VIRUS STRUCTURE

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INTRODUCTION

Viruses are the intracellular obligate microorganisms having either DNA or RNA, but not the both. Viruses are the molecular nanomachine that comes in variety of shapes, size and require host machinery to complete its life cycle.

During the last couple of years after the concept given by Caspar and Klug regarding virus construction, high resolution structural studies using X-ray crystallography and cryo-EM techniques have build up the knowledge and understanding of structural organization in viruses.

Viruses are metastable macromolecular assemblies composed of the viral genome enclosed within a proteinaceous coat i.e. capsid. Irrespective of their shape and size, the ultimate motive of all the virus structure is designed to contain and protect the viral genome and deliver it to a specific host cell for subsequent replication of the virus. The viral genome, in addition to encoding the proteins that constitute the capsid, also encodes other proteins referred to as nonstructural proteins, so called because they are not part of the final capsid organization. These nonstructural proteins are essential for viral replication inside the host cell. In some viruses, particularly of bacterial origin, viral genome encodes a protein called scaffolding protein that may not

be part of the mature capsid but may be a critical factor in facilitating the capsid assembly (Prasad *et al.* 2012).

The size of the virus is proportional to the size of the genome. But capsid proteins contribute more than viral genome towards total mass of the virion. Capsid formation involves both a single gene and multi gene products depending upon viruses. Studies on capsid assembly helps in designing various antiviral strategies.

TECHNIQUES USED TO STUDY VIRUS STRUCTURE

Basically two important techniques are used in the structural studies of viruses such as electron microscopy and X-ray crystallography. Early electron microscopic studies of viruses by Ruska in 1939-1941 involved metal shadowing of purified virus preparations and later improvised to ultrathin sectioning and negative staining (Murphy *et al.*). X-ray crystallography studies of viruses reveled virion organization and assembly, localization of antigenic sites on the surface of virions and aspects of virion attachment and penetration into cells. This also helped in determining the structure of hemagglutinin molecule of influenza virus and the placement and variation of neutralizing epitopes on this molecule.

Electron microscopy involving negative staining technique of virus specimens provided the first glimpse of viruses and led to early classification of viruses based on shape and form (Prasad *et al.* 2012). Using computer image analysis protocol EM images of virus particle can be used to redefining the 3D structure of viruses which

advances the virus structural studies. Most of the information regarding subunit interactions in a viral capsid at the atomic level has come from X-ray crystallographic structure of spherical viruses. The closely related technique of X-ray fiber diffraction has been used to study viruses that have helical symmetry. Cryo-EM technique has allowed visualization of a variety of spherical viruses at subnanometer to near-atomic resolutions. When the virus capsid could not be crystallized, a lower resolution structure could be determined by cryo-EM, as this technique does not require the specimen in a crystalline form. Independently determined X-ray crystallographic structures of the capsid components are fitted into lower-resolution cryo-EM map of the capsid. This technique is useful in studying capsid–receptor, capsid–antibody interactions and in studying capsid-associated structural dynamics. In addition to the X-ray crystallographic and cryo-EM structural techniques, other diffraction techniques such as neutron diffraction, low-angle X-ray scattering and spectroscopic techniques have been useful in understanding the capsid organization in viruses.

STRUCTURE OF VIRUSES

A virus is composed of mainly two parts i.e. the protein coat called capsid and the genetic material enclosed within it.

CAPSID STRUCTURE

Capsid is the protein coat that surrounds the genetic material of the viruses. The capsid and enclosed nucleic acid together constitute the nucleo-capsid. The nucleocapsid of certain viruses is surrounded by a lippoprotein membranous structure

called envelope. The structural units of capsid is capsomeres seen on the surface of virion by EM and are folded polypeptide chains specified by viral genome.

Function of capsid

1. protects the nucleic acid from digestion by enzymes

2. contains special sites on its surface for attachment of virion to host cell.

3. provides proteins that help in penetration of virion to host cell membrane and in some cases, inject nucleic acid to host cell cytoplasm.

Generally three types of symmetry come into account while describing the structure of viruses. These are---icosahedral (Cubic), helical and complex symmetry.

ICOSAHEDRAL SYMMETRY

Most of the spherical viruses have this type of symmetry and the number of arrangement of subunits is determined by T number which is derived from quasi equivalence theory proposed by Casper and Klug in 1962. (Caspar & Klug, 1962). Crick & Watson (1956) observed that the majority of viruses exhibits symmetry in the structural organization of their capsids. The icosahedral symmetry implies that the capsids exhibits 6 fivefold, 10 threefold and 15 twofold discrete rotational symmetry axes and that their surface structures are hence highly ordered. Virions with icosahedrons symmetry have 12 vertices (corners), 30 edges and 20 faces, each face an equilateral triangle. Icosahedra have axes of 2, 3 and 5 fold rotational symmetry ,

which pass through their edges, faces and vertices respectively (Figure-1 and 2, Murphy, Vet. Virology).

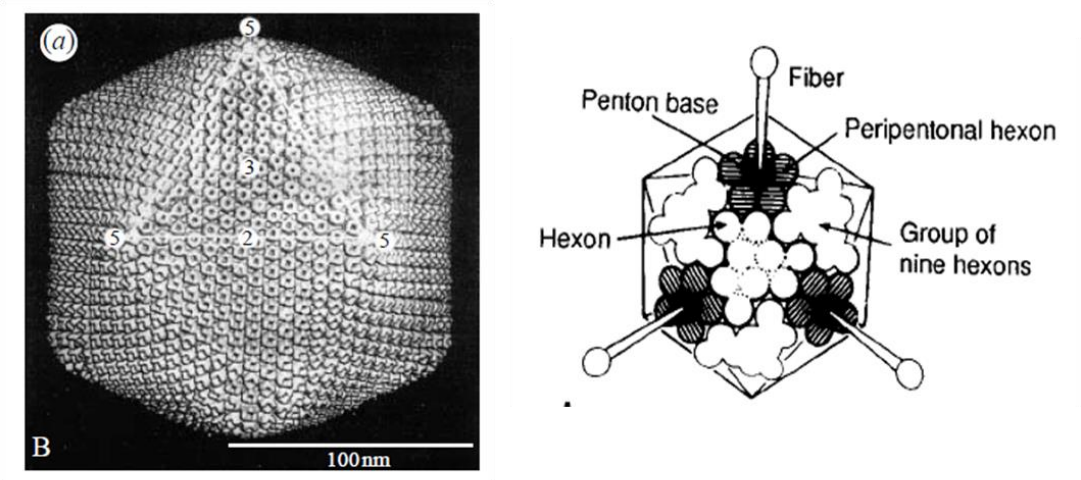

Fig-1: Icosahedral symmetry (Murphy, Vet. Virology)

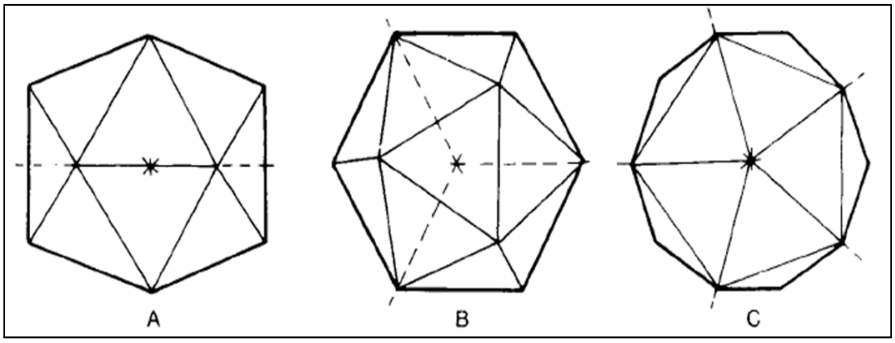

Fig-2: An icosahedron viewed along (A) twofold (B) threefold (C) fivefold axes of symmetry (Murphy, Vet Virology)

Quasi equivalence theory

This theory is one of the major tools in modern virology, universally accepted for the classification of viral capsids and the three-dimensional reconstructions of viral capsids from experimental data. But Papovaviridae group of virus doesn't obey this theory. So viral tiling theory has been introduced in 2004 to close this gap (Twarock 2004).

The quasi-euivalence theory developed by Caspar & Klug (1962) is the first approach to the prediction of viral capsid architecture. It is applicable to icosahedral viruses that exhibit protein subunits organized according to a hexagonal surface lattice. According to this theory icosahedral viral capsid consists of pentamers and hexamers. Hexamers are flat and pentamers are convex forming the twelve apexes of the icosahedrons. The same protein molecules form the pentamer as well as hexamer, but the bonding relation and their environment are not identical. Thus one subunit has quasi-equivalent relationship with the adjoining subunit justifying the name of the theory.

Triangulation Number (T- Number)

Pentamer occupy the twelve apexes in an icosahedrons thus only twelve pentamers are present but the number of hexamers varies depending upon the size of the viruses. T number is used to describe the relation between pentamer and hexamer present in the capsid. T number can be formulated as below—

$$T = h^2 + hk + k^2$$

Where T denotes the Triangulation number

where h and k are the distances between the successive pentagons on the virus surface for each axis and either 0 or positive integers. The larger the T-number the more hexagons are present relative to the pentagons(Wikipedia). T number can take only some discrete value such as 1, 3, 4, 7 etc.

Q number

Some viruses like bacteriophages have prolate shaped head which is icosahedrons elongated along the five fold axis. An icosahedrons is consists of three parts namely top and bottom cap and middle cylindrical part. This cylindrical part consists of ten triangles and characterized by this Q number which is similar to T number but it can take any positive value except 0 (Casens, 2009).

Viral tiling theory (Twarock, 2006)

This theory was proposed by Twarock. Dr Twarock developed a new mathematical description using techniques known from the study of quasicrystals i.e. alloys with atomic configurations that are non-periodic but exhibit long-range order, such as Penrose tilings. Viral tiling theory differs from the Caspar–Klug theory by the introduction of more general types of surface lattices. The tiling approach not only encodes the locations, types and relative orientations of the protein clusters in papovaviridae capsids, but it also determines the locations and types of the interactions between them. The viral tiling theory incorporates the Caspar–Klug theory, but enhances its spectrum of applications crucially.

HELICAL SYMMETRY

Tobacco mosaic virus (TMV) is the representative virus studied for helical symmetry. The simplest way to arrange multiple, identical protein subunits is to use rotational symmetry and to arrange the irregularly shaped proteins around the circumference of a circle to form a disc. Multiple discs can then be placed on top of one

another to form a cylinder, with the virus genome contained in the hollow centre of the cylinder. Closer examination of the TMV particle by X-ray crystallography reveals that the structure of the capsid actually consists of a helix rather than a pile of stacked disks(Figure-3).

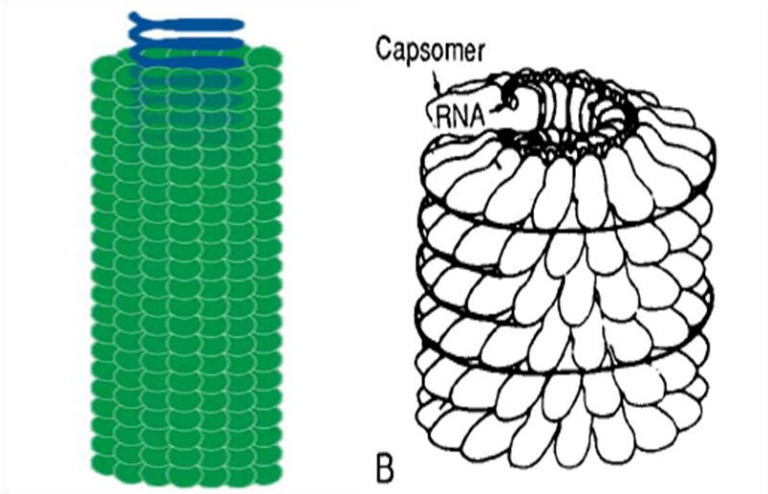

Fig-3: Helical symmetry (Murphy, Vet. Virology)

A helix can be defined mathematically by two parameters: **amplitude** (diameter) & **pitch** (the distance covered by each complete turn of the helix) [Figure-4]. In helically symmetrical nucleocapsids the genomic RNA forms a spiral within the nucleocapsid.

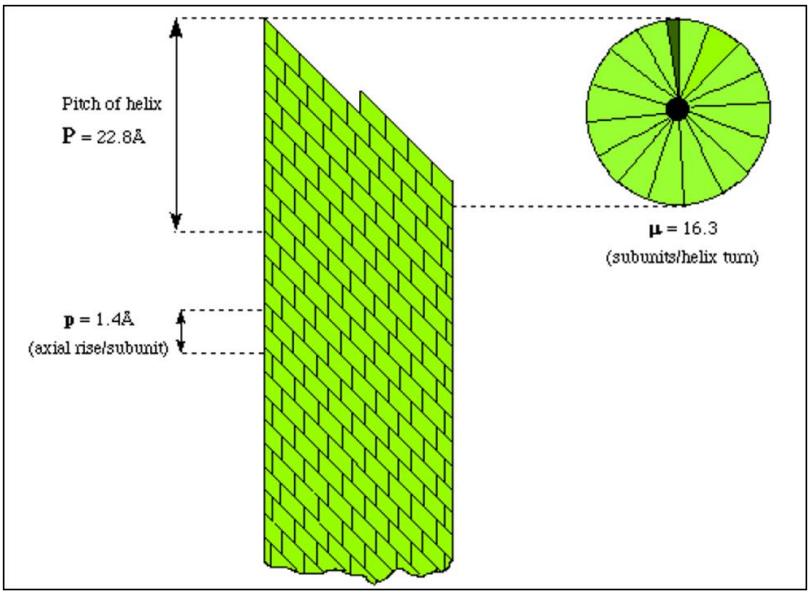

Fig-4: Pitch and amplitude of helix (Microbiologybytes, 2007)

COMPLEX SYMMETRY

It is the combination of both icosahedral and helical symmetry. Pox viruses along with bacteriophages have shown this type of symmetry. In case of bacteriophage the head comprises of icosahedral symmetry and tail has helical symmetry (Figure-5).

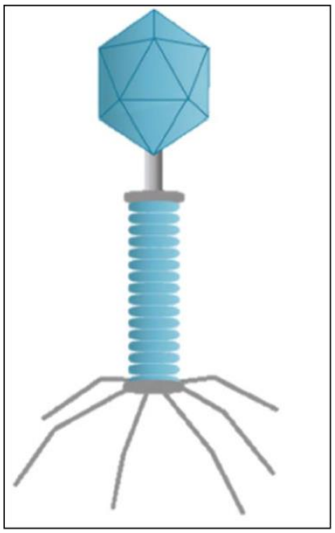

Fig-5: Structure of bacteriophage having complex symmetry (Microbiologybytes, 2007)

VIRAL ENVELOPE

During the formation of mature virus particle, several viruses acquire a lipid envelope derived from host cell organelle through the process of budding (Figure-7). Viruses such as influenza viruses, herpes viruses, corona viruses, bunya viruses, and HIV, the lipid envelop is externally located and is studded by various viral proteins, whereas in others such as alphaviruses and flaviviruses, the lipid envelop is internally located underneath the outer proteinaceous capsid layer (Prasad *et al.* 2012). With the exception of alphaviruses and flaviviruses, many of the enveloped viruses lack highly symmetric organization and are less amenable for high-resolution structural analysis. The lipids of the viral envelope are derived directly from the cellular membrane, but the proteins associated with the envelope are virus coded.

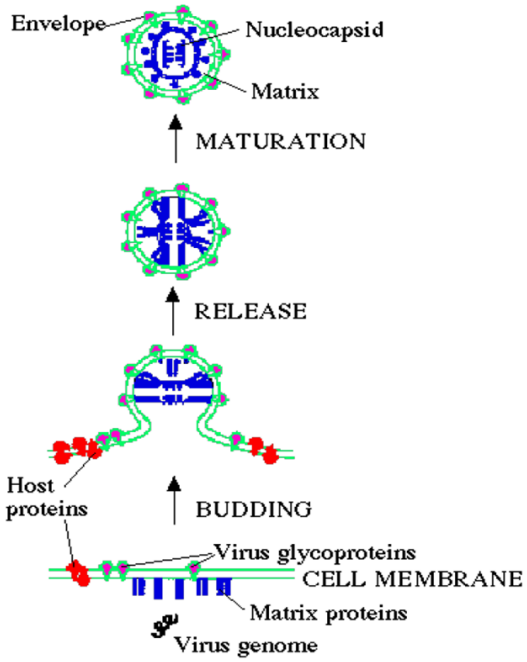

Figure-7: Viral envelope formation through budding (Microbiologybytes, 2007)

Envelope associated protein (Murphy, Vet. Virology)

These are virus encoded and help in receptor binding, membrane fusion, uncoating and receptor destruction.

Glycoproteins (Figure-6) are occur usually in the form of dimers or trimers, assemble into the virion peplomers (peplos = envelope) or spikes seen in electron micrographs on the surface of orthomyxoviruses, paramyxoviruses, rhabdoviruses, filoviruses, coronaviruses, bunyaviruses, arenaviruses, and retroviruses. Glycoproteins are transmembrane proteins, anchored to the membrane by a hydrophobic domain and on the basis of their function can be of two types as follows-

External Glycoproteins – it has single transmembrane domain help in anchoring to the envelope. Most of the structure of the protein is on the outside of the membrane, with a relatively short internal tail. Individual monomers associate to form the 'spikes' on the surface of many enveloped viruses and such proteins are the major antigens of enveloped viruses (Microbiologybytes, 2007).

Transport Channels - This class of proteins contains multiple hydrophobic transmembrane domains, forming a protein-lined channel through the envelope, which enables the virus to alter the permeability of the membrane, e.g. ionchannels (microbiologybytes, 2007).

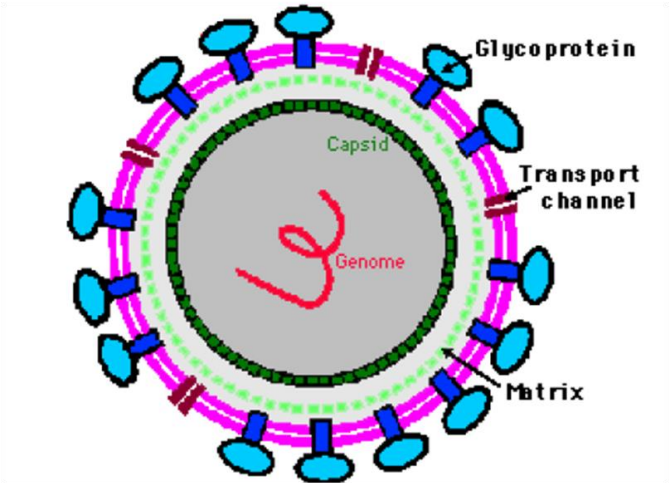

Fig-6: Viral envelope protein (Microbiologybytes, 2007)

Fusion proteins are glycosylated and are also associated with peplomers; they are involved

in key steps in viral entry and viral release.

Matrix proteins are non glycosylated and are found as a layer on the inside of the envelope of orthomyxoviruses, paramyxoviruses, rhabdoviruses, filoviruses, and retroviruses, but not coronaviruses, bunyaviruses, and arenaviruses. Matrix protein provides added rigidity to the virion; for example, the helical nucleocapsid of rhabdoviruses is closely apposed to a rather rigid layer of matrix protein, which in turn is tightly bound to the viral envelope and the internal domain of the surface glycoprotein peplomers.

Envelope Lipid

This lipid is host derivated. Lipid constitute about 20-35% of the dry weight of most enveloped viruses. 50-60% of the lipid is phospholipid and rest are of cholesterol (Murphy,).

Viral Proteins

The virions contain several different proteins but broadly classified as structural and non-structural proteins. Structural proteins are used to construct the capsid and other components of the virion and non structural are involved in various aspect of viral replication process. Capsid proteins like scaffold protein, glue protein etc. are part of viral structural proteins.

VIRAL NUCLEIC ACID

Unlike other living organisms viruses have only one type of the genetic material i.e. either DNA or RNA. The nature of either type of nucleic acid may be of single stranded or double stranded. The nucleic acid may be of monopartite (all viral genes contained in a single molecule of nucleic acid) or multipartite/ segmented (viral genes distributed in multiple segments of nucleic acid). All the viral genomes are haploid except retroviruses. Similarly hepadnaviruses have partial double stranded DNA i.e. 85% of the genetic material is of double stranded and rest are single stranded. Segmented genetic material is seen in the viruses family like reoviridae, birnaviridae, orthomyxoviridae, bunyaviridae and arenaviridae.

Viral genomic DNA

All viral DNA are monopartite. Except circoviridae and parvoviridae rest of the viruses have double stranded DNA as their genetic material. The DNA may be linear or circular. Circular DNA is seen in only papovaviridae, hepadnaviridae and circoviridae.

Viral genomic RNA

Except reoviridae and birnaviridae all othe viruses have single stranded RNA. Single stranded RNA can be defined according to its sense also called as polarity.

positive sense---if it is of the same sense as mRNA i.e. it can direct the synthesis of protein. This type is seen in picornaviruses, caliciviruses (Polyadenylated at 3' end), togaviruses, flaviviruses, coronaviruses and retroviruses (capped at its 5' end).

Negative sense----if genomic nucleotide is complementary to that of mRNA. Viruses include under this are paramyxoviruses, rhabdoviruses, filoviruses, orthomyxoviruses, arenaviruses, bunyaviruses.

Ambisense—partly positive and negative sense. Seen in case of some genus of arenaviruses and bunyaviruses.

Reverse Transcriptase Viruses

Reverse transcriptase is the RNA dependent DNA polymerase enzyme. The Hepadnaviruses and Retroviruses have this unique enzymes where a DNA strand is synthesized from a mRNA strand.

VIRAL STRUCTURE DATABASES

Due to the advance in molecular biology field structure of viruses studied in detail. So in recent times several databases are created for virus structure which will helpful for various studies. ViperDB, VIDA, VirusMint and PhEVER are the few databases available online for viral protein structural databases. ViperDB archives the icosahedral virus capsid structures, whereas VIDA incorporates open reading frames of animal virus

proteins. The VirusMint database contains protein interactions between viral and human proteins from 490 unique viral proteins of more than 110 different strains. PhEVER is a recently developed database that aims to provide accurate evolutionary and phylogenetic information for the analysis of virus-virus and virus-host lateral gene transfer (Sharma *et al*. 2011).

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