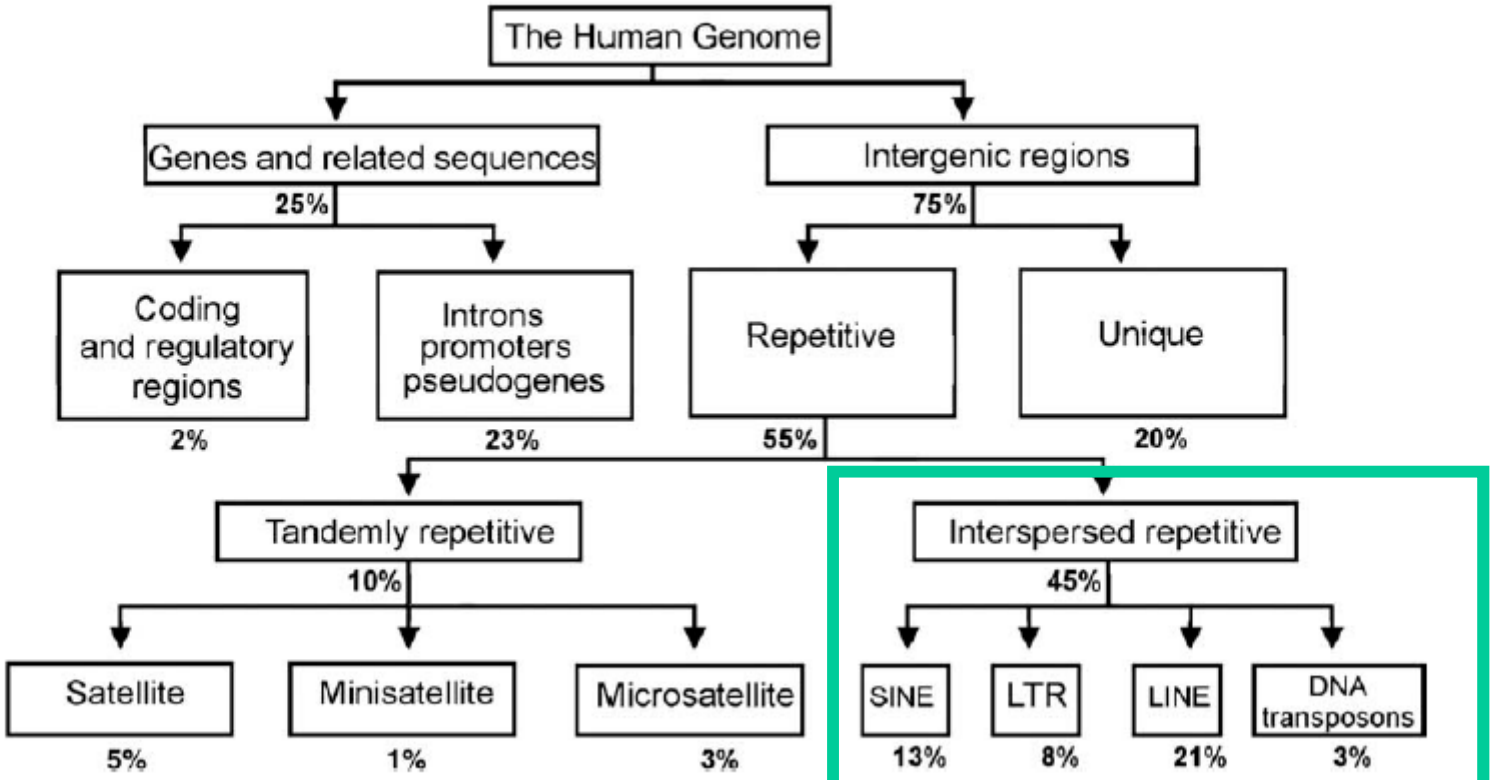
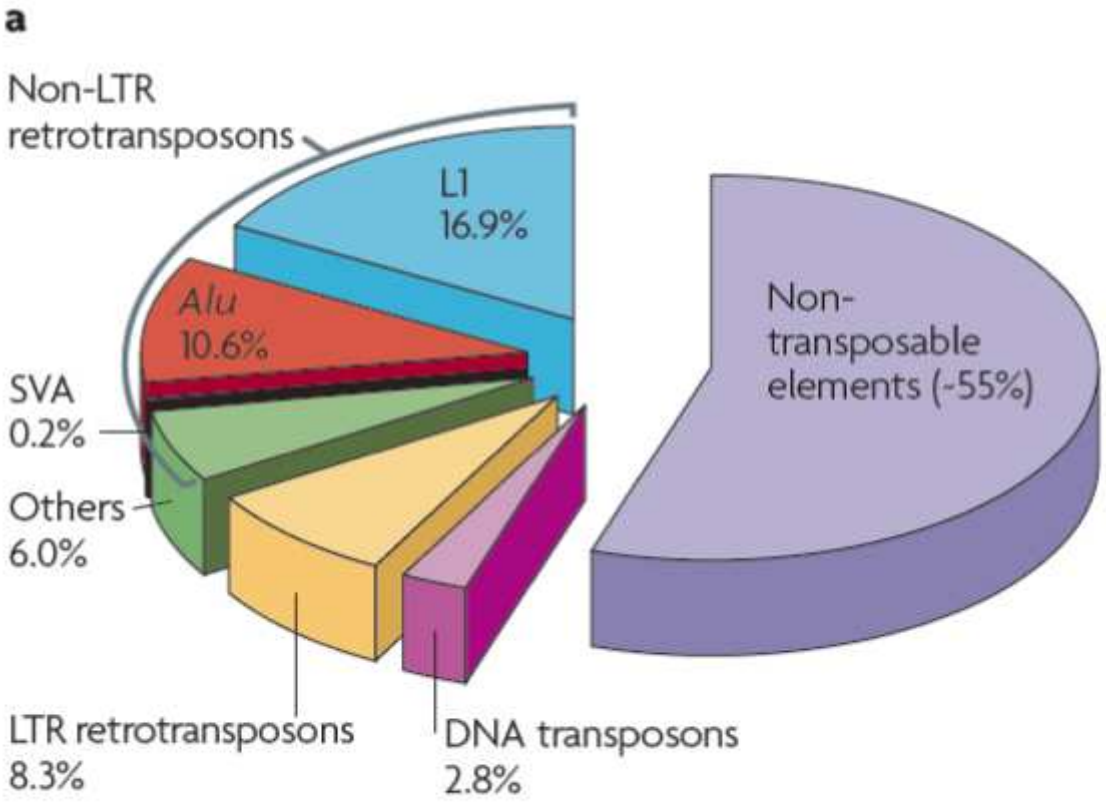
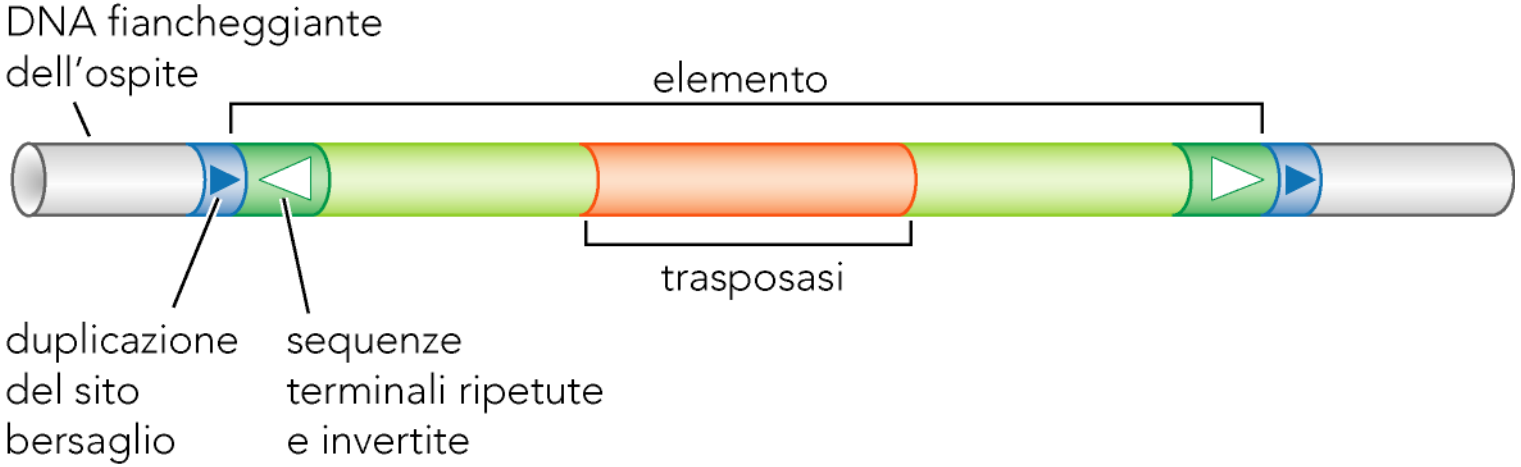


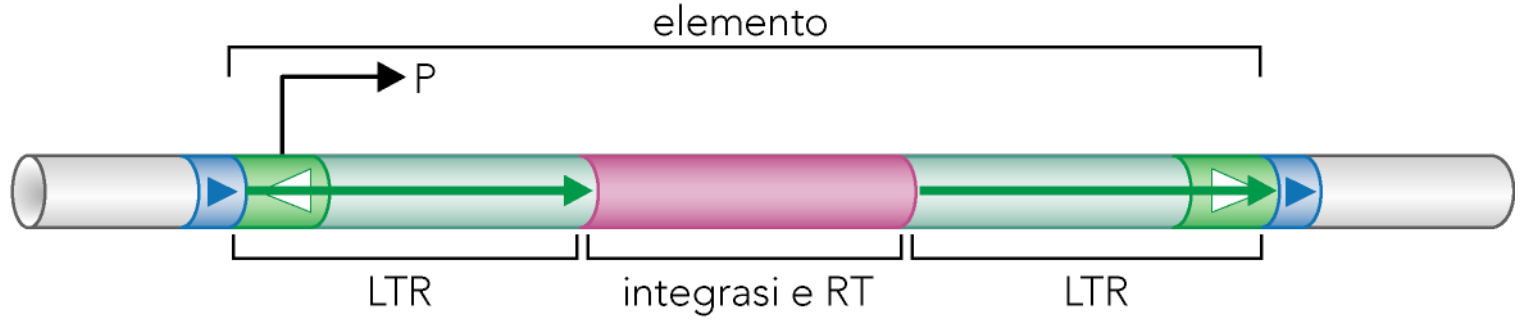
Fig. 1. Composition of the human genome. The percentage shares of various functional and non-functional sequences are shown.


a
Non-LTR retrotransposons
L1 16.9%
Alu 10.6%
SVA 0.2%
Others 6.0%
LTR retrotransposons 8.3%
DNA transposons 2.8%
Non-transposable elements (~55%)

a trasposoni a DNA



b retrotrasposoni tipo virus/retrovirus



c retrotrasposoni poli-A

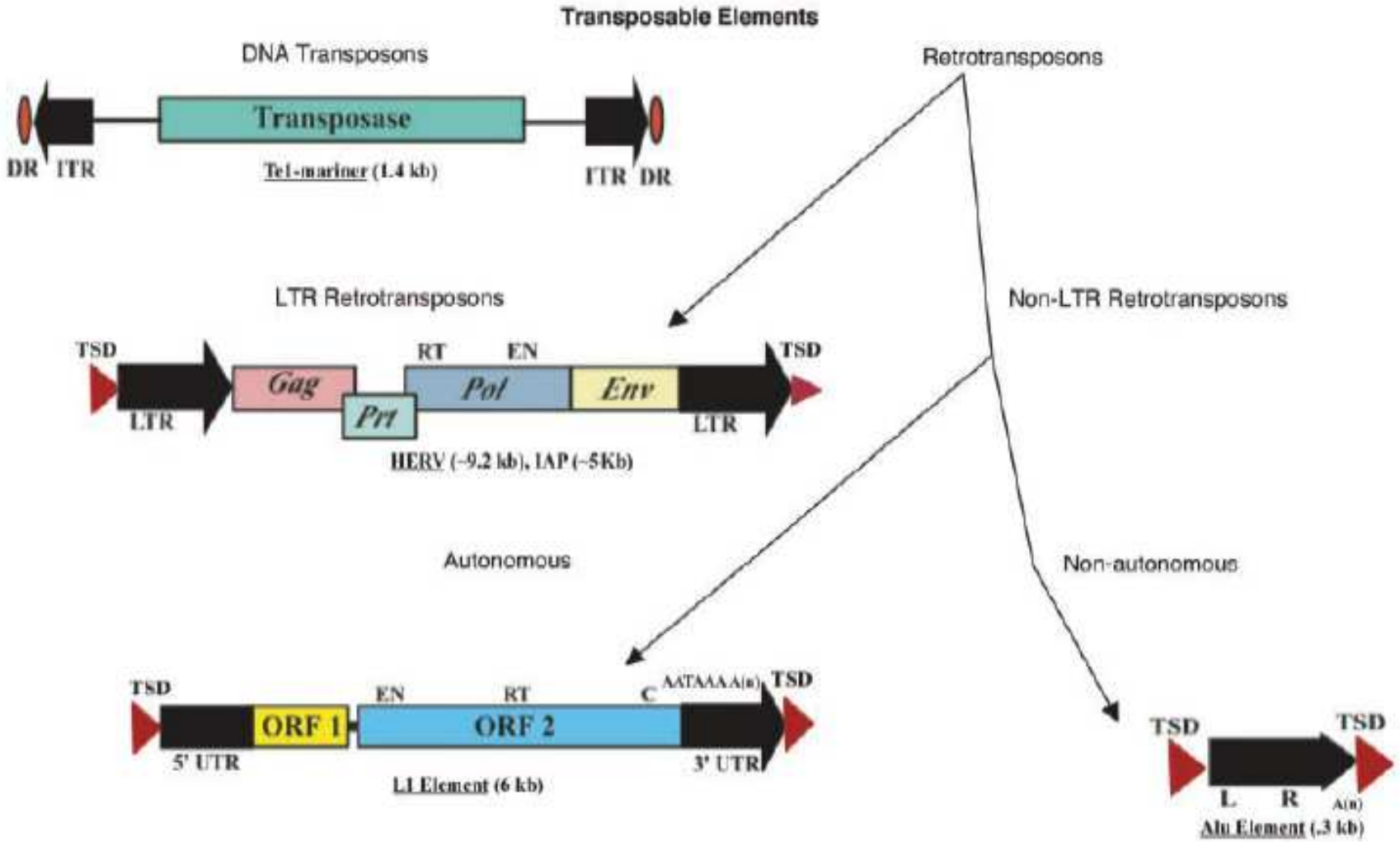
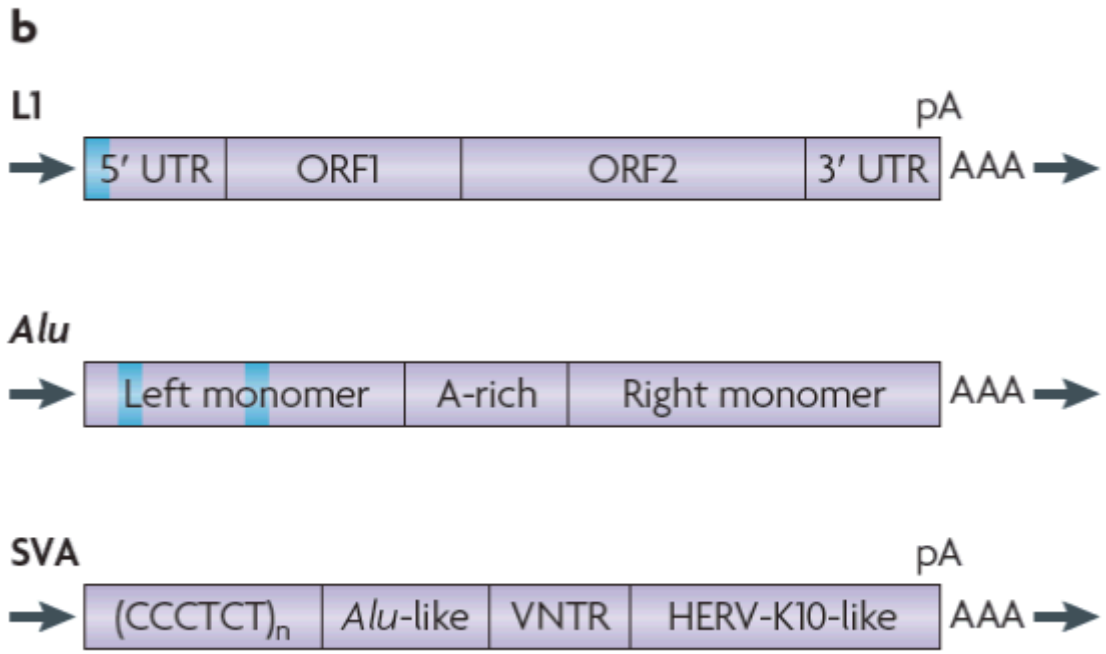


Fig. 1. Classes of mobile elements. DNA transposons, e.g., Tc-1/mariner, have inverted terminal inverted repeats (ITRs) and a single open reading frame (ORF) that encodes a transposase. They are flanked by short direct repeats (DRs). Retrotransposons are divided into autonomous and nonautonomous classes depending on whether they have ORFs that encode proteins required for retrotransposition. Common autonomous retrotransposons are (i) LTRs or (ii) non-LTRs (see text for


b
L1
pA
5′ UTR
ORF1
ORF2
3′ UTR
AAA
Alu
Left monomer
A-rich
Right monomer
AAA
SVA
pA
(CCCTCT)n
Alu-like
VNTR
HERV-K10-like
AAA

REVIEW

Mobile Elements: Drivers of Genome Evolution

Haig H. Kazazian Jr.*

Mobile elements within genomes have driven genome evolution in diverse ways. Particularly in plants and mammals, retrotransposons have accumulated to constitute a large fraction of the genome and have shaped both genes and the entire genome. Although the host can often control their numbers, massive expansions of retrotransposons have been tolerated during evolution. Now mobile elements are becoming useful tools for learning more about genome evolution and gene function.

Mobile, or transposable, elements are prevalent in the genomes of all plants and animals. Indeed, in mammals they and their recognizable remnants account for nearly half of the genome (*1*, *2*), and in some plants they constitute up to 90% of the genome (*3*). If, as many believe, the origins of life are in an "RNA world" followed by reverse transcription into DNA, then mobile elements could

Because sequence specificity of integration is limited to a small number of nucleotides—e.g., TA dinucleotides for Tc1 of *Caenorhabditis elegans*—insertions can occur at a large number of genomic sites. However, daughter insertions for most, but not all, DNA transposons occur in proximity to the parental insertion. This is called "local hopping." Active transposons encode a transposase enyme

residues, then a glutamate) and a handlike three-dimensional structure (*6*, *8*).

Although these elements generally transpose to genomic sites less than 100 kb from their original site (e.g., the *Drosophila* P element), some are able to make distant "hops" (e.g., the fish Tc1/mariner element; see below).

LTR Retrotransposons

Retrotransposons are transcribed into RNA, and then reverse transcribed and reintegrated into the genome, thereby duplicating the element. The major classes of retrotransposons either contain long terminal repeats at both ends (LTR retrotransposons) or lack LTRs and possess a polyadenylate sequence at their 3′ termini (non-LTR retrotransposons).

LTR retrotransposons and retroviruses are

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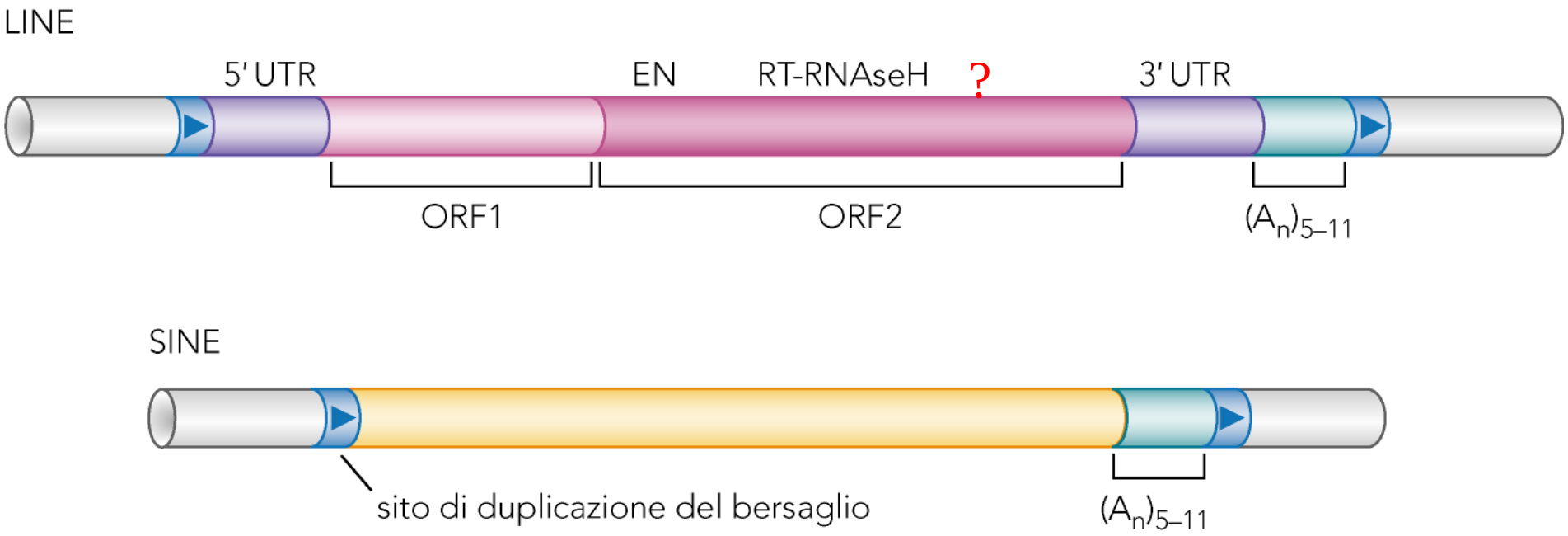
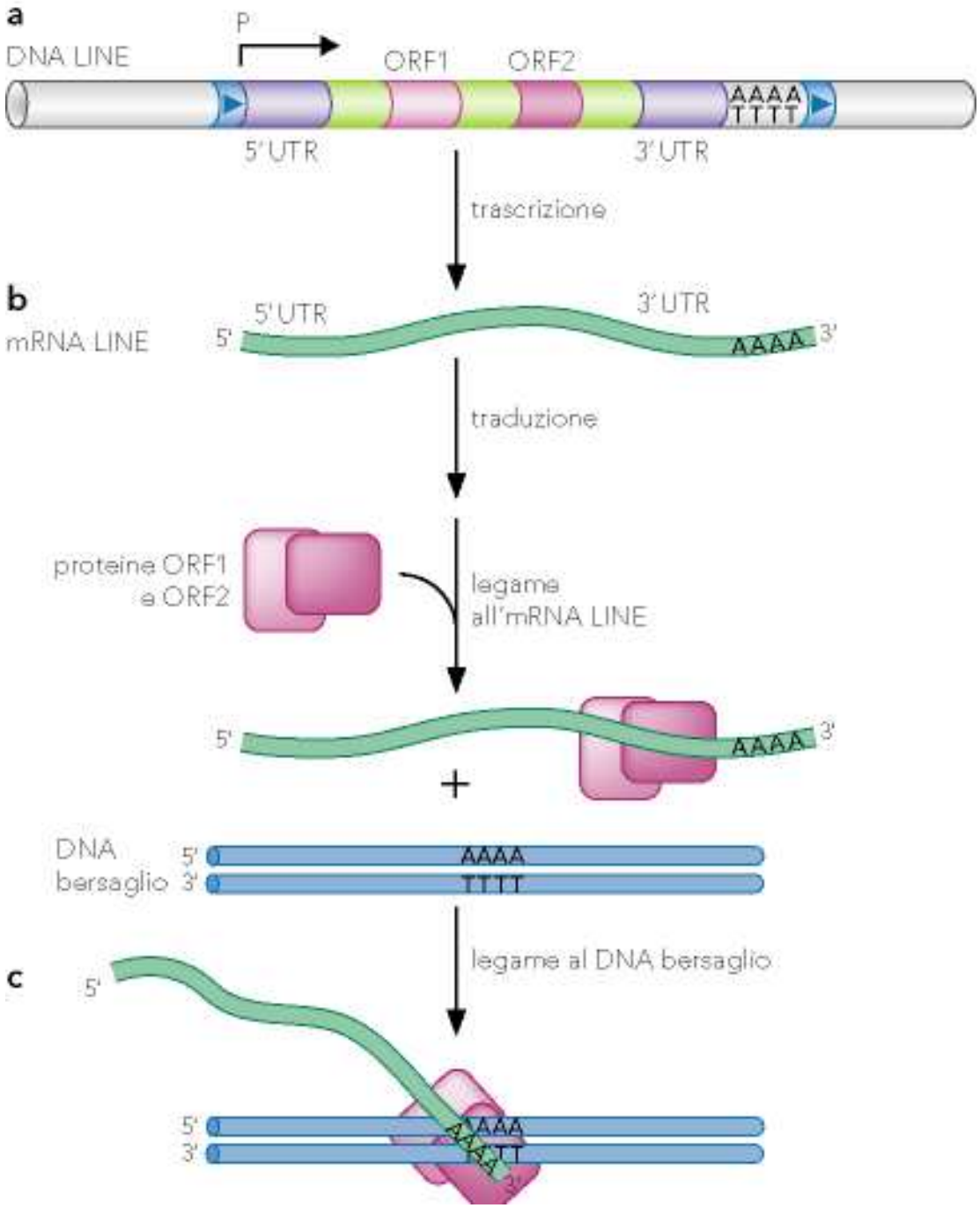
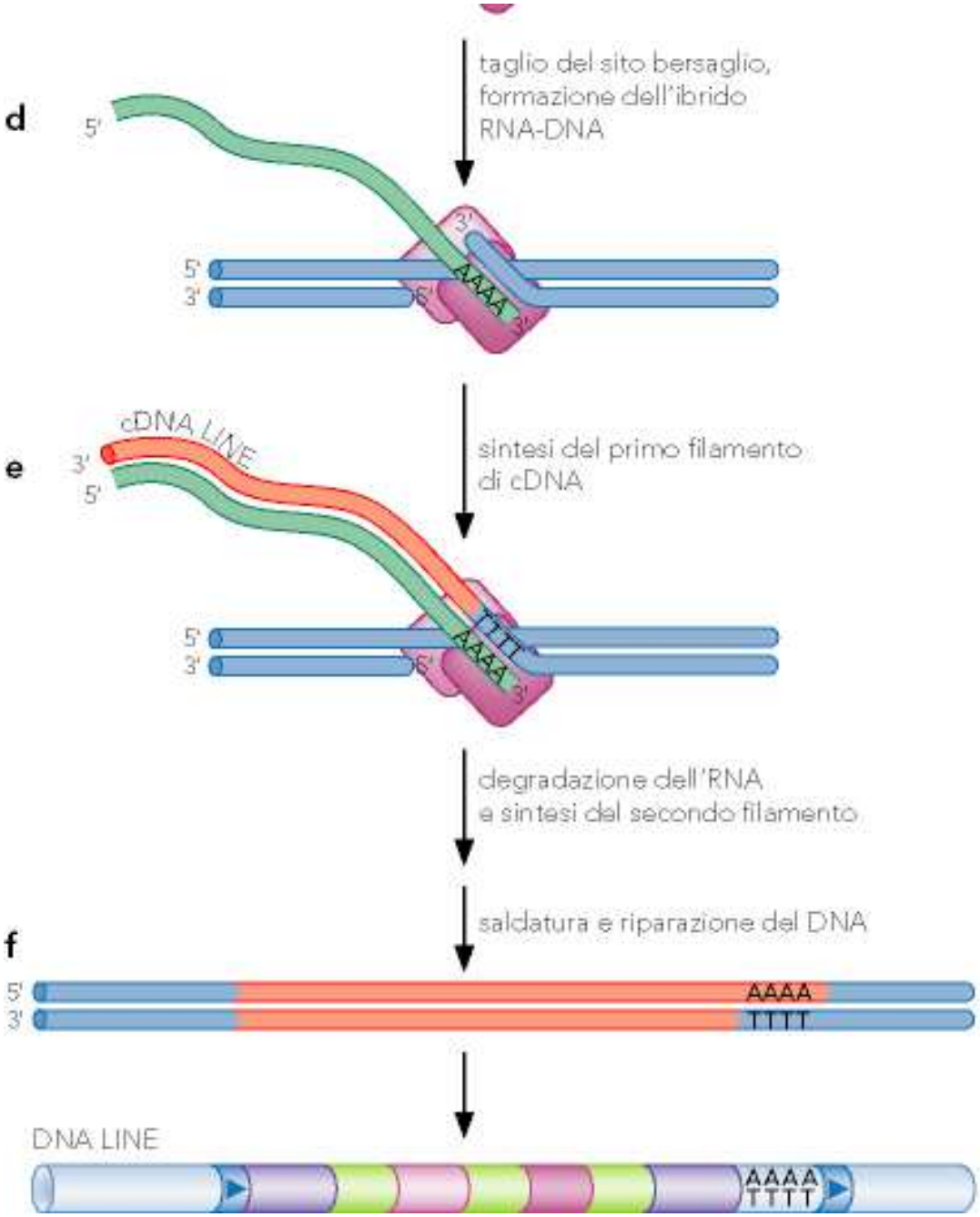
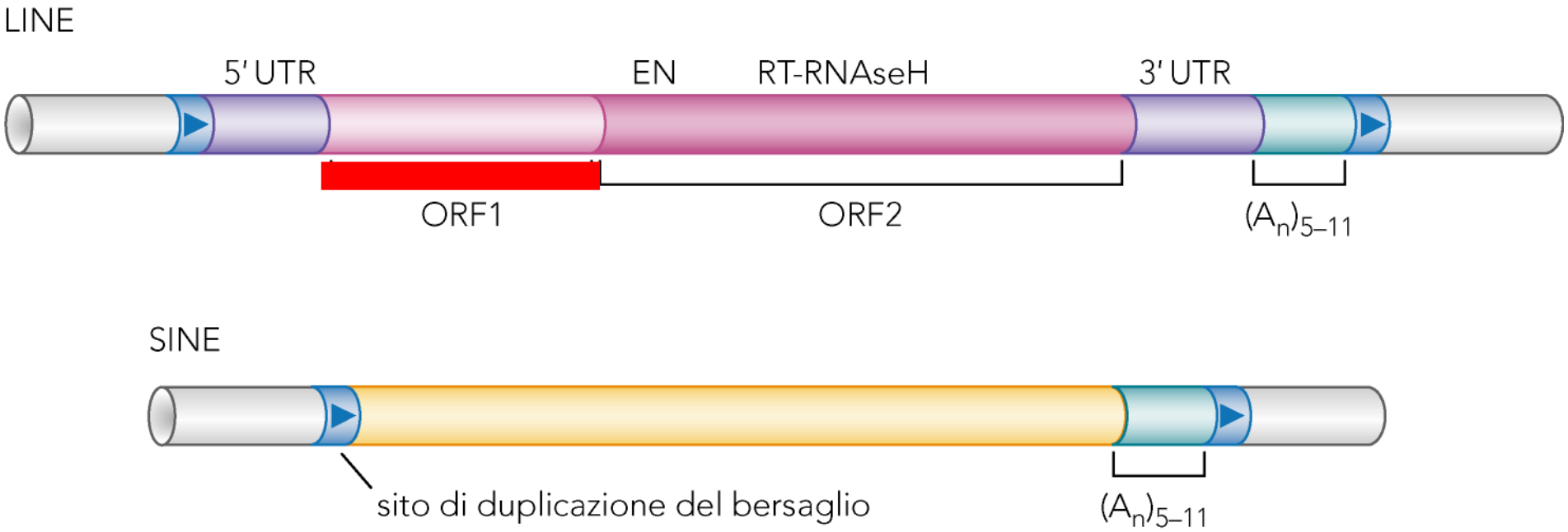
Watson BM Gene Capitolo 11


LINE
5′ UTR
EN
RT-RNAseH
?
3′ UTR
ORF1
ORF2
$(A_n)_{5–11}$
SINE
sito di duplicazione del bersaglio
$(A_n)_{5–11}$

Le proteine della trasposizione


a
DNA LINE
P
ORF1
ORF2
AAAA
TTTT
5' UTR
3' UTR
trascrizione
b
mRNA LINE
5' UTR
3' UTR
5'
AAAA
3'
traduzione
proteine ORF1
e ORF2
legame
all'mRNA LINE
5'
AAAA
3'
+
DNA
bersaglio
5'
3'
AAAA
TTTT
legame al DNA bersaglio
c
5'
5'
3'
AAAA
TTTT
3'


d
taglio del sito bersaglio,
formazione dell'ibrido
RNA-DNA
5'
3'
5'
3'
AAAA
e
cDNA LINE
sintesi del primo filamento
di cDNA
TTTT
degradazione dell'RNA
e sintesi del secondo filamento
saldatura e riparazione del DNA
f
AAAA
TTTT
DNA LINE



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Trimeric structure for an essential protein in L1 retrotransposition

Sandra L. Martin*†, Dan Branciforte*, David Keller‡, and David L. Bain§

Watson et al., BIOLOGIA MOLECOLARE DEL GENE, Zanichelli editore S.p.A.

The function of the protein encoded by the 5-most ORF, ORF1p, is incompletely understood,

the ORF1p from mouse L1 is known to bind single-stranded nucleic

acids (L1 RNA and DNA) and function as a nucleic acid chaperone.

Structural features are compatible with the nucleic acid binding

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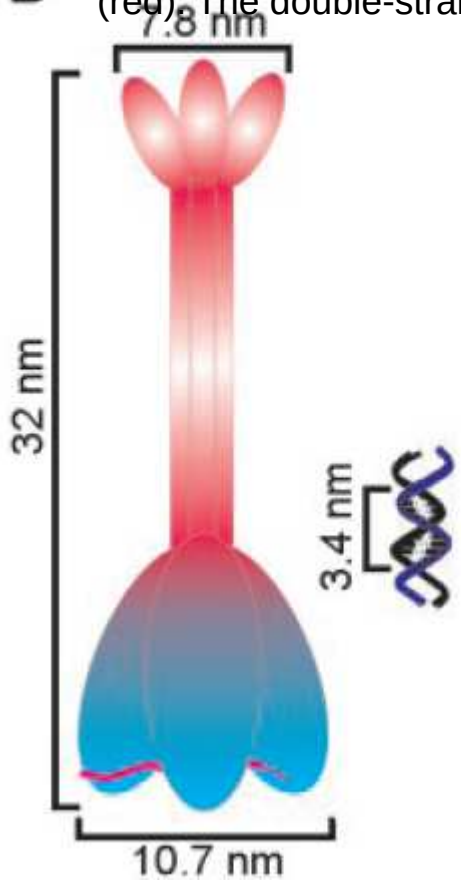
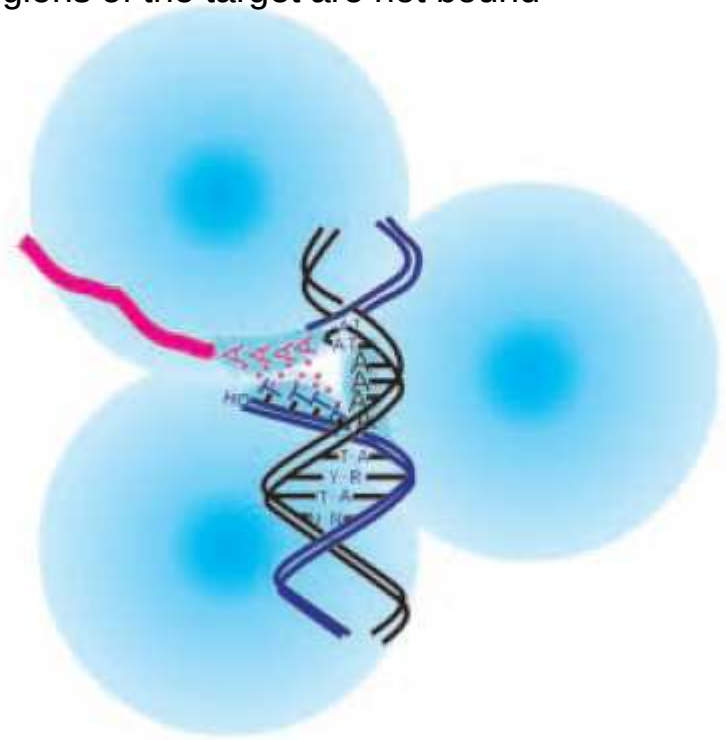
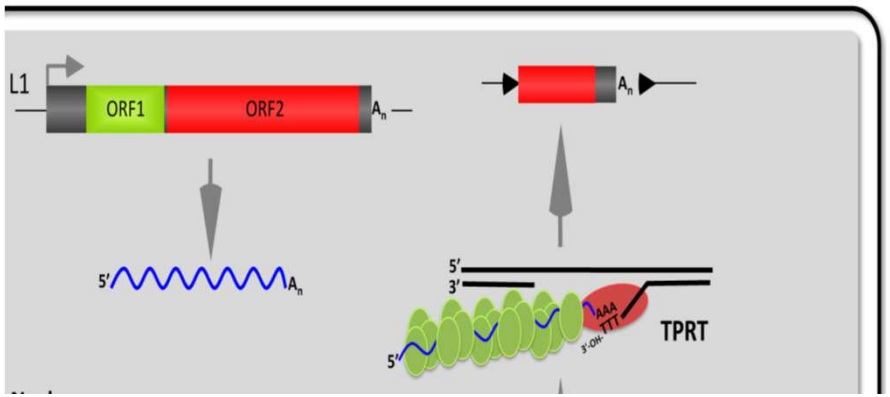
acidic(red) basic (blue)

Each subunit of the trimer contains one single-stranded nucleic acid binding interface which is bound with one of the DNA target strands or the polyA tail of the L1 RNA (red). The double-stranded regions of the target are not bound



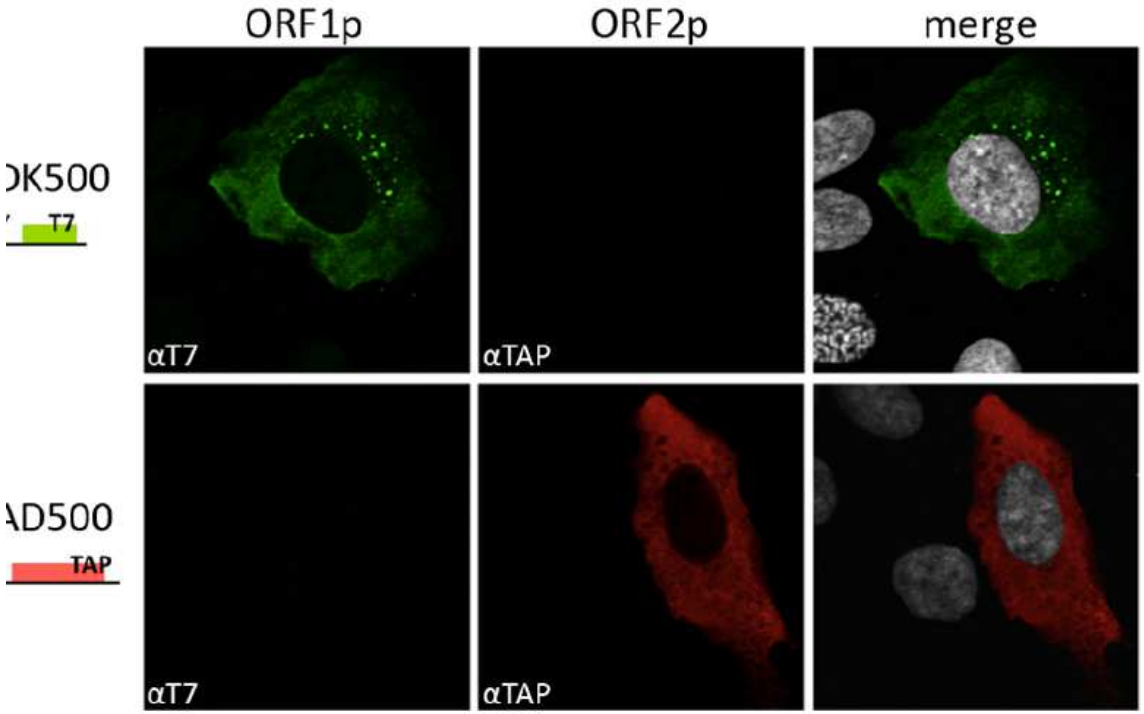


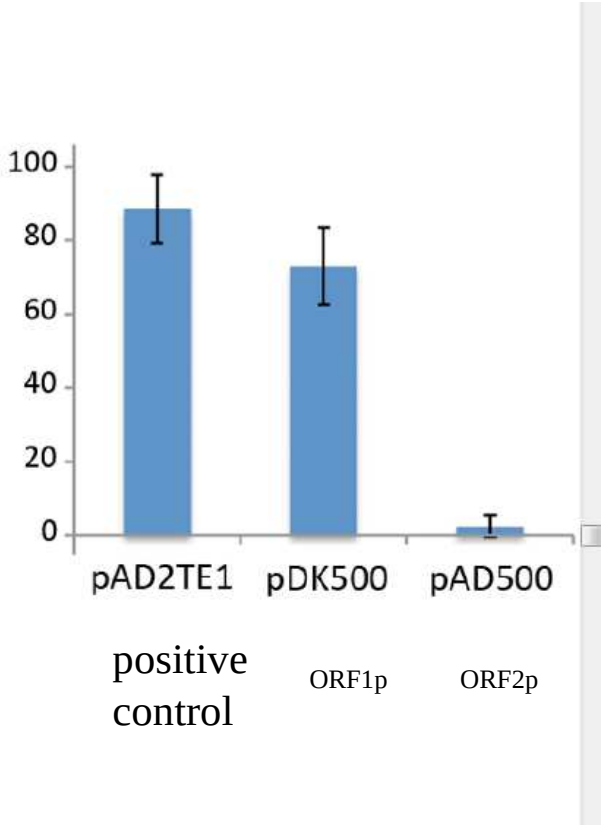
ORF1p lcoats the entire 7-kb L1 RNA to form a ribonucleoprotein particle
The nucleic acid chaperone activity of ORF1p melts the DNA and then facilitates formation of the RNA:DNA hybrid



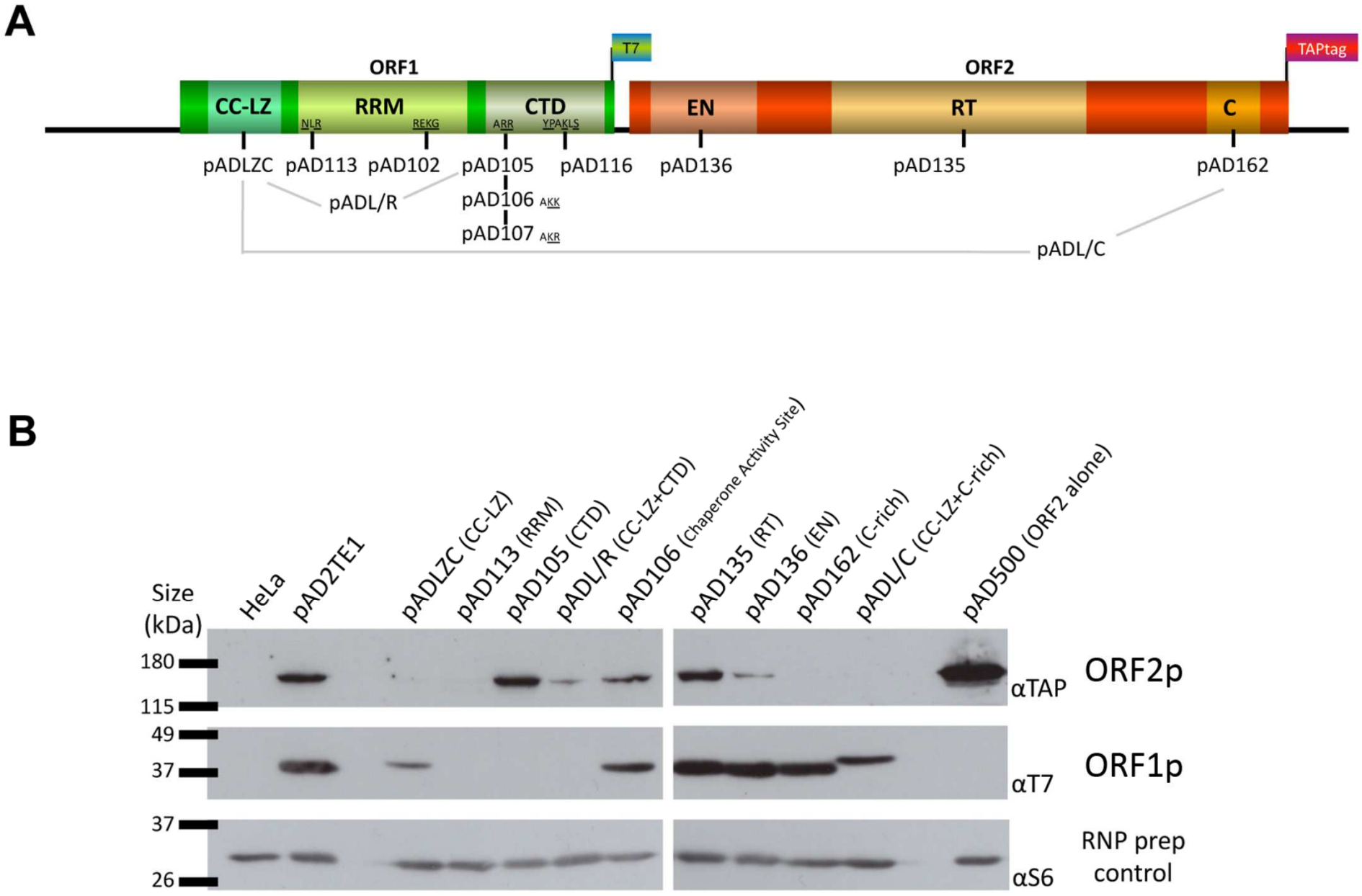
target-site primed reverse transcription

ORF1p is necessary and sufficient for L1 cytoplasmic foci formation





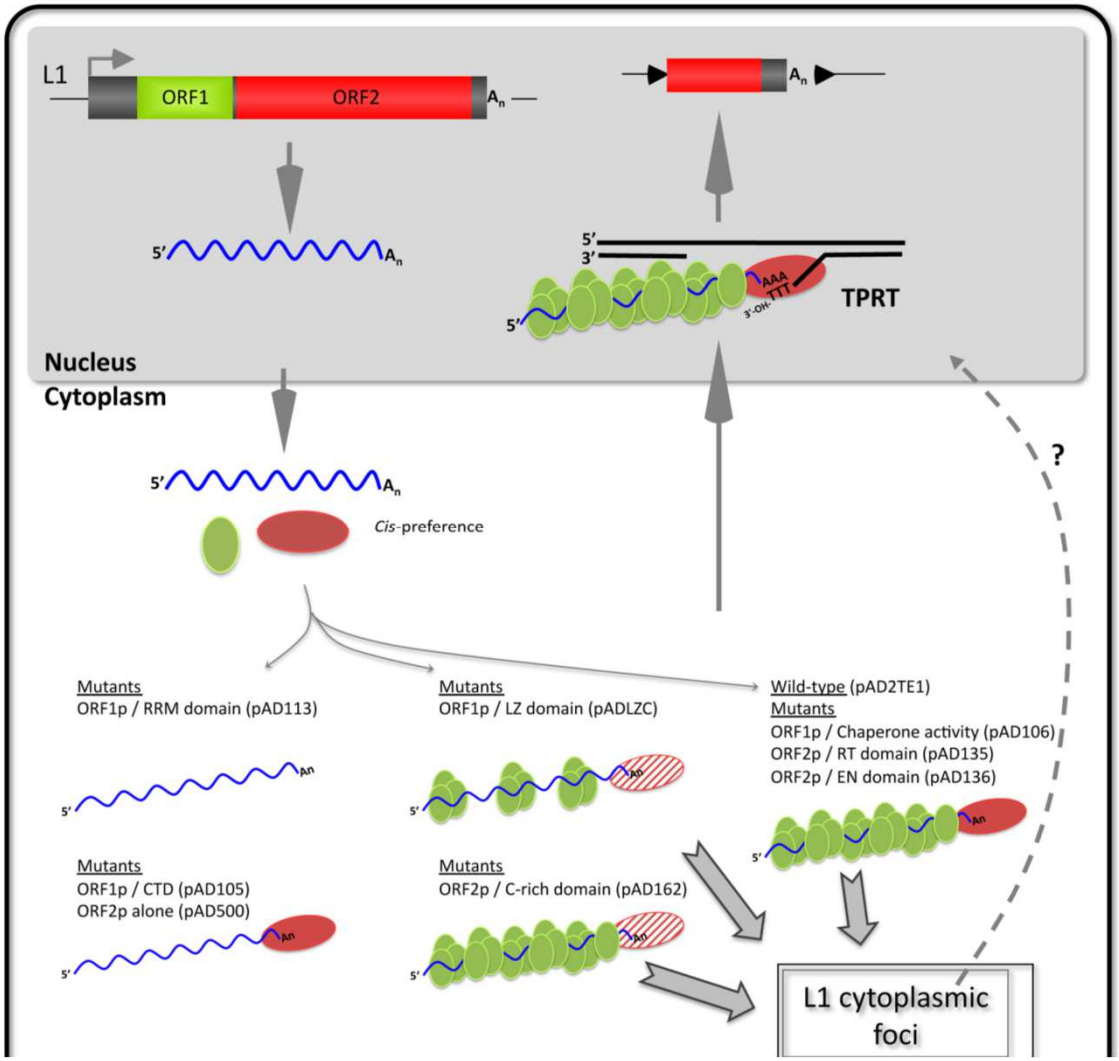
T7-tagged ORF1p green
TAP-tagged ORF2p red;



T7 gene 10 epitope tag

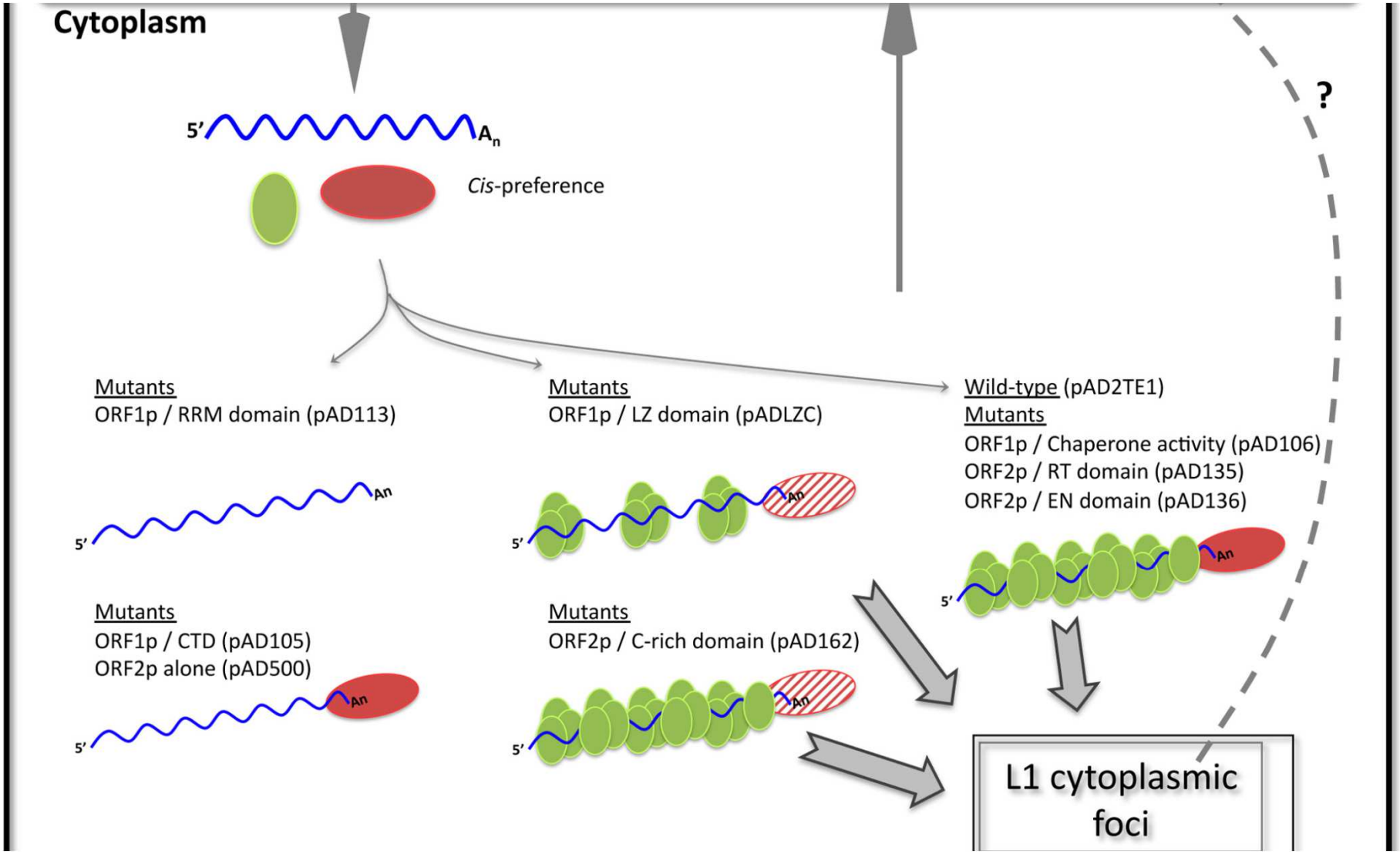
Tandem Affinity Purification epitope tag (TAP tag)

Mutations in the ORF1p RNA recognition motif (pAD113) disrupt L1 cytoplasmic foci formation and lead to a severe reduction of ORF1p and ORF2p in cytoplasmic RNP complexes

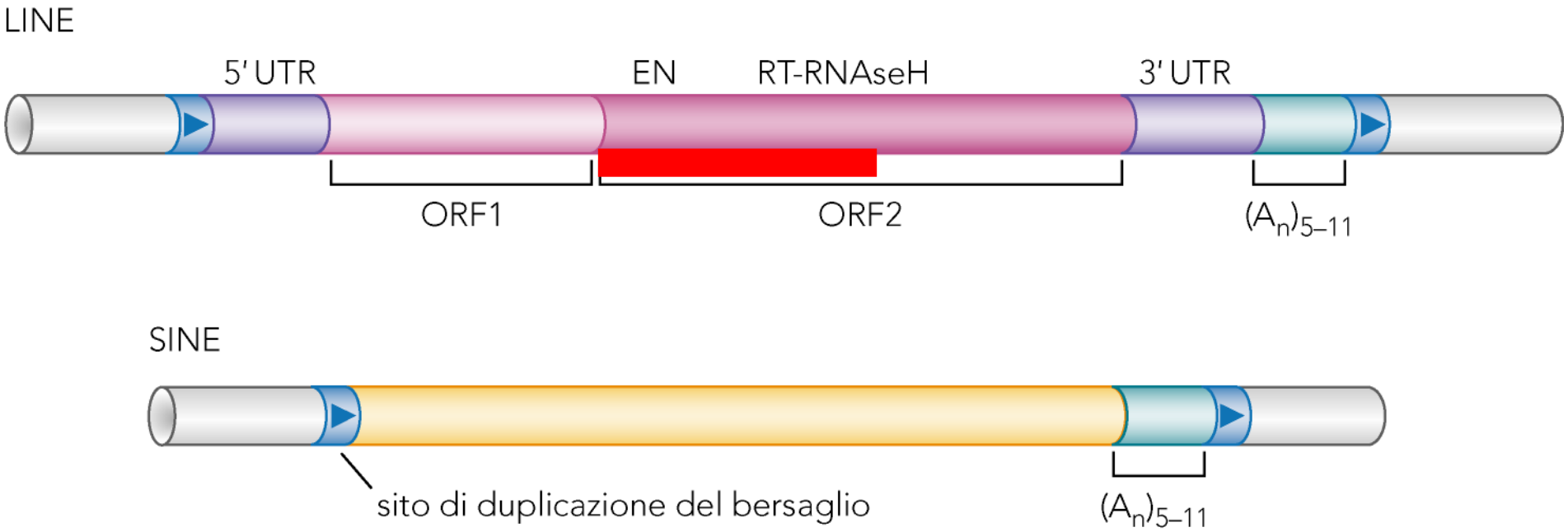


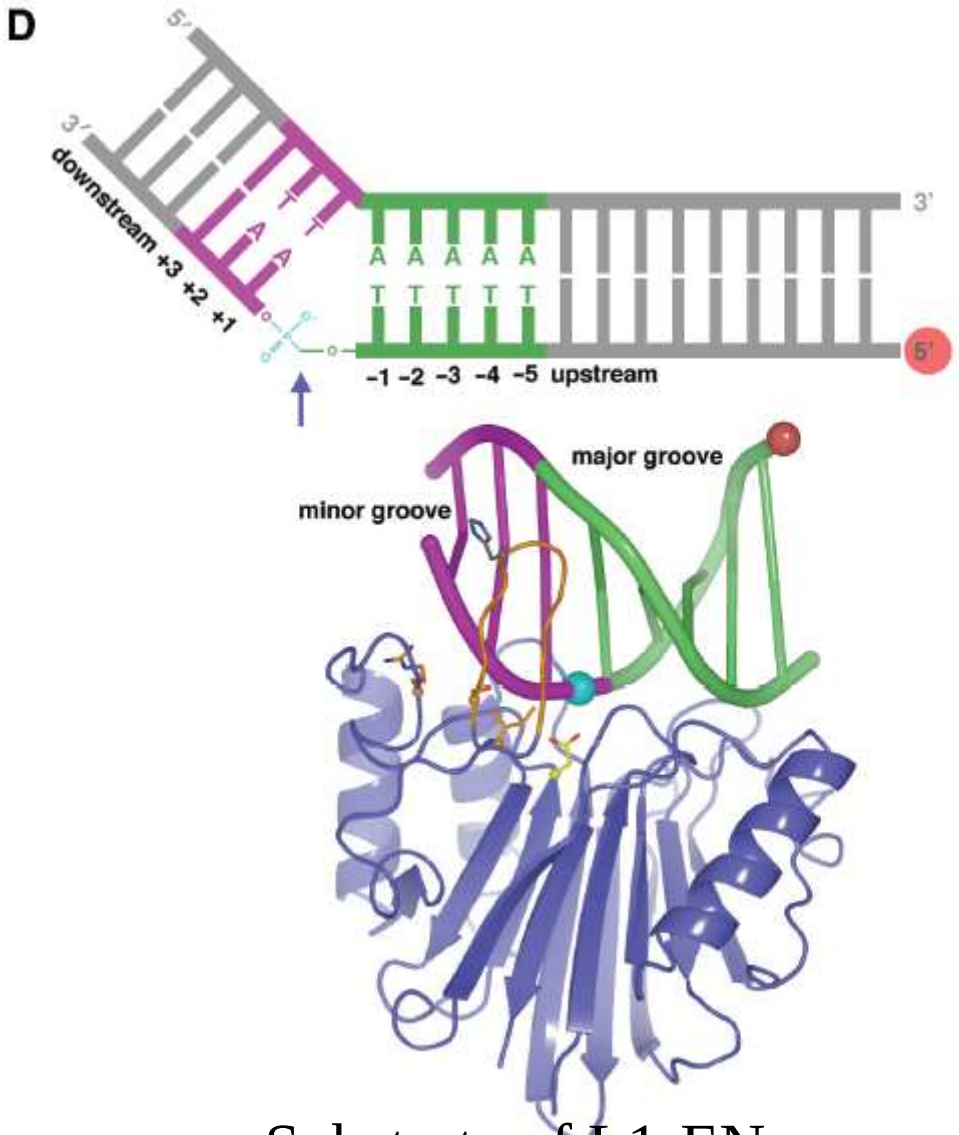
target-site primed reverse transcription (TPRT)

Mutations in the ORF1p RNA recognition motif (pAD113) disrupt L1 cytoplasmic foci formation and lead to a severe reduction of ORF1p and ORF2p in cytoplasmic RNP complexes



target-site primed reverse transcription (TPRT)

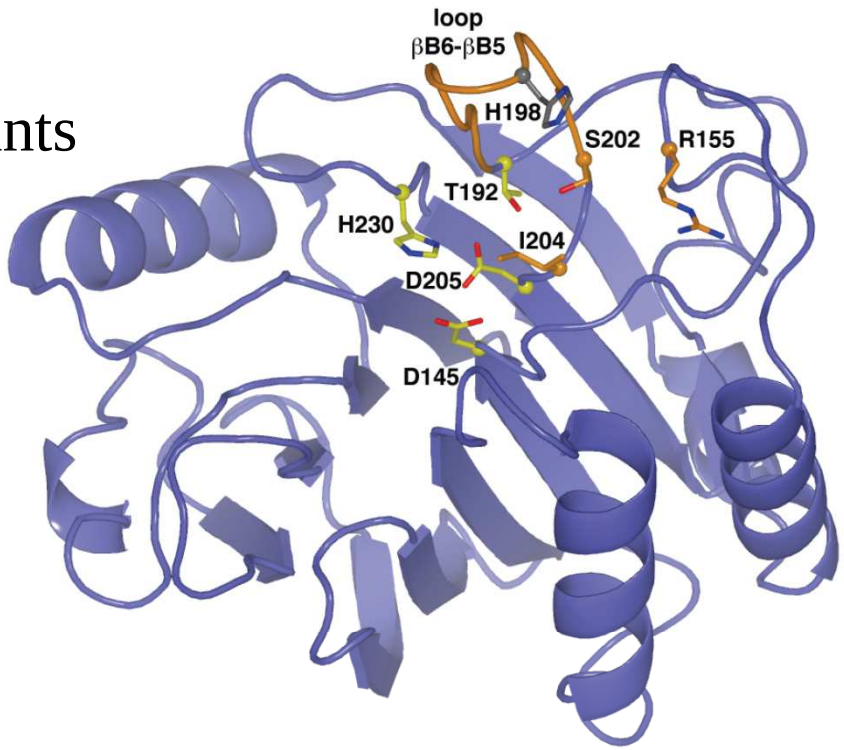

LINE
5′ UTR
EN
RT-RNAseH
3′ UTR
ORF1
ORF2
$(A_n)_{5–11}$
SINE
sito di duplicazione del bersaglio
$(A_n)_{5–11}$



Substrate of L1-EN

L1-EN point mutants and bB6–bB5 hairpin loop variants

A



orange: residues
potentially contacting DNA

B

	191	192	193	194	195	196	197	.	.	198	199	200	201	202	203	204	205
L1-EN	Y	T	F	F	S	A	P	.	.	H	H	T	Y	S	K	I	D
Tx1L-EN	F	T	Y	V	R	V	R	D	G	H	V	S	Q	S	R	I	D
R1Bm-EN	P	T	F	S	T	A	.	.	.	.	N	G	E	S	Y	V	D
Tras1-EN	P	T	F	D	T	I	R	G	G	K	R	Y	Q	S	R	V	D

	195	196	197	198	199	200	201	202	203	204	205	206	207	208	209	210	211

Substrate requirements of L1-EN

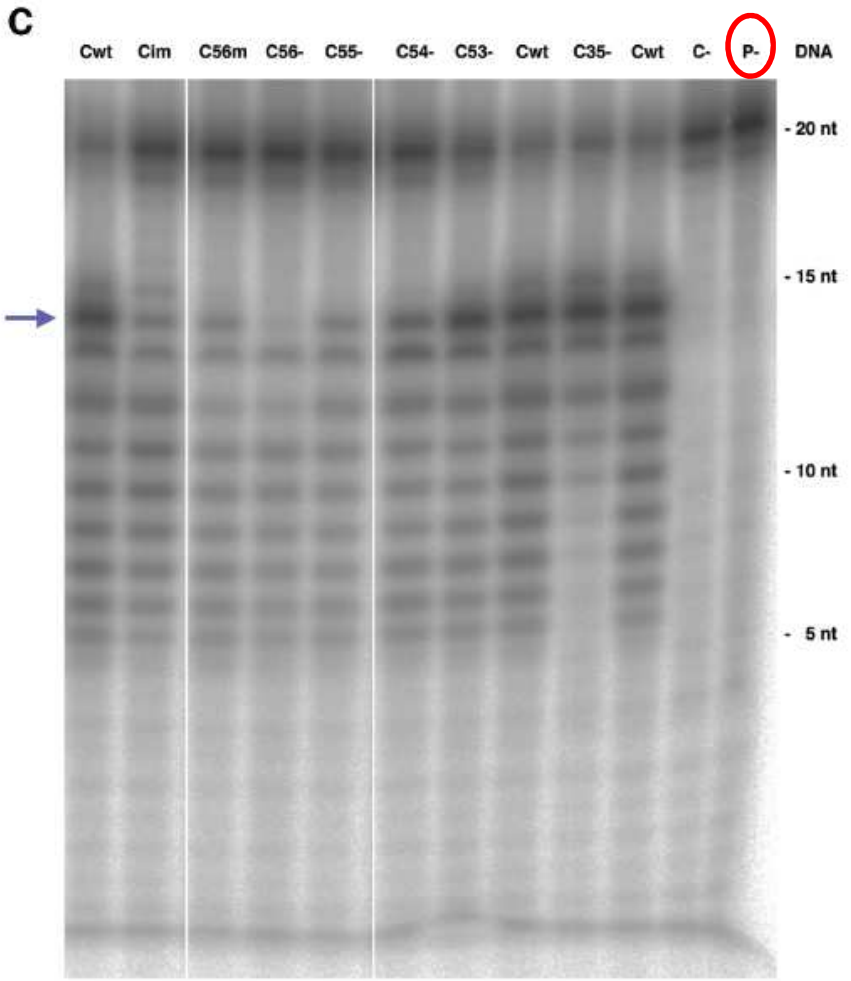
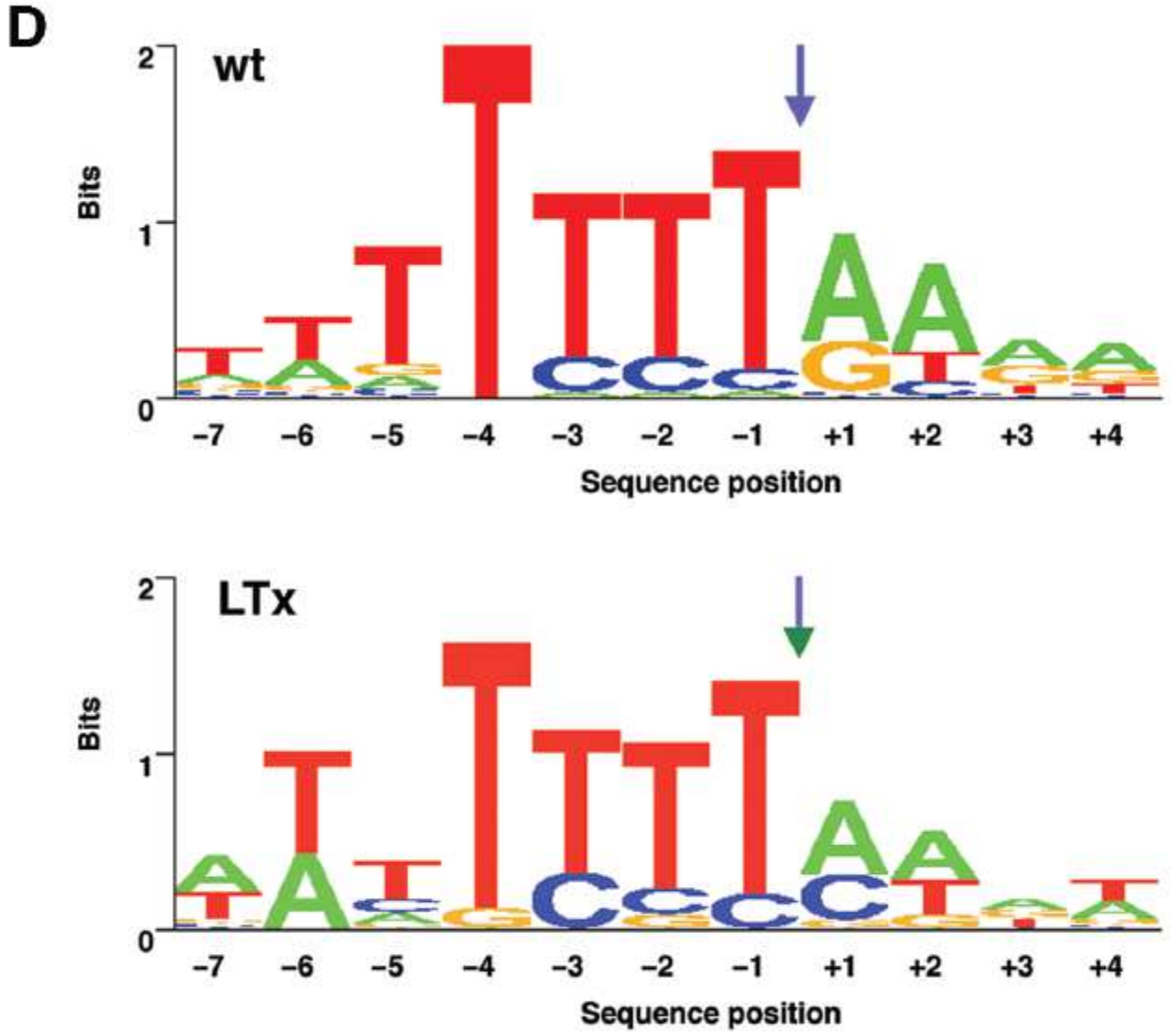


Table 1. Comparison of retrotransposition frequencies *in vivo* and plasmid nicking activities *in vitro*

L1-EN variant	Retrotransposition frequency[a], %	Plasmid nicking activity[b], %
wt	100 ± 17.1	100 ± 0.8
LTx	21 ± 2.4	29 ± 2.6
LR1	2 ± 2.3	6 ± 0.8
L3G	0 ± 2.2	10 ± 1.8
D145A	0[c]	3 ± 1.0
R155A	12 ± 3.3	19 ± 3.4
T192V	5 ± 3.0	–
S202A	32 ± 7.8	28 ± 2.2
I204Y	1 ± 1.1	4 ± 1.2
H230A	0	–

[a]Corrected for background activity (≤5%); for details see Supplementary Data.
[b]Normalized to L1-EN (wt) activity, (–) not analyzed.
[c]As a D145A/N147A double mutant.

Specificity of L1-EN



Come inibire l’espressione e trasposizione del Trasposone

Large parts of eukaryotic genomes are composed of transposons.

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)

Come inibire l'espressione e trasposizione del Trasposone

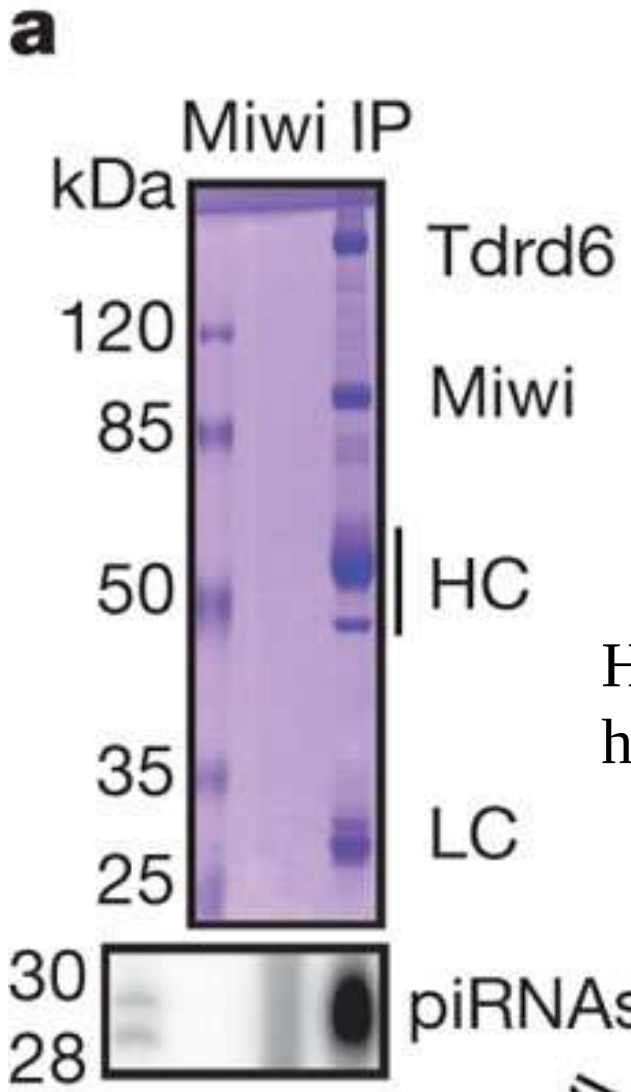
In mice, the piRNA pathway is mainly active in the male germ line where all of the three Piwi proteins (MILI, MIWI, and MIWI2) are expressed.

Nuclear MIWI2 is implicated in establishing transcriptional silencing in embryonic germ cells by deposition of DNA methylation marks on target transposon loci

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

The H3 dimethylated K9 modification cosuppresses L1 expression

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

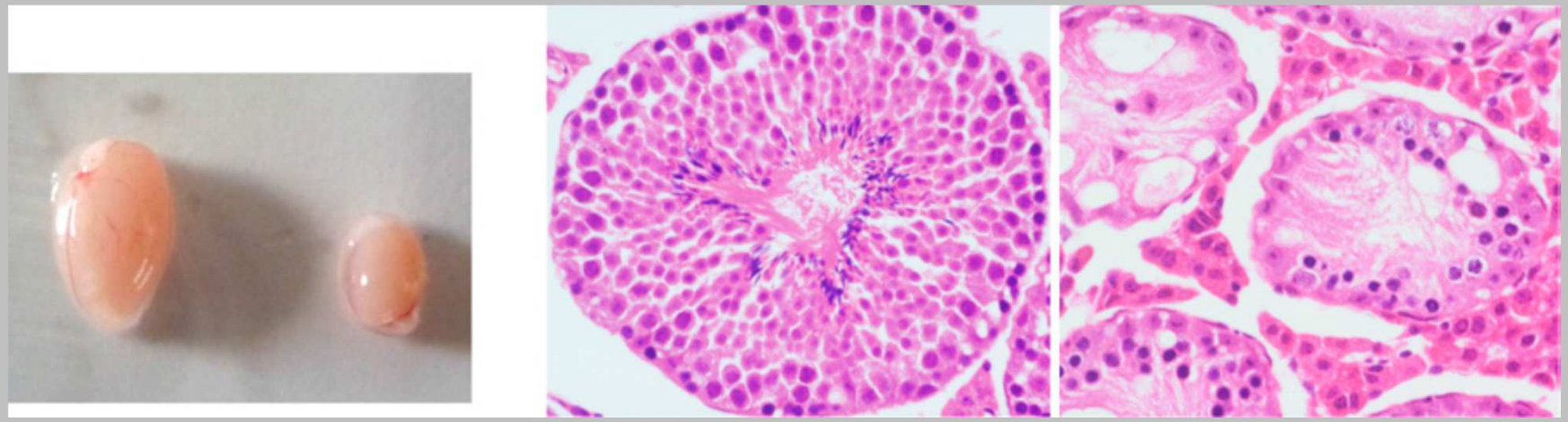


TDRD Tudor domain: role for the multidomain protein in mediating Complex formation with other participants during secondary piRNA biogenesis.

HC and LC: antibody heavy and light chains

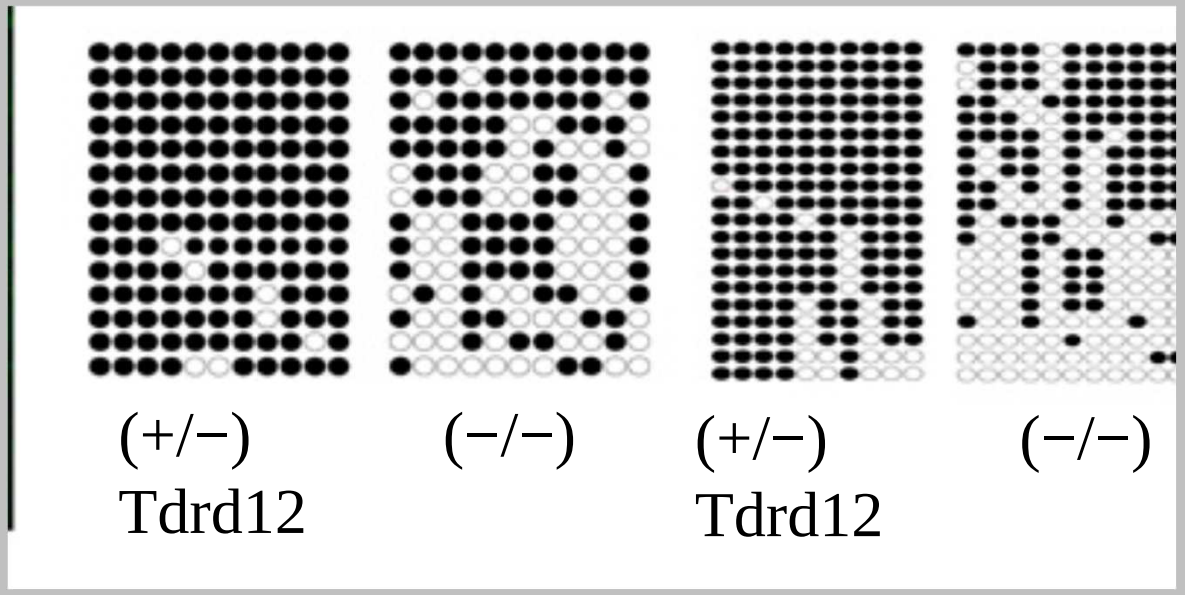
M Reuter *et al*. *Nature* **000**, 1-4 (2011) doi:10.1038/nature10672

Tdrd12 mutant male mice are infertile and display derepression of retrotransposons



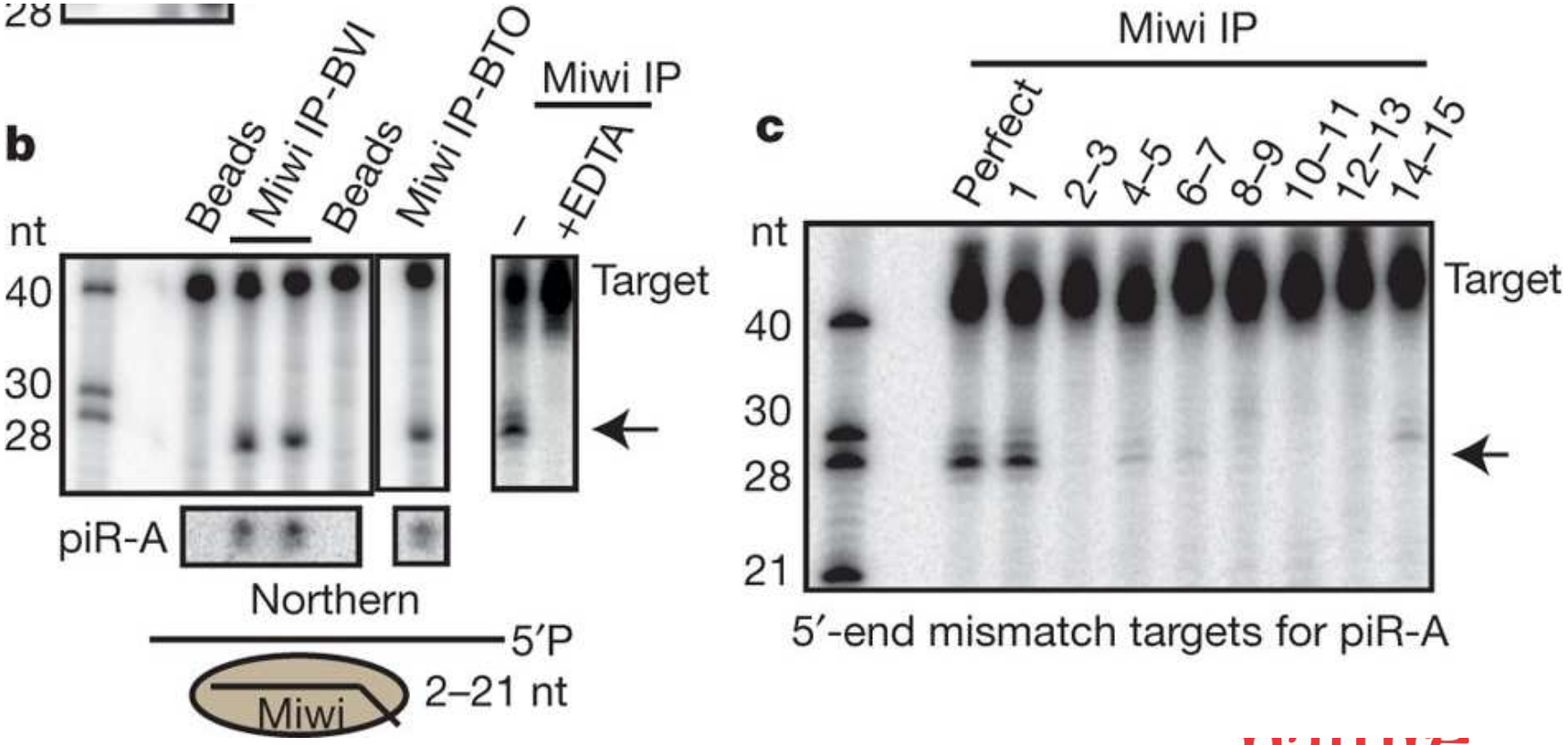
Atrophied testes of homozygous
(−/−) Tdrd12 mutants

Promoter CpG DNA methylation (indicated as filled circles) on transposon promoters



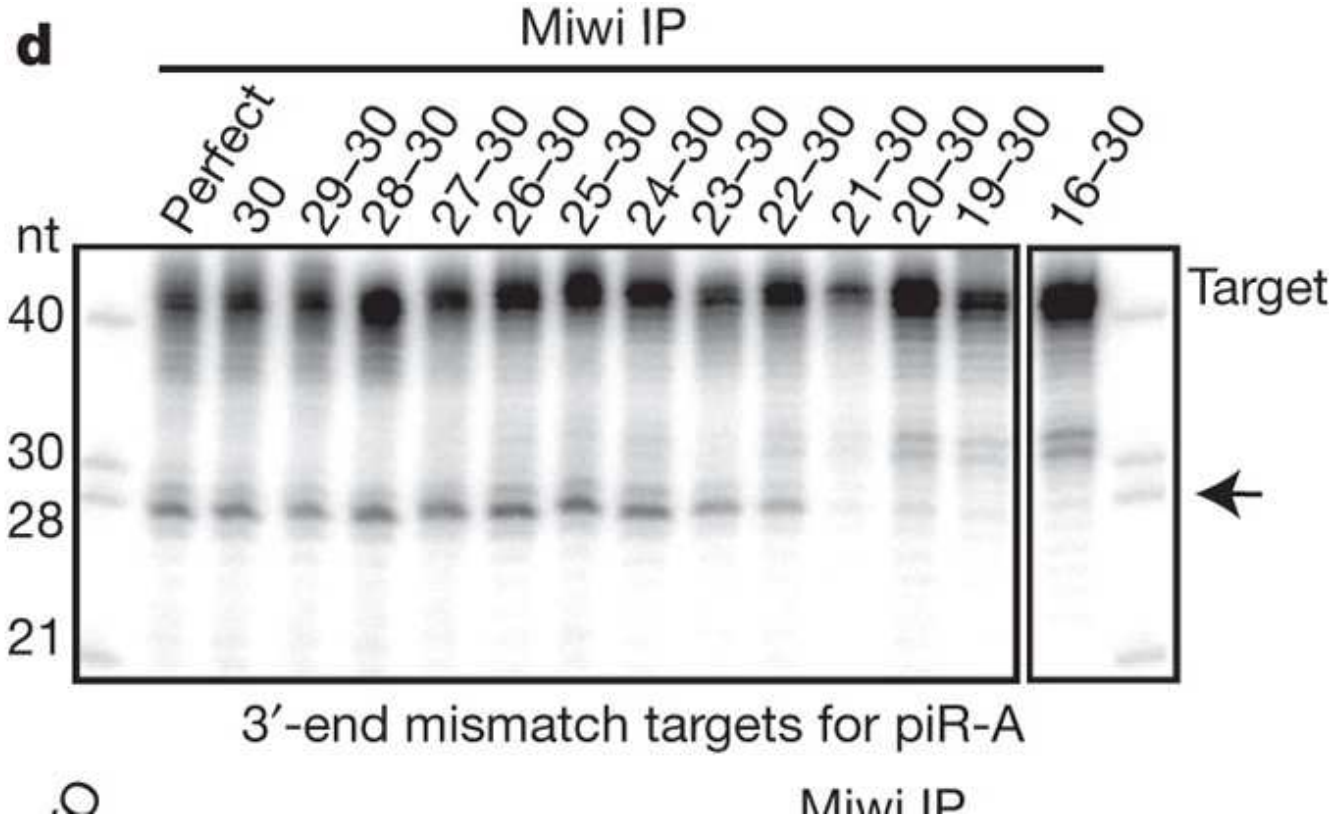
TDRD12 (Tudor Domain) is detected in complexes containing Piwi protein MILI(PIWIL2), its associated primary piRNAs, and TDRD1, all of which are already implicated in secondary piRNA biogenesis. Male mice carrying either a nonsense point mutation (reproductive mutant23 or repro23 mice) or a targeted deletion in the Tdrd12 locus areinfertile and derepress retrotransposons.
We find that TDRD12 is dispensable for primary piRNA biogenesis but essential for production of secondary piRNAs that enter Piwi protein MIWI2(PIWIL4).
Cell-culture studies with the insect ortholog of TDRD12 suggest a role for the multidomain protein in mediating complexformation with other participants during secondary piRNA biogenesis.

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



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

Miwi is a slicer requiring extensive complementarity for target cleavage.



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

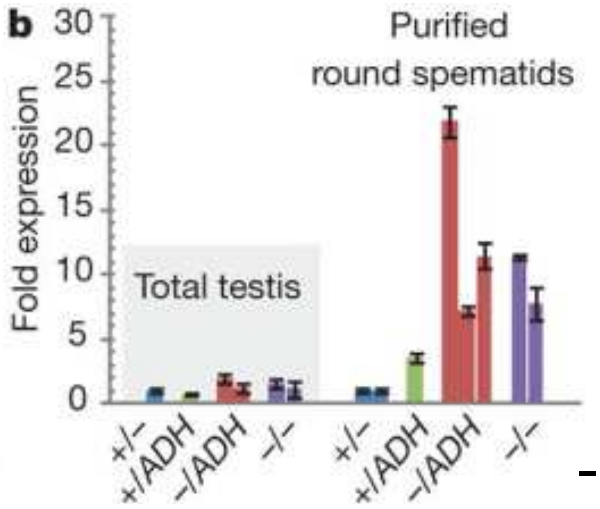
The slicer activity depends on the presence of a catalytic motif (Asp-Asp-His; DDH motif)

mice (Miwi ADH) with a point mutation in Miwi at the first aspartate (D633A) of the catalytic motif. Miwi1/ADHmale mice were sterile

To obtain males exclusively expressing the MiwiADH protein, we generated Miwi2/ADH mice

Miwi piRNAs target L1 transposons.

RT–PCR analysis of L1 transcripts in whole testis and purified round spermatids



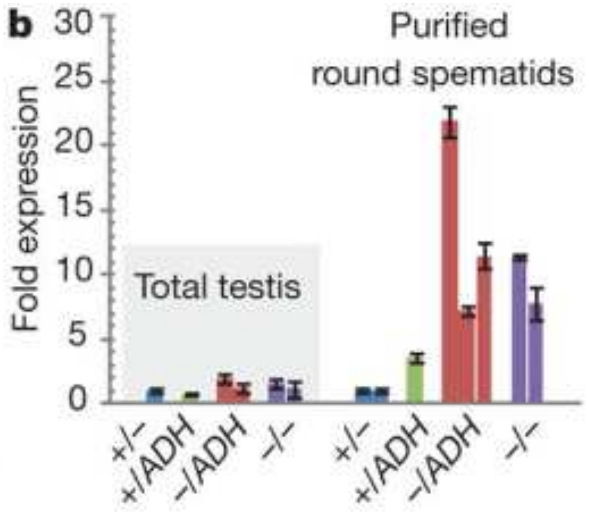
_ MIWI
Miwi ADH
DDH->ADH

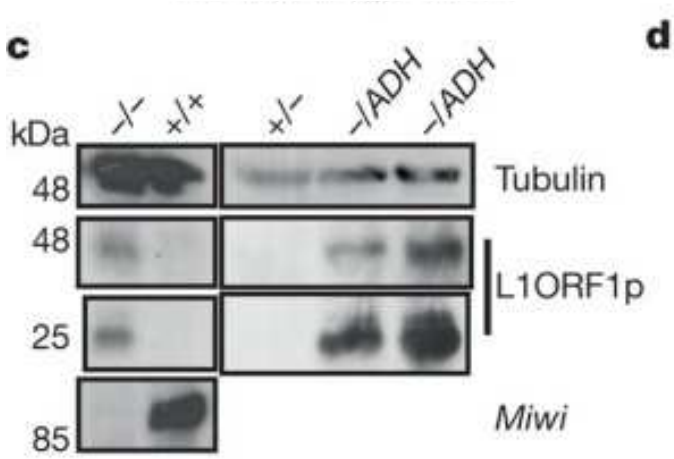
- Disruption of Miwi catalytic activity

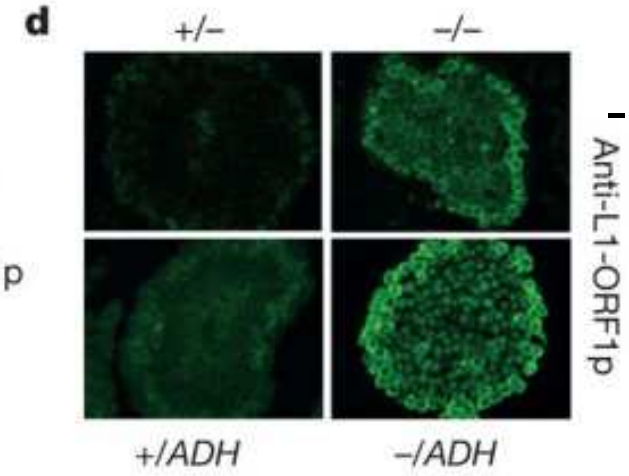
M Reuter *et al*. *Nature* **000**, 1-4 (2011) doi:10.1038/nature10672

Miwi piRNAs target L1 transposons.

RT–PCR analysis of L1 transcripts in whole testis and purified round spermatids







- Disruption of Miwi catalytic activity

nature

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