Virology (CC - 5)

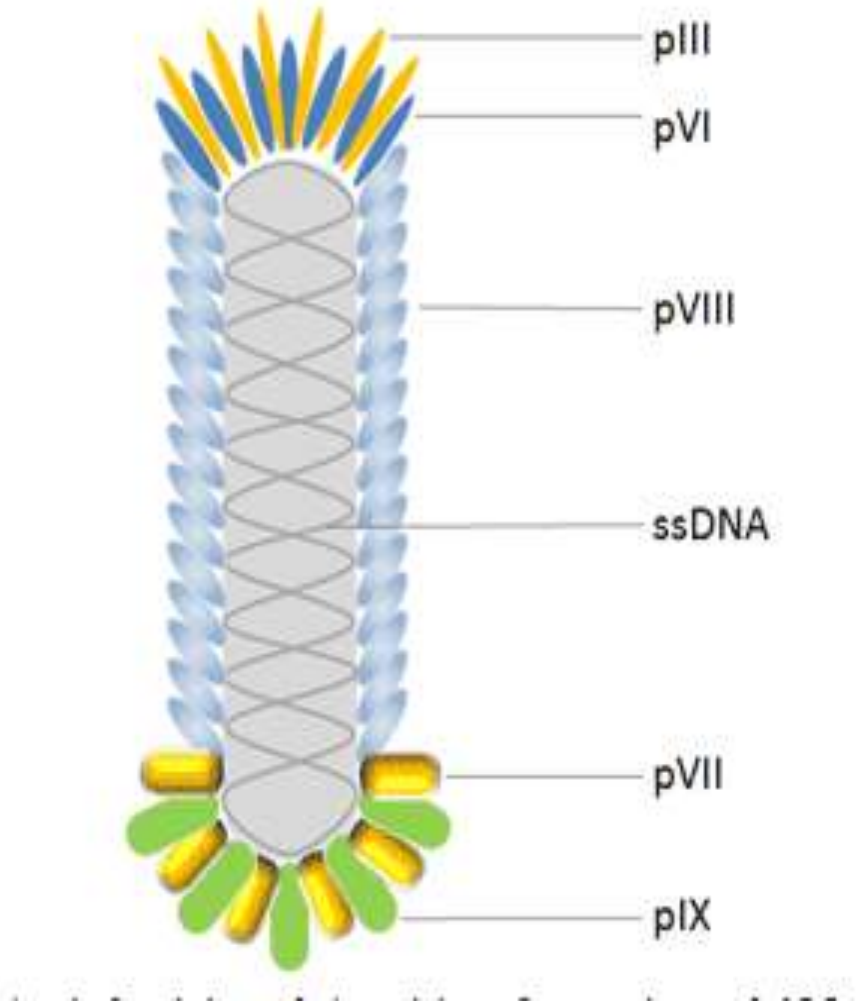
Applications of Virology (Unit - 4)

Phage Display

- Phage display was first developed by G. Smith in 1985 as a method of presenting polypeptides on the surface of lysogenic filamentous bacteriophages
- In phage display technique, a gene encoding a protein of interest is inserted into a phage coat protein gene, causing the phage to display the protein on the outside and the phage contains the gene for the protein inside, resulting in a connection between genotype and phenotype.
- These displaying phages can be screened for other proteins, peptides or DNA sequences, in order to detect interaction between the displayed protein and those other molecules

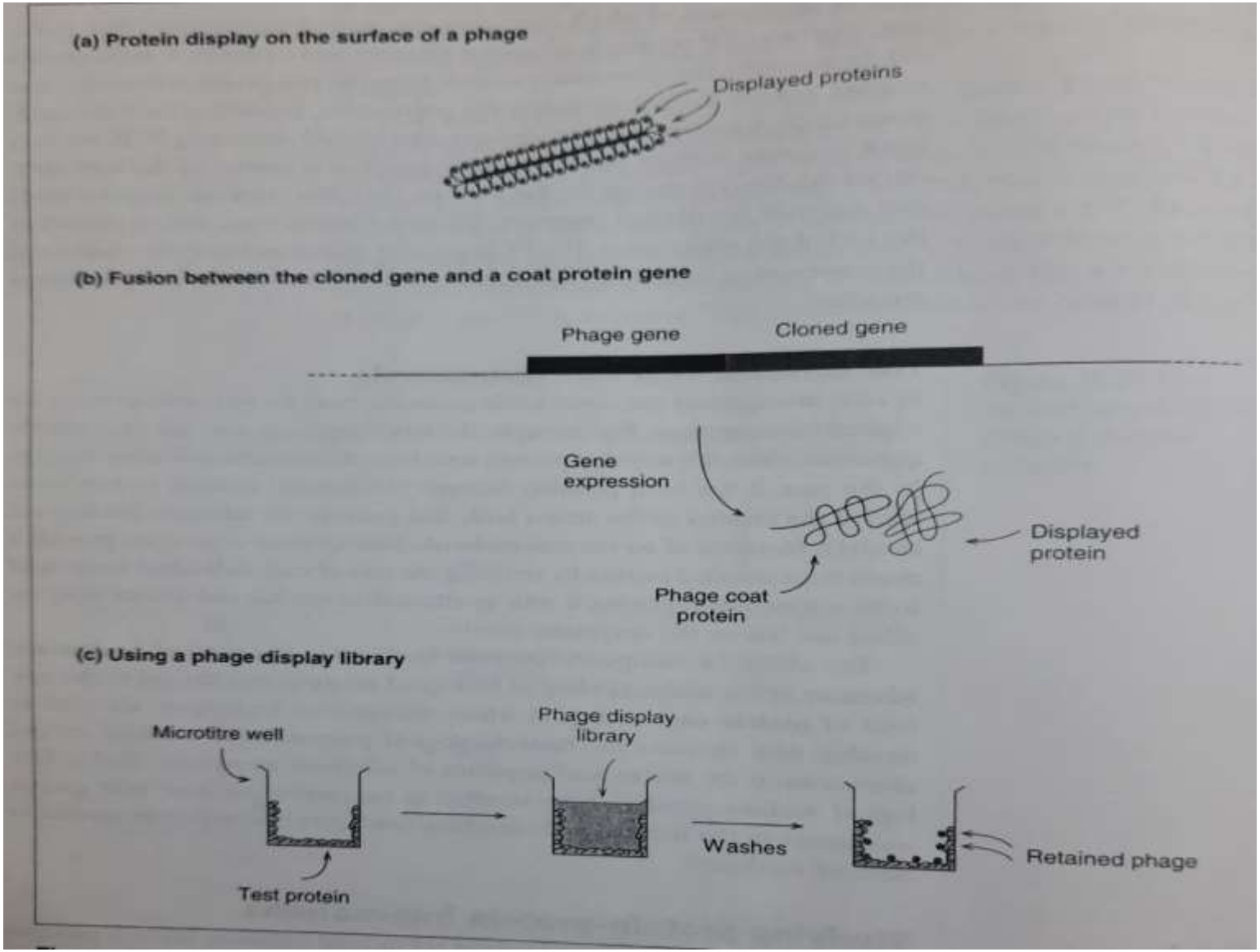
- So, it has become one of the most powerful and widely used laboratory technique for the study of protein-protein, protein-peptide and protein-DNA interactions.
- Phage display is used to obtain information about the function of a protein that has not been previously studied, like to which proteins it works with in the cell
- In this way, large libraries of proteins can be screened and amplified in the process called *in vitro* selection, which is analogous to natural selection.
- Phage display is also an effective way for producing large amounts of peptides, proteins and antibodies
- The most common bacteriophages used in phage display are *E. coli* filamentous bacteriophages f1, fd, M13, though T4, T7, and λ phages have also been used.

- M13 bacteriophage has a cylindrical shape with a length of 880nm and a diameter of 6nm. It encapsulates a single-strand genome that encodes five different capsid proteins which comprise two groups - major coat proteins (pVIII) and minor coat proteins (pVII, pIX, pVI and pIII)


pIII
pVI
pVIII
ssDNA
pVII
pIX

- The gene for the protein is first cloned in a vector (M13)
- This allows the protein coding gene to be fused with a gene for a phage coat protein
- After transfection of *E. coli*, this gene fusion directs synthesis of a hybrid protein, made up partly of the coat protein & partly of the product of the cloned gene
- When this hybrid protein will be inserted into the phage coat, the product of the cloned gene is located on the surface of the phage particles
- This technique is carried out with a phage display library made up of many recombinant phages, each displaying a different protein

- The library consists of phages displaying a range of different proteins and is used to identify those molecules that interact with a test protein
- This test protein could be a pure protein or one that is itself displayed on a phage surface
- The test/target protein is immobilized in the wells of a microtitre tray or on particles that can be used in an affinity chromatography column & then mixed with the phage display library
- Phages that are retained in the microtitre tray or within the column after a series of washes are ones that display proteins that interact with the immobilized test protein


(a) Protein display on the surface of a phage
Displayed proteins
(b) Fusion between the cloned gene and a coat protein gene
Phage gene
Cloned gene
Gene expression
Displayed protein
Phage coat protein
(c) Using a phage display library
Microtitre well
Phage display library
Washes
Retained phage
Test protein

Steps involved in Phage Display

STEP1: Construction of phage display library

- Recombinant DNA technology is used to incorporate foreign cDNA into viral DNA. Different sets of genes are inserted into the genomes of multiple phages. The protein will be displayed on the outside of phage particles, and these separate phages will only display one protein, peptide, or antibody.
- Collections of these phages can comprise libraries, such as antibody phage library, protein phage library, or random phage library

STEP2: Binding

- These libraries are exposed to selected targets and only some phages will interact with targets. The target is that specific protein (ligand) planned to be identified such as immobilized protein, cell surface protein or vascular endothelium

STEP3: Washing

- Unbound phages are washed away, and only the phages showing affinity for the receptors are retained

STEP4: Elution

- Target bound phages are recovered by elution.

STEP5: Amplification

- Eluted phages showing specificity are used to infect new host cells for amplification

Applications

1. Epitope mapping and mimicking

- Upon encountering antigen, host humoral immunity activates and triggers production of antibodies which directed against foreign protein epitopes. Knowledge of these protein epitopes is pivotal in understanding the pathogenesis of pathogen infections and in developing diagnostic reagents, therapeutic antibodies, and effective vaccines.
- Peptide phage display libraries are useful tools for identification of continuous or linear epitopes involving in interaction with antibody. This improve immunological studies in order to design and develop vaccine candidate

2. Identification of new receptors & ligands

- Phage display method using either gene-specific libraries, or random peptide libraries, provides a powerful technique for an approach to epitope identification. The technique can identify amino acids on protein antigens that are critical for antibody binding.
- The random peptide sequence is displayed on the surface of the phage to obtain the phage display polypeptide library. Polypeptides identifying specific cells are obtained by differential screening using cells as screening targets. By studying the polypeptide sequence, one can obtain the receptor protein expressed specifically on the cell surface

3. Drug discovery

- Peptides as biologically active molecules in hormones, neurotransmitters, cytokines, antigens, and growth factors are involved in a wide variety of biological processes.
- So, peptides are extensively used as therapeutic and diagnostic agents in the medical fields such as oncology, endocrinology, urology, and obstetrics.
- The peptide phage libraries present a huge number of different peptides mimicking the genuine epitopes play a key role in the development of new therapeutic peptides. Until now, several peptide drugs have been developed using phage display technology

4. Protein-protein interaction studies
5. Protein directed evolution
6. In vitro diagnostic
7. Recombinant antibody production