

# Molecular Virology

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An essay in the Dec 2001 issue of ASM News subtitled "So what is life?". It is written as a post script to a book by Erwin Schrodinger in 1944 entitled "What is Life?" This was a book that influenced many young scientists of the time.

In the article in the ASM News by Franklin Harold, the author make the point that in general we have no problem distinguishing living from non living objects, and he quotes others to the effect that living organisms are distinguished by two properties:

- 1) they are able to reproduce themselves and
- 2) they are capable of evolution by natural selection

This is a good point at which to begin because viruses are agents that cannot easily be classified as living or non living. Whereas in the context of a living cell or organism, they do satisfy the two criteria that I just listed, outside of a living cell, they do not, and exist only as an inert complex of macromolecules.

So 60 years after Schrodinger, although we can usually tell living objects from non living objects, it is still not an easy matter to clearly define life.

## 1. Historical Perspective:

1. Viral diseases were known in antiquity. We know from writings and drawings that diseases such as polio, rabies, and probably smallpox existed in ancient times.
2. Learned that certain diseases caused by viruses could be prevented, by the use of vaccines, long before anyone had any concept of what a virus is.
  - 18<sup>th</sup> century: Jenner and vaccination with cowpox to prevent smallpox.
  - 19<sup>th</sup> century: Pasteur developed a vaccine for the prevention of rabies.
3. Golden age of medical microbiology, in the last three decades of the 19<sup>th</sup> century.
  - Pasteur, Koch, Lister: the demonstration that infectious diseases of man and animals were associated with specific bacterial species.
4. Study of plant diseases in the 1890's led to the first preliminary definition of what we now call viruses.

Tobacco mosaic (TM) disease. A disease that can be transmitted from plant to plant.

In 1892 Ivanowsky in Russia, and in 1898 Beijerinck in Holland, working on TM disease showed that the agent that caused this disease could pass through porcelain filters that retained bacteria. Hence it was very small, smaller than any bacteria known at the time. But what was the nature of this agent. Was this **filtrable agent** particulate, or soluble, e.g. a toxin? Whatever it was, it was clearly able to replicate.

Historically this work is considered to be the first demonstration of an infection caused by what we now know to be a virus, although at the time there was no idea of the nature of this filtrable agent.

5. The first filtrable agent shown to cause a disease in animals was foot and mouth disease virus (FMDV).

6. The first filtrable agent shown to cause a human disease was yellow fever (YF) virus.

7. TMV was the first virus to be crystallized, the first virus shown to have RNA as its genetic material, and the first virus for which it was shown that the RNA was infectious. We must acknowledge the debt of animal virology to work with plant and bacterial viruses.

8. Medical virology progressed rapidly during the first half of this century, as many diseases were shown to be caused by filtrable agents. These included for example, measles, mumps, chickenpox, influenza, polio.

Progress in learning about the nature of viruses, however, was slow.

Half way through the 20<sup>th</sup> century, in textbooks, viruses were still classified on the basis of the organ system that was affected, or the type of disease they produced. Viruses had to be grown in animals or in embryonated eggs.

9. Beginning in the mid century, the pace began to accelerate, largely because of critical advances in methodology. **Landmarks** along the road to the rapid progress of animal were

- 1) the development of the electron microscope (1940s)

- 2) the ability to grow animal cells in culture
- 3) the ability to grow viruses in cultured cells.(Enders). One of the first fruits of this accomplishment was the development of the polio vaccine. The ability to grow animal viruses in cultured cells also made possible the development of the plaque assay for animal viruses, and thus made possible the more ready quantitation of animal viruses.
- 4) the study of bacteriophage and the development of molecular biology, and finally recombinant DNA technology led to an explosion of knowledge about viruses. It is now possible to sequence a viral genome, and study its proteins without even being able to grow the virus in culture. See, for example, hepatitis C virus.

## **2. What is the essence of a virus, how do we define a virus, and where do we find viruses?**

They are found in organisms in all three domains of life: Archaea, Bacteria, and Eukarya. Viruses challenge our definition of life.

Whether they are living or non-living, they have at least one attribute of living organisms: they are able to faithfully reproduce themselves. Yet, by themselves they have no intrinsic metabolism of their own.

Recently, a new family of viruses has been described, Mimiviridae. The mimivirus is closer to the threshold of a cell (or of life) than any other known virus. In fact, because of its size it was first thought to be a bacterium.

Mimiviruses were first found in the cytoplasm of an amoeba.

What is striking about the mimiviruses are their size: 400 nm in diameter or twice the size of the smallest bacterium (mycoplasma), the size of their genome ( $2 \times 10^6$  bp) and the number of genes they encode (about 1200). The smallest mycoplasma encodes about 500 genes. Poliovirus has about 7300 nucleotides.

Mimiviruses somewhat resemble other large DNA viruses that replicate in the cytoplasm, such as poxviruses.

Some of the enzymes they encode have rarely if ever been described in viruses before.

Some examples are enzymes involved in nucleotide biosynthesis, DNA repair, translation, glutamine metabolism, polysaccharide biosynthesis. As well, they encode proteins which act as protein chaperones.

The diversity of viruses is undoubtedly much greater than we have ever imagined.

For example, it is estimated that in the ocean there are about  $10 \times 10^9$  viral particles/ per liter. Most of them are probably unknown to us.

Were there transitional forms between viruses and cells?

### 3. The Nature and Properties of viruses:

Which of these properties are unique to viruses?

1. **Size:** Viruses are filtrable agents and are therefore small. Range from 25-30 nm to 250-300 nm in diameter. Some viruses are spherical, but some are rod shaped and can be very long. But, Chlamydiae and mycoplasma can be as small as 300 nm. So largest viruses overlap in size with smallest bacteria.
2. **Ability to replicate, given a suitable host:** If we put a small amount of virus into a suitable host, (an animal, an egg, cultured cells), depending on the virus and the host, after a certain time the amount of progeny virus we obtain may represent a 100-1000 fold or more increase over the viral inoculum.
3. **Viruses are obligate intracellular parasites:** However, most Rickettsiae and the Chlamydiae are also obligate intracellular parasites.
4. **Usually viruses contain only one type of nucleic acid, RNA or DNA,** but now we know there are exceptions to this rule.
5. **Composition of viruses:**

All viruses contain at the minimum, nucleic acid and protein. Viruses which contain only nucleic acid and protein are said to be “naked”. The protein(s) serves to protect the nucleic acid, and in this case would also contain sites which interact with the receptors for the virus on the cell surface. The viral nucleic acid and the protein in which it is enclosed are together called the **nucleocapsid (NC)**. In many cases, the NC is equivalent to the viral particle.

Some viruses contain, in addition to nucleic acid and protein, a membrane or envelope, in which the NC is enclosed. Such viruses would contain lipid and CHO, in addition to nucleic acid and protein. Virus-coded glycoproteins are embedded in the membrane. These envelope glycoproteins function to bind the virus to specific cell receptors; they also contain fusion sequences responsible for fusion of viral and cellular membranes.

#### **6. Manner by which viruses replicate;**

All forms of life that we know of grow by binary fission: one cell divides into two. The cell as a unit is always present; it never ceases to exist.

When a virus enters a cell, it ceases to exist as an intact virion. It is partially or completely disassembled. The viral genes then direct the synthesis of the components needed to make many new progeny virus particles. These components include the viral genetic material, RNA or DNA, and the viral proteins. The genetic material and the viral proteins are often made independently of each other, both spatially and temporally. For example, viral proteins, like cellular proteins are always made in the cytoplasm; but the viral genetic material, depending on the virus, may be made either in the cytoplasm or the nucleus.

Once sufficient amounts of the viral nucleic acid and the viral proteins are made, these components begin to be assembled into viral particles. This process is sometimes referred to as a process of **self assembly**, but it is undoubtedly a complex process.

Contrast this with how cellular organisms, eukaryotic or prokaryotic, form new cells. All cells arise from pre-existing cells, and the cell as such never disappears.

We know now that there are infectious agents which can replicate, and which are even simpler than viruses. I refer, for example, to viroids about which you will hear later and to prions,,

#### **4. Steps in viral replication, and the idealized viral growth curve:**

1. **adsorption:** specific cell receptors may be involved.
2. **entry:** delivery of the viral NC into the cytosol, or into the nucleus, where the first steps in replication occur. Enveloped vs non-enveloped viruses.
3. **synthesis of proteins needed for replication of the genome, RNA or DNA.** Often these are non-structural proteins.
4. **replication of the genome**
5. **synthesis of structural proteins**
6. **packaging of the genome** i.e. formation of nucleocapsids
7. **assembly and/or release** of virus: with some viruses this step may involve the acquisition of an membrane or envelope.

This is a general overview, and the specifics will vary from virus to virus.

The latent period is defined as the period from the time of infection until the time when progeny extracellular virus can be detected.

#### **5. Taxonomy of viruses and features of viruses used in classification:**

. Below are listed the properties of viruses which we are used for the classification of viruses. Taxonomy helps us group viruses.

1. **type of nucleic acid in the genome:** RNA or DNA, ss or ds, segmented or nonsegmented. In the case of ss RNA genomes, the polarity of the RNA.
2. **size and organization of genome** and strategy of replication.
3. **symmetry and structure of nucleocapsid**  
helical vs icosohedral (or sometimes complex)
4. whether virus is **enveloped or naked.**
5. **(sequence relationships)**
6. **(morphology and size)**
7. **(number and location of proteins)**
8. **(antigenic properties)**

## 9. (biological properties)

-latency of Herpes viruses.

-arthropod transmission of flaviviruses

1. **Family names** end in -viridae e.g. Picornaviridae

2. names of **genera** end in -virus e.g. enterovirus

3. **within genera**, have specific viruses, e.g. poliovirus, and specific strains of viruses

Thus poliovirus is in the family Picornaviridae, genus enterovirus, and there are three antigenic strains of poliovirus, 1,2, and 3.

Viruses in a given family resemble each other, with respect to the properties, we have just gone over.

4. Once viral genomes began to be sequenced, it became apparent that there were significant, although often limited, sequence homologies between genomes of viruses that were in quite different families and had been thought to be completely unrelated e.g. between the genomes of certain plant viruses and certain animal viruses. These sequence homologies, as well as certain similarities in genome organization, were sometimes seen even between viruses that had completely different physical structures.

Thus the concept of **superfamilies** was proposed.

## 6. Effect of viruses on cells:

1. Some viruses can kill cells. In tissue culture we say that they cause cytopathic effect or CPE. In a whole organism, or whole animal, we say a virus may be virulent, i.e. it causes disease, or avirulent, it either does not cause disease, or it causes only a mild form of the disease.

2. Some viruses have a temperate relationship with their host cells. The viruses multiply, and the cells show no evidence of any harmful effects on cell growth or function, at least in tissue culture. In a whole animal, however, the appearance of foreign viral antigens may trigger an immune response which may lead to severe cell damage.

3. Some viruses transform cells, that is they confer a neoplastic phenotype on

their host cell. Such cells develop properties of tumor cells.

### **7. Measurement and quantitation of viruses:**

1. The plaque assay remains the gold standard, because it measures the titer of infectious particles. We use the term, pfu, for plaque forming units.
2. Many viruses agglutinate red blood cells. Thus, can describe a virus stock in terms of hemagglutinating units or HAU/ml (for example: influenza virus). But viral particles which are capable of hemagglutination are not necessarily infectious. Also, viral subunits can agglutinate rbc's.
3. If no plaque assay available, sometimes have to use an animal model, and measure some endpoint, such as disease or mortality. Calculate LD50/ml.
4. Many immunologically based tests. Complement fixation; ELISA test. Again, these measure viral antigens, not infectious virions.
5. Measurement of viral nucleic acids. Quantitative PCR, or RT-PCR in the case of RNA viruses. Determine concentration of viral RNA molecules in the blood. Indication of viral load in case of HIV.

### **8. Diagnosis of viral infections:**

1. Look for the virus or viral components: see above. These might include infectious virus, viral antigens (proteins or viral glycoproteins), viral nucleic acid.
2. Look for immune response to the virus: Usually there is a humoral and a cellular immune response, but for practical reasons, it is much easier to measure antibody response.

To associate an acute illness with a given virus, must show an increase (usually at least 4-fold) in antibody titer, when comparing the acute and convalescent sera.

### **9. What are the new frontiers of virology?**

1. We will continue to find new viruses that cause human disease: e.g. Nipah virus in SE Asia, SARS, H5N1 influenza virus
2. Development of new and better vaccines.
3. Better understanding of the interactions between the virus and the host

organism, not only in terms of the classical immune response, but also in terms of our newer understanding of innate immunity, the interferons and chemokines. With the more complex viruses, we are learning how viruses have evolved strategies to circumvent the host defense systems.

4. Relationship between viruses and chronic degenerative diseases.

**The Baltimore classification of viruses** relates to their genomes and how they generate their mRNAs.

Seven groups of viruses, but each group contains multiple families.

1) ds DNA genome

2) ss DNA genome

3) ds RNA genome

4) ss (+) or positive-strand RNA genome

5) ss (-) or negative-strand RNA genome

6) reverse transcribing RNA viruses: have a ss (+) strand RNA genome, but replicate via a DNA-intermediate.

7) reverse transcribing DNA viruses: have a gapped circular ds DNA genome, but replicate via an RNA intermediate.

Families of RNA Viruses				
Genome			Envelope	Family Name
ss or ds	sense	segmented		
ds		yes	no	Birnaviridae Reoviridae
ss	positive	no	yes	Coronaviridae Flaviviridae Togaviridae
ss	positive	no	no	Caliciviridae Picornaviridae
ss	positive (diploid) reverse transcribing		yes	Retroviridae
ss	negative	yes	yes	Orthomyxoviridae
ss	negative	no	yes	Filoviridae Paramyxoviridae Rhabdoviridae
ss	negative and ambisense	yes	yes	Arenaviridae Bunyaviridae

Families of DNA Viruses		
ds or ss genome	envelope	Family Name
ds	yes	Herpesviridae Poxviridae
ds	no	Adenoviridae Papovaviridae
ss	no	Parvoviridae
ss/ds reverse transcribing	yes	Hepadnaviridae