

The non-coding genome evolution



Genome	5×10^6 bp	1×10^8 bp	3×10^9 bp
Chromosomes	1	6	23
Coding genes	6692	20541	21995
ncDNA	5%	60%	98%

Un grande parte del genoma umano consiste di elementi genetici mobili – genetic mobile elements

TRANSPOSONS

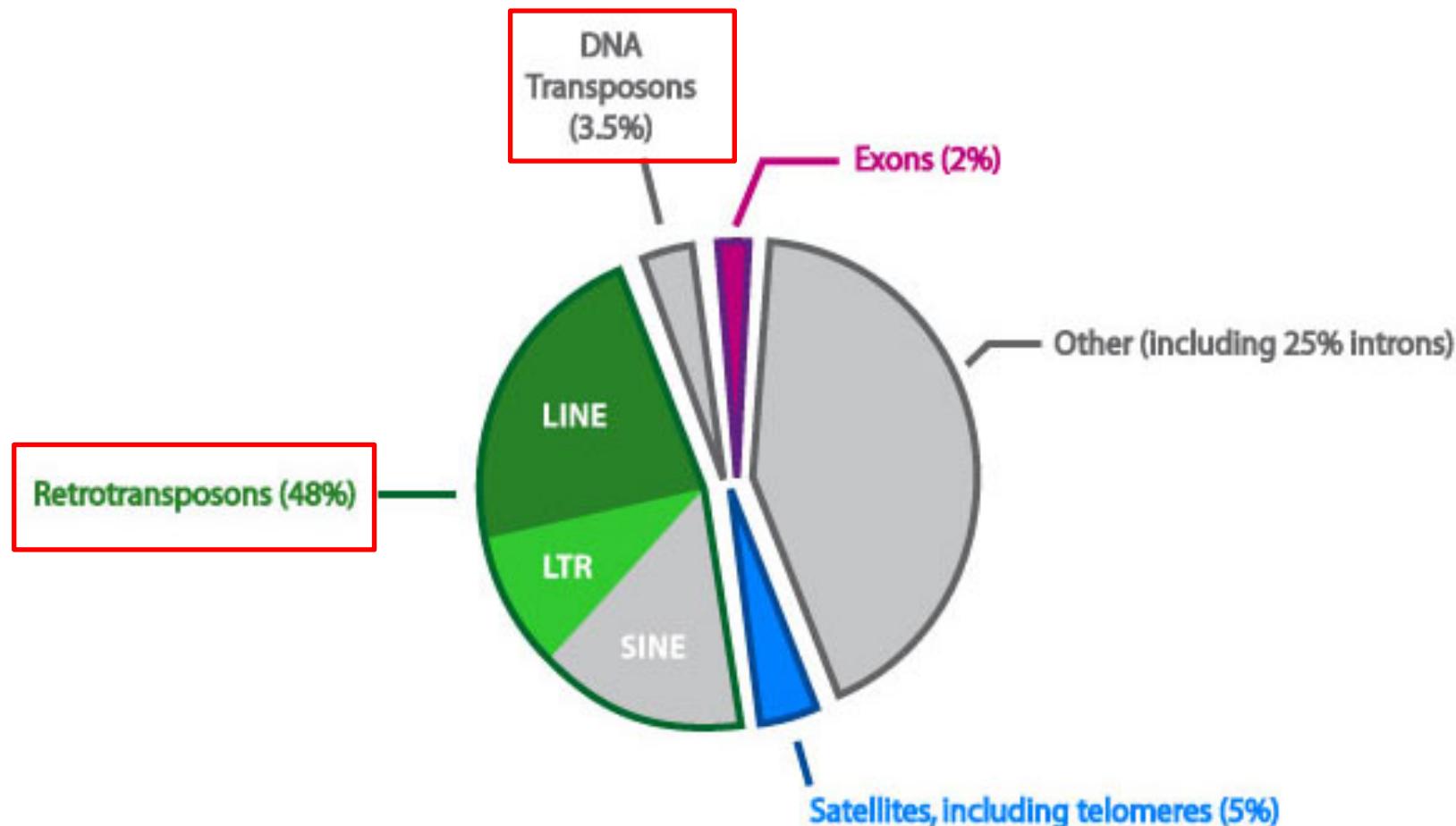


Fig. 1 - Composition of the human genome
Cristofari's lab 2010 - Adapted from Rollins et al. *Genome Res.* (2006)

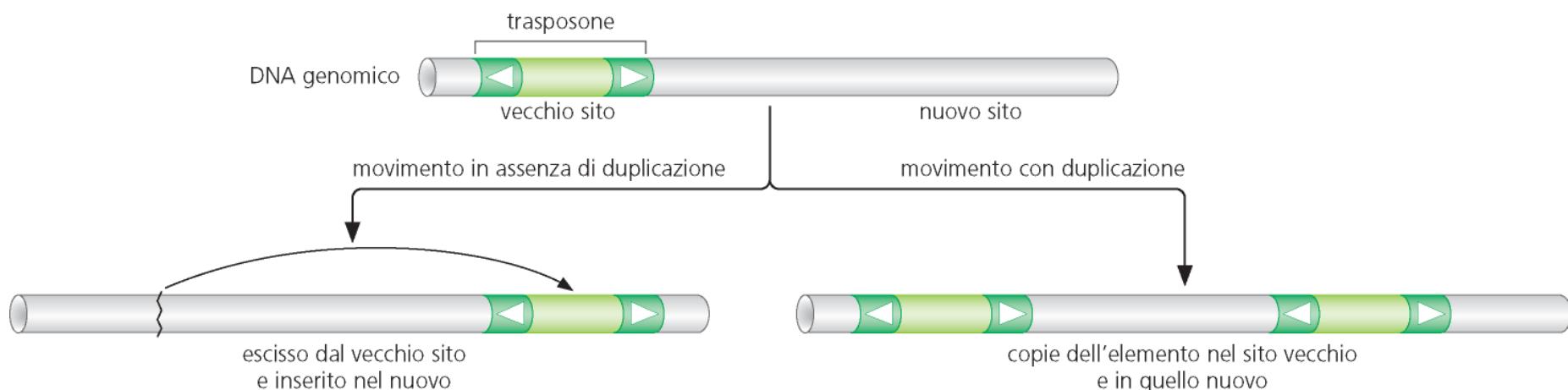
Trasposoni

Presente in tutte le speze: Fagi → vertebrati

Phages	<i>E. Coli</i>	<i>Yeast</i>	<i>Drosophila</i>	<i>Maize (Plants)</i>	<i>Human</i>
Mu <small>(repoduces via transposition)</small>	IS1 IS10 Tn3 Tn5 Tn7 Tn10	Ty1	P-Element Copia F-Element Hermes	Ac-Dc BS1 Cin4	Ty1 THE-1 L1 LINE
					<small>Mechanism used for V(D)J recombination (maturation of immunoglobilins)</small>

La trasposizione

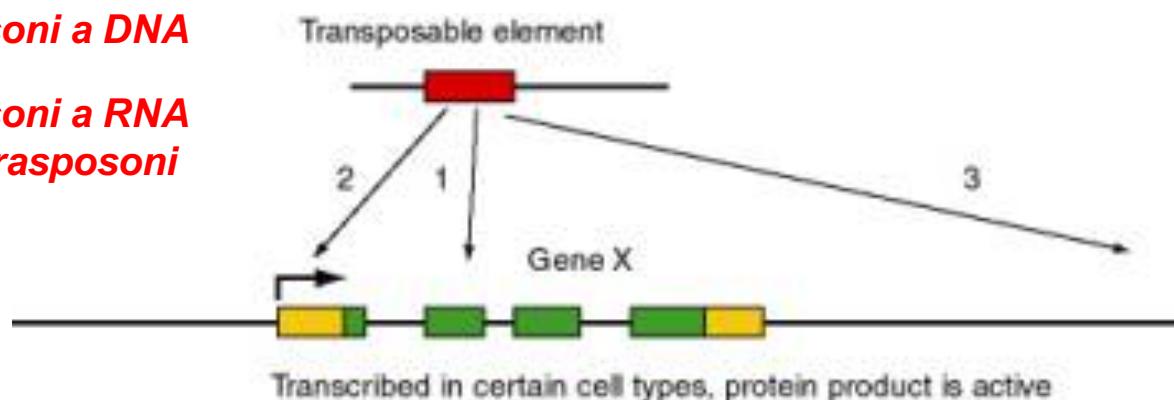
- Elementi trasponibili o trasposoni.
- Sposta elementi genetici da un sito ad un altro
- Il movimento avviene per una **ricombinazione** tra le **estremità dell'elemento trasponibile** e una **sequenza di DNA** della cellula.



Trasposizione: scarsa selettività nella scelta del sito d'inserzione

Trasposoni a DNA

**Trasposoni a RNA
=Retrotrasposoni**



Mobilità di trasposoni provoca malattie umane genetiche:

Hemophilia A/B (i.e. LINE 1 insertion into coagulation factor (Factor VIII))

Duchenne muscular dystrophy

Cancer

Severe combined immunodeficiency

Neurodegenerative disease

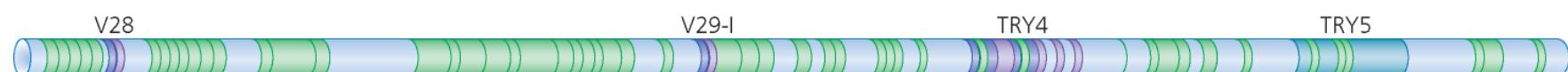
Haemophilia, also spelled hemophilia, is a mostly inherited genetic disorder that impairs the body's ability to make blood clots, a process needed to stop bleeding.[1][2] This results in people bleeding longer after an injury, easy bruising, and an increased risk of bleeding inside joints or the brain. Those with mild disease may only have symptoms after an accident or during surgery. Bleeding into a joint can result in permanent damage while bleeding in the brain can result in long term headaches, seizures, or a decreased level of consciousness.[3]

I trasposoni nei genomi: frequenza e distribuzione

a Mais



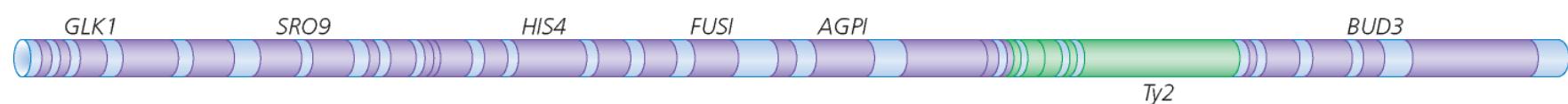
b Uomo 90% DNA non codificante; 2% codificante x proteine; 50% trasposoni; resto fragmenti tronchi, ncRNA genes, sequenze ripetute



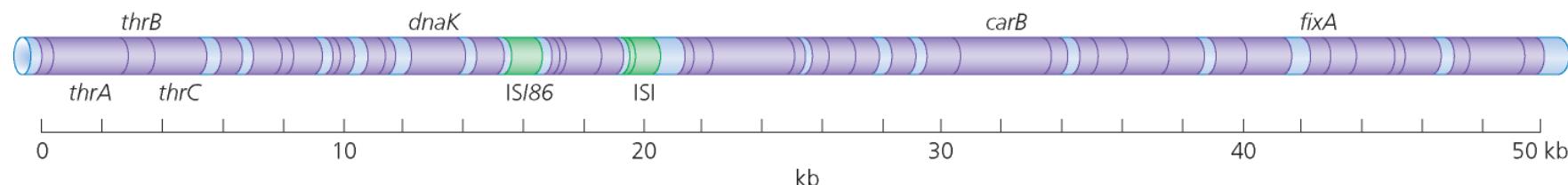
c *Drosophila melanogaster*



d *Saccharomyces cerevisiae*



e *Escherichia coli*

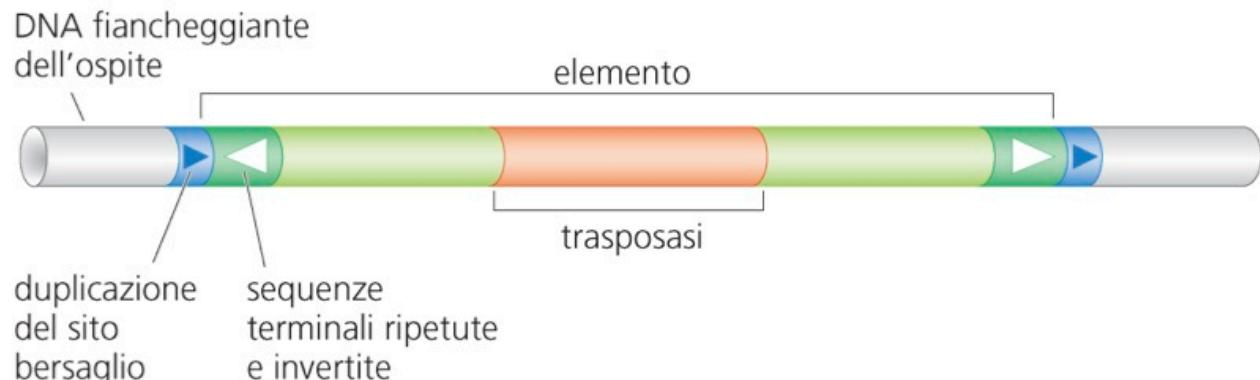


Tre classi di elementi trasponibili

Trasposoni a DNA

3.5% of human genome

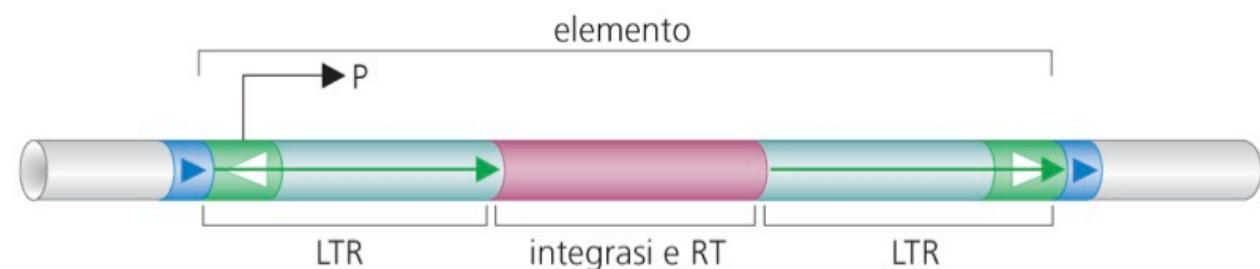
a trasposoni a DNA



Retrotrasposoni simile ai virus

48% of human genome

b retrotrasposoni tipo virus/retrovirus



Retrotrasposoni poli-A

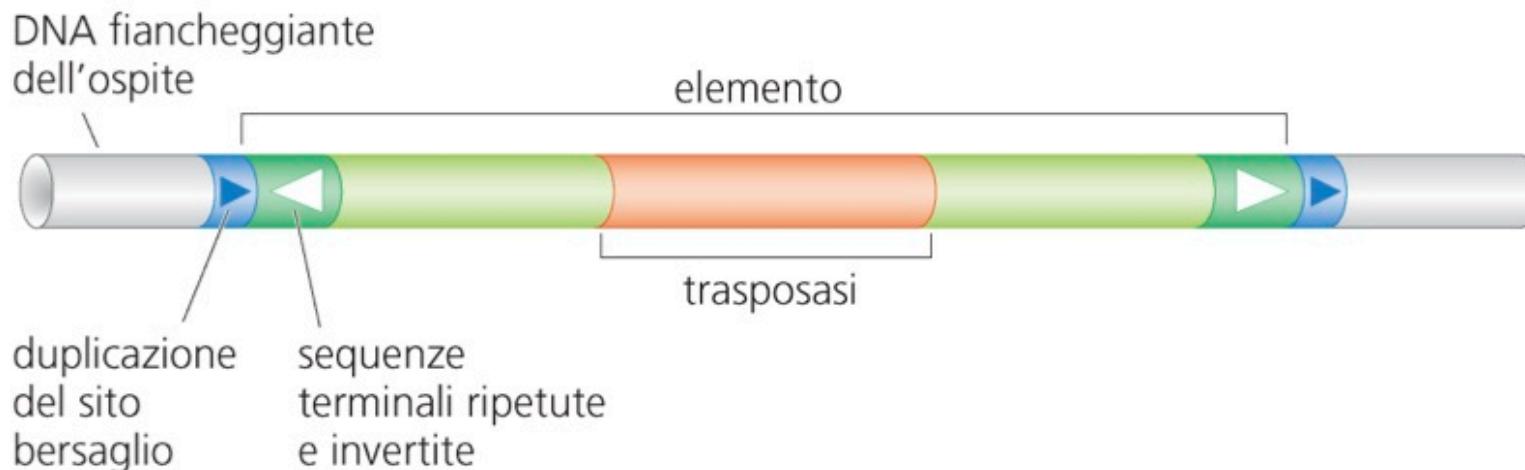
c retrotrasposoni poli-A



Trasposoni a DNA – DNA transposons

- Portano sia delle sequenze di DNA (invertite e ripetute da 25 a centinaia di pb) che servono come siti per la ricombinazione
- Portano geni che codificano proteine che partecipano alla ricombinazione come la trasposasi (integrasi) ma anche altre proteine (resistenza ad antibiotici).

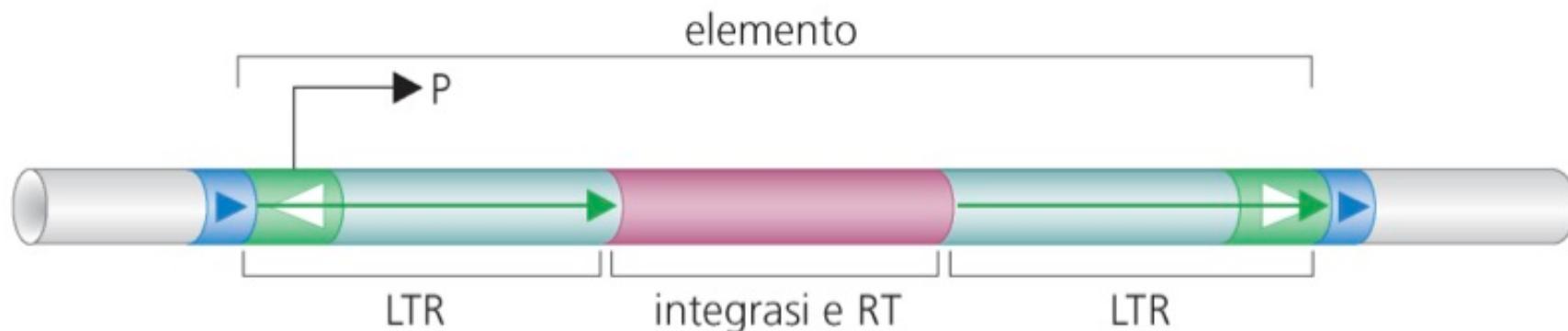
a trasposoni a DNA



Retrotrasposoni (tipo virus/retrovirus) - Retrotransposons

- Portano siti per ricombinasi all' interno di sequenze LTR (LONG TERMINS (DIRECT) REPEATs!!).
- Trasposizione: RNA → cDNA
- Geni per Integrasi (trasposasi), RT: trascrittase inversa (reverse transcriptase)

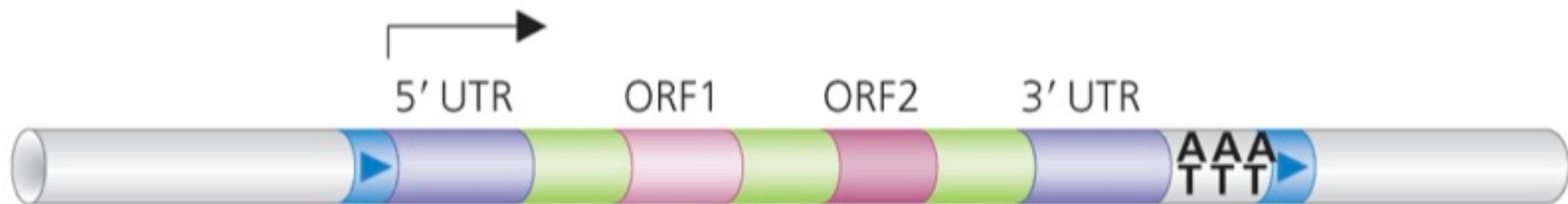
b retrotrasposoni tipo virus/retrovirus



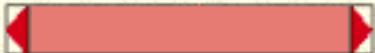
Retrotrasposoni poli-A – Poly-A retrotransposons

- Senza ripetizioni terminali invertite
- Trasposizione: RNA – cDNA
- Sequenza 5' UTR, 3' UTR e poli-A.
- ORF1 codifica per RNA binding protein
- ORF2 fattore con att. Trascrittasi inversa ed endonucleasica.

C retrotrasposoni poli-A



Meccanismi di Trasposizione

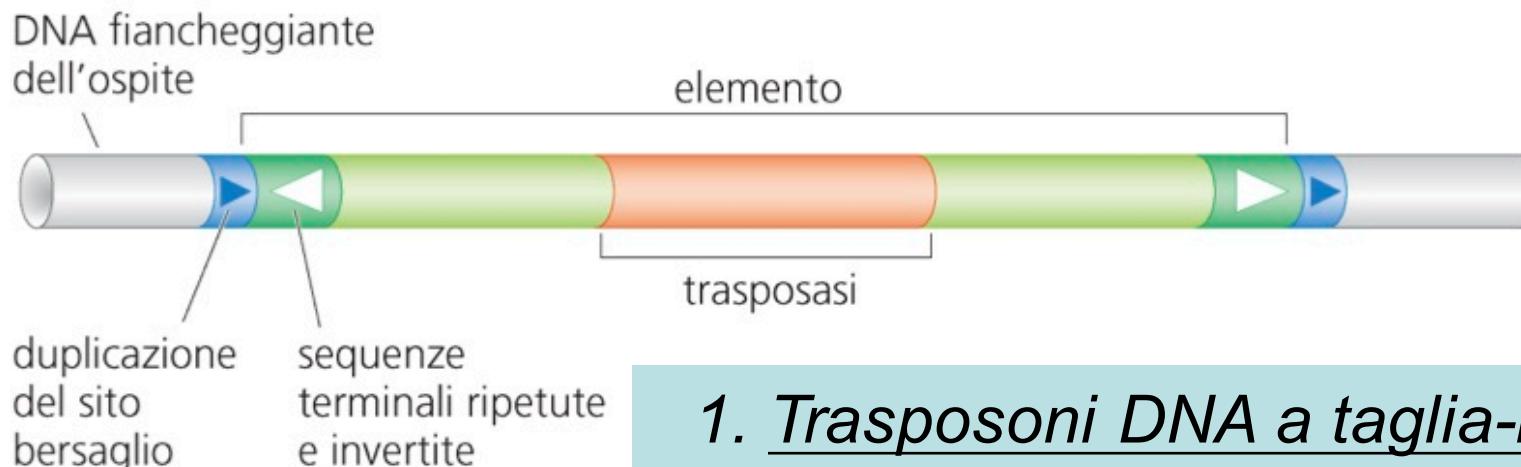
CLASS DESCRIPTION AND STRUCTURE	GENES IN COMPLETE ELEMENT	MODE OF MOVEMENT	EXAMPLES	
DNA-only transposons 	short inverted repeats at each end	encodes transposase	moves as DNA, either excising or following a replicative pathway	P element (<i>Drosophila</i>) Ac-Ds (maize) Tn3 and IS1 (<i>E.coli</i>) Tam3 (snapdragon)
Retroviral-like retrotransposons 	directly repeated long terminal repeats (LTRs) at ends	encodes reverse transcriptase and resembles retrovirus	moves via an RNA intermediate produced by promoter in LTR	Copia (<i>Drosophila</i>) Ty1 (yeast) THE-1 (human) Bs1 (maize)
Nonretroviral retrotransposons 	Poly A at 3' end of RNA transcript; 5' end is often truncated	encodes reverse transcriptase	moves via an RNA intermediate that is often produced from a neighboring promotor	F element (<i>Drosophila</i>) L1 (human) Cin4 (maize)

These elements range in length from 1000 to about 12,000 nucleotide pairs; each family contains many members, only a few of which are listed here. In addition to transposable elements, there are selected viruses that can move in and out of host cell chromosomes; these viruses are related to the first two classes of transposons.

MECHANISMO: Trasposoni a DNA – “DNA transposons”

- Portano sia delle sequenze di DNA (invertite e ripetute da 25 a centinaia di pb) che servono come siti per la ricombinazione
- Portano geni che codificano proteine che partecipano alla ricombinazione come la trasposasi (integrasi) ma anche altre proteine (resistenza ad antibiotici).

a) trasposoni a DNA

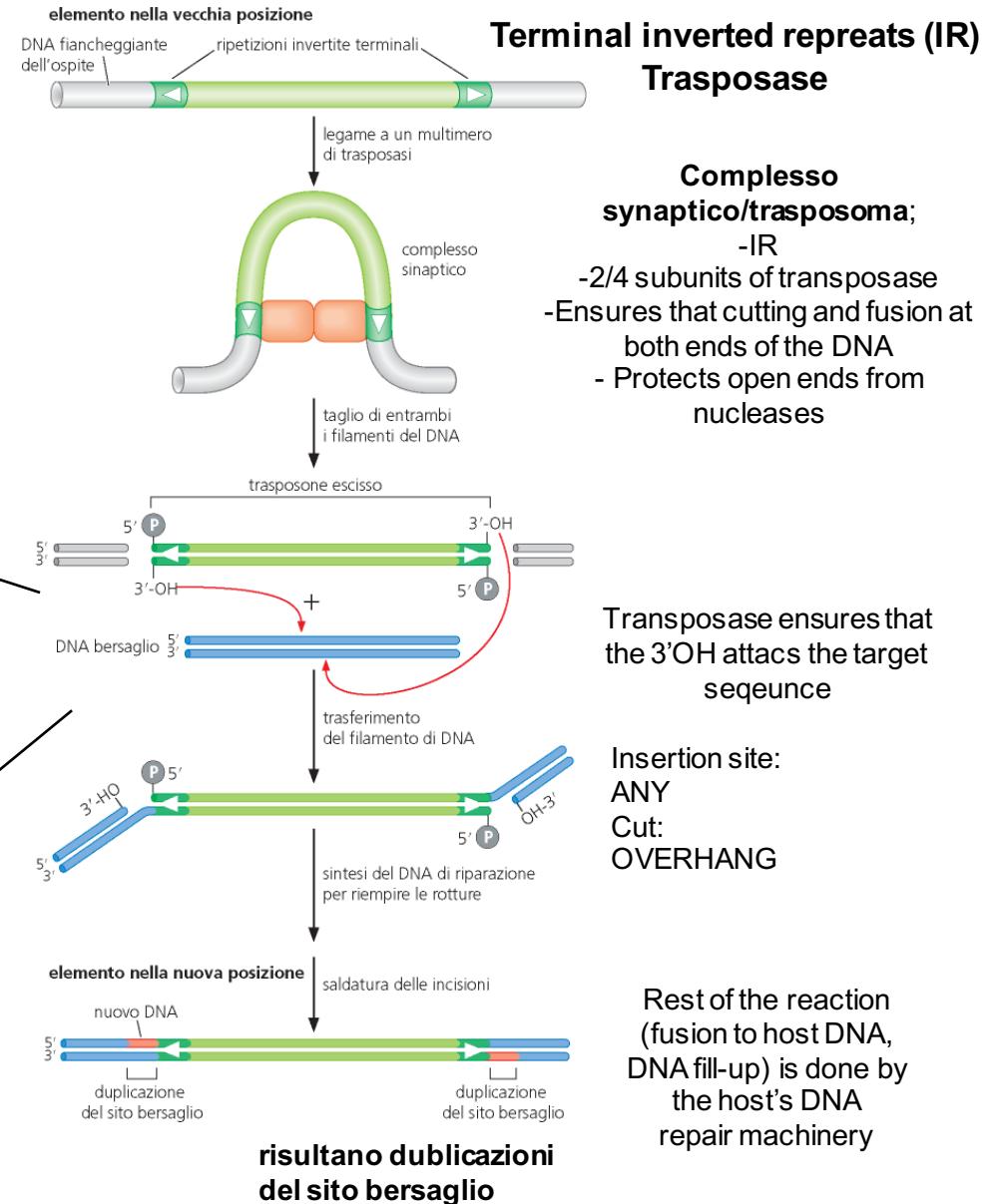
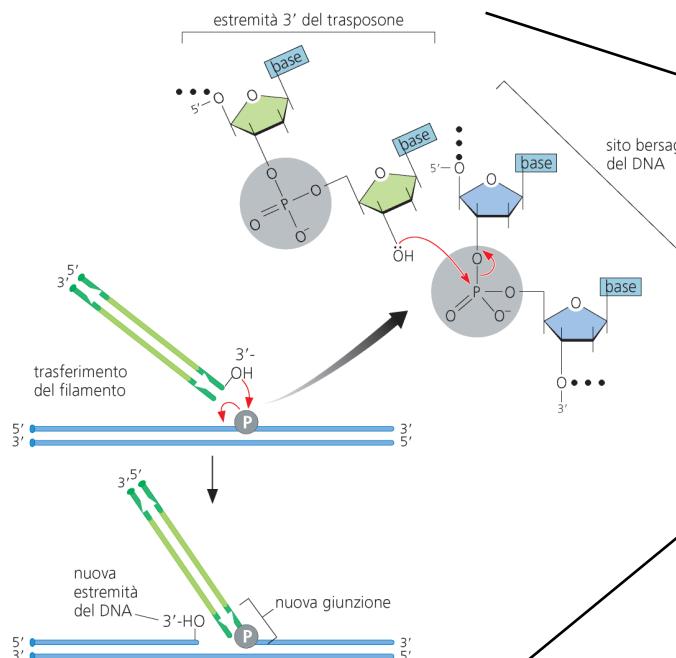


1. Trasposoni DNA a taglia-incolla
2. Trasposoni DNA con meccanismo Replicativo (Fago Mu)

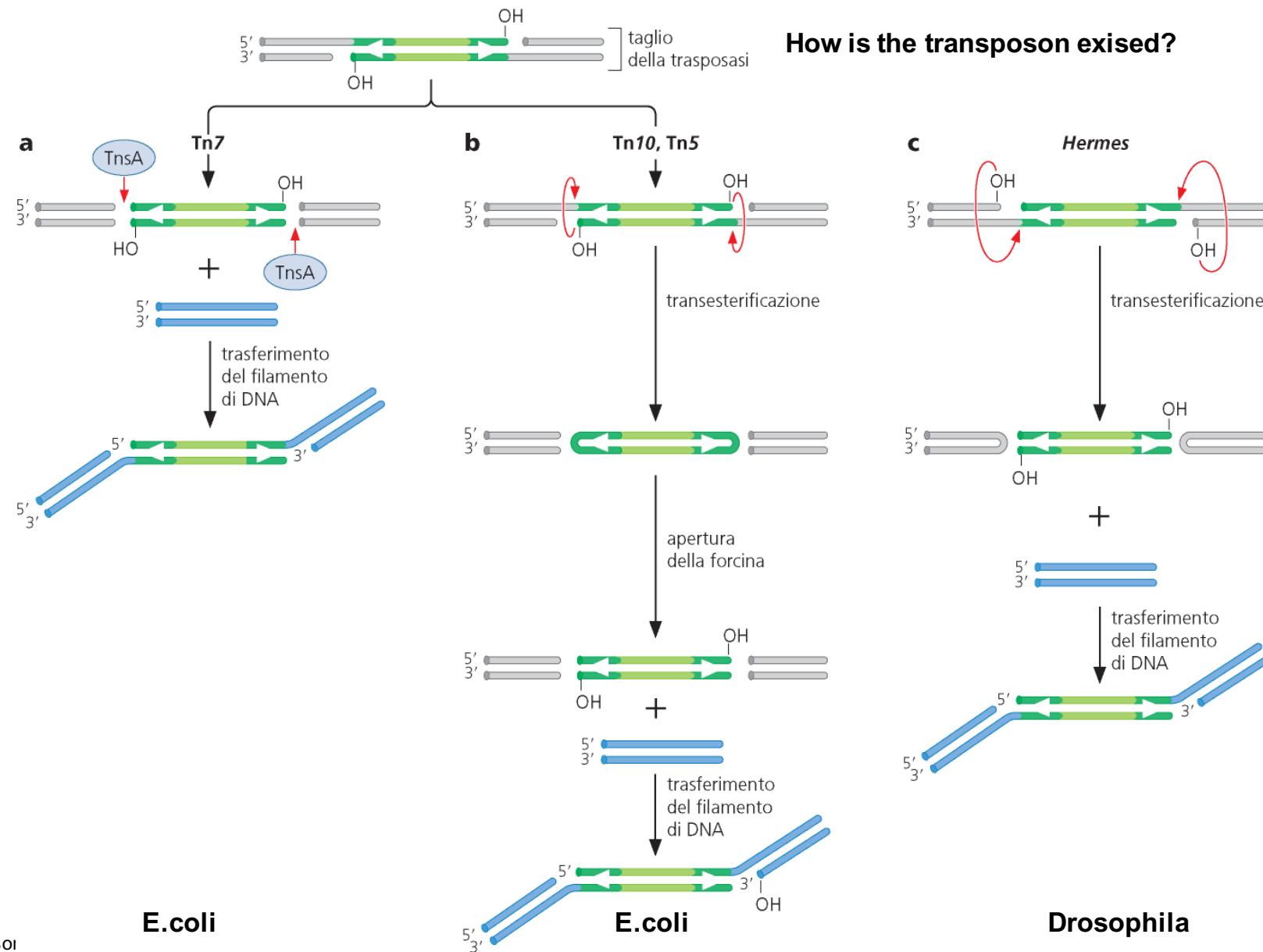
Trasposoni a DNA

1. taglia e incolla

- Il DNA con ripetizioni invertite viene escliso.
- Si forma il complesso sinaptico o trasposoma.
- Nel DNA bersaglio viene fatto un nick, e si fa una trans-esterificazione: trasferimento del filamento di DNA; poi seconda trasesterificazione
- E' un movimento di trasposizione con meccanismo **non replicativo**.

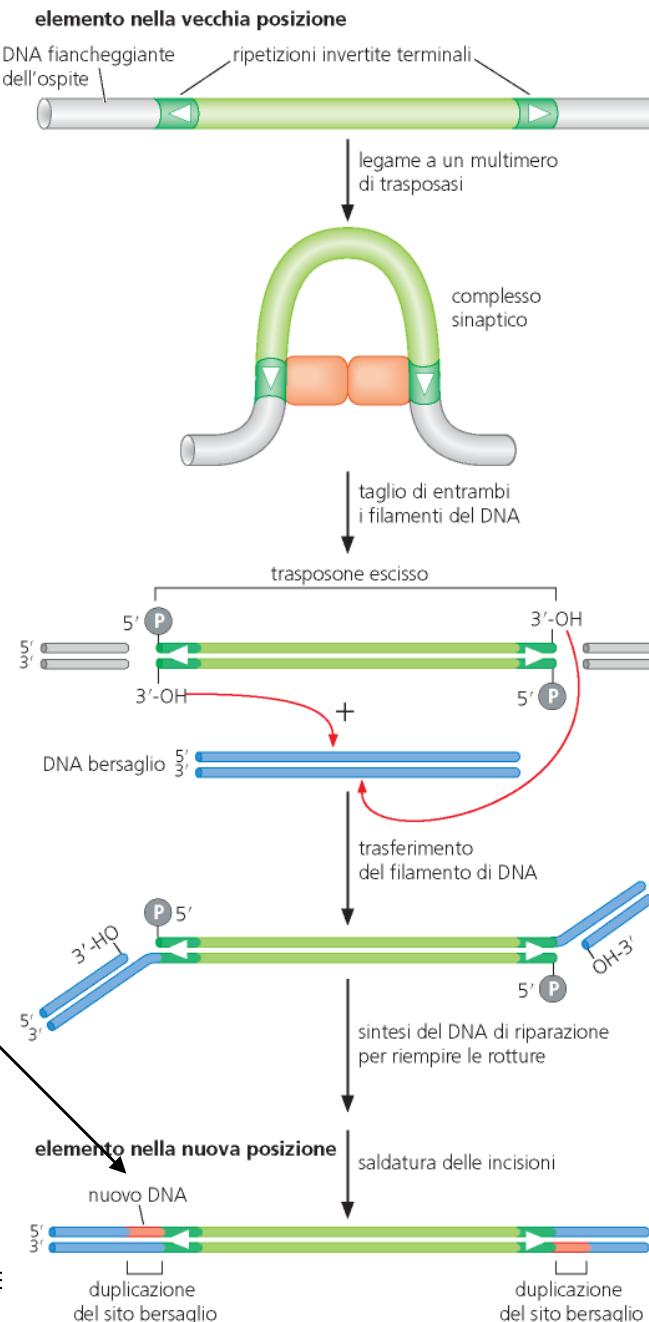


Meccanismi di taglio del filamento non trasferito



Il sito bersaglio: inserzione di trasposoni causa una duplicazione del sito bersaglio = “Footprint”

TARGET SITE DUBLICATION

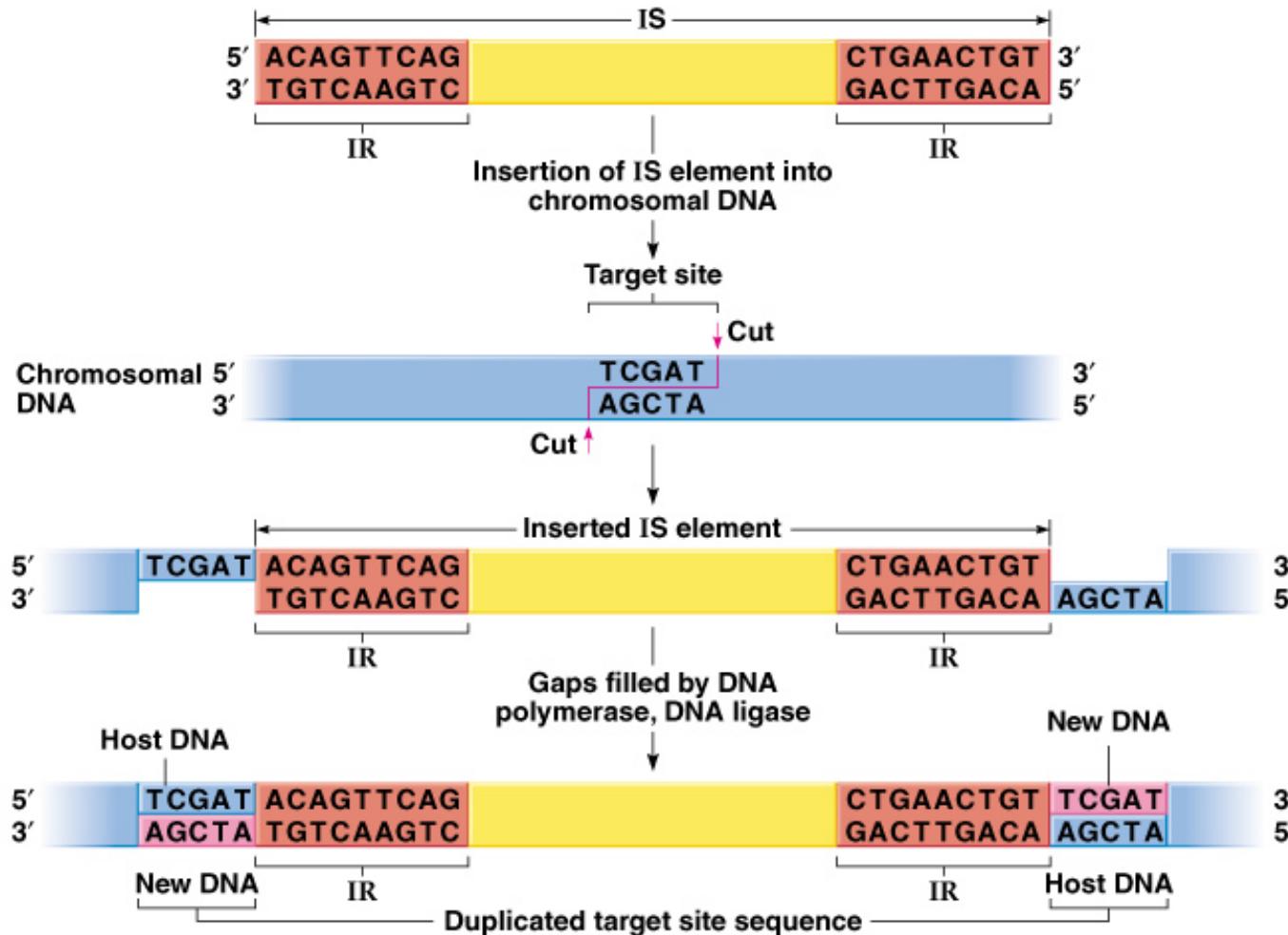


Insertion site:
ANY
Cut:
OVERHANG

Rest of the reaction
(fusion to host DNA,
DNA fill-up) is done by
the host's DNA
repair machinery

Il sito bersaglio: inserzione di trasposoni causa una duplicazione del sito bersaglio = “Footprint”

Esempio: IS (ospite: E. coli)

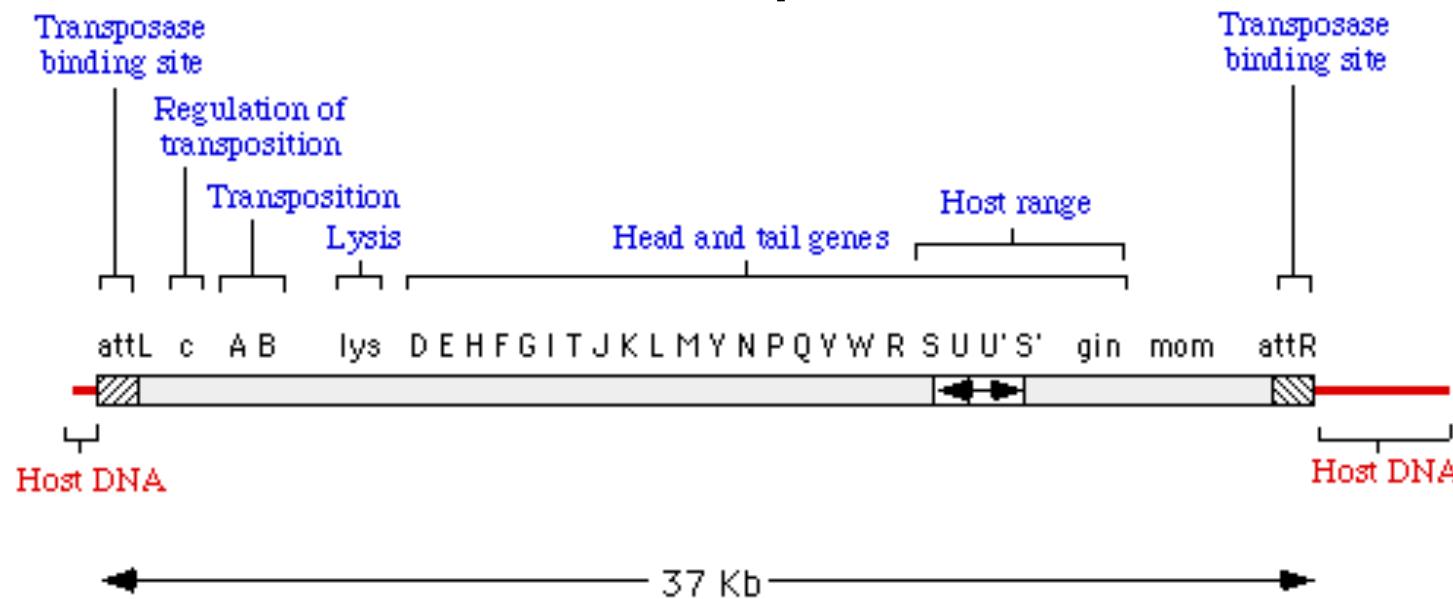


utilizzabile per identificare i siti di transposizione nel genoma

Transposoni a DNA

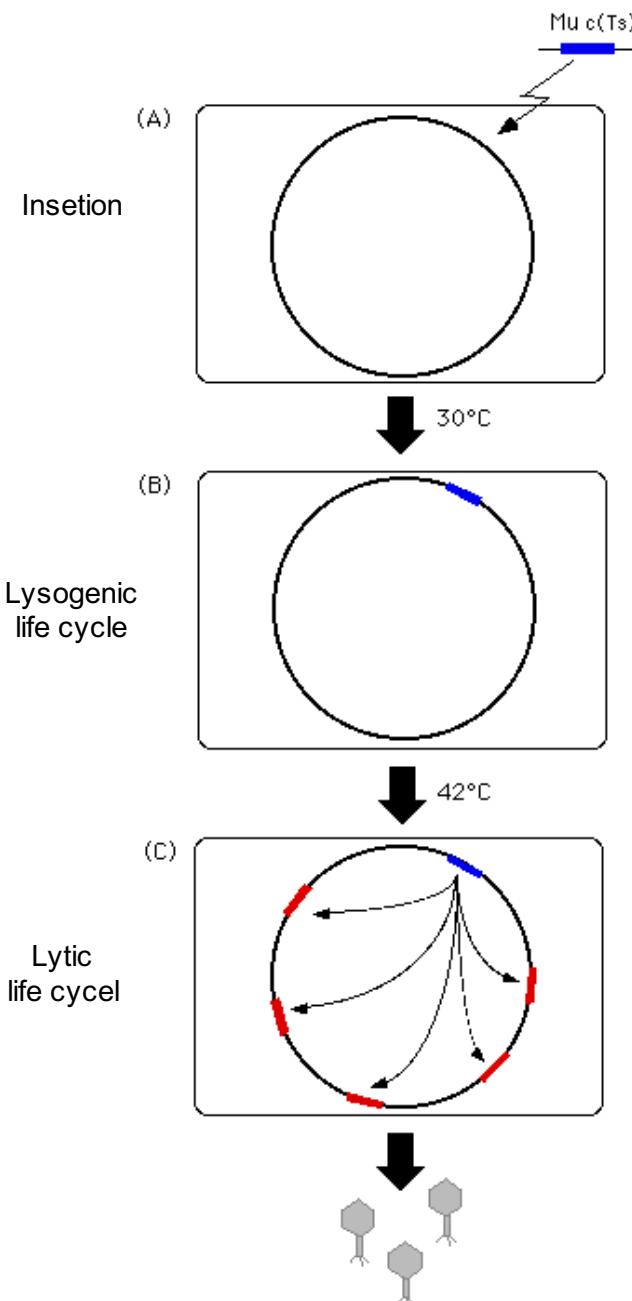
2. con meccanismo replicativo

ESEMPIO: The phage Mu uses a DNA transposon mechanism for insertion and amplification



The A and B gene products encode transposase -- the **A protein is required for all transposition** events, but the **B protein is only required for replicative transposition** events. Expression of the transposase genes is repressed by the c gene product. Transposition requires the two ends of Mu, labeled attL and attR (sometimes called MuL and MuR). When Mu DNA is packaged into a phage head it includes about 50-150 bp of host DNA at the left end and a variable amount of host DNA on the right end. For wild-type Mu the amount of host DNA on the right end is about 2 Kb but, because of the headful packaging mechanism shown below, the length of host DNA on this end increases if part of the Mu genome is deleted. Each Mu is packaged from a different site in the host genome, so the host DNA on the ends of Mu is unique in every different phage head.

The phage Mu uses a DNA transposon mechanism for insertion and amplification



The life cycle of phage Mu is shown in the cartoon below. (A) **When Mu infects a sensitive host, the linear DNA enters the cell and the Mu DNA (i.e. not including the variable sequences of DNA acquired from the previous host) is inserted into the recipient genome via a non-replicative, "cut and paste" mechanism.** (B) Lysogens of wild-type Mu are quite stable and are not induced by UV or other DNA damaging agents. However, derivatives of Mu with a temperature sensitive repressor -- Mu c(Ts)-- can be induced by shifting the lysogen to 42 C. (C) When the repressor is inactivated, **the A and B proteins are expressed and Mu transposes by a replicative mechanism to 50 - 100 new sites on the chromosome.** Meanwhile, late phage gene products are made (including phage heads, tails, lysis proteins, etc). The phage DNA is packaged by a headful mechanism, beginning by cutting the dsDNA in host sequences located about 100 bp from the left end of Mu. The length of Mu DNA is about 37 Kb and about 39 Kb are packaged into each head, so about approximately 2 Kb of host DNA is included on the right end of the packaged DNA. After assembly of the phage, the host is lysed, releasing 50-100 phage particles.

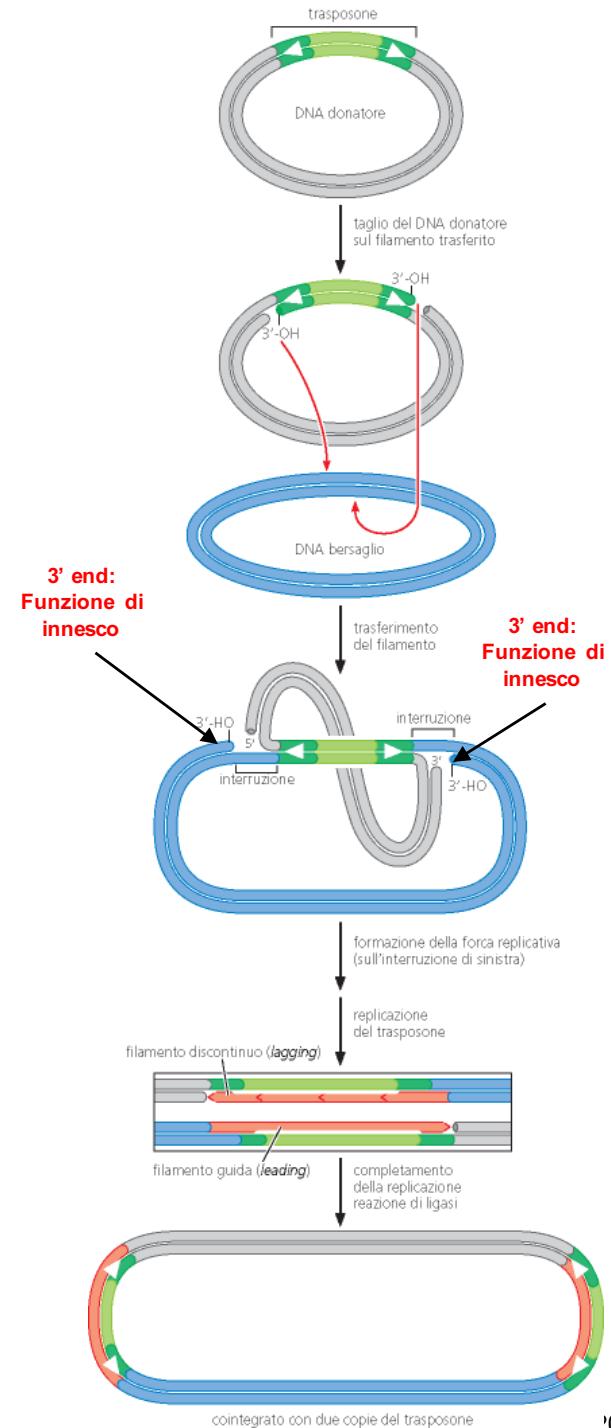
Insertion: taglia - incolla
Amplification: meccanismo replicativo
→ Phage particle contains host DNA!!!
→ After new infection host DNA is transported into new host!!
Increases genome variability

Transposoni a DNA

2. con meccanismo replicativo

Esempio: Mu batericophage

- Trasposoma/compesso synaptico: taglio (nick) DNA ospite
- 3'OH attacco al sito bersaglio
- Dopo il trasferimento del filamento
- Formazione di una struttura con due ramificazioni, che può fungere da forca replicativa (direzione de-novo sintesi: $5' \rightarrow 3'$ = **extension of 3' ends**).
- La replicazione termina sulla seconda forca e produce due copie del transposone
- → trasposizione + dublicazione del trasposon



A “nobel model” for transposons: the Ac/Ds (DNA type) transposons in maize

I genomi delle piante sono molto ricchi di trasposoni

Trasposizione cause una variazioni della pigmentazione

Nel 1940 Barbara McClintock
(Nobel per la Medicina nel 1983) scoprì gli “elementi di controllo” nel mais.

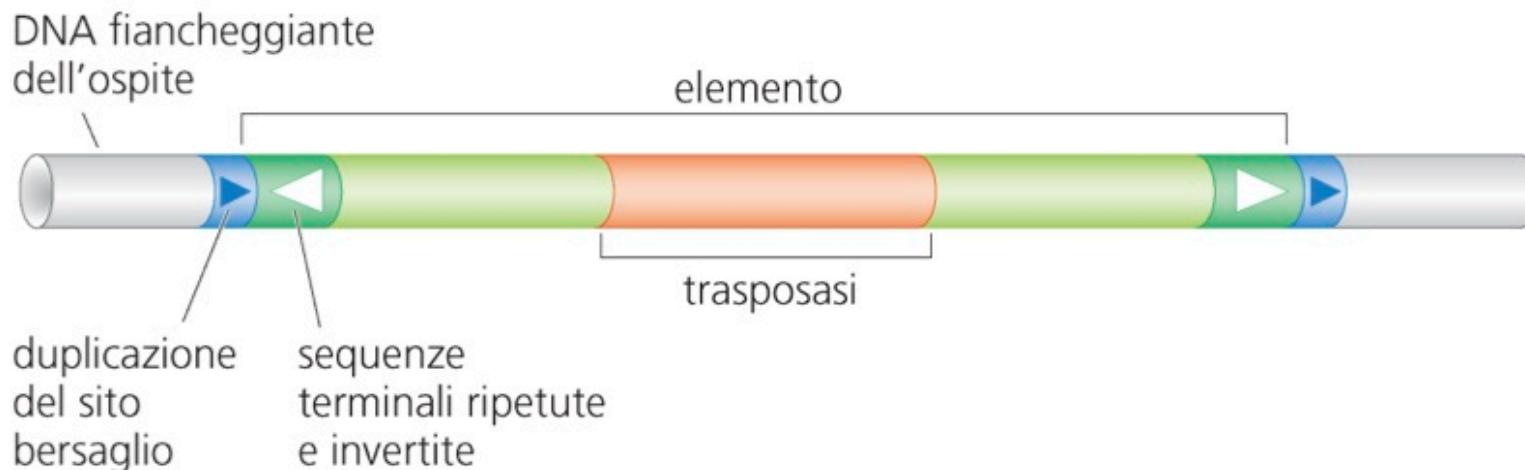


Indian corn

Trasposoni a DNA – DNA transposons

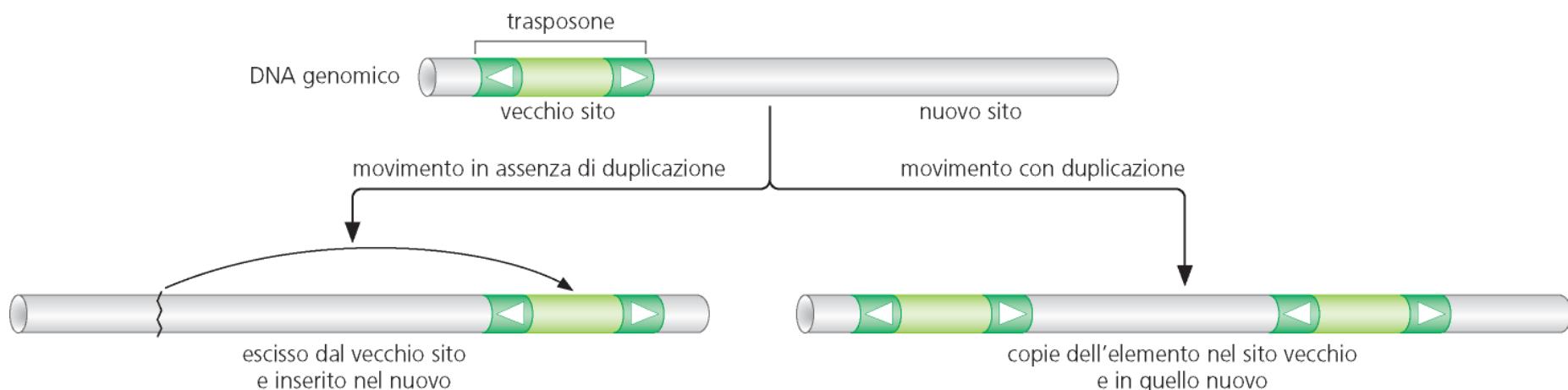
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a trasposoni a DNA

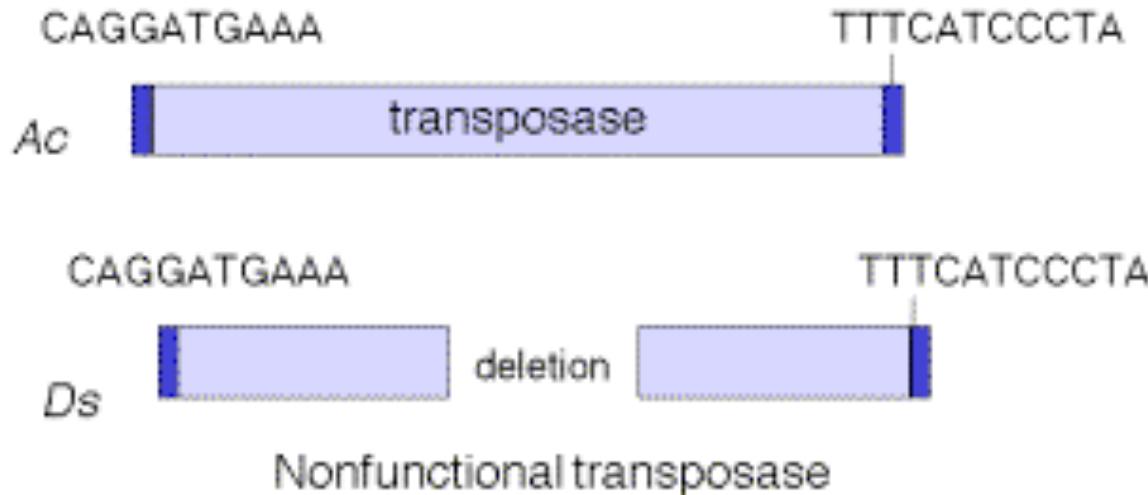


La trasposizione

- Elementi trasponibili o trasposoni.
- Sposta elementi genetici da un sito ad un altro
- Il movimento avviene per una **ricombinazione** tra le **estremità dell'elemento trasponibile** e una **sequenza di DNA** della cellula.



A “nobel model” for transposons: the Ac/Ds transposons in maize

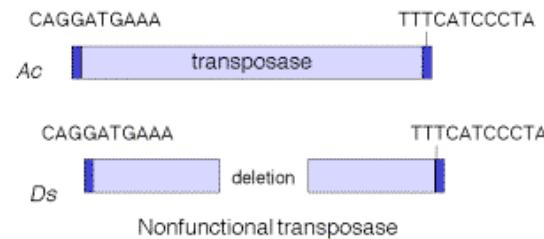


Ac: trasposone a DNA autonomo (trasposase)

Ds: trasposone a DNA non-autonomo (no trasposase)

Richiede Ac (activator) trasposase per trasposizione utilizzando le proprie inverted repeats.

A “nobel model” for transposons: the Ac/Ds transposons in maize



Ac: trasposone a DNA autonomo (trasposase)

Ds: trasposone a DNA non-autonomo (no trasposase)

Richiede Ac (activator) trasposase per trasposizione utilizzando le proprie inverted repeats.

Phenotypes

Pigmented



C-gene: pigment gene in maize

Top row: Wild type pigmented kernel. Second row: Ds is inserted into pigment gene (C) permanently, disabling it. By itself, it can't move. It's stuck. **Ds is a non-autonomous element**.

Second row: Ds is inserted into pigment gene (C) permanently, disabling it. By itself, it can't move. It's stuck. **Ds is a non-autonomous element**.

Third row: Ds and Ac both present, Ds can now excise from the C gene in some cells (i.e., it can transpose) during development, creating developmental fields that can produce pigment. **This is because Ac has provided the elements needed for Ds to transpose**.

Fourth row: Ac is inserted into pigment gene, but not permanently, as it can provide the elements that allow its removal from the gene. **Ac is an autonomous element**.

C gene (wild type)

c-m(Ds) (no Ac)

c-m(Ds) (+Ac)

c-m(Ac)

Colorless



Spotted kernels



Spotted kernels



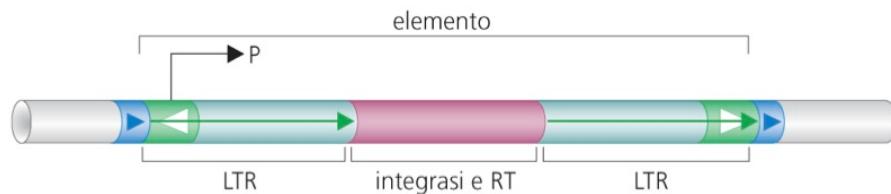
Figure 14-4
Introduction to Genetic Analysis, Ninth Edition
© 2008 W.H. Freeman and Company

Retrotransposons - Retrotrasposoni

Retrotrasposoni (tipo virus/retrovirus) -

- Portano siti per ricombinasi all' interno di sequenze LTR.
- Trasposizione: RNA – cDNA
- Geni per Integrasi (trasposasi) RT, **trascrittase inversa** (reverse transcriptase)

b retrotrasposoni tipo virus/retrovirus



Retrotrasposoni poli-A

- Senza ripetizioni terminali invertite
- Trasposizione: RNA – cDNA
- Sequenza 5' UTR, 3' UTR e poli-A.
- ORF1 codifica per RNA binding protein
- ORF2 fattore con att. **Trascrittasi inversa** ed endonucleasica.

c retrotrasposoni poli-A



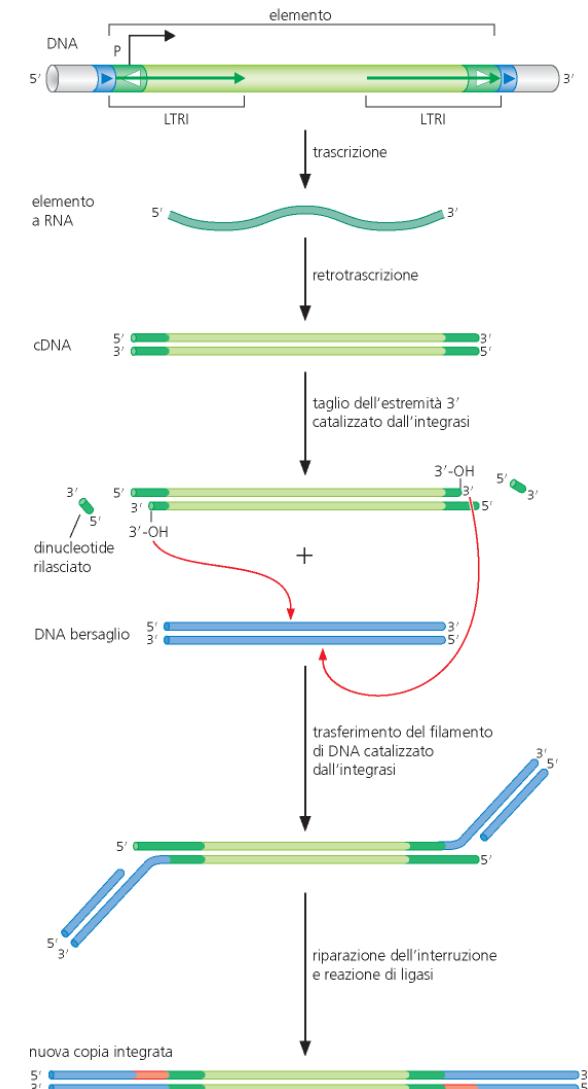
Retrotrasposoni

1. Retrotrasposoni simili ai virus

- Provengono di retroviri che hanno perso la capacità di formare un capsid
 - non escono della cellula ospite

Codificano una reverse transcriptase ed integrase

- Hanno un RNA intermedio
- Il promotore è su LTR ed l'RNA viene copiato in cDNA
- Un'integrase riconosce le estremità e dirige il trasposoma al bersaglio. Una trascrittasi inversa sintetizza il DNA



A current hypothesis proposes that retroviruses evolved from retrotransposons

Retrovirus Classification

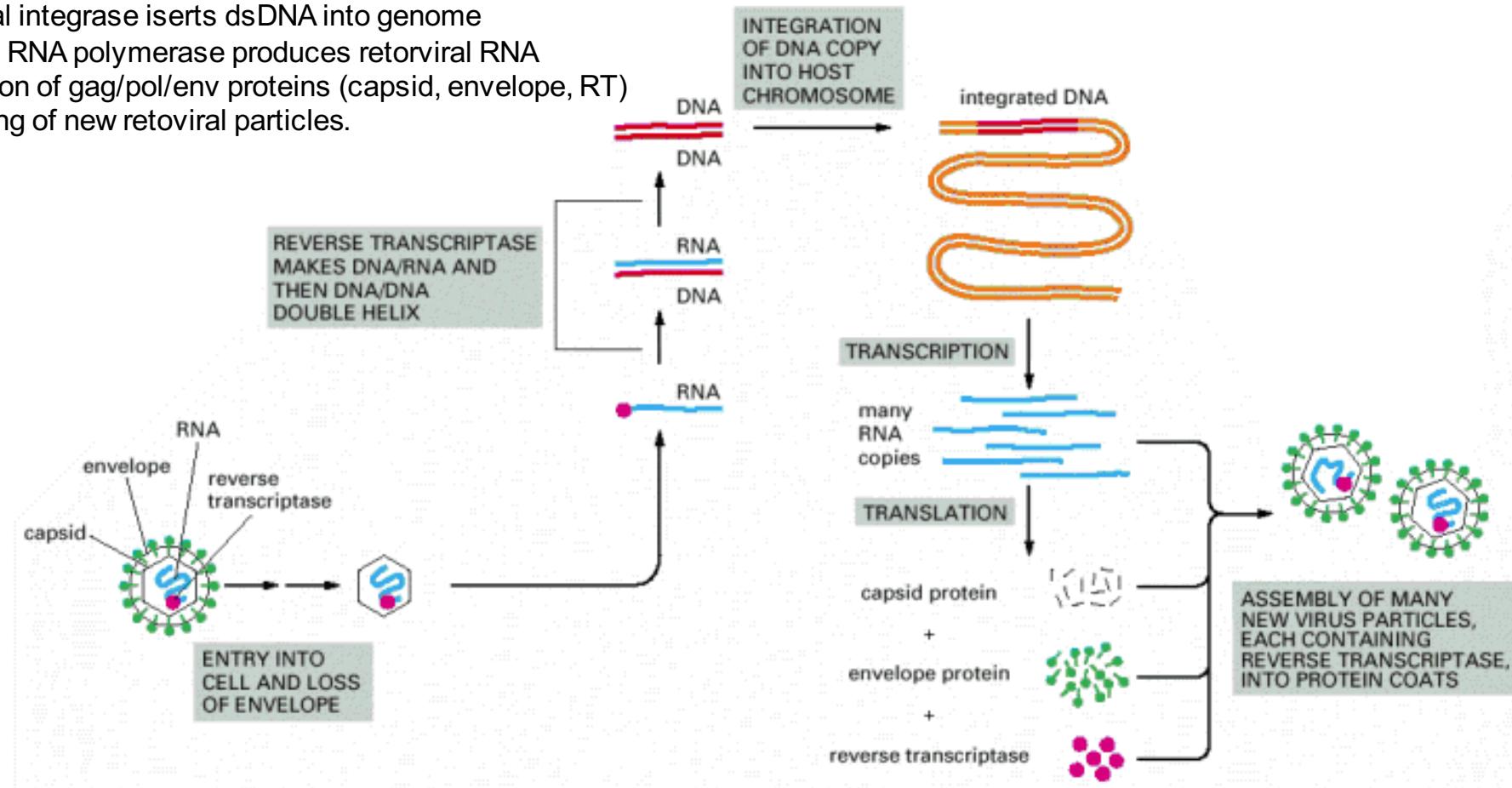
<u>Genus</u>	<u>Example</u>	<u>Genome</u>
Alpharetrovirus	Avian leukemia virus	Simple
Betaretrovirus	Mouse mammary tumor virus	Simple
Gammaretrovirus	Murine leukemia virus Feline leukemia virus Xenotropic murine leukemia-related virus	Simple
Deltaretrovirus	Human T-cell leukemia virus	Complex
Epsilonretrovirus	Wall-eyed sarcoma virus	Complex
Lentivirus	HIV, SIV, FIV	Complex
Spumavirus	Human foamy virus	Complex
Metavirus	Yeast TY-3	
Errantvirus	Drosophila melanogaster Gypsy	9

A current hypothesis proposes that retroviruses evolved from retrotransposons

Retrovirus = RNA virus

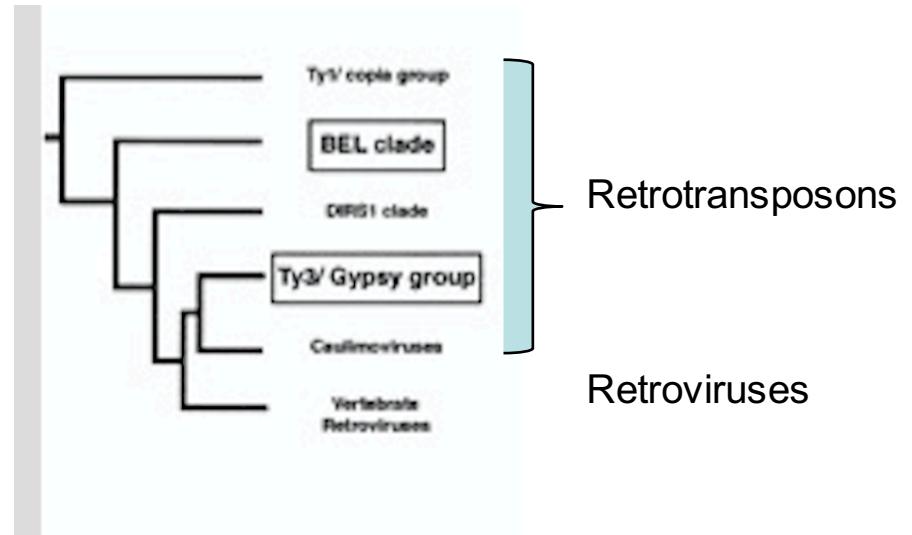
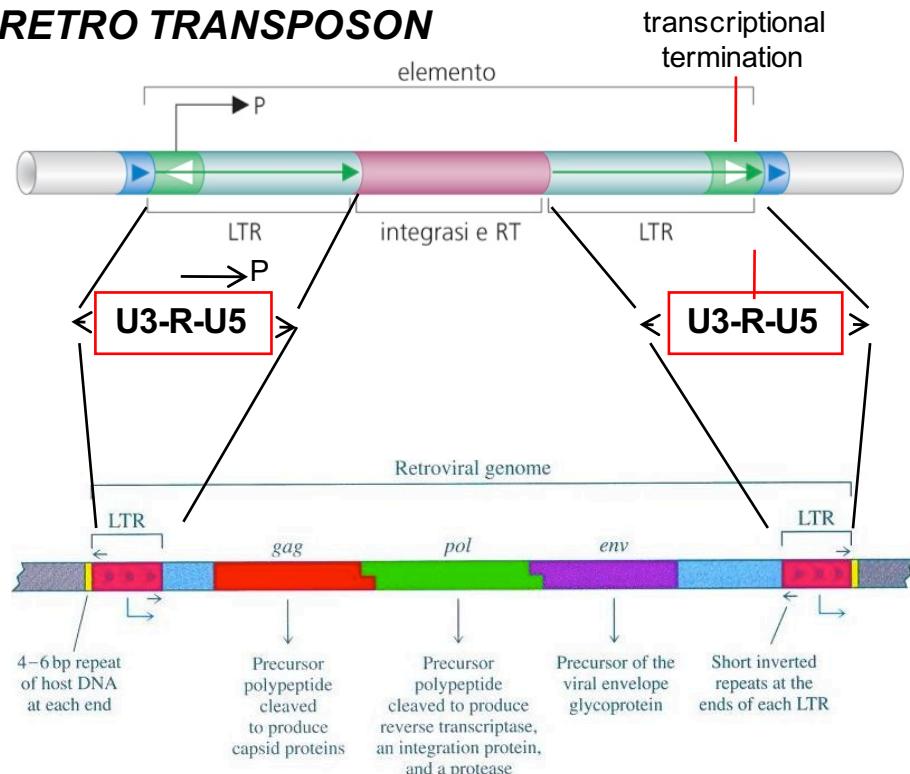
- retrovirus RNA genome: 8500 nucleotides
- Retroviral RNA genome + reverse transcriptase are packaged/viral particle.
- after infection RNAs leave capsid
- *The enzyme reverse transcriptase first DNA/RNA hybrid,*
- Followed by dsDNA synthesis → ready for genome insertion
- Retroviral integrase inserts dsDNA into genome
- Host cell RNA polymerase produces retroviral RNA
- Generation of gag/pol/env proteins (capsid, envelope, RT)
- Packaging of new retroviral particles.

Retrotransposons do not encode capsid and envelope proteins



A current hypothesis proposes that retroviruses evolved from retrotransposons

RETRO TRANSPOSON



RETROVIRUS

> = short inverted repeats

PBS = primer binding site

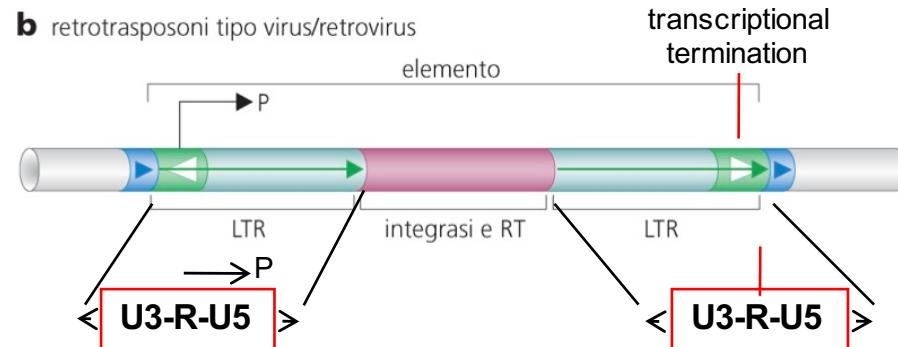
U3 = estremità unica al 3'

R = ripetizione

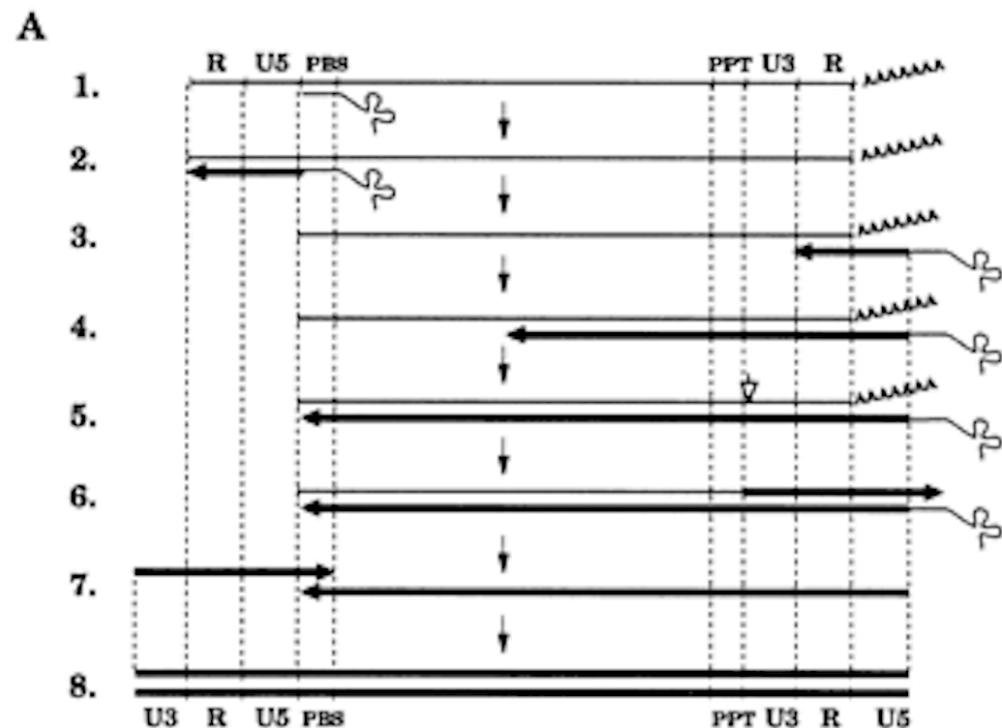
U5 = estremità unica al 5'

Reverse transcription of retrotransposons

RETRO TRANSPOSON

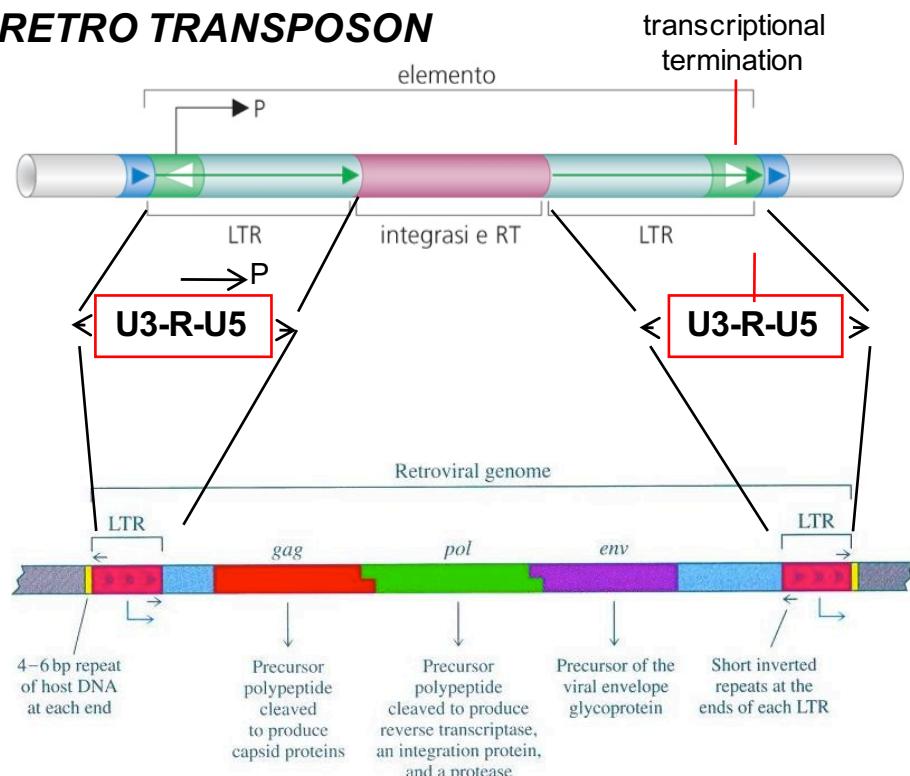


—Model for the generation of the deletion in the *Stonor* element. Thin lines are RNA, and thick lines are DNA. (A) Steps involved in normal reverse transcription of LTR-retrotransposons or retroviruses. U3, R, and U5 comprise the LTR. (1) Annealing of a Met-tRNA molecule (the clover-leaf structure) to the element-encoded RNA. (2) The Met-tRNA serves as a primer for synthesis of a DNA fragment corresponding to the R and U5 regions (called minus-strand, strong-stop DNA). (3) Degradation of the R and U5 regions of the template with RNaseH and transfer of the minus-strand, strong-stop DNA to the other end of the RNA template via homology to the R repeat. (4) Minus-strand DNA synthesis continues. (5) RNaseH nicks the RNA template at the PPT (location of the nick shown as a white arrow). (6) After removal of RNA downstream of PPT with RNaseH, plus-strand DNA synthesis initiates at PPT and stops in the Met-tRNA after synthesis of the plus-strand, strong-stop DNA (containing U3, R, U5, and PBS). (7) Removal of RNA by RNaseH followed by plus-strand strong-stop DNA transfer. (8) Completion of plus-strand and minus-strand DNA syntheses by reverse transcriptase. (B) Model for the generation



Modello della retro-trascrizione di RNA retrovirale e retro-trasposonale

RETRO TRANSPOSON



RETROVIRUS

> R = short inverted repeats

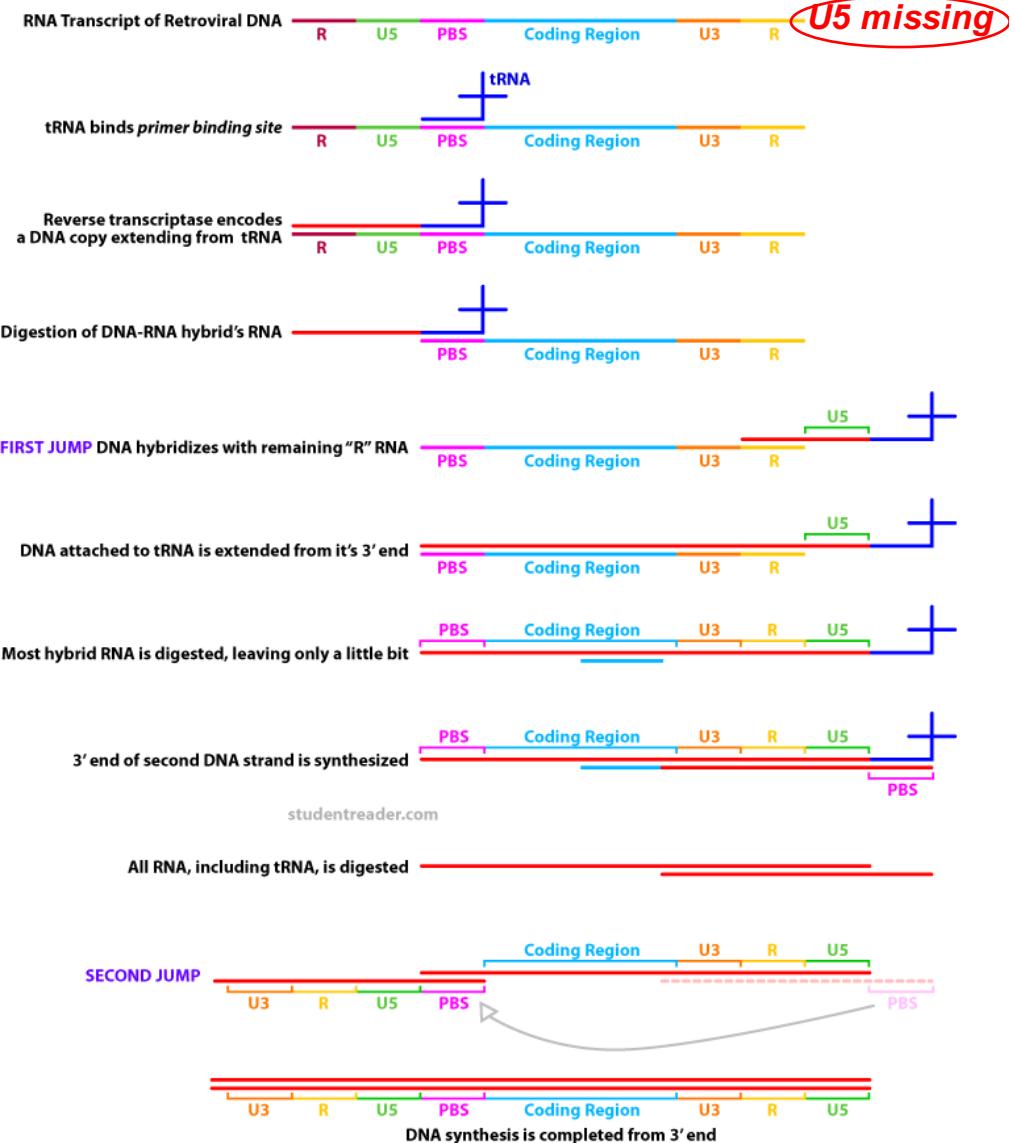
PBS = primer binding site

U3 = estremità unica al 3'

R = ripetizione

U5 = estremità unica al 5'

RETROVIRUS



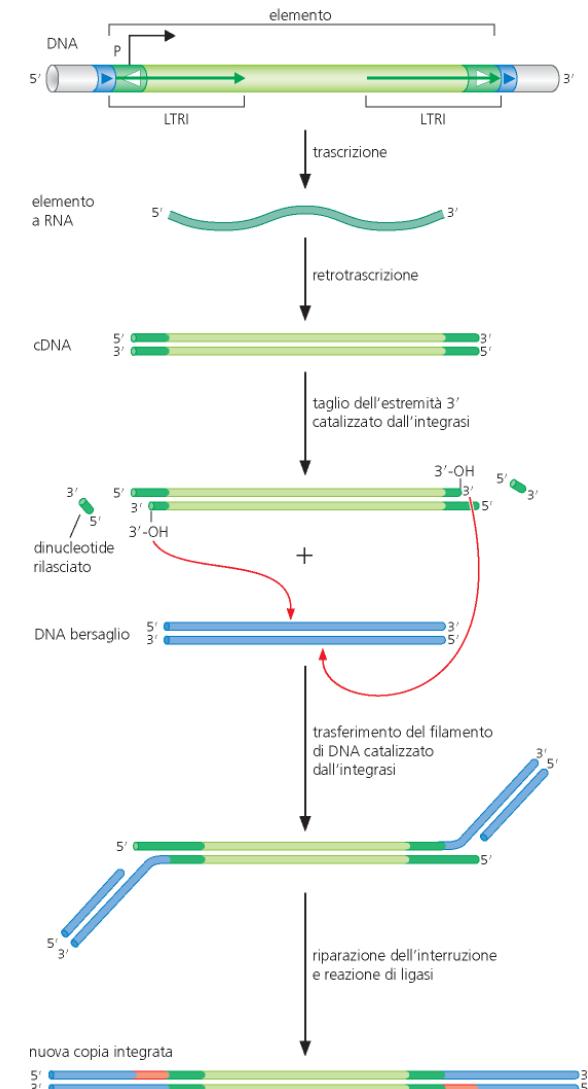
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1. Retrotrasposoni simili ai virus

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 - non escono della cellula ospite

Codificano una reverse transcriptase ed integrase

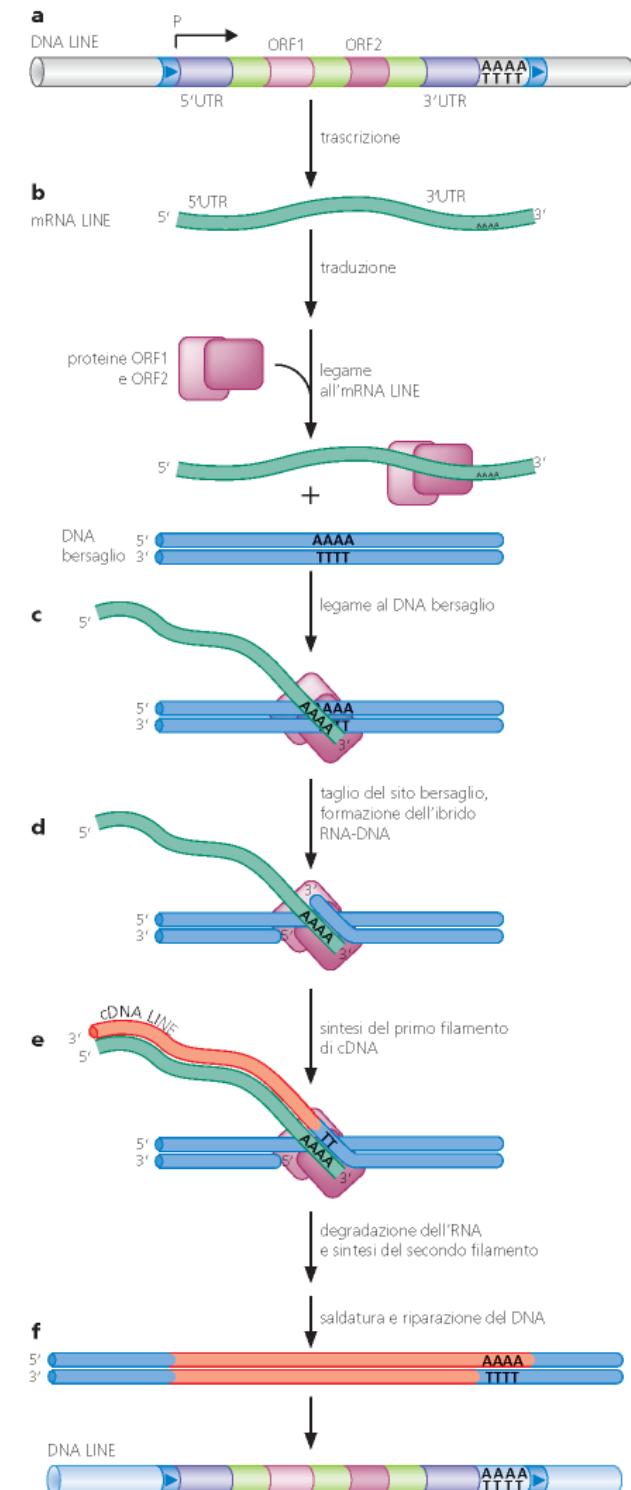
- Hanno un RNA intermedio
- Il promotore è su LTR ed l'RNA viene copiato in cDNA
- Un'integrase riconosce le estremità e dirige il trasposoma al bersaglio. Una trascrittasi inversa sintetizza il DNA



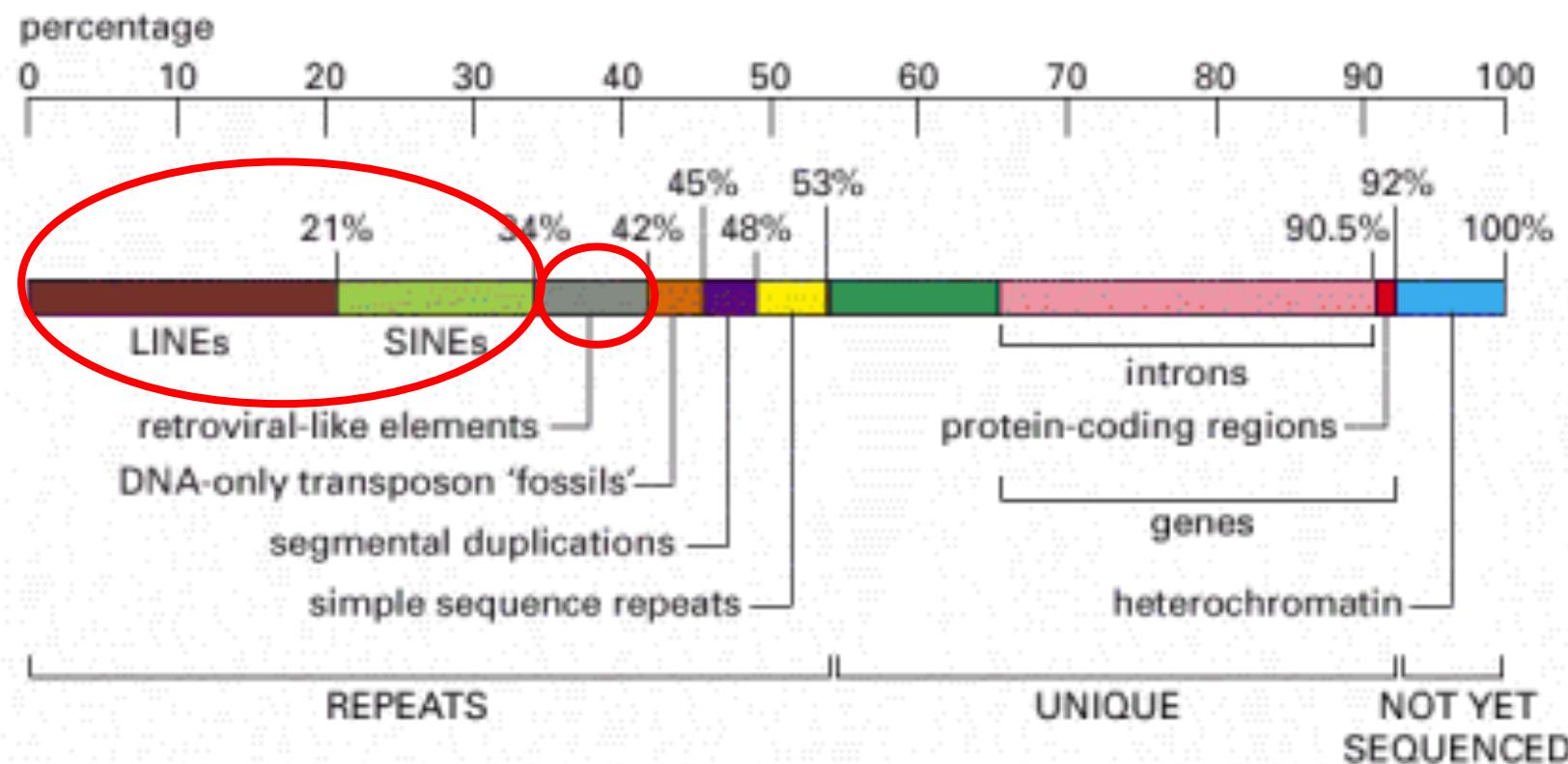
Retrotrasposoni

2. Retrotransposoni poli-A

- Usano un meccanismo chiamato **trascrizione inversa innescata dal sito bersaglio**
- Il RNA codifica per due proteine che portano l'RNA sul bersaglio :
 - ORF1 codifica un RNA binding protein che lega l'RNA,
 - ORF2 ha attività di **trascrittasi inversa e endonucleasi**.
- Dopo il taglio il 3'OH del DNA fa da primer e RNA da templato.
- Siti ricchi di T sono preferenziali perché la coda di poli-A si può appaiare.



Retrotrasposoni con rilevanza per vertebrati/uomo



Representation of the nucleotide sequence content of the human genome.

LINE, SINE, retroviral-like elements, and DNA-only transposons are all mobile genetic elements that have multiplied in our genome by replicating themselves and inserting the new copies in different positions. Simple sequence repeats are short nucleotide sequences (less than 14 nucleotide pairs) that are repeated again and again for long stretches. Segmental duplications are large blocks of the genome (1000–200,000 nucleotide pairs) that are present at two or more locations in the genome. Over half of the unique sequence consists of genes and the remainder is probably regulatory DNA. Most of the DNA present in heterochromatin, a specialized type of chromatin that contains relatively few genes, has not yet been sequenced.

Retrotrasposoni senza LTR; retrotrasposoni a poli A

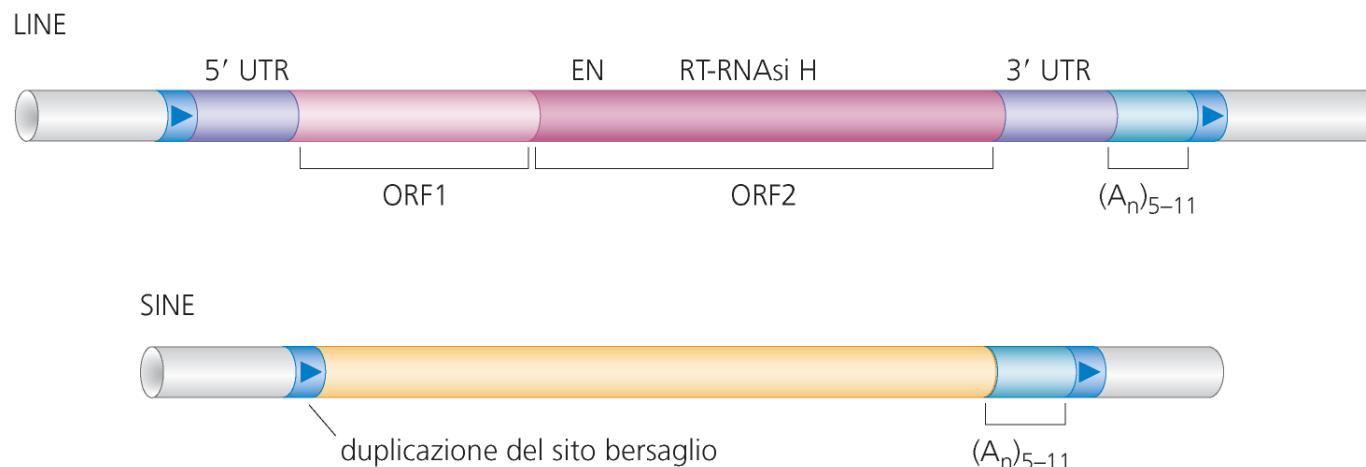
Chiamati anche retrotransposoni non virali; retotrasposon a poliA.

Formano due classi:

- Long interspersed nuclear elements (**LINE**) (6 kb), autonomi promuovono la loro mobilità e forniscono le proteine per la mobilità di SINE.
- Short interspersed nuclear elements (**SINE**) (300 bp); non autonomi.

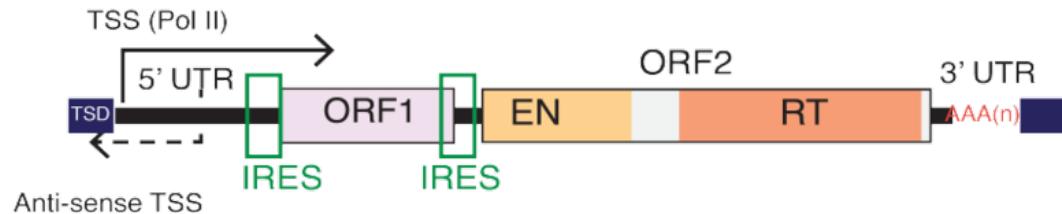
Hanno struttura simili ai geni: un promotore e poliA.

Nel genoma esistono retropseudogeni (geni processati) derivano da mRNA che vengono trasposti grazie a proteine codificate da LINE (poco efficiente perché si legano ad alta affinità a RNA LINE).



Retrotrasposoni senza LTR; retrotrasposoni a poli A

mouse LINE1 (~ 6 kb)



Because LINEs (and other class I transposons, e.g. LTR retrotransposons and SINEs) move by copying themselves (instead of moving by a cut and paste like mechanism, as class II transposons do), they enlarge the genome. The human genome, for example, contains about 500,000 LINEs, which is roughly 17% of the genome. Of these, approximately 7,000 are full-length, a small subset of which are capable of retrotransposition.

SINEs (~ 100-300 bp)

7SL RNA head



Alu - 280bp (human)



B1 - 135pb (mouse)

tRNA head



B2 - 175pb (mouse)

EN: Endo nuclease domain. Reverse transcriptase domain

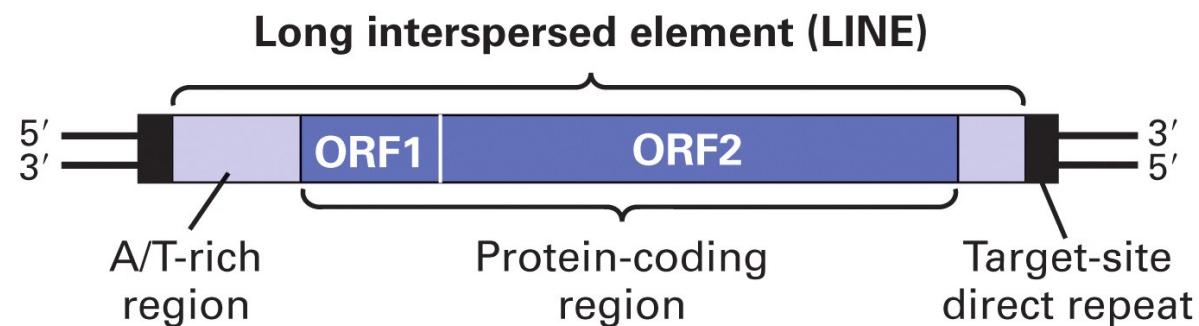
IRES: Internal ribosomal entry site **TSD:** tandem site duplication. **A** and **B:** RNA pol III promoter box

SINEs are the only TEs that are non-autonomous by nature, meaning that they did not evolve from autonomous elements. They are small (80- 500 bases) and rely in trans on functional LINEs for their replication, but their evolutionary origin is very distinct. SINEs can be found in very diverse eukaryotes, but they have only accumulated to impressive amount in mammals, where they represent between 5 and 15% of the genome with millions of copies.

Alu and B1 elements, with their 1.1 million and 650,000 copies in the human and mouse genomes, respectively, harbor a 7SL promoter. The 350,000 copies of B2 SINEs in the mouse are on the other hand tRNA-related.

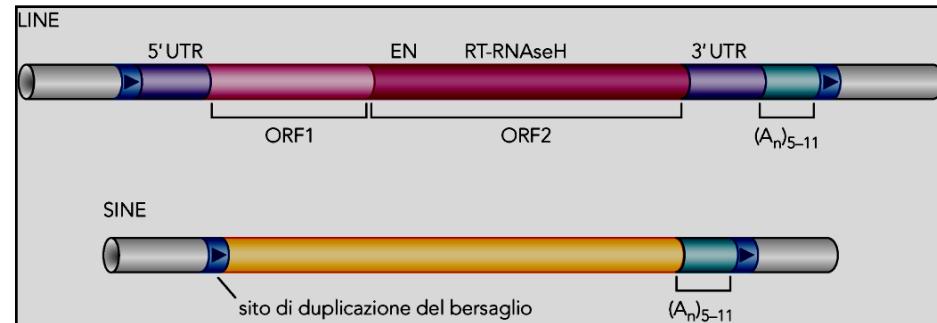
1. LINE – Long interspersed nuclear elements

- Tre maggiori famiglie: L1, L2 ed L3
- L1 è la più abbondante (21% DNA totale)
- Codifica:
RNA-binding protein (ORF1) e
rev transcriptatase/DNA endonuclease (ORF2).

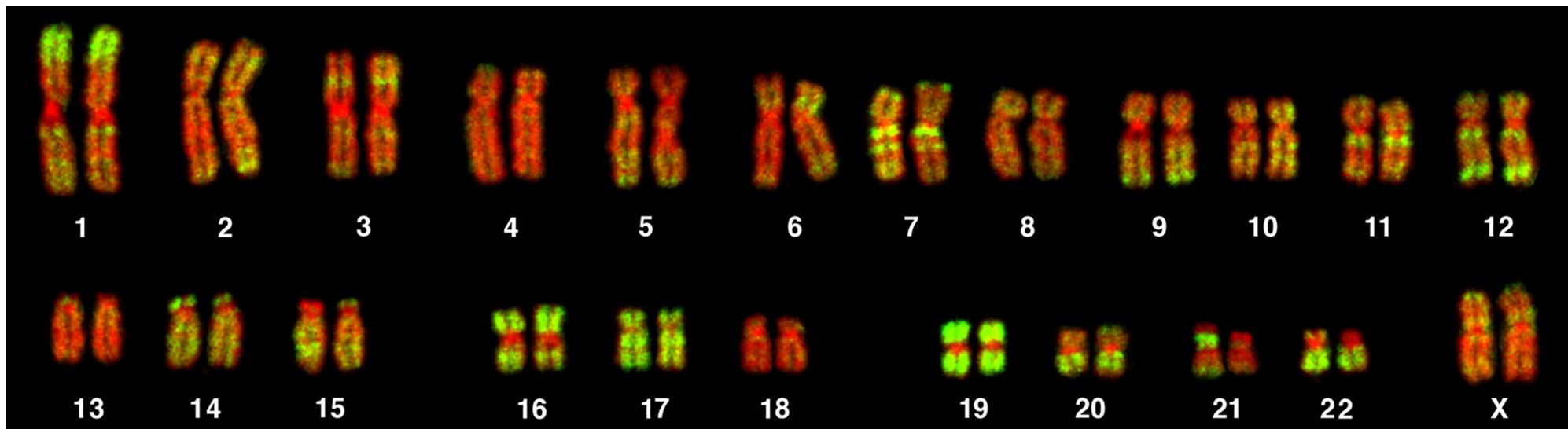


2. SINE (Short interspersed nuclear elements)

- 13% DNA umano
- 100-400 bp. Non codificano proteine.
- Hanno una sequenza ricca in A/T, come LINE.
- 1.6 milioni di siti, di cui 1.1 sono elementi Alu

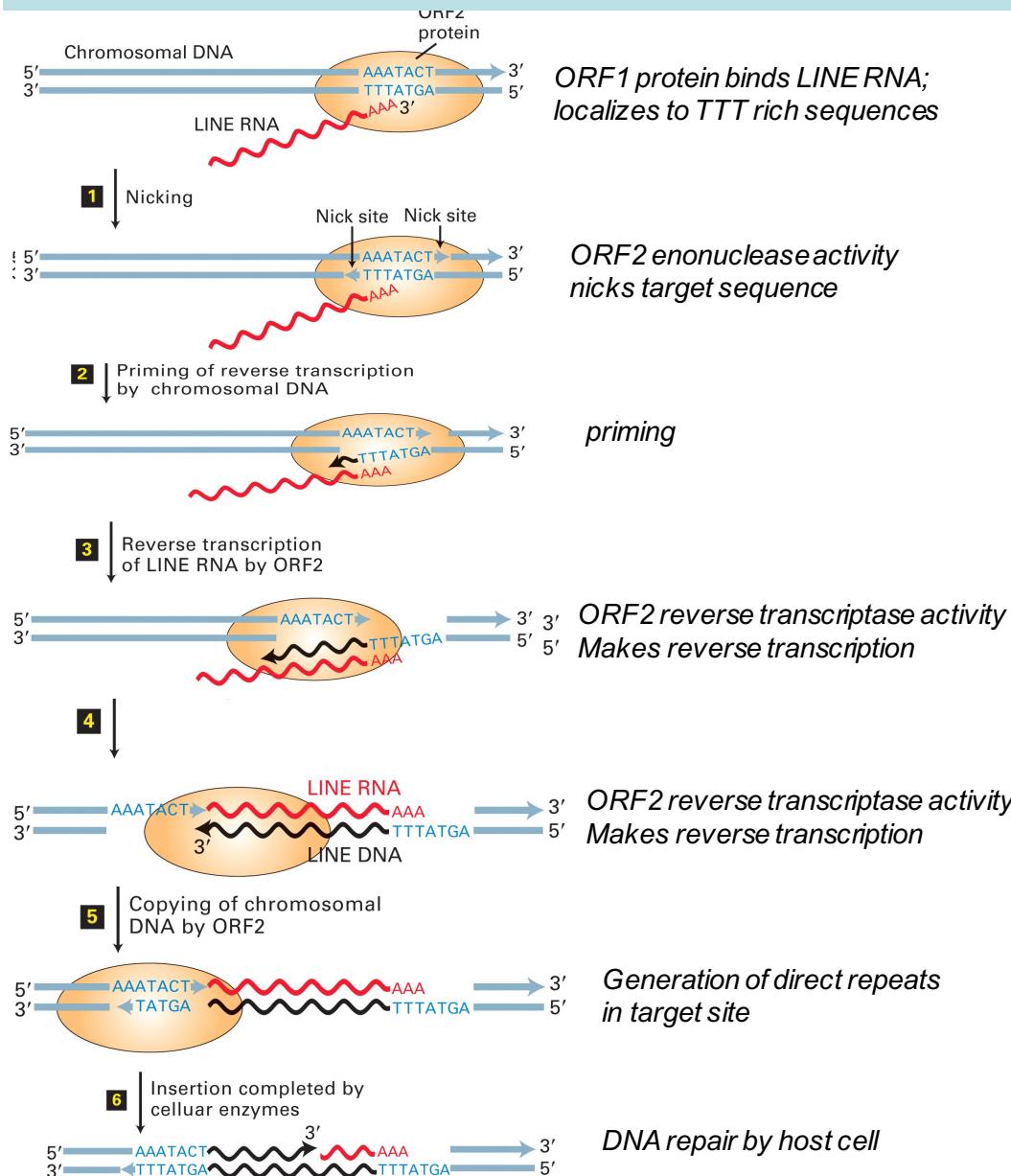


ALU repeats (sottogruppo di SINEs): Una sequenza Alu è una breve sequenza interspersa di DNA (SINE) originariamente caratterizzata come sito di taglio riconosciuto dall'endonucleasi Alu, un enzima di restrizione. Sequenze Alu di diversi tipi sono presenti in gran numero nei genomi dei primati. In particolare le sequenze Alu sono gli elementi mobili più abbondanti nel genoma dell'uomo e per questo possono essere utili per distinguere DNA umano per esempio da DNA di topo. Queste sequenze derivano dall'RNA 7SL, un componente del signal recognition particle.



Cariotipo di un linfocita di un individuo umano di sesso femminile (46, XX). I cromosomi sono stati ibridizzati con una sonda per sequenze Alu (verde). DNA_ rosso

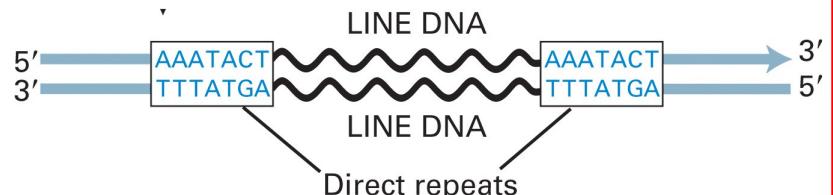
Transposons can generate pseudogenes



PSEUDOGENE:

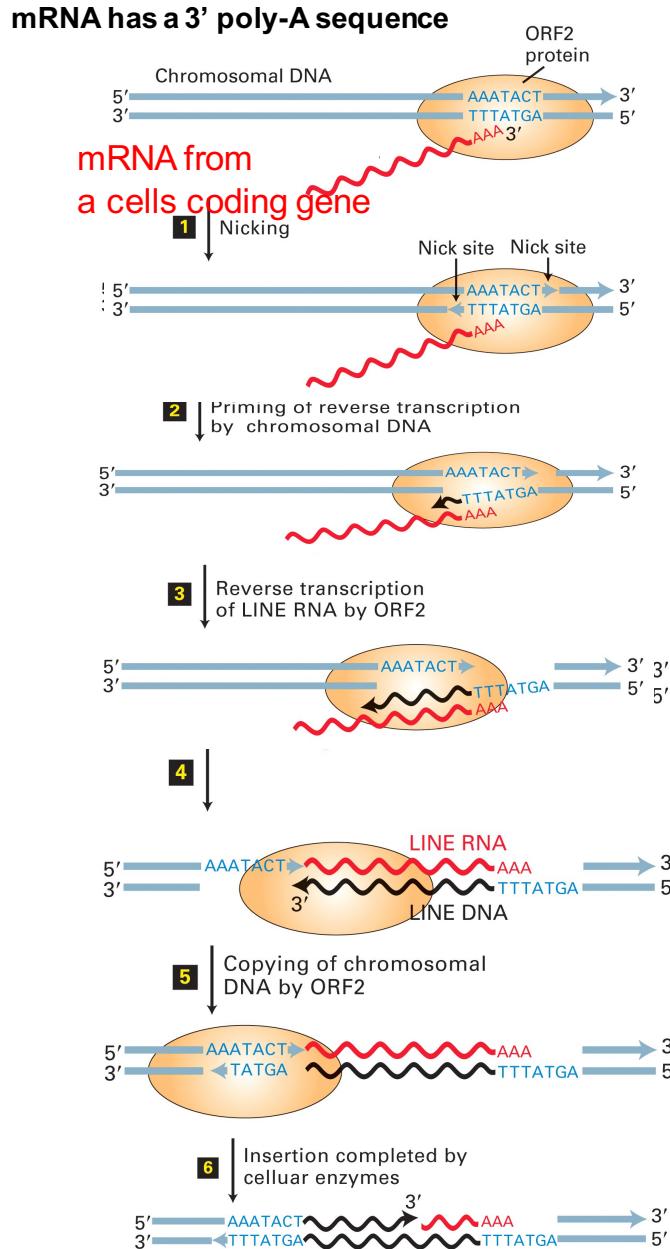
Con il termine pseudogene si intende una sequenza di nucleotidi simile ad un gene (a livello di struttura), ma priva di alcuna espressione proteica all'interno della cellula. **Solitamente si tratta di geni ancestrali che hanno perso la capacità di essere espressi (proteina)**. Sebbene mantengano a volte alcune strutture tipiche dei geni (promotore, isole CpG o siti di splicing), queste sequenze non sono in grado di generare un prodotto proteico funzionale, spesso a causa di mutazioni genetiche consolidate durante l'evoluzione: in questi casi sono spesso presenti mutazioni che generano trascritti nonsenso, che non possono essere tradotti a proteina. Se i geni codificano per una molecola attiva come RNA, è possibile che si siano consolidate mutazioni tali da renderlo del tutto inefficace.

FINAL PRODUCT



Mechanism of non-LTR (mRNAlike) retrotransposon insertion

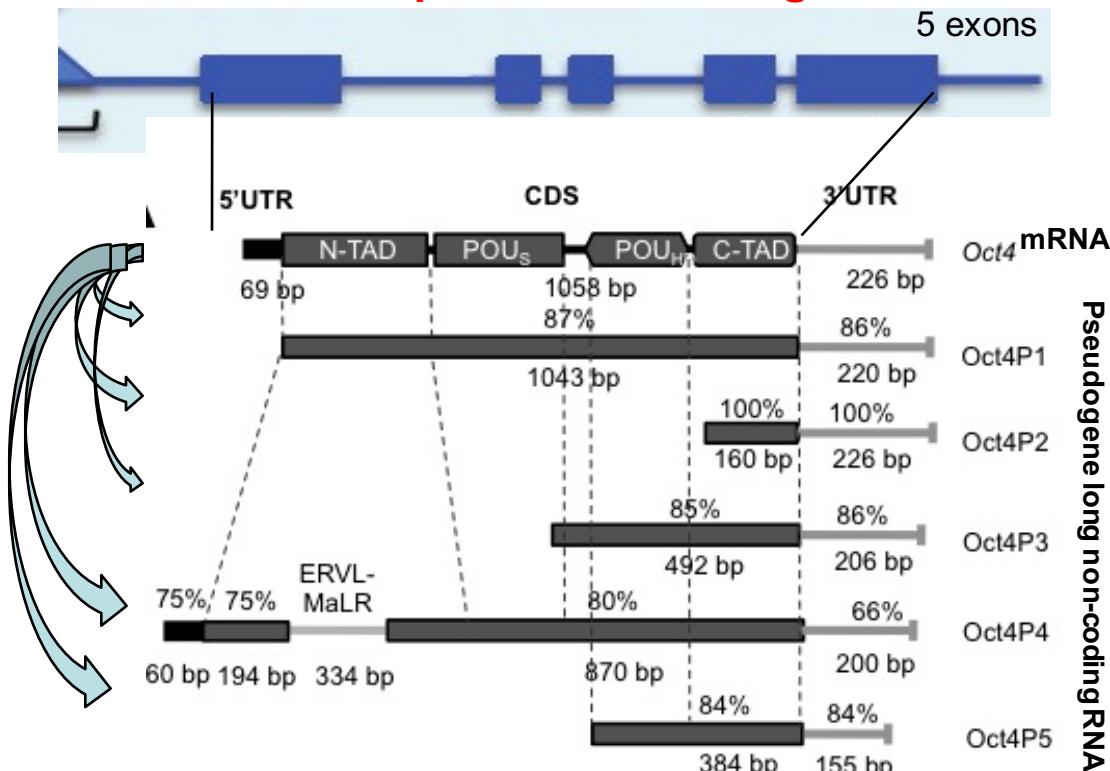
Vertebrate RNA transposons can create pseudogenes



Pseudogenes are mutant copies of ancestral genes

- Mutations
- Truncations
- Deletions
- Frameshift
- etc
- do not encode functional protein
- can be processed
(e.g. after splicing; most frequently)
- can be non-processed

An example: mouse Oct4 gene



pseudogenes can adopt new functions

Regulate chromatin structure

sponge miRNAs

HUMAN GENOME: ca 10.000 pseudogenes

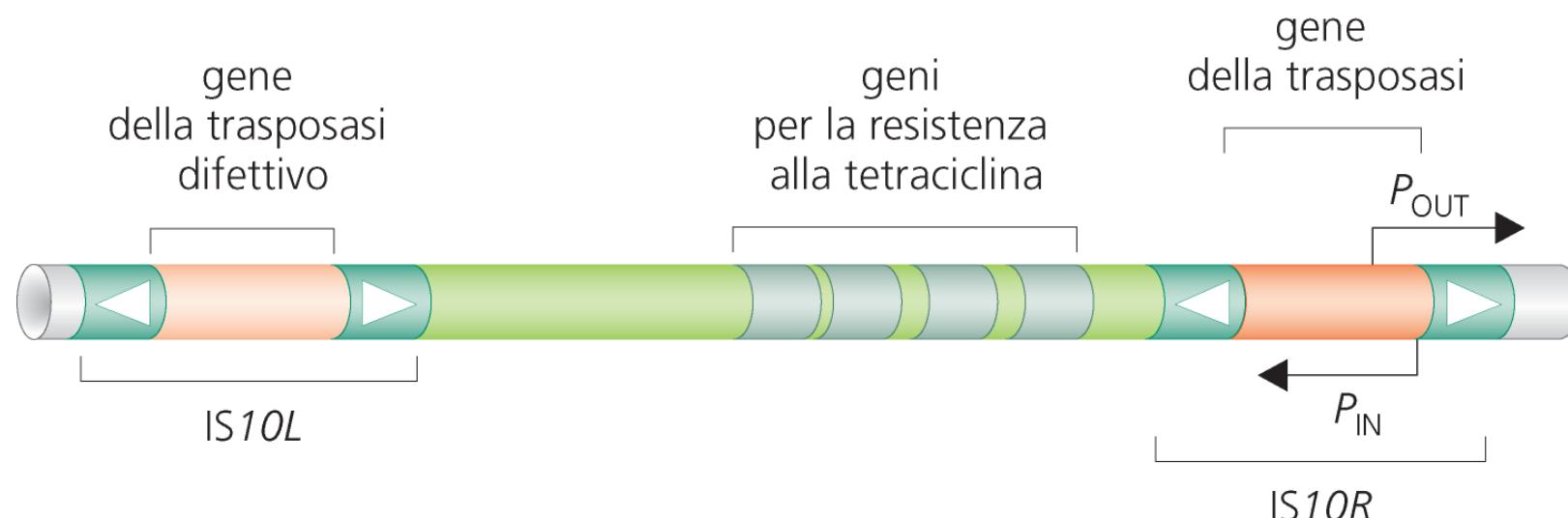
Esempi di elementi trasponibili e della loro regolazione

- I trasposoni sono presenti nei genomi di tutte le forme viventi.
- Questo “successo” è dovuto anche al fatto che la trasposizione è regolata in modo da non compromettere la cellula ospite.
- Sono importanti per la plasticità e l’evoluzione del genoma ma possono essere “distruttivi” -> equilibrio!!!!

Regolazione di trasposizione

- I trasposoni controllano il numero delle proprie copie -> limitano l’impatto sul genoma.
- I trasposoni controllano la scelta del sito bersaglio (siti non “pericolosi” per l’ospite).
- L’ospite reprime trasposoni

Regolazione di trasposizione – Tn10 (batterie)



- Appartiene alla famiglia IS4, con sistema *taglia e incolla*
- Di 9 kb, contiene la trasposasi e la resistenza alla tetraciclina
- Alle estremità ha IS10R (con la trasposasi) e IS10L (IS = insertion sequence; R = right).
- Trasposabile elementi: IS10L (non autonous)
IS10R (autonomous)
Tn10 (IS10L, resistenza, IS10R)
- Usa RNA antisenso per regolare la espressione della trasposasi

Regolazione antisenso dell' espressione di Tn10

P_{IN} : promotore che promuove l'espressione del gene della trasposasi
 P_{OUT} : promotore che promuove la trascrizione in orientazione anti-sense
Overlap 36nt con transposase mRNA

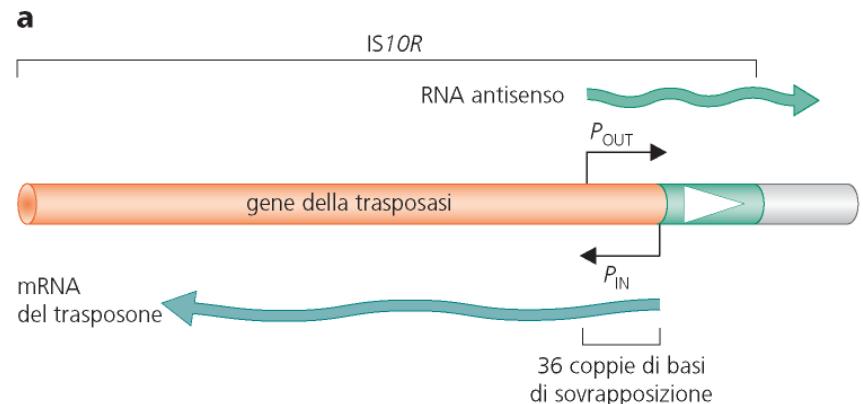
P_{OUT} : RNA ha una vita media più lunga.

In cellule con numero alto di Tn10, i livelli di P_{OUT} sono molto alti e si forma un appaiamento RNA-RNA

Traduzione bloccata

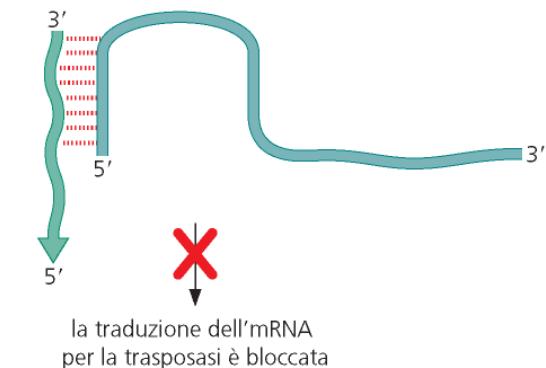
In cellule con numero basso di Tn10, i livelli di P_{OUT} sono bassi.
Traduzione della trasposase

RESULT: when Tn10 copy number is too high, transposons gets inactivated



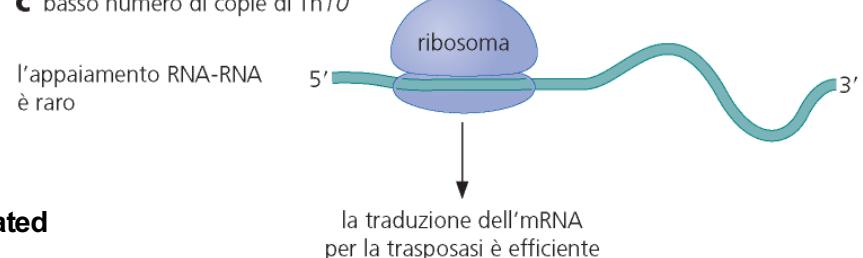
b alto numero di copie di Tn10

l'appaiamento RNA-RNA è frequente

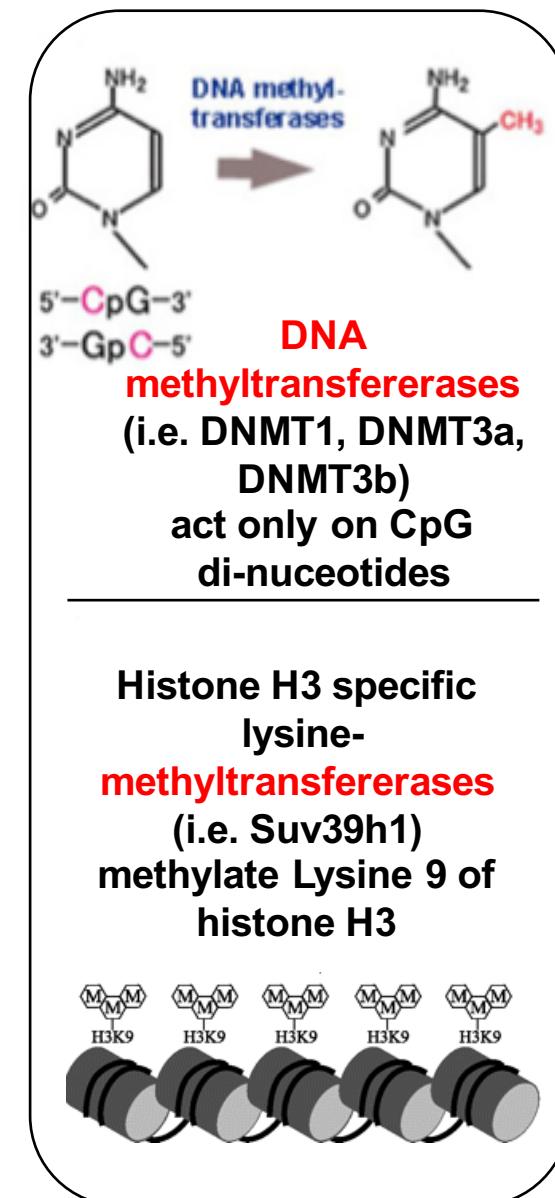
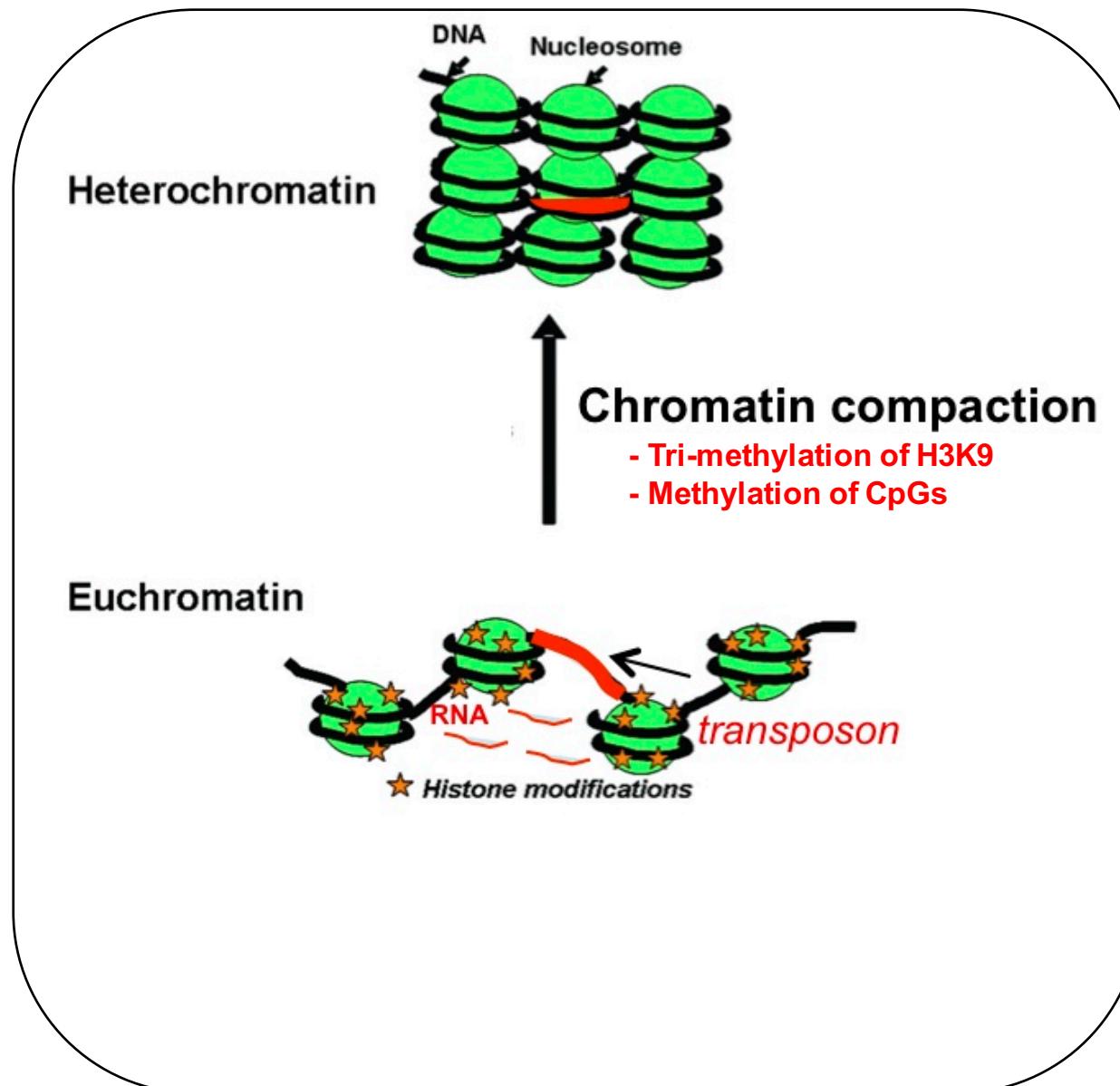


c basso numero di copie di Tn10

l'appaiamento RNA-RNA è raro

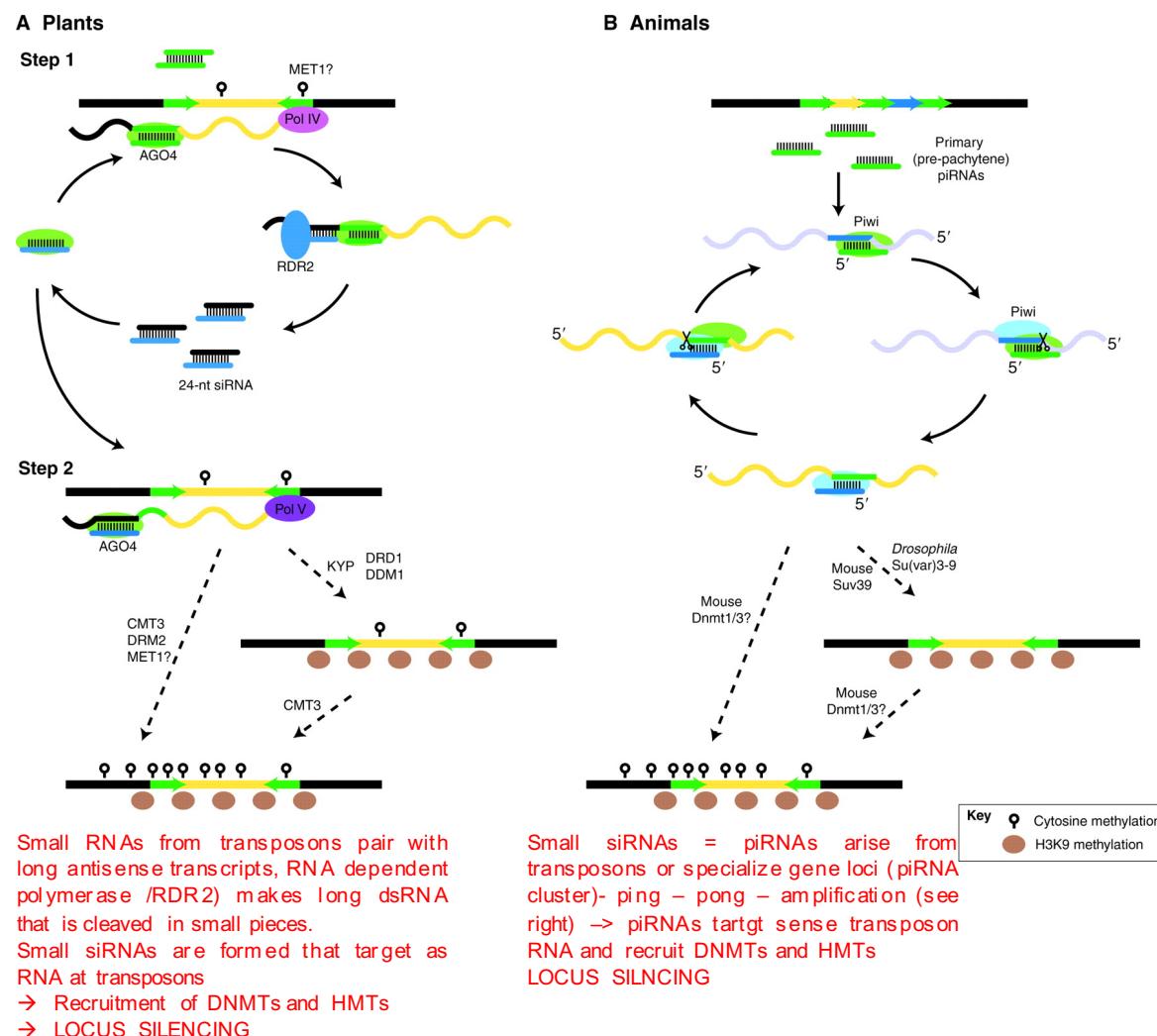


Cells can “silence” transposons by compacting chromatin structure



Mechanisms of EPIGENETIC SILENCING OF transposons in plants and animals.

(A) In plants, transposons are silenced by 24-nucleotide (nt) siRNAs, which are synthesized through an **amplification cycle involving Pol IV, AGO4, RDR2 and DCL3**. How Pol IV identifies a target locus is currently unclear, but it is possible that these sites are premarked by low levels of DNA methylation or by low levels of siRNAs that are not sufficient to mount a silencing response by themselves and require Pol IV to amplify their silencing effect. The siRNAs then require Pol V for recognition of their target locus, after which the locus is marked by **H3K9 methylation (brown) and DNA methylation (black)**. The order of these methylation events is unclear (as indicated by dashed arrows)

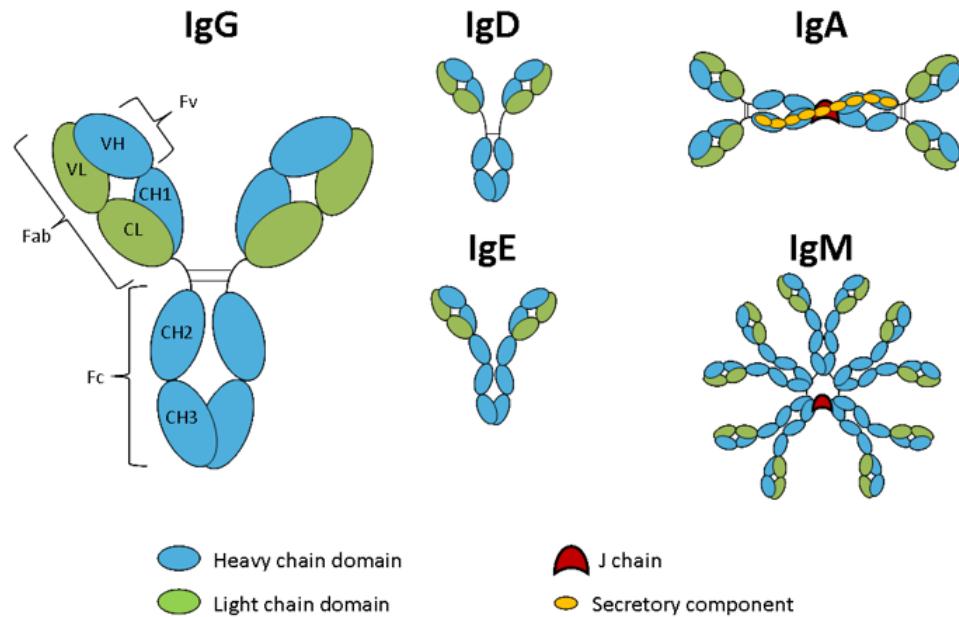


(B) In animals (DROSOPHILA): Piwi-interacting RNA (piRNA), the largest class of the small RNAs, are between 26 and 31 nucleotides in length and function through interactions with piwi proteins from the Argonaute protein family (gene silencing proteins). Many piRNAs are derived from transposons and other repeated elements, and therefore lack specific loci. Other piRNAs that do map to specific locations are clustered in areas near the centromeres or telomeres of the chromosome (piRNA clusters). piRNA clusters make up ~1% of the genome. **piRNAs mediate transposon silencing in the germ line.** The piRNAs are produced through an amplification cycle involving two Piwi proteins. One Piwi protein is loaded with primary piRNA transcripts with a 5' uridine (depicted in green) and can target complementary transcripts (transposon, blue). Slicer activity of the green Piwi protein results in the production of a secondary (blue) piRNA that has an adenine at position 10.

The secondary piRNA is bound by a second Piwi protein (shown in blue) and, upon targeting of a transcript from the piRNA locus (yellow), gives rise to a piRNA that is identical to the initial primary piRNA. **The piRNAs direct H3K9 methylation in *Drosophila*, and both H3K9 methylation and DNA methylation in mammals.** The order of these methylation events is unclear (dashed arrows).

Transposons hijack cells – cells hijack transposons

A transposon based mechanisms of V(D)J recombination in B and T cells



Antibodies are produced by B-lymphocytes

B-lymphocytes can expose antibodies on cell surface (5000 – 10.000) or secrete antibodies
Each cell produces only one type of antibody.

Even in the absence of antigen stimulation, a human can probably make more than 10^{12} different antibody molecules—its preimmune antibody repertoire.

	IgG	IgM	IgA	IgD	IgE
Structure					
	Monomer	Pentamer	Dimer with J chain and secretory component	Monomer	Monomer
Antibody percentage in the plasma	75%-85%	5%-10%	10%-15%	0.001%	0.002%
Presence in sites other than blood, connective tissue, and lymphoid organs	Fetal circulation in pregnant women	B lymphocyte surface (as a monomer)	Secretions (saliva, milk, tears, etc)	Surface of B lymphocytes	Bound to the surface of mast cells and basophils
Known functions	Activates phagocytosis, neutralizes antigens	First antibody produced in initial immune response; activates complement	Protects mucosae	Antigen receptor triggering initial B cell activation	Destroys parasitic worms and participates in allergies

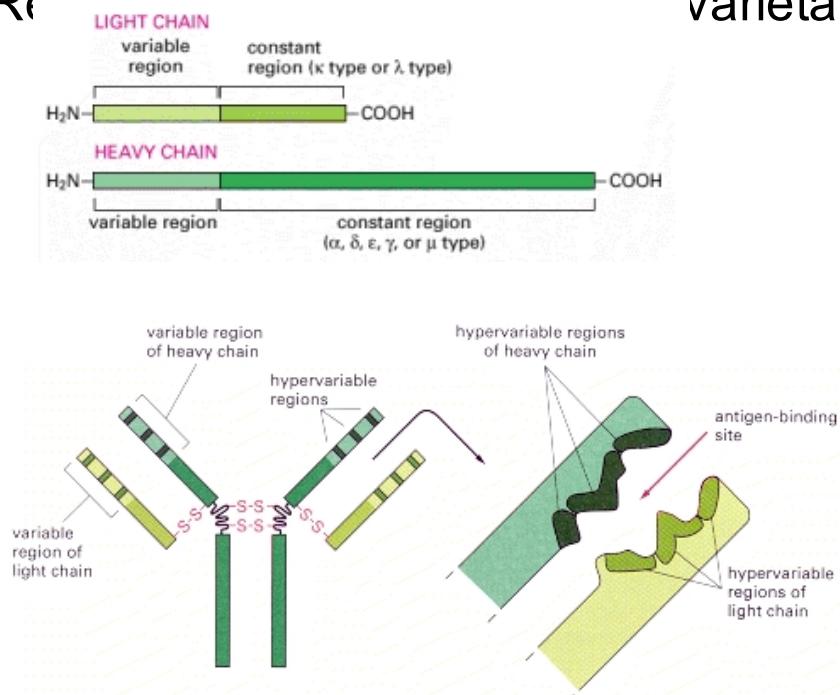
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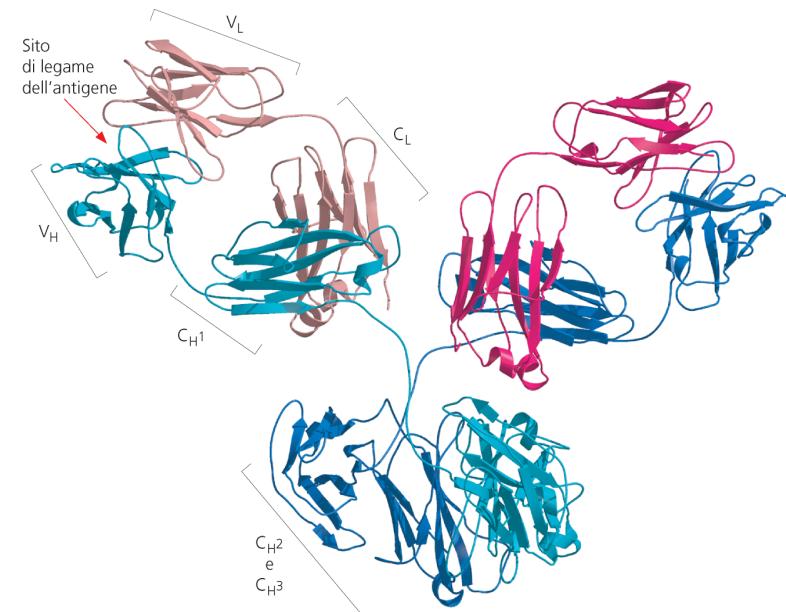
→ Avviene nelle cellule del sistema immunitario: cellule B (antibodies) and T (T-cell receptors)

→ Gli anticorpi ed i recettori delle cellule B/T devono riconoscere molti antigeni diversi (mechanismo identico)

→ Re-

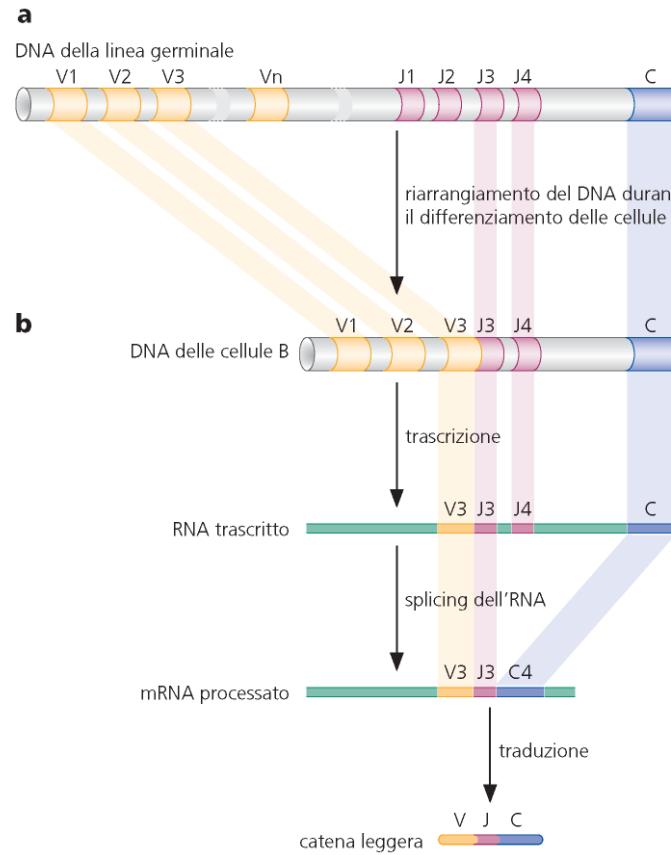


varietà di anticorpi



Ricombinazione V(D)J

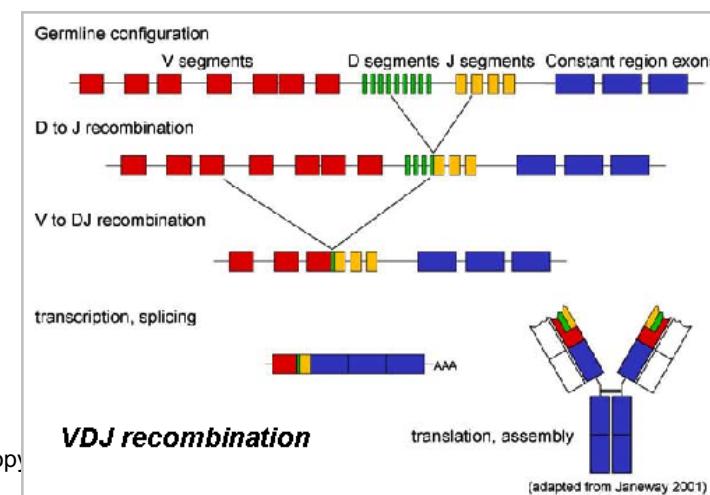
Light chain



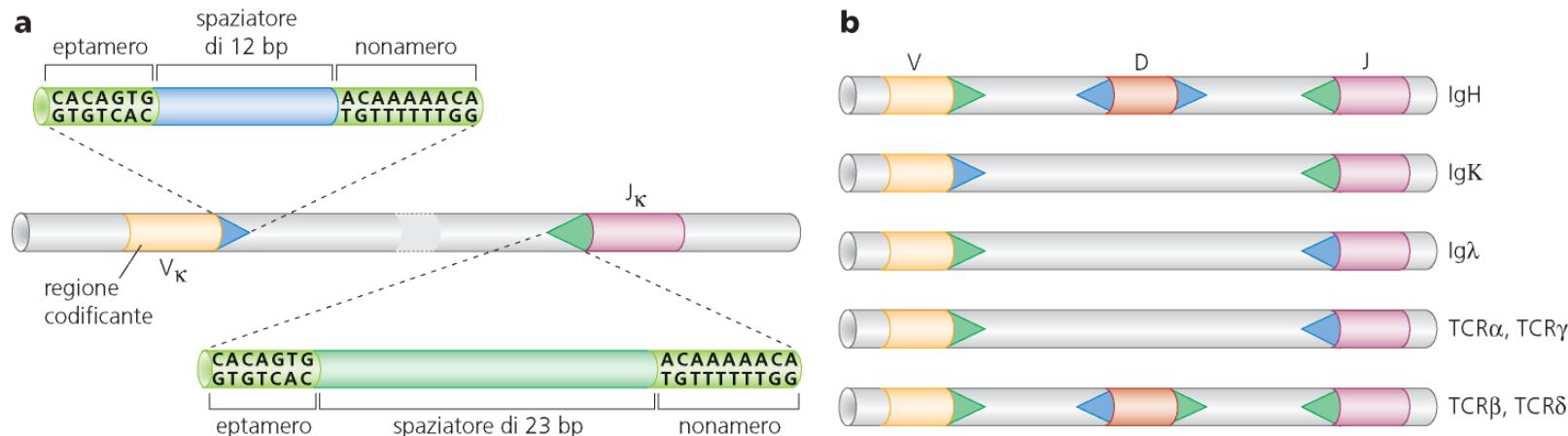
Heavy chain



- Regione genomica codificante una catena leggera di un anticorpo, locus kappa nel topo. Circa 300 segmenti genici V, 4 J ed 1 C -> 1200 versioni diverse di catene leggere a partire da questa unica regione genomica attraverso la ricombinazione.
- Per la catena pesante in aggiunta c'e' il segmento genico D (diversity). Un locus nel topo 100 segmenti V, 12 D e 4J -> 4800 combinazioni. L'anticorpo si forma fra qualsiasi catena pesante e qualsiasi leggera -> elevata diversità.

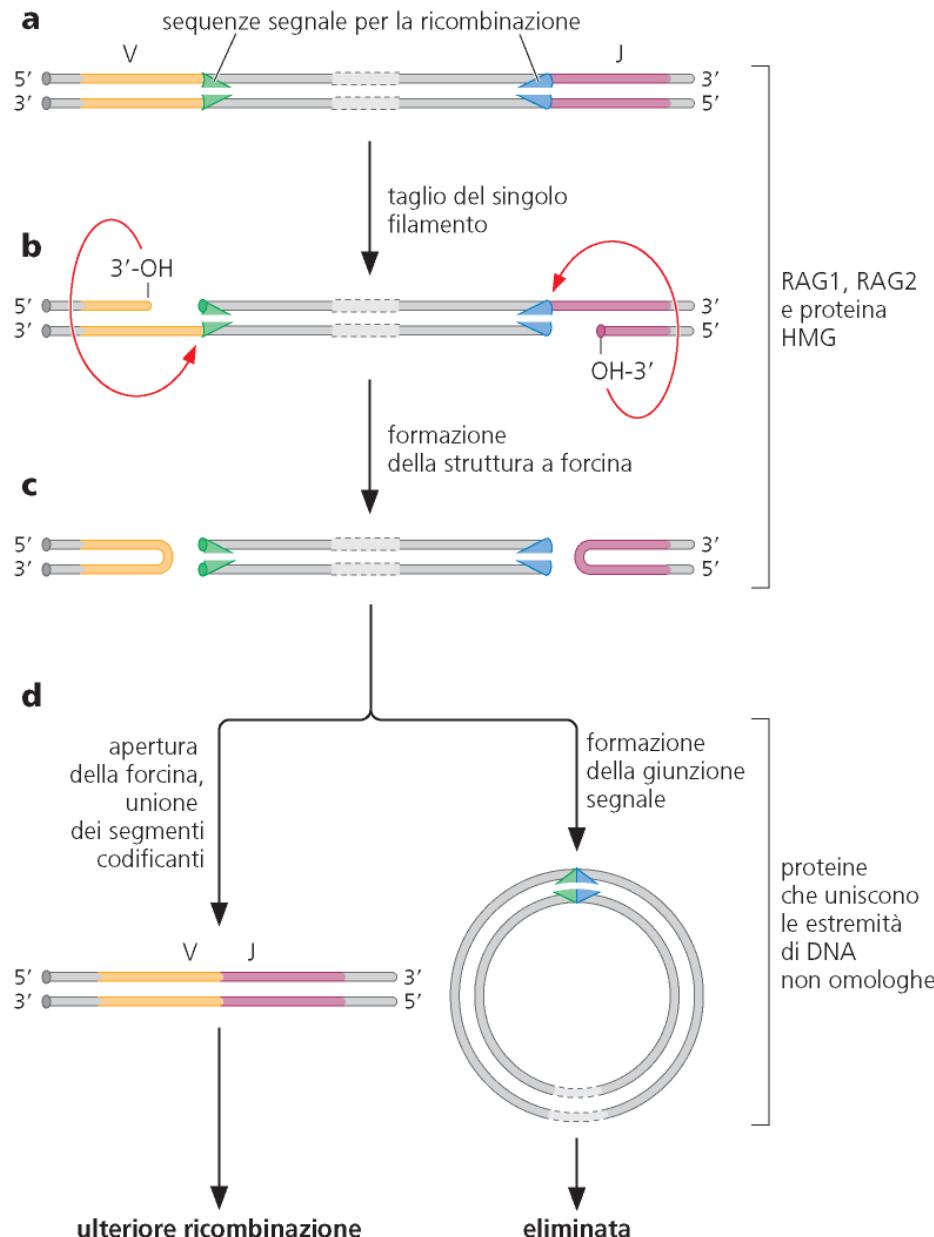


Ricombinazione V(D)J



- Adiacenti ai segmenti genici V(D)J vi sono le sequenze segnale per la ricombinazione: l' eptamero e il nonamero
- Terminal inverted repeats like DNA transposons
- Ci sono 2 classi di sequenze segnale per la ricombinazione (recombinazione sempre tra sequenze segnale per la ricombinazione con spaziatore da 12 e spaziatore da 23) → **La ricombinazione avviene sempre fra classi diverse.**
- Le ricombinasi sono **RAG1** e **RAG2** (**recombination-activating gene**) → funzionano molto simile a una trasposase (meccanismo del taglio)
- Durante l'unione del DNA si ha spesso aggiunta o delezione di nucleotidi -> ulteriore fattore di diversità.

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This process creates an enormous diversity of antibodies and T-cell receptors that is necessary the recognition of pathogen antigen