Discipline Courses-I
Semester-I
Paper: Phycology and Microbiology
Unit-IV
Lesson: Economic importance of- Virus
Lesson Developer: Pamela Singh
College/Department: Dept. of Biotechnology, Deen Bandhu Chhotu Ram University of Science and Technology

Table of contents

Chapter: Economic importance of Viruses

Introduction

A virus, as we now know is a very small sized acellular infectious agent which can replicate only inside a living host. Viruses are so small in size that they can easily pass through bacterial filters .They either have a limited number of enzymes or none at all as their metabolic machinery. Thus they have to depend completely on their host for survival and replication. The viruses have a very broad host range i.e. they can infect humans, animals, plants, bacteria, fungi,etc. Viruses are common human pathogens causing a number of deadly diseases like AIDS,Herpes,Cancer etc.

Viruses have been primarily identified as pathogens but they also have significant economic importance.

- Viruses act as simple systems, which can be used as tools for research and analysis. Studies on recombinant DNA technology were developed using bacteriophages (Bacterial Viruses).
- In addition, the viral genomes were the first ones to be sequenced (øx174 and MS 2).
- The lysogenic life cycle of certain viruses allows easy manipulation of the viral genome. Viruses are used as vectors to deliver therapeutic genes into humans in gene therapy. They are also used to cure cancer in a number of ways like virotherapy and VDEPT (virus directed enzyme pro drug therapy).
- Viruses are also the primary sources of vaccines used to prevent the viral infections.
- Some of the viruses can also be used for the control of insect pests of our crop plants. However, in contrast a number of viruses are important plant pathogens as will be discussed in the subsequent text.

Medicine and Diagnostics

Vaccine production: Viral vaccines confer immunity against infection by the pathogenic strains of the same viruses. The initial research on vaccines by Edward Jenner (1796) started with a virus. Jenner used the cowpox virus (now known as vaccinia virus) as a live vaccine for preventing smallpox. The conventional vaccines synthesized using live attenuated viruses or killed viruses are easy to produce and economic but have a number of disadvantages. Reversion of the live attenuated viral

vaccine to the initial virulent form has also been observed in some vaccinated subjects e.g. live polio vaccines. Besides, live vaccines might also contain certain undesirable and undefined components, as they are usually produced in animal cell culture systems. In addition some of the viral vaccines are difficult to produce, because they do not grow very well in culture system eg. Hepatitis B virus. The inactivated viral vaccines are ineffective in some disease thus live attenuated vaccines serve as a better option. However, the attenuated vaccines can cause disease in comprised hosts. Besides inactivated and live attenuated viral vaccines, recombinant - vector vaccines are nowadays being made for prevention of a number of viral infections. The genes coding for the major antigenic proteins of a virus are isolated and inserted into a vector, which can either be an avirulent virus or bacteria. This vector is inserted into human host where the vector replicates and expresses the protein products of the recombinant gene, generating either humoral or cell-mediated immune response. Viruses commonly used as vectors include vaccinia virus, adeno virus, canarypox virus and attenuated poliovirus. Out of these viruses, vaccinia virus is most commonly used as a vector.

Vaccinia virus has a broad host range and remains stable for a very long time period. However, the main drawback with the virus is its large size and lack of unique restriction sites, thus foreign genes can be inserted only by homologous recombination. A number of genes coding for major antigen of pathogenic viruses and even bacteria have been inserted into vaccinia virus DNA and expressed in animal cell culture. The important viral antigens include hepatitis B surface antigen, influenza virus hemagglutinin proteins, rabies virus G protein and *herpes simplex* virus glycoproteins. It is also possible to simultaneously clone genes for 2-3 viral antigens in a given *vaccinia* virus, thus opening the possibility of vaccination with multiple antigens at one go.

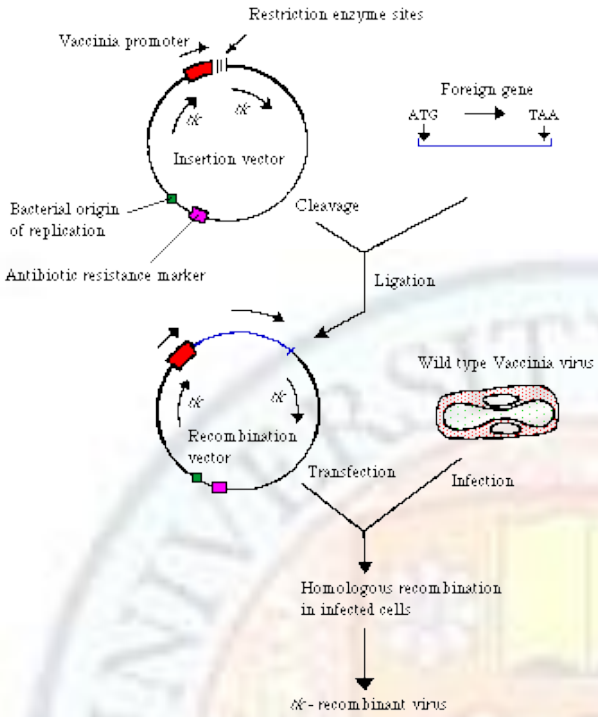


Figure: Strategy for the use of Vaccinia virus as vector

Source: http://www.bio.davidson.edu/Courses/Molbio/MolStudents/01teparakh/Pox3.gif

Recently, viruses have also been used to produce DNA vaccines, however DNA vaccines are still in infancy and research is underway. In a DNA vaccination, a plasmid preparation with genes of pathogenic virus and a strong viral promoter are injected into the muscles of human host. The antigenic proteins of the pathogens are now produced by the muscle cell evoking both humoral and cell mediated immune response.DNA vaccines against AIDS, viral cancers, herpes, hepatitis B virus etc are now being synthesized and tested.

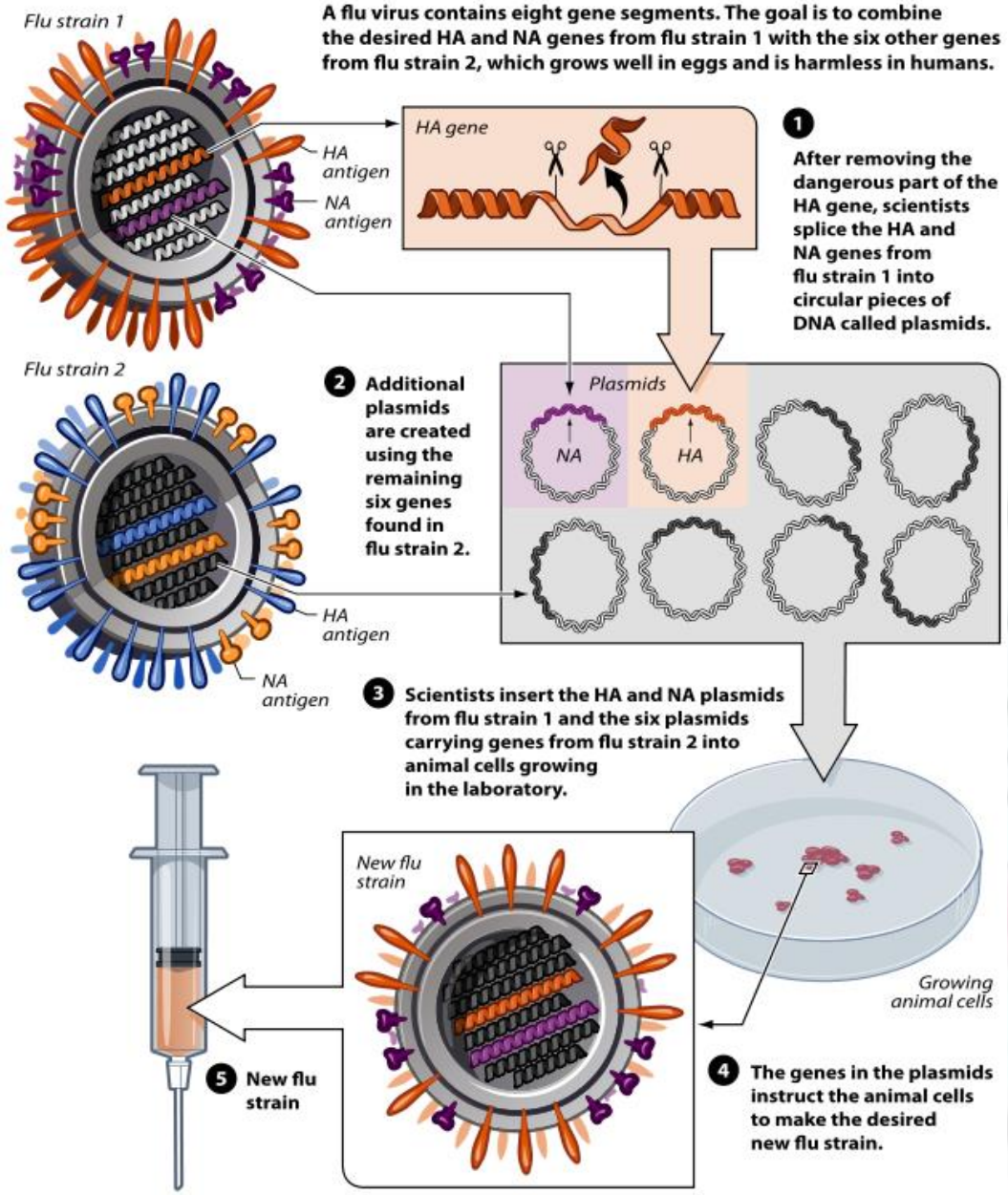


Figure: Strategy for avian flu vaccine

Source:https://upload.wikimedia.org/wikipedia/commons/thumb/0/05/ReverseGeneticsFlu.svg/629px-ReverseGeneticsFlu.svg.png

Gene therapy: The introduction of functional genes into human cells to correct defective genes by replacing them is known as Gene therapy. Initially gene therapy was

used for treatment of patients with genetic disorders, however the applications of gene therapy have increased widely now .

Gene therapy is effective in cases where introduction of either a single gene or limited numbers of genes are required to correct a given disorder. Besides these other important requirements of gene therapy include availability of a functional copy of defective genes (known as therapeutic gene) and a method of gene delivery. Gene therapy is largely used in cancer treatment. However, lately it is also being used in the treatment of some infectious diseases. An overview of gene therapy strategies is shown in given figure below.

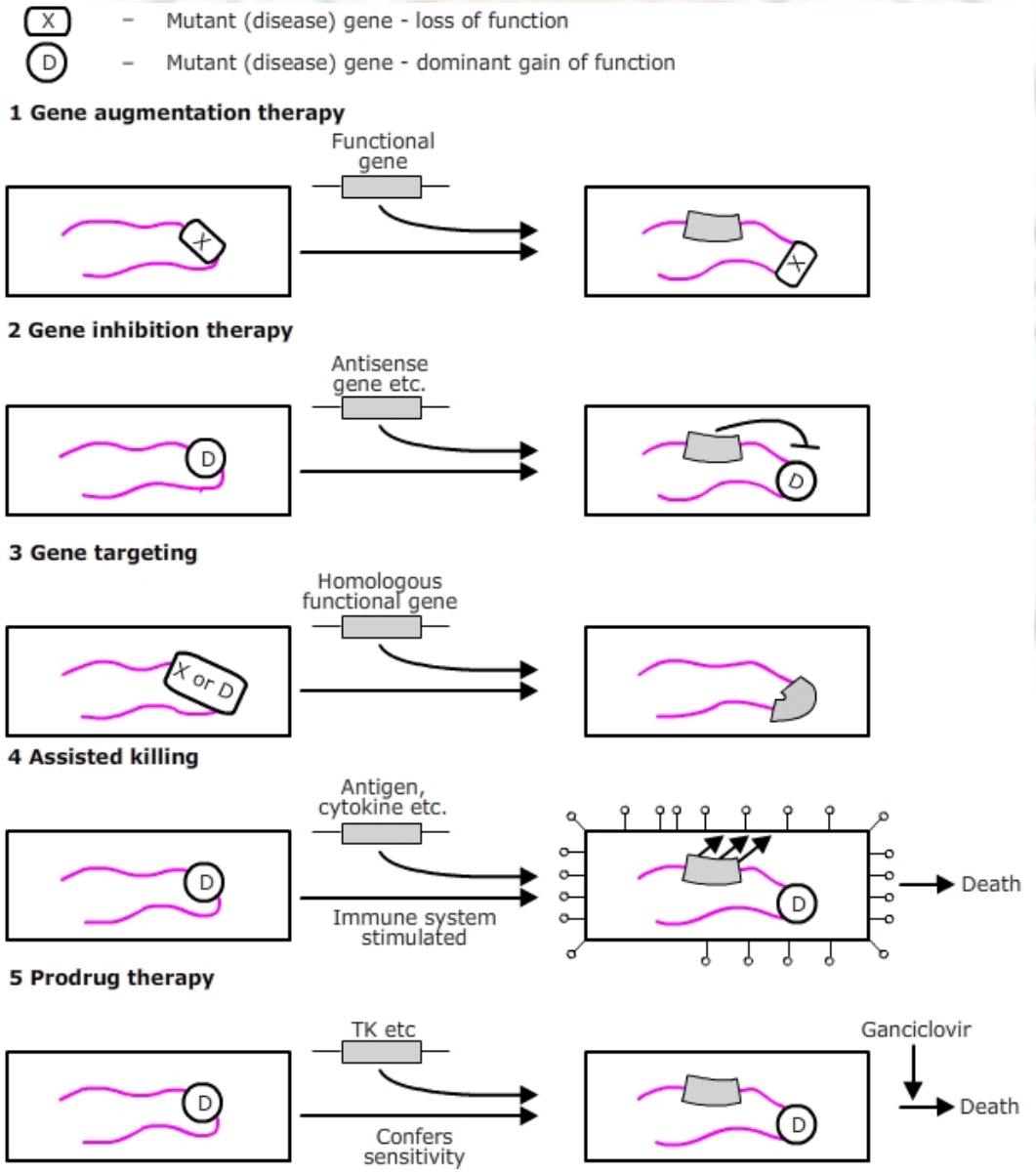


Figure: Overview of gene therapy strategies

Source: Author

Basically there are three types of gene therapy procedures-

Ex-vivo somatic gene therapy- It involves removal of cells with defective genes from the body, followed by introduction of therapeutic gene outside the body. The treated cells are then re-introduced into the patients.

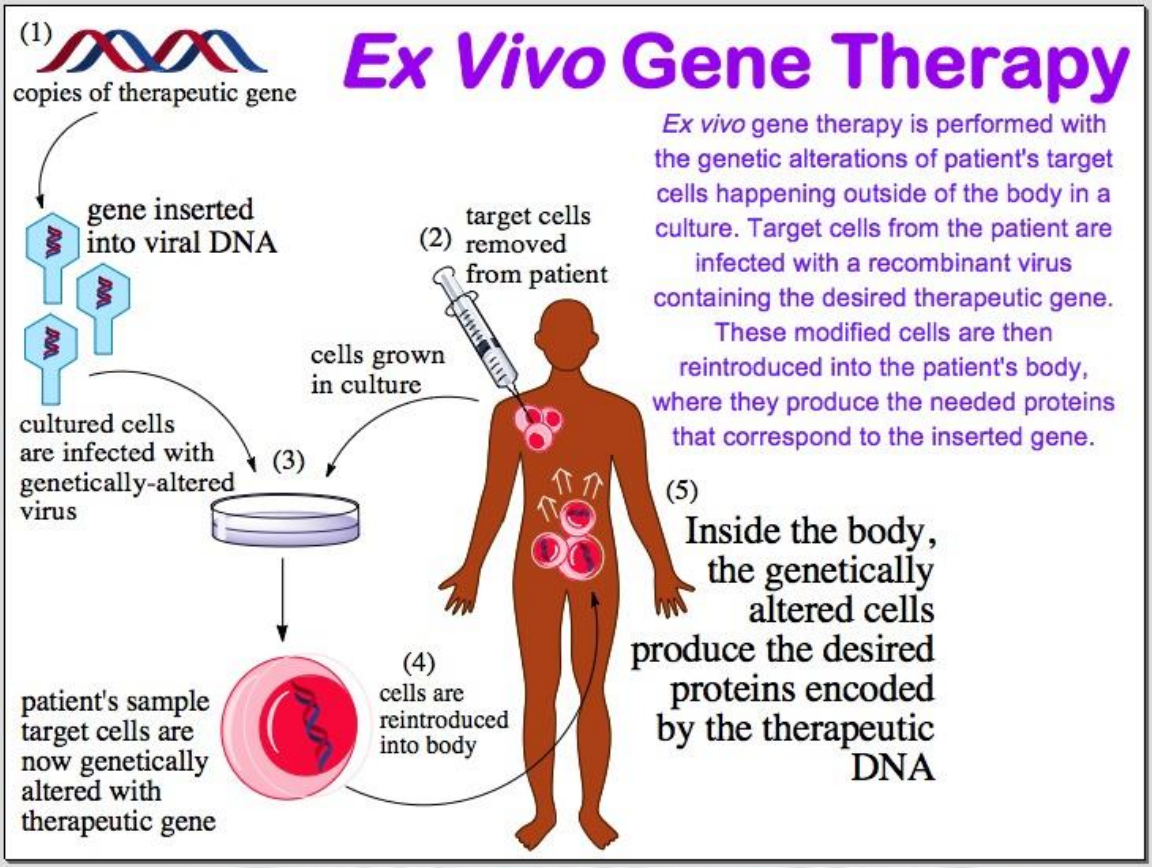


Figure: Procedure of *ex-vivo* gene therapy

Source:http://gene-therapy.yolasite.com/resources/ExVivoGeneTherapy.jpg?timestamp=1270176588750

In-vivo somatic gene therapy involves introduction of therapeutic gene directly into the target cells of the patient.

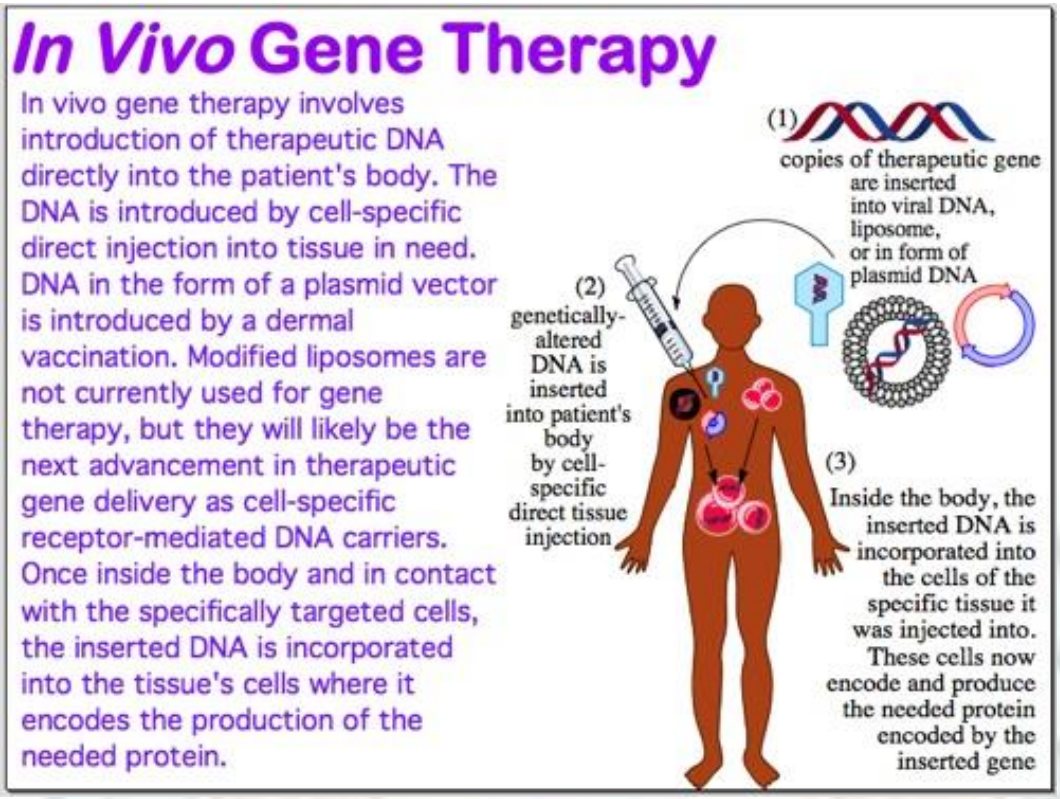


Figure: *In vivo* gene therapy

Source: http://gene-therapy.yolasite.com/resources/In_vivo_gene_therapy.jpg?timestamp=1270176498017

Germ line gene therapy includes the introduction of therapeutic genes into germ cells, aimed at correcting defects in the next generation. Germ line gene therapy is currently not being used in humans and is restricted only to laboratory.

In gene therapy vectors are used to carry the genes of interest into target cells. Both viral and non-viral approaches (microinjection, Gene gun etc) are used for gene delivery. Viruses act as a very specific and efficient gene carrier systems for both ex-vivo and in-vivo gene therapy modules. There are a number of viruses which are being used as vectors in gene therapy. The important ones being Adenovirus, Adeno-associated viruses , Retroviruses, Vaccinia virus , Poxvirus, Herpes simplex virus etc.

Adenoviruses are the most plausible candidates for gene therapy because of their high specificity and easy manipulation. They show efficient nuclear invasion and heightened expression. These viruses are especially suited for treatment of respiratory disorders

because of their high affinity for respiratory cells and can be used in treatment of cystic fibrosis. However some types of adenovirus may be cytotoxic with narrow host range.

Adeno associated viruses, belonging to family *Parvoviridae* are another important group of vectors used for gene therapy.

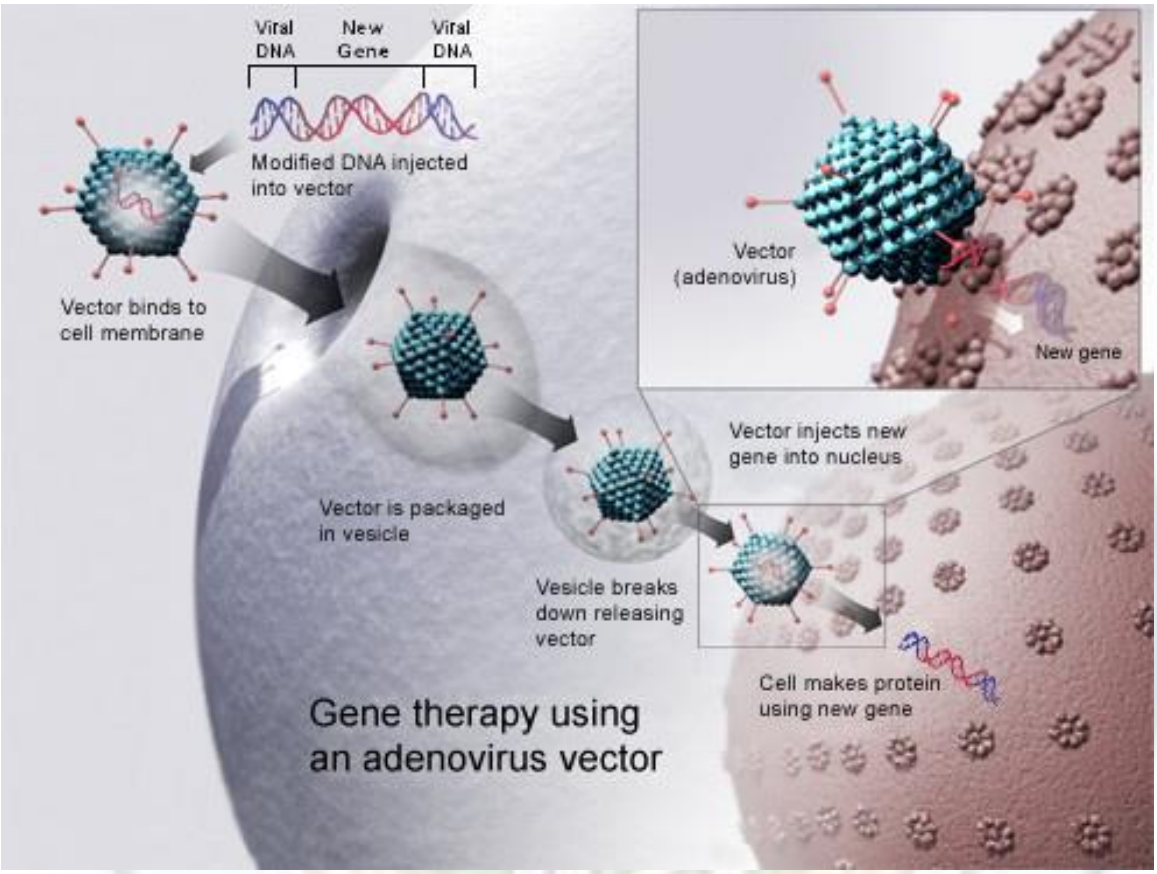


Figure: Adenovirus based gene therapy

Source: https://upload.wikimedia.org/wikipedia/commons/3/3d/Gene_therapy.jpg

These viruses are easy to manipulate and have a single stranded genome and low immunogenicity. They require a co-infection with a helper virus for replication. Gene therapy trials for pancreatitis are currently carried out using this virus as a vector. The only disadvantage of these viruses appears to be the fact that they can carry only a limited size of DNA insert. Amongst the retroviruses, the murine-leukemia virus has been very commonly used for gene therapy trials. HIV based vectors are also being designed currently. The retroviruses show high efficiency of gene transfer and can efficiently integrate into host genome. However, they could be oncogenic, thus unsafe

for use. In contrast vaccinia virus can carry large size of DNA insert, are easy to store and manipulate, thus act as potential model vector candidates even for gene therapy.

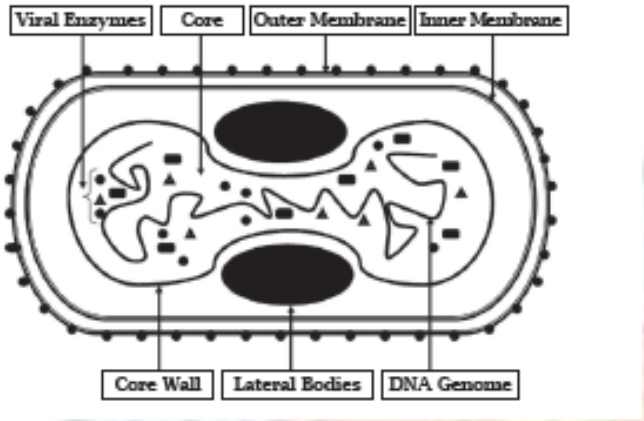


Figure: The morphology of Vaccinia virus

Source:http://www.scielo.br/img/revistas/bjid/v14n2/a03fig01.gif

Gene therapy requires frequent repetition of the process because of elimination of target cells by routine immune processes. Thus, in future germ line gene therapy if possible appears to be an answer to our current maladies.

Cancer therapy: Viruses can be directly used to prevent cancer by being the source of **anti cancer vaccines** eg. vaccines against hepatitis B virus (causes hepatic cancer) and human papillomavirus (cervical cancer) are commercially available. During carcinogenesis six fundamental properties are altered in cells to give rise to the destructive phenotype of cancer as illustrated in given anti viral vaccines are prophylactic in nature and stimulate immune system to synthesize and recruit specific antiviral molecules and cells. Cancerous cells express characteristic viral specific proteins, thus these could be targeted using vector to enhance the specificity of the vaccines. Such **therapeutic vaccines** are under clinical trials and are still not approved for human use.

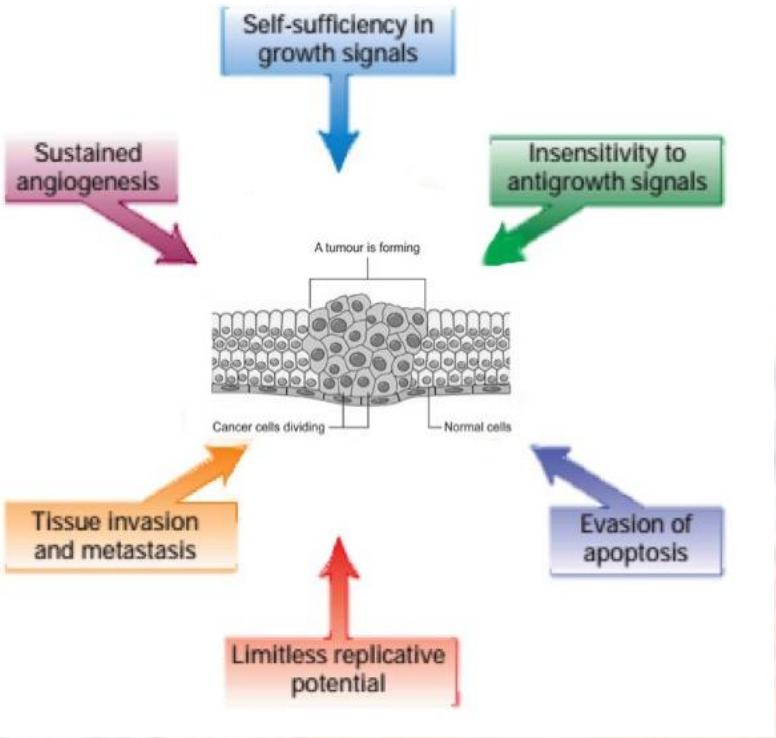


Figure: Overview of changes in cell that cause cancer

Source: Author

Viruses, especially the RNA viruses have been found to have an inherent anti-cancer effect. They have been found to have a direct cytotoxic effect on cancer cells without involving the immune system. The direct therapeutic approach of cancer treatment using viruses is called **virotherapy.** The RNA viruses like reovirus, vesicular stomatitis virus New Castle disease virus are being evaluated as viro therapeutic agents. These studies are at clinical trial stage. Genetically altered DNA viruses with enhanced specificity and cytotoxicity are also being investigated for virotherapy. Adenoviruses,Herpesviruses and Vaccinia virus are being studied for their anticancer effects.

Virus-directed enzyme prodrug therapy (VDEPT) uses viruses to deliver an enzyme into target cancer cell. This enzyme can alter an inactive precursor of a cytotoxic drug into an active form. This treatment module is in research stages and is being evaluated for its therapeutic efficacy.

Bacteriophage Therapy

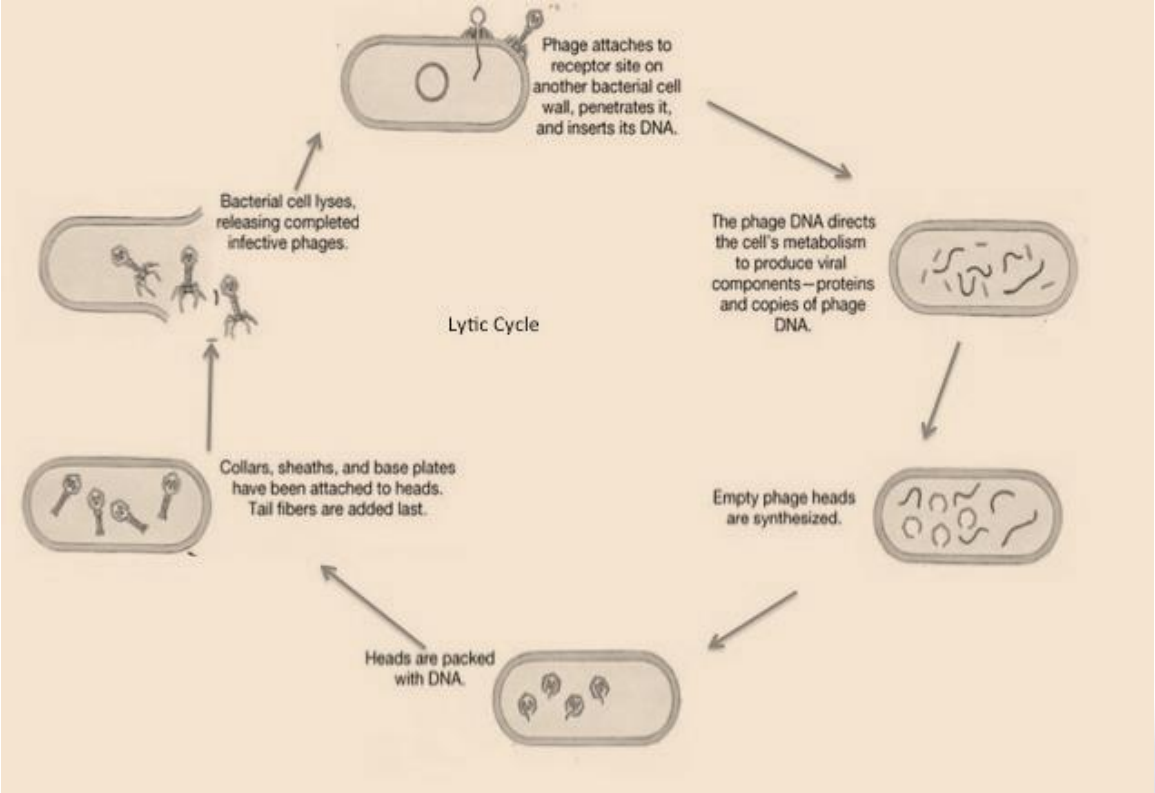


Figure: Lytic cycle - Bacteriophages infect and destroy the bacterial cells after using the host machinery for replicating itself. This property is used in phage therapy to destroy pathogenic bacteria using phages.

Source: Author

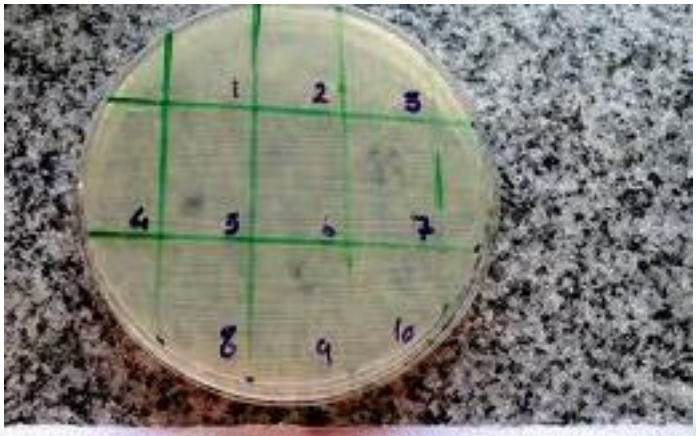
The use of bacterial viruses to infect and destroy pathogenic bacteria is known as bacteriophage therapy. This bacteriophage can be used as biocontrol agents to destroy their host bacteria. The bacteriophages undergo two types of life cycles lytic and lysogenic, which have an important bearing on their pathogenesis. The role of bacteriophages as therapeutic agents is known since earlier times. Bacteriophage preparations were used against bacterial dysentery, staphylococcal skin infections etc. However, with the discovery of antibiotics, the bacteriophage therapy was ignored. Resistance to antibiotics and emergence of methicillin-resistant *Staphylococcus aureus*(MRSA) , vancomycin –resistant *Enterococcus* (VRE) etc again opened the doors for research in bacteriophage therapy. This therapy has been used to control the bacterial pathogen *Listeria monocytogenes* in food products. The clinical trials for human pathogens have been conducted for *Enterococcus*, *E.coli, Pseudomonas aeruginosa*, MRSA etc. Bacteriophages are highly promising as therapeutic agents, however intensive systematic studies are required in this subject.

Role of Viruses in diagnosis

Viruses play a pivotal role in diagnostic procedures commonly employed in various biological sciences viz. microbiology, molecular Biology, immunology, genetic engineering etc. Besides diagnosis and treatment of disease viruses have contributed significantly to research and development of various scientific disciplines.

Most of the diagnostic techniques like southern blotting, northern blotting , dot blot, DNA and RNA sequencing, construction of genomic libraries etc. require probes for identification of DNA/RNA molecules. These probes are oligonucleotides [10-30 bases] of DNA or RNA used to detect the presence of complementary sequence in clinical or research samples. The probes are appropriately labeled with either radioactive or nonradio-active compounds for detection and are extremely sensitive, specific and convenient to use. Probes may be either double stranded or single stranded DNA or RNA (single stranded).The probes are DNA/RNA segments isolated from genome of bacteria or viruses can be used to prepare probes. cDNA (complementary DNA) probes are prepared using mRNA which have been cloned in *E.coli* using Bacteriophage vectors like λ or M13. Single stranded DNA probes can be prepared by using vector like M13. The enzyme ***reverse transcriptase*** produce by retroviruses has been extensively used in the generation of **cDNA** which is very frequently required both in research and clinical diagnosis. Viruses T4 phage also produce the enzyme ligase which can be used either in recombinant DNA technology or even in technique such as **ligase chain reaction**.

Bacteriophage typing is a technique, which employs bacterial viruses for identification of pathogenic bacteria in the clinical samples as well as for research purposes. The pathogenic bacterium to be identified is cultured on a nutrient medium contained in a petridish. The plate is then marked into squares and each square is inoculated with a specific phage. This is followed by incubation for 24 hours after which plates are observed for plaque formation. Results of phage typing are recorded according to phage sensitivity of the bacterium.



Phage typing plaque formation(e.g for staphylococcus aureus)

Figure: Phage typing

Source:http://upload.wikimedia.org/wikipedia/commons/thumb/d/dd/LambdaPlaques.jpg/220px-LambdaPlaques.jpg

Role of viruses in research

These are a number of ways in which viruses have been used for research. Some of the applications are as under. Viruses are commonly used as vector in recombinant DNA technology because of their ability to insert their content into host cells. A vector is an autonomously replicating DNA fragment into which genes of interest can be integrated for cloning. Vector should be DNA molecules which are amenable to manipulation, i.e., they should be easy to isolate and insert into host. They should carry target sites for restriction endonucleases as well as selectable markers for easy identification.

Bacteriophages are the routinely used viral vectors in genetic engineering. The lambda and M13 phage are the most commonly used *E.coli* phages. Phage vector have some advantages over the bacterial plasmid vector. Phages are naturally infective, can accommodate large DNA fragments for cloning and identification of recombinant vector is comparatively easier. The lambda bacteriophages are used extensively in DNA cloning research. The lambda genome contains an origin of replication genes for DNA replication etc. This is a temperate phage and can enter into both lytic and lysogenic cycle. In the host *E.coli*, the linear lambda genome becomes circular. The lambda genome has been modified to accommodate larger DNA insert, thus generating different types of λ vectors like λgt10, λgt11 etc.

The M13 viral vectors are used for obtaining single strands of cloned DNA. These single stranded DNA are routinely used for DNA sequencing. M13 genome is used to produce a variety of modified vectors like M13mp1, M13mp8, M13mp9 etc. The M13 viral vector can be used to clone very large sized DNA inserts. Other viral vectors used in recombinant DNA technology include cosmid vectors (containing the cos sites and pac sites of lambda genome) and phagemid vectors which has an origin of replication from phage.

These bacterial viruses have played a major role in development of genetics and molecular biology. Besides, viruses are also used for elucidation of various biochemical events. Microbiological research extensively uses viruses as both tools and model system.

Viruses have been sources of number of enzymes that are routinely used in molecular biology and genetic engineering. Reverse transcriptase an enzyme coded by the retroviruses is used for the generation of cDNA which is extensively required for construction of genomic libraries etc.

Viruses are routinely used in research in the field of animal and plant biotechnology for production of transgenics. Gene of interest from animal is initially cloned in bacterium *E.coli* and then expressed in animal cell culture or embroys using shuttle vectors derived from viruses e.g. pc DNA1.1/Amp. The animal cell cultures are transfected with viruses for research on gene therapy and vaccine production.

Research on plant genomes also uses plant viruses as vectors. The virus vector are used to produce specific products from plants. Baculoviruses are used to design vectors for production of recombinant proteins in insect cell lines eg. AcMNPV, BmNPV.

Viruses as biopesticides

The most commonly used microbial biopesticide is produced from the bacterium *Bacillus thuringiensis. However,* viruses are also used as a pesticide for killing a number of insect species like moths,bollworms,fruitworms etc. The Biopesticidal agents can either prey on pests , be parasitic on them,compete with insects or are insect pathogens .Viruses usually are pathogenic on various insect species.

Baculoviruses constitute a large viral group which infect insects and other arthropods. The Baculoviruses are extremely host specific .They form occlusion bodies, that are thick protein shells which are formed outside the nucleocapsid .The occlusion bodies are in turn surrounded by a carbohydrate covering which makes them resistant to denaturation . Baculoviruses includes two genera Nuclearpolyhedrosis virus [NPV] and Granulosis virus [Gv]. In the NPV a single occlusion body contains a number of nucleocapsids . These nucleocapsids may be packed either singly or in groups. The occlusion body in NPV is composed of virus polyhedrin protein. In case of GV the occlusion body [OB] contains only one nucleocapsid. The OB in GV is composed of viral granulin protein. The insect larvae ingest baculoviruses reach their gut and proliferate in it. From the gut viruses spread to the entire body of insect,destroying it. Death usually occurs in 4-5 days. A number of progeny virions are released, which can further infect new insect larva.

Some other viruses like cytoplasmic polyhedrosis viruses [family *reoviridae*] and entomopox viruses [family *poxviridae*] can also be used as biopesticides. However, limited work has been done on them.

Viruses as causal organism of plant disease

Plant diseases cause by viruses lead to massive agronomic losses .Viruses causes a number of diseases in plants, however there are limited studies in this field because it is difficult to cultivate and purify viruses. Some plant viruses like tobacco mosaic virus can be grown on plants protoplasts but most of the other viruses have to be cultured on either whole plant or tissue preparations, thus making it difficult to study their life cycle.

Most of the viruses infecting plants are rod shaped or spherical.They are usually RNA viruses , containing single stranded RNA in a protein coat .However ,there are a few exceptions like cauliflower mosaic virus ,which is a double stranded DNA viruses. Penetration of viruses into plant cell is difficult because of the presence of complex and thick cell wall .Therefore mechanical damage of plant cell wall is important which can be

caused by either insects or animals .Thus the most common mode of transmission of plants viruses is by insects like aphids, leafhoppers etc they pick up viruses on their mouth parts and transmit it to plants. There are basically two kinds of infections caused by plant viruses- the mosaic disease and the curling and dwarfing disease.

The mosaic diseases are the most prevalent type of diseases caused by plant viruses .This is characterized by spoiling of leaves by production of either yellowish or necrotic spots on leaves .Tobacco mosaic disease caused by Tobacco mosaic virus is the most common example of such type of disease pattern. The curling and dwarfing disease of plants also include symptoms like tumor formation, yellowing etc.

Few of the plant disease caused by plant viruses are discussed below:

Tobacco mosaic virus (TMV)

TMV is a thermal resistant virus which infects tobacco and other plants as well. It is transmitted through cell sap injury caused during clipping of the shoot .It has a positive sense RNA and codes for protein like RNA polymerase, replication proteins and movement protein etc. The symptoms of TMV infection are mosaic and even blisters on leaves. Control of this disease can be carried out by crop rotation, sanitation and use of resistant varieties.

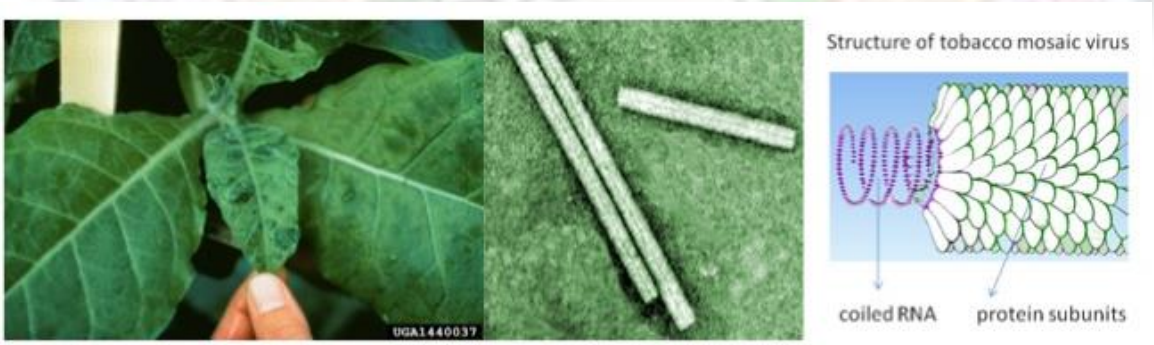


Figure: Leaves infected with tobacco mosaic virus

Source:https://d3r4ecz8hnfnqf.cloudfront.net/50a32064e4b0dc9d3964a477/full/6312327066-d562b0d95d-o.jpeg,
http://www.forestryimages.org/images/768x512/1440037.jpg

Potato virus

This plant virus is present worldwide and has a wide host range vizpotato ,tomato etc. The symptoms include mosaic and necrosis associated with dwarfing .Genome is composed of single stranded positive sense RNA .The virus is transmitted through infected tubers and insects like aphids .Infection of crops with this virus cause huge economic losses.

Figure: Potato plant infected with mosaic virus

Source:http://upload.wikimedia.org/wikipedia/commons/b/b0/Alfalfa_mosaic_virus.jpg

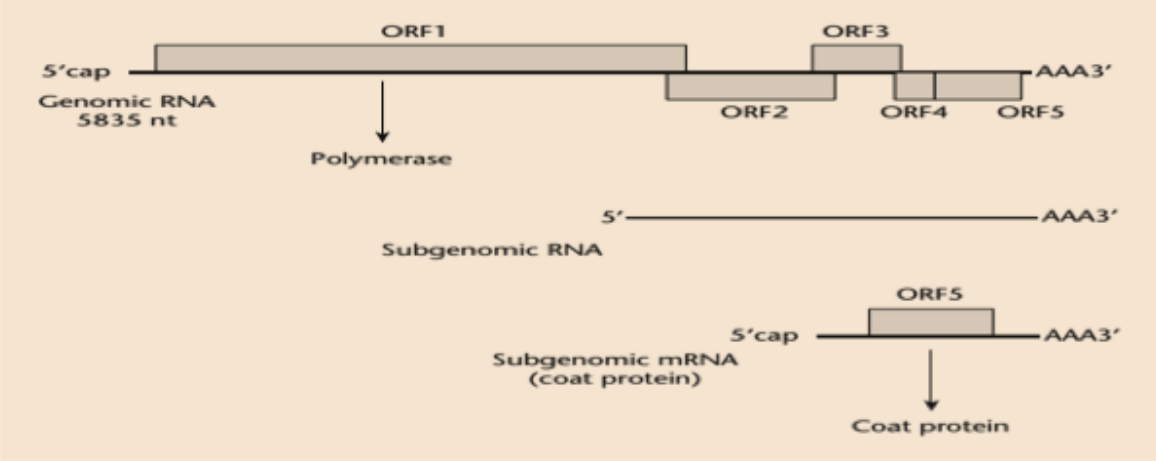


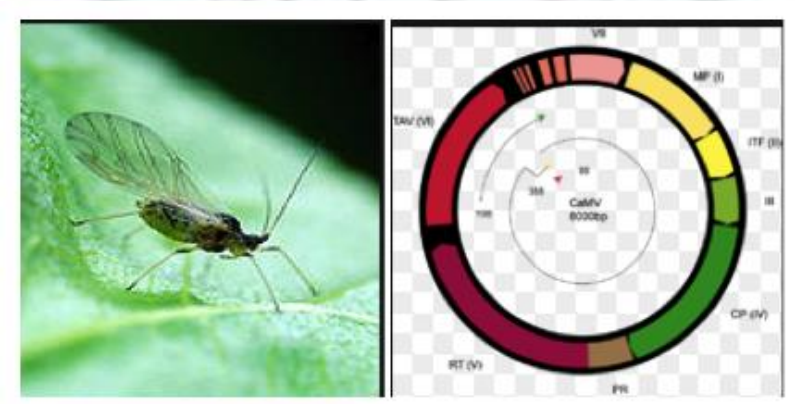
Figure: Genome map and expression of potato virus

Source:Author

Cauliflower mosaic virus

These viruses are the only group of plant virus with double stranded DNA as genetic material. This virus infects no. of plant species. The symptoms of this plant disease include leaf mottling and curling of edges .The fruits also show mottling with yellow and white patches. Cauliflower mosaic virus is transmitted by insects.

Besides viruses, viriods are cause number of plant disease. Viroids are smaller, circular RNA molecule with a rod like secondary structure with notable absence of a protein coat. They do not code for any protein and use the host cell enzymatic machinery.



A. **B.**

Figure: A. The aphid *Myzuspersicae* transmits the CaMVB. Map of cauliflower mosaic virus genome

Source:http://upload.wikimedia.org/wikipedia/commons/thumb/1/11/Myzus_persicae.jpg/140px-Myzus_persicae.jpg, http://upload.wikimedia.org/wikipedia/commons/thumb/4/46/CauliflowerMosaicRNA35S.png/200px-CauliflowerMosaicRNA35S.png

Summary

Viruses are extremely small sized infectious agents which are obligate parasitic. They are important as pathogens but have a number of benefits also. Viruses have a major role in medicine research and diagnostics . They are also important in environment management

Vaccine production: Viral vaccines confer immunity against infection with the pathogenic strains of same virus. The conventional vaccines synthesized using live attenuated viruses or killed viruses are easy to produce and economic.

Gene therapy: The introduction of functional gene into human cells to correct defective genes by replacing them is known as Gene Therapy. Gene therapy is largely used in cancer treatment.

Cancer therapy: Viruses can be directly used to prevent cancer by being the source of **anti cancer vaccines,** e.g., vaccines against hepatitis B virus (causes hepatic cancer) and human papillomavirus (cervical cancer) are commercially available.

Bacteriophage therapy: This is involve the use of bacterio phages for destruction of bacterial pathogens. This therapy has been used to successfully treat *staphylococcal* and *E.coli infections* and appears to be very promising .

Virus based diagnosis: virus play a pivotal role in diagnostic procedure commonly employed in various biological sciences viz. Microbiology, Molecular Biology, Immunology, Genetic Engineering etc.

Role of viruses in research: These are a number of ways in which viruses have been used for research. Viruses are commonly used as vector in recombinant DNA technology research procedure because of their ability to insert content into host cells. Bacteriophages are the routinely used viral vectors in genetic engineering. The lambda and M13 phage are the most commonly used *E.coli* phages.

Viral bio pesticides: They are much less significant than bacterial pesticides still research and development on various viruses as potential bio-control agents is going on. Baculoviruses are the most important group of viruses used as biopesticides

Plant viruses caused by viruses lead to massive agronomic losses. Viruses cause a number of diseases in plants. Few of the plant disease caused by plant viruses are- Tobacco mosaic virus, Potato virus and cauliflower mosaic virus.

Exercises

Answer the following questions

Q1.How can viruses be used to cure cancer? Elaborate.

Q2. Describe Bacteriophage therapy in detail.

Q3. Discuss the significance of viruses in Research and Diagnostics.

Q4.What are bio pesticides ? Elaborate the significance of baculoviruses as biopesticides?

Q5.Discuss any three important viral diseases of plants.

Objective Questions

a) The technique VDEPT uses viruses to deliver _____ into target cancer cell

1. Antibody 2. Enzyme

3.Antigen 4. Substrate

b)Bacteriophage typing is a technique for identification of?

1.viruses 2. fungi

3. bacteria 4. None of these

c)The*E.coli phages* used routinely as viral vectors in genetic engineering.

1.lambda,*Salmonella* 2. Lambda, M13

3.Both 1 and 2 4. None of these

d). Viruses which are used as Biopesticide belong to the family

1. Baculoviridae 2. PicornaViridae

3. Pox viridae 4. None of these

e) Genome of Potato virus is

1. +ive sense DNA 2. -ive sense RNA

3. –ive sense DNA 4. +ive sense RNA

Glossary

AIDS: A sexually transmitted disease, caused by human immunodeficiency virus [HIV]. The infection by HIV leads to extreme depression of the hosts immune response thus making him susceptible to a number of other opportunistic infections and some cancers

Vector: An autonomously DNA molecule like plasmid or bacteriophage , carrying marker genes for easy identification, which is used to deliver foreign gene of interest into a host cell.

Antigenic: The ability of a foreign substance to evoke an immune response

Humoral Immune response: The type of immune response which is characterized and mediated by soluble components, primarily anti bodies

Cell mediated immune response: The type of immune response which is mediated by cellular components primarily T-lymphocytes

Gene Gun: A device which is used to inject DNA into cells [transformation]. Gene gun either uses a high pressure gas or some other propellant for DNA injection

Retroviruses: RNA viruses which replicate through a DNA intermediate. They contain an enzyme reverse transcriptase which produces DNA from RNA template

Bacteriophages: Viruses which infect specifically use only bacteria as their host cells

Transgenics: A recombinant host organism [animal, plant] into which foreign genes [Transgenes] have been introduced.

References:

1. Bibek R. 1996. Fundamental of Food Microbiology, Third Edition. CRC Press.
2. Brown T.A. 2010. Gene Cloning and DNA analysis: An Introduction, (Fifth Edition). Wiley- Blackwell Publications.
3. Dimmock N.J., Easton A.J, and Leppard K.N. , 1988. Introduction to Modern Virology (Sixth Edition). Blackwell Publishing.
4. Lodish et. al. 2013.Molecular Cell Biology- (Fifth Edition) W.H. Freeman and Company.
5. Panicker A.: Textbook of Microbiology and Immunology.

6. Pelczar M. J., Chan E.C.S. and Krieg N. R. Microbiology (5th Ed.) 2008. Tata McgrawHill,New Delhi.
7. Prescott L.M.; Harley J.P. and Klein D.A. 2005. Microbiology (6th Edition). Mcgraw Hill Higher Education, New York.
8. Primrose S. B., Twyman , R.M. and Old,R.W. 2002. Principles of Gene manipulation:Sixth Edition. Blackwell Publishing.
9. Sambrook J., Fritzsch E.F., Maniatis T.1989. Molecular Cloning .A Laboratory Manual, second edition, vol1-3, Cold Spring Harbour Laboratory ,New York.
10. Tortora G.J.; Funke B.R. and Case C.L. 2008. Microbiology: An introduction (9th Ed.). Pearson Education New Delhi.