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

<table>
<thead>
<tr>
<th>Classes</th>
<th>Spontaneous</th>
<th>Induced</th>
<th>Fitness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Base Substitution</td>
<td></td>
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<td>Insertion/Deletion</td>
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<tr>
<td>Chromosome Aberrations</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Spontaneous Base Substitution

-AGTCTGAGCATTC-
-TCACTCTGTCAGG-

Transition vs Transversions

-AGTCTGAGCATTC-
-TCACTCTGTCAGG-

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

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 5X10^8 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

<table>
<thead>
<tr>
<th>BASE-PAIRS</th>
<th>WITH IS NOT A</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-exo-thymine</td>
<td>Tautomeric shift</td>
</tr>
<tr>
<td>3-exo-thymine</td>
<td></td>
</tr>
</tbody>
</table>
Tautomeric Shifts

<table>
<thead>
<tr>
<th>Common Tautomer</th>
<th>Rare Tautomer</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-T</td>
<td>A*-C</td>
</tr>
<tr>
<td>G-C</td>
<td>G*-T</td>
</tr>
<tr>
<td>C-G</td>
<td>C*-G</td>
</tr>
<tr>
<td>T-A</td>
<td>T*-G</td>
</tr>
</tbody>
</table>

Evidence for Role of Tautomeric Shift in Mutagenesis

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

Induced Based Substitution

- Alkylating Agents

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

<table>
<thead>
<tr>
<th>DNA</th>
<th>Missense</th>
<th>Silent</th>
<th>Nonsense</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAA</td>
<td>AAT</td>
<td>GAT</td>
<td>TAA</td>
</tr>
<tr>
<td>TCA</td>
<td>AGT</td>
<td>CAT</td>
<td>ATT</td>
</tr>
</tbody>
</table>

RNA 

<table>
<thead>
<tr>
<th>RNA</th>
<th>UUA</th>
<th>UCA</th>
<th>CUU</th>
<th>UAA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amino Acid</td>
<td>Leu</td>
<td>Ser</td>
<td>Leu</td>
<td>(Stop)</td>
</tr>
</tbody>
</table>

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

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

<table>
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<tr>
<th>Mutational hotspots</th>
<th>micro-satellite</th>
</tr>
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<tbody>
<tr>
<td>GATCAAGCCCCCCCCCCCGTAC</td>
<td>CTAGTTGGGGGGGGGGGCTG</td>
</tr>
<tr>
<td>GATCAAGTATATATATATATATATAC</td>
<td>CTAGTTCTATATATATATATATATAG</td>
</tr>
<tr>
<td>GATCAAGGTATGATGTATGATGTAC</td>
<td>CTACTTCTACTACTACTACTAG</td>
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Base Substitution

Insertion/Del

Transposon

Chromosomal Aberrations

Classes

Spontaneous

Induced

Mutational hotspots

micro-satellite

GATCAAGCCCCCCCCCCCGTAC
CTAGTTGGGGGGGGGGGCTG
GATCAAGTATATATATATATATATAC
CTAGTTCTATATATATATATATATAG
GATCAAGGTATGATGTATGATGTAC
CTACTTCTACTACTACTACTACTAG

Induced Insertion/Deletion

- Chemical Mutagens

Intercalation generates kinks that stabilize misalignments during replication

Hotspot Mechanism

Originally 14 C’s

During Replication

Breathing at end of DNA

Misalignment during reannealing

Strand elongation with kink

Insertion mutation

Kink

G

GATCAAGCCCC

CTAGTTGGGGGGGGGGGCTG

GATCAAG

CTAGTTGGGGGGGGGGGCTG

CCCCCC

CTAGTTGGGGGGGGGGGCTG

CTAGTTGGGGGGGGGGGCTG

GATCAAGGTATGATGTATGATGTAC
CTACTTCTACTACTACTACTACTAG

New strand 15 C’s

GATCAAGGATATATATATATATATAC
CTAGTTGGGGGGGGGGGCTG

GATCAAGTTATATATATATATATAC
CTAGTTGGGGGGGGGGGCTG

GATCAAGGATGATGATGATGATGTAC
CTACTTCTACTACTACTACTACTAG

Effect of Insertion/Deletion

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

Ser | Arg | Leu | Val | Leu | Ile
AGT GC ACT GTG ACT GT AC
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

ATGCCGTACCACTCATTGCAACGTCAUGAGTCAAAAGCGGGG---------------------

Met Pro Val Arg Pro Leu Gln Arg His Gln Ser Lys Ala Gly

Inserted nucleotide

ATGCCGTACCGACCTTCAACGTCAUGTCAAAGCGGGG---------------------

Met Pro Val Arg Thr Ile Ala Arg The (stop)

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**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>
<thead>
<tr>
<th>Trinucleotide Repeat</th>
<th>Number in Normal Individuals</th>
<th>Number in Affected Individuals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Huntington's disease</td>
<td>6-85</td>
<td>86-129</td>
</tr>
<tr>
<td>Myotonic dystrophy</td>
<td>5-37</td>
<td>51-708</td>
</tr>
<tr>
<td>Fragile X syndrome</td>
<td>20-230</td>
<td>150-230</td>
</tr>
<tr>
<td>Spinocerebellar atrophy</td>
<td>19-26</td>
<td>25-40</td>
</tr>
</tbody>
</table>

**Transposons**

<table>
<thead>
<tr>
<th>Class</th>
<th>Description</th>
<th>Example</th>
<th>Impact of Movement</th>
</tr>
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<td>DNA-only transposons</td>
<td>Intermolecular DNA transposons</td>
<td>Inserted nucleotide</td>
<td>Moves an RNA transcript from one cell to another</td>
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**DNA Transposon**

1. Transposase enzyme cuts transposon from donor site
2. Chromosome reaps chromosome ends
3. Transposase cuts target site and inserts Transposon

**Retrotransposon**

1. DNA transposon transcribes RNA transcript from chromosomal DNA
2. RNA transcript is reverse transcribed by a viral enzyme
3. DNA transposon is inserted into the genome
Transposable Elements

Classes of retrotransposons

Types of genome-wide repeats in the human genome

Chromosomal Aberrations

Spontaneous Chromosome Aberrations

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

Radiation causes chromosome breaks – repair of breaks can result in rearrangement
Nucleotide Excision Repair

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