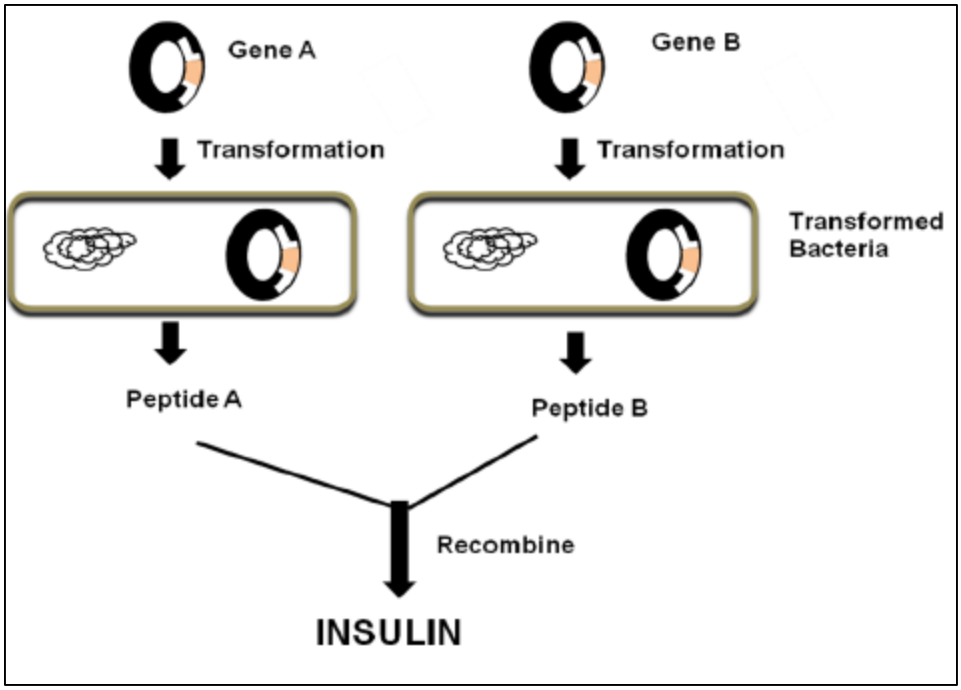
Biotechnology in medicine

Biotechnology utilizes transgenic micro-organism, plants or animals as living "factories" to produce pharmaceuticals for the use in humans or animals. Other medical applications include gene therapy and stem cells.

(A) Production of therapeutically proteins

A large number of genetic or metabolic diseases can be corrected by the supplying proteins or factors. Following the advancement in the biotechnology, many other proteins or factor are produced in different bacterial expression systems (Table1). In an approach, gene of the enzyme or proteins factor is cloned into the appropriate plasmid to produce recombinant clone, for example: production of **human insulin**. Insulin is a dimer of an A chain and B-chain linked by disulphide bonds, composed of 51 amino acids with a molecular weight of 5808 Dalton. A schematic presentation of steps in insulin production is given in Figure 1. In this process, gene A and B is cloned into the bacterial plasmid separately to produce two recombinant clones. Peptide chain A and B is over-expressed in the *E.coli* and recombined together to produce functional insulin.



Recombinant DNA technology to produce human insulin

The **human growth hormones**, if this hormone from the pituitary gland, is present in reduced quantities in children that may suffer from dwarfism. Today, recombinant gene technology uses bacteria in order to produce it on a large scale; and technology seem to work so well that dwarfism may be overcome in few years' time.

Factor VIII is very familiar to those people who suffer from hemophilia; again with the help of biotechnology, this factor is produced by bacteria; it has greatly reduced the likelihood of

hemophiliacs to contract AIDS, as previously applied substances originating from blood-plasma donors.

Erythropoitin is a hormone produced by the kidneys; it stimulates the production of red blood cells (erythrocytes). Patients with kidney failure do not produce this hormone anymore; therefore, they often suffer from anemia, are always tired, and apart from dialysis, the need a constant supply of fresh blood transfusions. Today, this hormone is made by a transgenic mammal, of the *Chinese hamster*. Extracting plasma from the animal, isolating the hormone, is a safer way to obtain this hormone, rather than relying again on human donors.

Table1 some recombinant proteins that are used therapeutically

Protein	**Clinical indication**
Hepatitis B vaccine	Prevention of hepatitis B infection
Interferonα_{2a}	Leukemia
Human DNase	Cystic fibrosis
Fibrinogen	Wound healing
Pro542	HIV infection
Collagen I	Tissue repair

(B) Gene therapy

As discussed before, production and supply of recombinant proteins is a temporarily solution for the treatment of a disease condition. In another approach, human expression system is used to produce the proteinous factor after inserting the recombinant clone into the human cells or inside the human body. Recombinant DNA is packed into the appropriate DNA delivery system (either a virus or liposome mediated) to deliver the gene into the human cells to correct the mutated genes or encode a therapeutic protein drug to provide treatment.

Liposomes *Artificially formed single-layer or multilayer spherical lipid bilayer structures. Made from solutions of lipids, etc. in organic solvents dispersed in aqueous media. Under appropriate conditions, liposomes form spontaneously. Often used as models of the plasma membrane. May also be used experimentally and therapeutically for delivering drugs etc. to cells, since liposomes can fuse with a plasma membrane and deliver their contents to the interior of the cell vary in size from submicron diameters to centimeters.*

There are two different types of gene therapy, **Somatic Gene therapy**: In this therapeutic approach, the therapeutic genes are transferred into the somatic cells as per the requirement of individual to treat the functional defects. This treatment does not move to the patient's offspring or next generations. **Germ line gene therapy**: In this therapeutic approach, germ cells (sperm or egg cells) are transformed by the introduction of the required gene to produce the protein or correct the mutated gene. This allows the treatment move to the patient's offspring or next

generations (Figure 2). Although the approach seems promising in providing long term solution to treat genetic disorder, but there are several ethical, technical reasons and possible future risks.

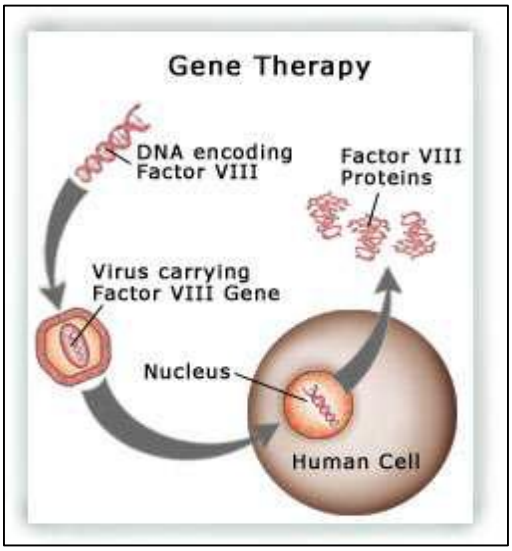


Fig2 the replacement of a defective gene or set of genes with a functional copy

The technical problems associated with the gene therapy are as follows

1. Short lived: Therapeutic gene delivery into the cells gives short term effects, either by rejection of recombinant DNA or suppression of the gene expression. Due to this problem, patient needs to go for several rounds of gene therapy.

2. Immune reaction: virus containing gene is treated as the foreign object, and immune system is stimulated to attack the invader. It is the main reason of reduced effectiveness of gene therapy.

3. Viral vector used as a vector to deliver the gene causes much adverse immune reactions and toxicity in patients.

4. Disturbance of host physiology: if the genes integrate to a wrong place in the genome, it may cause functional defects. In few cases, it may disrupt the function of the tumor suppression genes results into the development of the tumor.

(C) Monoclonal antibodies production:

Antibodies are used in the adjuvant therapy to remove the infectious agents or toxic substances. Monoclonal antibodies are generated by the hybridoma technology (see previous lecture).

(D) Vaccine

Vaccine is given to develop immunity against the disease in the human or other vertebrate animals. Vaccines are dead, attenuated organism or proteins derived from them. There are different strategies to enhance the immunological response to give long lasting protecting against the disease with minimum adverse effects. Different types of vaccine developed for human vaccination is as follows-

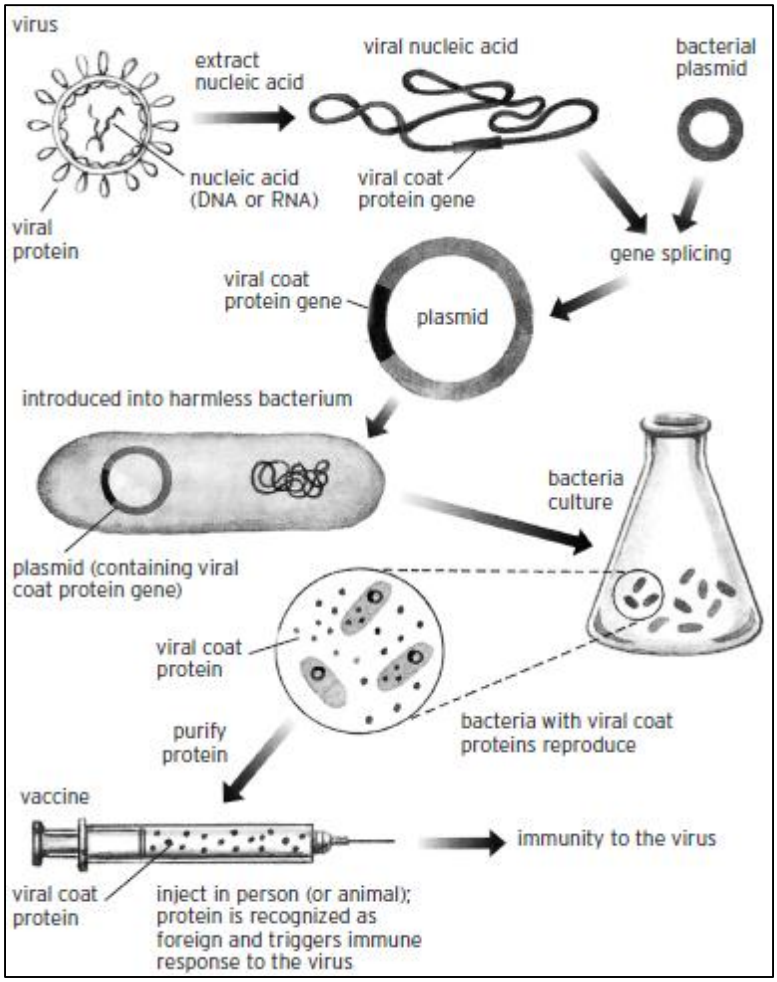


Fig3 strategy of vaccines constructing a safe vaccine against viral disease can be produced by engineering the gene for the virus's protein coat into bacteria. The bacteria manufacture the viral coat protein, which is then injected to stimulate the body to make antibodies against the virus.

Killed: In this vaccine preparation, pathogenic organism is killed by chemical or UV treatment and used as an immunogenic. It is mixed with the adjuvant to enhance immunological responses and long memory.

Attenuated: In this vaccine preparation, organism is treated with the chemical to destroy its ability to cause disease. As a result, organism grows and gives stimulation to the immune system for long term immunological memory.

Toxoid: In this vaccine preparation, inactivated toxic compounds are used as an immunogenic.

Subunit: In this vaccine preparation, a pure protein or antigen is given as an immunogenic. It is the safest form so vaccine with minimum adverse allergic reactions.

Conjugate: In this vaccine preparation, bacterial coat is tagged with the immunogenic protein to induce production of immune response against the bacterial coat.

Vectors in gene therapy

• A **virus** is a small infectious agent that can only replicate within living cells.
– Millions of different types infecting every organism on the planet.
• Have three possible components:
– Genome (ssDNA, dsDNA, ssRNA, dsRNA).
– Protein coat.
– Lipid membrane (optional).

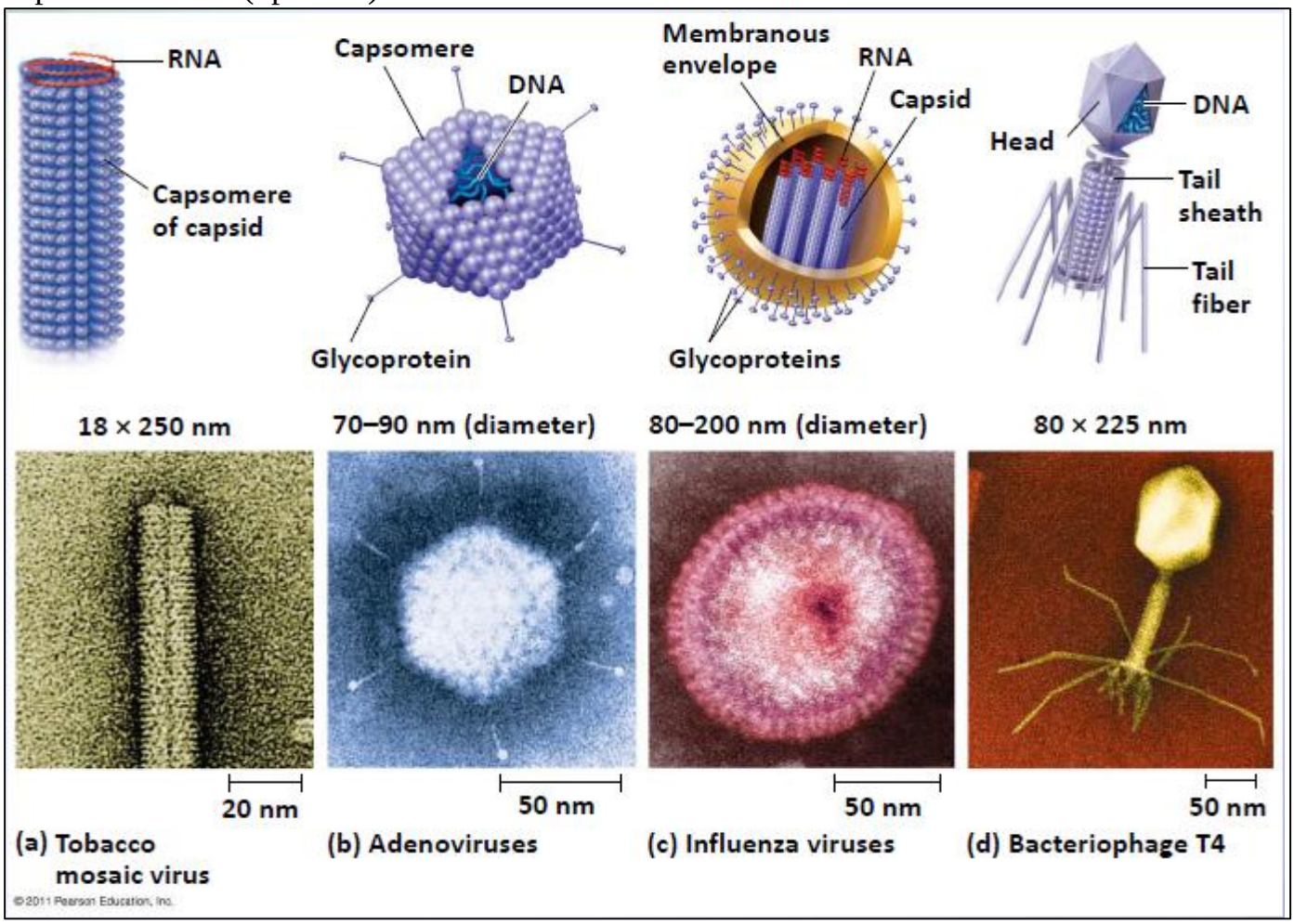


Fig4 types of viruses used as vector in gene therapy

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