

Come inibire l'espressione e trasposizione del Trasposone

Negative Control of the transcriptional activity of L1 in ES cells

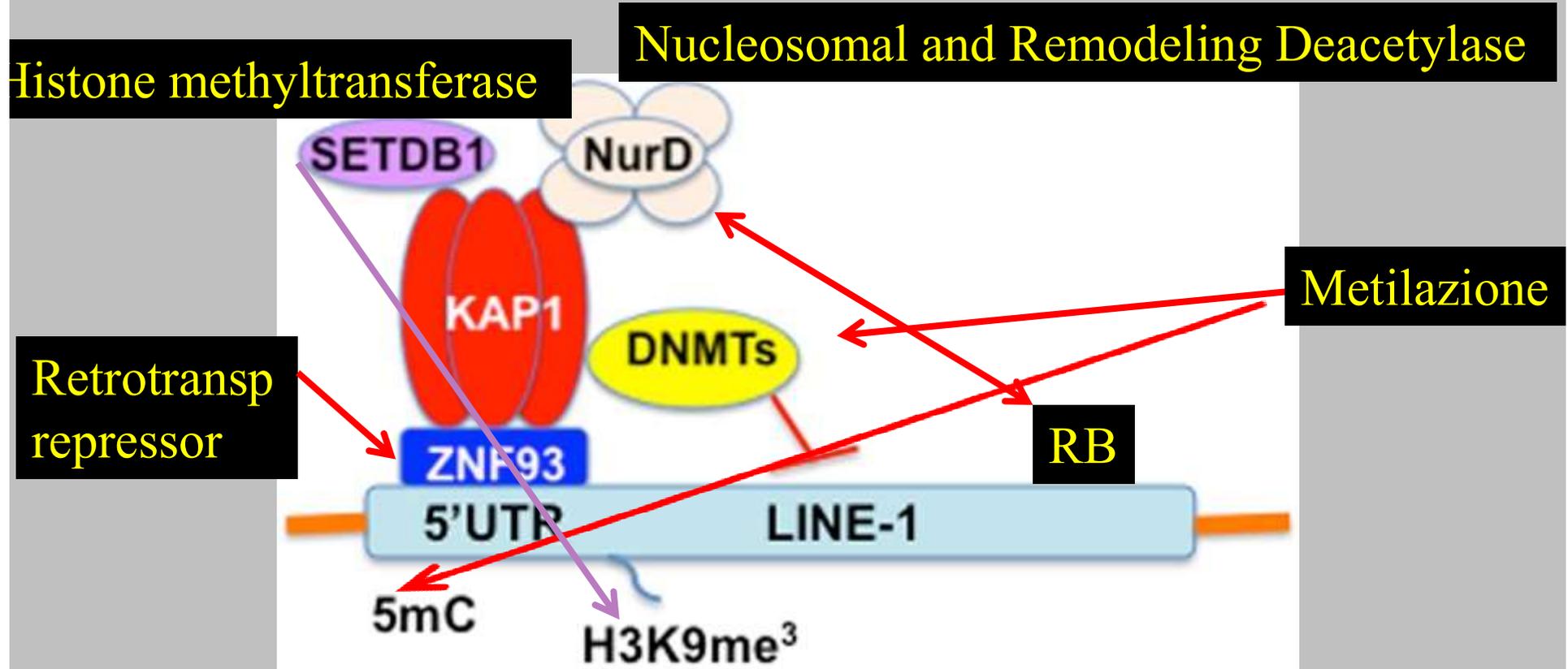
Histone methyltransferase

Nucleosomal and Remodeling Deacetylase

Metilazione

Retrotransp
repressor

Negative Control of the transcriptional activity of L1 in ES cells



KAP1 serves as a scaffold for heterochromatin complexes

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Riconoscimento delle sequenze L1

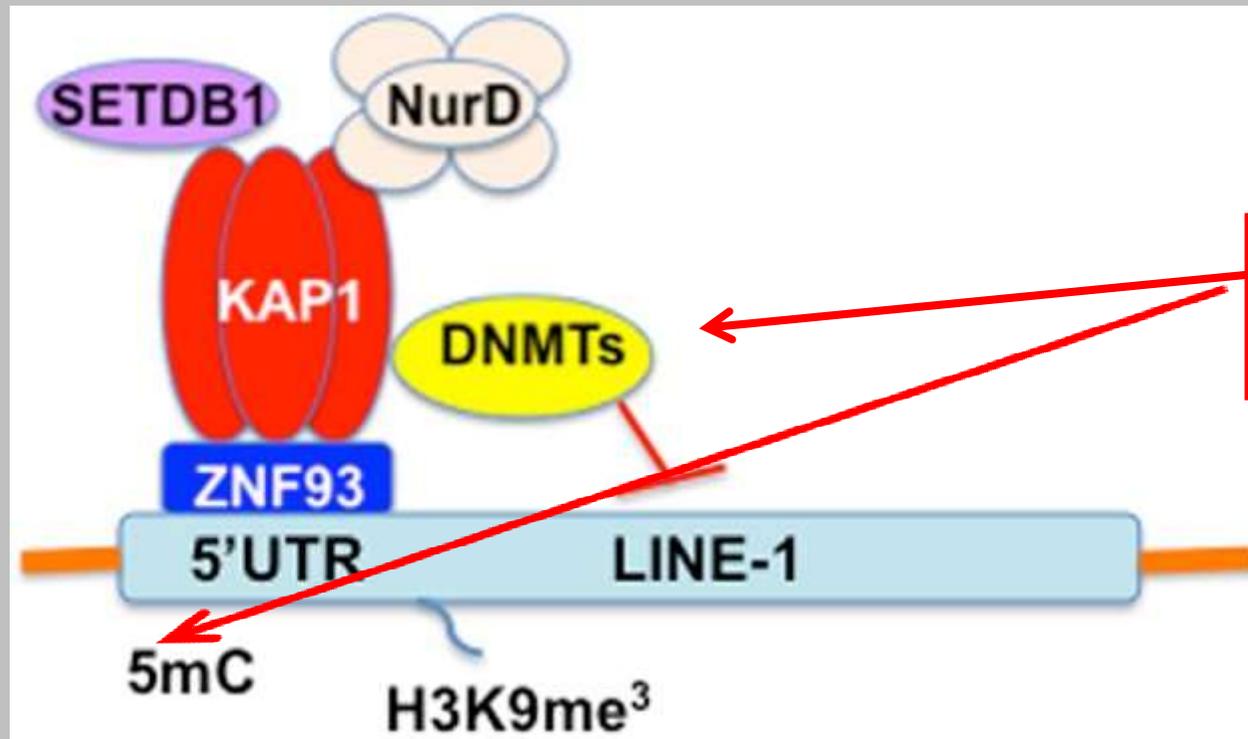
Le GUIDE - piRNA

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.
- ❑ Complex intracellular events from transcription of piRNA sources to piRNA maturation

Negative Control of the transcriptional activity of L1 in ES cells



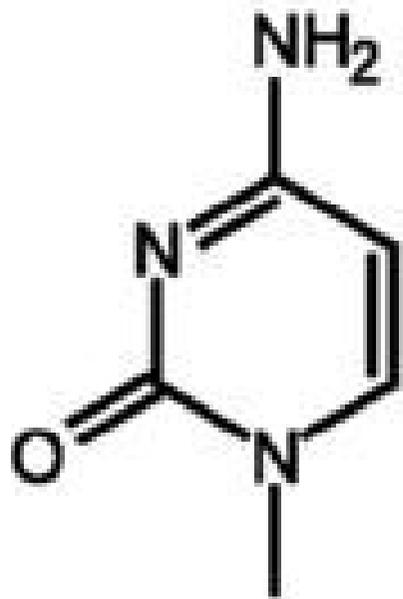
Metilazione
DNA

Come inibire l'espressione e trasposizione del Trasposone

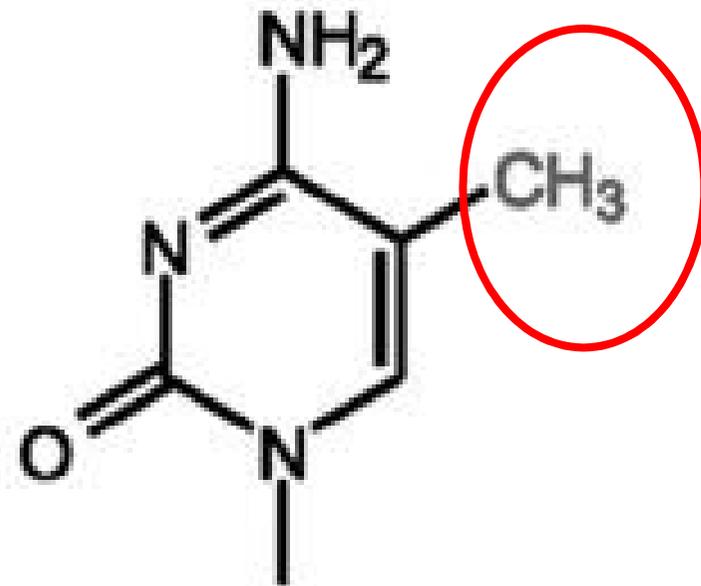
Mammalian genomes use CpG DNA **methylation** to silence these genomic parasites.

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)**



Cytosine



5-Methylcytosine

Rivelazione indiretta delle metil-citosine

Bisulfite-mediated conversion of cytosine to Uracil

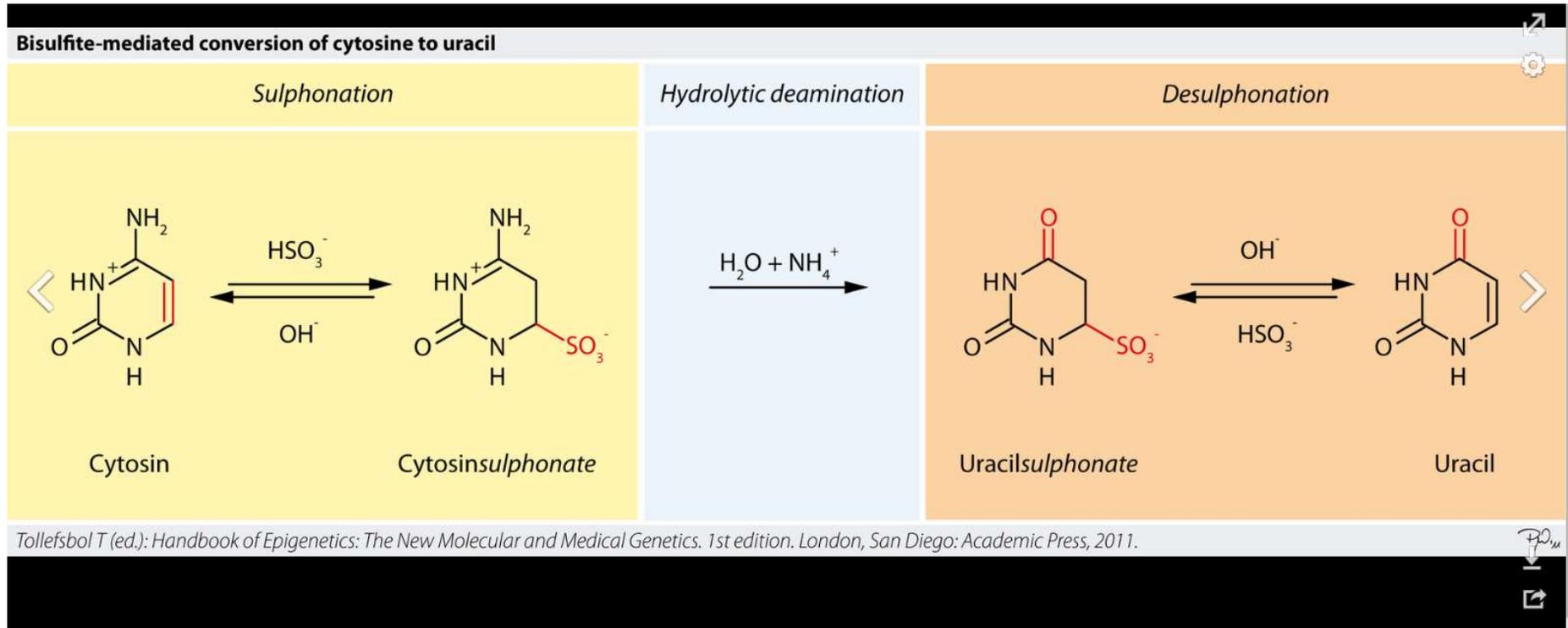


Figure 2: Outline of the chemical reaction that underlies the bisulfite-mediated conversion of cytosine to uracil.

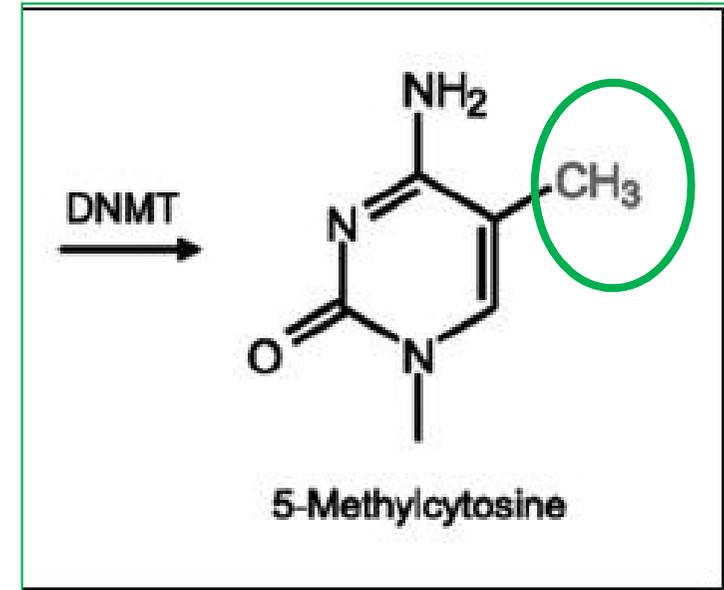
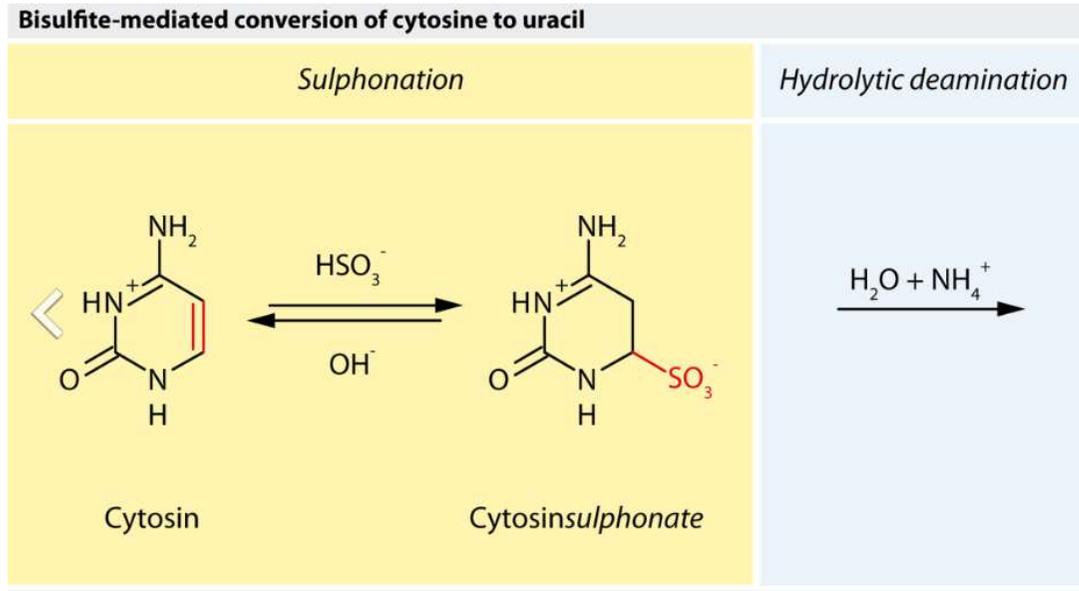
[More details](#)

C
G

->

U
A

Bisulfite-mediated conversion of cytosine to Uracil



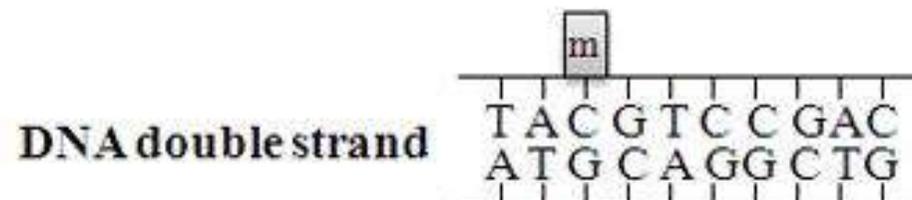
MC

G

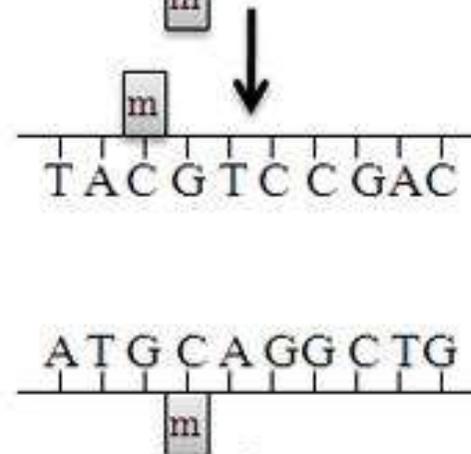
MC

G

Bisulfite does **NOT** convert 5MC-to Uracil

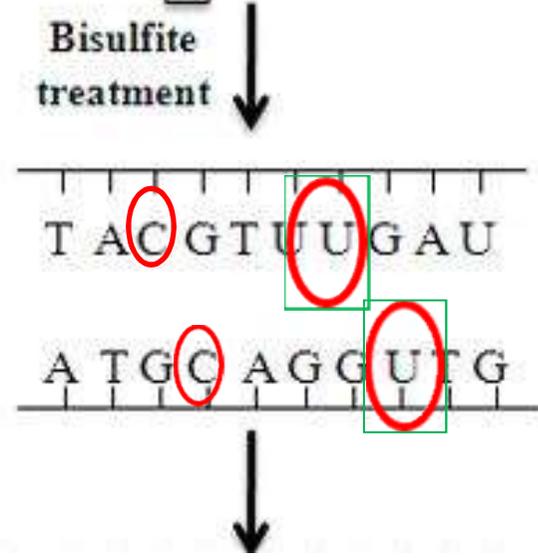


Denaturation

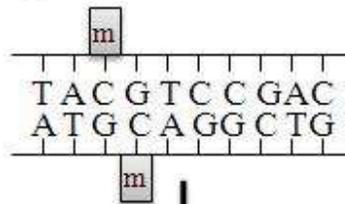


Bisulfite-mediated conversion

DNA conversion



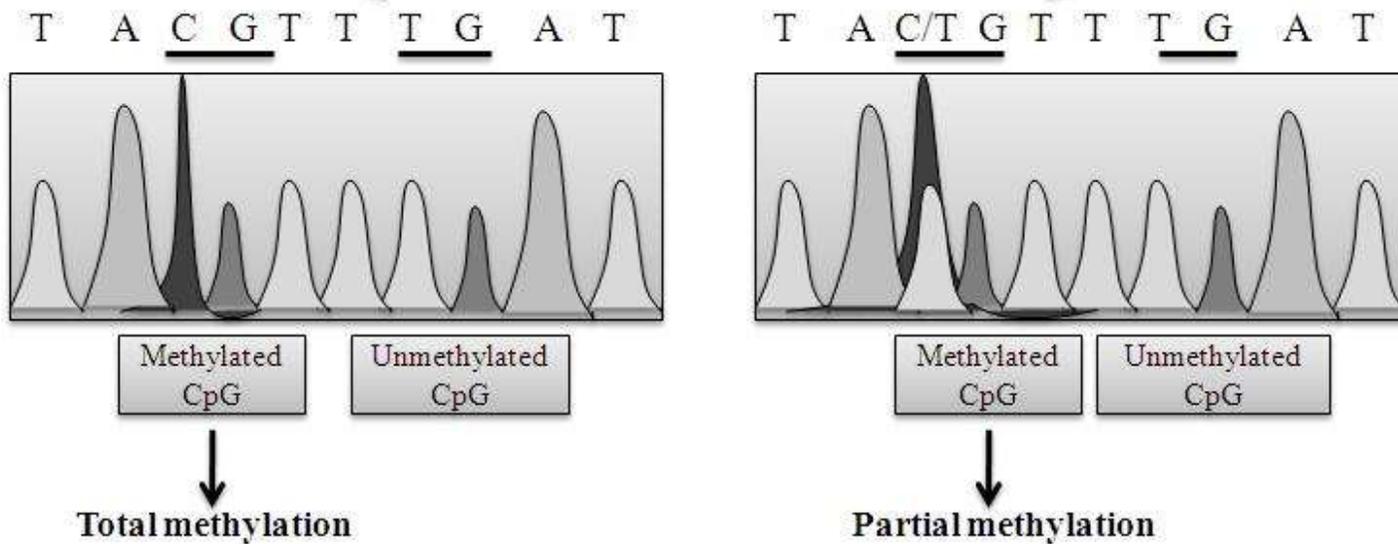
Original DNA double strand



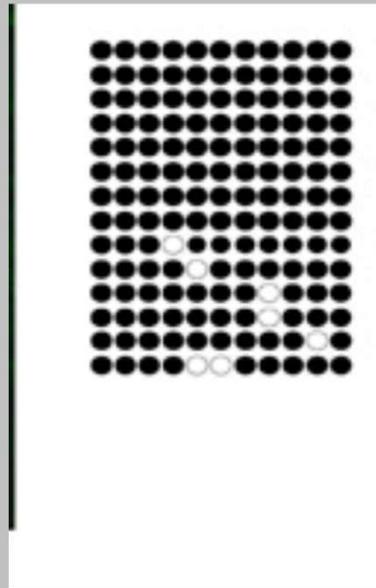
Bisulfite conversion

PCR amplification

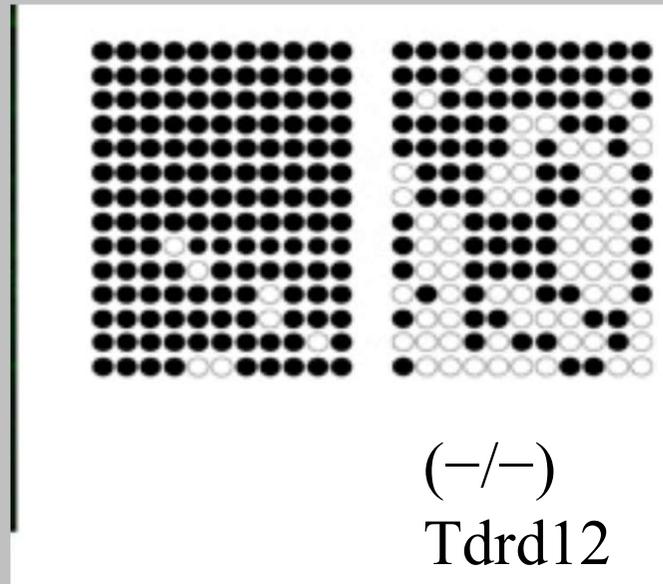
Interpretation of sequencing data



CpG DNA methylation (filled circles) on transposon L1 Promoters

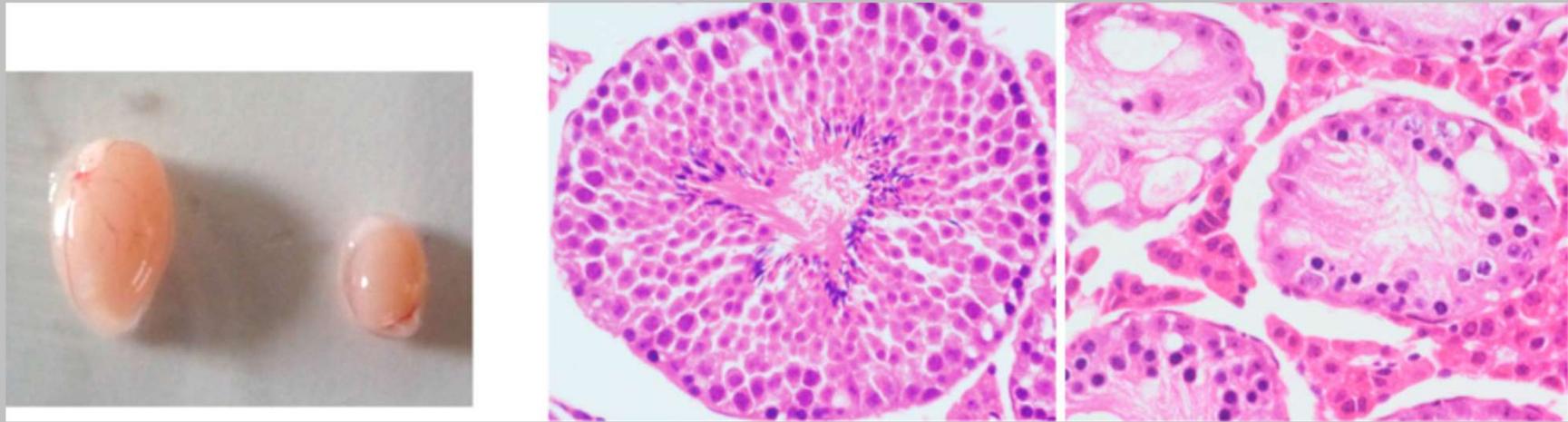


CpG DNA methylation (filled circles) on transposon L1 Promoters



Tdrd12 mutant -

Tdrd12 mutant male mice are infertile and display derepression of retrotransposons



Atrophied testes of homozygous
(-/-) Tdrd12 mutants

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

Tdrd family proteins (Tudor domain **Tdrd**) participate in **piRNA biogenesis**

The **Tdrd12** interacts with the piRNA pathway and regulates piRNA biogenesis

TDRD12 (Tudor Domain) is detected in complexes containing

Piwi protein **MILI**,

piRNAs,

TDRD1 (piRNA biogenesis)

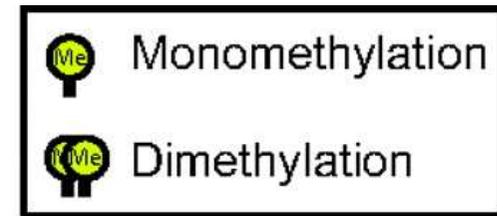
Male mice carrying either a nonsense point mutation or a targeted deletion in the *Tdrd12* locus are **infertile** and **derepress retrotransposons**.

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

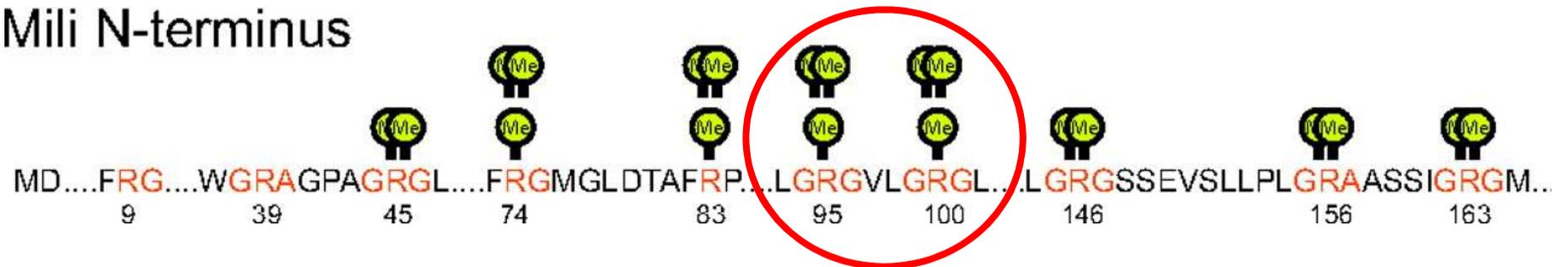
METILAZIONE DELLE PROTEINE

Arginine methylation sites detected on endogenous Miwi and Mili by mass spectrometry.

Miwi N-terminus



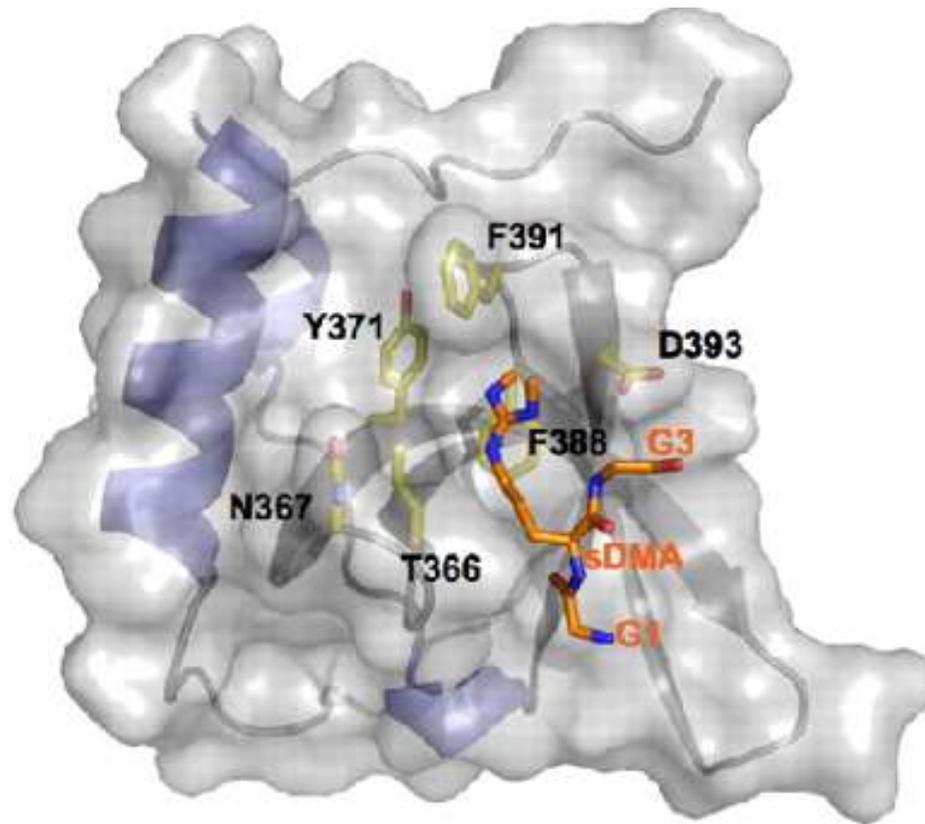
Mili N-terminus



Chen Chen et al. PNAS 2009;106:48:20336-20341

PNAS

Crystal structure of the Tudor domain



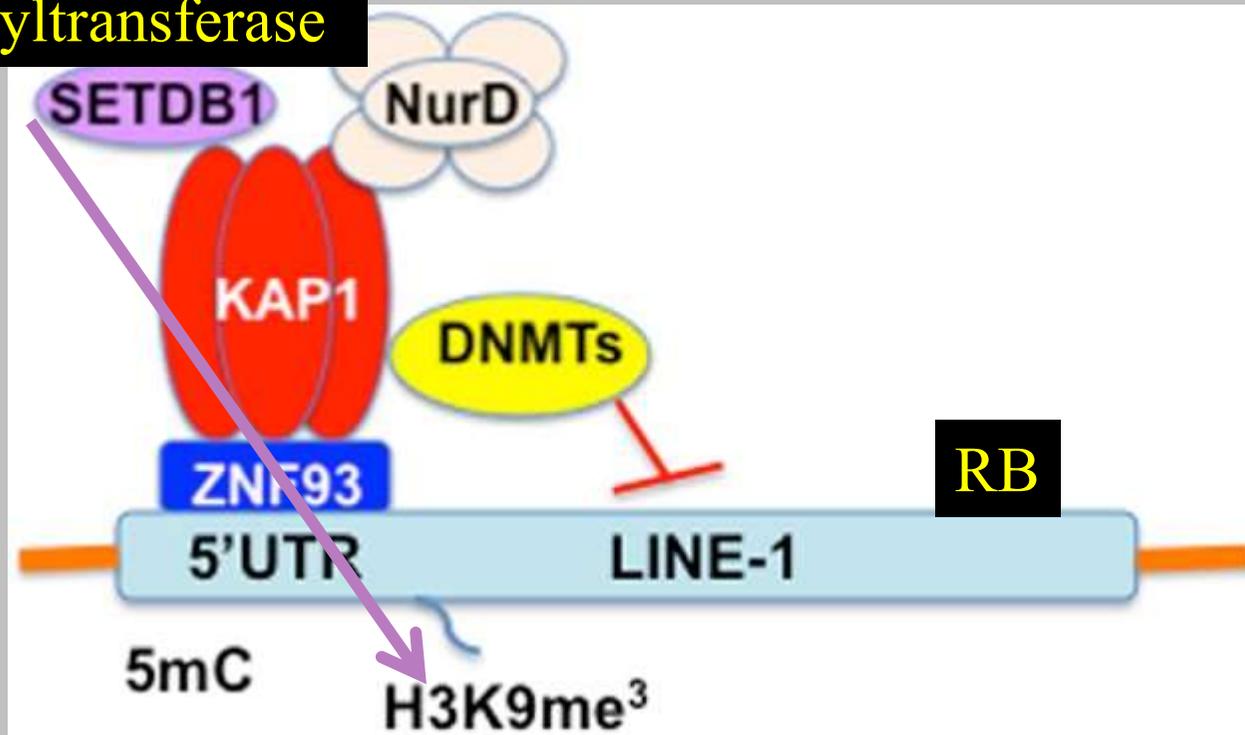
Molecular docking of a GRG peptide

Chen Chen et al. PNAS 2009;106:48:20336-20341

PNAS

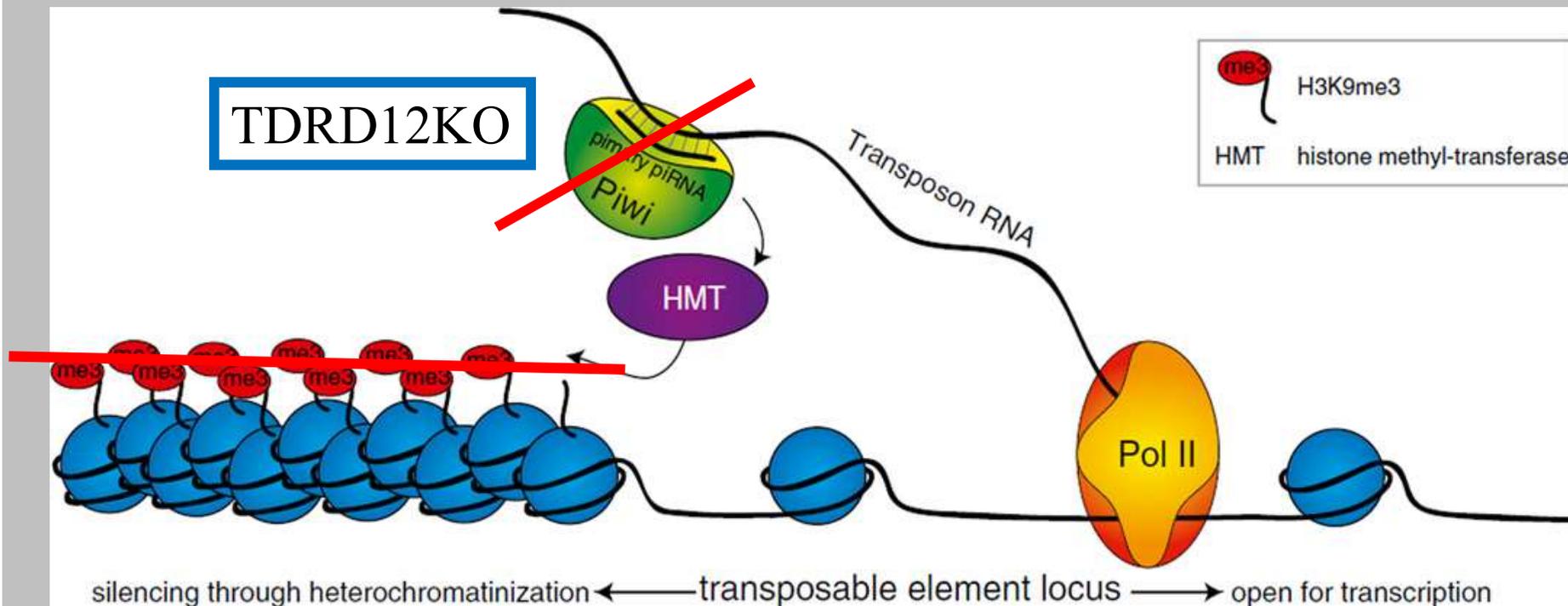
Negative Control of the transcriptional activity of L1 in ES cells

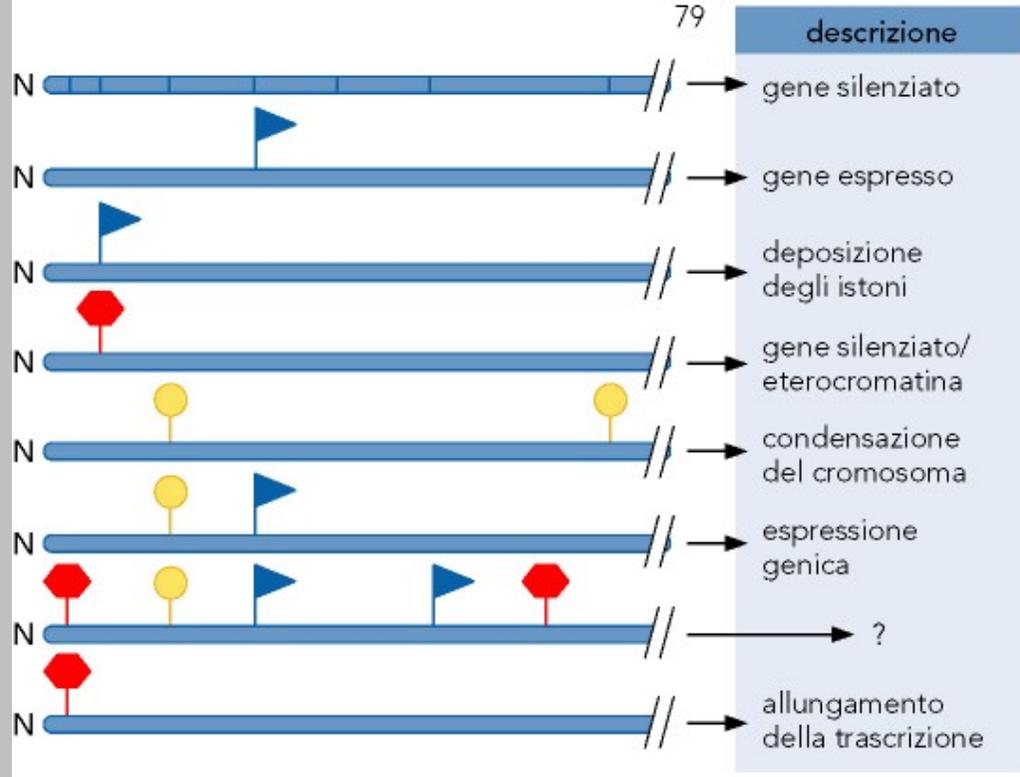
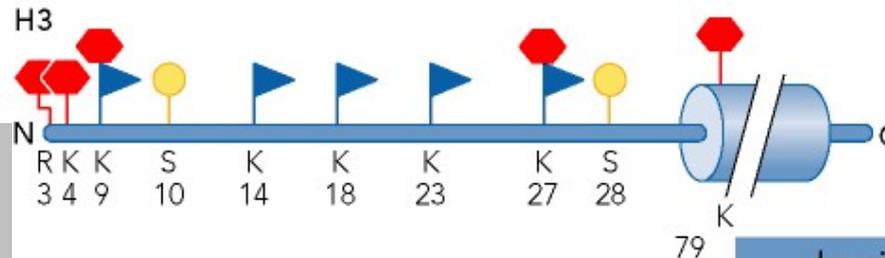
Histone methyltransferase

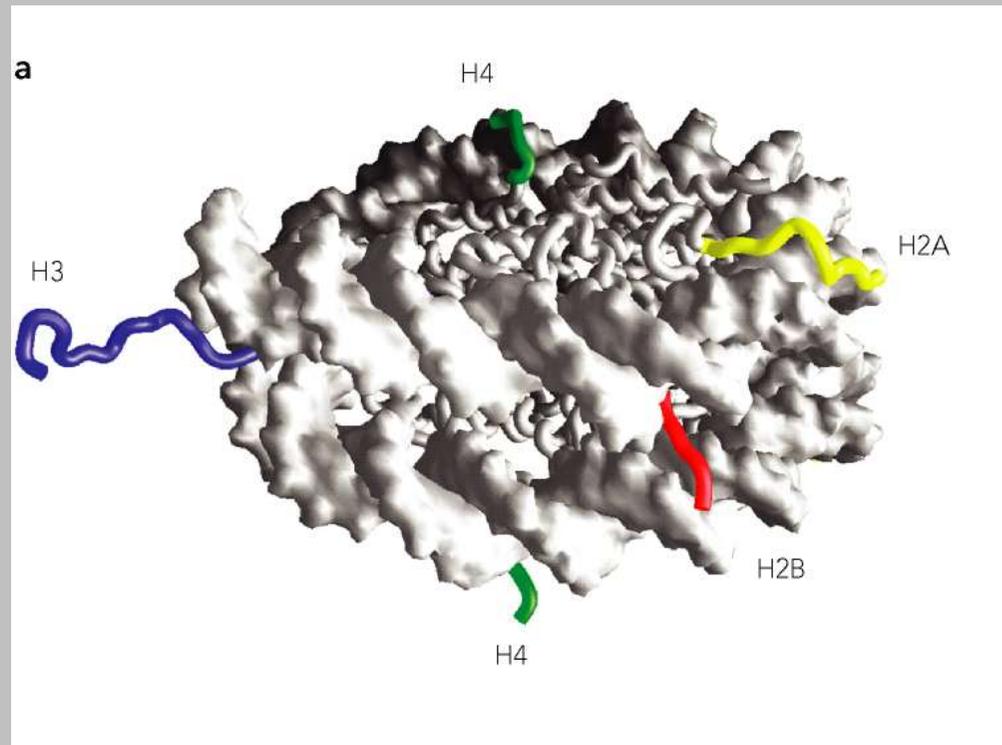


KAP1 serves as a scaffold for heterochromatin complexes

nuclear function of the piRNA pathway







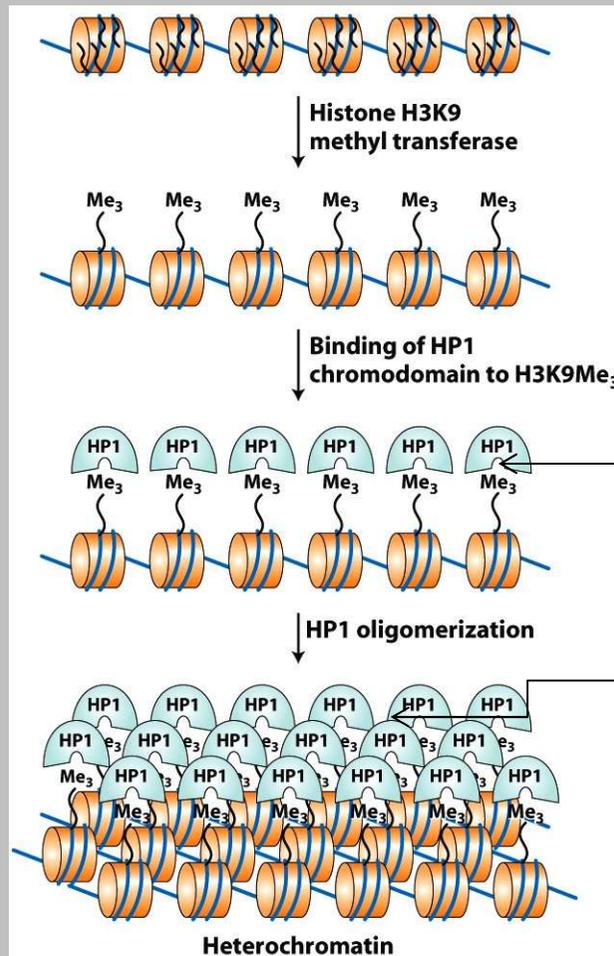
Heterochromatin (inactive/condensed)



Euchromatin (active/open)



Figure 6-33b
Molecular Cell Biology, Sixth Edition
© 2008 W. H. Freeman and Company



Cromodominio

Figure 6-34a
Molecular Cell Biology, Sixth Edition
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Come inibire l'espressione e trasposizione del Trasposone

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

three Piwi proteins

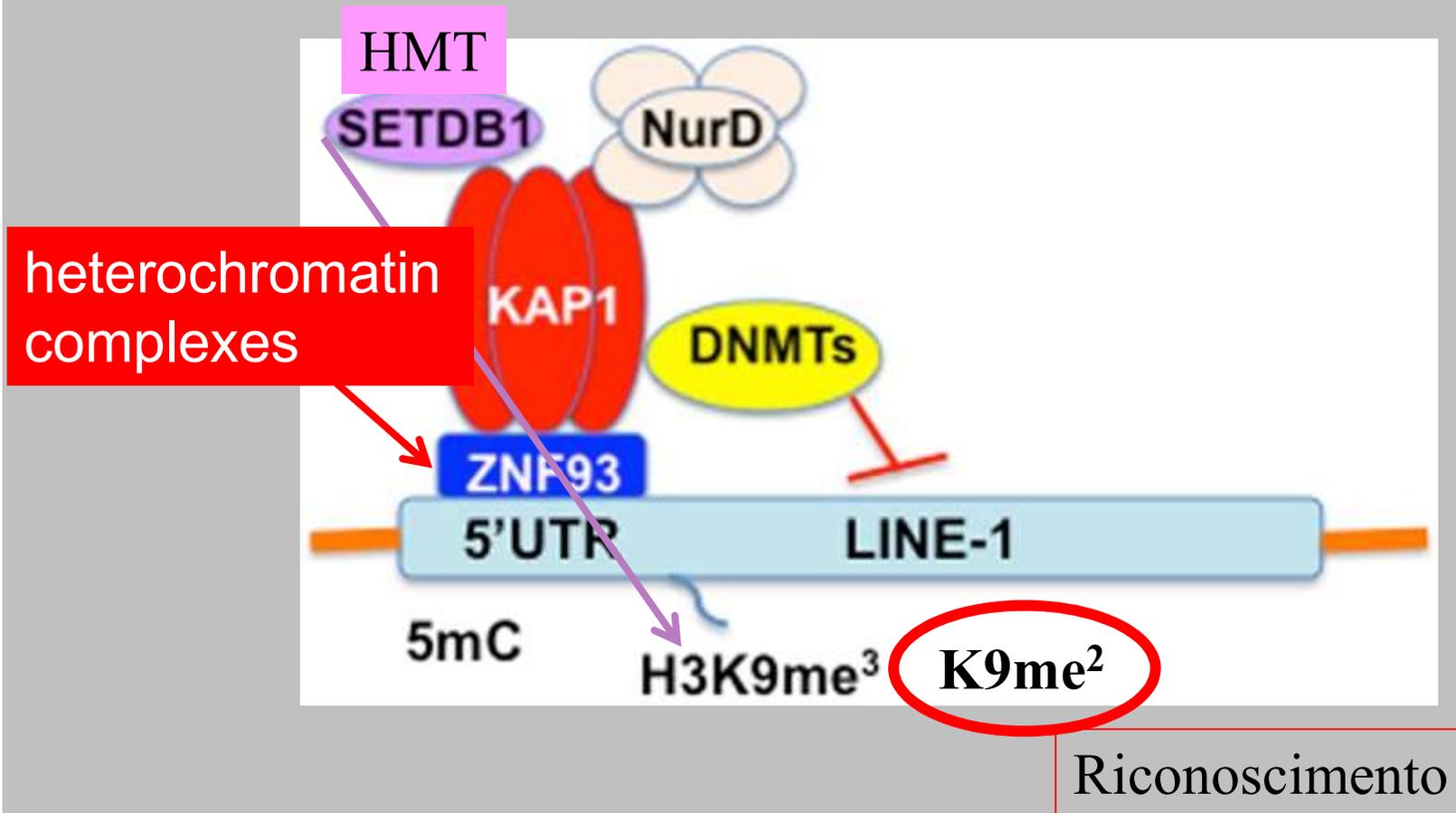
MILI Chromatin

MIWI

MIWI2

Nuclear

Negative Control of the transcriptional activity of L1 in ES cells



HMT istone metiltransferasi

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

three Piwi proteins

MILI

MIWI

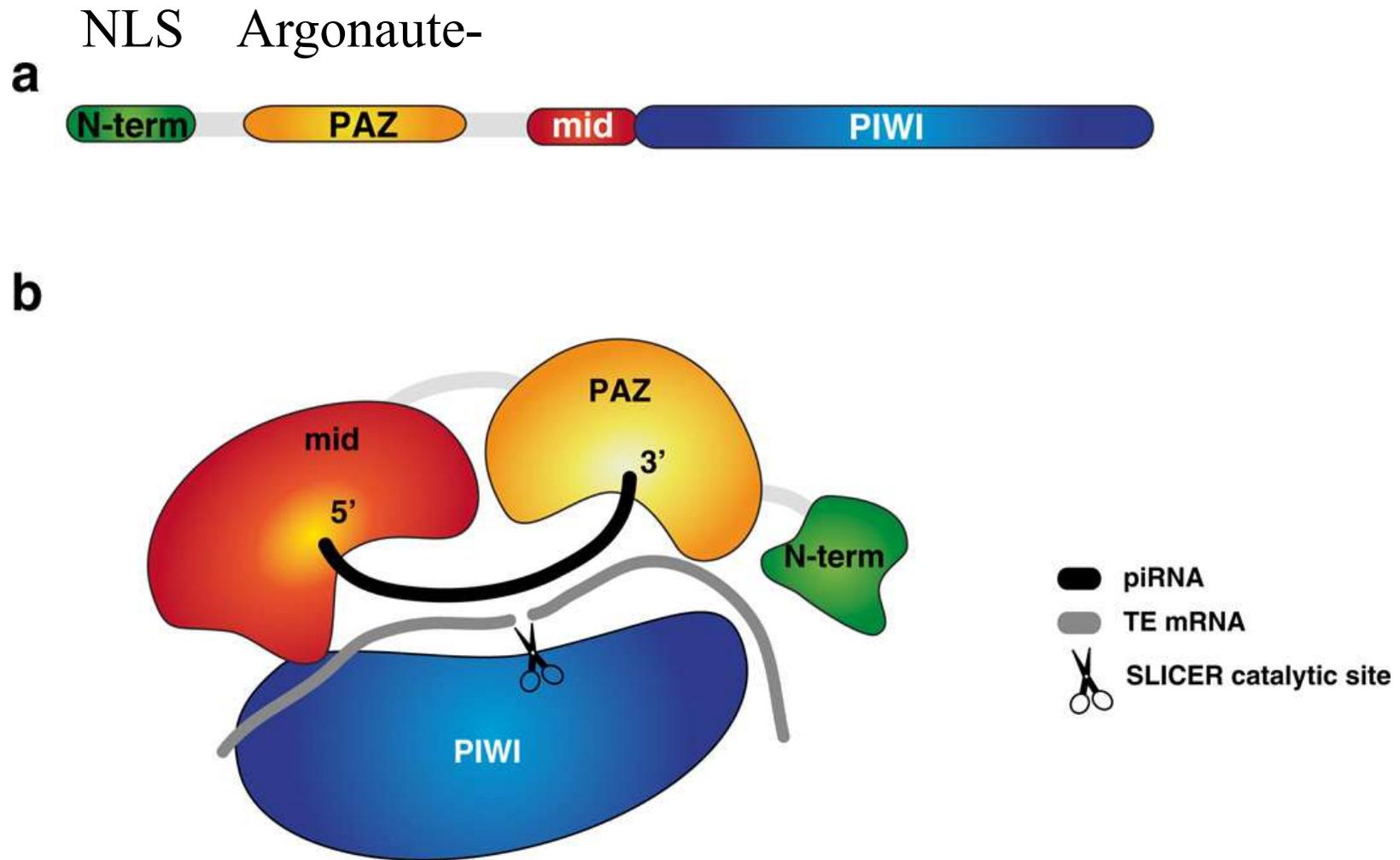
MIWI2

Cytoplasmic **MIWI** have a role in maintaining repression by **direct cleavage** of transposon transcripts using their endonucleolytic

(**Slicer**) cleavage activity

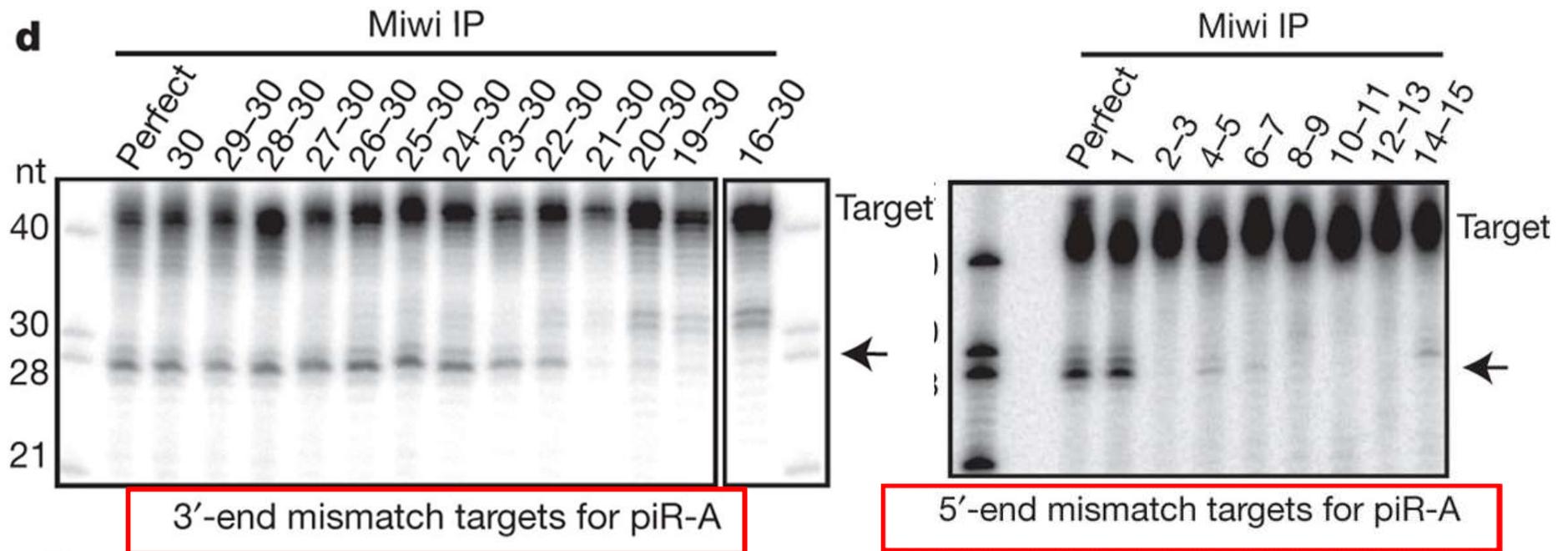
SLICER

- 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



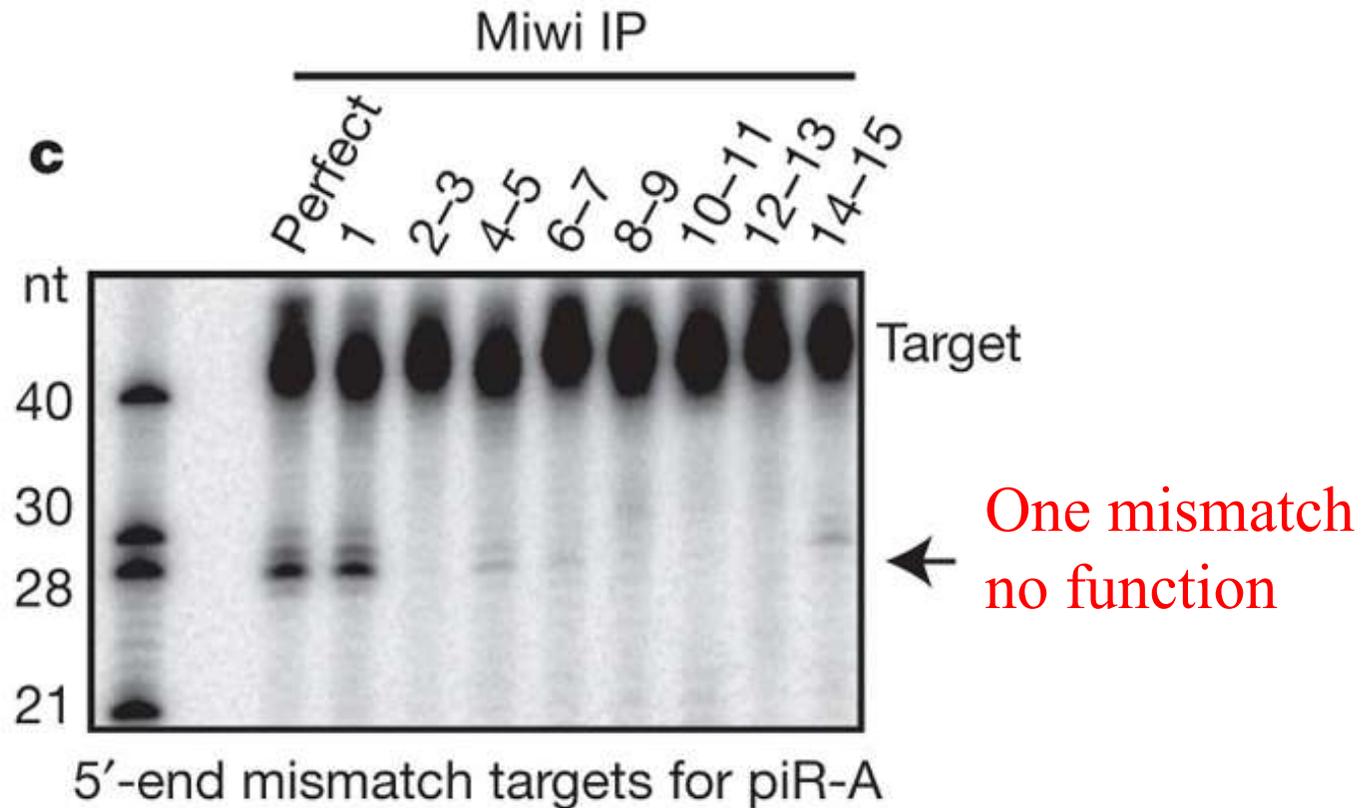
domain structure of PIWI - argonaute proteins

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



nature

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



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