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Special Topics on Genetics

Section 9: Transposable elements

Drosopoulou E
School of Biology

Ευρωπαϊκή Ένωση
Ευρωπαϊκό Κοινωνικό Ταμείο
ΕΠΙΧΕΙΡΗΣΙΑΚΟ ΠΡΟΓΡΑΜΜΑ
ΕΚΠΑΙΔΕΥΣΗ ΚΑΙ ΔΙΑ ΒΙΟΥ ΜΑΘΗΣΗ
επένδυση στην κοινωνία της γνώσης
ΥΠΟΥΡΓΕΙΟ ΠΑΙΔΕΙΑΣ & ΘΡΗΣΚΕΥΜΑΤΩΝ, ΠΟΛΙΤΙΣΜΟΥ & ΑΘΛΗΤΙΣΜΟΥ
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ΕΣΠΑ
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πρόγραμμα για την ανάπτυξη
ΕΥΡΩΠΑΪΚΟ ΚΟΙΝΩΝΙΚΟ ΤΑΜΕΙΟ
Με τη συγχρηματοδότηση της Ελλάδας και της Ευρωπαϊκής Ένωσης

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Section Contents

- Introduction
- Transposable elements in prokaryotes
- Transposable elements in eukaryotes
- Biological significance of transposable elements

Introduction (1/10)

<u>Transposable elements</u>: DNA sequences, which can move from one location to another or from one DNA molecule to another(“jumping genes”).

1930-1950’s: Barbara Mc Clintock conducted studies on corn and discovered a genetic element capable of directing the transfer of itself into the genome.

Introduction (2/10)

Observations of B. Mc Clintock (1930-1950)

- The genetic factor Ds (dissociation) frequently causes breaking of the short arm of chromosome 9

- In the same strain the following also occur:

    - High frequency of gene deactivations
    - Reactivations of genes accompanied by deactivation of other genes
    - Frequent production of seeds with significant phenotypic differences (unstable phenotype) e. g. Colorless with spots.

Introduction (3/10)

Observations of Mc Clintock (1930-1950)

- The frequency of chromosome breaking and deactivation – reactivation of genes was related to the presence of genetic factor Ac (Activator).
- **The Ds and Ac loci can not be mapped. They change positions in the genome.**

Conclusion-proposal of B. McClintock (1930-1950)

There are elements-genes which "jump" around the genome

Introduction (4/10)

Schematic:

When the Ac is absent, the Ds can not move and the normal phenotype is expressed → **Purple** seed color

Chromosome carrying the **Ds** factor

Normal gene producing the pigment of the seeds

http://www.mun.ca/biology/scarr/Ac-Ds_instability.html

Introduction (5/10)

When Ac is present:

Chromosome carrying the Ac and Ds factors

a) the Ds can move close to the gene

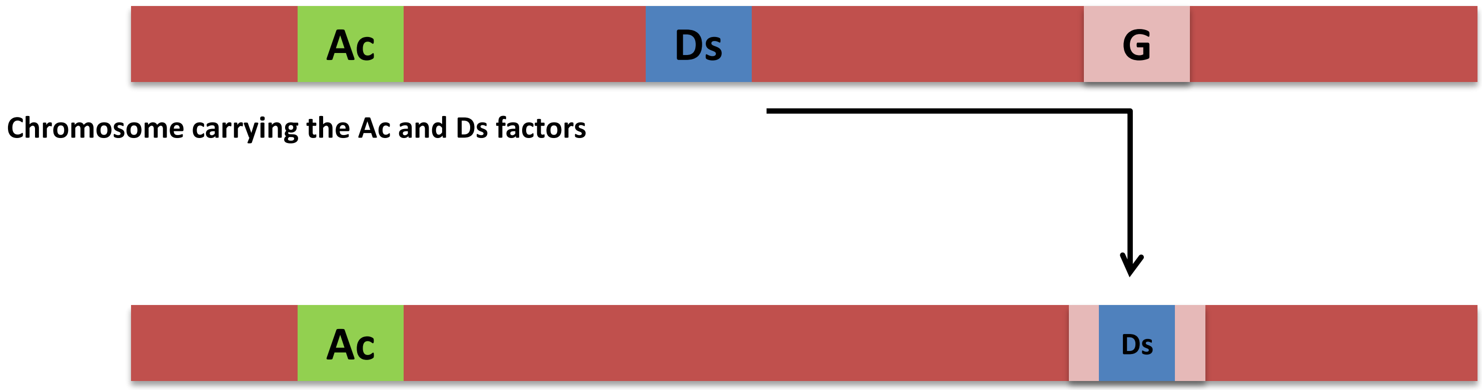
and cause chromosome breakage and loss of a fragment

In this case expression of the mutant phenotype is observed → **Yellow** seed color

http://www.mun.ca/biology/scarr/Ac-Ds_instability.html

Introduction (6/10)

b) the Ds can move within the gene

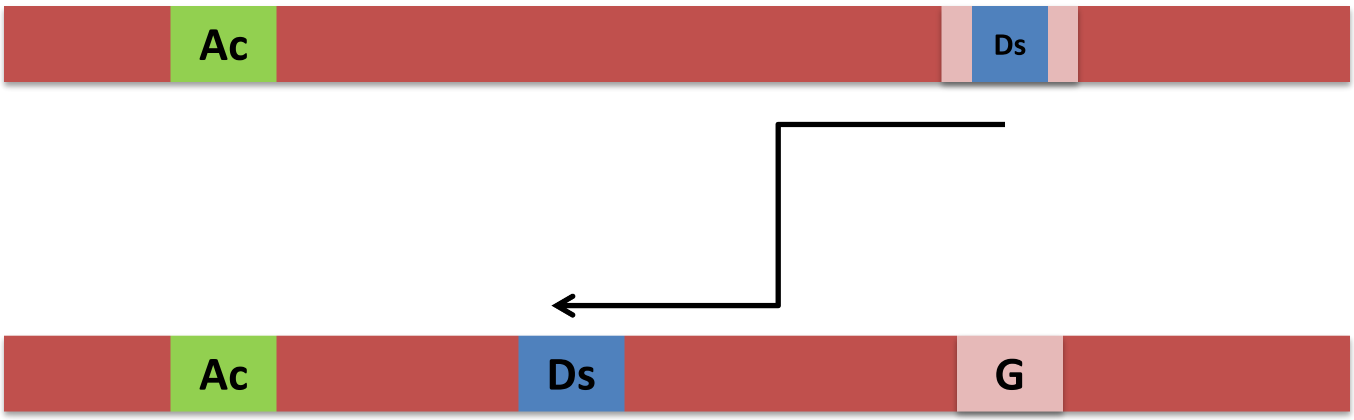


In this case the gene is inactivated, and expression of the mutant phenotype is observed → **Yellow** seed color

http://www.mun.ca/biology/scarr/Ac-Ds_instability.html

Introduction (7/10)

c) in some cells the Ds may again move away from the gene



In these cells the gene is reactivated
and the expression of the wild phenotype is restored →
Seeds with **spots**

http://www.mun.ca/biology/scarr/Ac-Ds_instability.html

Introduction (8/10)

Conclusion-proposal of B. McClintock (1930-1950)

There are two types of transposable elements :

Autonomous elements : contain the information required for their movement and the movement of other elements of the family (e.g. Ac).

Non-autonomous elements : can not move except they coexist with autonomous elements of the same family (e.g. Ds).

Introduction (9/10)

More systems of mobile genetic elements were found in corn:

- Dotted (Dt) from *Marcus Rhoades*
- Enhancer/Inhibitor (En/In) from *Mc Clintock- Peter Peterson*

However the question arises

Are there transposable elements only in corn?

Introduction (10/10)

•1960΄s: Isolation of transposable elements from *Escerichia coli*

•1970's: Isolation of transposable elements from *Drosophila*, the yeast and other organisms

The presence of transposable elements is **universal**

They are an important component of all genomes

1983: Barbara Mc Clintock was honored with the Nobel Prize

Transposable elements in prokaryotes (1/15)

There are two types transposable elements in prokaryotes :

- Insertion Sequences-IS
- Transposons-Tn

Transposable elements in prokaryotes (2/15)

Bacterial Insertion sequences

- The simplest transposable elements in prokaryotes
- They contain only the genes necessary for their movement
- They are widespread, both in bacterial chromosomes and plasmids
- Size: 700-5000 bp

Transposable elements in prokaryotes (3/15)

Bacterial Insertion sequences

The insertion sequence IS*1*. The size of this transposable element is 768 bp and carry inverted repeats (IR) in its ends. The length of each is 23 bp.

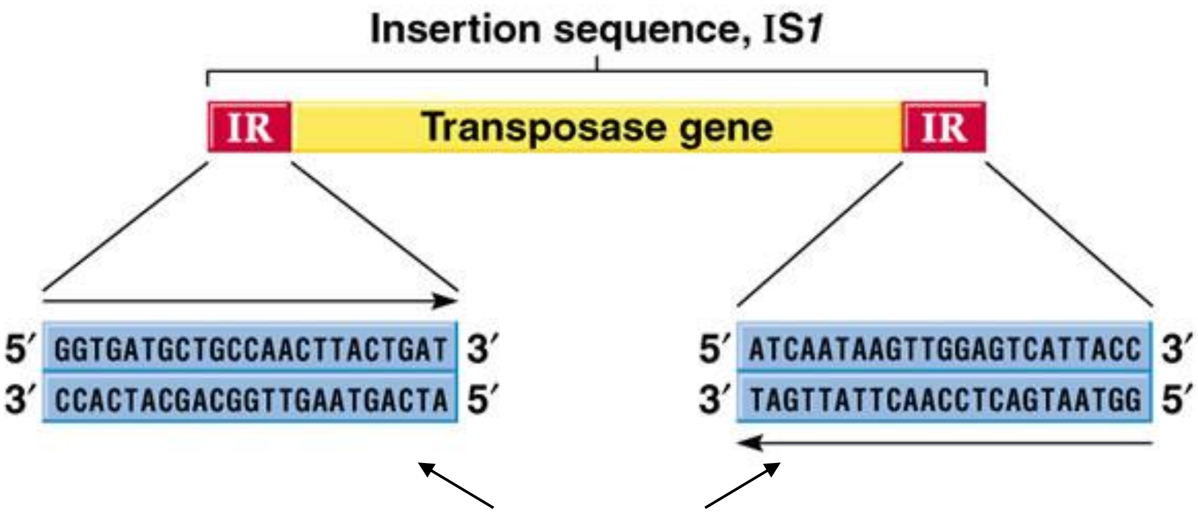


Figure 1: Transposase recognition sequences

Transposable elements in prokaryotes (4/15)

Insertion sequence	Length (bp)	Inverted repeat (length in bp)	Target area (length in bp)	Number of copies in the chromosome of *E. coli*
IS1	768	23	9 ή 8	6-10
IS2	1327	41	5	4-13
IS3	1400	38	3-4	5-6
IS4	1428	18	11 ή 12	1-2
IS5	1195	16	4	10-11

Transposable elements in prokaryotes (5/15)

Transposons-Tn

They contain, in addition to the genes necessary for their movement, other genes as well (e.g. antibiotic resistance genes).

There are two types of prokaryotic transposons:

- **Composite transposons**
- **Simple transposons**

Each transposon may comprise different antibiotic resistance gene.

http://www.eplantscience.com/index/genetics/plasmids_is_elements_transposons_and_retroelements/transposons_in_prokaryotes.php

Transposable elements in prokaryotes (6/15)

Transposons-Tn

➢ The bacterial transposons (Tn elements) are larger than the IS elements which contain genes coding for proteins, including the transposase.

➢ Due to the inverted repeats, transposons can form loops «**lollipop**», which are distinguishable under the electron microscope, after denaturation and renaturation of the molecules.

http://bio3400.nicerweb.com/Locked/media/ch15/bacterial_transposon.html

http://bio3400.nicerweb.com/Locked/media/ch15/bacterial_transposon2.html

Transposable elements in prokaryotes (7/15)

Composite transposons

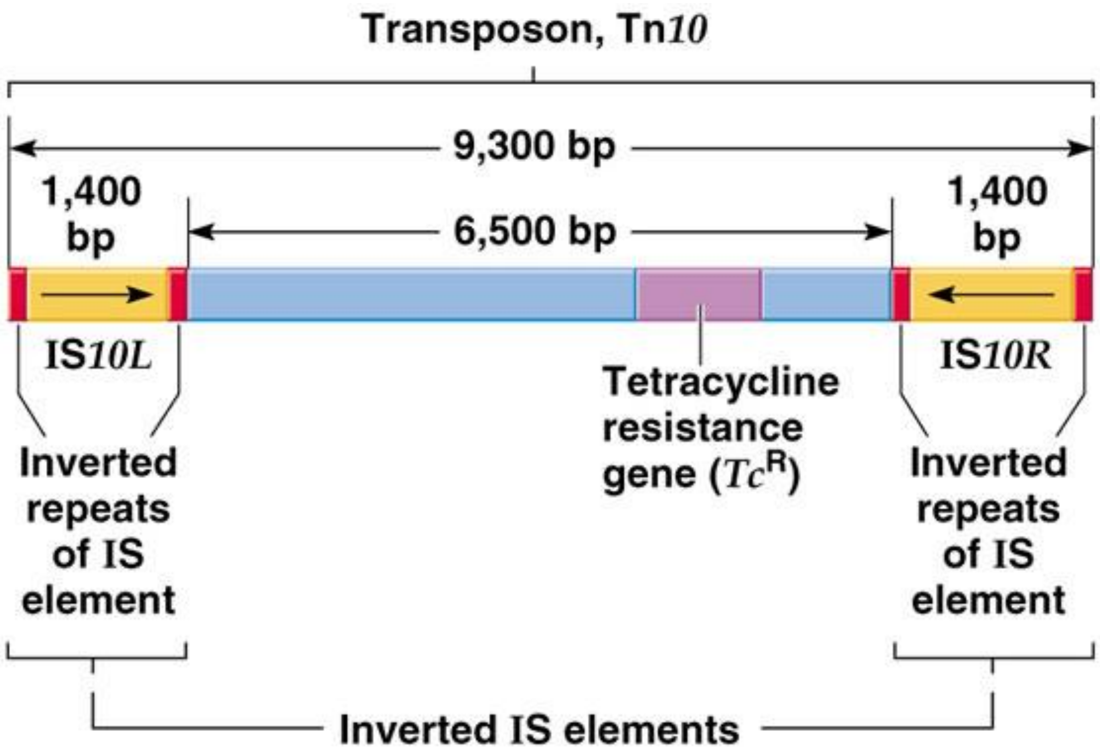
✓ They usually contain antibiotic resistance genes framed by IS insertion sequences in parallel or antiparallel arrangement.

✓ The IS provides the transposase (the enzyme that moves the transposable element) and the recognition sequences for moving.

Transposable elements in prokaryotes (8/15)

Composite transposons

Figure 2: The structure of a bacterial transposon

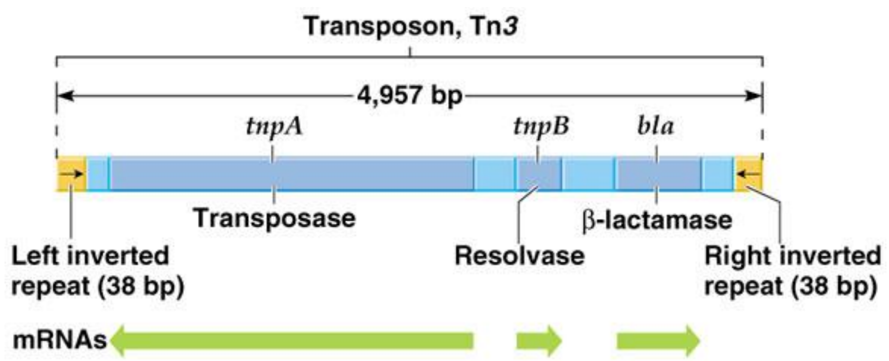


Transposable elements in prokaryotes (9/15)

Simple transposons

- ✓ They also contain genes (e.g. for resistance to antibiotics)
- ✓ Framed by short (<50bp) inverted repeats (IR) and not by IS insertion sequences
- ✓They contain the transposase gene and the resolvase (an enzyme involved in the process of recombination during moving of transposons)

Figure 3: The structure of a bacterial transposon



Transposable elements in prokaryotes (10/15)

Plasmids of multiple resistance

✓ They result from the movement-introduction of multiple transposons in them

✓ Each transposon carries its own antibiotic resistance gene

✓ Therefore, the plasmid can confer resistance to various antibiotics

Transposable elements in prokaryotes (11/15)

Moving mechanisms

- **Conservative:** cut from the initial position and insertion to the new one

- **Replicative:** Replication and insertion to a new position while the original copy remains in the starting position

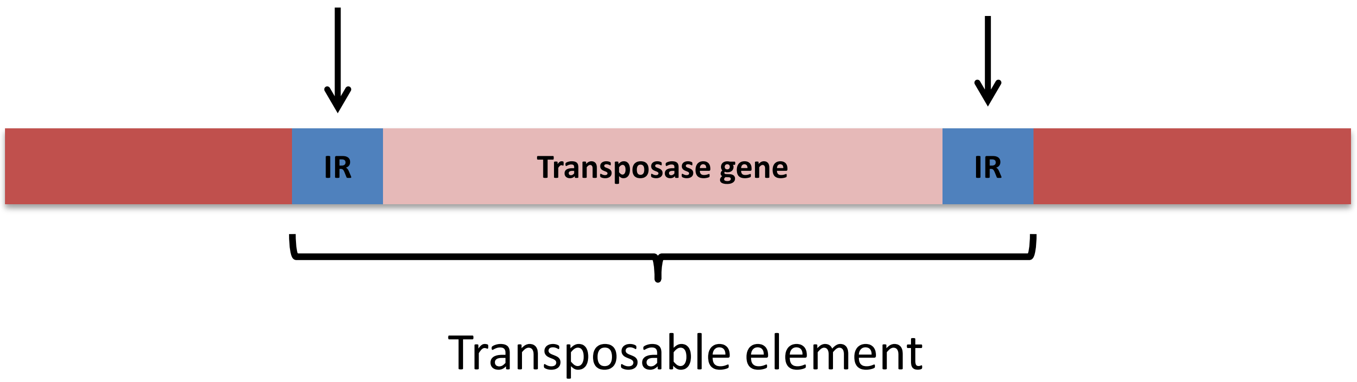
In any case

Replication of target sequence in the insertion site occurs

Transposable elements in prokaryotes (12/15)

Moving mechanisms

The transposase recognizes the inverted repeats and cuts the transposable element



Transposable elements in prokaryotes (13/15)

Conservative moving mechanism (Cut and paste)

- The transposase creates a double stranded cutting point on the donor DNA at the ends of the transposon and stepwise cutting points in the receiver DNA
- Each end of the DNA donor is then joined to a protruding end of the DNA receiver
- The DNA polymerase fills the protruding sequences

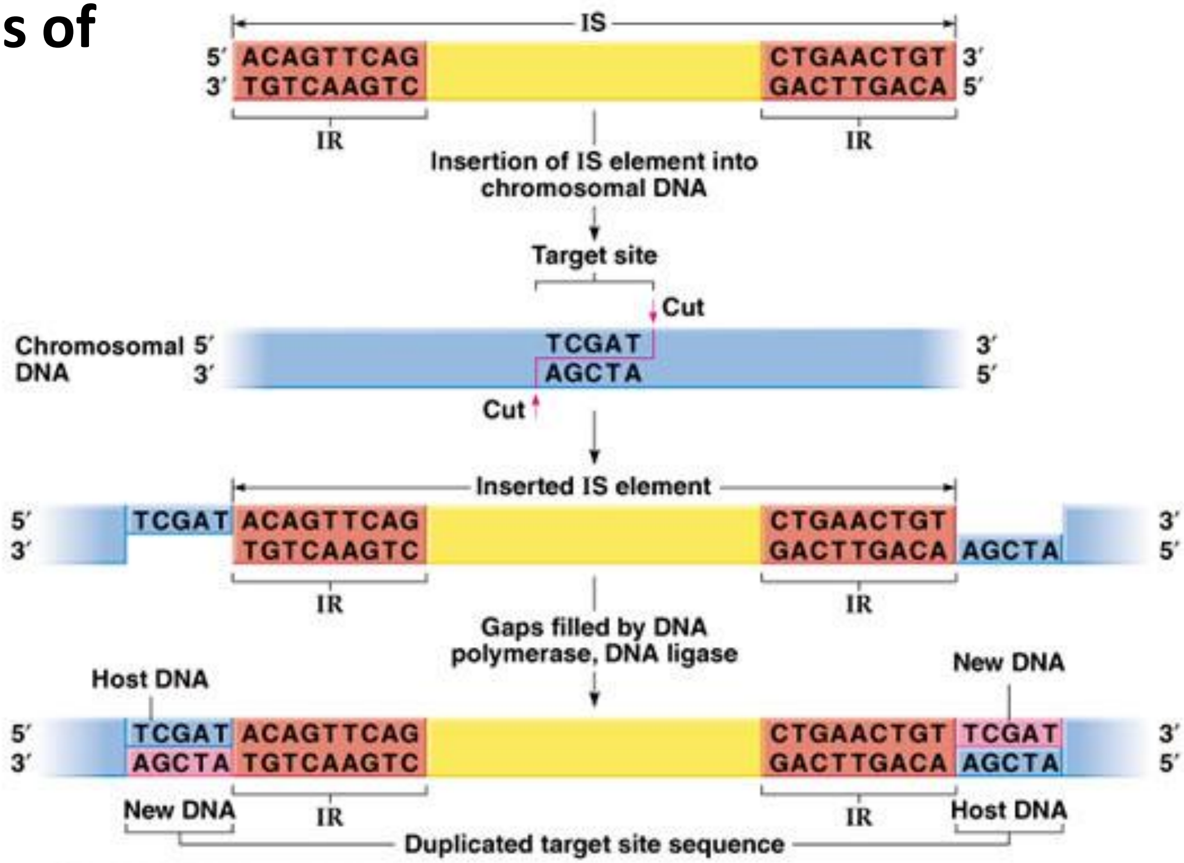
http://www.sci.sdsu.edu/~smaloy/MicrobialGenetics/topics/transposons/non-repl-tpn.html

Transposable elements in prokaryotes (14/15)

Figure 4: Insertion process of an IS element in chromosomal DNA

Conservative moving mechanism

Cut and paste



Transposable elements in prokaryotes (15/15)

Replicative moving mechanism

Copy and paste

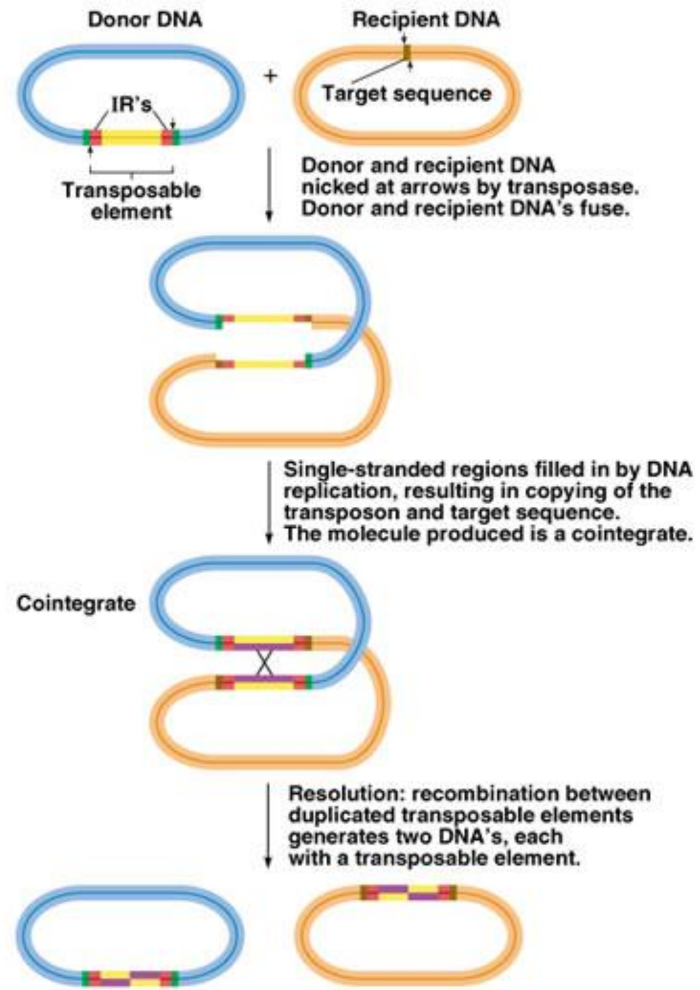


Figure 5: Model of cointegration for translocation of a transposable element by copying

Transposable elements in eukaryotes (1/13)

- **DNA Transposons**
- **Retrotransposons**
  - **LTR**
  - **Non LTR**

Transposable elements in eukaryotes (2/13)

DNA Transposons

Mobile genetic elements that move with mechanisms

similar to bacterial transposable elements

Transposable elements in eukaryotes (3/13)

DNA Transposons in plants

Ac-Ds transposable elements:

- Ac autonomous element
  - Length: 4,563bp
  - Includes inverted repeats with length 11 bp
  - Target site duplication 8 bp
  - It encodes the transposase
- Ds non-autonomous element
  - The size varies
  - Includes the same inverted repeats with Ac
  - Incomplete and / or rearranged forms of Ac
  - It does not encode the transposase

Transposable elements in eukaryotes (4/13)

DNA Transposons in *Drosophila*

P- Transposable elements

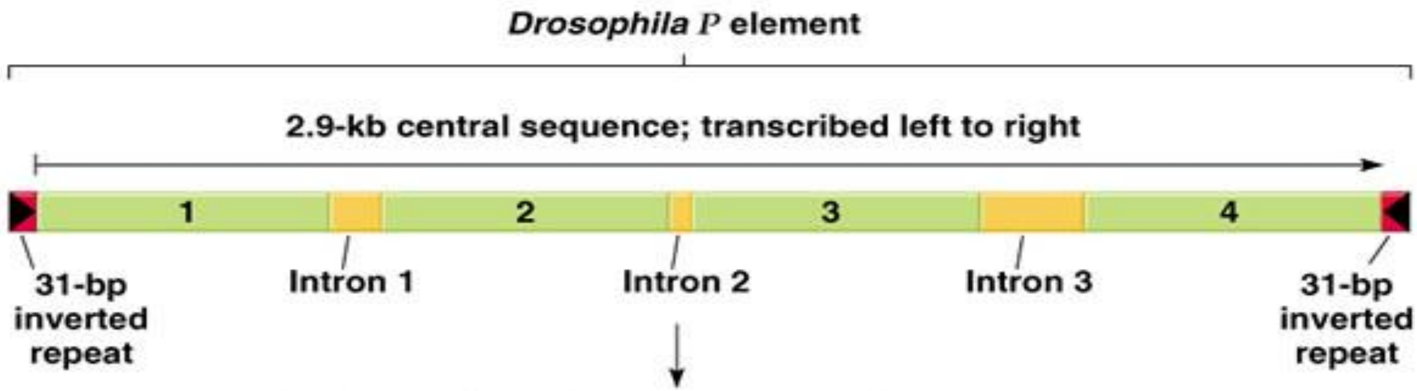
- Complete elements
  - Length: 2.900 bp
  - Includes inverted repeats with length 31 bp
  - Target site duplication 8 bp
  - They encode the transposase
  - Moving control with repressors encoded by the element itself
- “defective“ elements
  - The size varies
  - Include the same inverted repeats with the complete elements
  - Incomplete and / or rearranged forms of P
  - It does not encode the transposase

Moving only in the germ cells

Transposable elements in eukaryotes (5/13)

DNA Transposons in *Drosophila*

Figure 6: Structure of the autonomous P transposable element found in *Drosophila melanogaster*



Following transcription and polyadenylation the coding sequences 1 to 4, give various polypeptides through alternative splicing, including the transposase and repressor.

Transposable elements in eukaryotes (6/13)

DNA Transposons in *Drosophila*

P transposable elements – hybrid dysgenesis

- The P elements were first discovered because of a phenomenon that was observed in controlled crossings, known as **hybrid dysgenesis**
- hybrid dysgenesis: high mutation rate, high frequency of chromosomal rearrangements and non-separation - Not stable mutations
- This phenomenon appeared in offspring coming from crossing females of laboratory strains and wild males, but not in the reverse
-

http://www.bio.miami.edu/dana/250/250SS13_17.html (Figure 15.17)

Transposable elements in eukaryotes (7/13)

DNA Transposons in *Drosophila*

P- transposable elements– hybrid dysgenesis

Laboratory strains M: They do not contain P transposable elements
female gametes -M cytotype:
they do not contain an inhibitor of translocation

Wild populations P: They contain P transposable elements
female gametes - P cytotype:
they contain an inhibitor of translocation

Female M X Male P
↓
Translocation of P elements
↓
Dysgenetic hybrids

Female P X Male M
↓
No translocation of P elements
↓
Normal hybrids

http://www.bio.miami.edu/dana/250/250SS13_17.html (Figure 15.18)

Transposable elements in eukaryotes (8/13)

Retrotransposons

Mobile genetic elements that their translocation occurs through an RNA intermediate stage and reverse transcription

Transposable elements in eukaryotes (9/13)

LTR Retrotransposons

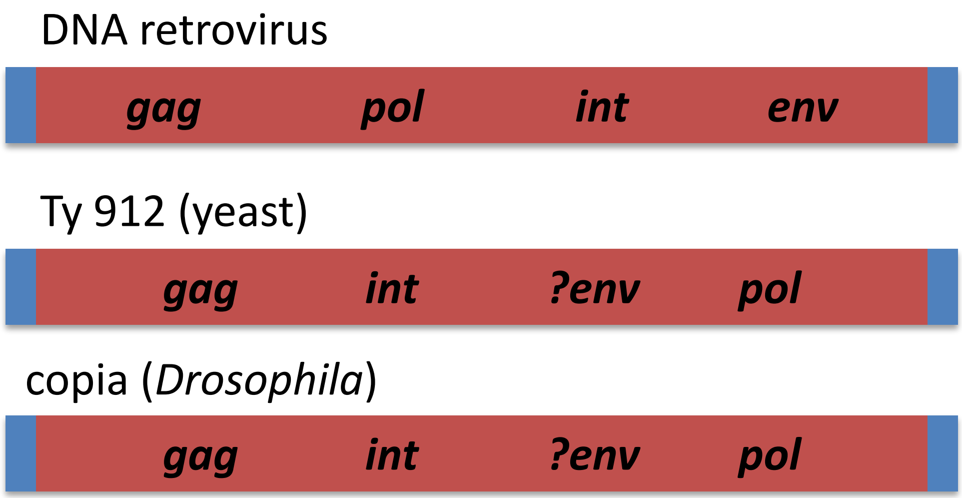
Similarities in the structure and sequence with retroviruses

gag: RNA maturation

Pol: reverse transcriptase

Int: incorporation

Env: envelope



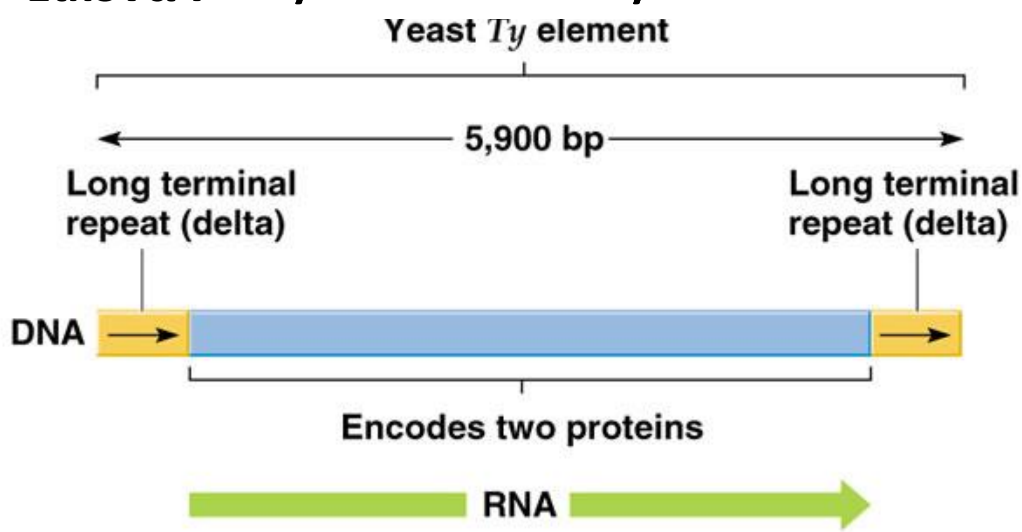
LTR specific for each transposon

Transposable elements in eukaryotes (10/13)

Retrotransposons

Yeast – Ty elements: - Length: 5900 bp
- LTRs: 334 bp
- 35 copies in the genome are identified

Εικόνα 7 Ty element of the yeast



Transposable elements in eukaryotes (11/13)

Retrotransposons –Ty elements in the yeast

Translocation of Ty elements through an intermediate RNA stage

1. The Ty elements present in the genome, are transcribed (by the enzymes of the host cell) into mRNA.
2. The mRNA is then reverse transcribed by reverse transcriptase, encoded by the Ty element (by the *pol* gene).
3. The single-stranded DNA is converted into double-stranded DNA.
4. The new DNA molecule is incorporated into a new location. Production of a new element.

http://www.bio.miami.edu/dana/250/250SS13_17.html

Transposable elements in eukaryotes (12/13)

Retrotransposons

Drosophila - copia elements:

⦿ Length: 5000 bp

⦿ LTRs: 267 bp

⦿ ITRs: 17 bp

⦿ There are 10-100 copies in the genome

☞ There are 7 families of copia transposable elements (copia-like elements)

http://bioinformatica.uab.cat/base/base3.asp?sitio=ensayosevolucion&anar=dnaegoista&item=

Transposable elements in eukaryotes (13/13)

Transposable elements in human

Almost half of the human genome consists of transposable elements

- LINES (Long interspersed elements): retrotransposons, they have no terminal repeats, 20% of genomic DNA
  Autonomous >5000 bp, reverse transcriptase
  Non-autonomous incomplete
  L1: 6.5 kb, 50,000-100,000 copies, ~5% of the total genome

- SINES (short interspersed elements): non-autonomous, depend on enzymes encoded by LINEs
  Alu family: approximately 200 bp, straight repeats 7-20 bp,> 1,000,000 copies, 10% of the total genome

http://genome.wellcome.ac.uk/doc_WTD020733.html

Biological significance of transposable elements (1/9)

What is the biological significance of transposable elements?

✓ Is it “selfish DNA”?

✓ There is no example of a component that plays a role in normal development

✓They are a source of genetic change and diversity

Significant participation in the evolution of genomes

Biological significance of transposable elements (2/9)

The transposable elements are a common cause of mutations in many organisms

- Entry in coding region → Absence of product → Production of alternative products
- Entry in regulatory region → Increase or decrease of transcription

E.g. In *Drosophila* the entry of P, copia and other transposable elements in regulatory, intronic and exonic regions of the gene of the eyes red pigment, lead to the production of a variety of phenotypes with different shades and intensities in eye color.

Biological significance of transposable elements (3/9)

Transposable elements - source of mutations

In human, at least 39 cases of *de novo* transposable elements insertion (L1 και Alus) are known to cause diseases

Examples:

-**Hemophilia A: insertions of** L1 element in the 14th exon of the factor VII gene in two unrelated patients (Kazazian et al. 1988, Nature)

- **Breast cancer**: insertion of the Alu element in the BRCA2 gene (Miki et al. Nature Genetics 1996)

Biological significance of transposable elements (4/9)

Transposable elements - source of mutations

The mutations are not always harmful.

They can offer adaptive advantage under certain conditions.

E.g. In Drosophila the insertion of the Doc transposable element in the choline kinase gene, confers resistance to organophosphate insecticides.
Science: Aminetzach et al 2006

http://petrov.stanford.edu/pdfs/39.pdf

Biological significance of transposable elements (5/9)

Transposable elements - Cause of chromosomal rearrangements

- Homologous recombination between transposable elements → deletions, inversions
- Unequal crossing over between transposable elements → deletions, duplications

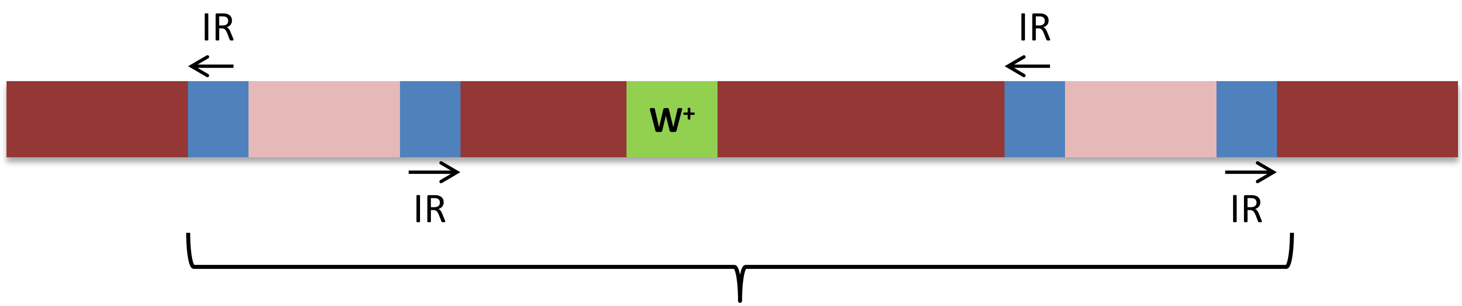
E.g. In Drosophila recombination between two adjacent Doc elements results in inversion of the intermediate part containing the promoters of two adjacent genes (*antennapedia* and *rfd*). This way each gene is being placed under the control of the promoter of the other gene and developmental abnormalities are observed.

Biological significance of transposable elements (6/9)

Transposable elements - Cause of chromosomal rearrangements

- Moving of genomic fragments between two adjacent transposable elements → deletions, insertions

Two transposons can create a large composite transposon



The composite transposon can be moved to a new position

Biological significance of transposable elements (7/9)

Transposable elements constitute a large part of the genome of many organisms

10% in nematodes, 20% in Drosophila, ~50% in human, 70% in corn; >90% in salamanders

How do the species survive by the presence of such mobile DNA in their genome?

Biological significance of transposable elements (8/9)

How do the species survive by the presence of so much mobile DNA in their genome?

- Transposable elements are mainly found in **intronic regions** – negative selection for insertions in regulatory regions
- The largest part consists of inactive transposable sequences – not able to move due to accumulated mutations
- Host mechanisms that control the translocation of transposable elements. e.g. nematodes: strains in which transposable elements are suppressed by RNAi

Biological significance of transposable elements (9/9)

<u>Successful transposable element:</u> one that can be held at a high copy number without “being harmful“ for the host

- ➢Endogenous mechanisms that control the translocation of transposable elements.
  - ❑Self-regulation mechanism

    e.g. repressor of P elements

  - ❑ Entry in "safe locations"

    e.g. Other transposable, heterochromatic regions

  - ❑ Entry in specific areas?

    e.g. Yeasts Ty3: insertion near but not within tRNA genes

    Drosophila R1, R2: insertion only in rRNA genes

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