Lecture 3: Mutations

Recall that the flow of information within a cell involves the transcription of DNA to mRNA and the translation of mRNA to protein. Recall also, that the flow of information between generations involves DNA replication and distribution to two daughter cells. Therefore, we would expect a change in DNA to be replicated and passed on to future generations and to affect protein structure and function if the change occurred in a gene that coded for that protein. These changes are called mutations.

Point Mutations
Point mutations are the most common type of mutation.
A single point mutation, also called a base substitution, occurs when a single nucleotide is replaced with a different nucleotide. A point mutation results in a base pair substitution after replication and possibly a mutant protein after transcription and translation. There are three types of point mutations:

1. Silent Mutation: causes no change in the activity of the protein; is usually the result of a substitution occurring in the third location of the mRNA codon. Because the genetic code is degenerate (most amino acids are coded for by several alternative codons), the resulting new codon may still code for the same amino acid.

2. Missense Mutation: A missense mutation is a nucleotide substitution that changes a codon so that it codes for a different amino acid in the protein. This usually results in a change of the activity of the protein. The change may be harmful or beneficial to the protein.

3. Nonsense Mutation: A nonsense mutation is the same as a missense mutation except the resulting codon codes for a STOP signal. The result is a premature termination of translation. The protein is shorter than usual (or nonexistent) and does not contain all the amino acids that it should. Therefore, this protein is most likely nonfunctional.
Frameshift Mutations

**Frameshift mutations** are caused by the insertion or a deletion of a base pair. An inserted or deleted nucleotide alters the triplet grouping of nucleotides into codons and shifts the reading frame so that all nucleotides downstream from the mutation will be improperly grouped. The result is a protein with extensive missense ending sooner or later in nonsense. Frameshifts can also come about by mutations, which interfere with mRNA splicing. The beginning and end of each intron in a gene are defined by conserved DNA sequences. If a nucleotide in one of the highly conserved positions is mutated then the site will no longer function with predictable consequences for the mature mRNA and the coded protein product. There are many examples of such mutations, for instance, some beta thalassemia mutations in the beta globin gene are caused by splice junction mutations.
Trinucleotide expansion
The commonest inherited cause of mental retardation is a syndrome originally known as Martin-Bell syndrome. Patients are most usually male, have a characteristic elongated face and numerous other abnormalities including greatly enlarged testes. The pattern of inheritance of this disease was, at first, puzzling. It usually behaved as an X linked recessive condition but sometimes manifested itself in females and occasionally nonaffected transmitting males were found. In 1969 it was discovered that if cells from patients were cultured in medium deficient in folic acid their X chromosomes often displayed a secondary constriction near the end of the long arm. The name of the syndrome was changed to the fragile X syndrome. The puzzling genetics remained unclear. Eventually the mutation was tracked down to a trinucleotide expansion in the gene now named FMR1 (Fragile site with Mental Retardation) at the site of the secondary constriction. As in the case of myotonic dystrophy symptomless premutations could occur (and were the cause of the transmitting males). Only when the premutation chromosomes were transmitted through females did expansion to the full mutant allele and phenotype occur. A number of diseases have now been ascribed to trinucleotide expansions. These include Huntington's disease.
Fragile X

Causes mental retardation due to trinucleotide expansion

FMR-1 genes in unaffected people have fewer than 50 CGG repeats.

Unstable “premutation” alleles have between 50 and 200 repeats.

Disease causing alleles have > 200 CGG repeats.

Pedigree shows X- linkage

Nucleotide expansion inactivates FMR-1 and causes chromosome fragility..
Kinds and mechanisms of mutation

(a) Substitution
   Transition: Purine for purine, pyrimidine for pyrimidine
   Transversion: Purine for pyrimidine, pyrimidine for purine

(b) Deletion

(c) Insertion

(d) Inversion
   Site of inversion
   5' 3' 5' 3' 5' 3' 5' 3'
   3' 5' 3' 5' 3' 5' 3' 5'

(e) Reciprocal translocation
   Chromosome 1
   Chromosome breaks
   Translocation
   Chromosome 2

Translocation (example: Philadelphia chromosome!)
**Somatic vs. germinal**
Most of our cells are somatic cells and consequently most mutations are happening in somatic cells. New mutation is only of genetic consequence to the next generation if it occurs in a germ line cell so that it stands a chance of being inherited. That is not to say that somatic mutation is unimportant, since cancer occurs as a direct consequence of somatic mutation and aging too may be caused at least in part by the accumulation of somatic mutations with time.

**Mosaicism**
If a mutation such as a chromosome loss occurs early in development, the descendents of the cell may represent a significant fraction of the individual who, being composed of cells of more than one genotype is a genetic *mosaic*.

**Recessive mutations**
Because most genes code for enzymes, if one gene is inactivated the reduction in the level of activity of the enzyme may not be as much as 50% because the level of transcription of the remaining gene can possibly be up regulated in response to any rise in the concentration of the substrate. Also, the protein itself may be subject to regulation (by phosphorylation for instance) so that its activity can be increased to compensate for any lack of numbers of molecules. In any case, if the enzyme does not control the rate-limiting step in the biochemical pathway a reduction in the amount of product may not matter.

**Dominant mutations**
**Haploinsufficiency.** In this case, the amount of product from one gene is not enough to do a complete job. Perhaps the enzyme produced is responsible for a rate-limiting step in a reaction pathway.

**Dominant negative effect.** The product of the defective gene interferes with the action of the normal allele. This is usually because the protein forms a multimer to be active. One defective component inserted into the multimer can destroy the activity of the whole complex.

**Gain of function** It is possible to imagine that by mutation a gene might gain a new activity, perhaps an enzyme active site might be altered so that it develops a specificity for a new substrate. Many genes have duplicated and subsequently the two duplicates have diverged in their substrate specificities. **Dominance at an organismal level but recessive at a cellular level.** Some of the best examples of this are tumor suppressor genes, e.g., retinoblastoma.
In 1946 Nobel Prize winner Hermann J. Muller (1890–1967) coined the terms amorph, hypomorph, hypermorph, antimorph and neomorph to classify mutations based on their behavior in various genetic situations.

Key: In the following sections, alleles are referred to as + = wildtype, m = mutant, Df = gene deletion, Dp = gene duplication. Phenotypes are compared with: > phenotype is more severe than.

Amorphic describes a mutation that causes complete loss of gene function. Amorph is sometimes used interchangeably with "genetic null". An amorphic mutation might cause complete loss of protein function by disrupting translation ("protein null") and/or preventing transcription ("RNA null"). An amorphic allele elicits the same phenotype when homozygous and when transheterozygous with a chromosomal deletion (deficiency) that disrupts the same gene. This relationship can be represented as follows: m/m = m/Df. An amorphic allele is commonly dominant to its wildtype counterpart. It is possible for an amorph to be dominant if the gene in question is required in two copies to elicit a normal phenotype (i.e. haploinsufficient).

Hypomorphic describes a mutation that causes a partial loss of gene function. A hypermorph is a reduction in gene (protein, RNA) expression, but not a complete loss. The phenotype of a hypomorph is more severe in trans to a deletion allele than when homozygous: m/Df > m/m. Hypomorphs are usually recessive, but occasional alleles are dominant due to haploinsufficiency.

Hypermorphic mutations cause an increase in normal gene function. Hypomorphic alleles are dominant gain of function alleles. A hypermorph can result from an increase in gene dose (a gene duplication), from increased mRNA or protein expression, or constitutive protein activity. The phenotype of a hypermorph is worsened by increasing the wildtype gene dose, and is reduced by lowering wildtype gene dose. m/Dp > m/+ > m/Df.

Antimorphs are dominant mutations that act in opposition to normal gene activity. Antimorphs are also called dominant negative mutations. Increasing wildtype gene function reduces the phenotypic severity of an antimorph, so the phenotype of an antimorph is worse when heterozygous than when in trans to a gene duplication: m/+ > m/Dp. An antimorphic mutation might affect the function of a protein that acts as a dimer so that a dimer consisting of one normal and one mutated protein is no longer functional.

Neomorphic mutations cause a dominant gain of gene function that is different from the normal function. A neomorphic mutation can cause ectopic mRNA or protein expression, or new protein functions from altered protein
structure. Changing wildtype gene dose has no effect on the phenotype of a neomorph.

**Mutations: Larger scale changes**

(a) Unequal crossing over

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<table>
<thead>
<tr>
<th>Homologous chromosomes</th>
<th>Unequal crossing-over</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red-photoreceptor gene</td>
<td>Green-photoreceptor gene</td>
<td></td>
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</tbody>
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One chromosome has one hybrid gene,
Other chromosome has two normal genes and one hybrid

**Homologous Recombination**

- Identical sequences may “align” and may then recombine....

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acggcattagccatgcatgcgccccc

agtgtagcctagcgcg

acggcattagccatgcatgcgccccc

agtgtagcctagcgcg
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RecA protein promotes homologous pairing
“Allelic” versus “non-allelic” homologous recombination.