Quadrant II- Transcript and Related Materials

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Notes

Missense mutations- involves a single base substitution that changes a codon for one amino acid into a codon for another eg. Codon TCC which codes for serine if changed to TGC will code for lysine. The effects of missense mutations vary and they may range from complete los of protein activity to no change at all depending on the location of the amino acid substitution. Replacement of a non polar amino acid in the interior of the protein by a polar amino acid can affect its three dimensional structure and also its function, so also the replacement of a critical amino acid at the active site of an enzyme often destroys its activity but replacement one polar amino acid by another at the protein surface may show little or no effect. Missense mutations are not often lethal and therefore remain in the gene pool thus playing an important role in evolution.

Nonsense mutations – are called thus because here a sense codon is changed to a stop codon or nonsense codon eg. The codon AAA which codes for lysine is changed to TAA which is a stop codon. Depending on the location of the mutation the phenotype may be more or less severely affected. If the affected protein is shortened by one or two amino acids it can retain normal function but if the mutation occurs close to the beginning or the middle of the protein it can have drastic results.

Silent mutations – change the nucleotide sequence of a codon but do not change the amino acid encoded by that codon. This is possible because of degeneracy of the genetic code by virtue of which there may be multiple codons for one amino acid eg. If the codon ACA which

codes for the amino acid threonine was changed to ACC it would still code for the same amino acid. Thus even though a mutation has occurred it can still be detected only in the DNA or mRNA.

Conditional mutation – is a mutation that is only expressed under certain environmental conditions for example temperature sensitive mutants. For some mutations to be expressed, the individual needs to be placed in a specific environment. This is called the restrictive condition. But if the individual is grown in any other environment (permissive condition), the wild type phenotype is expressed. These are called conditional mutations. Mutations that are only expressed at a specific temperature (temperature sensitive mutants), usually elevated, can be considered to be conditional mutations.

Supressor mutation– is a mutation that counters the phenotypic effect of a previous mutation. It alleviates or reverts the effect of an existing mutation. This restores the wild type phenotype seen prior to the original mutation.

Intragenic Supressor mutation – is when a altered phenotype caused due to an original mutation is changed back to the wild type phenotype due to a second mutation occurring in the same gene. The suppressing mutation may be a true revertant restoring the original DNA sequence or it may be an alteration of a base in the same codon resulting in a less detrimental amino acid or else it may be a change in a different codon causing an amino acid change in another position that restores the function of the protein. Such suppressor mutations are in close proximity to the original mutation and located in the same gene.

Extragenic Supressor mutations – also known as intergenic suppressor mutation is when a phenotypic change due to a mutation located in one gene is compensated by another mutation occurring elsewhere on the genome in another gene.For eg. In case of physiological suppressors a defect in one chemical pathway due to a mutation can be circumvented by another mutation which opens up another pathway to the same product or which enables that the chemical produced is taken up more efficiently from the environment. In case of nonsense suppressors the gene for tyrosine tRNA undergoes a mutational change in its anticodon region which enables it to recognize and bind to a mutant nonsense codon and insert tyrosine thus ensuring completion of translation.

Large deletions – Deletion mutations occur when part of a chromosome or a sequence of DNA is lost during replication. This can be due to errors in chromosomal crossover during meiosis and can lead to serious genetic disorders eg. Turners syndrome, Williams syndrome. Large deletions are mostly fatal.

Site directed mutagenesis – is a molecular biology method that is used to make specific and intentional changes to the DNA sequence of a gene and gene products. It is also called site

specific mutagenesis and is used for studying structure and biological activity of DNA , RNA and protein molecules and in protein engineering. In 1978 Clyde Hutchinson and Michael Smith used oligonucleotides in a primer extension method with DNA polymerase to carry out site directed mutagenesis.

Process - A short DNA primer is synthesized which contains the desired mutation and which is complementary to the template DNA around the mutation site so that it can hybridize with the DNA in the gene of interest. The single stranded primer is then extended using a DNA polymerase which copies the rest of the gene. The copied gene contains the mutated site and is then introduced into the host cell via a vector and cloned. Mutants are then selected by DNA sequencing to verify that they contain the desired mutation. Some of the new modified methods which give better results are as follows:

1. Kunkels method – which makes use of M13mp18 phagemid and *E.coli* which is deficient in dUTPase and uracil deglycosidase which are part of DNA repair mechanism.
2. Cassette mutagenesis – does not use primer extension of DNA polymerase however a fragment of DNA containing the mutation is synthesised and inserted into a plasmid.
3. PCR Site directed mutagenesis – for generation of larger fragments containing the mutation as well as restriction endonuclease recognition sites.