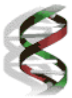


Elementos genéticos móviles

2014

DNA móvil

Movilidad intracelular

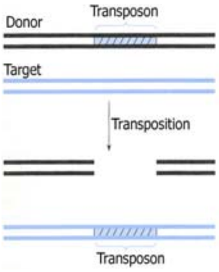
transposones a DNA, integrones, retroelementos móviles (varios), incluidos intrones móviles (tipo I y II).

Movilidad intercelular

virus, plásmidos (ambos son vehículos de transferencia horizontal de información genética)

Elementos Genéticos Móviles y Transposones

Un EGM es un segmento de DNA que puede moverse de un lugar a otro en el genoma de un organismo...



Puede salir de un lugar e insertarse en otro

El movimiento de un EGM puede producir mutaciones o reorganizaciones (*rearrangements*) cromosómicas y afectar de esa manera la expresión de otros genes …

Nina Fedoroff, 1984.

Mobile DNA

- Moderately-repeated, mobile DNA sequences are interspersed throughout the genomes of prokaryotes, higher plants and animals
- These sequences range in size from hundreds to a few thousand base pairs [x 10^2 – x 10^3 bp]
- The sequences are copied (or excised) and inserted into a new site in the genome by the process of transposition
- These sequences appear to serve no useful function

Transposable element: mobile genetic elements of a chromosome that have the capacity to move from one location to another in the genome.

- **Normal and ubiquitous** components of prokaryote and eukaryote genomes.

  Prokaryotes-transpose to/from cell's chromosome, plasmid, or a phage chromosome.

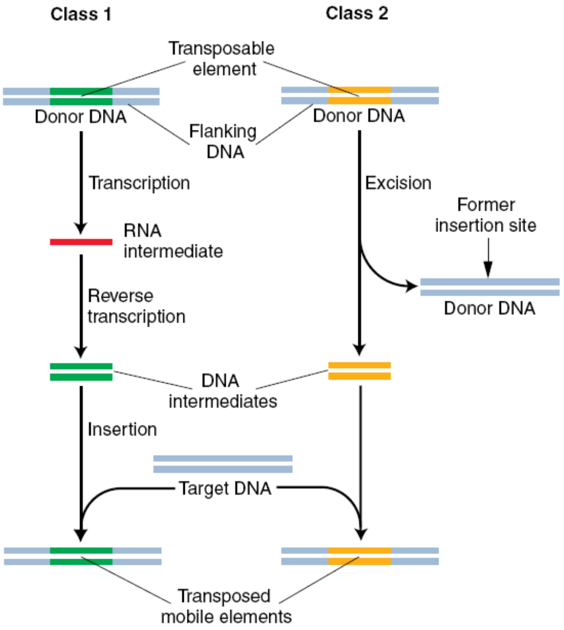
  Eukaryotes-transpose to/from same or a different chromosome.

- **Nonhomologous recombination**: transposable elements insert into DNA that has no sequence homology with the transposon.

- Transposable elements **cause genetics changes** and make important contributions to the **evolution of genomes**:

  •Insert into genes.
  •Insert into regulatory sequences; modify gene expression.
  •Produce chromosomal mutations (rearrangements).

The two classes of mobile elements



Two classes of **transposable elements / mechanisms of movement:**

class 2
Encode proteins that (1) move DNA directly to a new position or (2) replicate DNA and integrate replicated DNA elsewhere in the genome (prokaryotes and eukaryotes).

class 1
Retrotransposons encode reverse transcriptase and make DNA copies of RNA transcripts; new DNA copies integrate at different sites (eukaryotes only).

Tipos de transposones

Dos clases principales de elementos móviles **autónomos**:

Aquellos que codifican proteínas que mueven al DNA directamente a una nueva posición o duplican el DNA para producir un elemento nuevo que se integra en otro lugar. Estos se encuentran tanto en procariotes como en eucariotes (transposones clase II).

Los que codifican una transcriptasa reversa con la que sintetizan DNA a partir de un RNA molde. Dicho DNA se integra luego en nuevos sitios del genoma (transposones clase I, retrotransposones, retroposones, etc.).

Transposable Elements in Prokaryotes

a. Insertion sequence (IS) elements.

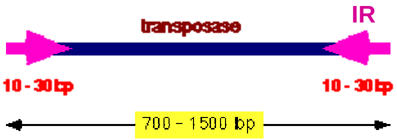
b. Transposons (Tn).

c. Bacteriophage Mu

Insertion Sequences

IS elements are the simplest transposable elements found in prokaryotes, **encoding only genes for mobilization and insertion of its DNA**. IS elements are commonly found in bacterial chromosomes and plasmids.

IS elements were first identified in *E. coli's* galactose operon, where some mutations' were shown to result from insertion of a DNA sequence now called **IS1**



Prokaryotic IS elements range in **size** from **768 bp** to over **5 kb**

E. coli IS elements include:

IS1 is 768 bp long, and present in 4 –19 copies on the *E. coli* chromosome.

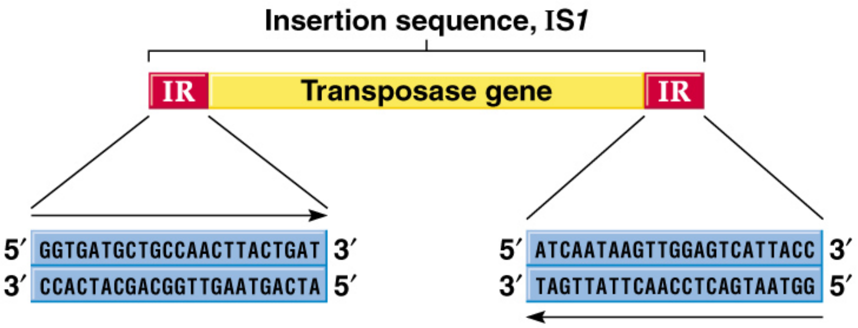
IS2 has 0–12 copies on the chromosome, and 1 copy on the F plasmid.

IS10 is found in R plasmids.

The **ends of all sequenced IS elements** show **inverted terminal repeats** (**IR**s) of 9 – 41 bp

(*e.g.*, IS1 has 23 bp of nearly identical sequence)

Insertion Sequences (IS)



Las repeticiones terminales invertidas de IS1
son secuencias casi idénticas de 23 bp (repeticiones imperfectas).

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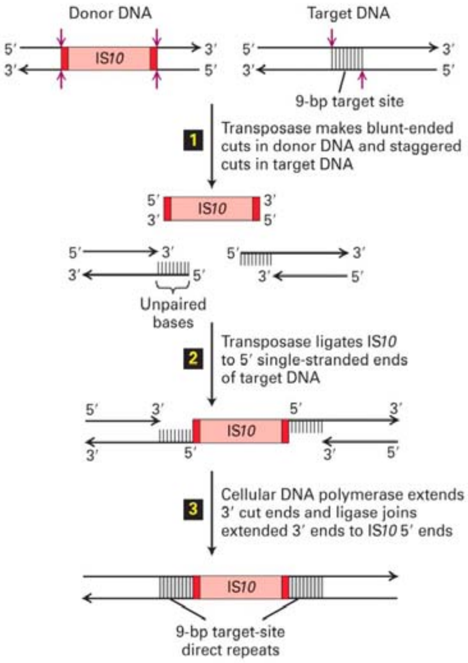
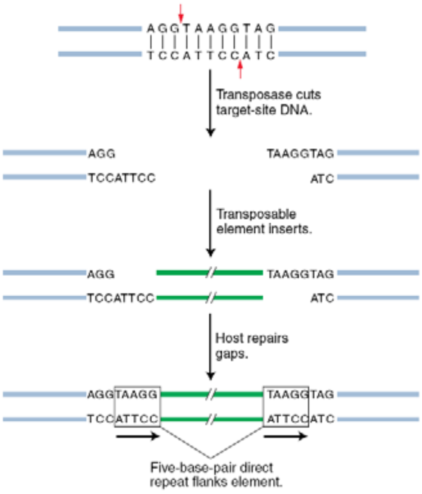
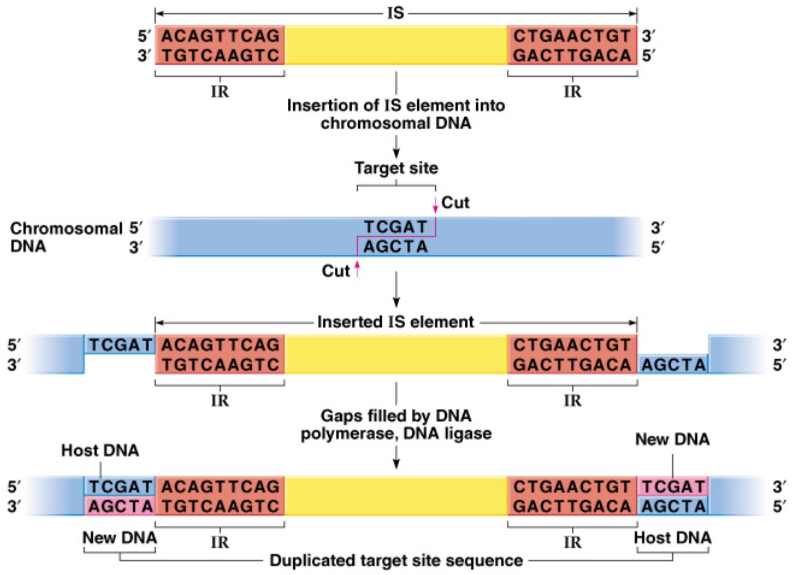
Duplicación de las secuencias del sitio de inserción (*target*): repeticiones directas –**DR**– a ambos lados del **transposón**







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When an IS element transposes:

- a. The original copy stays in place, and a new copy inserts randomly into the chromosome.
- b. The IS element uses the host cell replication enzymes for precise replication.
- c. Transposition requires transposase, an enzyme encoded by the IS element.
- d. Transposase recognizes the IR sequences to initiate transposition.
- e. IS elements insert into the chromosome without sequence homology (illegitimate or non-homologous recombination) at target sites.
  - i. A staggered cut is made in the target site, and the IS element inserted.
  - ii. DNA polymerase and ligase fill the gaps, producing small direct repeats of the target site flanking the IS element (target site duplications).
- f. Mutational analysis shows that IR sequences are the key

Integration of IS elements may:

- a. Disrupt coding sequences or regulatory regions.
- b. Alter expression of nearby genes by the action of IS element promoters.
- c. Cause deletions and inversions in adjacent DNA.
- d. Serve as a site for crossing-over between duplicated IS elements.

Frecuencia de transposición de IS

1. Rate of transposition:

   ~10^{-3}-10^{-4} per element per generation

2. Rate of spontaneous mutation:

   ~10^{-5}-10^{-7} per generation

3. Rate of reversion (by *precise excision* of the IS element)

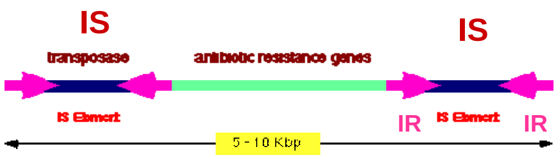
   ~10^{-6}-10^{-10} per generation

   ~10^{3} times less frequent than insertion

Transposons

Transposons are similar to IS elements, but carry additional genes, and have a more complex structure. There are two types of prokaryotic transposons:

Composite transposons carry genes (e.g., antibiotic resistance) flanked on both sides by **IS elements** (IS modules).



The **IS** elements are of the same type, and called ISL (left) and ISR (right).

ISL and **IS**R may be in direct or inverted orientation to each other.

Tn10 is an example of a composite transposon. It is 9.3 kb, and contains:

(1) 6.5 kb of central DNA with genes that include tetracycline resistance (a selectable marker).

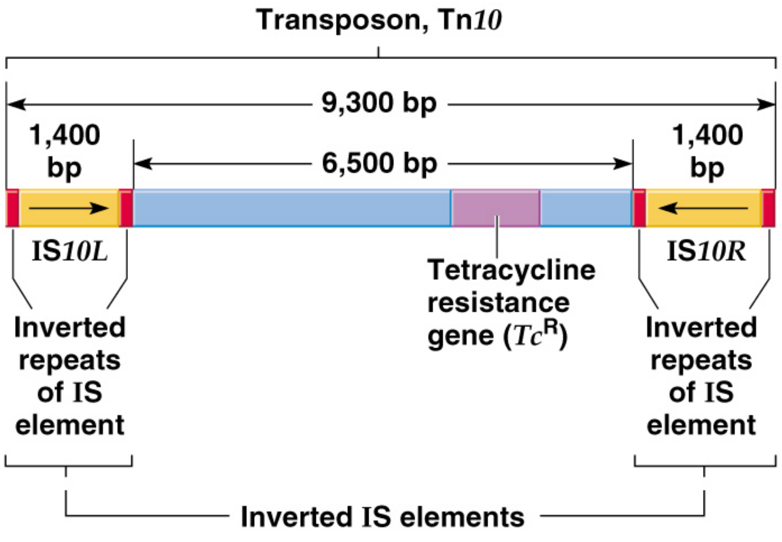
(2) 1.4 kb IS elements (IS10L and IS10R) at each end, in an inverted orientation.

Transposition of composite transposons results from the **IS elements**, which supply **transposase** and its recognition signals, the **IR**s.

(1) Tn10's transposition is rare, because transpose is produced at a rate of 0,1 molecule/generation.

(2) (2) Transposons, like IS elements, produce target site duplications (e.g., a 9-bp duplication for Tn10). (Table 20.1)

Transposón compuesto Tn*10*



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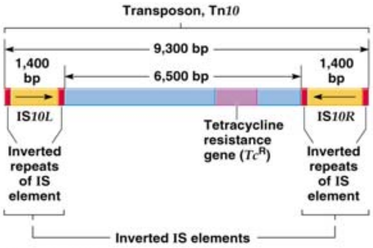
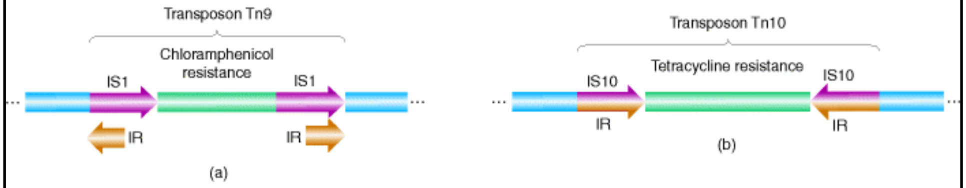
Transposones compuestos





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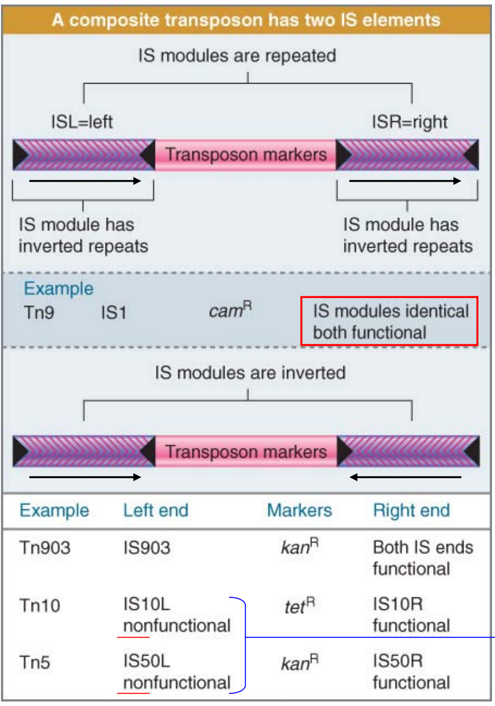


Example	Left end	Markers	Right end
Tn903	IS903	kan^R	Both IS ends functional
Tn10	IS10L nonfunctional	tet^R	IS10R functional
Tn5	IS50L nonfunctional	kan^R	IS50R functional

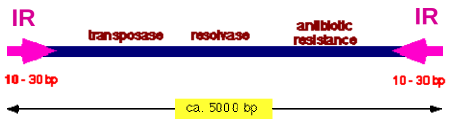
NOT identical but closely related

Transposones compuestos

- *Either* one *or both* of the IS elements of a composite transposon may catalize transposition.
- A functional IS module can transpose *either* itself *or* the entire transposon
- An active IS element at *either end* may also transpose independently.

Genes IX, Ch21. Lewin (2008)

Noncomposite transposons also carry genes (e.g., drug resistance) but do <u>not</u> terminate with IS elements



Transposition proteins are encoded in the central region.

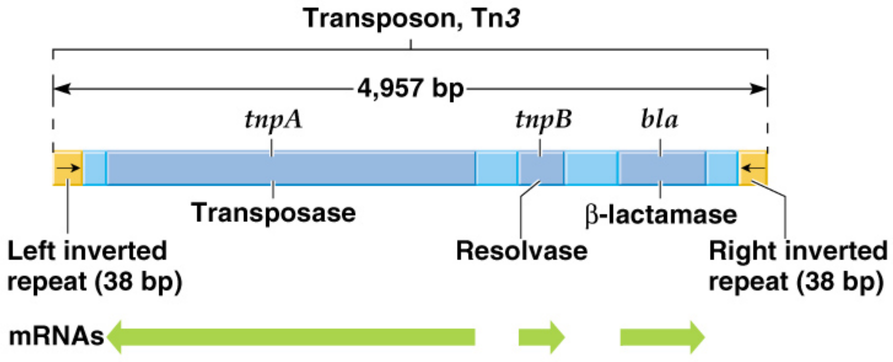
The **ends** are **repeated sequences** (but not IS elements).

Noncomposite transposons cause target site duplications (like composite transposons).

An example is Tn3.

- (1) Tn3’s length is about 5 kb, with 38-bp inverted terminal repeats.
- (2) It has three genes in its central region:
  - (a) *bla* encodes β-lactamase, which breaks down ampiciliin.
  - (b) *tnpA* encodes transposase, needed for insertion into a new site.
  - (c) *tnpB* encodes resolvase, involved in recombinational events needed for transposition (not found in all transposons).
- (3) Tn3 produces a 5-bp duplication upon insertion.

Transposon no compuesto Tn*3*



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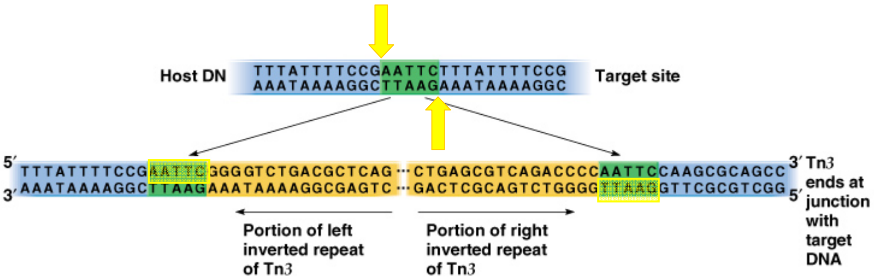
DNA sequence of a target site of Tn*3*



Transposons cause the same sorts of mutations caused by IS elements:

a. Insertion into a gene disrupts it.
b. Gene expression is changed by adjacent Tn promoters.
c. Deletions and inversions occur.
d. Crossing-over results from duplicated Tn sequences in the genome

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Mecanismos de transposición

Transposición conservativa (no replicativa) :

El Tn se pierde de su ubicación original cuando se traspone a un nuevo sitio (ej. Tn10).

Transposición replicativa:

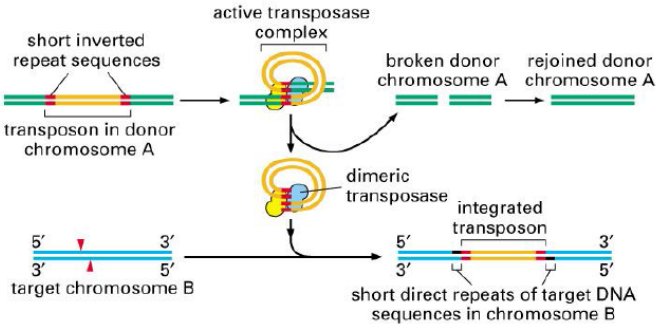
Queda una copia del Tn cuando se inserta otra copia en un nuevo sitio blanco (ej. Tn3)

Cointegration is an example of the replicative transposition that occurs with Tn3 and its relatives

i. Donor DNA containing the Tn fuses with recipient DNA.
ii. The Tn is duplicated, with one copy at each donor-recipient DNA junction, producing a cointegrate.
iii. The cointegrate is resolved into two products, each with one copy of the Tn.

Mecanismos de transposición

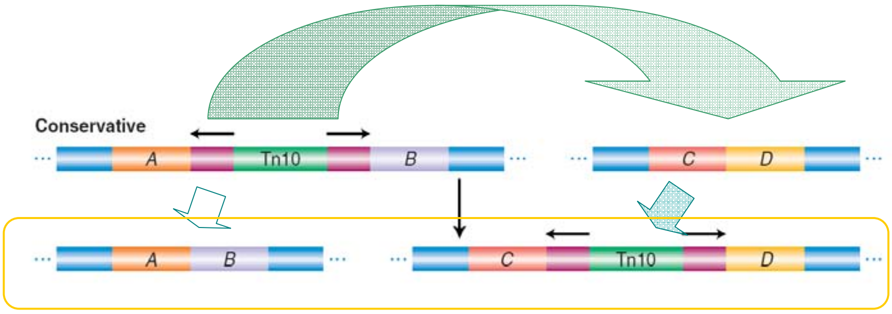
Transposición conservativa (no replicativa)



Mecanismos de transposición

Transposición conservativa (no replicativa) :

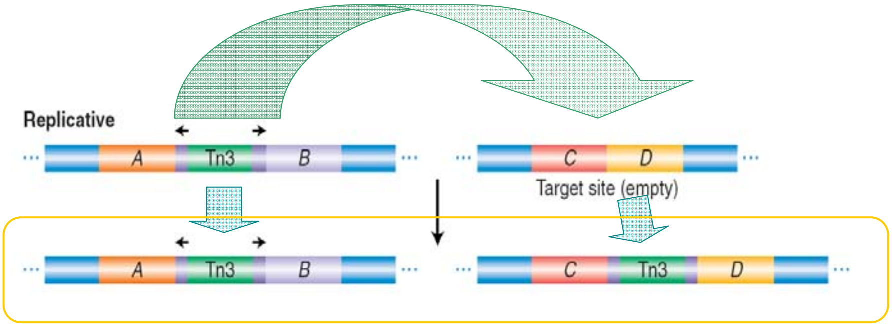
El Tn se pierde de su ubicación original cuando se traspone a un nuevo sitio (ej. Tn10).



Mecanismos de transposición

Transposición replicativa:

Queda una copia del Tn cuando se inserta otra copia en un nuevo sitio blanco (ej. Tn3)



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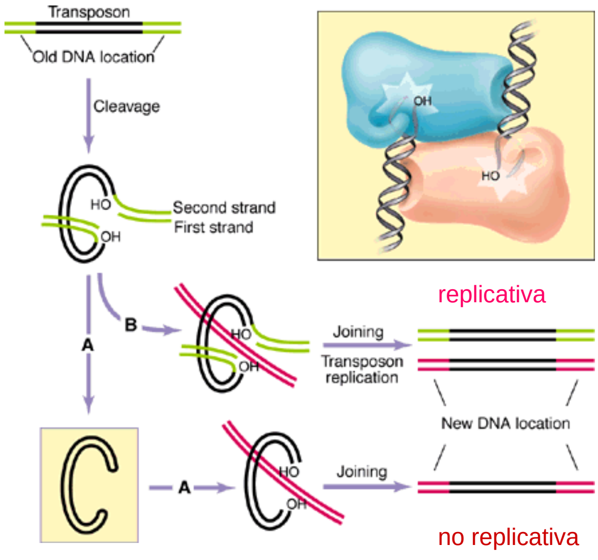
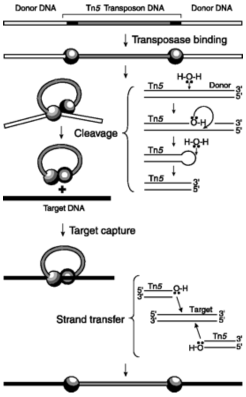
transposición conservativa (no replicativa)

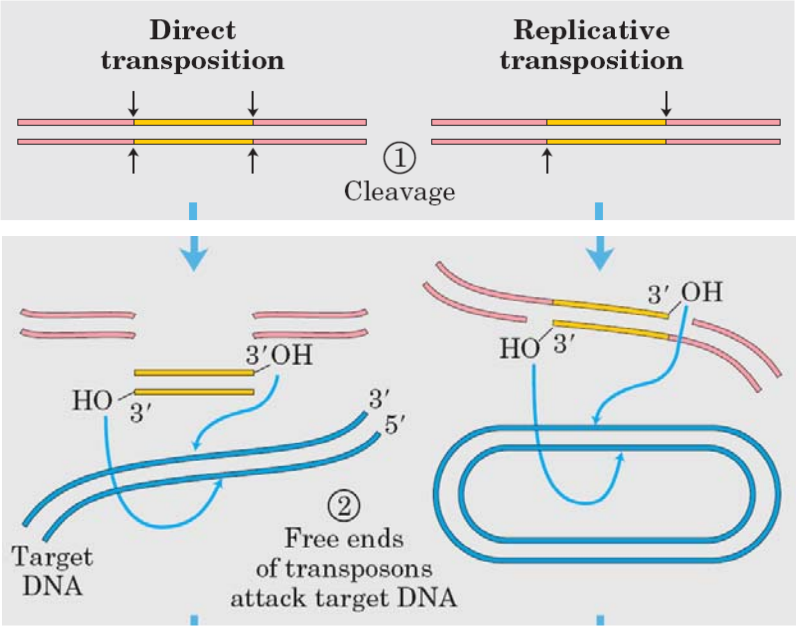


replicativa

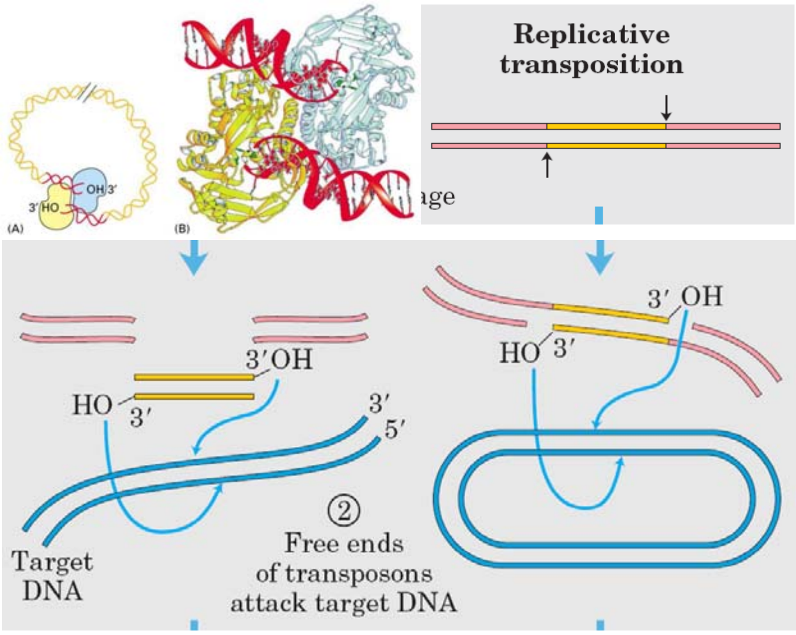
no replicativa



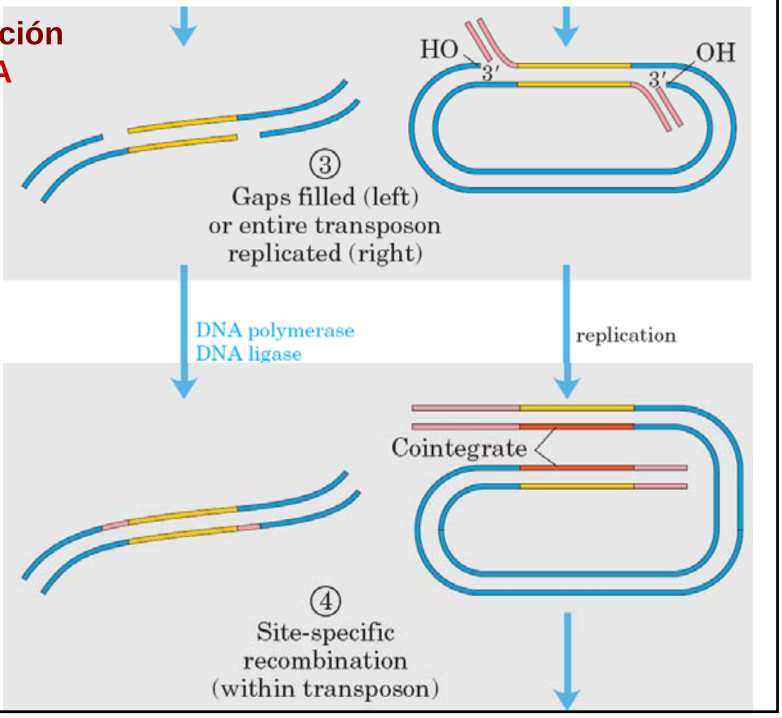
Transposición de DNA



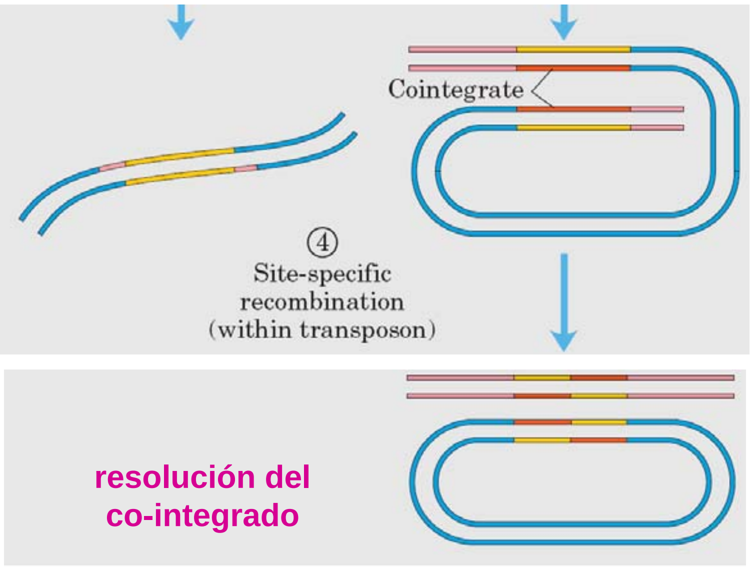
Transposición de DNA



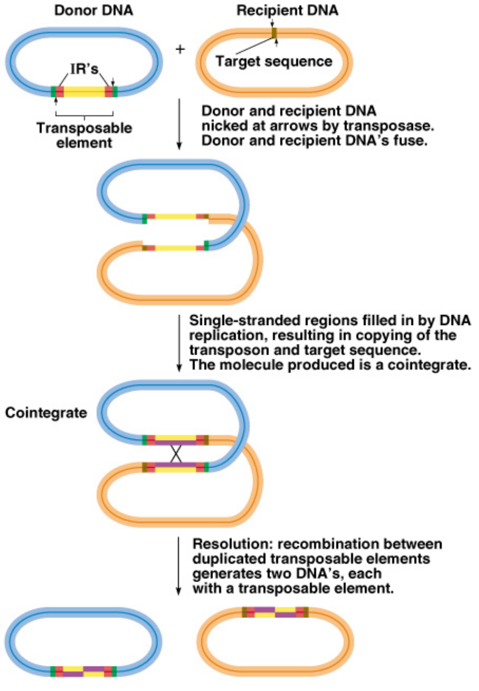
Transposición de DNA



Transposición de DNA



resolución del co-integrado



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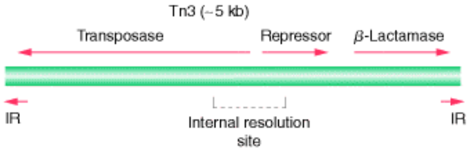
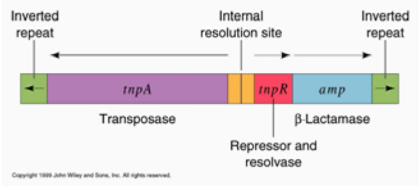
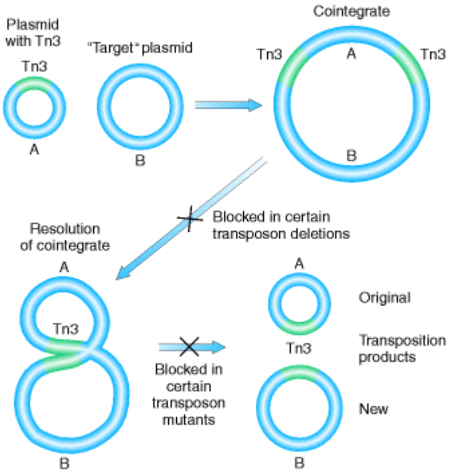
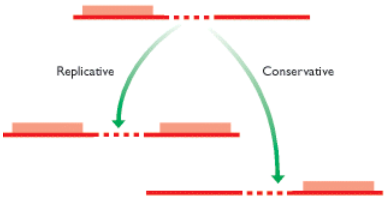
Cointegration model
for transposition of a
transposable element by
replicative transposition

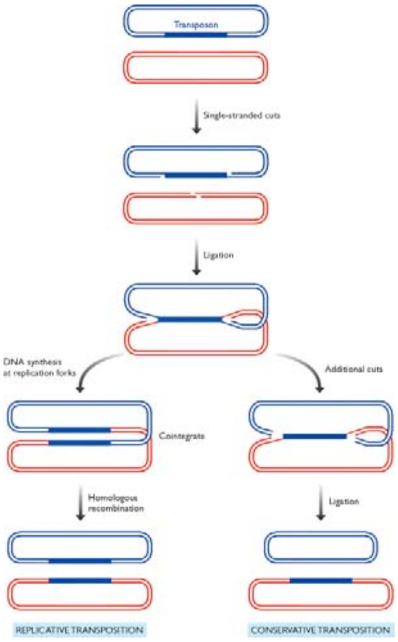






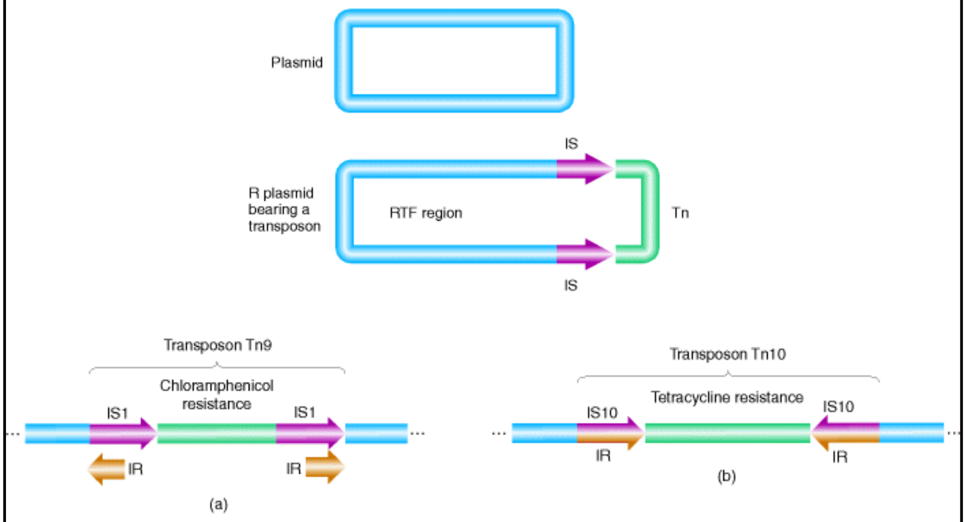
Replicative and conservative transposition of DNA transposons

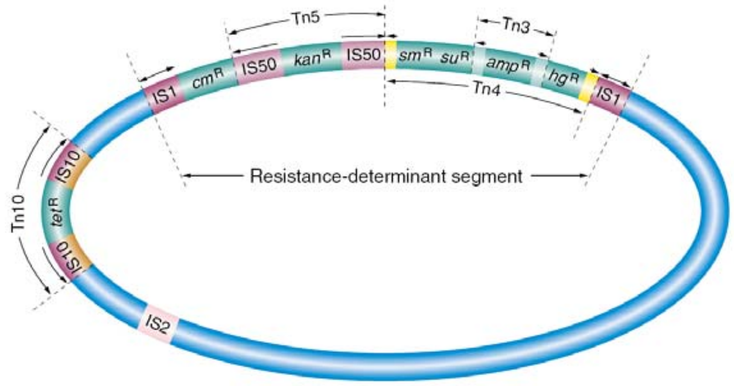




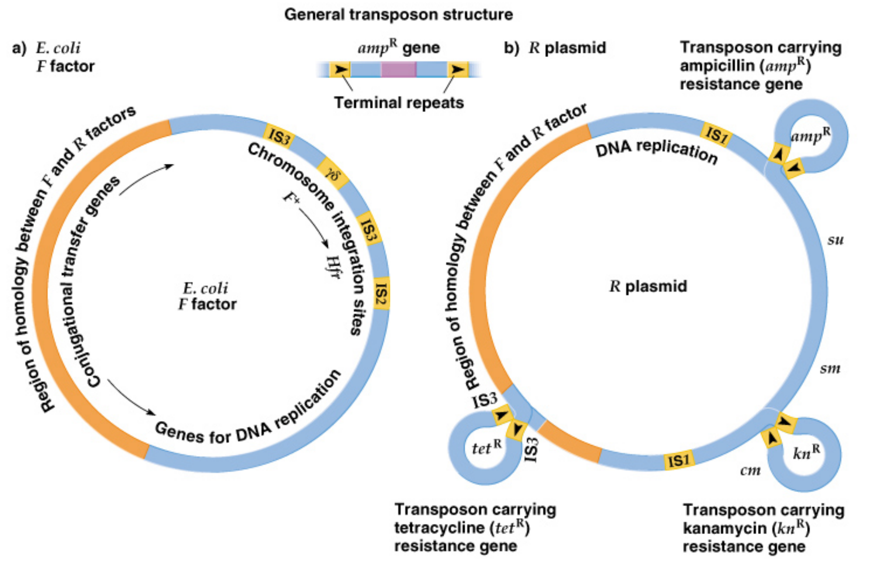
IS Elements and Transposons in Plasmids

1. Bacterial plasmids are extrachromosomal DNA capable of self-replication. Some are episomes, able to integrate into the bacterial chromosome. The *E. coli F* plasmid is an example:
   a. Important genetic elements of the *F* plasmid are:
      i. *tra* genes for conjugal transfer of DNA from donor to recipient.
      ii. Genes for plasmid replication.
      iii. 4 IS elements: 2 copies of IS3, 1 of IS2, and 1 of γδ (gammadelta). All have homology with IS elements in the *E. coli* chromosome.
   b. The *F* factor integrates by homologous recombination between IS elements, mediated by the *tra* genes.
2. *R* plasmids have medical significance, because they carry genes for resistance to antibiotics, and transfer them between bacteria.
   a. Genetic features of *R* plasmids include:
      i. The resistance transfer factor region (RTF), needed for conjugal transfer. It includes a DNA region homologous to an *F* plasmid region, and genes for plasmid-specific DNA replication.
      ii. Differing sets of genes, such as those for resistance to antibiotics or heavy metals. The resistance genes are transposons, flanked by IS module-like sequences, and can replicate and insert into the bacterial chromosome.
   b. *R* plasmids are clinically significant, because they disseminate drug resistance genes between bacteria.


Plasmid
IS
R plasmid bearing a transposon
RTF region
Tn
IS
Transposon Tn9
Chloramphenicol resistance
IS1
IS1
IR
IR
(a)
Transposon Tn10
Tetracycline resistance
IS10
IS10
IR
IR
(b)


Tn5
Tn3
IS1
cm^R
IS50
kan^R
IS50
sm^R
su^R
amp^R
hg^R
IS1
Tn4
Resistance-determinant segment
Tn10
IS10
tet^R
IS10
IS2

Organizational maps of bacterial plasmids with transposable elements



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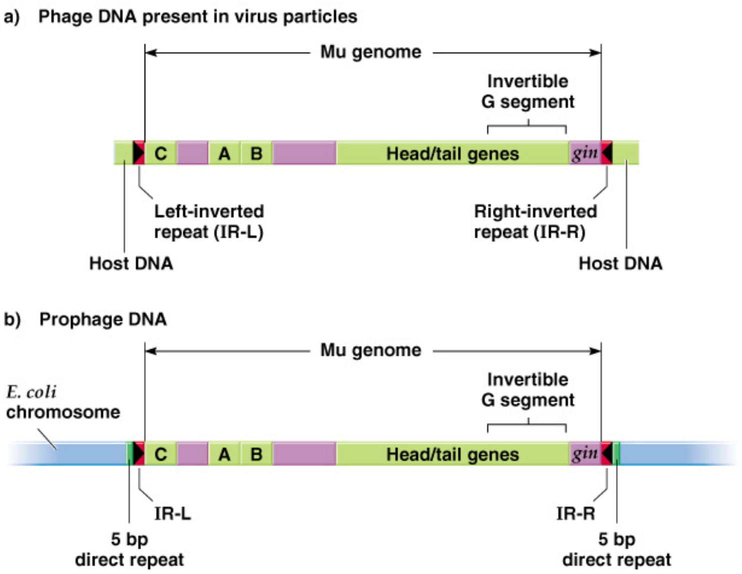
OJO: dibujo del “rulo” mal hecho

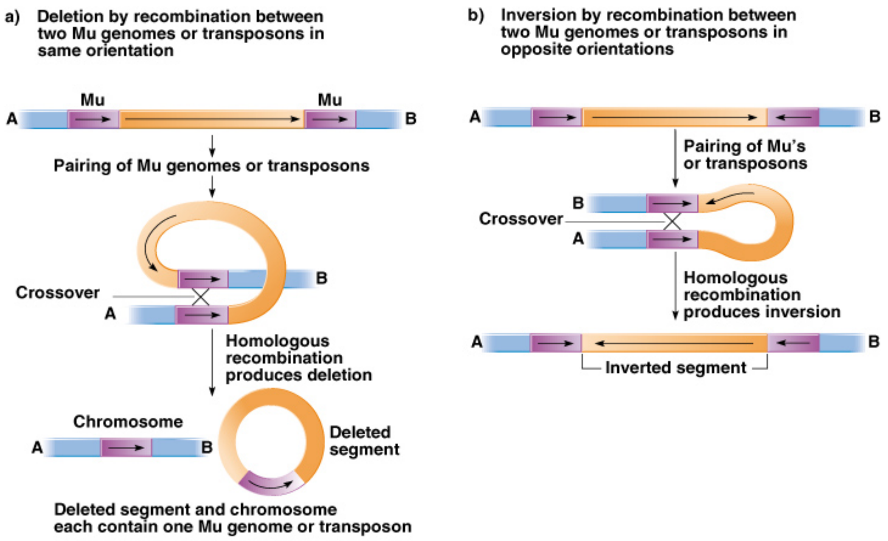
Bacteriophage Mu

1. Temperate bacteriophage Mu (mutator) can cause mutations when it transposes. Its structure includes:
   a. A 37 kb linear DNA in the phage particle that has central phage DNA and unequal lengths of host DNA at the ends.
   b. The DNA’s G segment can invert, and is found in both orientations in viral DNA.
2. Following infection, Mu integrates into the host chromosome by conservative (non-replicative) transposition.
   a. Integration produces prophage DNA flanked by 5 bp target site direct repeats.
   b. Flanking DNA from the previous host is lost during integration.
   c. The Mu prophage now replicates only when the *E. coli* chromosome replicates, due to a phage-encoded repressor that prevents most Mu gene expression.
3. Mu prophage stays integrated during the lytic cycle, and replication of Mu’s genome is by replicative transposition.
4. Mu causes insertions, deletions, inversions and translocations

Temperate bacteriophage Mu genome shown in (a) as in phage particles and (b) as integrated into the *E. coli* chromosome as a prophage



Production of deletion or inversion by homologous recombination between two Mu genomes or two transposons



General structure of **bacterial IS elements**

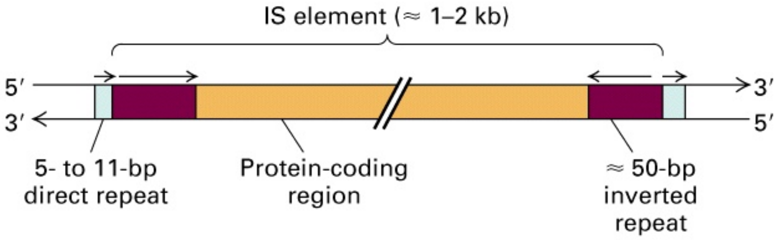
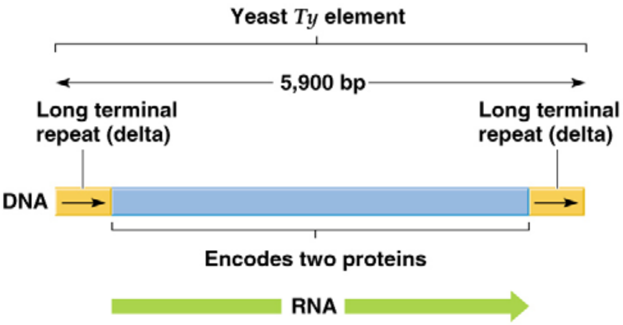


Figure 9-11

Ty elements in yeast:

- Similar to bacterial transposons; terminal repeated sequences, integrate at non-homologous sites, with target site duplication.
- Ty elements share properties with retroviruses, retrotransposons:
  - Synthesize RNA copy and make DNA using reverse transcriptase.
  - cDNA integrates at a new chromosomal site.



Viral retrotransposons contain LTRs and behave like retroviruses in the genome

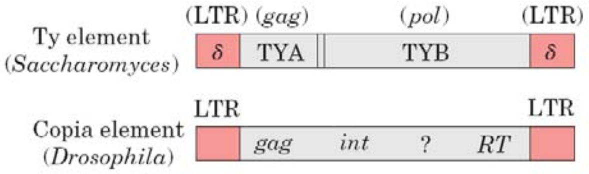
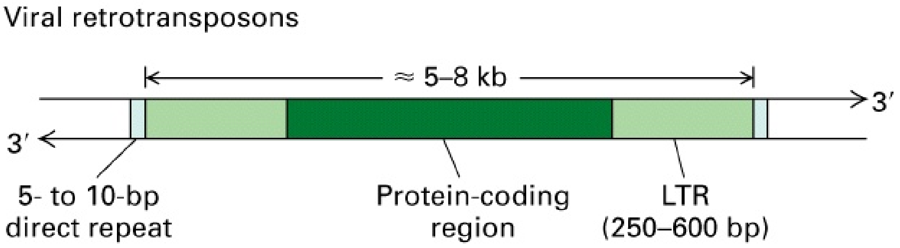
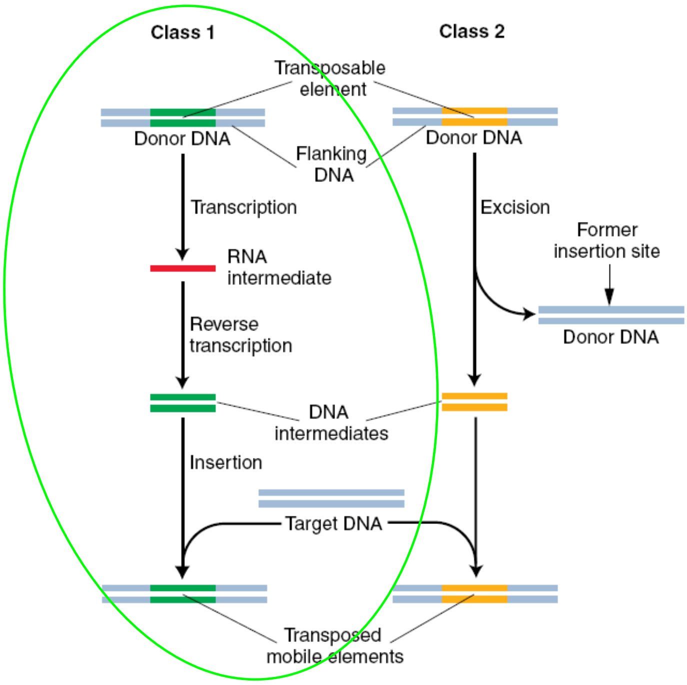


FIGURE 26-33 **Eukaryotic transposons.** The Ty element of the yeast *Saccharomyces* and the copia element of the fruit fly *Drosophila* serve as examples of eukaryotic transposons, which often have a structure similar to retroviruses but lack the *env* gene. The δ sequences of the Ty element are functionally equivalent to retroviral LTRs. In the copia element, *int* and *RT* are homologous to the integrase and reverse transcriptase segments, respectively, of the *pol* gene.

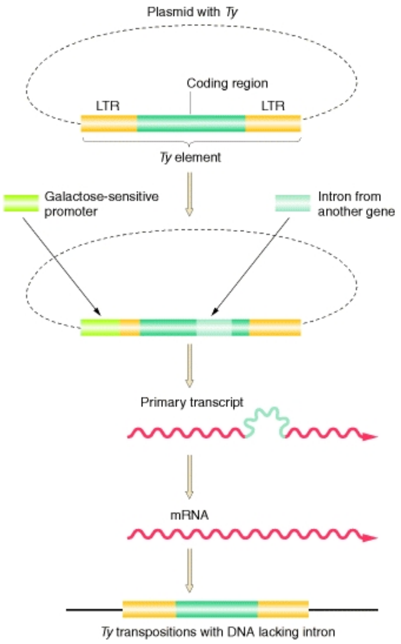
Viral retrotransposons contain LTRs and behave like retroviruses in the genome



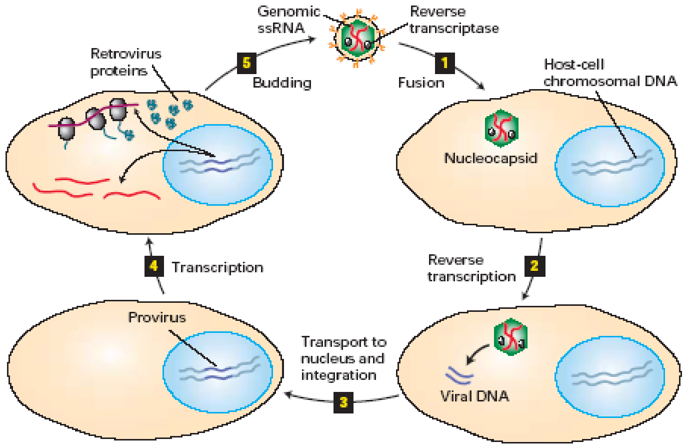


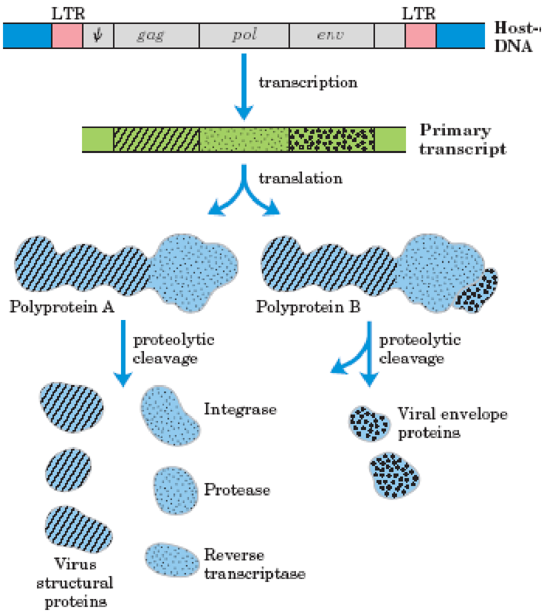
Demonstration of transposition through an RNA intermediate.

A *Ty* element is altered by adding a promoter that can be activated by the addition of galactose. Activation of the promoter will increase transcription through the *Ty* element. Then an intron from another gene is inserted into the *Ty* element. Because the final product of transposition contains no intron, the intron must have been spliced out of an RNA transcript (see Chapter 10). This splicing must have taken place as shown here, where the primary transcript contains the intron but the final processed mRNA does not. This RNA is then copied by reverse transcriptase and integrated into the chromosomal DNA. [after Lodish et al., 1995]



Infección por retrovirus





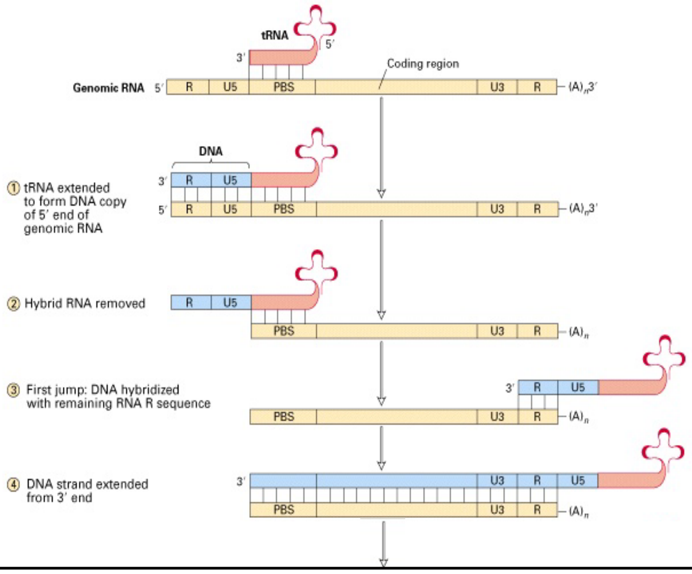
Provirus:

DNA producido por transcripción reversa del RNA retroviral e integrado en un sitio al azar del genoma de la célula huésped.

El LTR de la izquierda funciona como promotor de la RNA pol II: se transcribe y procesa de diferentes maneras el mRNA.

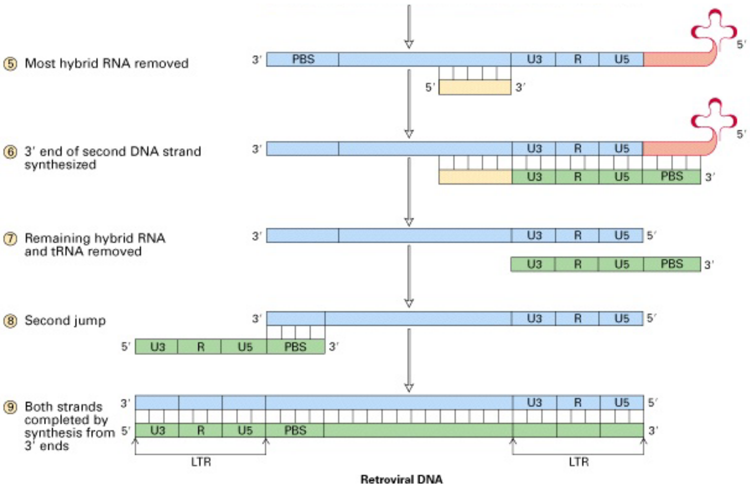
El mRNA, a su vez, se traduce para dar diferentes poliproteínas que se procesan proteolíticamente y permiten construir nuevas partículas de virus, que brotan de la célula infectada y son capaces de infectar nuevas células.

Generation of LTRs during reverse transcription of retroviral genomic DNA (1)



Lodish

Generation of LTRs during reverse transcription of retroviral genomic DNA (2)

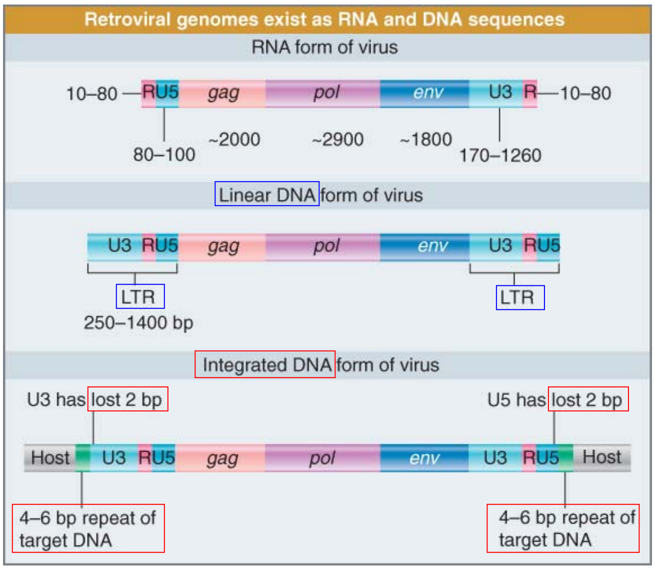


Lodish

Viral DNA Is Generated by Reverse Transcription

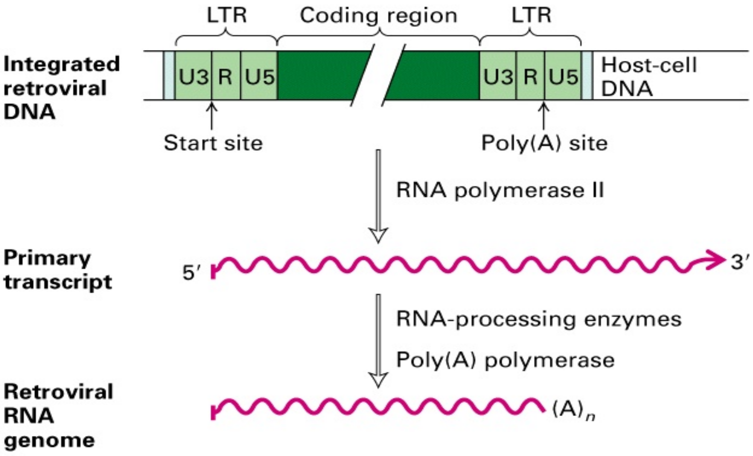
A short sequence (R) is directly repeated at each end of the viral RNA

The 5' and 3' ends are R-U5 and U3-R, respectively



Genes IX, Ch.22. Lewin (2008) Figure 22.5. Retrovirial RNA ends in direct repeat (R), the free linear DNA ends in LTR and the provirus ends in LTRs that are shortened by two bases each.

Generation of retroviral genomic RNA from integrated retroviral DNA



Nonretroviral retrotransposons lack LTRs

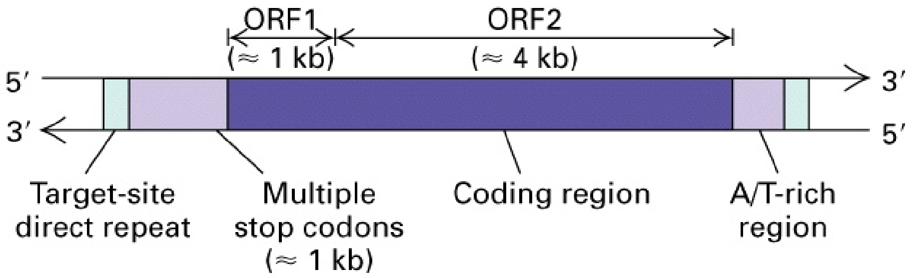
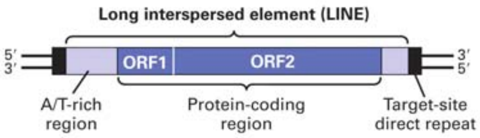
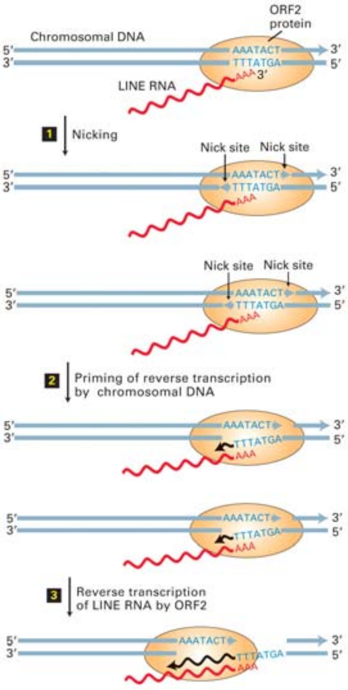


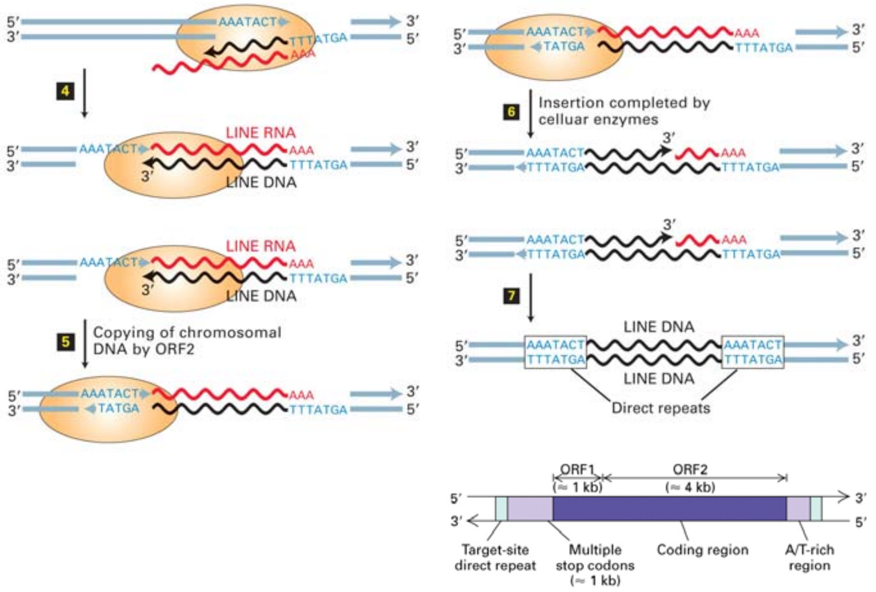
Figure 9-18

LINE

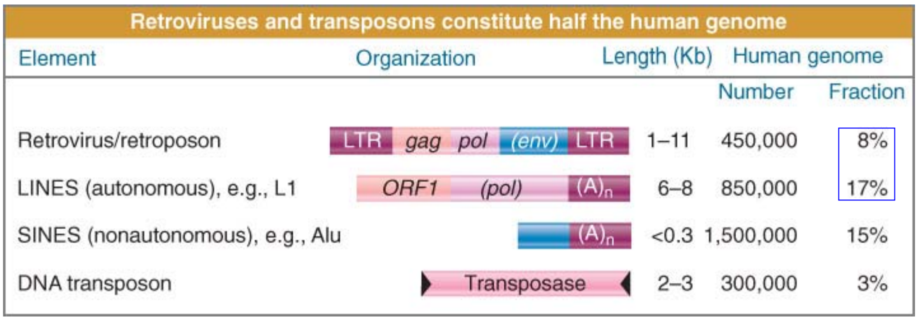
Lodish Figs.10.15 & 10.16







Transposons and *retroposons*
constitute almost half of the human genome

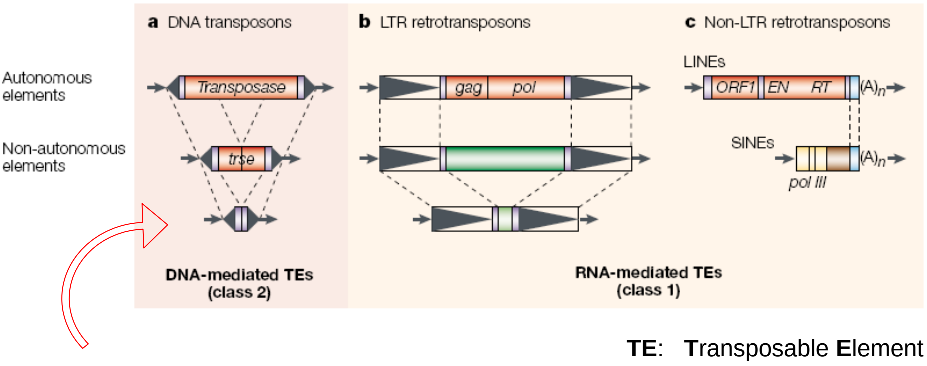


Retroviruses and transposons constitute half the human genome

Element	Organization	Length (Kb)	Human genome Number	Human genome Fraction
Retrovirus/retroposon	LTR *gag* *pol* *(env)* LTR	1–11	450,000	8%
LINES (autonomous), e.g., L1	*ORF1* *(pol)* $(A)_n$	6–8	850,000	17%
SINES (nonautonomous), e.g., Alu	$(A)_n$	<0.3	1,500,000	15%
DNA transposon	Transposase	2–3	300,000	3%

Lewin. Figure 22.18

Características estructurales y clasificación de elementos genéticos móviles en eucariotas

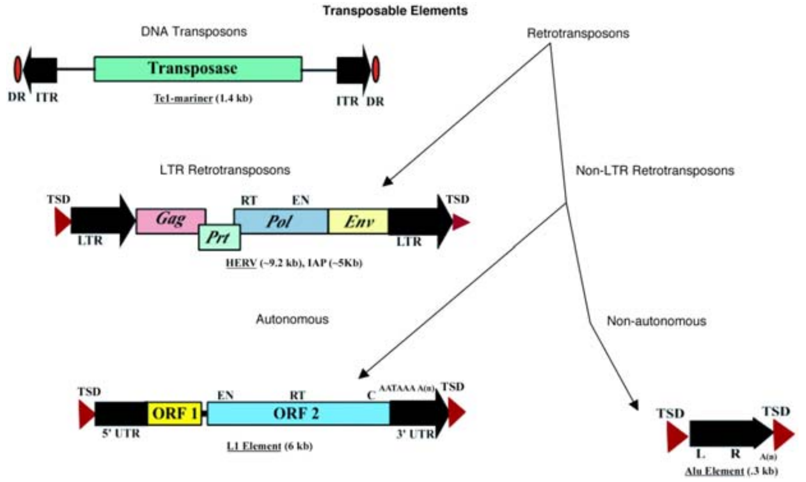


TE: **T**ransposable **E**lement

Miniature inverted-repeat transposable elements

MITEs are sequences of about 400 base pairs and 15 base pair inverted repeats that vary very little. They are found in their thousands in the genomes of both plants and animals (over 100,000 were found in the rice genome). MITEs are too small to encode any proteins.

*Inverted repeats: **IR*** *Direct repeats: **DR*** *Long Terminal Repeats: **LTR***



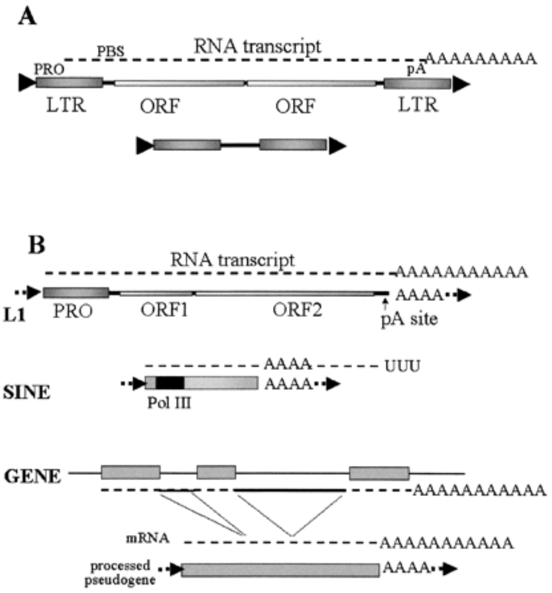
Science, Vol 303, Issue 5664, 1626-1632 , 12 March 2004, **Mobile Elements: Drivers of Genome Evolution**, Haig H. Kazazian, Jr.*

Fig. 1. Classes of mobile elements. DNA transposons, e.g., Tc-1/mariner, have inverted terminal 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 a discussion of other **retrotransposons** that do not fall into either class). Examples of LTR **retrotransposons** are human endogenous retroviruses (HERV) (shown) and various Ty elements of *S. cerevisiae* (not shown). These elements have terminal LTRs and slightly overlapping ORFs for their group-specific antigen (*gag*), protease (*prt*), polymerase (*pol*), and envelope (*env*) genes. They produce target site duplications (TSDs) upon insertion. Also shown are the reverse transcriptase (RT) and endonuclease (EN) domains. Other LTR **retrotransposons** that are responsible for most mobile-element insertions in mice are the intracisternal A-particles (IAPs), early transposons (Etns), and mammalian LTR-**retrotransposons** (MaLRs). These elements are not present in humans, and essentially all are defective, so the source of their RT in trans remains unknown. L1 is an example of a non-LTR retrotransposon. L1s consist of a 5'-untranslated region (5'UTR) containing an internal promoter, two ORFs, a 3'UTR, and a poly(A) signal followed by a poly(A) tail (A*n*). L1s are usually flanked by 7- to 20-bp target site duplications (TSDs). The RT, EN, and a conserved cysteine-rich domain (C) are shown. An Alu element is an example of a nonautonomous retrotransposon. Alus contain two similar monomers, the left (L) and the right (R), and end in a poly(A) tail. Approximate full-length element sizes are given in parentheses. [Modified from (*31*)

Classes of retroelements.

(*A*) LTR-retrotransposons. The LTR-retrotransposons have long-terminal repeats at both ends of the elements that contain sequences that serve as transcription promoters, as well as terminators. These sequences allow the element to code for an mRNA molecule that is processed and polyadenylated. There are at least two genes coded within the element to supply essential activities for the retrotransposition mechanism. The RNA contains a specific primer binding site (PBS) for initiating reverse transcription. A hallmark of almost all mobile elements is that they form small direct repeats formed at the site of integration.

(*B*) NonLTR retrotransposons. L1 elements in humans represent the most abundant class of these elements. They have an unusual RNA polymerase II-promoter structure in which the promoter is included within the final transcript. These elements create a polyadenylated mRNA which codes for a bicistronic mRNA. The consensus poly A addition site is relatively weak, resulting in transcripts that commonly extend into downstream sequences, resulting in transduction of those downstream sequences to new chromosomal loci. Integration of a nonLTR element into a new chromosomal location results in a chromosomal duplication of variable length forming relatively short, flanking direct repeats. The mechanism for expression of the second open reading frame (ORF) is also uncertain. SINEs represent elements that are independently derived from RNA polymerase III-transcribed RNA genes (tRNAs and 7SL RNA). SINEs are transcribed by RNA polymerase III and encode a poly A, or A-rich region, at the 3' end of the element. However, transcription extends into unique flanking sequences downstream of the poly A stretch. These elements have no protein coding capacity and share flanking direct repeats with properties similar to those of L1 elements and are thought to be dependent on the L1 proteins for their retroposition. Processed pseudogenes are derived from the mature mRNAs (spliced) from numerous genes. These are also likely to be dependent on the L1 retrotransposition mechanism.



Genome Research 12 (10), 1455-1465 (October 2002) REVIEW: Mammalian Retroelements; P.L. Deininger and M.A. Batzer

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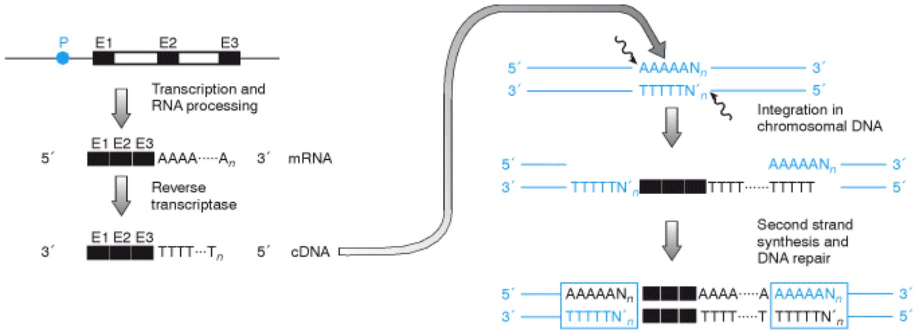
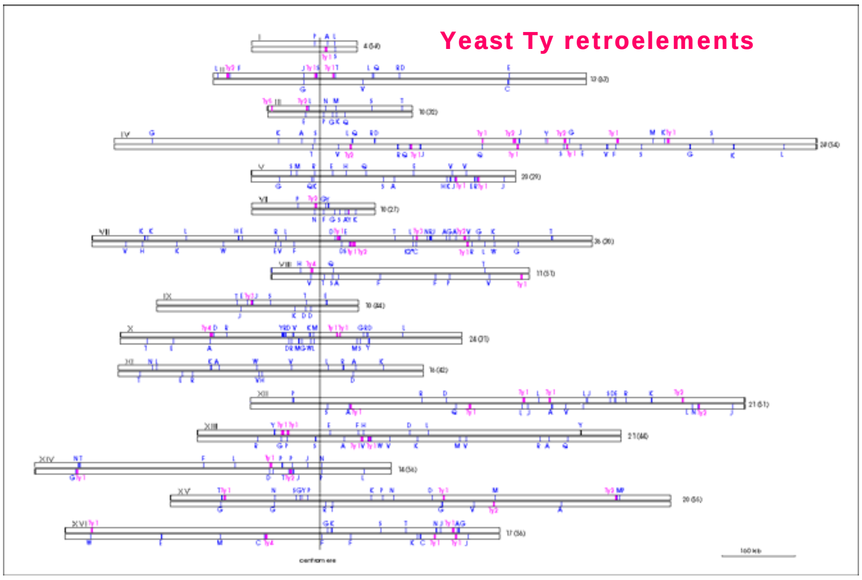
Processed pseudogenes



Yeast Ty retroelements



- **Location of the tRNA genes and the Ty elements** to the single chromosomes. The two open bars for each chromosome represent the 'Watson' (upper) strand, transcribed from left to right, and the 'Crick' (lower) strand, transcribed into the opposite direction. Blue symbols indicate the tRNA genes, the one-letter code is used to identify the resepective amino acid specificity. Ty1 through Ty5 represent the different classes of the retroelements (shown in red). The numbers of tRNA genes for each chromosome are specified (average kb per tRNA gene in brackets).

General Features of Transposable Elements

1. Transposable elements are divided into two classes on the basis of their mechanism for movement:
    a. Some encode proteins that move the DNA directly to a new position or replicate the DNA to produce a new element that integrates elsewhere. This type is found in both prokaryotes and eukaryotes.
    b. Others are related to retroviruses, and encode reverse transcriptase for making DNA copies of their RNA transcripts, which then integrate at new sites. This type is found only in eukaryotes.
2. Transposition is nonhomologous recombination, with insertion into DNA that has no sequence homology with the transposon.
    a. In prokaryotes, transposition can be into the cell’s chromosome, a plasmid or a phage chromosome.
    b. In eukaryotes, insertion can be into the same or a different chromosome.
3. Transposable elements can cause genetic changes, and have been involved in the evolution of both prokaryotic and eukaryotic genomes.

    Transposons may:
    a. Insert into genes.
    b. Increase or decrease gene expression by insertion into regulatory sequences.
    c. Produce chromosomal mutations through the mechanics of transposition.