# An Expanded View of Viruses

Urs F. Greber 1) & Ralf Bartenschlager 2)

1) Department of Molecular Life Sciences, University of Zurich, Winterthurerstrasse 190, 8057 Zurich, Switzerland
2) Department of Infectious Diseases, Molecular Virology, Heidelberg University, Im Neuenheimer Feld 345, 69198 Heidelberg, Germany

Correspondence to:

urs.greber@imls.uzh.ch

Ralf.Bartenschlager@med.uni-heidelberg.de

1 figure

**Keywords:**

Zoonosis; Pandemics; Dengue, Ebola; Influenza; Entry; Endocytosis; Uncoating; Transport; Filovirus; Comparative genomics; Computational analyses; Virus-host interactions; Filamentous virus; Bacterial biofilm; Phage; Bacteriophage; Glycan; Immunity; Infection; Disease; Evolution; Variability; Giant virus; Ferret; Guinea pig; Emerging disease; Pandemic; Host range; Enveloped virus;

Viruses are ubiquitous, and are important in medicine, biology, biotechnology and ecology. All kinds of cells can be infected with viruses, and sometimes, a particular cell is infected with different viruses at the same time. The virus particle, 'virion' is composed of the viral coat proteins sheltering the viral genome, and is often surrounded by a lipid "envelope". A virion is small compared to cells, and when it enters cells gives rise to infection distinct from an intracellular bacterial pathogen (Lwoff, 1957). A virus-infected cell has a profoundly altered homeostasis due to numerous interactions between cellular and viral components. This leads to evolutionary pressure on both virus and host, and argues that viruses are a part of life (Ludmir & Enquist, 2009).

Our cells can be infected by viruses causing acute disease, such as respiratory disease by Influenza virus or rhinoviruses, or chronic disease, such as hepatitis or immune deficiency. However, most viral attacks on cells are fend off, or the spread of viruses in an infected organism is restricted, and infection abrogated. By virtue of triggering host defence, viruses have been major drivers of host evolution, and contributed an estimated 30% of the adaptive changes in the human proteome (Enard *et al.*, 2016). This is much more than the well investigated innate immunity factors, such as interferon or interferon stimulated genes arguing for the existence of a network of proteins that protects cells from viral attack. Other anti-viral networks are based on DNA and RNA immunity mechanisms, and may complement the protein network (Forsdyke *et al.*, 2002, Guo & Steitz, 2014, Lander, 2016). When viruses, such as filoviruses or avian influenza change their hosts, they hit networks not adapted to these pathogens and cause extensive human disease. Thus, a key challenge is to understand the nature of the protective networks, and sort out their strengths, weaknesses and adaptability.

In this series of FEMS Microbiology Reviews, a set of nine articles provides insights into the wealth and diversity of virus research. The articles cover

filamentous bacteriophages attacking biofilms (Mai-Prochnow *et al.*, 2015), and phage-bacteria co-evolution (Koskella & Brockhurst, 2014). The articles bring together results from computational and wet lab studies of bacteriophage-host interactions (Edwards *et al.*, 2016), and provide insights into comparative Ebola virus genomics (Jun *et al.*, 2015). The issue also features an impressive layout of the currently understudied and potentially high risk filoviruses (Burk *et al.*, 2016). Other articles discuss zoonotic viruses and their potential pandemic threats (Van Breedam *et al.*, 2014, Cruz-Oliveira *et al.*, 2015, Richard & Fouchier, 2016). The issue concludes with a discussion of the recently discovered giant viruses, which started to blur the boundaries between viruses and cells (Abergel *et al.*, 2015).

Virions have many different shapes – spherical, icosahedral, helical, bullet-shaped or pleomorphic. Single-stranded DNA or RNA viruses and phages can be as small as a ribosome of about 25 nm, and giant viruses as big as a bacterium of several micrometers. Among the simplest viruses are bacteriophages, arguably the most abundant and diverse biologics on Earth. Bacteria - phage relationships have far-reaching consequences, which include massive effects on the carbon cycle in the deep-sea by turning over bacterial biomass (Danovaro *et al.*, 2008). They also include bacterial virulence in eukaryotic hosts. Mai-Prochnow and colleagues describe how filamentous phages contribute to disease phenotypes caused by bacterial hosts (Mai-Prochnow *et al.*, 2015). These phages infect gram-negative bacteria, and can integrate into the host genome as prophages. For example, the CTXphi phage of Vibrio cholerae encodes, and horizontally transfers genes that are genetically modulated in the host. In this way, new variants of cholera toxin genes are created causing epidemic cholera disease. Despite their relatively small genomes, these phages exert significant influence on their hosts. They enhance biofilm development, cell motility or fitness to metal resistance, and contribute to the emergence of new pathogenic bacterial strains.

While phages modulate features of their hosts, bacterium - phage coevolution is an important driver of ecological and evolutionary processes in microbial communities with impact on particular bacterial traits. Koskella et al. discuss the dynamics of gene networks of coevolving bacteria and phages, and the impact on host communities and environments (Koskella & Brockhurst, 2014). This relatively new field offers unique opportunities for studying in real time co-evolutionary processes in both host and parasite under laboratory and natural conditions, and provides glimpses into the impact of environmental factors on co-evolution.

Metagenomics is the analyses of viral genetic material isolated directly from the environment. Although it identifies viral genomes in ecosystems, the nature of the hosts remains unknown in many cases. Edwards et al. describe how the use of sequence homology approaches aids in identifying known phage - host pairs (Edwards *et al.*, 2016). Together with compositional and abundance-based methods they argue that this is a powerful approach to identify the unknown sequences in viral metagenomes, and aids in illuminating interactions between phages and their hosts. The hope is to use this and similar approaches to predict unknown phage - host interactions with relevance for medical and biotechnological applications.

An increasingly important impact on human health comes from zoonotic viral transmissions which lead to the infection of humans with animal viruses, such as Ebola, or Influenza virus infections. Zoonotic infections are dangerous, as they break through the protective host networks in unprecedented ways, and have the potential for world-wide pandemic virus spreading. A most recent demonstration of this threat is the outbreak of Ebolavirus having caused an estimated 29,000 infections with ~11,300 deaths. Ebolavirus belongs to the family *Filoviridae*, negative strand RNA viruses, which cause hemorrhagic

fever in humans and primates. Typical for RNA viruses, Ebolavirus is not a single strain, but rather a huge number of variants differing substantially at the nucleotide and amino acid sequence levels. Thus, identifying conserved targets for antiviral drugs and highly conserved epitopes informing vaccine development is a major challenge. However, with the advent of novel sequencing methods and bioinformatics approaches this challenge can now be met. Jun et al. apply bioinformatics tools and reveal considerable sequence diversity between filoviruses that fall into three distinct genera forming this virus family (Jun *et al.*, 2015). Of note, sequence variation is scattered throughout the genome unevenly, allowing the prediction of regions that might correspond to relevant B and T cell epitopes as well as possible determinants of virulence. This analysis also underscores that the 2014 West African Ebolavirus strain is clearly different from those having caused earlier outbreaks.

The best studied filoviruses are Ebola and Marburg virus, but a review by Robin Burk and coworkers competently summarizes that there is much more. In fact, there is a substantial number of filoviruses falling into three genera and a total of eight species (Burk *et al.*, 2016). These viruses share an overall comparable genome organization and presumably replication strategy. However, owing to high press coverage and the fact that outbreaks have predominantly been caused by Ebolavirus (and to lesser extent by Sudan and Marburg virus), other filoviruses are largely neglected. This emphasis is short-sighted, because it remains unknown where and how filoviruses occur in nature and how they cause zoonotic outbreaks. It is possible that future outbreaks might be caused by these neglected filoviruses, but our knowledge about their biological properties is poor. Part of the reasons is the lack of adequate tools, but also the current focus on Ebolavirus. Thus, as discussed by Burk and colleagues, more investment is required to decipher the possible zoonotic potential of neglected filoviruses.

The potential for zoonotic transmission and subsequent spread is also a major concern with influenza viruses as witnessed by several pandemics, the last one in 2009. The hallmark of influenza virus is the segmented genome allowing high genetic plasticity by the exchange of segments in cells co-infected with two different influenza virus strains. One of the genome segments encodes the envelope glycoprotein HA (haemagglutinin) responsible for binding to and entry into host cells. The specificity of HA to attach to distinct receptors on the surface of human cells or cells of reservoir animal species such as pigs or various birds is a major determinant for efficient virus spread (Richard & Fouchier, 2016). However, *airborne* transmission, critically determining *pandemic* spread, requires additional features such as HA stability and mutations in the genes for viral polymerase activity. Moreover, the driving forces leading to adaptation of influenza virus to novel hosts have to take into account host and environmental factors. In this way, and by including analogies to other airborne respiratory viruses, such as MERS coronavirus or parainfluenza virus, we might be able to better predict key determinants for airborne transmission within newly emerging pathogenic respiratory viruses.

An important prerequisite for zoonotic transmissions of viruses is that the virion enters both animal donor and human acceptor cells. The article by van Breedam et al. discusses how mosquito-borne virions, such as Dengue virus use polysaccharides on glycoproteins and glycolipids of insect vectors and mammalian cells to modulate host interactions (Van Breedam *et al.*, 2014). These so called glycans can serve as receptors for viral lectins, that is, proteins that bind to carbohydrates, or viruses use glycans to bind to host lectins. Glycan-lectin interactions can directly affect virus entry and endocytic uptake, if receptor pairs are involved, or glycans and lectins alone can indirectly act as facilitators of entry (Mercer & Greber, 2013, Boulant *et al.*,

2015, Yamauchi & Greber, 2016). The review by Van Breedam et al. provides numerous examples for glycan-lectin interactions in the context of viral infection and antiviral immunity, and discusses consequences of their variability.

Along similar lines, Cruz-Oliveira and colleagues competently summarize our current knowledge how the most frequent arbovirus, Dengue virus enters host cells (Cruz-Oliveira *et al.*, 2015). Several candidate entry molecules have been reported mediating viral entry into mammalian and insect host cells, including glycosaminoglycans, the mannose receptor of macrophages, the adhesion molecule of dendritic cells, the lipopolysaccharide receptor CD14, or heat-shock proteins. Equally promiscuous is the entry route used by Dengue virus that appears to depend on the particular cell system applied to study this process, and that might include a direct entry of the viral RNA genome by capsid protein-mediated RNA translocation at the plasma membrane and the endosome. This breadth of entry factors and pathways likely is a reflection of the broad host range and tropism of this virus replicating in various species and tissues. A speciality of Dengue virus is the requirement for proteolytic cleavage of the envelope “cofactor” prM. Proteolytic cleavage is mediated by the host cell enzyme furin, and can occur both during exit in the Golgi compartment or during entry in the early endosome, thus increasing infectivity of immature (non-infectious) Dengue virus particles. The latter process appears to be of high importance for the entry of virion - antibody complexes that are taken up into immune cells via the Fcγ-receptor potentially triggering a cytokine storm, which is the hallmark of fulminant Dengue virus disease.

The compilation ends up with an update on the so called ‘giant viruses’, which are much larger and more complex than common viruses and bacteriophages. Abergel and coworkers describe the history, biogenesis and genetic make up of four new families of ‘giant viruses’, mimivirus, mollivirus, pithovirus and

pandoravirus (Abergel *et al.*, 2015). These viruses can be infected with smaller viruses, and are thought to contain anti-viral and perhaps even anti-bacterial defence systems. The authors end up by providing ideas for new biology, and speculate about the evolutionary origin of DNA viruses.

In conclusion, collective insights from studies summarized in this issue of FEMS Microbiology Reviews, and many other data argue for the fact that viruses are virtually without enemies, and may become extinct only if their hosts die out. We have to accept the notion that viruses are part of a tree of life owing to their ever evolving genetic material, which is capable of self-regulation, adaption and preservation of viral structure and function. The contributions in this issue of FEMS Microbiology Reviews remind us of the dormant potential of viruses to cause unpredictable infectious disease, as has been amply documented in human history (Lederberg, 2000).

## References

Abergel C, Legendre M & Claverie JM (2015) The rapidly expanding universe of giant viruses: Mimivirus, Pandoravirus, Pithovirus and Mollivirus. *FEMS microbiology reviews* **39**: 779-796.

Boulant S, Stanifer M & Lozach PY (2015) Dynamics of virus-receptor interactions in virus binding, signaling, and endocytosis. *Viruses* **7**: 2794-2815.

Burk R, Bollinger L, Johnson JC, Wada J, Radoshitzky SR, Palacios G, Bavari S, Jahrling PB & Kuhn JH (2016) Neglected filoviruses. *FEMS microbiology reviews* **40**: 494-519.

Cruz-Oliveira C, Freire JM, Conceicao TM, Higa LM, Castanho MA & Da Poian AT (2015) Receptors and routes of dengue virus entry into the host cells. *FEMS microbiology reviews* **39**: 155-170.

Danovaro R, Dell'Anno A, Corinaldesi C, Magagnini M, Noble R, Tamburini C & Weinbauer M (2008) Major viral impact on the functioning of benthic deep-sea ecosystems. *Nature* **454**: 1084-1087.

Edwards RA, McNair K, Faust K, Raes J & Dutilh BE (2016) Computational approaches to predict bacteriophage-host relationships. *FEMS microbiology reviews* **40**: 258-272.

Enard D, Cai L, Gwennap C & Petrov DA (2016) Viruses are a dominant driver of protein adaptation in mammals. *eLife* **5**.

Forsdyke DR, Madill CA & Smith SD (2002) Immunity as a function of the unicellular state: implications of emerging genomic data. *Trends Immunol* **23**: 575-579.

Guo YE & Steitz JA (2014) Virus meets host microRNA: the destroyer, the booster, the hijacker. *Mol Cell Biol* **34**: 3780-3787.

Jun SR, Leuze MR, Nookaew I*, et al.* (2015) Ebolavirus comparative genomics. *FEMS microbiology reviews* **39**: 764-778.

Koskella B & Brockhurst MA (2014) Bacteria-phage coevolution as a driver of ecological and evolutionary processes in microbial communities. *FEMS microbiology reviews* **38**: 916-931.

Lander ES (2016) The Heroes of CRISPR. *Cell* **164**: 18-28.

Lederberg J (2000) Infectious history. *Science* **288**: 287-293.

Ludmir EB & Enquist LW (2009) Viral genomes are part of the phylogenetic tree of life. *Nat Rev Microbiol* **7**: 615; author reply 615.

Lwoff A (1957) The concept of virus. *Journal of general microbiology* **17**: 239-253.

Mai-Prochnow A, Hui JG, Kjelleberg S, Rakonjac J, McDougald D & Rice SA (2015) 'Big things in small packages: the genetics of filamentous phage and effects on fitness of their host'. *FEMS microbiology reviews* **39**: 465-487.

Mercer J & Greber UF (2013) Virus interactions with endocytic pathways in macrophages and dendritic cells. *Trends Microbiol* **21**: 380-388.

Richard M & Fouchier RA (2016) Influenza A virus transmission via respiratory aerosols or droplets as it relates to pandemic potential. *FEMS microbiology reviews* **40**: 68-85.

Van Breedam W, Pohlmann S, Favoreel HW, de Groot RJ & Nauwynck HJ (2014) Bitter-sweet symphony: glycan-lectin interactions in virus biology. *FEMS microbiology reviews* **38**: 598-632.

Yamauchi Y & Greber UF (2016) Principles of Virus Uncoating: Cues and the Snooker Ball. *Traffic* **17**: 569-592.

**Fig. 1: Virus-host interaction dynamics.**

A series of nine articles in this issue of FEMS Microbiology reviews sheds light onto selected features of virus-host interaction dynamics. It describes viral zoonosis, transmission, entry, evolution, and the potential of viruses to cause pandemic disease, based on genomics in ecosystems, and novel bioinformatics approaches. Other articles discuss the impact of bacteriophages on human disease caused by bacterial, hemorrhagic fever causing filoviruses, or the complexity and evolutionary trajectories of giant viruses. This series illustrates the enormous diversity of viruses and their dynamics.