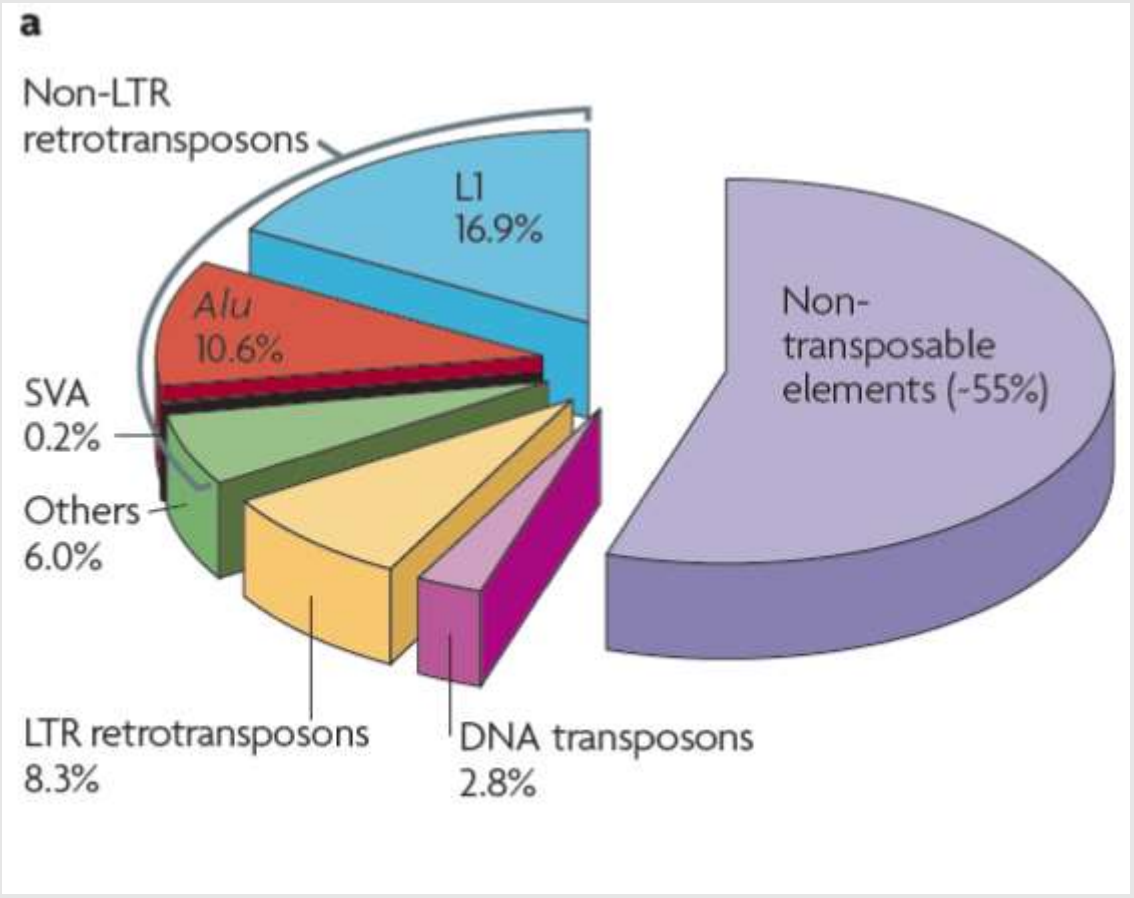
Corso di laurea in Scienze Biologiche
Corso di laurea magistrale in Scienze Biomolecolari e dell'Evoluzione

Materiale didattico di supporto

Tutto il materiale fornito a supporto delle lezioni e reperibile nel minisito dell'insegnamento o sulla piattaforma online UniFE deve essere inteso come traccia degli argomenti svolti e non sostituisce il libro di testo.


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%)

LINE-1

The non-LTR retrotransposon Long INterspersed Element-1 (or L1) is the **only active autonomous TE**.

In addition to mobilizing its own RNA to new genomic locations via a "copy-and-paste" mechanism, LINE-1 **is able to retrotranspose other RNAs including** Alu, **SVA**, and occasionally cellular RNAs.

EVOLUZIONE RECENTE DEI TRASPOSONI
Retrotrasposizione di SVA

SVA

SINE-VNTR-Alu (SVA) elements are **nonautonomous**, hominid-specific non-LTR retrotransposons

They represent the evolutionarily youngest, currently active family of human non-LTR retrotransposons

SINE-VNTR-Alu (SVA)

«composite mobile elements»

Portion of endogenous retrovirus HERV-K10
(env gene + 3′ LTR)

Intact L1 is required for trans-mobilization of SVA

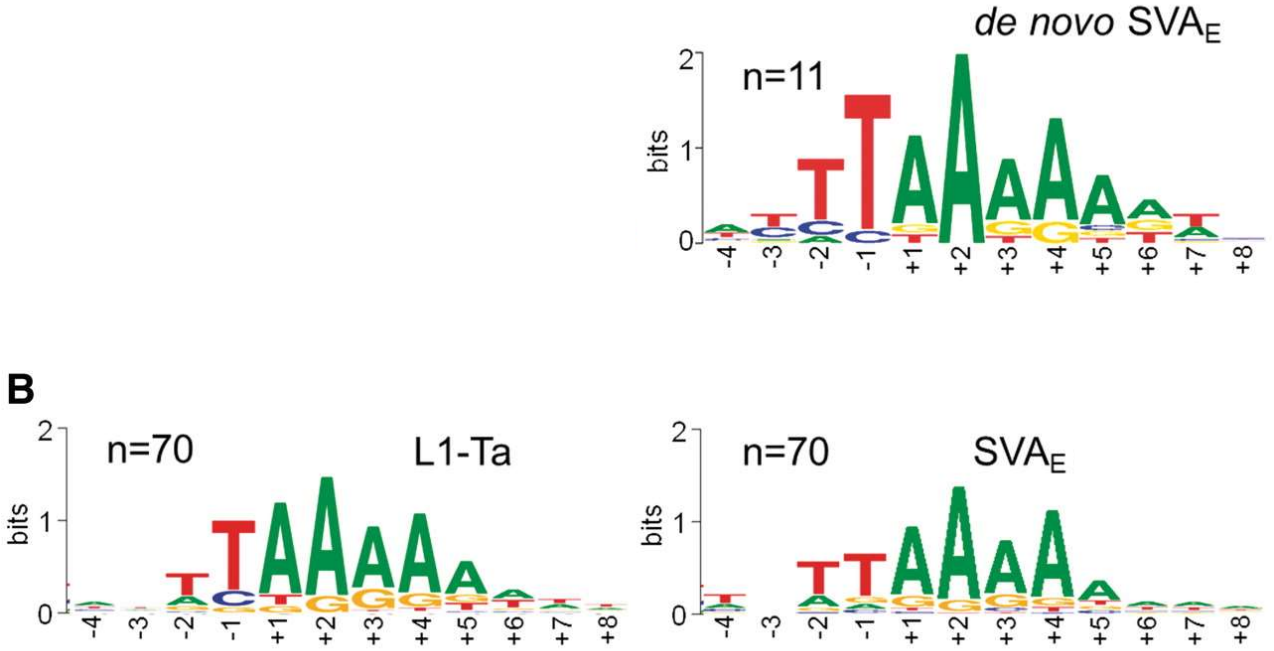
SVA con resistenza
Neomicina

pCEP4 empty vector	1.3 ± 0.67
intact (L1RP)	197 ± 13
mutant (ΔORF1)	3.2 ± 2.3

Raiz J et al. Nucl. Acids Res. 2011;nar.gkr863

Nucleic Acids Research

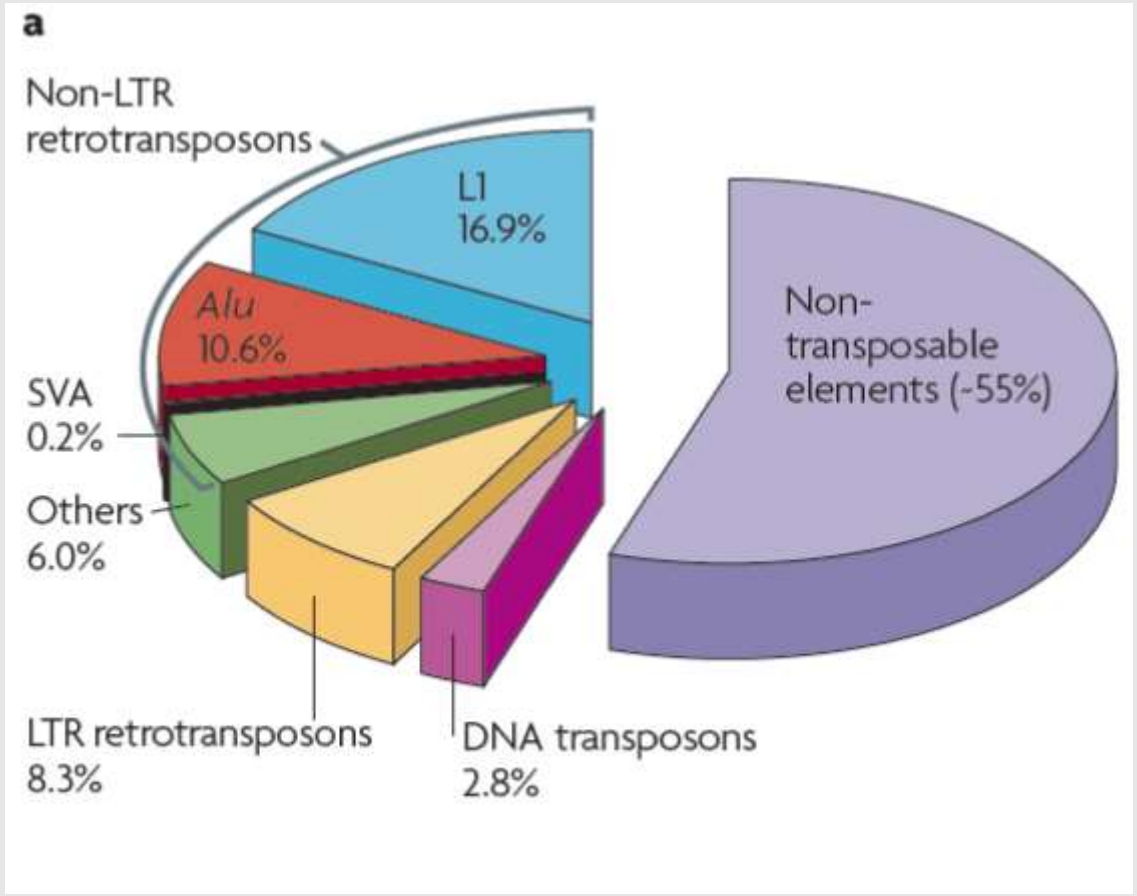
The nucleotide profile of SVAE de novo insertion sites resembles the consensus target sequence of pre-existing human-non-LTR retrotransposons.



Raiz J et al. Nucl. Acids Res. 2011;nar.gkr863

Nucleic Acids Research

EVOLUZIONE RECENTE DEI TRASPOSONI
La Retrotrasposizione di SVA dipende dalle proteine codificate da L1 (ORF1p e ORF2p)


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

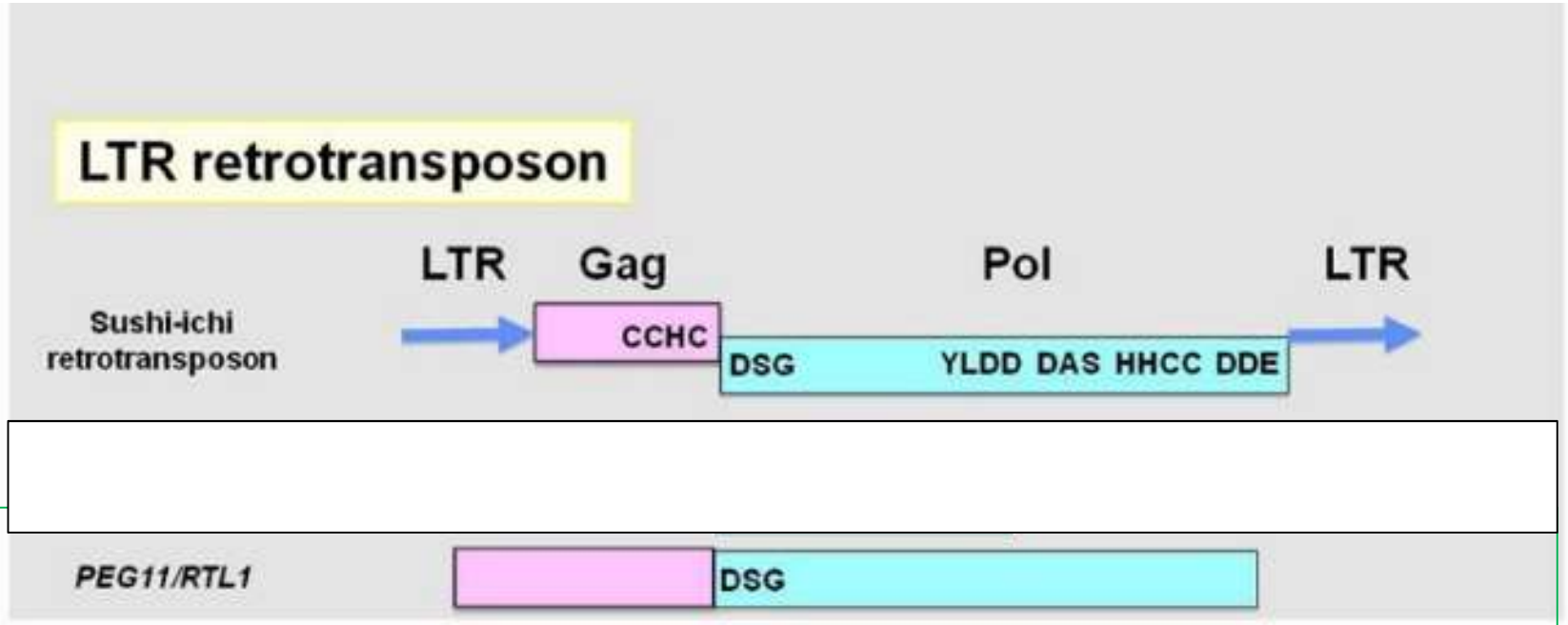
RTL1 is one of the LTR retrotransposon-derived genes specific to eutherian mammals

These elements contain a gag-pol-like structure characteristic of retroviruses

Have lost their ability to retrotranspose into the mammalian genome

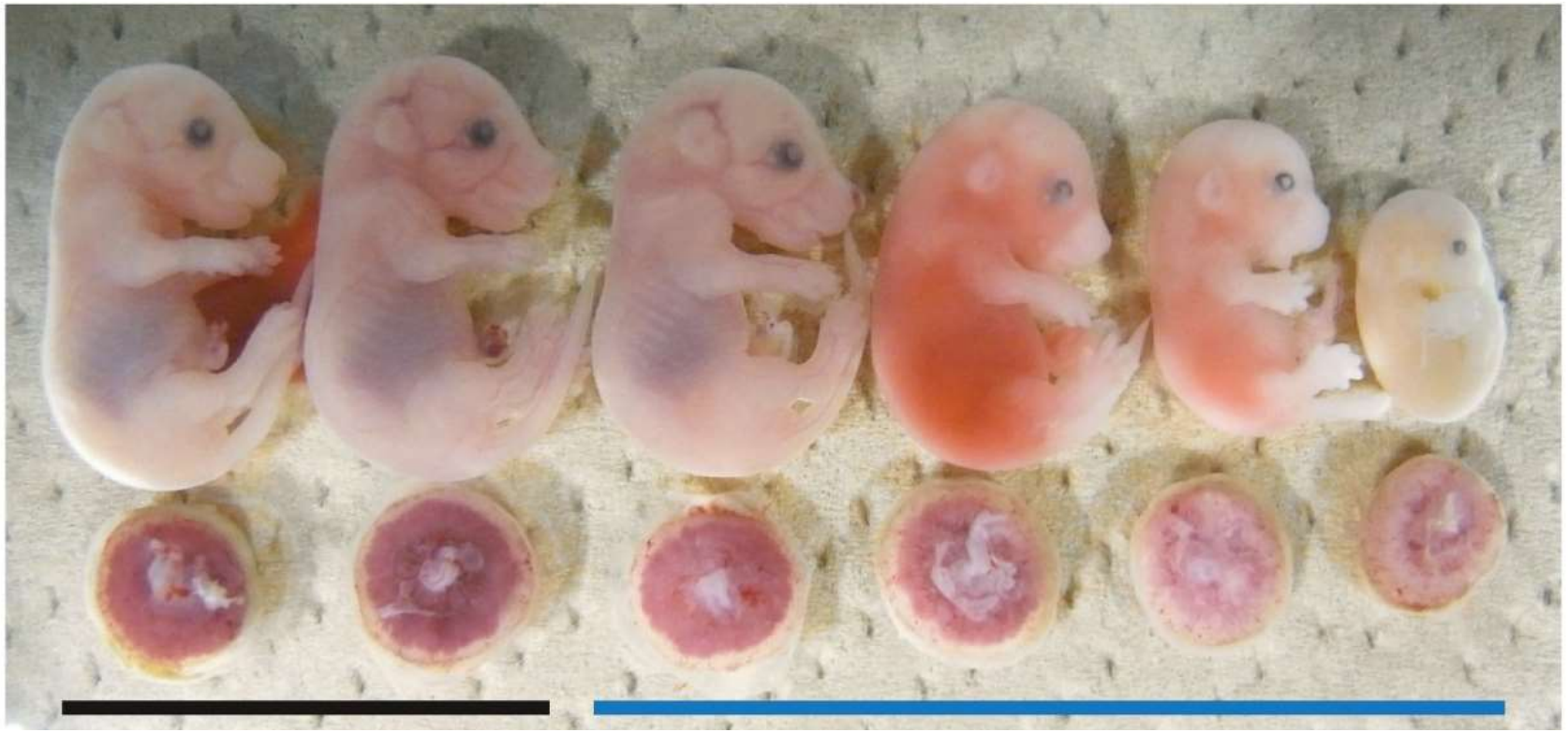
Are thought to be inactive relics of ancient retrotransposition events.

One of these retrotransposon-like elements is RTL1/PEG11



DSG, protease active site

■ : WT ■ : *Peg11/Rtl1* KO



Dawei Yu et al. PNAS 2018;115:47

PNAS

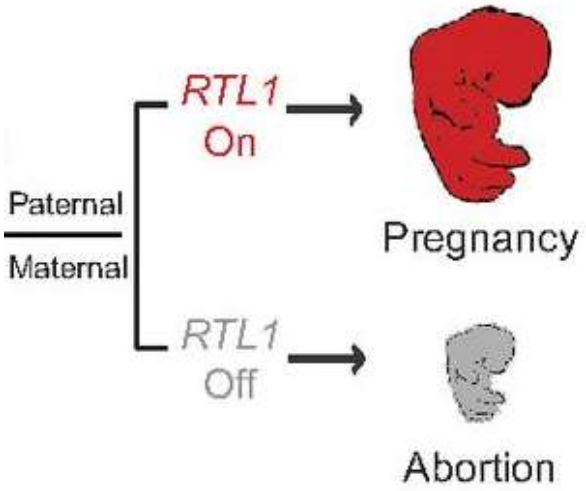
What is the function of Peg11/Rtl1 in the maintenance of fetal capillaries? How does it regulate the interaction between the endothelial cells and the surrounding cells

As Peg11/Rtl1 protein possesses a protease domain derived from a retrotransposon Pol protein, it is possible that this protease activity is important for protecting the endothelial cells

RTL1 rescues excessive loss of Pluripotent Stem Cell (iPSC)-derived fetuses

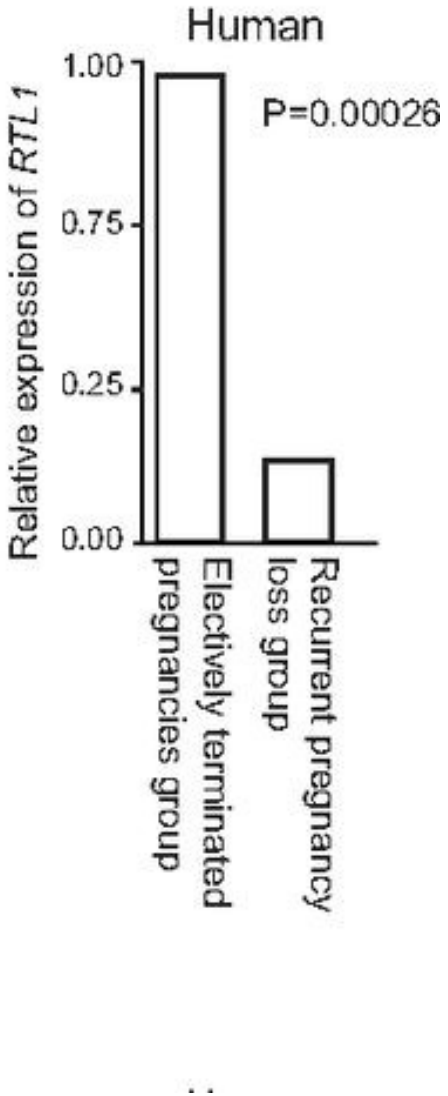
plasmid for RTL1 overexpression

RTL1-iPSC embryos were transferred into 10 recipients and 9 were pregnant



Dawei Yu et al. PNAS 2018;115:47:E11071-E11080

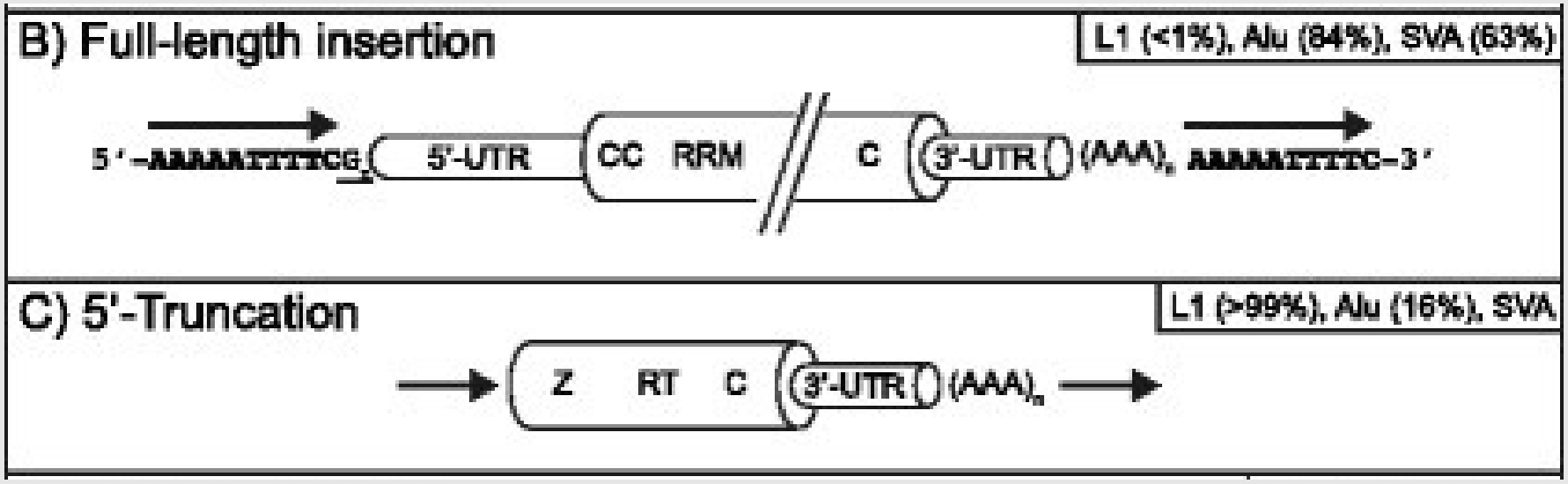
relative expression of RTL1 in human first trimester placental chorionic villi



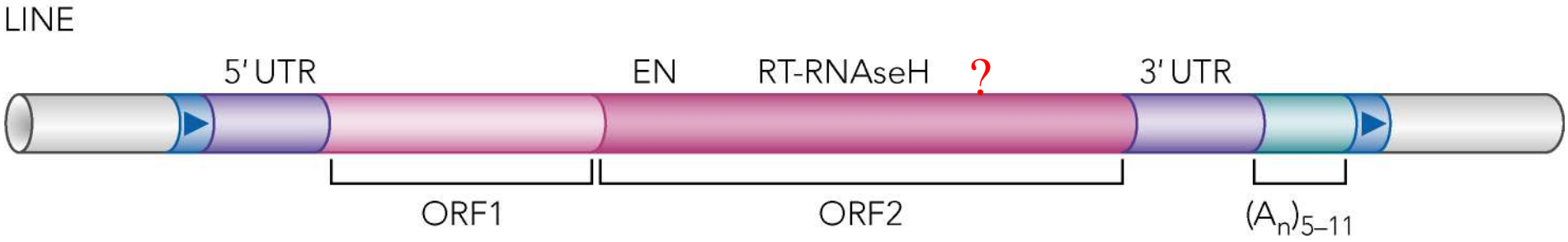
Dawei Yu et al. PNAS 2018;115:47:E11071-E11080

L’espressione di proteine dei trasposoni (RTL1)
è coinvolta nello sviluppo fetale dei mammiferi placentati

retrotransposon insertions



100 copies/genome ACTIVE Full-length 6 KB



516000 copies

20% genome

0,9Kb mean length Truncated!

A large fraction of truncated L1 is located in introns

¼ of promoters contain repetitive sequences

REGOLAZIONE DELLA TRASCRIZIONE DA TRASPOSONI NEL GENOMA

L1 intragenica ed espressione genica

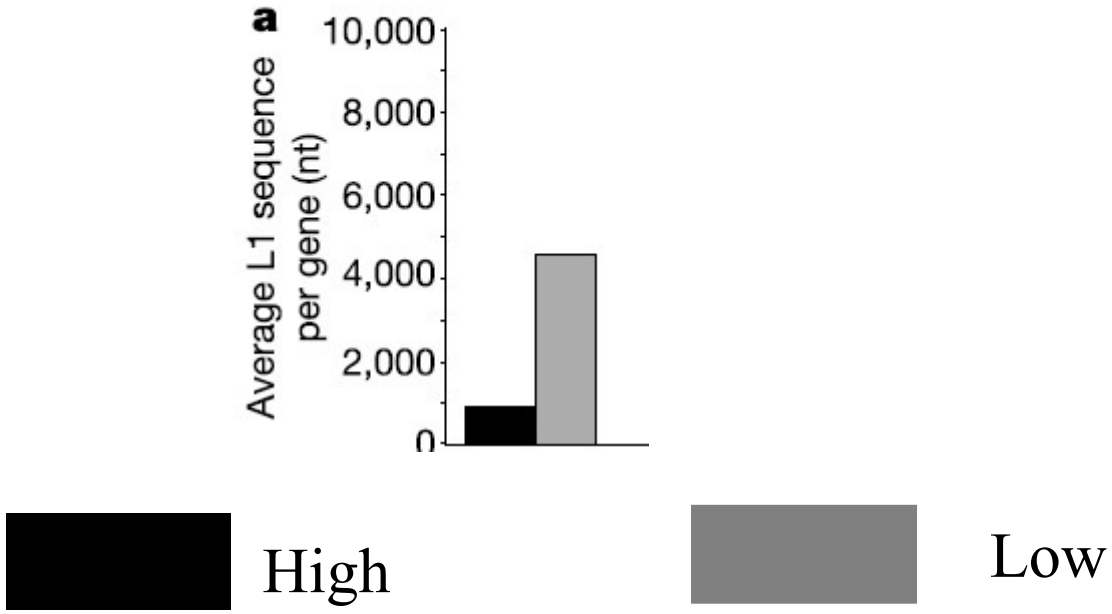
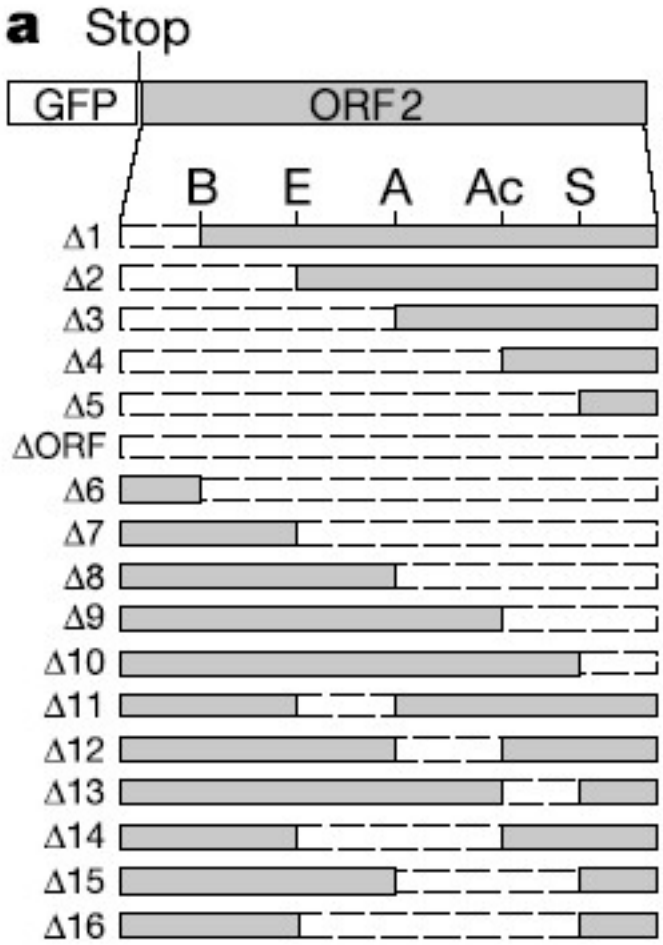


Figure 5 Bioinformatic analysis of L1 content in genes. **a**, Average L1 content of genomic loci of sets of highly (black bars) and poorly (grey bars) expressed genes (see Methods).

Nature. 2004 429:268-74 Han JS

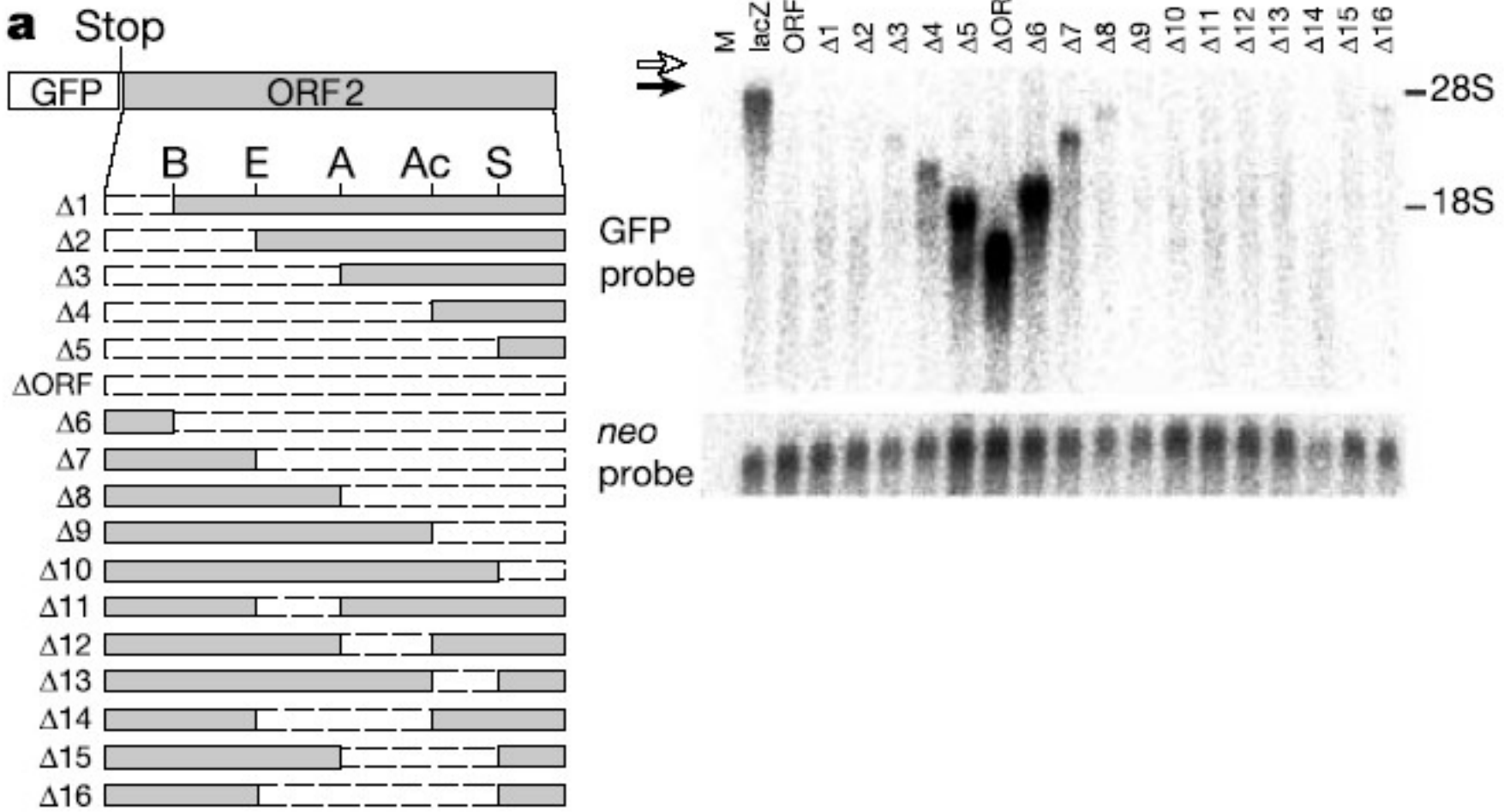
L1 mutants



Fusione di sequenze GFP con ORF2 Intatta o deleta

L1 mutants

RNA analysis



Decrease in expression depends on L1 length

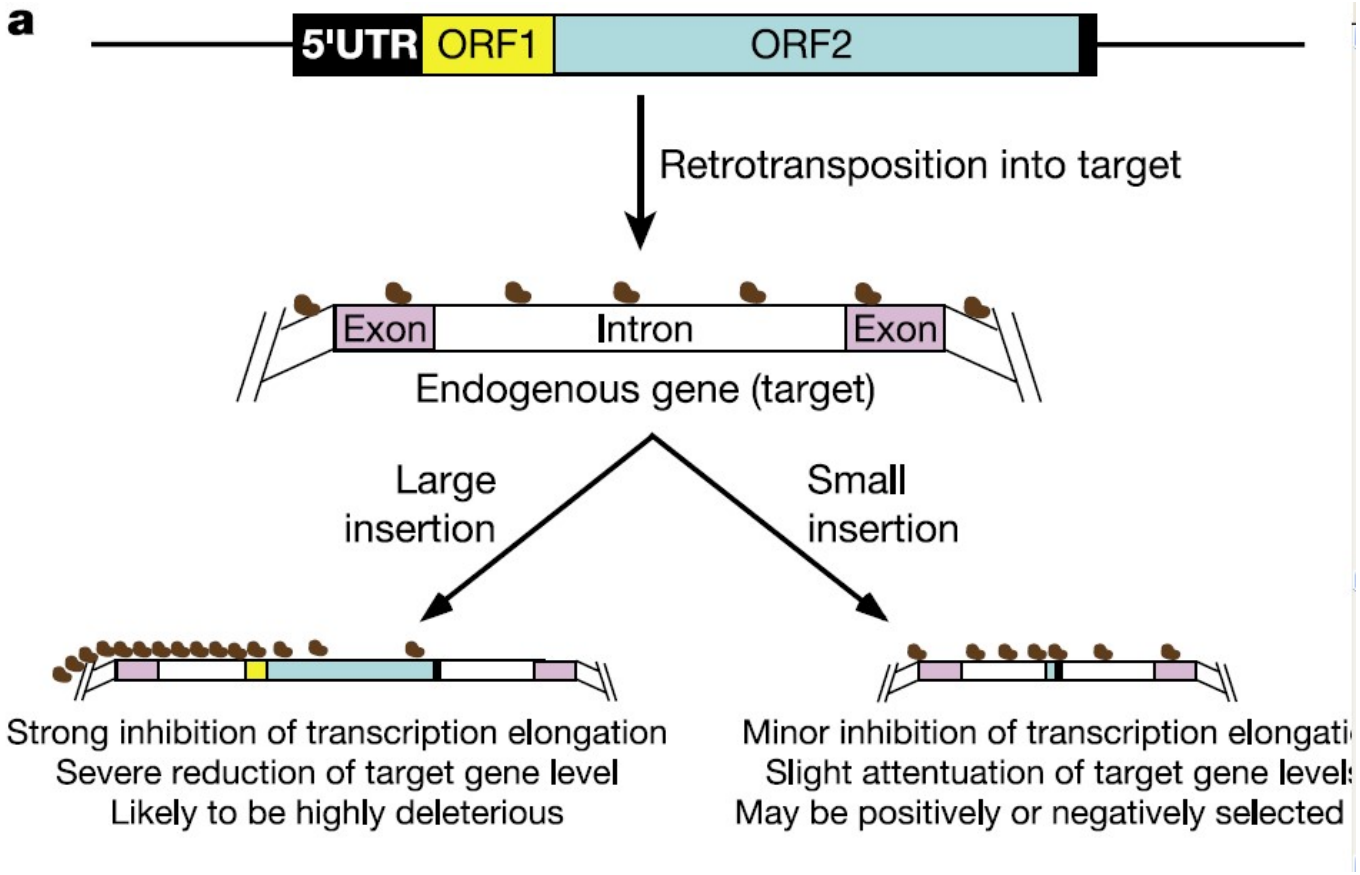


Figure 6 Models for L1-mediated modulation of gene expression/structure. **a**, Effects on transcription. Brown dots represent transcriptional complexes, which could be slowed, paused or dissociated from the templates on encountering significant amounts of L1

Transcriptional disruption by the L1 retrotransposon implications for mammalian transcriptomes.

Nature. 2004 429:268-74 Han JS

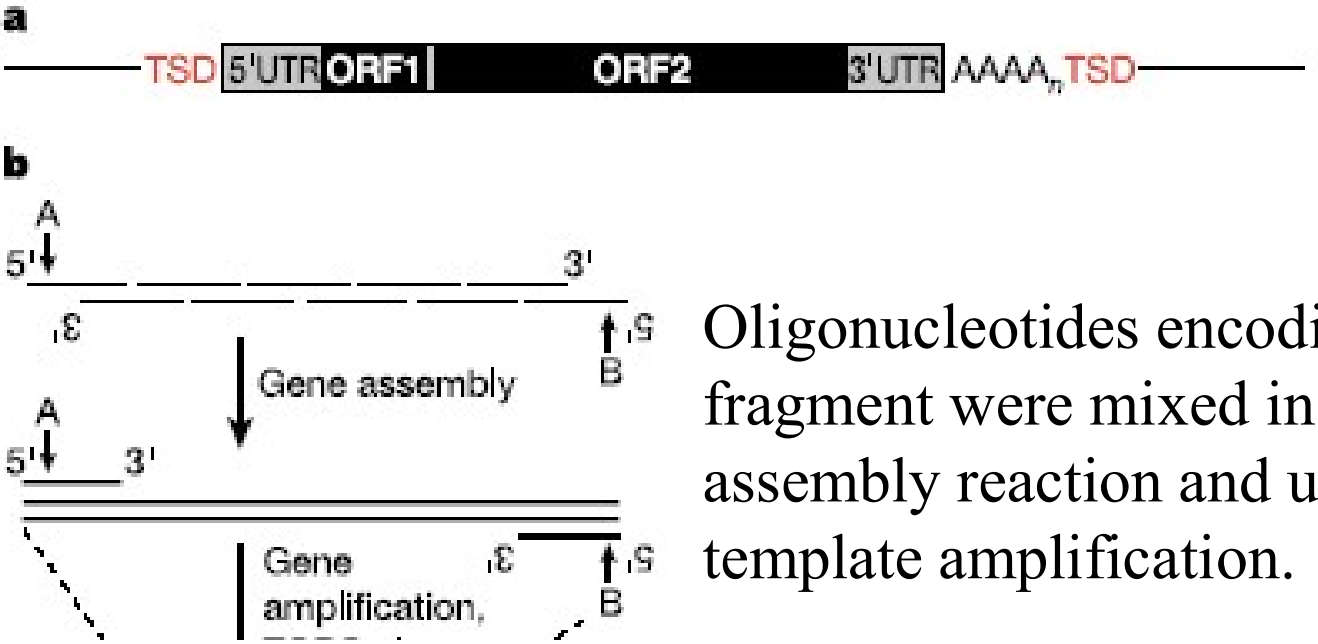
Inserting L1 sequences on a transcript significantly decreases RNA expression and therefore protein expression.

The poor L1 expression is primarily due to inadequate transcriptional elongation.

Because L1 is an abundant and broadly distributed mobile element, the inhibition of transcriptional elongation by L1 might profoundly affect expression of endogenous human genes.

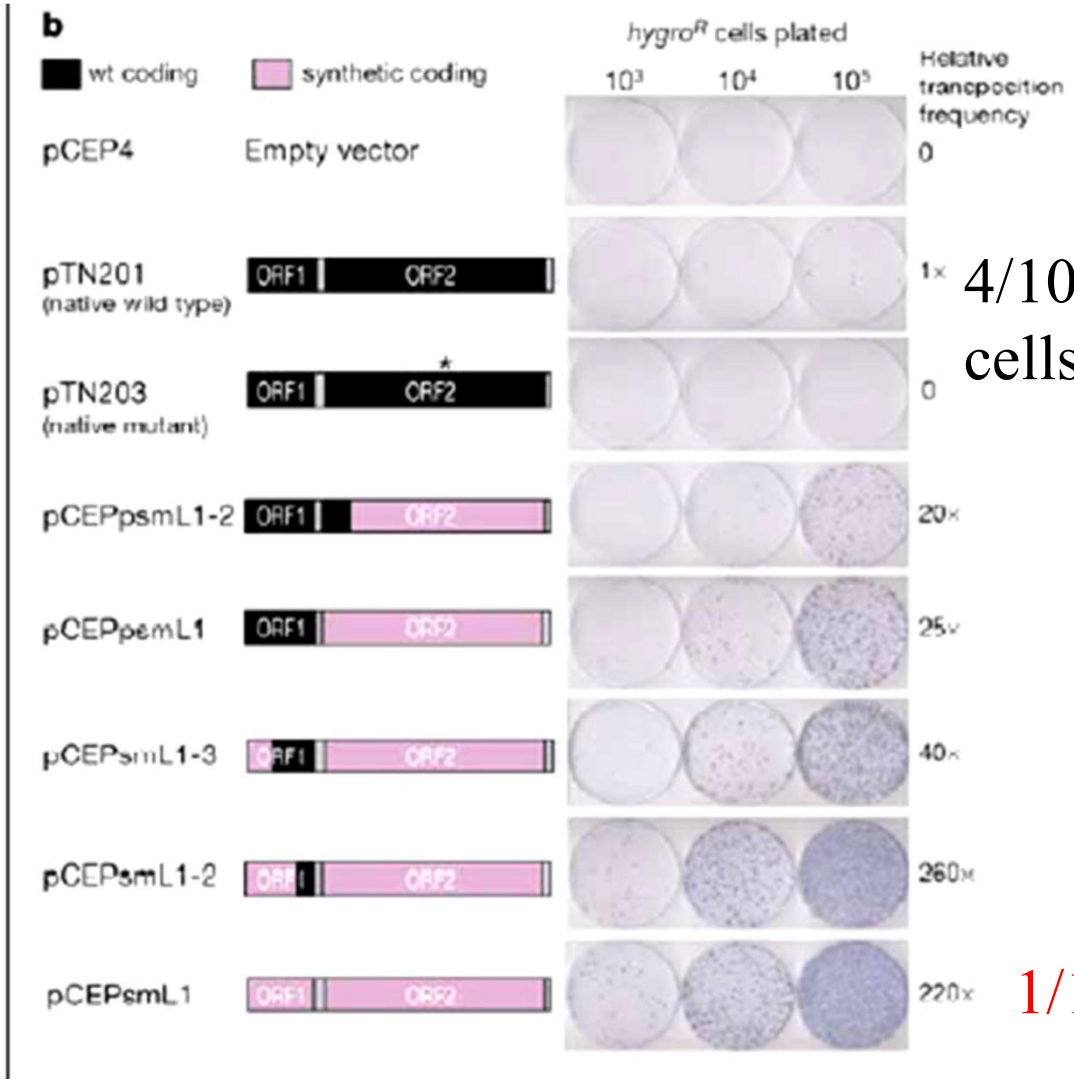
We propose a model in which L1 affects gene expression genome-wide by acting as a 'molecular rheostat' of target genes. L1 can serve as an evolutionary fine-tuner of the human transcriptome.

A highly active synthetic mammalian retrotransposon.
Han JS Nature. 429:314 2004



Oligonucleotides encoding each fragment were mixed in a PCR assembly reaction and used as template amplification.

Synthesis cloning and expression of synthetic mouse ORFs



4/10000 cells

selezione di cellule resistenti aseguito di retrotrasposizione

1/10 cells!

Figure 2 Retrotransposition of synthetic mL1. **a**, The retrotransposition assay. The L1 element contains an intron-interrupted *neo* reporter in the 3′ untranslated region with its own promoter and polyadenylation signal. Only when *neo* is transcribed from the L1 promoter, spliced, reverse transcribed and integrated into the genome does a cell become G418-resistant[4]. Blue lines represent probes for RNA analysis (Fig. 4). SD, splice donor; SA, splice acceptor. **b**, Retrotransposition was assayed in HeLa cells ($N = 3$). pTN201 contains only wild-type native mouse L1 sequence, and pTN203 contains wild-type native mouse L1 sequence with a D709Y reverse transcriptase point mutation[22]. The average absolute number of colonies for pTN201 was 440 events per 10^6 transfected cells.

A highly active synthetic mammalian retrotransposon.

Han JS Nature. 429:314

Transcription through L1 open reading frames is inefficient owing to an elongation defect

This elongation defect probably controls L1 transposition frequency in mammalian cells.

We report bypassing this transcriptional defect by synthesizing the open reading frames of L1 from synthetic oligonucleotides, altering 24% of the nucleic acid sequence without changing the amino acid sequence.

When the synthetic open reading frames were substituted for the wild-type open reading frames in an established retrotransposition assay, transposition levels increased more than 200-fold.

These synthetic retrotransposons are also the most highly active L1 elements known so far and have potential as practical tools for manipulating mammalian genomes