Model Answer (Paper Code-2533)

B. Pharm. (VII Semester) Examination, 2013

PHARMACEUTICAL BIOTECHNOLOGY

Section A

1. The production of additional copies of a DNA or gene sequence. These may be present as intrachromosal or extrachromosomal DNA. It is possible to amplify a DNA in vitro using PCR.
2. Transformation is the asexual transfer of genetic material as free DNA . The living bacterial cells may pick up fragment of DNA .or uptake of free nucleic acids or plasmid into a cell.
   Infection of a cell with nucleic acid from a virus, resulting in replication of the complete virus.
3. It is a technique to block expression of a specific gene, and hence interfering with the protein production. The targeted silencing of genes is done by (a) recombination to mutate or delete the unwanted functional gene, (b) anti-sense RNA technology.
4. The temperature at which half of the DNA molecule has open is referred as the Tm (melting of DNA).
5. An autonomous self-replicating extrachromosomal circular DNA. Present naturally in a number of prokaryotes and also in certain lower eukaryotes and plants .these extra-chromosomal elements are called plasmid.
6. Gene therapy paves way to either replace the missing or defective gene at the origin or arrest undesired gene expression at the origin.
7. Gene guns are said to utilize tissue bombardment with gold or tungsten microparticles coated with DNA plasmid-based gene expression system. This method has been successfully used to deliver DNA in vivo into liver, skin, pancreas muscle, spleen, and tumors.
8. Opsonization is the process by which a particulate antigen becomes more susceptible to phagocytosis by combination with an opsonin. Opsonin is an antibody, which when combined with a particulate antigen, increases the susceptibility of the antigen to phagocytosis. This process is termed opsonization . these antibodies are known as opsonizing antibodies.
9. Active immunity is the resistance developed by an individual in response to an antigenic stimulus, resulting in the synthesis of specific antibodies or production of immunologically active cells.

Passive immunity – the immunity that a non-immune individual acquires by receiving antibodies or sensitizing white blood cells from another immune individual is known as passive immunity.

10. Antigen specific.
11. Immunization is a process of rendering an individual resistant to infections. The resistance to infection can be brought about by two methods, namely active and passive immunization.
12. The cell produced by fusing two cells of different origin. In monoclonal antibody technology, hybridomas are formed by fusing an immortal cell and an antibody producing cell.

Section B

Long Answer Type Questions

Ans.2 Human health is greatly affected by the products emerging from biotech industry. The biotech industry has symbiotic relationship with pharmaceutical industry. Biotechnology has become a wing of pharmaceutical research. Because of the advances in molecular and cell biology like recombinant DNA and hybridoma technologies, control of gene expression, gene amplification by PCR, embryo stem cell manipulation, efficient methods of gene transfer (transfection), tissue and protein engineering; it is now possible to go into a cell and alter its genetic make-up and control cell differentiation.

Biotechnology uses body's own tools and weapons to fight against diseases. Assistance of plant/animal cells, viruses and yeast are taken in large scale production of human drugs by biotechnology. There are 4 primary areas of health care in which biotechnology is currently employed,

(a) Medicines, (b) vaccines, (c) diagnostics, (d) gene therapy

Disease itself in now being understood, diagnosed, prevented and treated in an increasingly higher order of genetic structure, function and regulation. Low molecular weight drugs will soon be replaced by recombinant therapeutic proteins, protein agonists or antagonists, anti-codon nucleotides and or cDNA cells. All these modern medicines are called so called targeted drugs. First therapeutic protein approved by US FDA is somatostatin. Almost 60 therapeutic proteins are registered and more than 200 are in clinical or investigational phase.

Conventional vaccine preparation includes killed or attenuated pathogen, which suffers from the drawbacks possibly by reversion of cells pathogenicity. The preparation contains contaminant

proteins other than the antigen required to stimulate immunological apparatus of the host. Biotechnology derived novel vaccines like recombinant subunit vaccines, gene deleted vaccines and raw DNA vaccines are potential in the field. They consists only antigen and not the actual pathogen either in killed or attenuated form. Adverse effects due to the presence of contaminant proteins and microbial cell components are thus omitted.

Biotechnology offers cost-effective, quick and precise solution for diagnosis of variety of disease and genetic conditions. Rapid detection kits for infectious diseases, cancers, auto-immuno diseases, genetic disorders, based on specificity of monoclonal antibodies are now available in the market. Home pregnancy tests and tests for demonstration of antenatal abnormalities are the examples of diagnostic tests offered by biotechnology.

The advent of gene therapy was sparked off by the case of bubble boy David, a five year boy suffered with severe combined immunodeficiency disease (SCID) due to the lack of enzyme adenosine deaminase (ADA). Amy Harper, a girl is a first human treated the treatment of single or multi-gene disorders. In gene therapy a faulty or missing gene is replaced with functional gene, ex-vivo or in-vivo. Because of the presence of functional gene the cell wall now produce the protein which was previously absent and will overcome the deficiency of protein, which is a root cause of the diseased condition.

Ans.3 Replication of DNA

DNA replication is the process of DNA duplication and its distribution in two daughter cells two possible scenarios.

One cell received the pre-existing DNA, while the other cell receives the newly synthesized DNA. This type of DNA replication will be called as the conservative mode of DNA replication.

Two strands of original DNA are separated, each is duplicated by the synthesis of the complementary strand, thus producing two exact replica of the parent DNA. This mode of replication is known as semiconservative mode of replication.

DNA replication continuous on 5'-3' strands while it is discontinuous on 3'-5' strands. DNA polymerase I capable of step-by- step addition of deoxyribonucleotide units to a DNA chain.

Unbinding is must for replication

The bacterial chromosome is circular in appearance therefore mode of replication is generally called Θ-replication. Since two chain of replicating molecules must undergo full rotation to unwinding of entire structure. The axis of rotation for the process of unwinding is provided by nicks made in the back bone of one the strands of the double helix during the course of replication. After unwinding each cut is repaired rapidly.

An enzyme that could generate cuts and nicks and repairs them quickly is called topoisomerase. The position just adjacent to cut or nicks is called replication origin and the region where parent strand tend to separate allowing the synthesis of new one is referred to as **replication fork.** The process responsible for generating new fork is termed as initiation.

DNA contains multiple origin of replication

The eukaryotic DNA is linear molecule that replicates bidirectionally. The replication could be initiated at a time at many sites in DNA. This result into multiple loops. This process reduced the replication time significantly.

DNA polymerase responsible for new DNA Strands

DNA polymerase is enzymes that form the sugar phosphate bond between adjacent nucleotides in a nucleic acid chain. DNA polymerase provides:
The 5'-triphosphate are dATP,dGTP, dTTP, dCTP
Single strand of DNA
The primer segment
The DNA polymerase possessing nuclease activities these are:
Exonucleases and Endonucleases

DNA synthesis proceeds in 5'to 3' Direction
DNA polymerase can add nucleotide only to the 3'-OH group. The 3' –OH terminals of continuously replicating strands proceed to be ahead of discontinuous strand hence referred as leading strand while in order to 5'-3' oriented growth one of the parent strand is replicating in small precursor fragment as lagging strand.
RNA as an initiator of new strand
This short stretch of RNA provides a primer over which DNA polymerase can subsequently add deoxy- nucleotides. The DNA primer producing RNA polymerase is called primase.
Percursor fragment
Finally combined to produce a continuous strand of DNA . Joining of fragmented DNA is accomplished by DNA ligase.
Proteins also participate in DNA replication
During replication nearly twelve different types of proteins participate in the activity.

Ans.4 Monoclonal antibodies in drug targeting

The use of monoclonal antibodies to target drugs to specific cell types is a promising approach. Drugs are coupled to an antibody, thereby creating a hybrid molecule with the specificity of the immunological ligands as well as retaining the therapeutic activity of the drug.

Antibody and antibody conjugates

Antibodies or their fragments are the ideal modules to augment the association of drug carrier with macrophages because of their ability to interact with Fc/C3b receptors which enhance their uptake in the system. It has been shown that for heat aggregated antibodies and for haptens expressed on the surface bound antibodies, opsonization and endocytic process mediated through Fc region of IgG. Without Fc receptors no binding occur.

On the other hand no receptor for IgM antibody on macrophages. Galactosyl ceramide system taken up by peritoneal macrophages in the presence of IgM, antigalactosyl antibodies and complement receptor. The access of MAbs to receptor bearing cells can be improved by [F(ab')2 and Fab'] fragments instead of complete IgG portion.

Palmitate derivatized antibody molecules on the cell membranes, where they function as surrogates receptor (SR) for facilitating specific cellular interaction. Cellular conjugation, enabling SR to cooperate with endogenous target recognition process, that is pivotal in receptor-ligand interaction at the cell-cell interface.

Suppresser deletion therapy

In this approach, targeted immunotherapy concept has been utilized. The T suppresser cells have been deleted by conjugating antibodies with hematoporphyrin which can be targeted towards specific tumor cells. Conjugation is brought about by using carbodiimide as a coupling agent.

Site- specific modification of MAbs

Site-specific modification refers to covalent chemical modification.MAbs either by direct labeling with isotopes or conjugation to drugs, chelators ot toxins .

Antibody – enzyme conjugates

The strategy namely antibody-directed enzyme prodrug therapy (ADEPT), targets the enzyme to the tumor site using a monoclonal follow by a non- toxic prodrug which can be converted by the enzyme to a potent anti-tumor agent.

Antibodies as receptor surrogates for mediating cell-cell interaction

The underspinning and mysteries of immunological competence are intimately tied to accomplish network of exquisitely specific intercellular encounter. Through these interaction host defense could serve as surrogate receptors where derivative forming ligand mediates cell-cell interaction.

Immunoliposomes based diagnostic kit

The liposomes are prepared encapsulating coloured dye into their aqueous domains. Dye loaded liposomes are sensitized by immobilizing specific antigen or antibody on their surface. Antibodies specific for target pathogen are immobilized on nitrocellular membrane in a

particular shape like triangle. The analyte with pathogenic antigen load on addition to such membrane selectively coate /binds to the immobilized antigen or antibody of the cellulose nitrate membrane. Thus the analyte gets linked immobilized. On addition of dye filled liposomes to such a test sample a triangle is generated indicative of positive reactions. However, the sample is negative if contains no target analyte as no binding or reaction is visualized. Liposomes based diagnostic kits are getting importance as a result for prompt diagnosis of various diseases.

Ans 5 Precipitation refers to an antigen- antibody reaction between a soluble antigen and its antibody resulting in the formation of insoluble precipitate. The antibody causing precipitation is called precipitin.

Mechanism of precipitation: precipitation is due to the formation of antigen- antibody complex. As the each antibody is a bivalent molecule, it can bridge two multivalent antigen molecules. This bridging leads to the formation of a lattice which forms the precipitate. When antigen and antibody are in optimal concentration, the precipitation is complete and a large lattice is formed.

Precipitin reaction in fluids

A quantitative reaction can be performed by placing a constant amount of antibody in a series of test tube and by adding a measured amount of antigen to the tubes. A precipitin curve is obtained by plotting the amount of precipitate against the antigen concentrations. Maximum precipitate occurs when the ratio of antibody to antigen is optimum. This is equivalent zone. The other two zones are the antibody excess zone and antigen excess zone . This test is a rapid quantitative test for determining the presence of antibody or antigen. This test is of value in detecting and identifying antigens having application in the typing of streptococcoi or pneumococci.

Precipitin reaction in gel or immunodiffusion reactions

The diffusion of antibody in antigen bearing agar or vice versa, resulting into the formation of a visible line of precipitate. This line occurs at the region of equivalence. these immunodiffusion reaction can be used in determining the relative concentration of antibodies or antigens to compare antigens or to determine the relative purity of an antigen preparation. Two technique which are used commonly .

1. Radial immunodiffusion
2. Double immunodiffusion

Immunoelectrophoresis

Immunoelectrophoresis is a quantitative technique that can be detect antibody concentration of 3-20 µg/ml. when large no of different antigen are present in a solution , it is difficulte to separate the precipitin bands . in such situation multicomponent analysis is required, electrophoresis could be used effectively. Immunoelectrophoresis is a technique widely used in detection of serum proteins. It is also helpful in determining whether a patient has an immunodeficiency or not.

Rocket electrophoresis the name of the technique so derived because of the shape of the precipitate formed due to antigen-antibody interaction which is in a rocket shape.

Two –dimensional immunoelectrophoresis a modified version of the rocket electrophoresis , for the quantitative estimation of antigen in a complex mixture. In this method, the antigen is separated into its components by electrophoresis. The gel is then laid over another agar gel containing antiserum and electrophoresis is repeated at right angle to the previously conducted. This appears in the form of precipitin peaks.

Ans 6. (i)

Cystic fibrosis is a complex, multi-system disease which is inherited in an autosomal recessive pattern. The goal of gene therapy in young children is to prevent the generation of infectious lung disease. CF is due to mutation in the CFTR gene, which perturbs the salt and water composition of secretions, slow the mucocilliary clearanceof airways and promotes infection. It has been found that transformed and primary cultures of human airway epithelia in non-polarized and polarized culture condition have been relatively easy to transduce with the help of adenoviral vectors containing CFTR, with correction of the CF Cl^- Transport defect.

New generation modulation in the treatment of CF utilize recombinant deoxyribonuclease which reduces the viscoelasticity of respiratory tract. A plasmid encoding for chloramphenicol acetyl transferase (pRSV2-CAT) complexe with lipofectin liposome has in vivo functioning. Similarly, delivery of CFTR to the respiratory tract using cationic liposomes have also reported.

Ans 6. (ii)

Genes for Bt toxins- The use of pesticides and insecticides is a common measure in plant protections programmes, since pests and insects cause appreciable damage to our crops. Most of these pesticides and insecticides are chemically synthesized. However, an exception is the Bt toxins produced by a bacterial species (*Bacillus thuringiensis*), so that a spore preparation of this bacterium has been used as a biological insecticide during the last 20 years. Insecticidal activity of this species depends on the protein (delta endotoxins) synthesized during sporulation. Since these toxins are very specific in their action, they are safe insecticides, but their use is limited due to high production cost and due to instability of crystal protein when exposed in the field.

The above toxin gene from B. thuringiensis has been isolated and used for Agrobacterium Ti plasmid mediated transformation of tobacco, cotton and tomato plants.

Genes for protease inhibitors- In cowpea (Vigna unguiculata), trypsin inhibitor (CpTI) level was shown to be responsible for its resistence to attack by the major storage pest of its seed(i.e. bruchid beetle= Callosobruchus maculates). CpTI gene was joined with CaMV 35S promoter, and one or more marker genes.

Gene for other insecticidal secondary metabolites –Secondary metabolites produced by plants have also been implicated in the resistance to insect attack. However, biosynthesis of each of these metabolites involves a series of steps(more than one biosynthetic pathway), each controlled by a separate gene. Furthermore, these genes are tissue specific in expression.