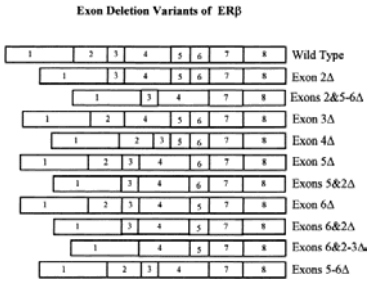
Estrogen Receptor β - splice variants



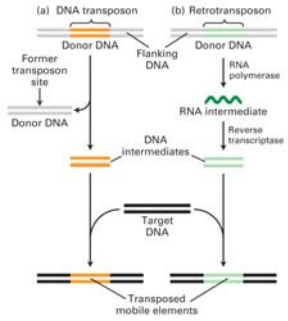
NEUROFIBROMAS: NF-1 Mobile Element

Mobile Elements

- 25-40% of the mammalian genomes consist of moderately repetitive DNA
- Transposition is the process by which these sequences are copied and inserted in the genome
- Scattered throughout genome
- "Selfish DNA" - What is this?

2 Major Classes of Mobile Elements

DNA – mediated



RNA-mediated

Fig 10-8

Features of Mobile Elements

- Excise from Genome
- Insert into genome
- Replicative or nonreplicative

Major Types of Transposable Elements

DNA-Mediated

- *Ac/Ds* elements from maize
- *P-element* from *Drosophila*

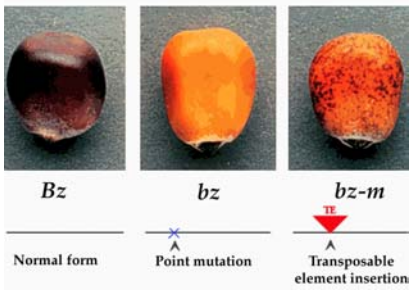
RNA-Mediated

- Viral retrotransposons
  - *Ty* elements from yeast
  - *Copia* elements from Drosophila
- Nonviral retrotransposons
  - *LINE/SINE* from mammals
  - *Alu* sequences from humans

Barbara McClintock Discovered Mobile Elements

Characterized genetic entities that could move into and out of genes affecting the color of corn kernels

Ac/Ds Transposition System



From http://mbclserver.rutgers.edu/~dooner/PGRPpage.html

Ac/Ds Transposable Elements

Ac : Activator
Autonomous
~4.5 kb, encodes a transposase

Ds : Dissociation
Non-autonomous
Ac element with internal deletions
Needs Ac activity to transpose

Move by a non-replicative mechanism

Ac Element

Transposase

No Transposase

Ds Element

Nonreplicative Transposition

Transposase recognizes and binds to inverted repeats

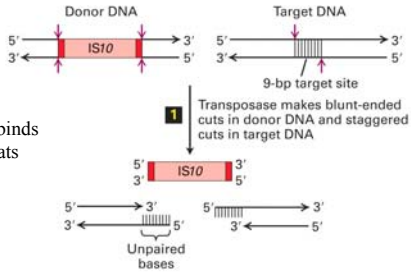


Fig 10-10

Nonreplicative Transposition

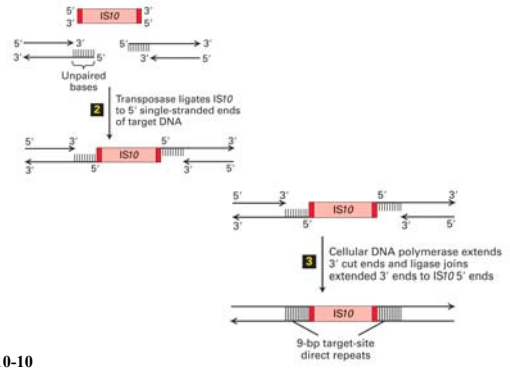
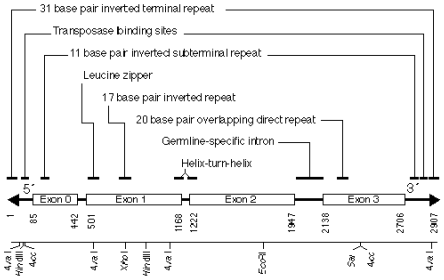


Fig 10-10

Drosophila P-elements

- 2907 bp, containing a 31-bp terminal inverted repeat
- Encodes a transposase with four exons
- P-elements are believed to have invaded the *D. melanogaster* genome < 100 years ago
- All natural populations have P-elements, only old laboratory strains are free from them
- Horizontal transfer between species

Drosophila P-elements

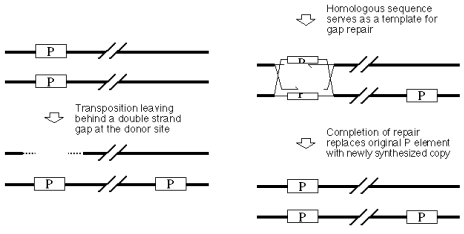


Exons code for transposase

http://www.wisc.edu/genetics/CATG/engels/Pelements/index.html

Drosophila P-elements

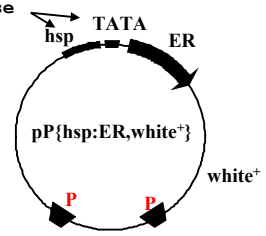
Amplification of P-elements by DNA repair mechanisms



http://www.wisc.edu/genetics/CATG/engels/Pelements/index.html

Example P-Element Plasmid

What are these for?



Each P element expression vector was microinjected into *Drosophila* embryos along with a transposase activity provided by an additional intact P element.

Retrotransposons

- Require reverse transcriptase for replication

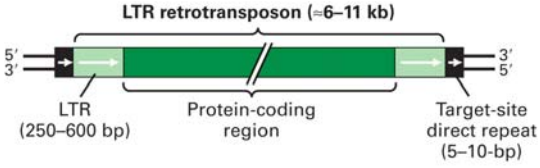
Viral

Abundant in yeast and *Drosophila*

Nonviral

Most common type of mobile elements in mammals

Typical Eukaryotic Viral Retrotransposon



LTR – Long Terminal Repeat

The Ty and Copia elements encode 3 of 4 proteins from retroviruses incl. transposase and integrase

Fig 10-11

Retrotransposons

- Similar mechanisms to retroviruses
- Retrotransposons may have formed from retroviruses
- Retroviruses like HIV may have evolved from retrotransposons

Generation of Retroviral Genomic RNA

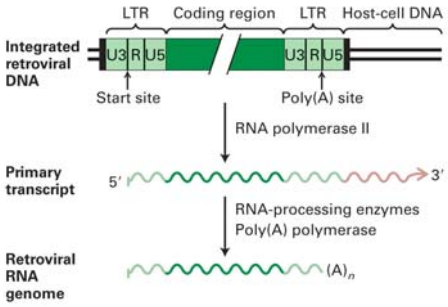


Fig 10-12

Reverse Transcription of Genomic RNA

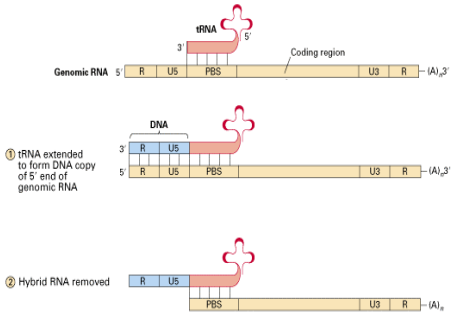


Fig 10-13

Reverse Transcription of Genomic RNA (cont)

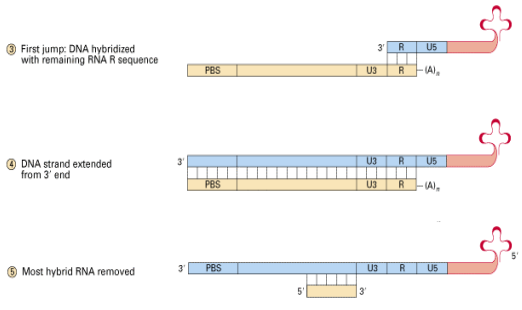


Fig 10-13

Reverse Transcription of Genomic RNA (cont)

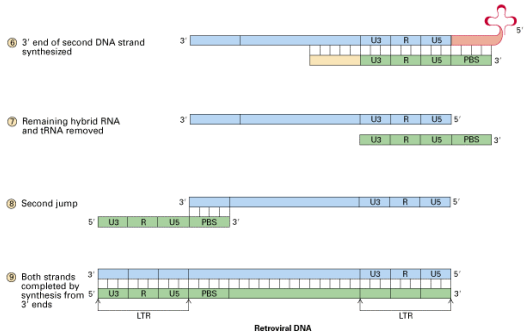
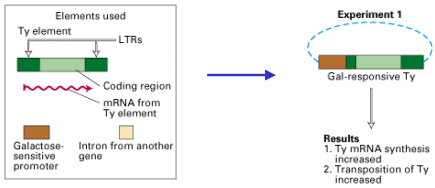


Fig 10-13

Reverse Transcriptase Movie

Movie

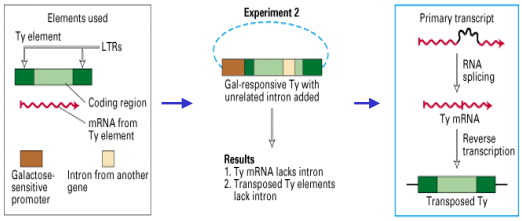
Yeast Ty Transposition



(1) Engineer recombinant plasmid
(2) Grow in presence of galactose
(3) What will inserting an intron test?

Fig 10-14

Yeast Ty Transposition (cont)



What would happen with Ac element introns?

Potential Test Question

Fig 10-14

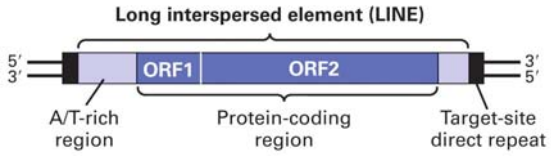
Most Mammalian Mobile Elements are Nonviral

LINES Long Interspersed Elements
6-7 kb, about 10 classes in humans
(15% of genome)

SINES Short Interspersed Elements
~300 bp, one major class in humans
(10% of genome)

May be present in *thousands of copies* per genome

A Typical LINE Element



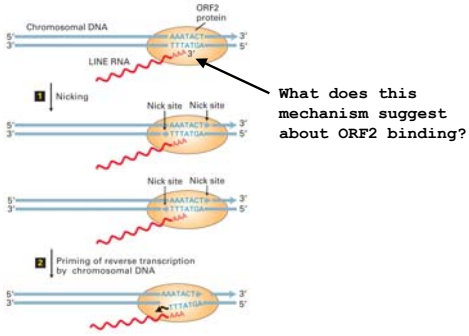
ORF1 Encodes an RNA binding protein
Multiple copies bind LINE 1 RNA, assist transport from nucleus

ORF2 Binds the poly A tail of mRNA transcript.
Also encodes a reverse transcriptase-like protein

Fig 10-15

LINE Transposition

No LTRs, therefore different mechanism



What does this mechanism suggest about ORF2 binding?

Fig 10-16

LINE Transposition (cont)

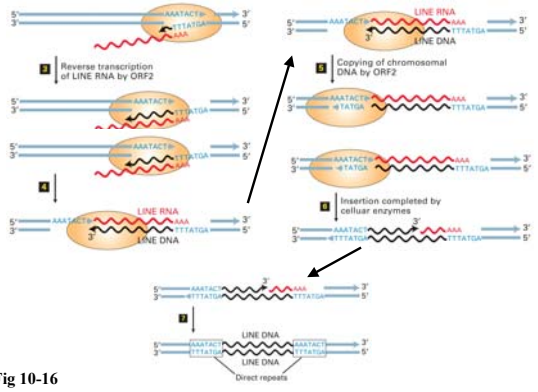
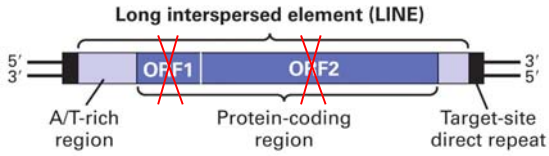


Fig 10-16

LINE Transposition



Most LINEs have accumulated mutations in their ORFs. Only a single, functional LINE element is needed to retrotranspose these mutated elements.

Only 0.01% of LINE elements in genome are functional.

Exon Shuffling via LINEs

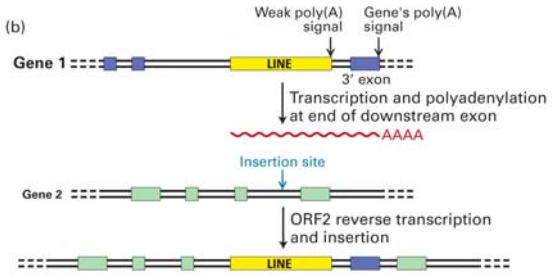


Fig 10-17

SINES and *Alu* sequences

- A particular SINE contains the recognition site for *AluI*
- *Alu* sequences are present at ~ 1 million sites in the human genome (10% of genome)
- *Alu* sequences probably evolved from the 7SL RNA a small RNA that is part of the signal recognition complex (translation).
- Flanked by direct repeats, may transpose like L1 LINE elements
- NF1: *Alu* insertion into an intron, results in deletion of the downstream exon during splicing and consequently shifts the reading frame (neurofibromatosis type 1)

Exon Shuffling via SINES

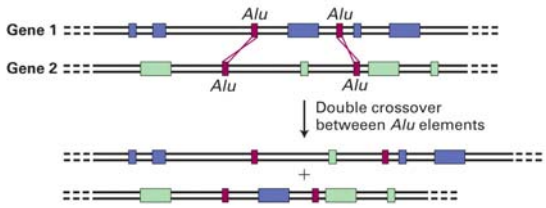


Fig 10-17

Retrotransposed mRNAS

- "Processed pseudogenes"
- Flanked by short, direct repeats probably a rare transposition event
- snRNAs and tRNAs also common
- How do we know a protein sequence is a pseudogene? How about a tRNA?
- 20,000 pseudogenes in human DNA
- Makorin gene regulated by its Pseudogene

Nature. 2003 May 1;423(6935):91-6

Importance of Mobile Elements

- Insertional mutagenesis: spontaneous mutation
- Recombination between elements: gene duplication
- Recombination between elements: "exon shuffling"
- Recombination between elements: "enhancer shuffling"