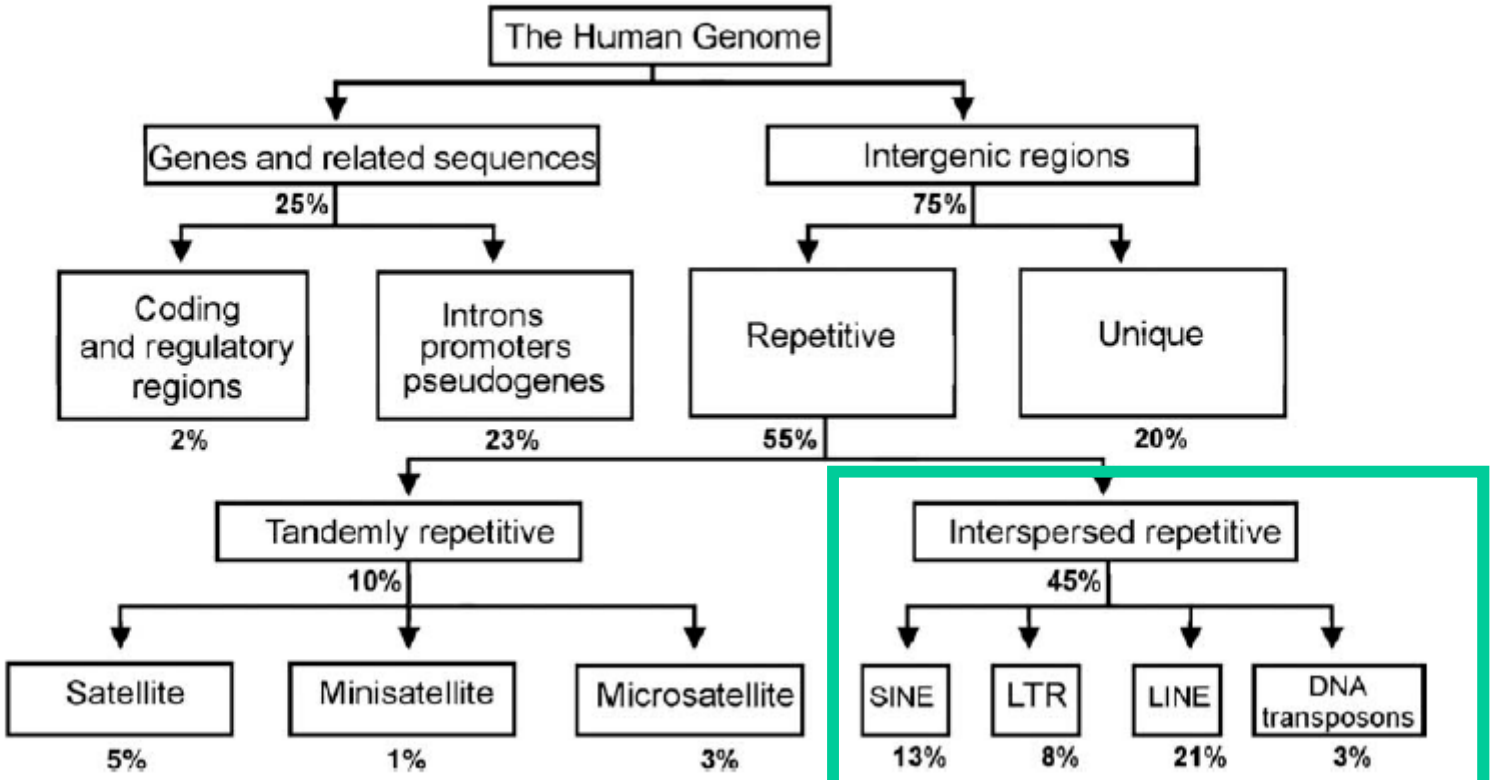
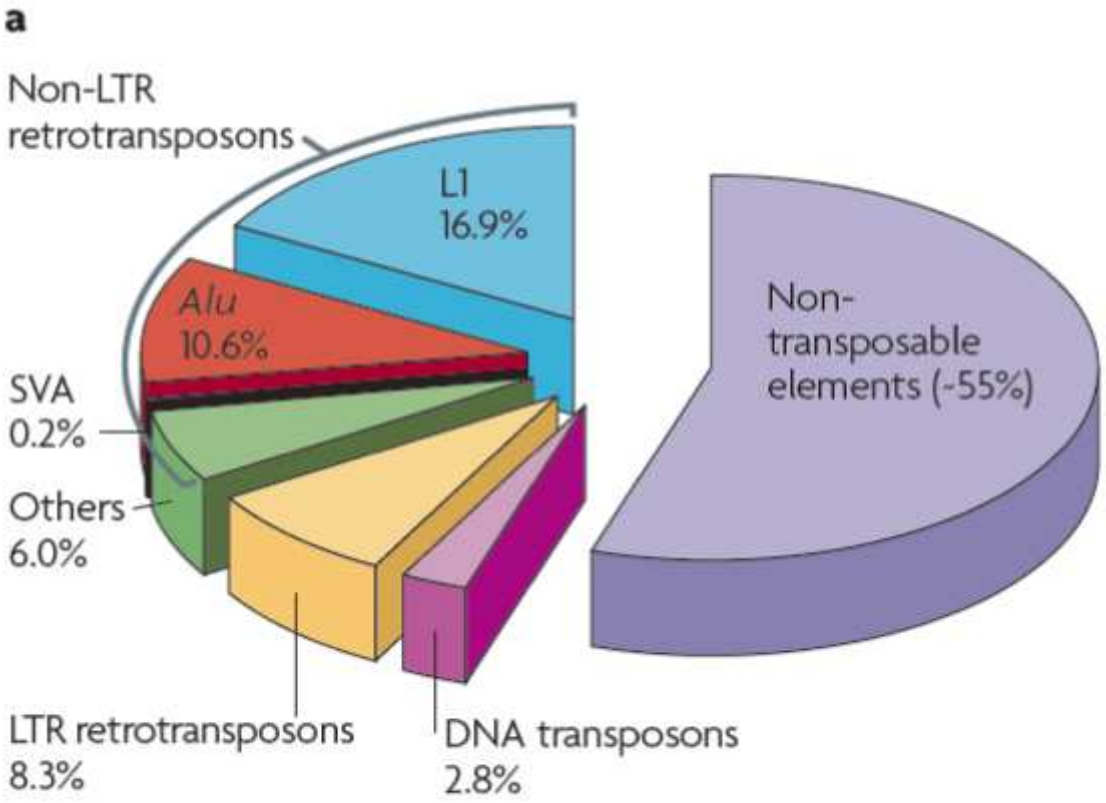
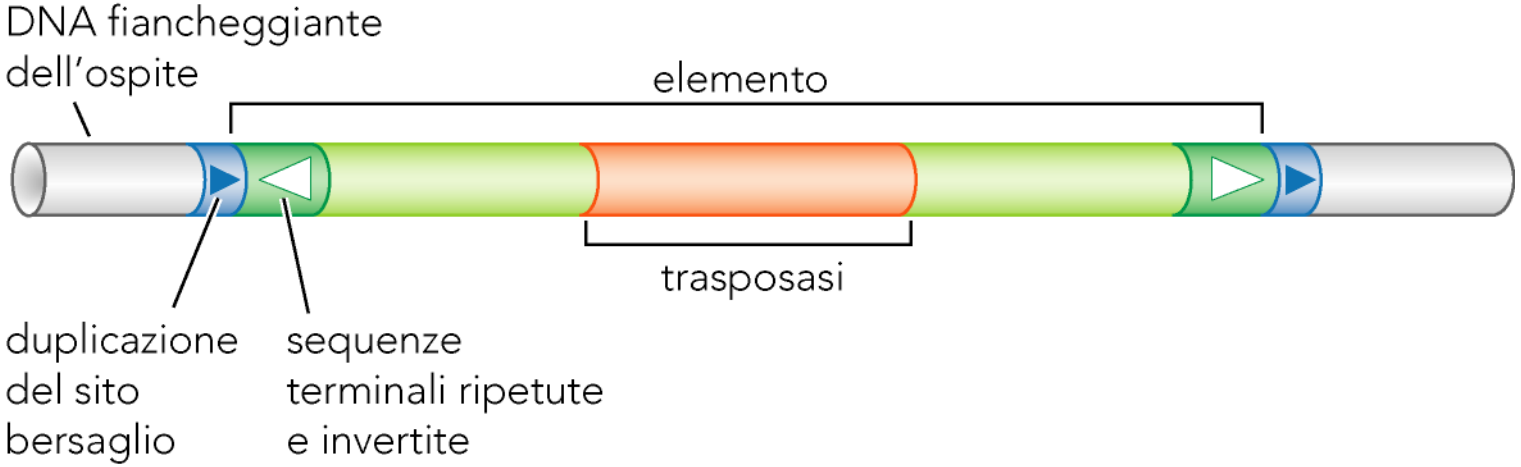


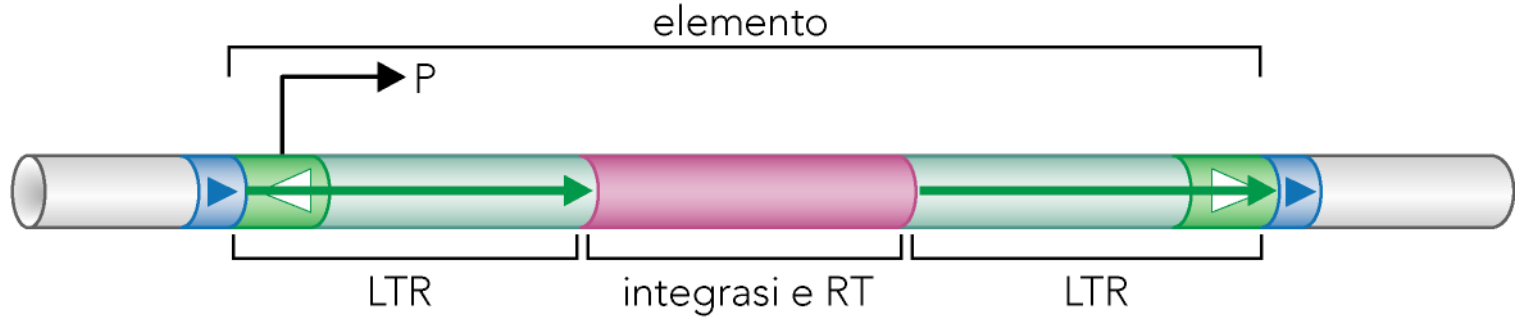
Fig. 1. Composition of the human genome. The percentage shares of various functional and non-functional sequences are shown.


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

REVIEW

Mobile Elements: Drivers of Genome Evolution

Haig H. Kazazian Jr.*

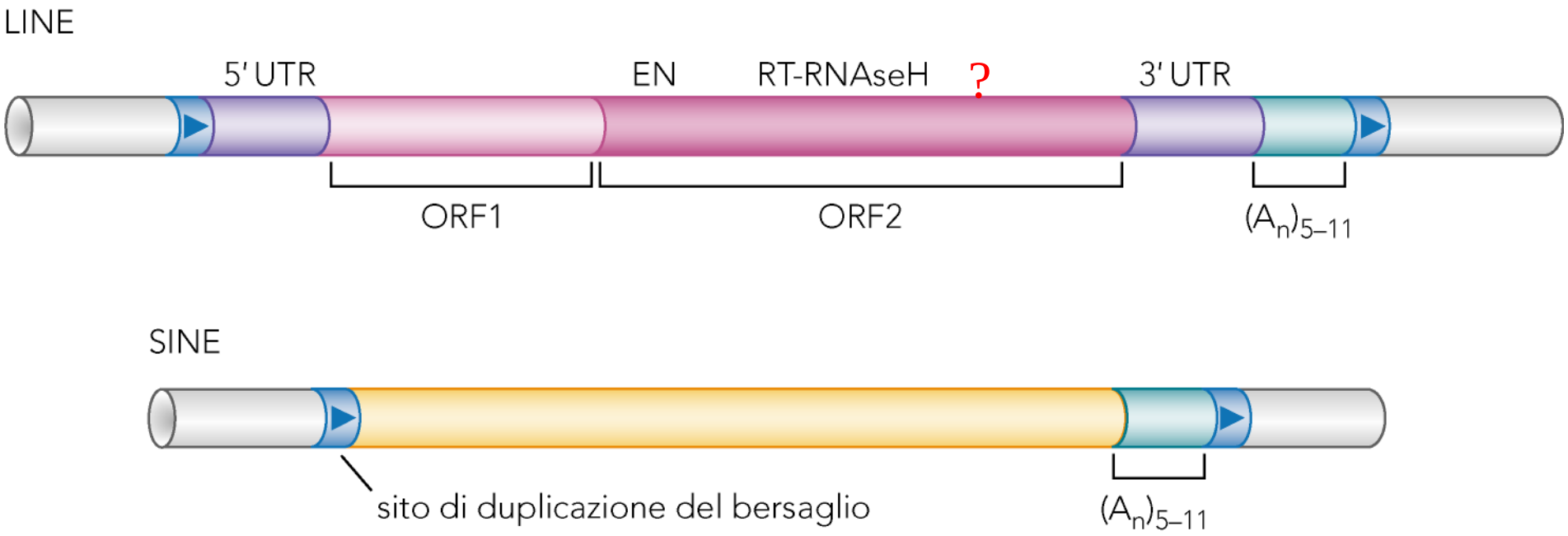
Mobile elements within genomes have driven genome evolution in diverse ways. Particularly in plants and mammals, retrotransposons have accumulated to constitute a large fraction of the genome and have shaped both genes and the entire genome. Although the host can often control their numbers, massive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

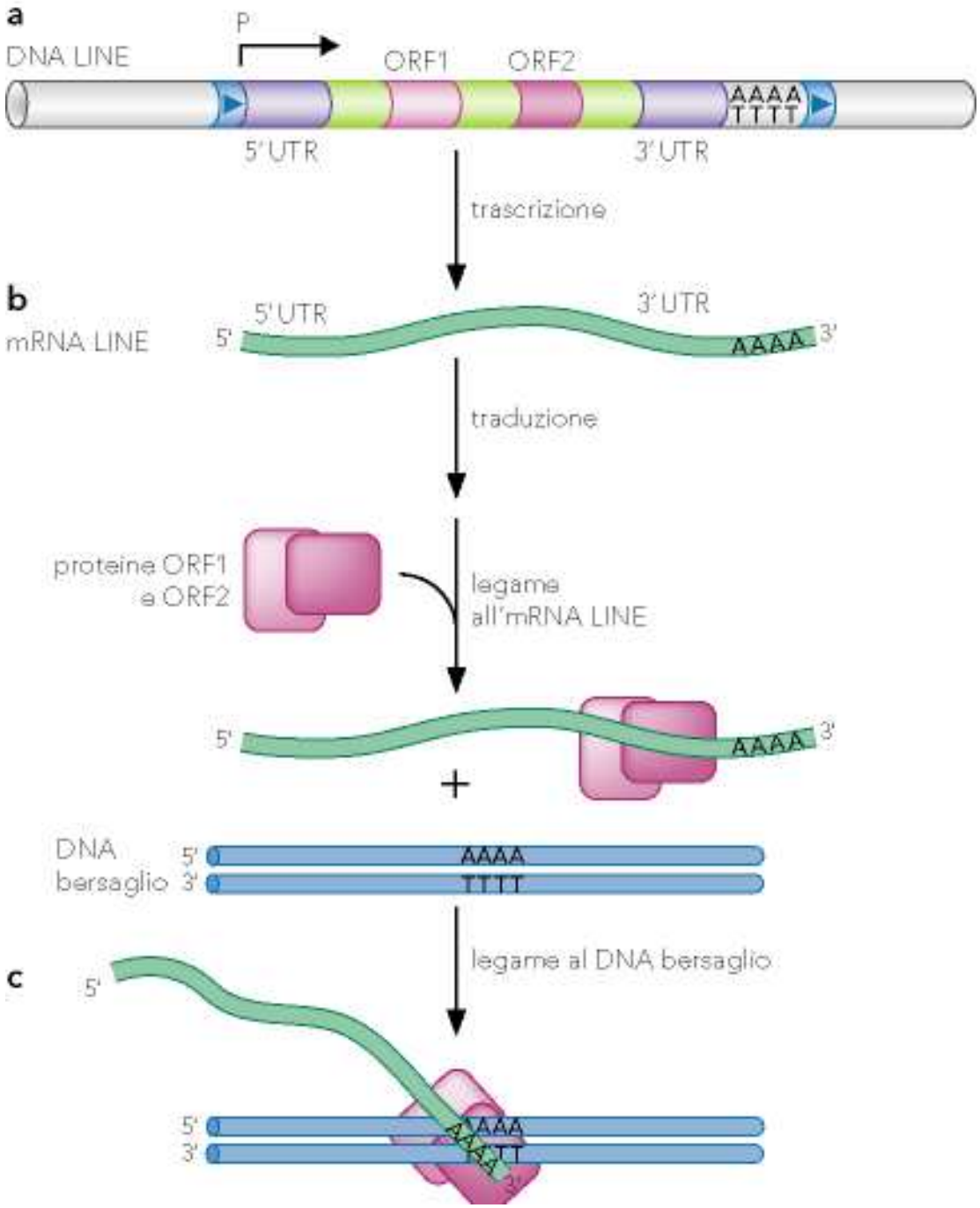
residues, then a glutamate) and a handlike three-dimensional structure (*6*, *8*).

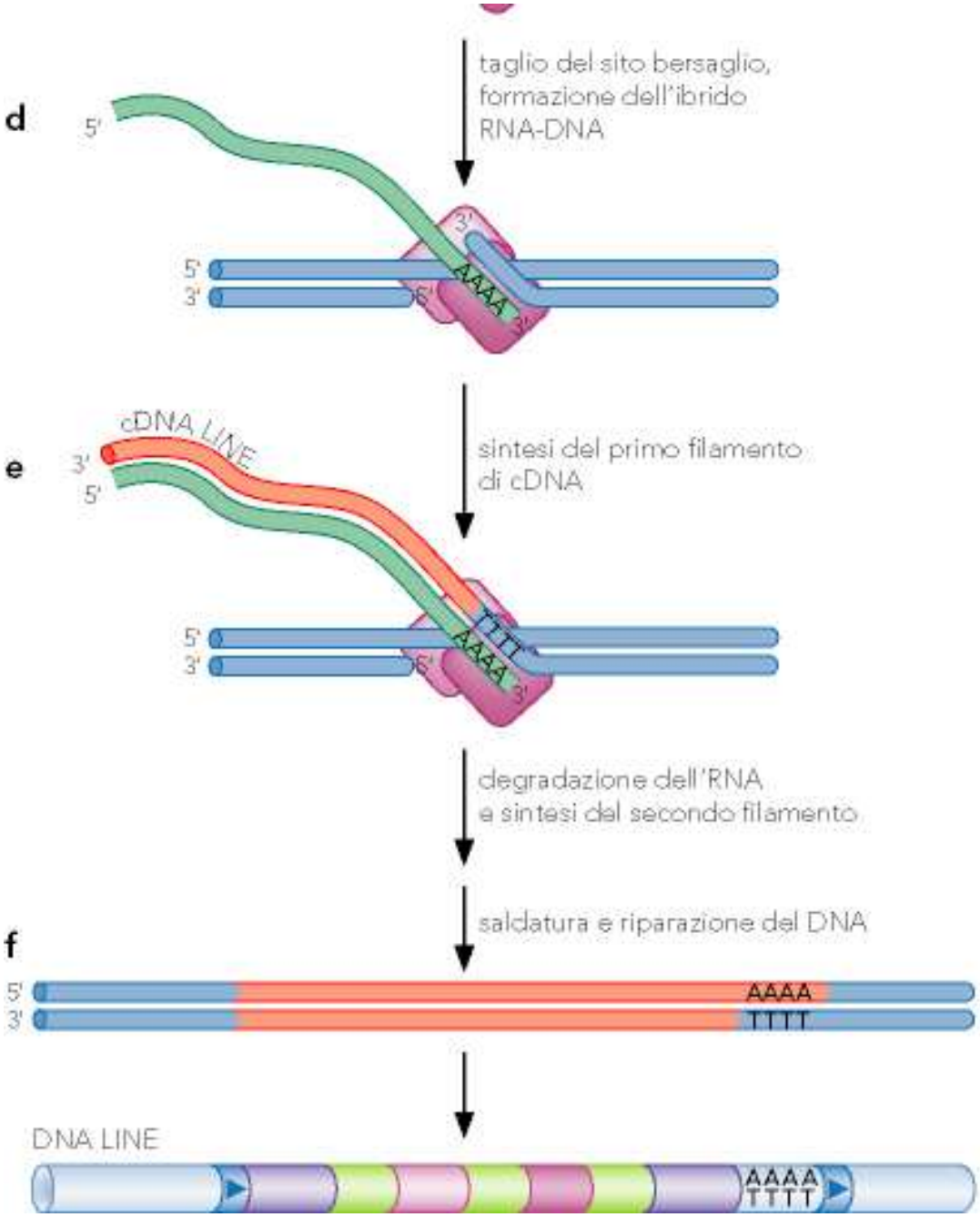
Although these elements generally transpose to genomic sites less than 100 kb from their original site (e.g., the *Drosophila* P element), some are able to make distant "hops" (e.g., the fish Tc1/mariner element; see below).

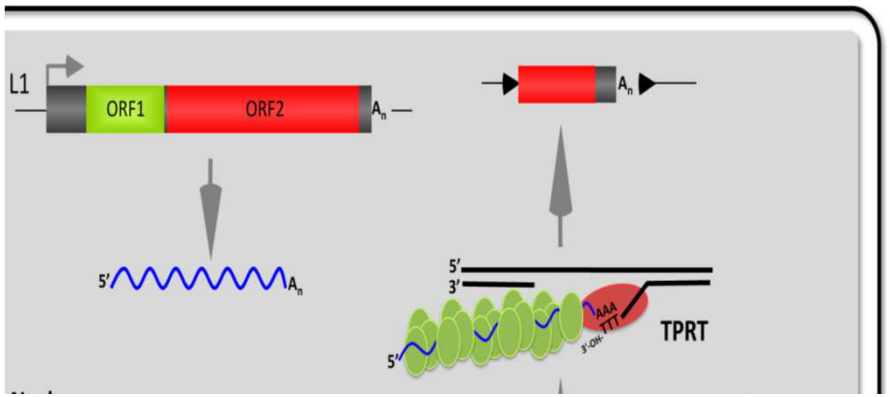
Watson BM Gene Capitolo 11

Le proteine della trasposizione

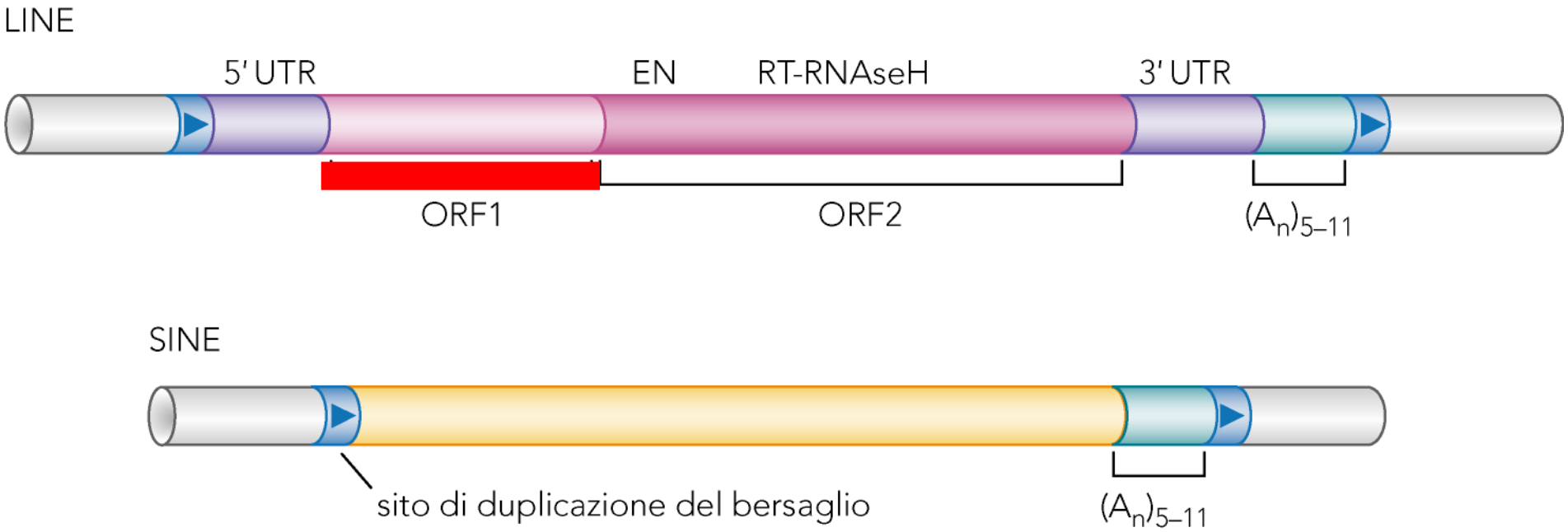

LINE
5′ UTR
EN
RT-RNAseH
?
3′ UTR
ORF1
ORF2
$(A_n)_{5–11}$
SINE
sito di duplicazione del bersaglio
$(A_n)_{5–11}$


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



target-site primed reverse transcription



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

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

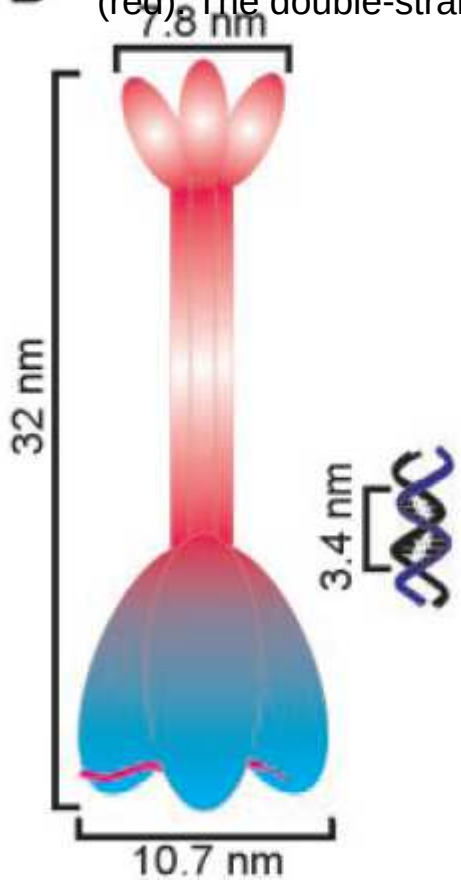
acids (L1 RNA and DNA) and function as a nucleic acid chaperone.

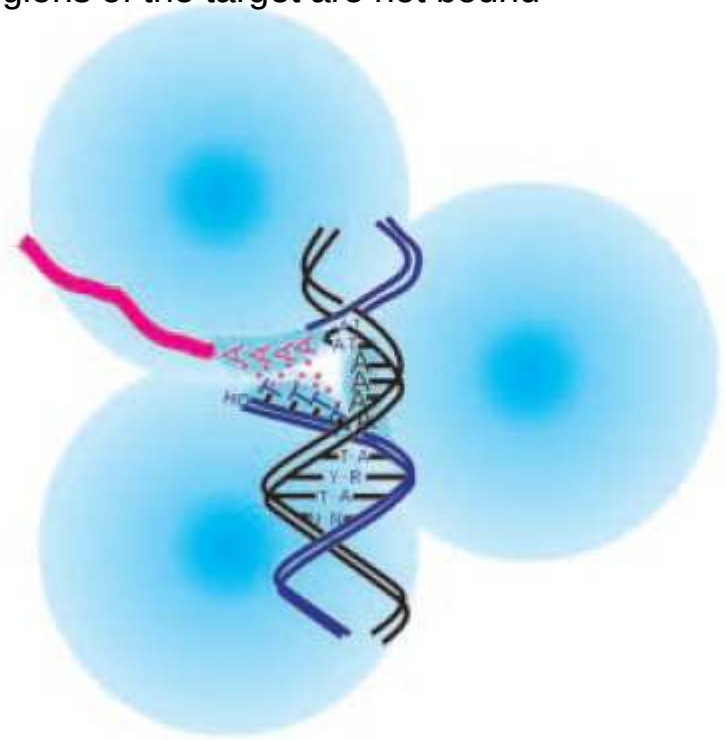
Structural features are compatible with the nucleic acid binding

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

acidic(red) basic (blue)

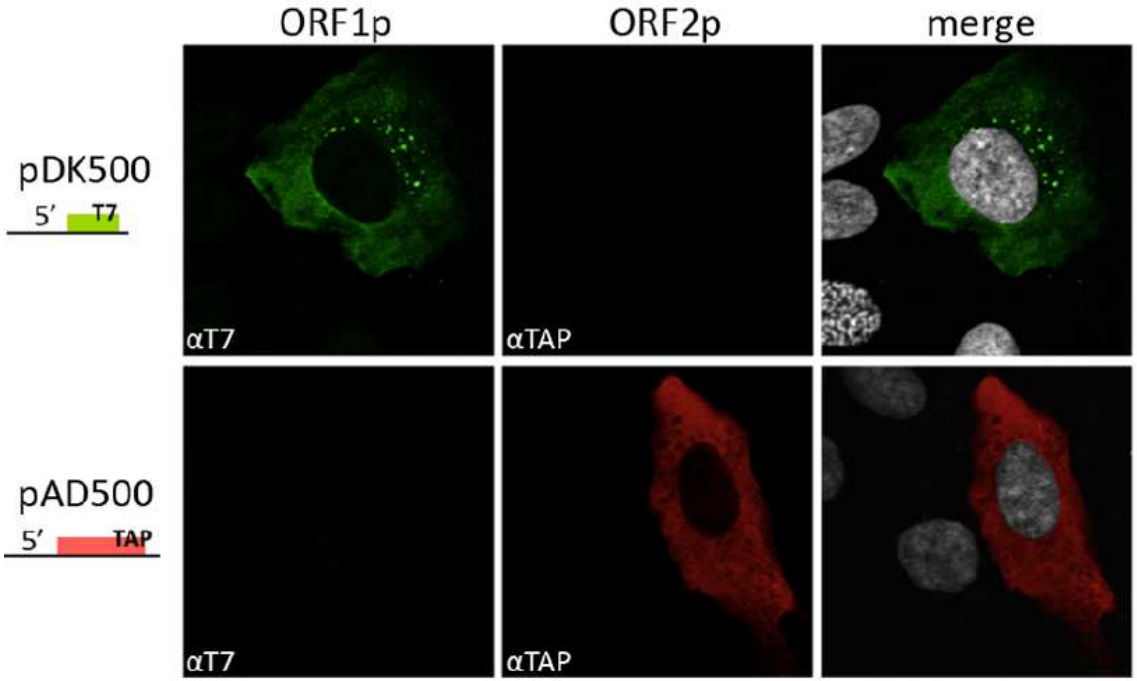
Each subunit of the trimer contains one single-stranded nucleic acid binding interface which is bound with one of the DNA target strands or the polyA tail of the L1 RNA (red). The double-stranded regions of the target are not bound

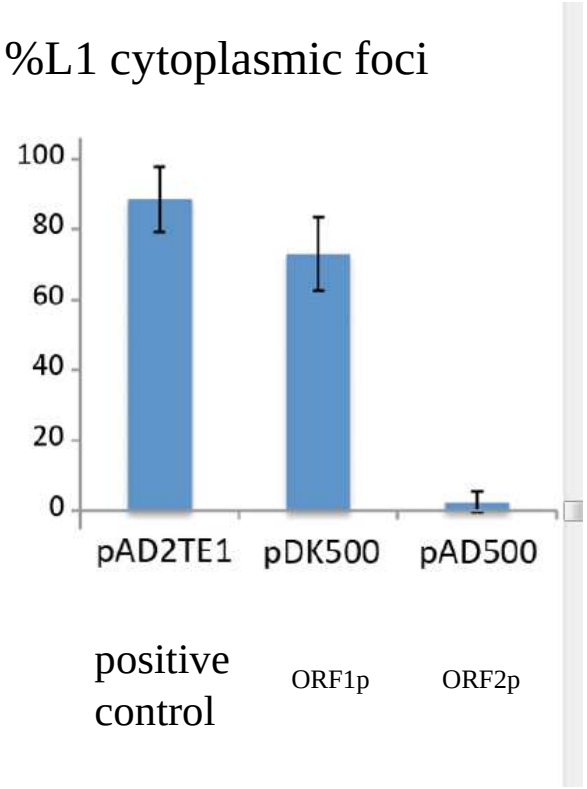




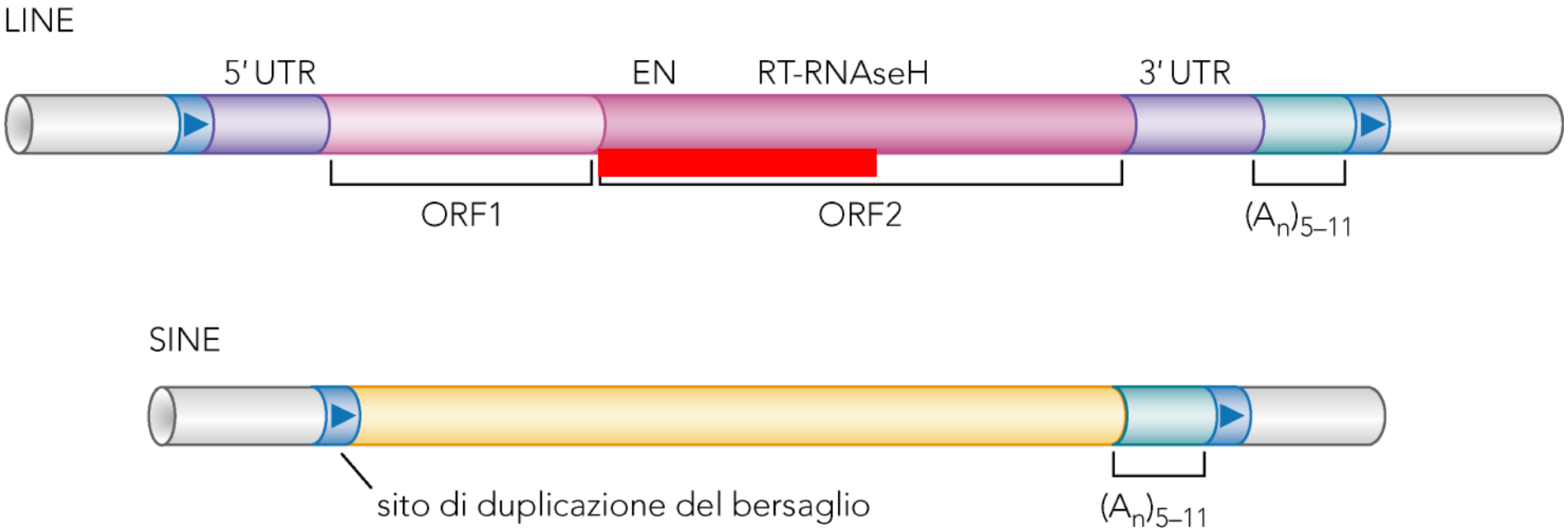
ORF1p lcoats the entire 7-kb L1 RNA to form a ribonucleoprotein particle
The nucleic acid chaperone activity of ORF1p melts the DNA and then facilitates formation of the RNA:DNA hybrid

ORF1p is necessary and sufficient for L1 cytoplasmic foci formation





T7-tagged ORF1p green
TAP-tagged ORF2p red;


LINE
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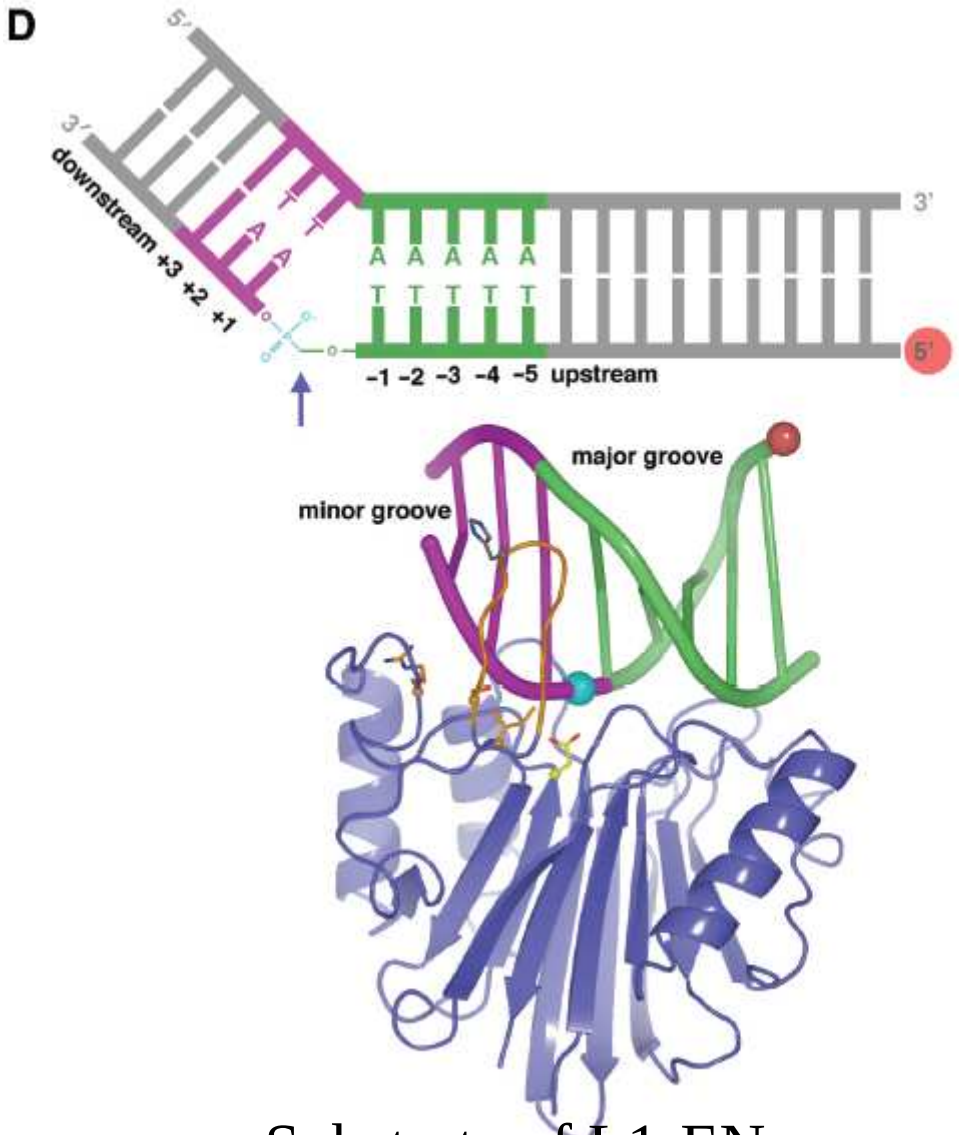
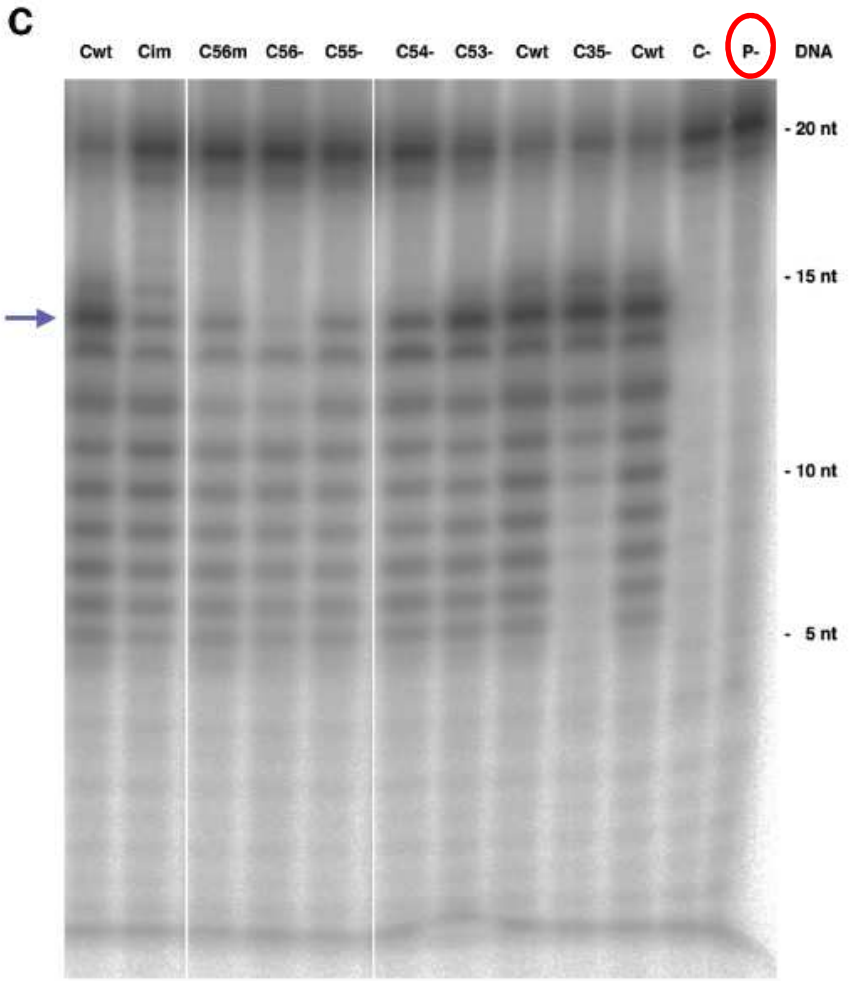


Substrate of L1-EN

Substrate requirements of L1-EN



Specificity of L1-EN

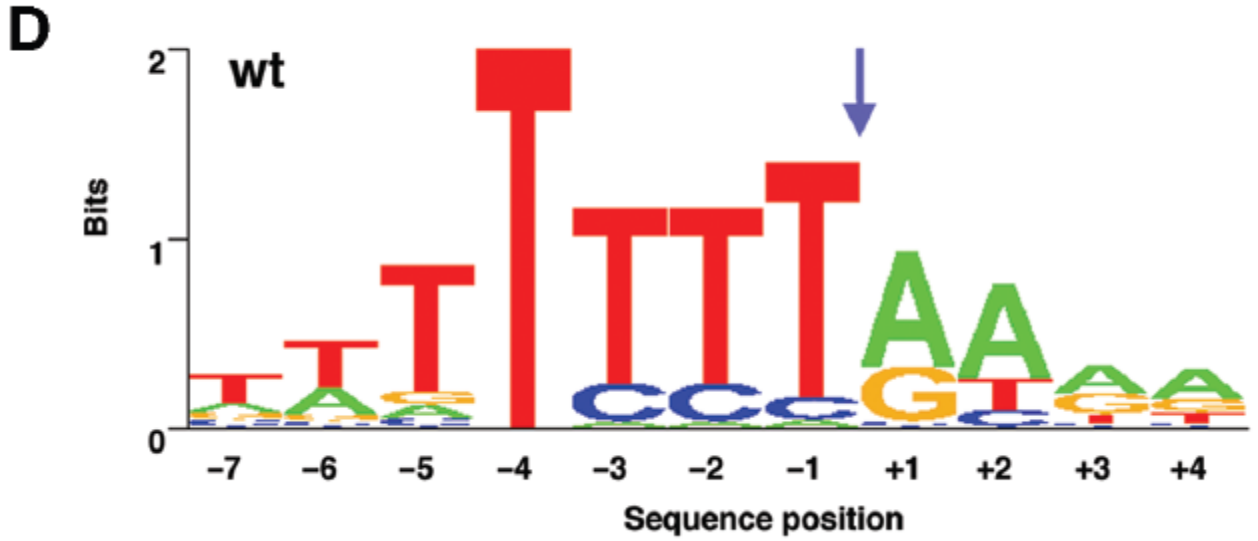


Table 1. Comparison of retrotransposition frequencies *in vivo* and plasmid nicking activities *in vitro*

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.

Come inibire l’espressione e trasposizione del Trasposone

Throughout the domains of life, transposon activity represents a serious threat to genome integrity and evolution has realized different molecular mechanisms that aim to inhibit the transposition of mobile DNA.

Small noncoding RNAs that function as guides for Argonaute effector proteins represent a key feature of so-called RNA interference (RNAi) pathways and specialized RNAi pathways exist to repress transposon activity on the transcriptional and posttranscriptional level.

Transposon transcription can be diminished by targeted DNA methylation or chromatin remodeling via repressive Histone modifications.
Posttranscriptional transposon silencing bases on degradation of transposon transcripts to prevent either reverse transcription followed by genomic reintegration or translation into proteins that mediate the transposition process.

In the germline of animals, these tasks are often assumed by a second subclass of Argonaute proteins referred to as Piwi-like proteins, which bind a distinct class of small noncoding RNAs named piwi-interacting RNAs (piRNAs).

Though the principals of RNAi pathways are essentially the same in all eukaryotic organisms, remarkable differences can be observed even in closely related species reflecting the astonishing plasticity and diversity of these pathways.

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

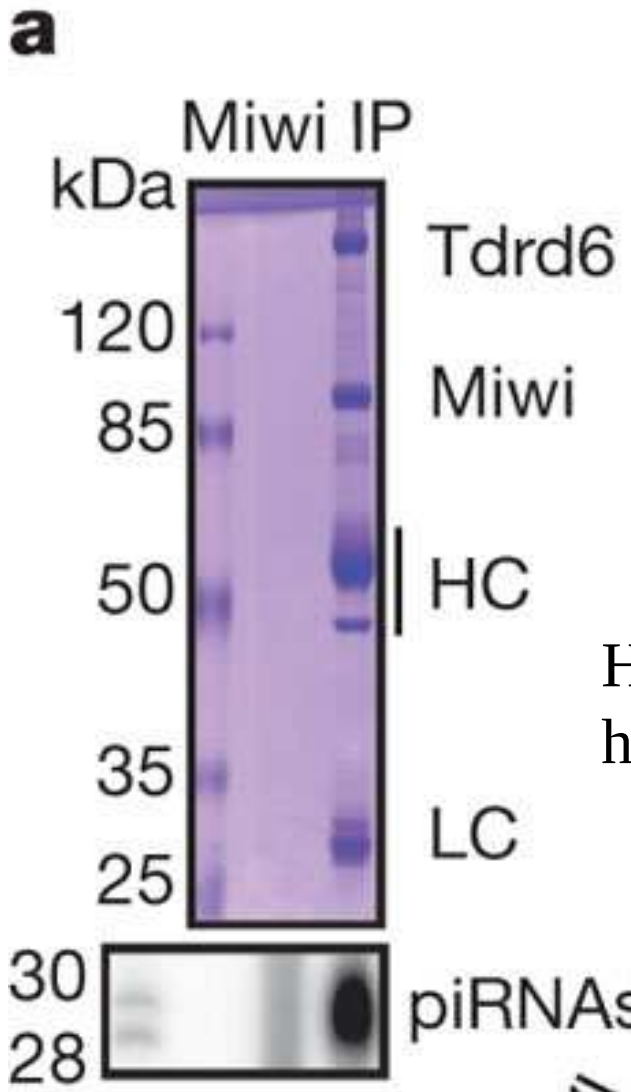
In mice, the piRNA pathway is mainly active in the male germ line where all of the three Piwi proteins (MILI, MIWI, and MIWI2) are expressed.

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).



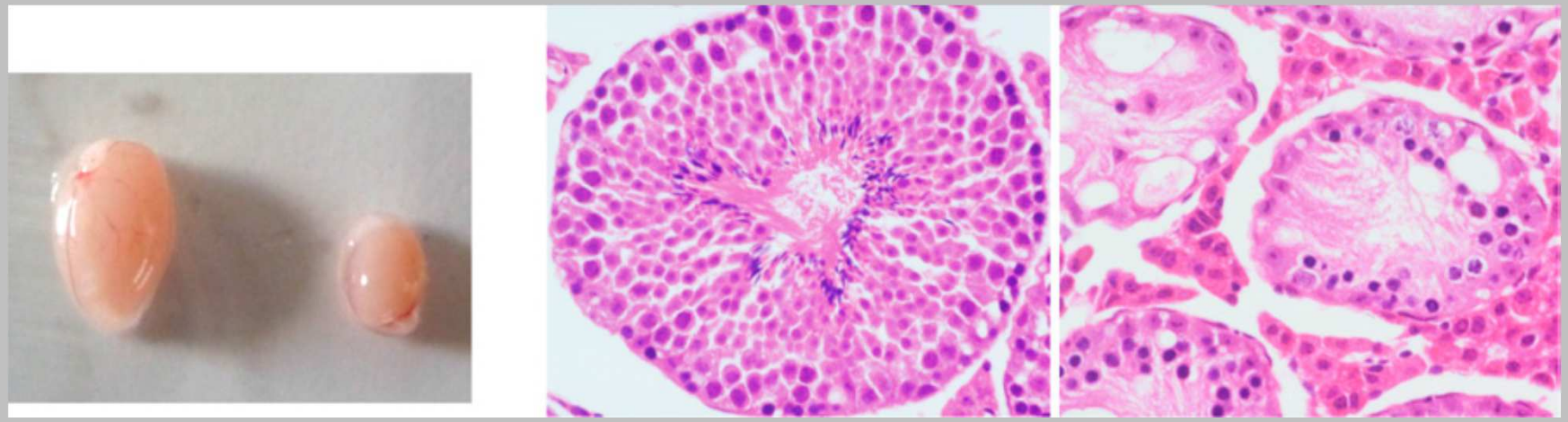
TDRD Tudor domain:
A multidomain protein mediating Complex formation with other participants in piRNA biogenesis.

HC and LC: antibody heavy and light chains

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

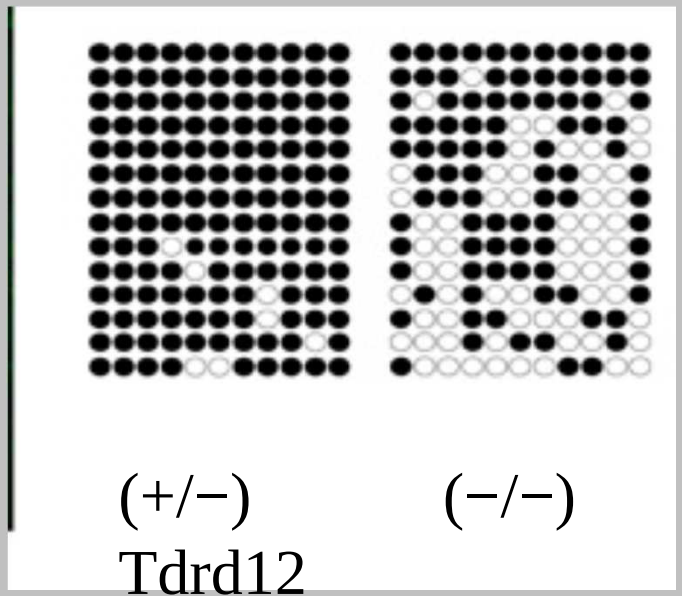
TDRD12 (Tudor Domain) is detected in complexes containing Piwi protein MILI(PIWIL2), its associated primary piRNAs, and TDRD1, all of which are already implicated in secondary piRNA biogenesis. Male mice carrying either a nonsense point mutation or a targeted deletion in the Tdrd12 locus are **infertile** and **derepress retrotransposons.**
We find that TDRD12 is essential for production of secondary piRNAs that enter Piwi protein MIWI2.

Tdrd12 mutant male mice are infertile and display derepression of retrotransposons

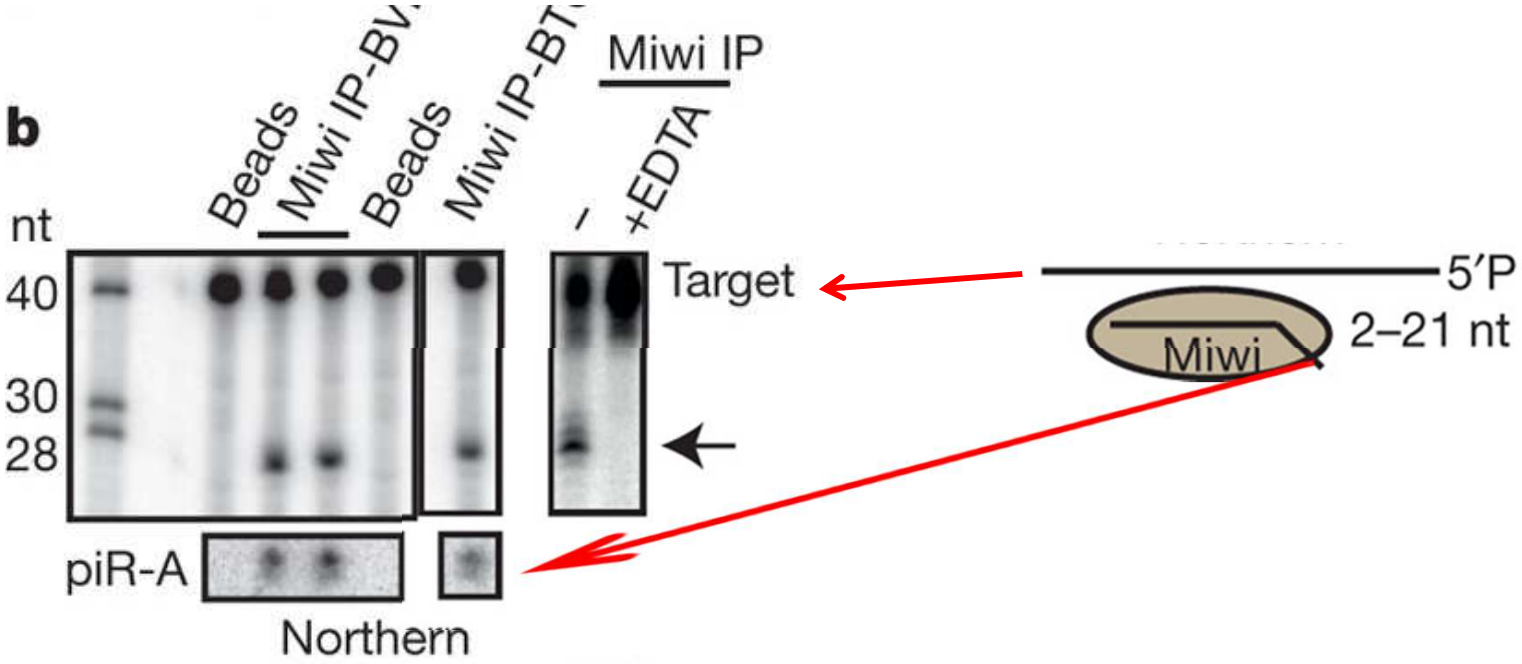


Atrophied testes of homozygous
(−/−) Tdrd12 mutants

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



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



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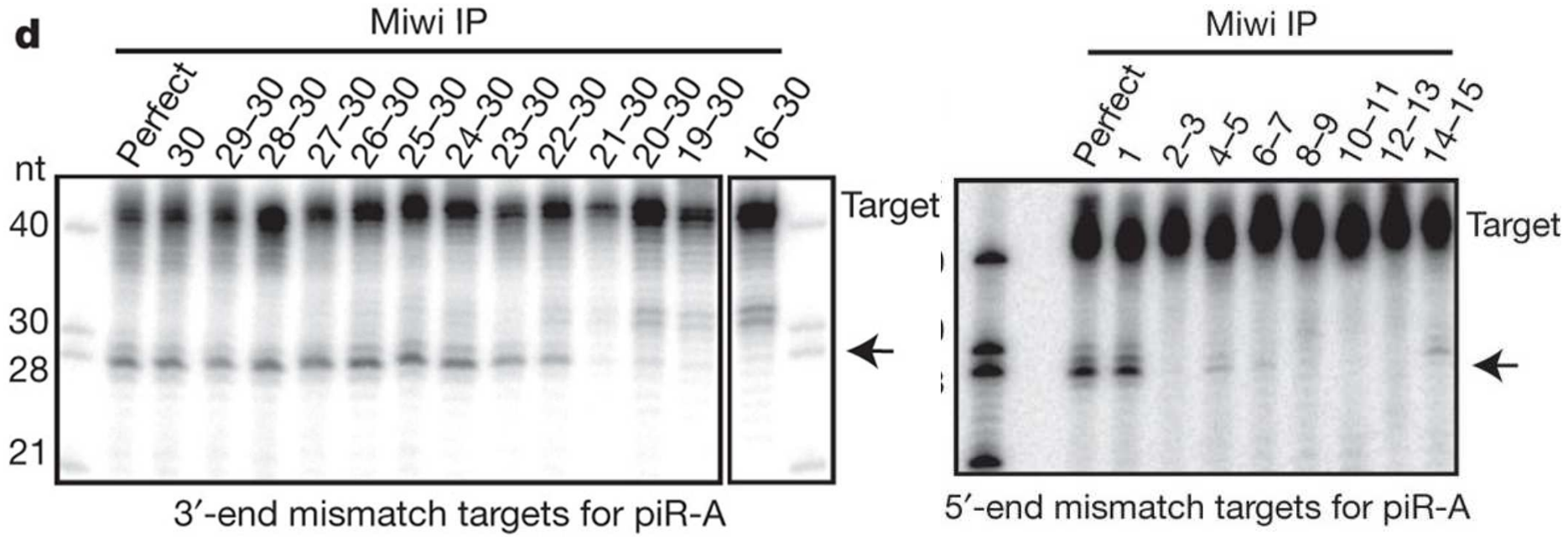
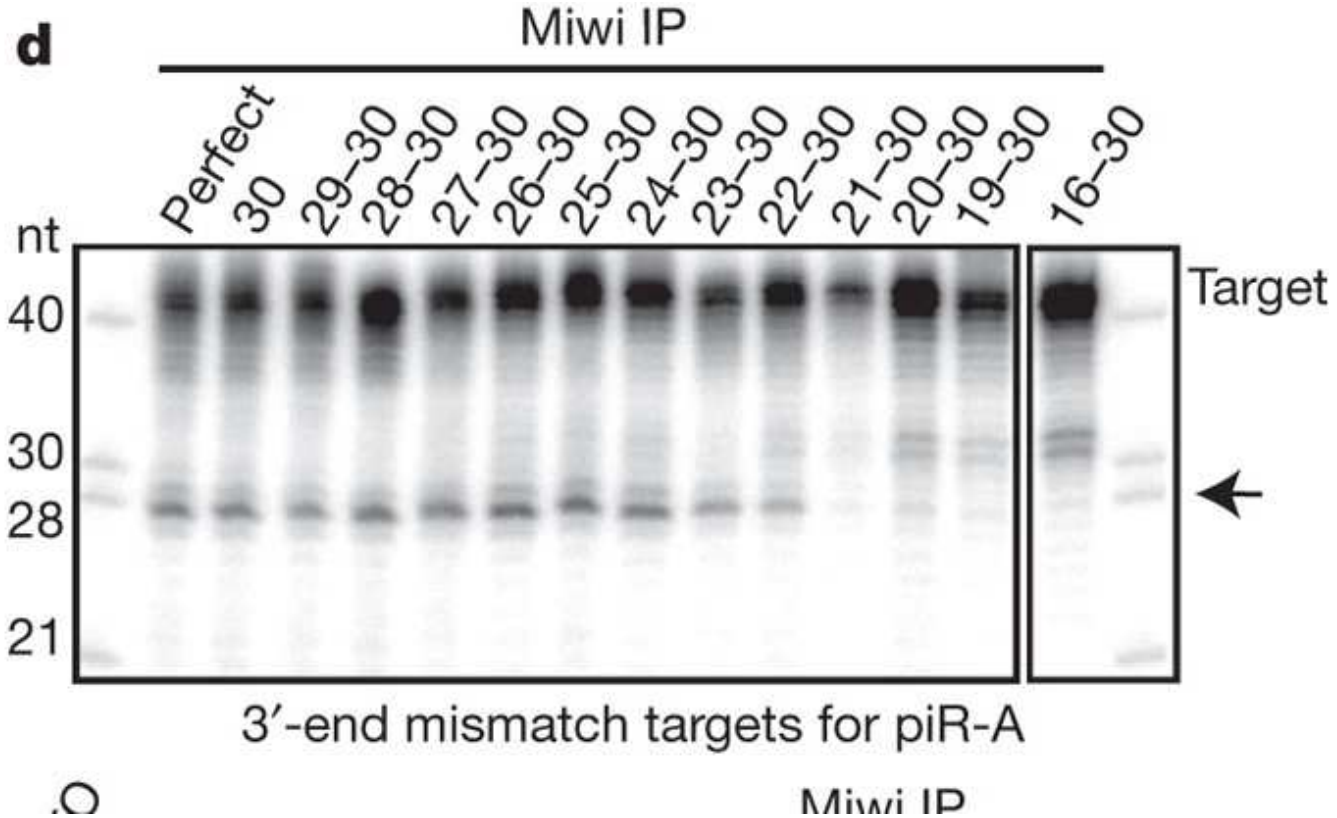


nature

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)

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

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

Loss of Karma transposon methylation underlies the mantled somaclonal variant of oil palm.

Nature. 2015 Sep 24;525(7570):533-7. doi: 10.1038/nature15365. Epub 2015 Sep 9.

Abstract

- Somaclonal variation arises in plants and animals when differentiated somatic cells are induced into a pluripotent state, but the resulting clones differ from each other and from their parents. In agriculture, somaclonal variation has hindered the micropropagation of elite hybrids and genetically modified crops.
- The oil palm fruit 'mantled' abnormality is a somaclonal variant arising from tissue culture that drastically reduces yield, and has largely halted efforts to clone elite hybrids for oil production.

- **DNA hypomethylation of a LINE retrotransposon related to rice Karma** is common to all mantled clones and is associated with alternative splicing and premature termination.
- Dense methylation near the Karma splice site (termed the Good Karma epiallele) predicts normal fruit set, whereas hypomethylation (the Bad Karma epiallele) predicts homeotic transformation and marked loss of yield.