Unit-III : Molecular Biology-II

BSc TY Botany Sneha My Sir

* Genes :-

"The hereditory units which are transmitted from one generation to the other are called as genes". A gene is fundamental biological unit, like the atom which is the fundamental physical unit. Its presence was first proposed by Mendel (1865) and he referred the gene as 'hereditory factor'. The term 'gene' was coined by Johannsen (1909) for Mendelian 'factor'.

* Classical Concept of gene :- (T. H. Morgan)

The classical concept of gene was proposed by T. H. Morgan (1911), during his study on Drosophila.

According to this concept, the genes are arranged in the linear manner on the chromosome. The genes are like beads & chromosome is like the string of beads. Several genes are present on each chromosome & all such genes of the same chromosome are called linked genes. Each gene has a definite position (place) in a particular chromosome. This position is called locus. The mutation can bring about change in locus. Each character is controlled by a pair of genes, one gene comes from father & other from mother.

The gene can be mutated. ie. it can be broken by mutagens, which avoids its expression. During meiosis, the gene can be exchanged with homologous chromosome during crossing over.

According to classical concept of gene, the part of chromosome as a whole (ie. DNA and protein) is responsible for expression of a particular character & considered as a gene.

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* Fine Structure of gene :- (Seymour Benzer)

The fine structure of gene was proposed by Seymour Benzer (1955).

According to this concept, the gene may be cistron or muton or recon. Benzer studied virus T4 - bacteriophage & bacterium E. coli.

(1) Recon :- It is the unit of recombination. It is the smallest unit of DNA, made up of one or two nucleotides.

As per Benzer's demonstration, ~~crossin~~ in T4 bacteriophage, crossing over takes place within the gene.

(2) Muton :- It is the unit of mutation. It is the smallest unit of DNA, which is capable of undergoing mutation. It is made up of one or many nucleotides within the DNA molecule. In many cases, gene mutation is a change in single nucleotide of DNA.

(3) Cistron :- It is a unit of function. It is the largest unit of DNA. It represents a segment of DNA molecule & consists of a linear sequence of nucleotides, which controls some cellular functions. In E. coli, cistron may contain about 1500 base pairs. The cistron starts with initiation codon & ends with termination codon. Each cistron is responsible for coding one messenger RNA molecule, which in turn serves for the formation of a polypeptide chain. It has been found that, hundreds of recons & mutons exist within each cistron. Therefore, cistrons occupy much greater chromosomal length than mutons or recons.

As a conclusion, Benzer's study has clearly shown that, not the whole chromosome, but only DNA fragments acts as genes. The fragment of DNA from chromosome acts as recon, muton or cistron.

* Regulation of gene expression in Prokaryotes :-

(Lac operon model)

Operon is a set of closely linked genes regulating a metabolic pathway in prokaryotes. The operon hypothesis was put forward by Jacob and Monod in 1961. For this discovery, they were awarded with Nobel Prize in 1965.

A bacterium contains thousands of genes. When all the genes are functioning at the same time, the cell will be flooded with enzymes and proteins. At any one time, the required enzymes alone are produced. Other enzymes which are not required are not synthesized. The genes for the required enzymes are switched on and other genes are switched off. This on & off mechanism was explained by the operon model.

Lac operon :-

1) Lac operon is a set of genes responsible for the metabolism of lactose in Escherichia coli. The lac operon was discovered by Jacob & Monod (1961).
2) The lac operon consists of 3 structural genes - z, y and a, and 3 control genes - Operator(o) promotor gene (P) & regulator gene (I).
3) The structural genes are responsible for the synthesis of three enzymes. The gene z synthesizes β-galactosidase, gene y synthesizes galactoside permease and gene a synthesizes enzyme thiogalactoside transacetylase.
4) The operator gene is closely related to the first structural gene z. When the operator gene is

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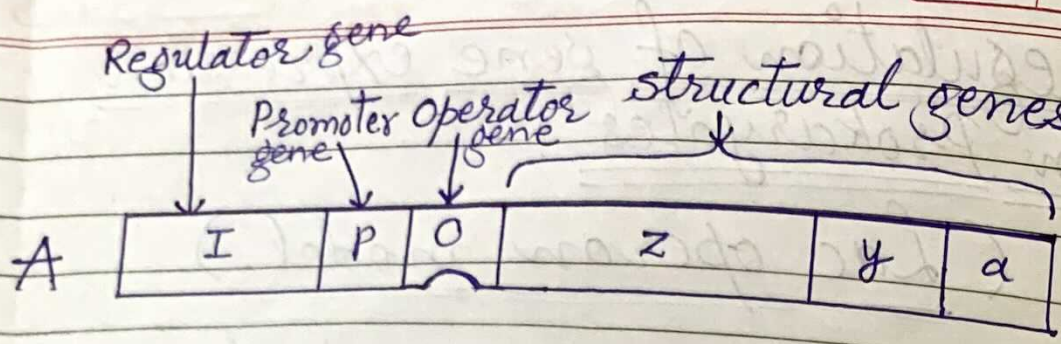

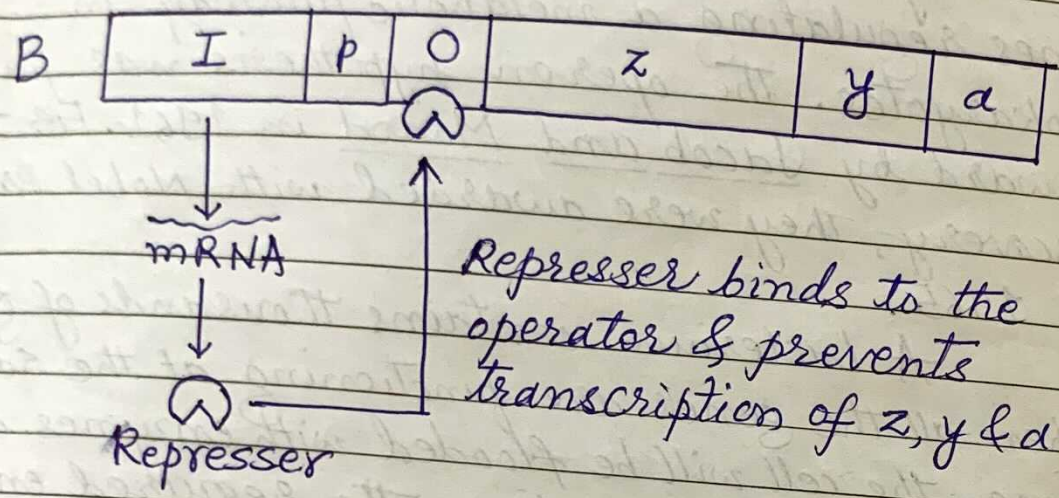

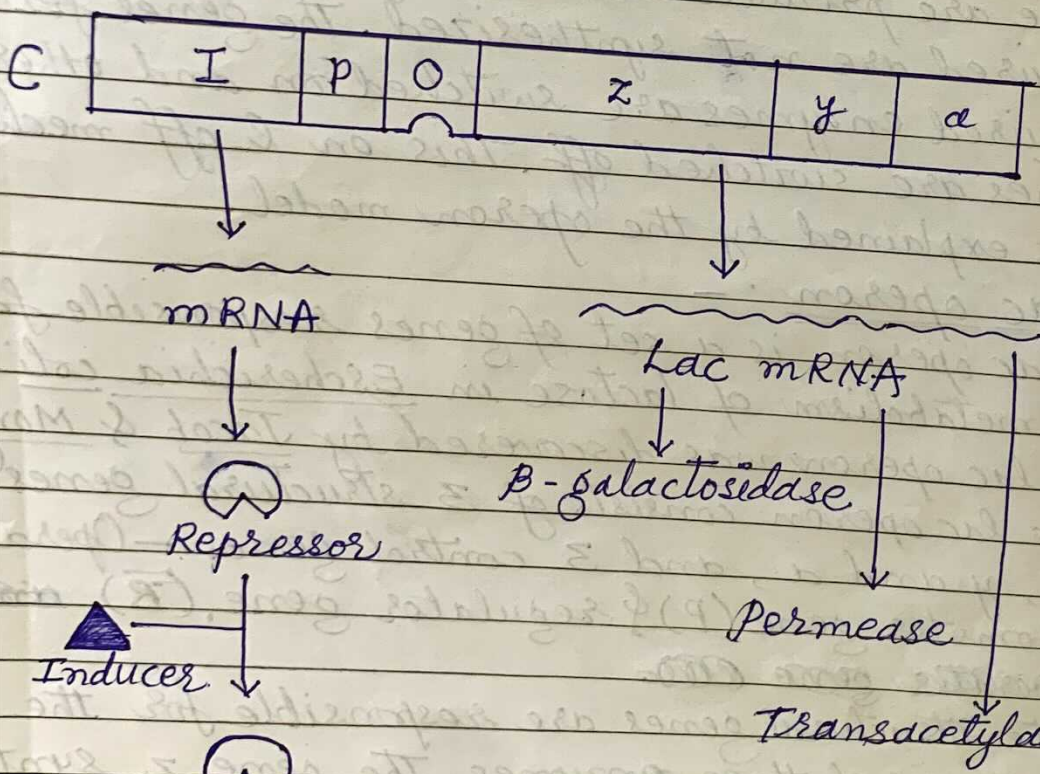

Fig: Lac operon
A - Structure of Lac operon
B - Repressed state of Lac operon
C - Depressed state of Lac operon

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active, the structural genes synthesize enzymes.

5) The activity of the operator gene is decided by a repressor (protein) synthesized by the regulator gene.

6) When the repressor binds to the operator gene, the operator gene is made non-functional. This state of operator gene is called repressed state and the phenomenon is called repression. During repression, the enzymes are not synthesized.

7) When the lactose is introduced into the medium, it diffuses into the cell and binds to the repressor protein to form an inactive inducer-repressor complex.

8) The inactive inducer-repressor complex can not bind to the operator gene & the operator gene is free to do the function. This state of operator gene is called depressed state and the phenomenon is called depression.

9) When the operator gene is depressed, the RNA polymerase binds to the promoter gene. This initiates the transcription of structural genes, which leads to synthesis of 3 enzymes, viz.- β-galactosidase, galactoside permease, and thiogalactoside transacetylase.

10) These three enzymes bring out metabolism of glucose. β-galactosidase splits lactose into glucose & galactose. Galactosidase permease facilitates the entry of lactose into the cell. The function of galactosidase transacetylase is not known.

11) In the lac operon system, the lactose functions as inducer for the synthesis of 3 enzymes. Hence, the lac operon system is called as an inducible system.

12) The lac operon system is a system of negative regulation, where protein repressor prevents gene transcription.

cAMP - Cyclic AMP
CAP - Cyclic AMP receptor protein.
cAMP - CAP complex enhances RNA polymerase attachment to the promoter gene.

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3) cAMP-CAP complex is functioning as a regulatory element of lac operon. It binds to a base sequence in the DNA of the promotor gene in order to start transcription. Thus, the cAMP-CAP complex acts as positive regulator.

4) Thus, lac operon acts independently both positively & negatively. The repressor protein acts as a negative regulator & cAMP-CAP protein acts as a positive regulator.

* Mutagens - Agents which cause mutations are called mutagens.

* Gene mutations & related diseases *

* Gene mutations :-

"A gene mutation is an abrupt inheritable change in the genetic material of an organism".

The term 'mutation' was first used by Hugo de Vries in (1901). He postulated mutation theory based on his observations on an ornamental plant called evening primrose (ie. Oenothera lamarckiana).

Mutations occur frequently in nature & have been reported in many organisms, like Drosophila, mice, rats, rabbits, guinea pigs & man. In Drosophila, mutation causes white & pink eyes, black & yellow body colors, & vestigial wings. In mice & other rodents, mutations are responsible for black, white & brown coats. In man, mutations cause variations in eye color, hair color, skin pigmentation & several somatic malformations. Various genetic diseases of human beings, such as haemophilia, colorblind-ness, phenylketonuria, etc. are other examples of mutation in human beings.

Mutations may be spontaneous or induced.

1) Spontaneous mutations :- The mutations which occur under natural conditions are called as spontaneous mutations.

(The mutagens include cosmic radiations, radioactive compounds, heat, etc.)

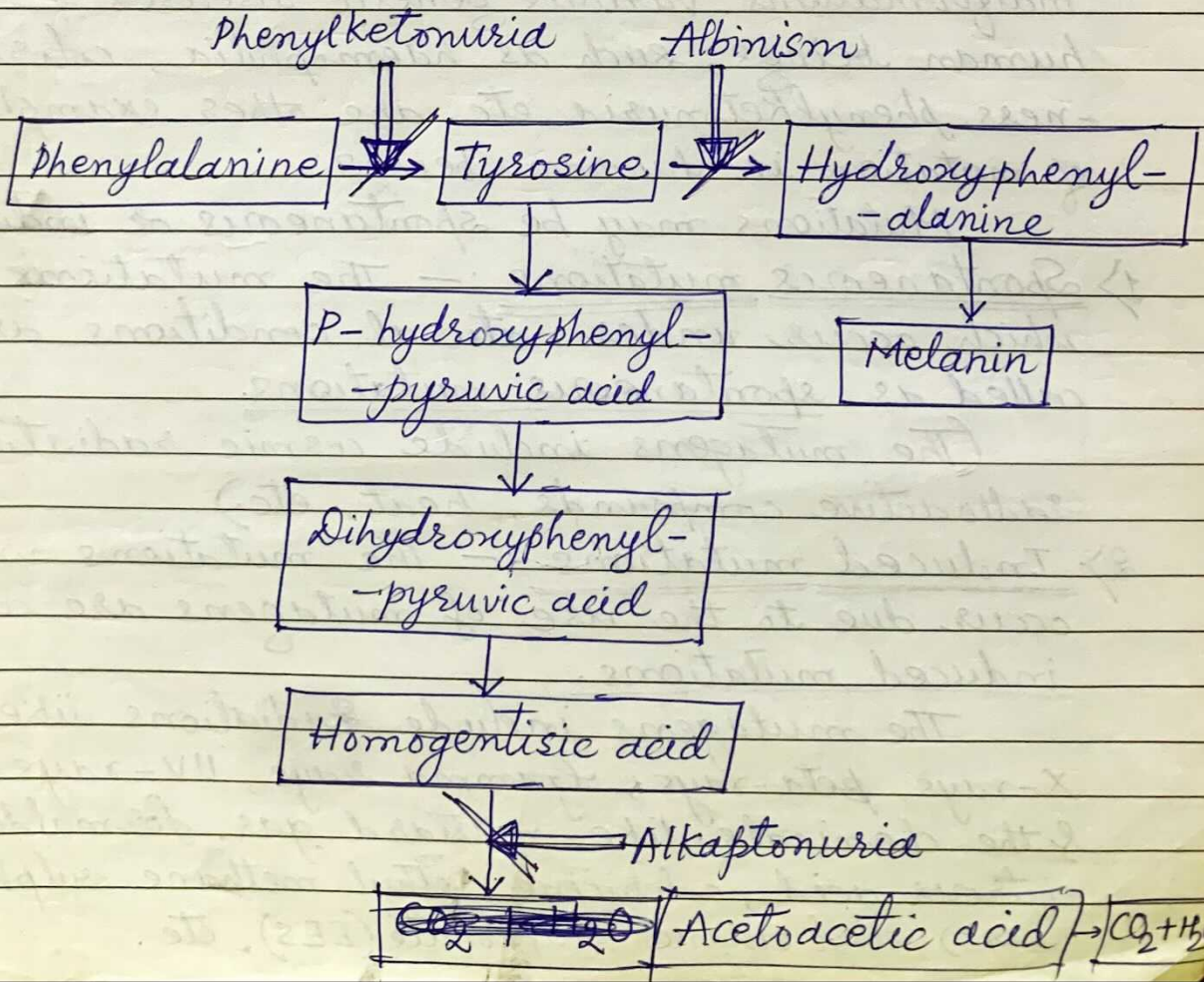
2) Induced mutations :- The mutations which occur due to the use of mutagens are called induced mutations.

The mutagens include Radiations like X-rays, beta-rays, Gamma rays, UV-rays etc. & the chemicals like mustard gas, formaldehyde, nitrous acid, colchicine, ethyl methane sulphonate (EMS), ethyl ethane sulphonate (EES), etc.

* Inborn errors of metabolism :—

In 1908, Archibald E. Garrod published book called 'Inborn Errors of Metabolism'. He proposed that, the genes produce enzymes. The mutant forms of these genes do not produce the enzymes & hence physiological abnormalities are resulted. He studied the metabolism of phenylalanine (an essential amino acid) and showed that, the metabolism of this amino acid involves a chain of enzyme-mediated reactions & a change or absence of an enzyme results in abnormality. There are three important diseases associated with metabolic breakdown of phenylalanine. They are phenylketonuria, alkaptonuria & albinism.

~~Phenyl Ketonuria~~



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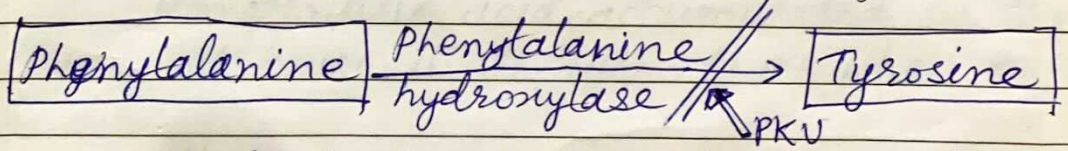
(1) Phenylketonuria (PKU) :—

Phenylketonuria (PKU) is an inborn error of metabolism. It is the genetic condition associated with abnormally high levels of phenylalanine in the body. This leads to increased level of phenylketones in the ~~body~~ blood, which are excreted in the urine, thus the name phenylketonuria.

Metabolic ~~pathways~~ processes occur along multiple steps called pathways. Each step in the pathway is catalyzed by specific enzyme.

Phenylalanine is one of the amino acids, which are important nutrients which we get from our diet. Amino acids are used to make proteins, which is essential for most physiological processes. Amino acids are also precursors which are converted by enzymes into other important compounds for the brain and other organs.

The metabolic pathway dealing with PKU is the conversion of phenylalanine into another amino acid tyrosine. The importance of this pathway is that, it removes excess phenylalanine & enables production of sufficient tyrosine. Tyrosine is important for production of neurotransmitters that function in the brain. The enzyme phenyl--alanine hydroxylase (PAH) is responsible for the conversion of phenylalanine to tyrosine.



Individuals with PKU have genetic defect in the ability to produce the enzyme PAH. Therefore, the phenylalanine they get from diet keeps accumulating rather than being converted to tyrosine. Two major consequences are—

i) Toxic levels of phenylalanine in the body and
ii) impairment of production of neurotransmitte

High levels of phenylalanine, cause brain damage & mental retardation. Early implementat of a low phenylalanine diet prevents mental retardation associated with this condition.

The deficiency of PAH in a person with PKU is a result of mutation in the gene that instructs our cells to make PAH. We have 2 copies of PAH gene, one comes from our mother & one from our father. To have PKU, both these copies must have mutation. Therefore, both parents must have at least one copy of defective gene. People with one normal PAH gene & one defective ~~gene~~ PAH gene are carriers. Because having one normal PAH gene is enough for the body to produce sufficient PAH, carriers do not have PKU.

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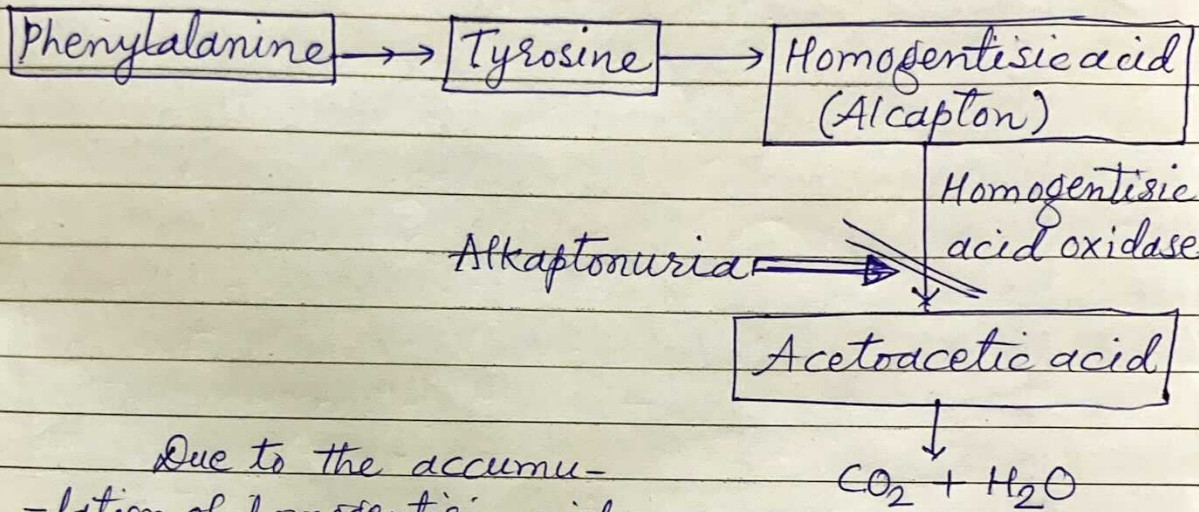
(2) Alkaptonuria (AKU) :-

Alkaptonuria (AKU) is an inborn error of metabolism. It results from a deficieney of enzyme homogentisic acid oxidase. This enzyme deficiency leads to accumulation of homogentisic acid in tissues of the body.



Due to the accumulation of homogentisic acid in cartilage, there is ~~progressive~~ premature progressive degeneration of the cartilage of joints. This results in osteoarthritis of joints throughout the body at an early age. Typical joints affected include the spine, knees, hips & shoulders. Joint pain symptoms include stiffness, pain, swelling & limited motion.

The person affected by alkaptonuria can note persistent, painless, bluish darkening of the outer ears, nose & whites of the eyes.

Homogentisic acid accumulated in the urine will cause it to turn black when exposed to air.

Alkaptonuria is also known as ochronosis or homogentisic acid oxidase deficiency. It is a classic recessive condition. The gene for it is on autosomal chromosome. Parents of the person with alkaptonuria each have one alkaptonuric

gene and one normal gene paired with it. They have no symptoms of alkaptonuria at all. Each of their children has a 25% chance to receive both normal genes, 50% to receive one alkaptonuric & one normal gene and 25% chance to receive both alkaptonuric genes & have alkaptonuria (Ochronosis).

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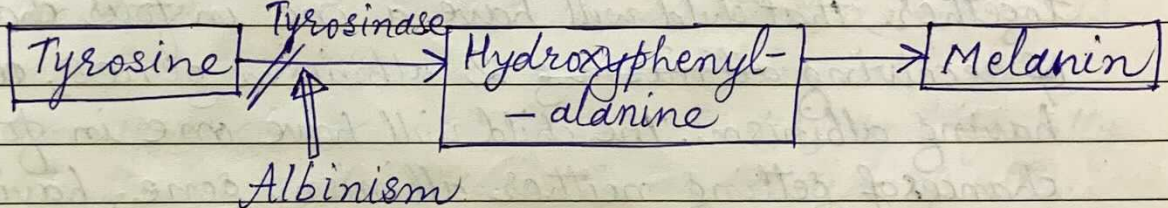
(3) Albinism :- (Latin albus = white.)

Albinism is an inborn error of metabolism. The affected persons lack pigmentation in skin, hairs & eyes. It is an inherited condition arising from the combination of recessive genes passed from both the parents of an individual. A variety of problems with photosensitivity in eyesight & skin usually result from the condition.

The gene which results in albinism prevents the body from making the usual amount of melanine from tyrosine. The enzyme Tyrosinase is not produced & tyrosine is not converted to melanin.



Melanin protects the skin from the ultraviolet light coming from the sun. People with albinism lack this pigment in their skin, & can burn easily from exposure to the sun. Lack of melanin in the eye often results in problems with vision, as the eye will not develop properly without the pigment.

Individuals with full albinism (called albinos) generally have flax-white hairs, blue eyes & pale white skin. The affected persons are paler in complexion than rest of the family.

Growth and development of the children with albinism is normal. Their life-span, intelligence & ability to have children is also normal. The chance of albino children resulting from the marriage of an albino with a non-albino is very low.

~~Albino~~ Oculocutaneous albinism is the most severe form of the condition. Several different genes are involved with pigment production, but it's not clear ~~that~~ exactly what role each of these plays in this condition. In most cases, there is no family history & the children are borne to parents with normal pigmentation. In oculocutaneous albinism, individuals inherit an 'albinism gene' from both the parents. When an individual receives one ~~alb~~ albinism gene & one normal gene, that person will not show outward signs of the condition, but will become carrier of the recessive gene. When two carriers of the recessive gene have a child together, that child will have a one in four chance of receiving ~~albinism~~ 2 albinism genes, and having albinism. The child will have one in four chances of getting neither albinism gene, having normal pigment & not being a carrier. The child has two in four chances of having one normal & one albinism gene, having normal pigment but being a carrier.

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★ Amniocentesis (Amniotic fluid test or AFT)

Amniocentesis is a medical procedure used in prenatal diagnosis of chromosomal abnormalities and fetal infections, in which a small amount of amniotic fluid, which contains fetal tissues, is extracted from amnion or amniotic sac surrounding a developing fetus. & the fetal ~~cells~~ DNA is examined for genetic abnormalities.

Before the start of procedure, a local anaesthetic can be given to the mother in order to relieve the pain felt during the insertion of the needle used to withdraw the fluid. After the local anaesthesia is in effect, a needle is inserted through the mother's abdominal wall, then through the wall of the uterus, and finally into the amniotic sac. With the ~~de~~ aid of ultrasound guidance, a physician punctures the sac in an area away from the foetus and extracts approximately 20 ml of amniotic fluid. After the amniotic fluid is extracted, the fetal cells are separated from the sample. The cells are grown in a culture medium, then fixed and stained. The chromosomes are observed under microscope for abnormalities. The most common abnormalities detected are Down's syndrome (Trisomy-21), Edward's syndrome (Trisomy-18) and Turner's syndrome (Monosomy-X). In regard to the fetus, the puncture heals & the amniotic sac replenishes the liquid over the next 24-48 hours.

Early in pregnancy, the amniocentesis is used for diagnosis of Down's syndrome (Trisomy-21), Trisomy-13, Trisomy-18, rare inherited metabolic disorders, neural tube defects (anencephaly & spina bifida) by α-fetoprotein

levels. It also can be used to detect problems, such as Rh incompatibility, prediction of lung maturity, etc.

Cartilage - Supporting connective tissue of joints.
Osteoarthritis -