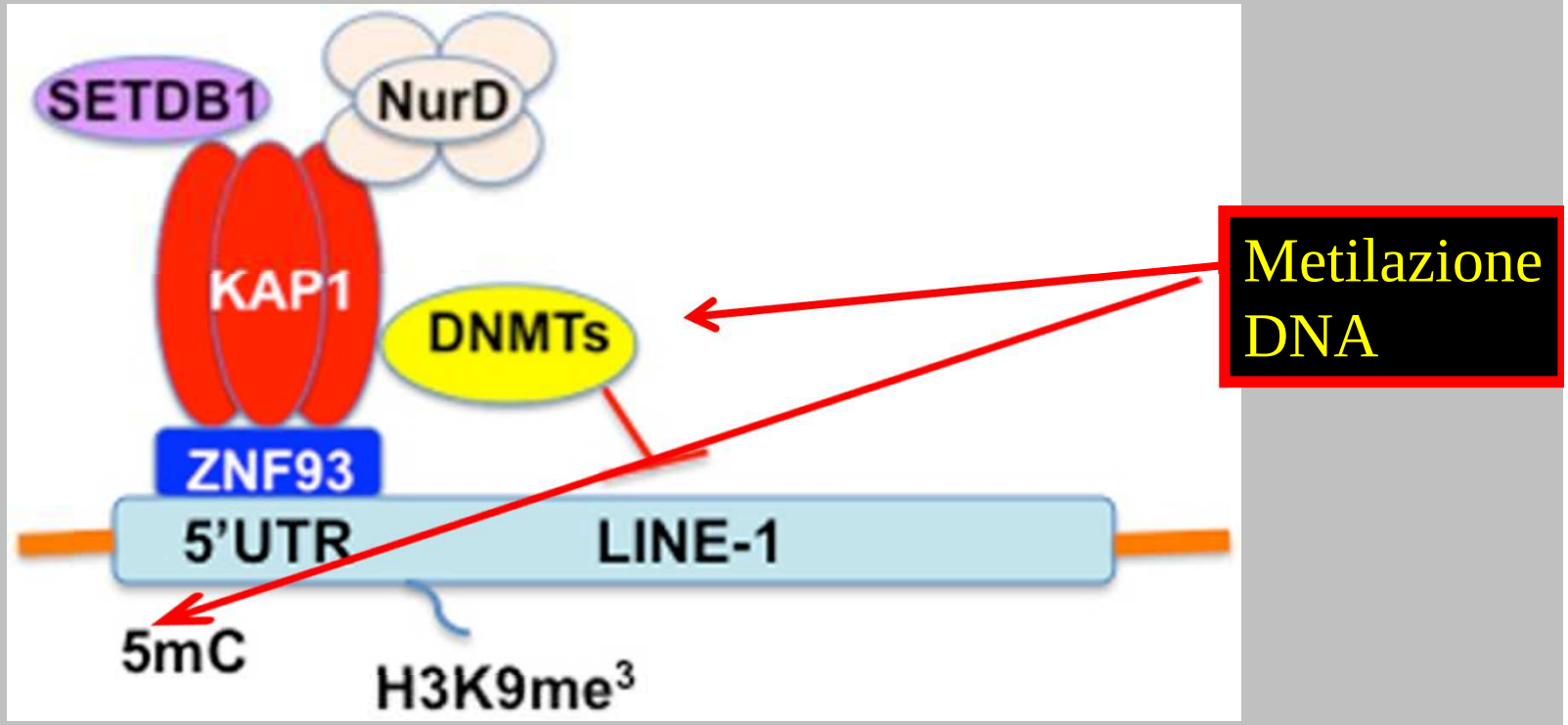
Come inibire l'espressione e trasposizione del Trasposone

Mammalian genomes use CpG DNA methylation to silence these genomic parasites.

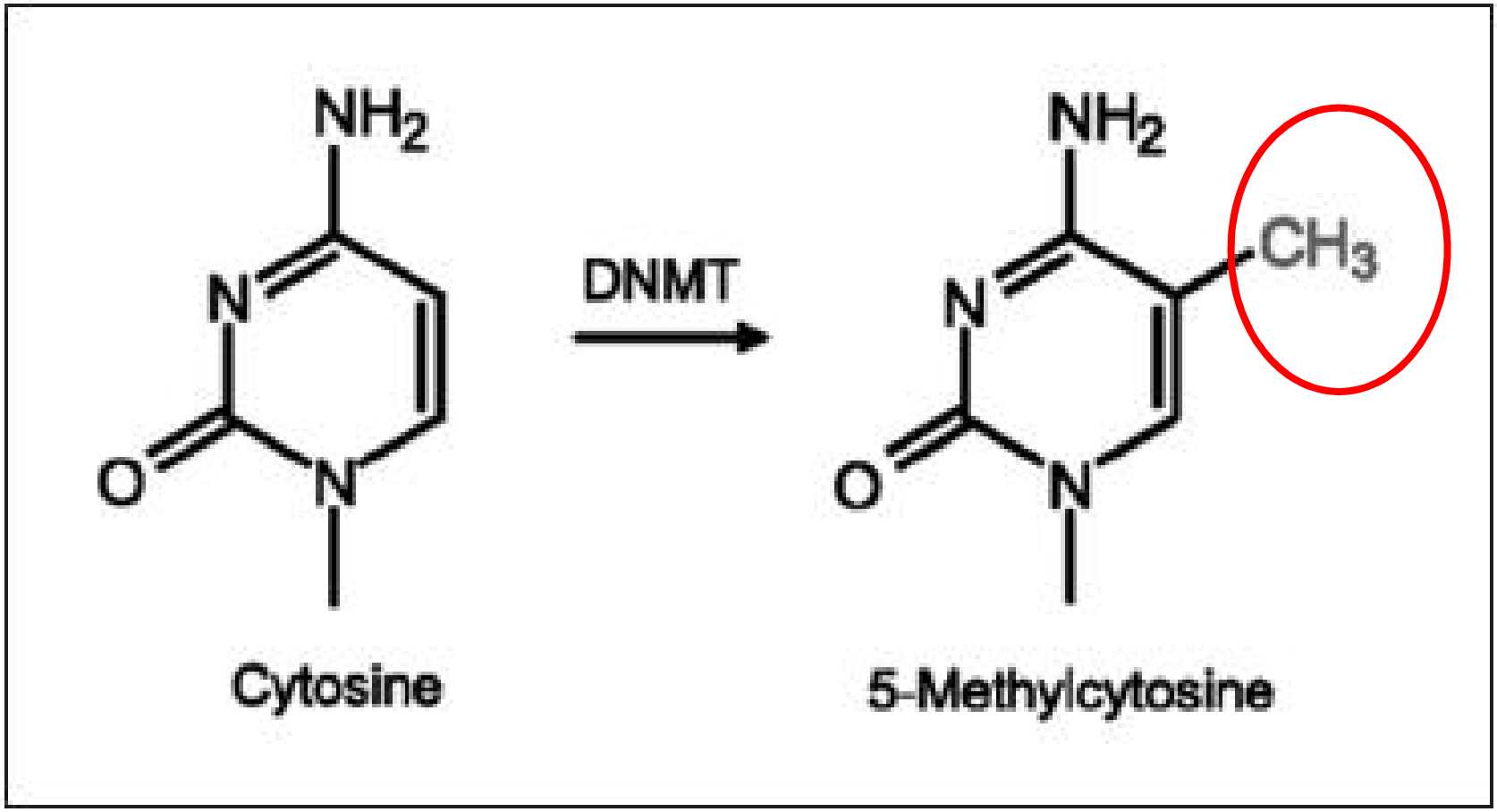
A class of small RNAs is used to specifically guide the DNA methylation machinery to the transposon DNA elements.

Animal germ lines have evolved a dedicated class of 24- to 30-nucleotide (nt)-long small RNAs called Piwi-interacting RNAs (piRNAs)

Negative Control of the transcriptional activity of L1 in ES cells



KAP1 serves as a scaffold for heterochromatin complexes


NH2
N
O
N
DNMT
NH2
CH3
N
O
N
Cytosine
5-Methylcytosine

Bisulfite-mediated conversion of cytosine to Uracil

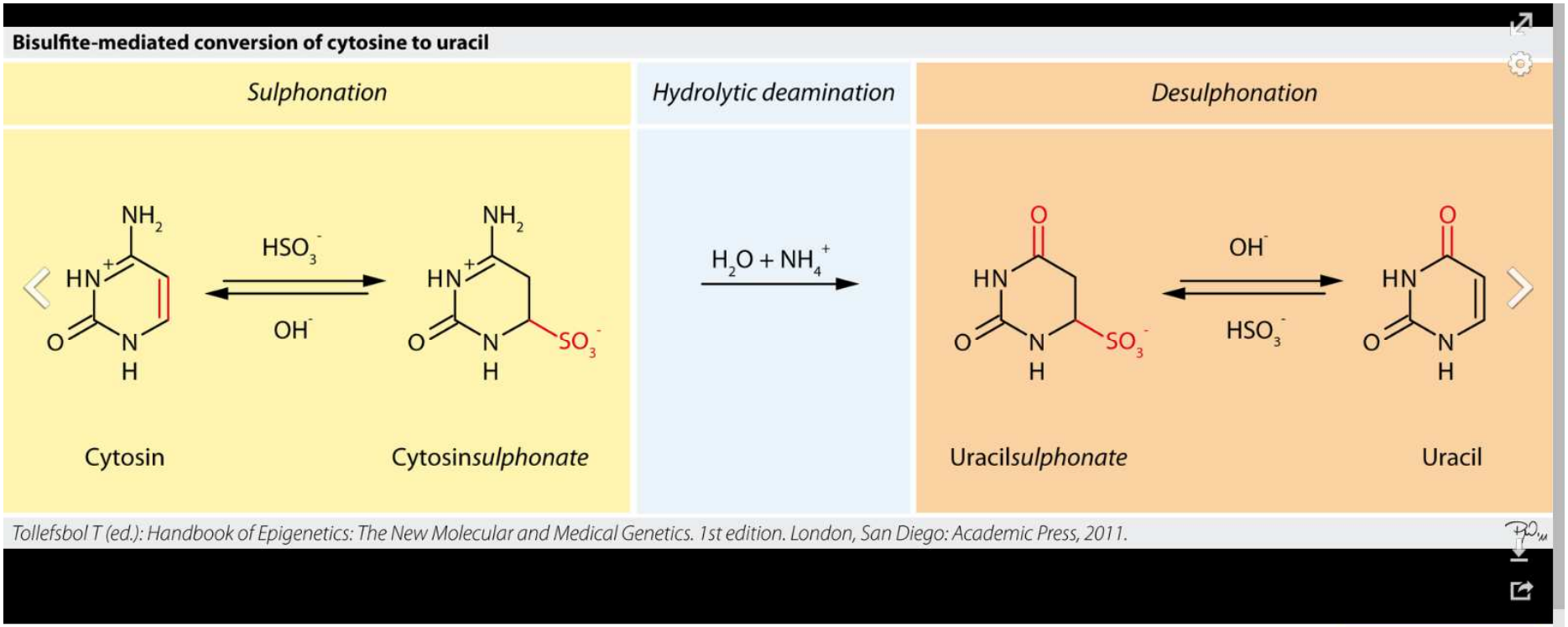


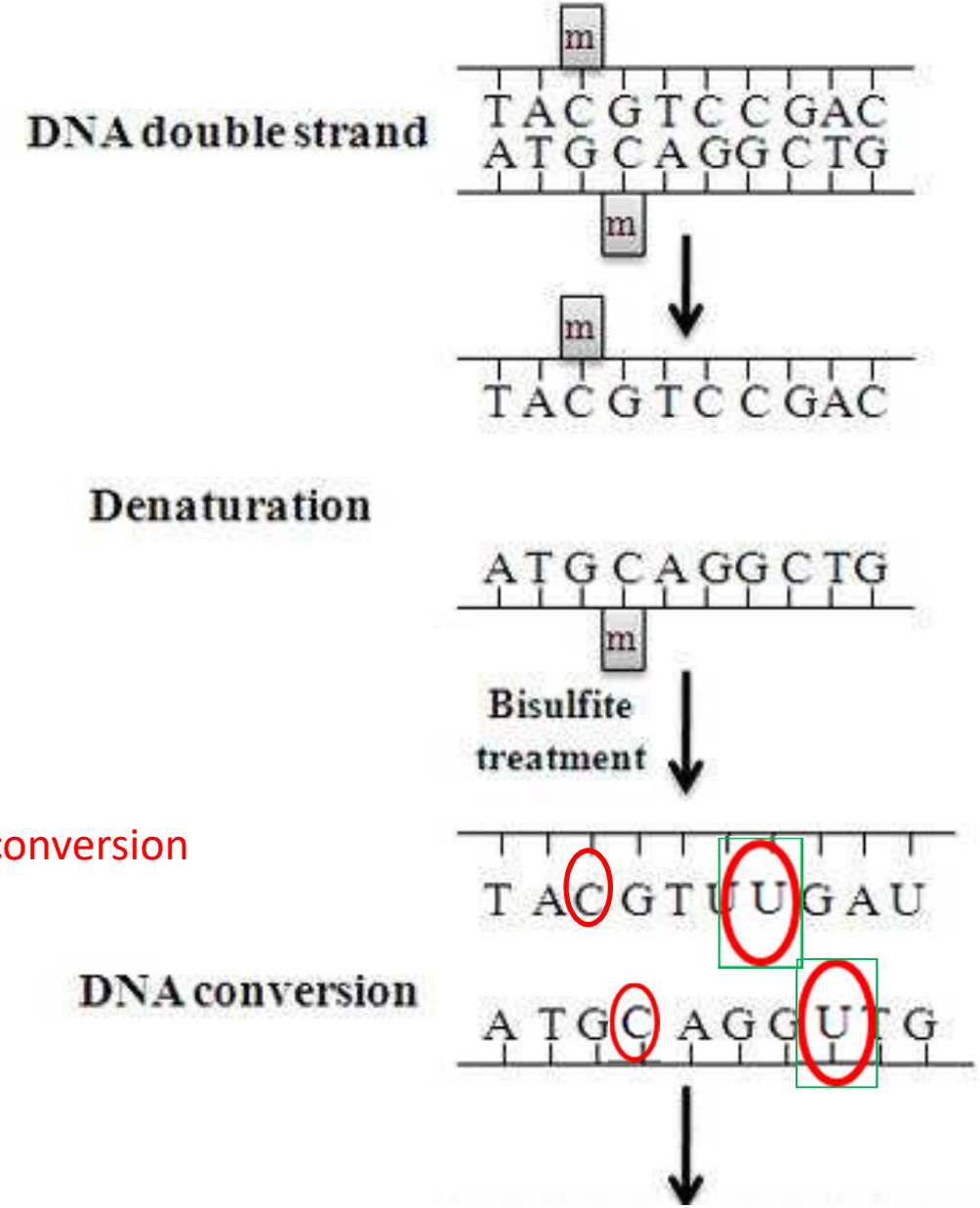
Figure 2: Outline of the chemical reaction that underlies the bisulfite-mediated conversion of cytosine to uracil.

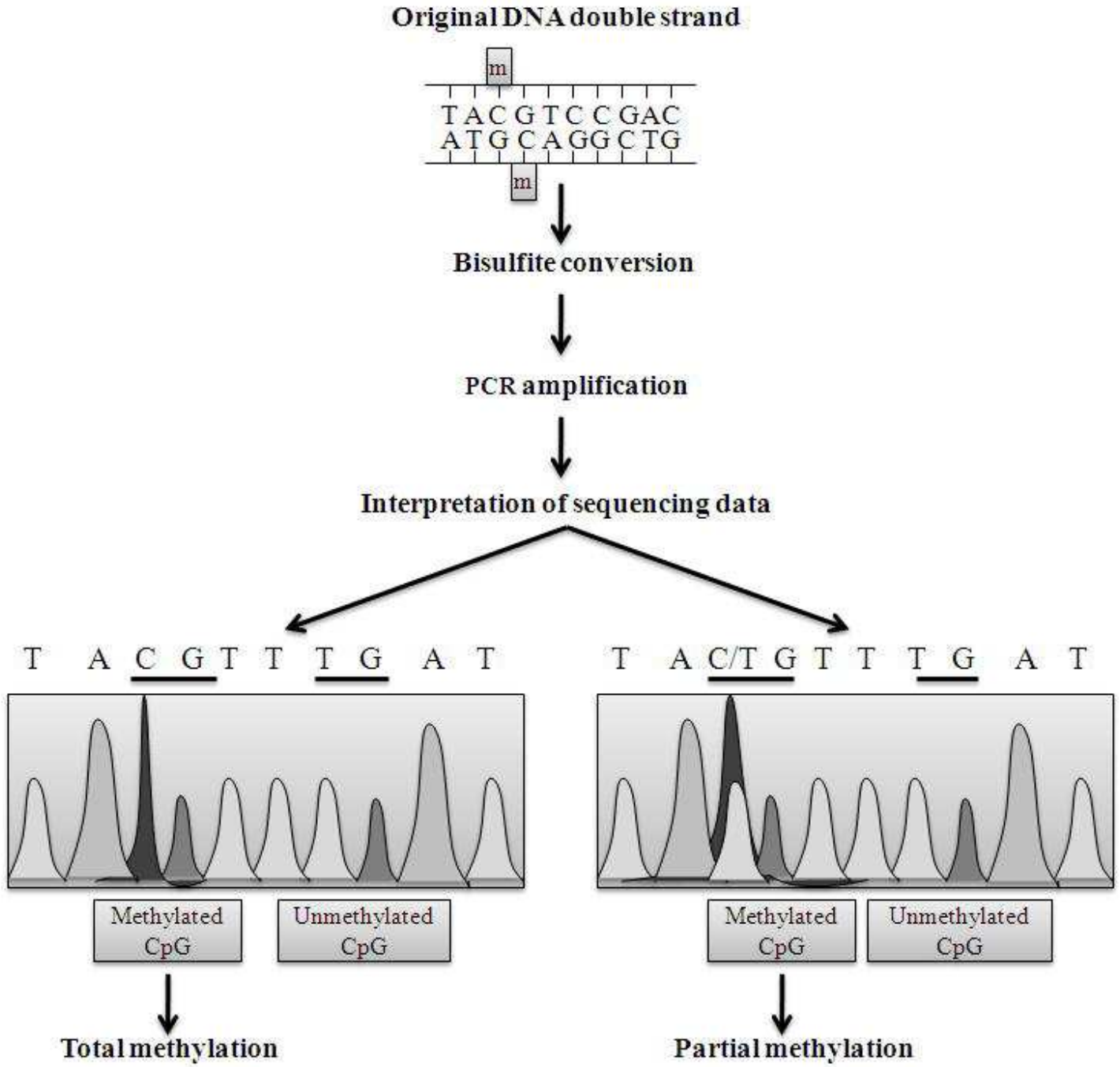
More details

C->U

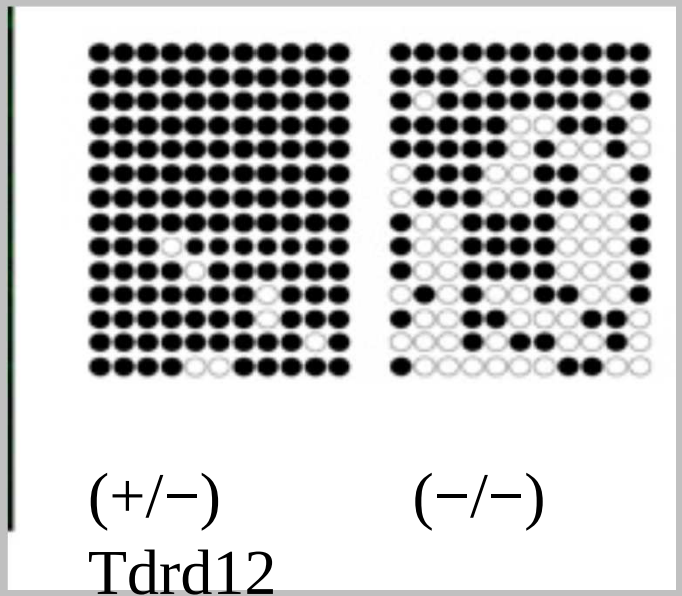
5MC -> 5MC

Bisulfite-mediated conversion




Original DNA double strand
m
TACGTCCGAC
ATGCAGGCTG
m
Bisulfite conversion
PCR amplification
Interpretation of sequencing data
T A C G T T T G A T
T A C/T G T T T G A T
Methylated CpG
Unmethylated CpG
Methylated CpG
Unmethylated CpG
Total methylation
Partial methylation

CpG DNA methylation (filled circles) on transposon L1 Promoters



Tdrd12 mutant -

Epigenetic status of LINE-1 predicts clinical outcome in early-stage rectal cancer

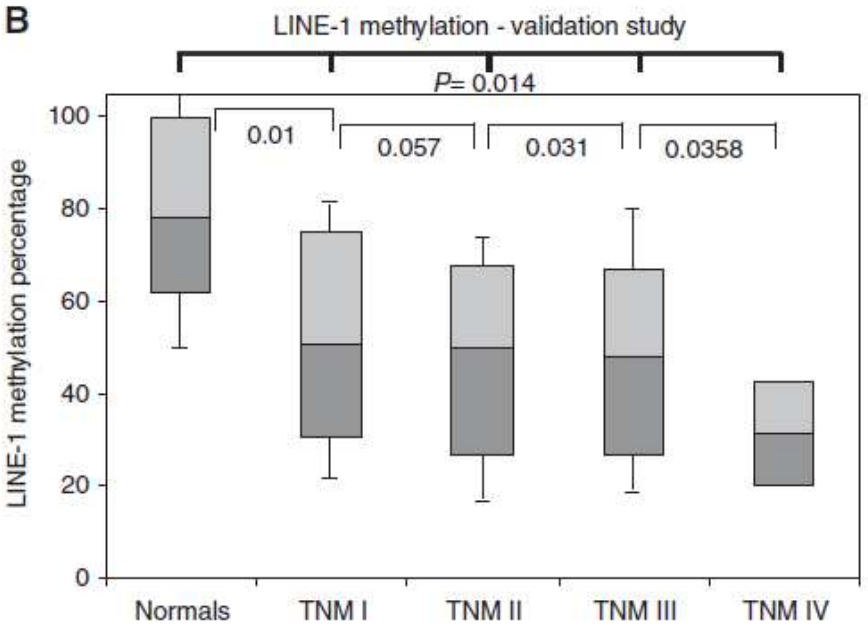
British Journal of Cancer (2013) 109, 3073

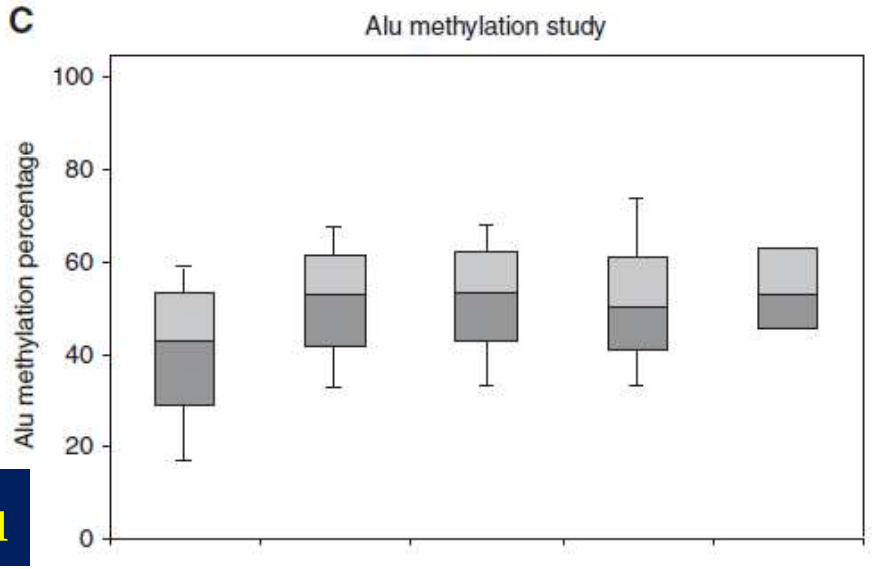
Method

Bisulfite conversion

quantitative real-time PCR with probes specific to the bisulfite-converted methylated or unmethylated LINE-1 sequence

L1



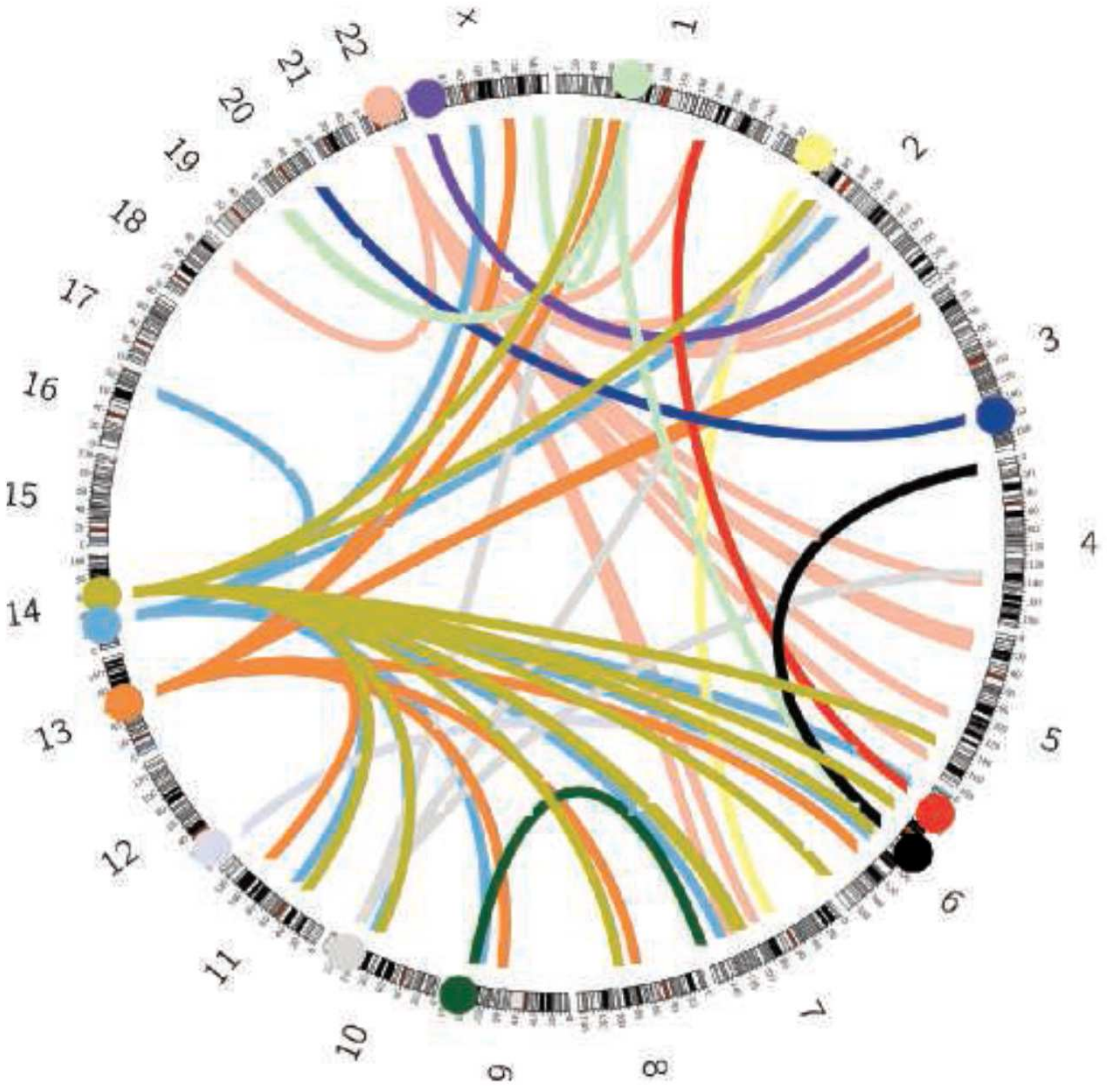


TNM (tumour node metastasis); early-stage rectal cancer (stage I-II),

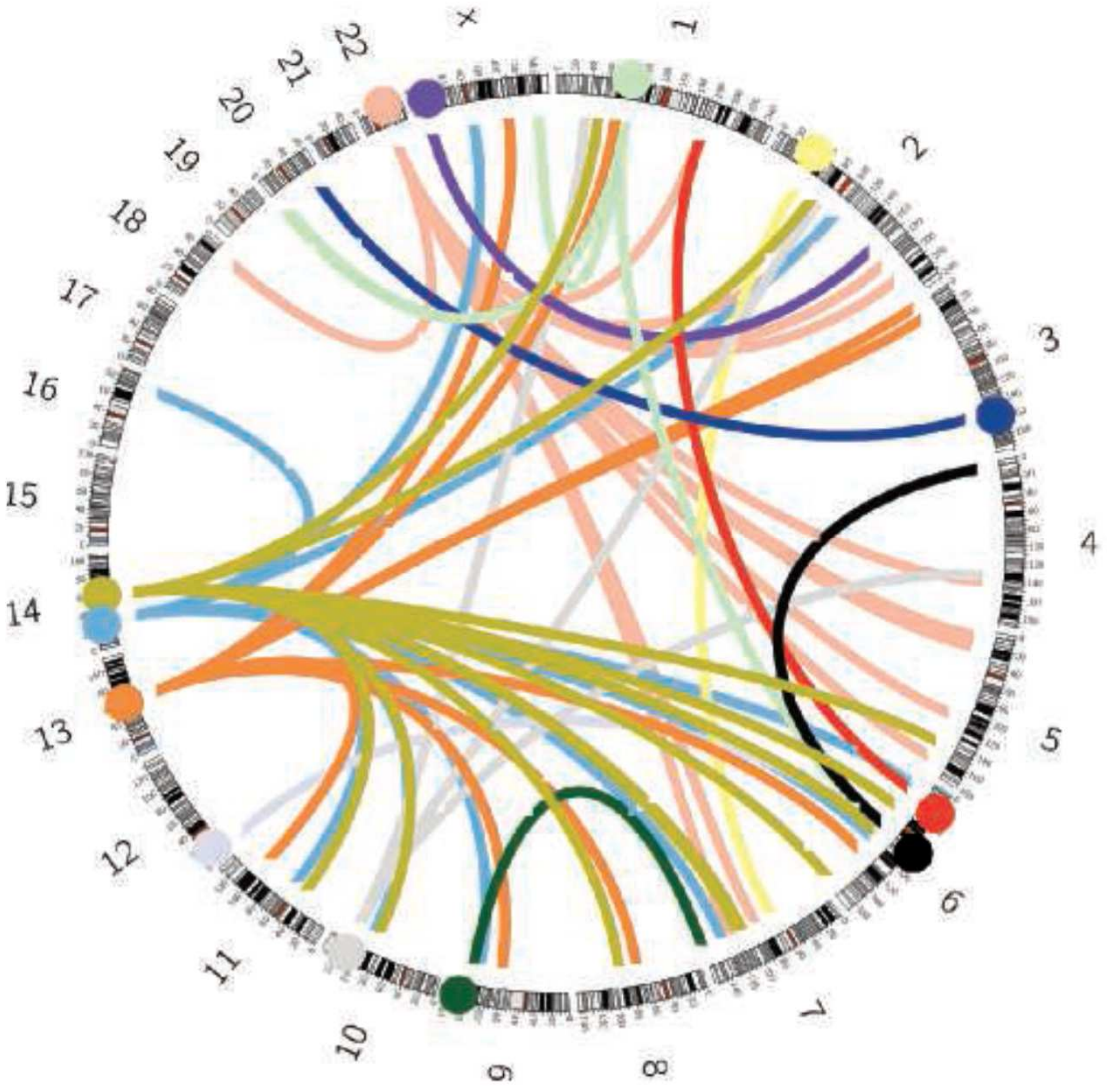
Alu

L1 and Tumor

In a lung tumor, hundreds of 3′ transductions arose from a small number of active L1 source elements (colored circles)



These somatic L1 insertions are not only potential mutagens in the development of tumor , but also useful markers of tumor clones



Interazione espressione L1 e mutageni

Benzopirene (genotossico) ed L1

- Cells

a) primary mouse embryo fibroblasts (MEFs) and
b) Retinoblastoma (RB) null MEFs (TKO)

- Treatment

medium (Control 1), 0.06 % DMSO (control2)

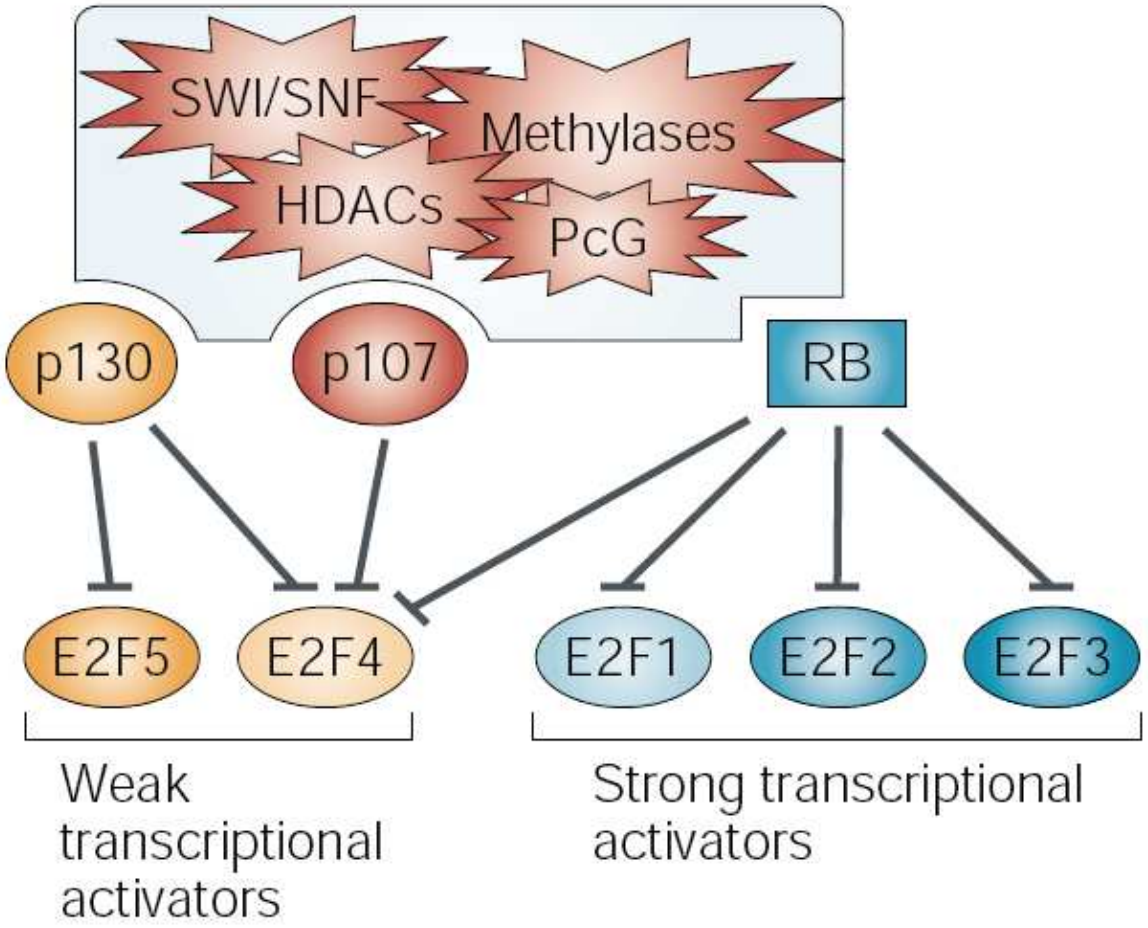
3 μM B(a)P for 16 h.

- Assay

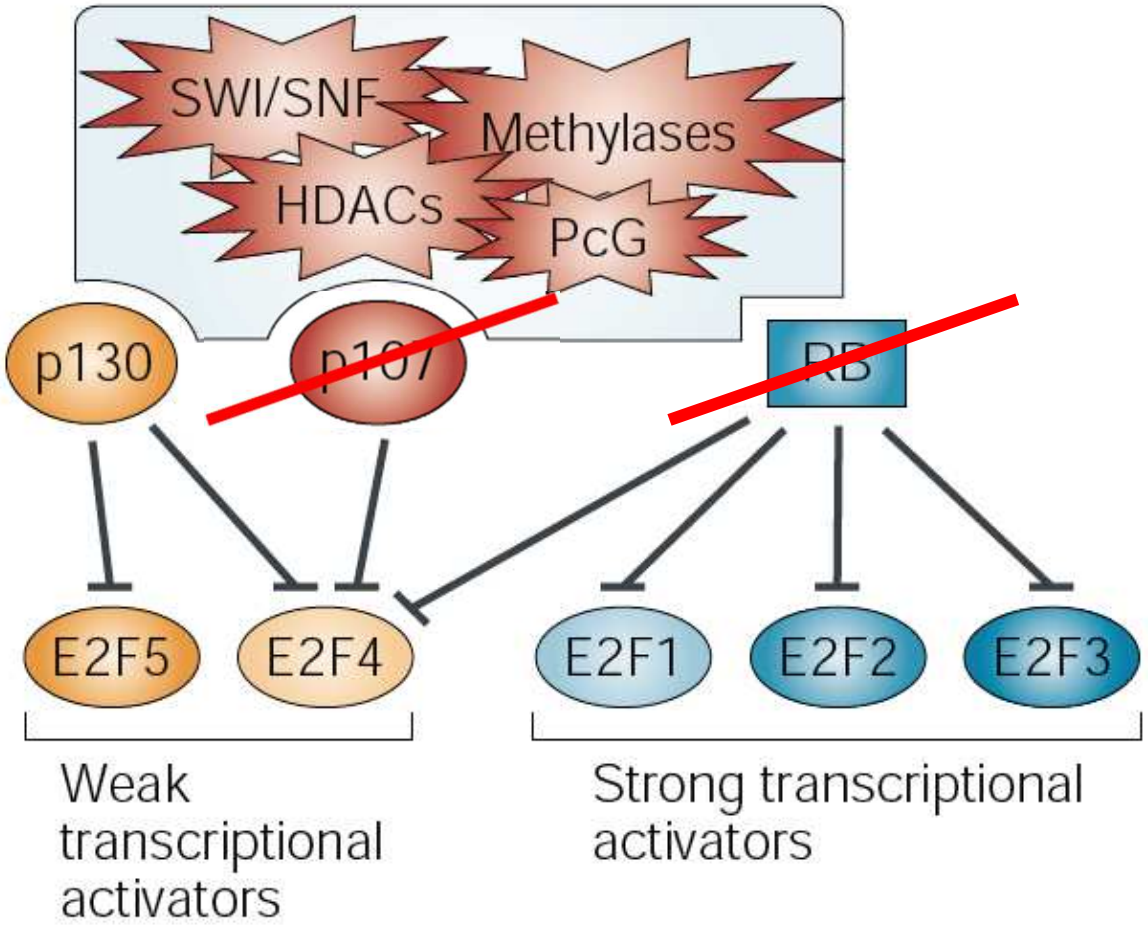
Total RNA -> cDNA -> qPCR

primers β actin or murine L1 ORF1

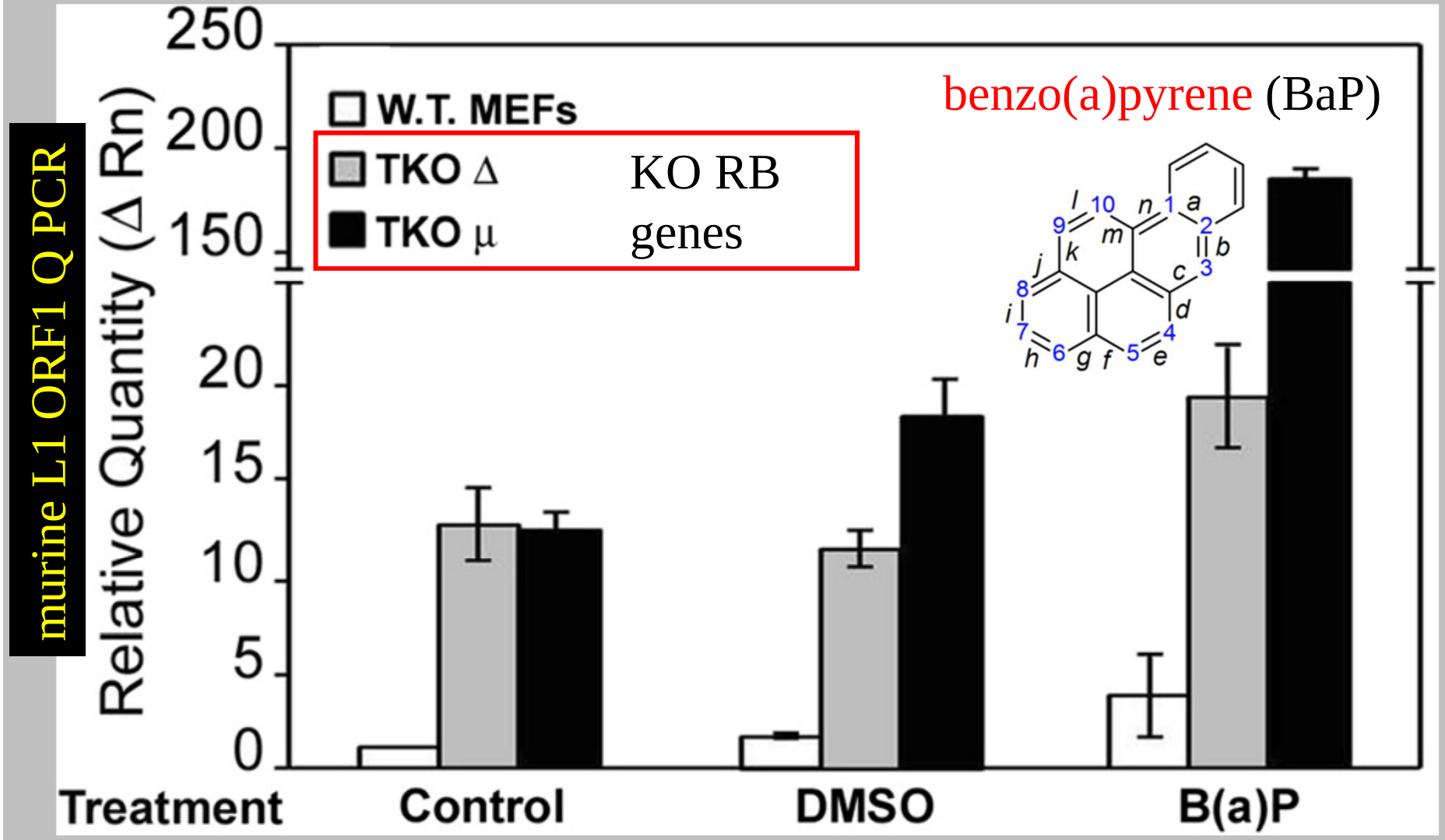
Retinoblastoma (RB) – una proteina oncosoppressore



Retinoblastoma (RB) – una proteina oncosoppressore



Genotoxic injury in the absence of the Retinoblastoma (RB) proteins upregulates L1 expression



mouse embryo fibroblasts (MEFs)

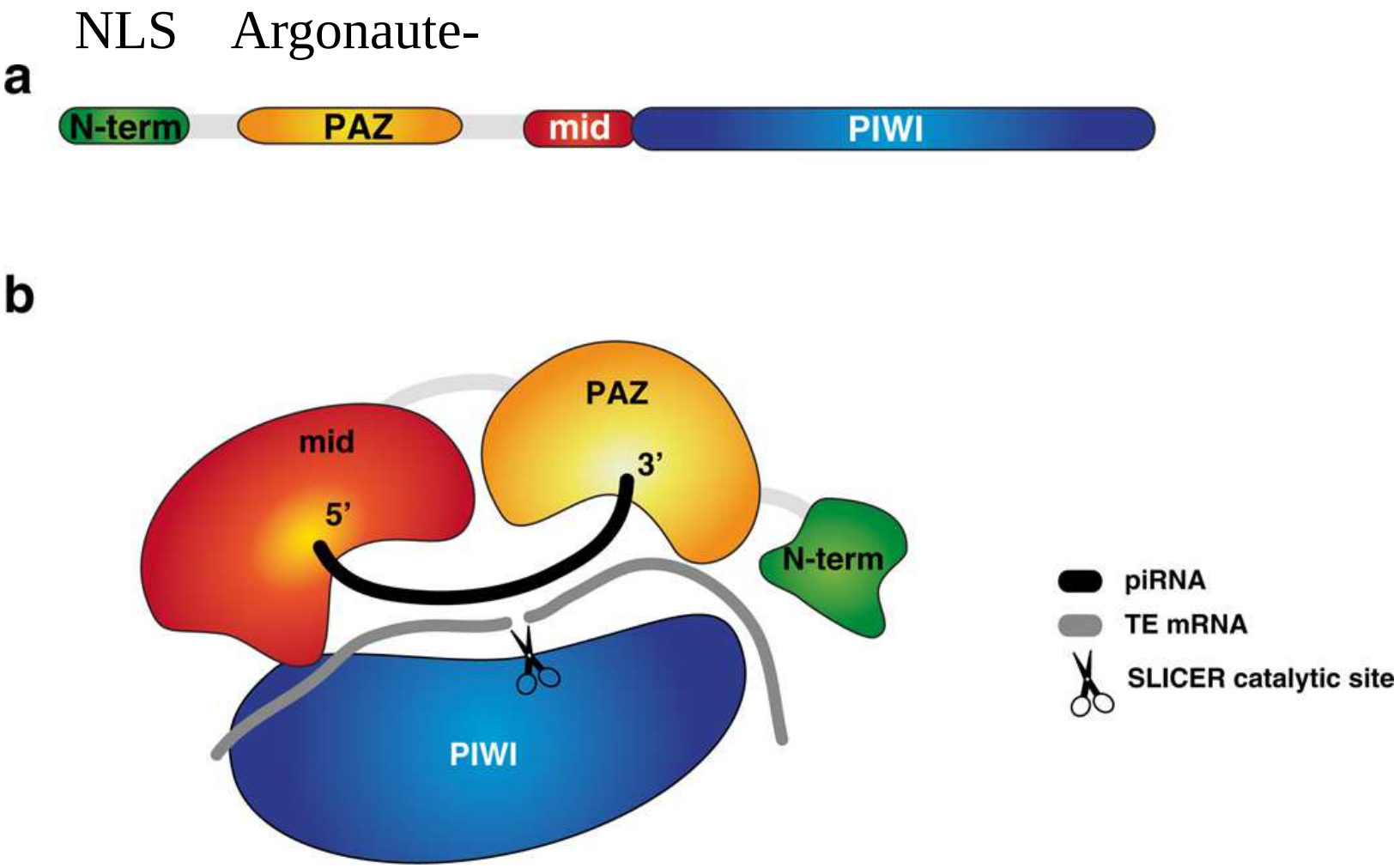
Come inibire l’espressione e trasposizione del Trasposone

In mice, the piRNA pathway is mainly active in the male germ line

three Piwi proteins

MILI
MIWI
MIWI2

Cytoplasmic MIWI have a role in maintaining repression by direct cleavage of transposon transcripts using their endonucleolytic (Slicer) cleavage activity



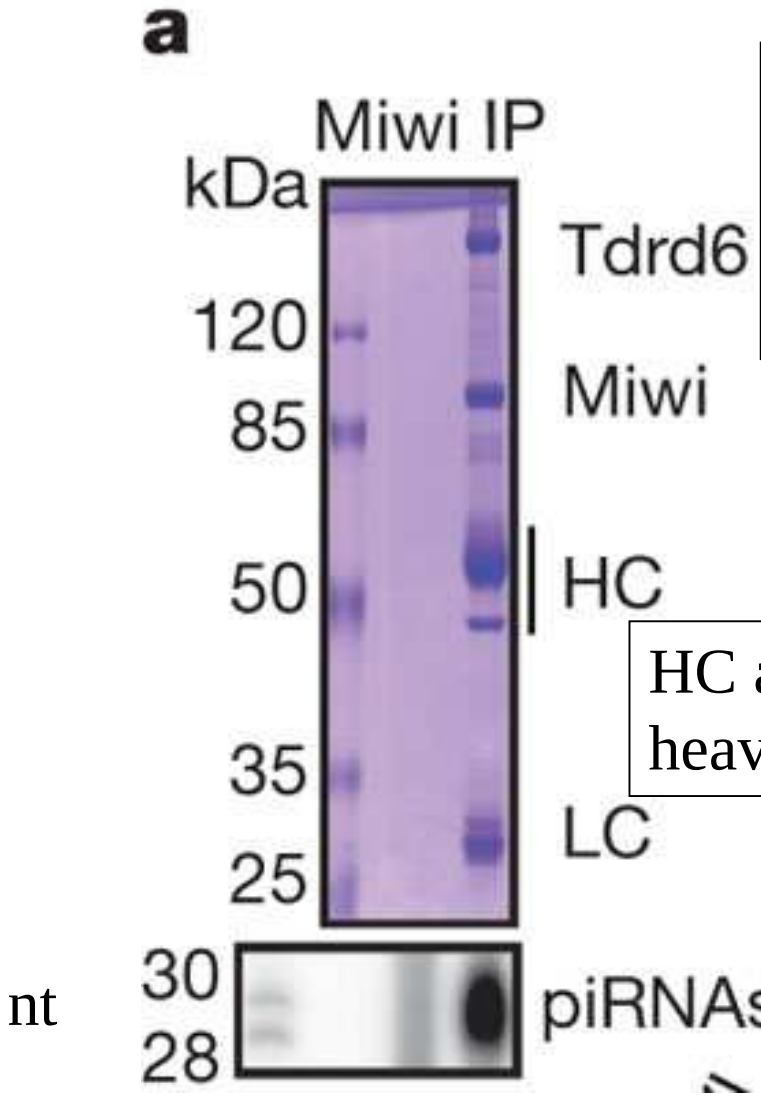
domain structure of PIWI - argonaute proteins

SLICER

- Posttranscriptional transposon silencing bases on degradation of transposon transcripts

- to prevent either reverse transcription followed by genomic reintegration or
- To prevent translation into proteins that mediate the transposition process

Miwi complexes and 5′-end-labelled small RNAs (piRNAs).



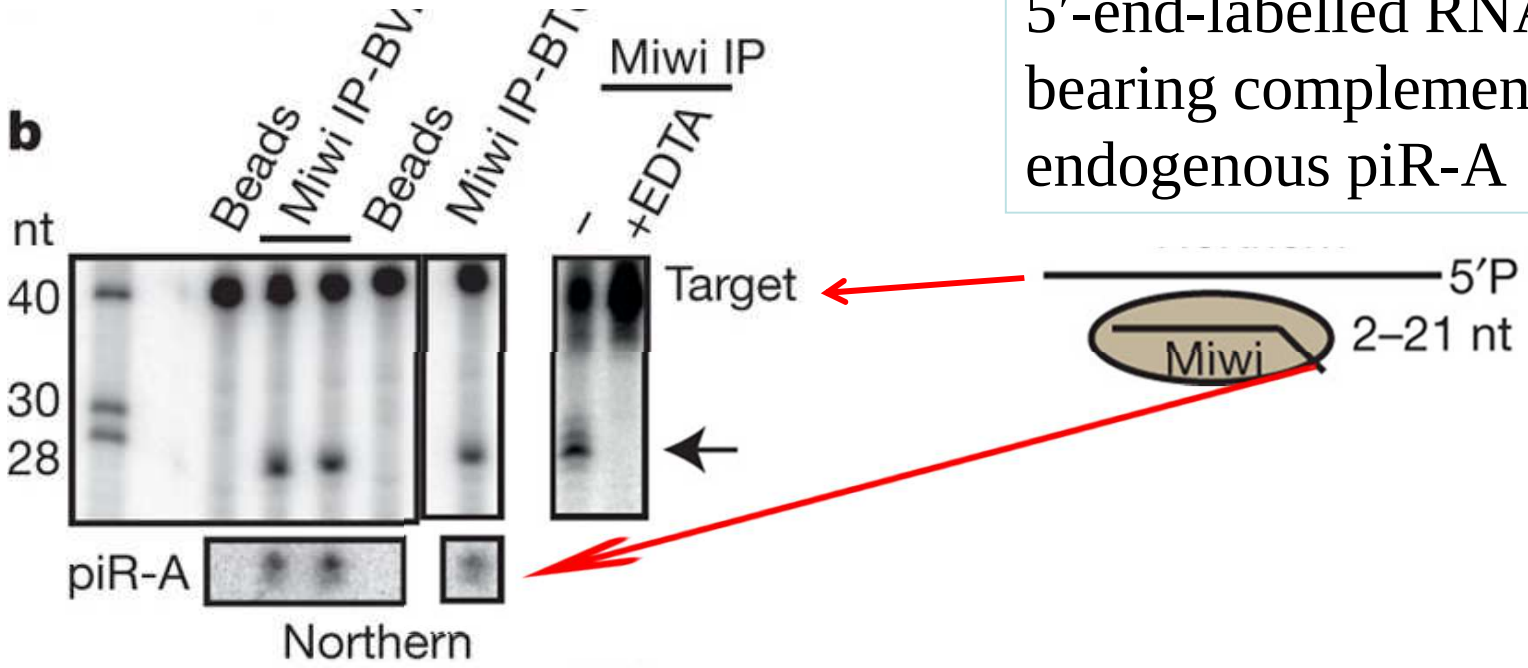
TDRD Tudor domain:
A multidomain protein mediating Complex formation with other participants in piRNA biogenesis.

HC and LC: antibody heavy and light chains

nature

M Reuter *et al*. *Nature* **000**, 1-4 (2011) doi:10.1038/nature10672

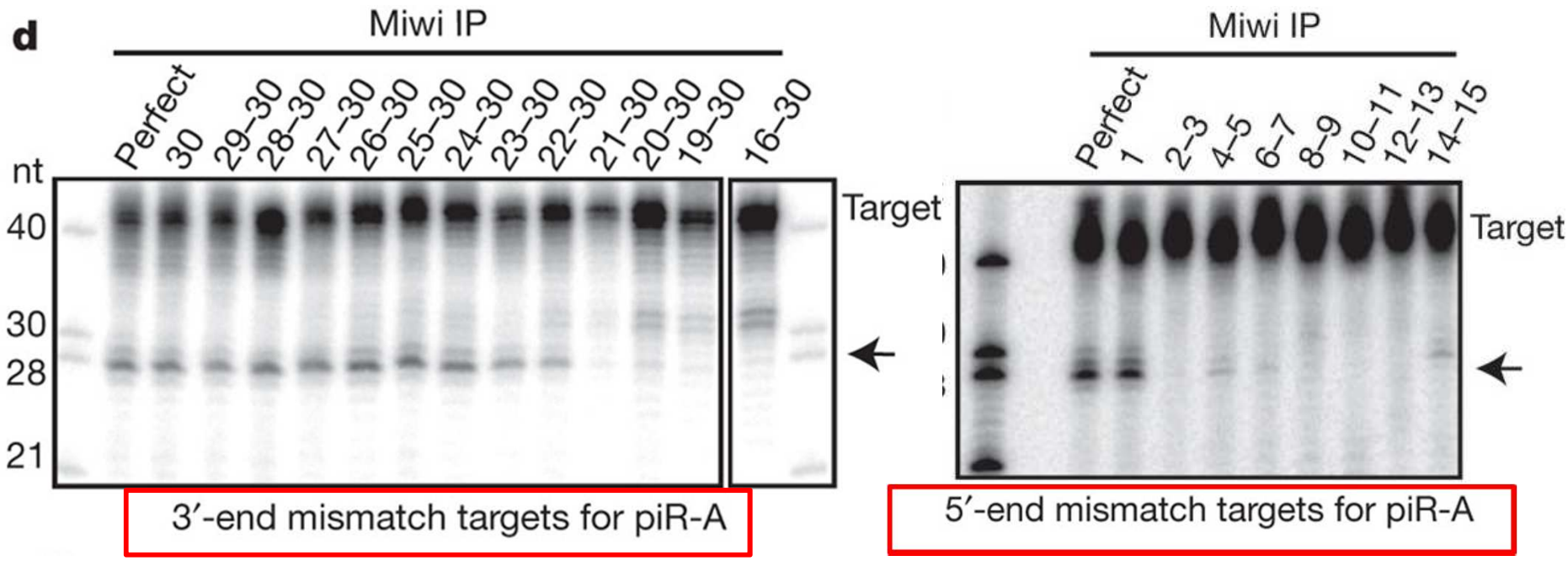
Slicer assays with Miwi complexes (immunopurified)
or beads (control)



5′-end-labelled RNA target bearing complementarity to endogenous piR-A

M Reuter *et al*. *Nature* **000**, 1-4 (2011) doi:10.1038/nature10672

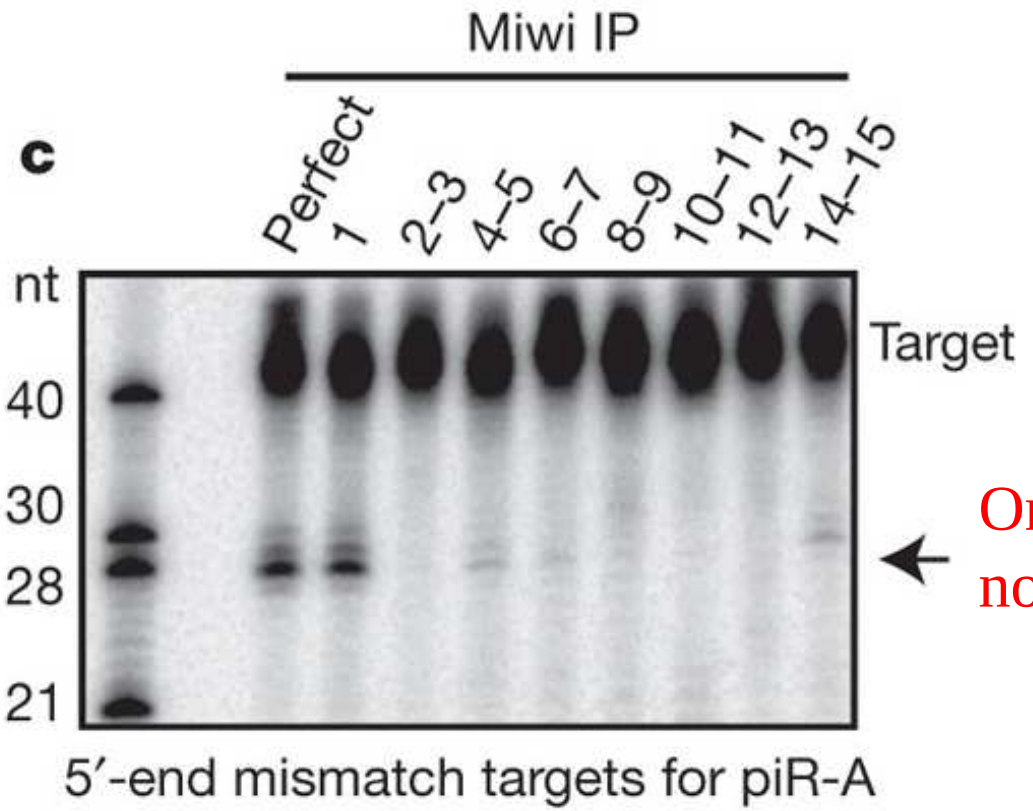
Miwi is a small RNA-guided RNase (slicer) that requires extensive complementarity for target cleavage



nature

M Reuter *et al*. *Nature* **000**, 1-4 (2011) doi:10.1038/nature10672

Miwi is a slicer requiring extensive 5' complementarity for target cleavage.



One mismatch
no function

The slicer activity depends on a catalytic motif (Asp-Asp-His; DDH motif)

Miwi mice heterozygous (Miwi1/ADH) for a point mutation in Miwi at the first aspartate (D633A, ADH) of the catalytic motif were sterile

nature

M Reuter *et al*. *Nature* **000**, 1-4 (2011) doi:10.1038/nature10672