Mutagenesis

1. Classification of mutation
2. Base Substitution
3. Insertion Deletion
4. Transposons
5. Chromosomal Aberration
6. Repair Mechanisms

Classification of mutation

1. Definition – heritable change in DNA sequence
   - Somatic vs Germline mutation (BRCA-2 example)
2. Classification by phenotypes
   - Morphological
   - Biochemical/Nutritional
   - Behavioral
   - Lethal
   - Conditional (temperature sensitive)
3. Importance of mutation
   - Source of all alleles
   - Raw material of natural selection
   - Source of new genes – duplication and divergence
     - Pseudogenes

Molecular Mutagenesis

Classes	Mechanism: Spontaneous	Mechanism: Induced	fitness
Base Substitution			
Insertion/Deletion			
Transposon			
Chromosome Aberrations			

Affect on: gene, protein, phenotype - fitness

Spontaneous Base Substitution

```
---AGTCTGAGCAGTTC---
---TCAGACTCGTCAAG---
```

Transition vs Transversions

```
---AGTCTGGGCAGTTC---
---TCAGACCCGTCAAG---
```

- Limits to DNA polymerase fidelity
- Tautomeric Shifts – inherent in DNA

Classes	Spontaneous	Induced
Base Substitution		
Insertion/Deletion		
Transposon		
Chromosome Aberrations		

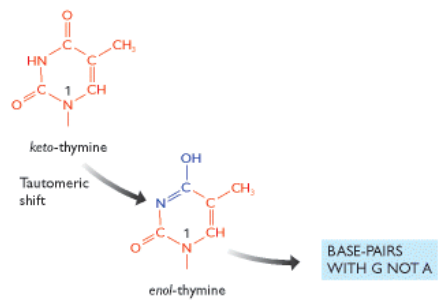
Limits to DNA Pol Fidelity

DNA Polymerases have different error rates

DNA Pol III	1 error every $5X10^9$ base copied
DNA Pol I	1 error every $5X10^7$ base copied
T7 Pol	1 error every $5X10^5$ base copied
Taq Pol	1 error every $1X10^4$ base copied
Reverse Transcriptase	1 error every $2X10^4$ base copied

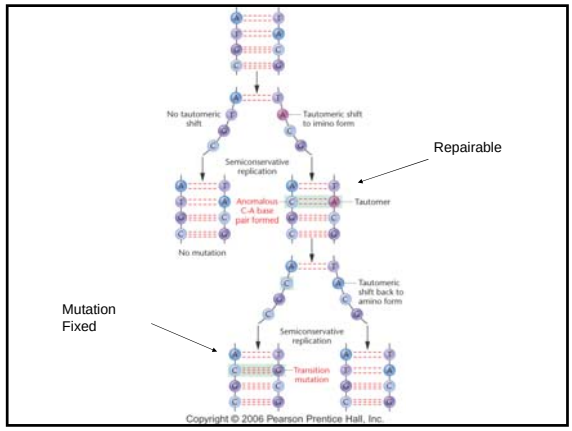
Influenced by active site discrimination and proof reading

Tautomeric Shifts

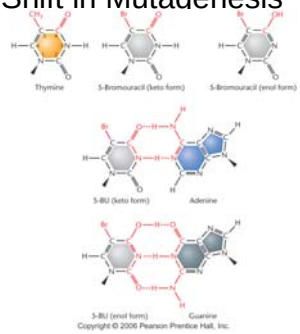


Tautomeric Shifts

Common Tautomer	Rare Tautomer
A-T	A*-C
G-C	G*-T
C-G	C*-G
T-A	T*-G



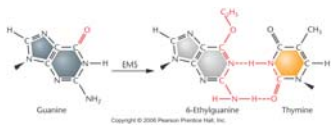
Evidence for Role of Tautomeric Shift in Mutagenesis



Induced Based Substitution

- Chemical Mutagens
  - Base analogues – eg. 5 Bromo Uracil
  - Chemically modify bases – eg. Alkylating Agent
  - Indirect – interfere with repair

Alkylating Agents



Classes	Spontaneous	Induced
Base Substitution		
Insertion/Deletion		
Transposon		
Chromosome Aberrations		

Affect of Base Substitutions

- Where in Gene
  - ORF
    - May affect protein structure
  - Regulatory Regions – e.g. Promoter
    - May affect protein abundance
  - Intragenic Region
    - Often Neutral

Base Substitution in ORF's

1. Missense mutations
2. Silent mutations
3. Nonsense Mutations

		Missense	Silent	Nonsense
DNA	TTA AAT	T**C**A A**G**T	**C**TA **G**AT	T**A**A A**T**T
RNA	UUA	UCA	CUA	UAA
Amino Acid	Leu	Ser	Leu	(Stop)

How might each class of mutation affect protein function or fitness?

Spontaneous Insertion Deletion

- Mechanism – misalignment during replication
- Evidence – insertion/deletion hotspots

Mutational hotspots micro-satellite

GATCAAGCCCCCCCCCCCCCGTAC
CTAGTTCGGGGGGGGGGGGGCATG

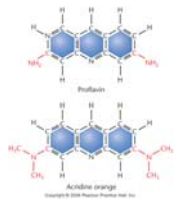
GATCAAGTATATATATATATATGTAC
CTAGTTCATATATATATATATACATG

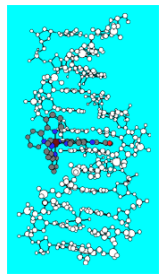
GATCAAGGATGATGATGATGATGTAC
CTACTTCCTACTACTACTACTACTAG

Classes	Spontaneous	Induced
Base Substitution		
Insertion/Deletion		
Transposon		
Chromosome Aberrations		

Induced Insertion/Deletion

- Chemical Mutagens





Intercalation generates kinks that stabilize misalignments during replication

Hotspot Mechanism

Originally 14 C's

GATCAAGCCCCCC
CTAGTTCGGGGGGGGGGGGGCATG

During Replication

GATCAAGCCCCCC
CTAGTTCGGGGGGGGGGGGGCATG

Breathing at end of DNA

Kink

GATCA A G C CCCCC
CTAGTTCGGGGGGGGGGGGGCATG

Misalignment during reannealing

GATCA A G C CCCCCCCCCCCGTAC
CTAGTTCGGGGGGGGGGGGGCATG

Strand elongation with kink

New strand 15C's

GATCAAGCCCCCCCCCCCCCCGTAC

Insertion mutation

Hotspot Mechanism

Originally 14 C's

GATCAAGCCCCCC
CTAGTTCGGGGGGGGGGGGGCATG

During Replication

GATCAAGCCCCCC
CTAGTTCGGGGGGGGGGGGGCATG

Breathing at end of DNA

GATCAAGCCCCCC
CTAGT GGGGGGGGGGGGCATG
T G
C

Misalignment during reannealing

GATCAAGCCCCCCCCCCCCCGTAC
CTAGT GGGGGGGGGGGGCATG
T G
C

Strand elongation with kink

New strand 13C's

GATCAAGCCCCCCCCCCCCCGTAC

Deletion mutation

Other Repeats

GATCAAGTATATATAT
CTAGTTCATATATATATATATACATG

GATCAAGTATATATAT
CTAGTT ATATATATATACATG
C T
AA
T

Misalignment results in gain or loss of two units two nucleotides

Effect of Insertion/Deletion

- In open Reading Frame – Frame Shift
- Consider 3 reading Frames

Ser Arg Leu Val Leu Ile
AGTCGACTGGTACTGATC

Val Asp Trp Tyr (stop)

- Only one open reading frame encodes functional proteins – the other reading frames encode non-functional strings of amino acids – often with early stop codons.

Insertion Mutation
Frameshift Mutation

Original Gene

ATGCCCGTACGACCATTGCAACGTCAUGAGTCAAAAGCGGGG----------
Met Pro Val Arg Pro Leu Gln Arg His Glu Ser Lys Ala Gly ----------

Inserted nucleotide

ATGCCCGTACGCACCATTGCAACGTCAUGAGTCAAAAGCGGGG----------
Met Pro Val Arg **Thr Ile Ala Arg The (stop)**

Trinucleotide Repeats in Humans

- Several human genetic diseases associated with trinucleotide repeats
- Expansion of repeats associated with disease causing alleles
- Results in "genetic anticipation"

TABLE 15.4 SUMMARY OF TRINUCLEOTIDE-REPEAT DISORDERS

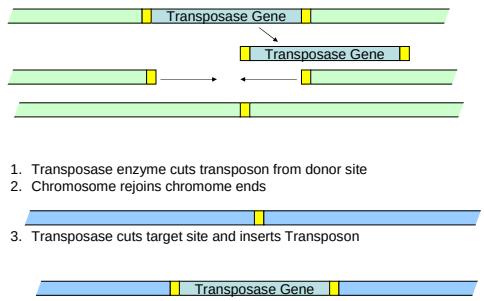
	Trinucleotide Repeat	Number in Normal Individuals	Number in Affected Individuals
Huntington disease	CAG	6–35	36–120
Myotonic dystrophy	CTG	5–37	37–1500
Fragile X syndrome	CGG	6–230	>230
Spinobulbar muscular atrophy	CAG	10–35	35–60

Transposons

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 (*Drosophila*) Ac-Ds (maize) Tn3 and IS1 (*E.coli*) 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 (*Drosophila*) 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 (*Drosophila*) 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.

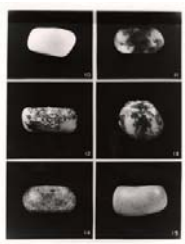
DNA Transposon

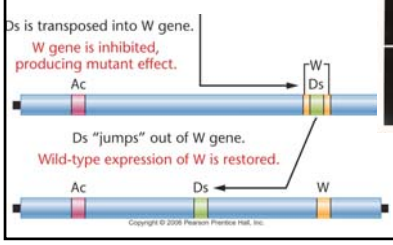


1. Transposase enzyme cuts transposon from donor site
2. Chromosome rejoins chromome ends

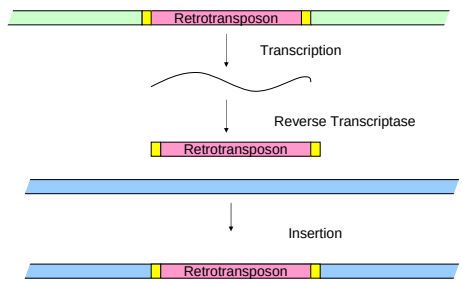
3. Transposase cuts target site and inserts Transposon

Barbara McClintock

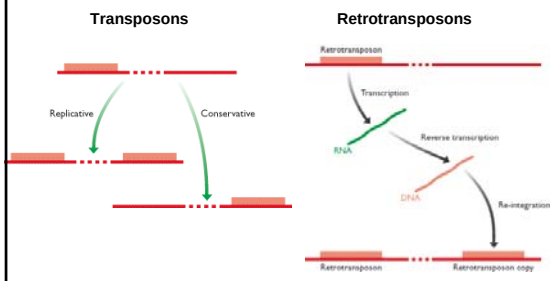




Retrotransposon

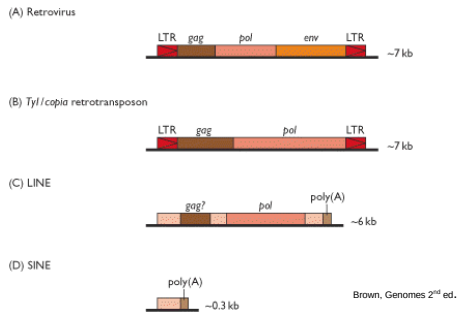


Transposable Elements



Brown, Genomes 2nd ed.

Classes of retrotransposons



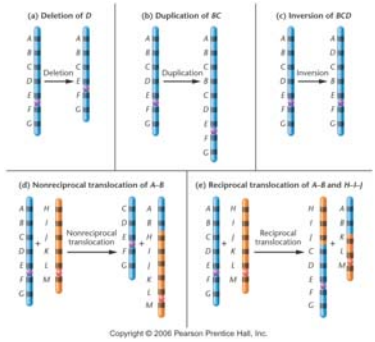
Brown, Genomes 2nd ed.

Types of genome-wide repeats in the human genome

Type of repeat	Approximate number of copies in the human genome
SINEs	1 558 000
LINEs	868 000
LTR elements	443 000
DNA transposons	294 000

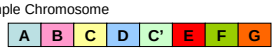
Taken from IHGSC (2001). The numbers are approximate and are likely to be under-estimates (Li *et al.*, 2001).

Chromosomal Aberrations

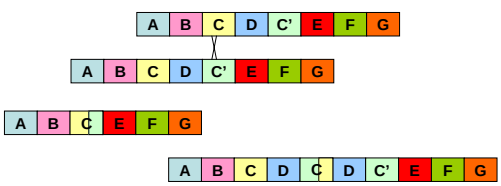


Spontaneous Chromosome Aberrations

Example Chromosome



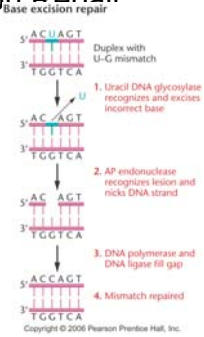
Unequal Crossing Over



Radiation causes chromosome breaks – repair of breaks can result in rearrangment

Repair Mechanisms
Base Excision Repair

1. DNA Glycosylase scans DNA for inappropriate base.
2. Glycosylase removes base.
3. Sugar without base is called AP site (apyrimidinic)
4. AP Endonuclease nicks DNA
5. DNA Polymerase replaces DNA using replacement nuclease activity
6. Ligase joins DNA Together



Nucleotide Excision Repair

- Nuclease recognizes distorted DNA and cleaves damage strand.
- DNA Polymerase replaces DNA, Ligase joins together strands.