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Molecular techniques used in field of Molecular oncology and Radiobiology.

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ABSTRACT – Recombinant DNA technology has revolutionized research in molecular biology of cancer, in fact this technology has invaded every field of advanced biological research thus it's important to know what is all about recombinant technology and how it works, in this research article goal is to provide overview about the same , Birth of molecular biology could be described in one page publication in NATURE in 1953 by Watson and Crick who described structure of DNA this leads the way to breaking the genetic codes and understanding the process of transcriptions of DNA to m-RNA AND m-RNA WITH THE HELP OF r-RNA does help in translation which further help in the synthesis of various kind of proteins.
Keywords – Molecular biology, Recombinant technology, Cancer.

DISCUSSION - Whole concept of molecular biology of cancer diagnosis and its treatment emerged that the sequence of Bases (Purine/Pyrimidine) which codes for particular kind of proteins which in turns ultimately determined specific functions of Cells Start of recombinant DNA technology was first successful cloning experiment of "Stanley cohen" he joined two DNA fragments together (a plasmid containing tetracycline and kanyamysin resistant genes) .

PLASMID – A circular DNA molecule capable of autonomous replications which typically carry one or more genes which having the property of drug resistance.

Further Stanley and Cohen introduce this recombinant molecule into bacteria Escherichia coli and demonstrated that now this bacteria having resistance for both tetracycline and kanamysin antibiotics but above process is not an simple as it appears this requires techniques for DNA culture as well as requires techniques for joining the fragments with help of enzyme DNA Ligase , culturing requires enzyme called as Restriction enzymes as well as using E Coli as host with ability to take up foreign DNA via help of plasmid vectors this was followed by development of methods start pieces of DNA and RNA by size using the methods called as GEL electrophoresis and Blotting methods.

THE STRUCTURE OF DNA- DNA composed of two anti parallel helices like ladder with twisted appearances, DNA contains sugar deoxyribose alternating with phosphates each rings is composed of nucleotides, base pairs held together by hydrogen bond. There are complementary arrangements between bases like Adenine pairs with thiamine and cytosine pairs with guanine. Thus nucleotide sequences of one stand of DNA helix determines the sequences of other stand of DNA .

FIGURE-1, Structure of DNA

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THE STRUCTURE OF RNA – Liked DNA which is located primarily in nucleus of the cell RNA found abundance in the cytoplasm of the cell as well as nucleolus , sugar molecule of RNA is ribose unlike DNA, RNA also having base made up of two purines and two pyrimidines , purines are Adenine , and Guanine , pyridines are cytosine and uracil unlike DNA having in place of uracil , thymidine .

TRANCRIPTION AND TRANSLATION - flow of genetic information from DNA up to protein synthesis are series of steps , first step is DNA coding given to m-RNA only exons of DNA coding taken up from DNA to m-RNA after taking coding information from DNA m-RNA goes to cytoplasm where r-RNA and t-RNA present helps in the process of protein synthesis .the genetic code Triplet m-RNA sequence specify each one of amino acid because there are 4 base thus possibilities for 3 codes calculated 3^4 means 4×4×4= 64 because nearly all proteins made up of amino acids its codons gives signal for protein synthesis .

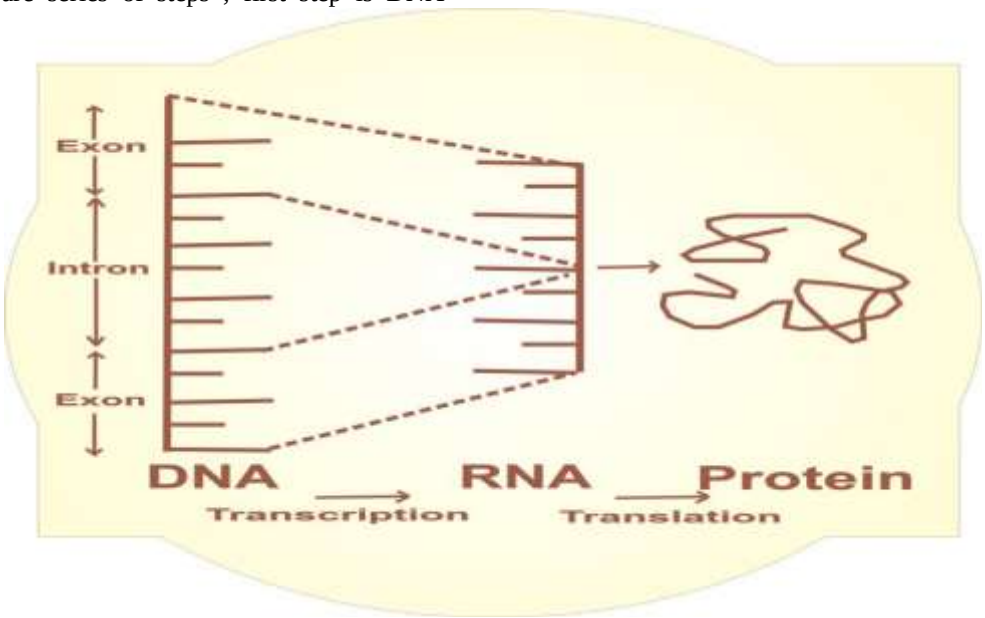

FIGURE-2, CONCEPTS OF PROTEIN SYNTHESIS

AMINOACIDS AND PROTIENS- Majority of proteins are made up of series of 20 amino acids each protein is made up of polypeptide chains and further made of unique sequences of its amino acids, vast majority of proteins are enzyme the act as catalysts inducing chemical changes in other substances but them self's remain unchanged, once polypeptide chains is being formed it appears as strings of amino acids it tends to fold up in 3D form, the shape of proteins is key to its functions.

RESTRICTION AND ENDONUCLEASE -

Restriction enzymes are endonuclease enzymes found in bacteria they have property of recognizing the specific DNS sequence and cleaving the DNA , these endonucleases are the enzymes can be grouped in three categories ,

Type- 1 , type -2, type -3 , the most commonly use enzymes among these are type -2 endonucleases enzyme till now more the 1000 types of type-2 endonclease enzymes have been isolated so far and out of 1000 enzymes 70 are commercially available they have been named according to the given below systems

1-First letter from genus of organism from which enzymes have been isolated 2- second and third letter follow organism's species like for example named "**HIND-3"** Means H is stands for genus heamophilus , IN stands for species INFLUENZA , D stands for strain , 3 stands for THIRD ENDONUCLEASES .

VECTORS – vectors are self replicating molecules of DNA that has ability to carry another foreign DNA into host cells , here objective is to insert human DNA fragments with the help of vectors in to bacteria so that it can be replicated and grow there for study there are five kind of vectors as given below –

1-PLASMIDS , 2- COSMIDS , 3-BACTERIOPHAGE , 4- VIRUSES , 5- YEAST ARTIFICIAL CHOROMOSOMES.

PLASMIDS – this is a simplest bacterial vectors which are having circular DNA molecules that can exist and replicate in the bacteria , independent of

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host chromosome a piece of foreign DNA can be inserted into plasmids after that this plasmid introduced in to bacterium , as bacteria grows and replicates so this plasmid also a gene for resistance to an antibiotic so that if bacteria are suitably grown in to culture medium containing antibiotic only those bacteria's having plasmid can be grown those are inside plasmid others bacteria will die .

Limitation of plasmid vectors are

1- Useful only for small DNA

2- Plasmids transfect bacteria with low efficiency.

BACTERIOPHAGE – Bacteriophage are the virus those infect bacteria's at much higher rate than Plasmids it can be accommodate large DNA molecules to transfect bacteria .

VIRUSES – viruses are highly effective vectors for introducing foreign DNA into mammalian cells , Retroviruses are ideal vectors for this , the genetic material of retroviruses are RNA so that if they infect cells their RNA genomes are converted into DNA form by the help of viral enzymes reverse transcriptase than viral genome replicating with host DNA cells with every cycle.

DNA MEDICATED GENE TRASFER – Gene transfer is now routine tool for studying gene structure and its functions, gene transfer in mammalian cells brings abundant source of transfected cells which contains transferred genes so that study of that very gene can be easily carried out.

But mammalian cells do not take up the foreign DNA naturally, although they to protect themselves from invading DNA, so several methods and tricks must be used to overcome these natural barriers.

A – Microinjections- this is the most direct method but most difficult procedure, here DNA can be injected directly into nucleus of the cell via fine glass niddle .

B—Calcium phosphate precipitation method of DNA transfer – concepts used here knowing the fact that cell take up DNA relatively more efficiently in form of calcium phosphate precipitate , here DNA is mixed with insoluble calcium phosphate as carrier in to cell in petridiscs.

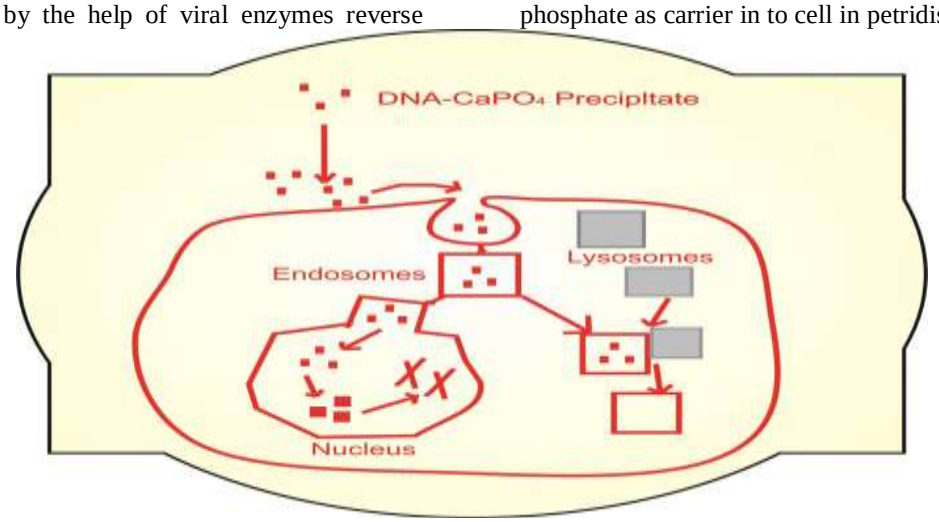

FIGURE-3 , Calcium phosphate precipitation method

C-VIRAL VECTORS—this the ultimate method of DNA transfer by the help of Retroviruses (Bacterioophage) Bactriophage infect bacteria to get DNA in to bacteria , since genetic material of retroviruses are RNA so when retrovirus infect mammalian cells their RNA genome converted into DNA by the help of enzyme reverse transcriptase this viral DNA effectively taken up in to host genome and starts replicating along with host DNA at every cell cycle , if a foreign gene incorporated in to retroviruses it permanently maintain into infected mammalian cells . Oncogenes causing cancer and their counterpart tumor suppresser gene can be studied by incorporating these genes into retroviruses.

POLYMERASE CHAIN REACTION -PCR – In PCR technique we use enzymatic amplification of DNA fragment, the principal is based by the help of DNA polymerase enzyme several copies can be made out of one DNA fragment, so the PCR technique is basically the method in which amplification of DNA fragments is being done, the number of DNA molecules get double in each cell cycle which take around 7 minutes, so that in matter of hours millions of copies of DNA fragment can be made and study as well.

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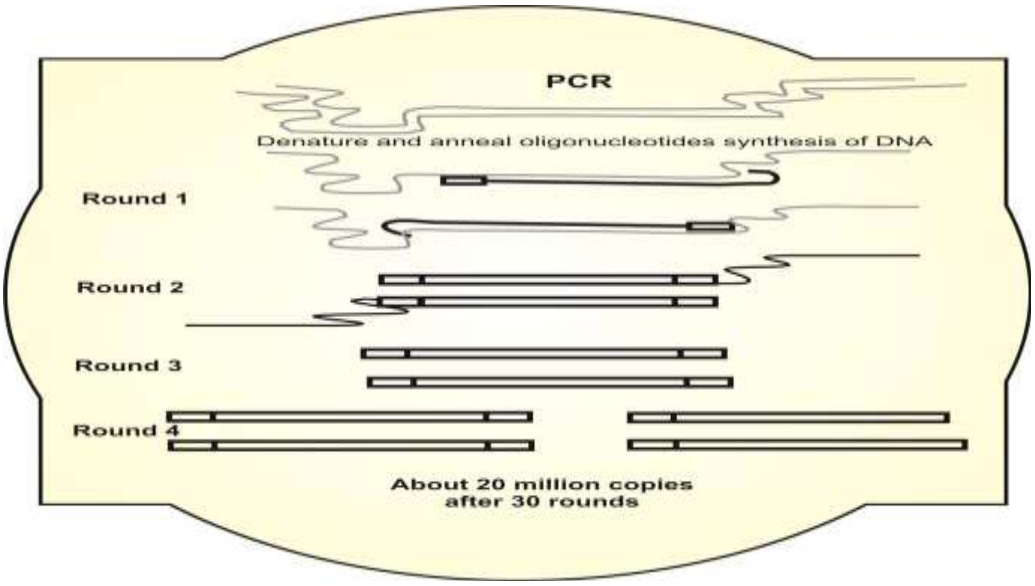

FIGURE-4 , POLYMERASE CHAIN REACTION

DNA HYBRIDIZATION – Double stranded DNA can be denatured (split) that is the hydrogen bonds between base pairs can be disrupted causing DNA strand to break and two complementary strands produced, DNA breaking can be done by various methods like presence of high PH , or high temperature under suitable environment from two complementary two more DNA can be formed so this process complementary single strand molecule lining up to form double strand DNA molecule is known as DNA Hybridization .

CONCLUSION

We are currently witnessing a revolution in medical research, which is attributed to the invention and rapidly increasing uptake of the next generation sequencing. NGS allows comprehensive description of the germ line DNA , analysis of somatic mutations and RNA profile in naturally occurring tumors , these advances may need to be considered while discussing the standers of clinical research, data dissemination and interaction between clinical and laboratory specialist.

Conflict of interest – The author declares no conflict of interest.

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