Editorial

An update on virology and emerging viral epidemics

Dimitrios Vlachakis, Karozou Argiro and Sophia Kossida

Bioinformatics & Medical Informatics Team, Biomedical Research Foundation, Academy of Athens, Soranou Efessiou 4, Athens 11527, Greece

Correspondence should be addressed to Dimitrios Vlachakis; Phone: +30 210 6597 647, Fax: +30 210 6597545, Email: dvlachakis@bioacademy.gr

Introduction

A virus is a small infectious agent that is able to replicate only inside the living cells of an organism, known as a host. Viruses can infect all types of organisms, from animals and plants to bacteria and archaea (Koonin *et al.* 2006). They have been characterized as obligate intracellular parasites since they lack the metabolic enzymes and equipment for making proteins (Reece *et al.* 2010). In other words, they can reproduce and carry out metabolic activities only within a host cell. Hence, lacking these basic mechanisms, most viruses are little more than genetic material contained in protein coats (Crawford 2011).

It is often debated whether a virus should be considered a living organism or a non-living chemical system. Some biologists claim that they are not "alive" but exist in a shady area between life-forms and chemicals. As a matter of fact, the Latin root for the word *virus* means “poison”. A simple phrase used recently by two researchers describes them aptly enough: Viruses lead “a kind of borrowed life” (Villarreal 2004). A virus will remain dormant until it is able to infect the next host, activate and replicate. Some viruses can live in an open place for a short time, in some cases, only a few hours. Viruses use the most efficient methods to locate a host, create copies, and spread to other hosts (Dimmock *et al.* 2007).

They are considered to be formidable pathogens of animals and plants. The damage that they can cause depends on the type of the virus and the host cells. In some cases, they can damage or kill cells by inflicting the release of hydrolytic enzymes from lysosomes. In addition, some viruses cause host cells to produce toxins that lead to disease states, and others have molecular components that are toxic, such as envelope proteins. How much damage a virus causes depends partly on the ability of the infected tissue to regenerate by cell division. For instance, damage inflicted by poliovirus to mature nerve cells is permanent, since these cells cannot normally be replaced. It should be clarified that many of the temporary symptoms associated with viral infections, such as fever and aches, result from the response of the immune system itself rather than from cell death caused by the virus (Reece *et al.* 2010).

Obviously, beyond their pathogenic role, viruses have experimental and research value. Molecular biology, biotechnology and a variety of medical applications were born in the laboratories of biologists studying viruses that infect bacteria. Their value as experimental systems contributed to a better understanding of the fundamental processes of DNA replication, transcription, and translation and provided evidence that genes are made of nucleic acids (Vlachakis 2009). Moreover, they led to the development of techniques related to gene manipulation i.e. in gene therapy viruses are used as agents of gene transfer.

The Discovery of Viruses: Scientific Inquiry

Scientists discovered viruses in the late 1800s by studying a plant disease; tobacco mosaic, which stunts the growth of tobacco plants and gives their leaves mosaic coloration. In fact, researchers were able to detect viruses indirectly long before they were actually able to see them. In 1883, Adolf Mayer, a German scientist, discovered that the disease could be transmitted from plant to plant by simply rubbing sap extracted from diseased leaves onto healthy ones (Mayer 1886). However, Mayer was not able to determine the infectious microbe. He just suggested that the disease was caused by unusually small bacteria that were invisible under a microscope.

In 1892, this hypothesis was tested by Dimitri lvanowsky, a Russian biologist who gave the first concrete evidence for the existence of a non-bacterial infectious agent. Specifically, he designed a filter to remove bacteria, known as Chamberland filter candles. He passed sap from infected tobacco leaves through it and showed that the sap still produced mosaic disease (Iwanowski 1903). But Ivanowsky remained rather

convinced that the causal agent was an unculturable bacterium. He reasoned the bacteria were too small to be retained on the employed filter or made a toxin that could do so. The Dutch botanist Martinus Beijerinck carried out experiments that ruled out the second possibility, showing that the infectious agent in the filtered sap was able to reproduce and multiply in the host cells of the tobacco plant (Beijerinck 1898).

Beijerinck is generally credited with being the first scientist to voice the concept of a virus. After a series of other experiments, he showed that the mysterious agent of mosaic disease could not be cultivated on nutrient media in test tubes or petri dishes, unlike bacteria used in the lab at the time. Beijerinck imagined a reproducing particle much smaller and simpler than a bacterium. Hence, he coined the term "virus" to indicate that the causal agent of tobacco mosaic disease was of non-bacterial nature.

In 1935, the American scientist Wendell Stanley crystallized the infectious particle, now known as tobacco mosaic virus (TMV), confirming Beijerinck's suspicions. In addition, he showed that this virus remains active even after crystallization (Iwanowski 1903). TMV was the first virus to be crystallized. Subsequently, TMV and many other viruses were actually seen with the help of the electron microscope (Kausche *et al.* 1939). The investigations of tobacco mosaic disease and discovery of its viral nature were fundamental in the establishment of the basic concepts of virology.

Structure, Lifecycle and Evolution

Examining the structure of viruses more closely reveals that the nucleic acid of a virion (the entire infectious virus particle outside the cell) is enclosed within a protein capsid and sometimes a membranous envelope composed of viral proteins that helps the virus enter the cell. In fact, the tiniest viruses are only 20 nm in diameter and millions could easily fit on a pinhead.

Viral Genomes

Viral genomes may consist of double or single stranded DNA (ds- or ssDNA), double or single stranded RNA (ds- or ssRNA); this depends on the type of virus. The term "DNA" or "RNA" virus is based on the kind of nucleic acid that makes up its genome. In addition, the genome is usually organized as a single linear or circular molecule of nucleic acid, although the genomes of some viruses consist of multiple molecules of nucleic acid (e.g. influenza virus).

Capsids and Envelopes

The capsid is the protein shell that encloses the viral genome. Capsids are composed of a large number of protein subunits called *capsomeres,* but the number of different *kinds* of proteins in a capsid is usually small. Capsomeres are closely associated with the nucleic acid and reflect its configuration; either a rod-shaped helix or a polygon-shaped sphere (Lidmar *et al.* 2003). Some viruses, such as bacteriophages (viruses that infect and replicate within bacteria), have developed more complex structures due to constraints of electrostatics and elasticity (Vernizzi *et al.* 2011).

The capsid performs three main functions. Firstly, it has a protective role as it prevents the nucleic acid from being digested by enzymes. Secondly, it allows the virion to attach to a host cell through its special sites on its surface. Finally, some proteins of the capsid enable the virion to penetrate the host cell membrane and inject the infectious nucleic acid into the cell's cytoplasm.

For instance, the tobacco mosaic virus has a helical capsid since it is made from over a thousand molecules of a single type of protein arranged in a helix with the overall shape of a rigid rod (Branden & Tooze 1991). Furthermore, adenoviruses, which infect the respiratory tracts of animals, have a capsid that is composed of 252 identical proteins organized in a polyhedral shape, with 20 triangular facets -an icosahedron; thus, these and other similarly shaped viruses are referred to as *icosahedral viruses (*Caspar & Klug 1962*)*.

Some viruses have a membranous envelope that surrounds their capsids. The envelope functions as an accessory structure that helps them infect their hosts. It is composed of two lipid layers interspersed with protein molecules (lipoprotein bilayer). Viral envelopes, which are derived from the membrane of the host cell, may contain its membrane proteins and phospholipids. The virus obtains the lipid molecules from the cell membrane during the viral budding process. They also contain materials (proteins and glycoproteins) of viral origin. It is worth noting that sometimes the virus creates a hybrid structure of cell-derived lipids and virus-derived proteins, replacing the proteins in the cell membrane with its own proteins. Additionally, many viruses develop spikes made of glycoproteins on their envelopes that contribute to viral binding.

General Features of Viral Reproductive Cycles

The term "host range of a virus" represents the variety of hosts that each type of virus can infect and it is, in fact, actually limited. Due to the evolution of viral recognition systems, a virus is able to identify host cells by a "lock-and-key" fit between viral surface proteins and specific receptor molecules on the membrane of the host cells. A viral infection begins when a virus

binds to a host cell and the viral genome makes its way inside. The viral entry is dependent on the host cell and the type of the virus. It can occur via three processes; membrane fusion, endocytosis and genetic injection. In the first case, the viral receptors attach to the receptors on the surface of the host cell and, thereafter, receptors may initiate the puncture of the cell membrane or fusion with the host cell (Campadelli-Fiume *et al.* 2007). During entry via endocytosis, the virus "tricks" the cell into thinking that the virus knocking at the door is nothing more than nutrients or "harmless goods" (Dimmock *et al.* 2007). Finally, in the case of genetic injection, the virus only injects its genome into the host cell after attaching to its surface. An example of the latter includes the phage viruses (Sebestyén *et al.* 2006).

Once the viral genome is inside, the proteins it encodes reprogram the cell to translate the viral genome and transcript viral proteins. The host provides the nucleotides for creating viral nucleic acids, as well as enzymes, ribosomes, tRNAs, ATPs, amino acids, and other components necessary for making viral proteins. The DNA polymerase of the host cell is used in order to replicate the genome of the DNA virus. In contrast, RNA viruses use their own RNA polymerase, encoded by its viral genes. Recent studies have employed bioinformatics techniques to study the diverse viral enzymes (Sellis *et al.* 2009, 2012, Vlachakis *et al.* 2013a, b).

Consequently, the viral nucleic acid molecules and capsomeres self-assemble into new viruses. The cell is now no longer useful to the virus; therefore it must find a new host. Hence, the simplest type of viral reproductive cycle ends with the exit of hundreds or thousands of viruses from the infected host cell, a process that is called shedding and often damages or destroys the cell. Such cellular damage and death, as well as the body's responses to this destruction, cause many of the symptoms associated with viral infections. The viral progeny that exit a cell have the potential to infect additional cells, spreading the viral infection (Reece *et al.* 2010).

It is worth noting that some viruses have the potential to "hide" inside the host cell, either to defend itself against the host cell defenses or immune system, or because the conditions are not appropriate for the virus to make more copies. This "hiding" is called latency. During this time, the virus will remain inactive until an external stimulus prompts it into activation, or the restart of the life cycle.

There are numerous variations of the viral reproductive cycle. For instance, in the case of phages two alternative mechanisms can be described: the lytic and the lysogenic cycle. The first one culminates in the death of the host cell as it triggers the bacterium lysis and the release of the phages. Contrary, the lysogenic cycle allows replication of the phage genome without destroying the host. The term *lysogenic* implies that prophages have the potential to generate active phages that lyse their host cells (Reece *et al.* 2010).

There are a lot of variations concerning the mechanisms of viral infection and production of new viruses. The nature of the viral genome (DNA/ RNA, single-stranded/double-stranded) is a principal variable. The broadest variety of RNA genomes is detected among viruses that infect animals, even though most plant and some phages viruses are RNA viruses.

More specifically, ssRNA viruses are further classified into three classes (lV-VI) depending on the function of the RNA genome in a host cell. The genome of class IV viruses can directly serve as mRNA and hence be translated immediately after infection. In class V viruses, the RNA serves as a template for RNA synthesis. In this way, it is transcribed into complementary RNA strands (cRNA) which is used both as mRNA and as template for the synthesis of additional copies of genomic RNA. Finally, the class VI viruses are equipped with the reverse transcriptase enzyme, thereby transcribing RNA to DNA. Of particular medical importance is HIV (human immunodeficiency virus); the well-known retrovirus that causes AIDS (acquired immunodeficiency syndrome).

Evolution of viruses

There is a scientific debate concerning the origins of viruses. Since they depend on cells, it seems likely that viruses are not the descendants of precellular forms of life but evolved after the first cells appeared (Iyer *et al.* 2006). There are three main hypotheses about the origins of viruses. According to the regressive hypothesis, viruses may have once been small cells that parasitized larger cells (Regenmortel 2010). The other is the cellular origin hypothesis, which refers to viruses that may have evolved from bits of DNA or RNA that "escaped" from the genes of a larger organism. The escaped DNA could have come from plasmids or transposons. In this regard, plasmids, transposons, and viruses all share an important feature: they are mobile genetic elements (Palaiomylitou *et al.* 2008, Vlachakis *et al.* 2013a).

Emerging Viruses

Newly discovered viruses that increase or have the potential to increase in incidence are often referred to as emerging viruses. The deadly viruses of AIDS, some types of influenza (like the H7N9 strain), Ebola, SARS (severe acute respiratory syndrome) and West Nile are representative cases of emerging viruses that

affect and cause death of millions of people.

There are three processes that attempt to explain how such viruses can burst on the human scene, giving rise to harmful diseases that were previously rare or even unknown. The first is mutations, which is very usual in RNA viruses. RNA viruses tend to have higher rates of mutation due to errors in RNA replication as RNA polymerase lacks proofreading abilities. Hence, some mutations lead to new genetic varieties (strains) that can cause disease, even in individuals who are immune to the ancestral virus. A representative case is that of general outbreaks of flu, or flu epidemics that are caused by new strains of the influenza virus.

The second factor is associated with the dissemination of a viral disease from a small, isolated human population. Technological, social and environmental factors could be involved in this case. For example, HIV was an unnoticed human disease for decades before suddenly beginning to spread around the world. This event was favored by numerous factors including affordable international travel, sexual promiscuity, blood transfusions, and the abuse of intravenous drugs.

A third source that leads to the emergence of viral diseases is the spread of existing viruses from other animals. These animals are said to act as a natural reservoir for that virus since they harbor and transmit a particular virus but are unaffected by it. It is estimated that about three-quarters of new human diseases originate in this way. SARS virus is a classic example: a species of bat has been identified as its likely natural reservoir. Bats are sold as food in China, and their dried feces are even sold for medicinal purposes; either of these practices could provide a route for transmission of the virus to humans.

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