

OUTLINE OF GENETICS LECTURE #1

A. TERMS

PHENOTYPE: Phenotype refers to the observable properties of an organism, such as morphology, growth rate, ability to grow under different conditions or media. For example, a mutant unable to grow on minimal medium, but able to grow on minimal medium supplemented with arginine is an Arg- mutant. Although we know that the mutation in this mutant has something to do with arginine metabolism/biosynthesis, the phenotype is not indicative of the gene that is actually affected.

GENOTYPE: Genotype refers to the genetic constitution of an organism. If we find out that the mutation that results in the Arg- phenotype in the mutant resides in the *argB* gene, then the genotype of the strain is *argB*.

MUTATIONS: A mutation is a heritable change in genotype that results from alterations in DNA sequence. Mutations in genes may or may not affect the phenotype of an organism.

MUTANT: A mutant is a strain that has suffered a mutation and exhibits a different phenotype from the parental strain.

LOCUS: A locus is a place in the genome of an organism that encodes a particular gene, for example, the *argB* locus.

ALLELE: The actual gene encoded by a locus, and which may vary between individuals and mutant/parental strains. For example, I have two mutants that both contain mutations at the *argB* locus and are unable to grow on media without arginine (they have an identical phenotype). One mutant has a base pair alteration and the second mutant contains a small deletion within the *argB* gene. These two genes are different alleles of the *argB* gene. They are therefore referred to with allele numbers, such as *argB4* and *argB15*. Different alleles may differ in DNA sequence, but may not affect phenotype of the strain. Mutations that result in loss of function are termed "null" alleles. Mutations may also result in partial-loss of function or altered function alleles, such as mutations that confer temperature sensitivity. Whether mutations occur in the same gene (and are therefore alleles) is determined by "allelism tests".

EPIGENETICS: Changes (such as methylation of DNA, or heterochromatin formation or modification of heterochromatin) that can affect expression of a gene and may result in a "mutant" phenotype, but these changes can be reversed (for example, by the loss of methylation). Epigenetic changes may or may not be inherited.

B. MECHANISM OF VARIATION

1. MUTATION

SPONTANEOUS MUTATIONS: These mutations occur through errors in DNA replication and mismatch repair due to tautomerism of bases (imino form of cytosine base pairs with adenine or imino form of guanine binds with thymine). Upon DNA replication, an incorrect base is incorporated into the daughter strand that used the mis-paired DNA strand as a template.

CHEMICAL MUTAGENS

The spontaneous mutation rate can be increased substantially by treatment of cells with mutagens. Examples are:

Base alterations: Base alterations are caused by chemical mutagens such as nitrous acid. Treatment of cells results in oxidative deamination of cytosine, which then resembles uracil, which will base pair with a T. This change will cause a mutation to occur during DNA replication whereby a C-G base pair is converted T-A.

Alkylating agents such as ethyl methane sulfonate which leads to depurination. SOS DNA repair allows replication to proceed past the gap with incorporation of incorrect bases.

Ultraviolet light: Exposure of cells to UV results in the formation of pyrimidine dimers in DNA. Two thymine dimers on the same strand are covalently linked and thus cannot be replicated. These type of events are fixed by error-prone mechanisms (SOS response) and results in the incorporation of incorrect bases.

2. DNA REPAIR

DNA replication is a high fidelity process that results in a very low overall mutation rate due to proof-reading processes. When the wrong base is incorporated or when thymine dimers are formed, the cell activates a repair mechanism called excision repair. Mutants defective in this pathway show an increased sensitivity to mutagens, such as ultraviolet light. A second way a cell has to deal with misincorporation of bases involves a post-replicative process that requires the RecA protein and proteins involved in recombinational repair.

Mutations are often associated with repeated sequences in genes/genomes because of mis-match during DNA replication.

3. MUTATIONAL TYPES

POINT MUTATION: The sequence of DNA has been altered at a single position. Mutations can be transition mutations (T-C or A-G) or transversion mutations (T-A or G-C). Point mutations may result in no change (silent mutation) in amino acid composition (because of third base pair wobble in the codon). Point mutations may result in an amino acid substitution (missense mutation). Missense mutations may or may not result in a change in protein function. Point mutations may also result in a change from an amino acid to a stop codon

(nonsense mutation). These mutations usually result in loss-of-function or altered function of the protein.

FRAMESHIFT MUTATION: A deletion or addition of nucleotides with a gene. These mutations, when they occur in open reading frames (ORFs), result in frameshift mutations from incorrect spacing of codons following the insertion or deletion. The new ORF often terminates at a new stop codon shortly after the frameshift mutation. These mutations have usually result in loss-of function mutants. However, deletions/insertion of multiples of 3 maintain the correct ORF and result in a missing amino acid(s). These types of mutations can often be tolerated.

DELETIONS: Deletions within genes often occur that are much larger than the few nucleotides. Deletions can occur via recombination between repeated elements. These mutations differ from other types of mutations in that they do not revert.

INVERSIONS: A segment of DNA is inverted relative to its original orientation. These types of mutations can occur during a recombination event between repeated elements, and result in DNA sequences that are reversed in order. Reversion of inversions is common.

DUPLICATIONS: Duplications can also occur because of recombination/replication errors and results in repetitions of DNA sequence. Whether a duplication affects a gene depends on where the duplication occurs. These type of mutations may result in a frameshift mutation. Duplications are unstable unless selected for and often revert.

TRANSLOCATIONS: Translocations occur when part of the genome is translocated to a new location. These types of events may or may not cause a phenotypic change, depending upon where the breakpoint/insertion occurs. However, translocations affect progeny types during a cross (i.e. partial duplication progeny and deficiency progeny).

INSERTIONS: Insertions are common in genomes from transposable elements (and genetic elements they may carry) and phage elements.

EXTRACHROMOSOMAL ELEMENTS: Differences in phenotype may occur because of the acquisition or loss of extrachromosomal elements, such as plasmids or phenotypes associated with organelles, such chloroplasts or mitochondria.

4. VARIATION BY RECOMBINATION

Mendel's laws

1. **EQUAL SEGREGATION OF MARKERS:** Two members of a gene pair segregate from each other into gametes (if diploid) or progeny (if haploid), so

that one-half carry one member of the pair and the other half carry the other member of the pair.

2. **INDEPENDENT ASSORTMENT OF MARKERS:** During gamete or progeny formation, the segregation of one gene (allele) is independent of the segregation of a different gene (allele), providing the loci are unlinked.

LINKAGE: Segregation of alleles will deviate from a 1:1 segregation if loci are linked (they do not assort independently). The more closely linked two loci are, the more often allele types (genes) will co-segregate with each other.

BACTERIAL RECOMBINATION: Recombination in bacteria uses molecular machinery similar to eukaryotic species. However, genetic exchange in bacteria does not involve two whole genomes, but usually between a complete genome and an incomplete one (plasmid, phage or part of the bacterial chromosome).

HOMOLOGOUS RECOMBINATION: Recombination occurs at region of homology between chromosomes or other DNA elements (phage, plasmids, etc) through breakage and reunion of DNA molecules.

GENE CONVERSION: Gene conversion results in aberrant segregation ratios. Aberrant ratios occur because of mismatch repair of regions of heteroduplex DNA in the process of crossing over. Mendelian (1:1) ratios are normally observed in crosses because it is only rarely that a heterozygous locus is the precise point of chromosome exchange.

C. TYPES OF MUTANTS

AUXOTROPHS: These mutants require a growth supplement to be able to grow. For example the Arg⁻ mutant above is an auxotrophic mutant.

INABILITY TO UTILIZE A SUBSTRATE FOR GROWTH: These mutants are able to grow on various media, but are unable to utilize various substrates for growth. For example, a mutant deficient in the ability to utilize lactose cannot grow on media with lactose as the sole carbon source. However, these mutants do just fine on minimal media containing glucose as a sole carbon source.

ANTIBIOTIC RESISTANCE: These mutants are resistant to treatment by antibiotics.

DEVELOPMENTAL MUTANTS: Mutations in these genes are generally not essential for growth of the mutant strain under normal laboratory conditions, but affect developmental process, such as sporulation or sexual development.

CONDITIONAL MUTANTS: Many processes are essential for growth. Mutations that knock out such genes result in strains that cannot grow and therefore cannot be analyzed. The isolation of conditional mutants, such as temperature-sensitive

mutants (normal or near normal growth at temperatures optimal for growth, but no growth at temperatures suboptimal for growth, either too cold or hot) has been useful in the genetic dissection of processes such as cell cycle regulation, cell division and cell wall biosynthesis. These mutants are recovered much less frequently than loss-of-function mutants in non-essential genes.

D. GENETIC ANALYSIS

GENETIC MAPPING: Genetic mapping is based on linkage and segregation analysis. The more closely-linked two markers are, the more likely they will segregate with each other (the less likely a recombination event will occur between them). Map distances are based on recombination frequencies between 2 or 3 markers. A map unit is defined as a recombination frequency of 1 percent. A three point testcross is used to determine order of markers.

ALLELISM/COMPLEMENTATION: An allelism test is performed to determine whether mutations in two different strains reside in the same gene (allelic mutations) versus different genes (non-allelic mutations). Allelism may be determined either by crosses, heterokaryons or partial diploid analysis.

DOMINANCE/RECESSIVE: Mutations may be either dominant or recessive, which is determined by a complementation test. Null mutations are generally recessive, that is, they can be complemented by a wild-type copy of the gene. Dominant mutations cannot be complemented by a wild-type copy of a gene.

REVERSION: Reversion occurs when the mutated base pair is converted back to the original base pair and the function of the protein/translation is restored.

SUPPRESSION: There are two types of suppression. One type occurs within the gene itself, for example, a mutation at a different place in *argB* restores original Arg⁺ phenotype (or nearly so). An example would be a second deletion that restores the correct ORF in a frameshift mutant. These mutations are called **intragenic** suppressor mutations. A mutation may also occur in a separate gene that restores the original phenotype of the parental strain. These type of mutations are referred to as **extragenic** suppressor mutations. An example would be a mutation in a tRNA synthetase gene that results in a tRNA being inserted in place of a stop codon, which was originally caused by a point mutation in a gene.

EPISTASIS: An allele at one locus eliminates expression of a phenotype caused by a mutation at another locus. For example, I have isolated a mutant (*pigA*) that secretes a black pigment into the medium and another mutant (*pigB*) that secretes a red pigment into the medium. I construct a *pigA pigB* double mutant and find that only the red pigment is produced. Thus, mutations at the *pigB* locus are "epistatic" to mutations in the *pigA* locus.