Virology in the 21st Century

Viruses remain at the center of science, agriculture, and medicine, providing some of our greatest challenges and triumphs

L. W. Enquist for the Editors of The Journal of Virology

Viruses and viral diseases have been at the center of science, agriculture, and medicine for millennia, and some of our greatest challenges and triumphs have involved virology. Smallpox is a prime example. This greatest killer of humankind changed the course of history during the European conquest of the New World and is also the only disease ever eradicated. This remarkable achievement began with Edward Jenner in 1796 and concluded by 1979 with a worldwide vaccination effort led by the World Health Organization. The smallpox vaccination breakthrough was only the first in a series of important investigations inspired by the study of viruses, while the numerous Nobel Prizes awarded to virologists are another measure of the impact of this discipline (see table).

Viruses as Disease Agents

Many viral infections are prevented or controlled through vaccination and other public health measures, making one-time scourges such as measles, poliomyelitis, rabies, and yellow fever now rare in the developed world. Numerous effective antiviral drugs are also in widespread use. We now recognize that some cancers are caused by viral infections, including hepatitis B virus and human papillomavirus, that also can be prevented by vaccination. However, substantial challenges remain.

New viruses periodically emerge and cause great personal and societal tragedy. Acquired immunodeficiency syndrome (AIDS), caused by human immunodeficiency virus-1 (HIV-1), remains the defining epidemic of our time. Although the severe acute respiratory syndrome (SARS) epidemic was brief, dengue and West Nile viruses continue to smolder, and Chikungunya virus, monkeypox virus, and Ebola and other hemorrhagic fever viruses crouch in the darkness. H5N1 avian influenza virus continues to sporadically infect humans in Southeast Asia and elsewhere, and this year an H1N1 flu strain emerged and spread rapidly across the globe. The emergence of a deadlier influenza pandemic or a viral bioterrorism attack could have catastrophic consequences on public health, commerce, and civic discourse.

Viruses also cause serious disease in plants and livestock. The 2001 epidemic of foot-and-mouth disease in the United Kingdom devastated its beef industry. Plum pox virus, which has decimated stone fruit trees in Europe since the early 1900s, has now spread to the United States and Canada. Viruses have been implicated in a disease

Summary

- Virology continues to flourish as a discipline at the center of science, agriculture, and medicine, providing great lessons in basic and applied biology.
- Viruses will be increasingly viewed as part of a complex microbial ecosystem where a single host is infected with a plethora of microbes, including many viruses.
- We can count on uncovering new or previously unrecognized viral infections in plants, animals, and humans, for whom such infections often prove zoonotic.
- One sea change in virology is that we now function in a cross-disciplinary environment, which is key to training the next generations of virologists.
### Landmarks in the study of viruses

#### Protovirology—before viruses were recognized
- **1796** Cowpox lesions used to vaccinate against smallpox (Jenner)
- **1882** Transmission of tobacco mosaic disease with cell-free extracts (Mayer)
- **1885** Development of rabies vaccine (Pasteur, Roux)

#### Auroravirology (after the Roman goddess of dawn)—the dawn of virology
- **1892** Description of filterable infectious agent (TMV) (Ivanovsky)
- **1898** Concept of the virus as a contagious element (TMV) (Beijerinck)
- **1901** First human virus (yellow fever virus) (Reed)
- **1902** Discovery of rabies virus (Remlinger, Riffat-Bay)
- **1903** First leukemia-causing virus (Ellerman, Bang)
- **1909** Discovery of poliovirus (Landsteiner, Popper)
- **1911** First solid tumor virus (RSV) (Rous)
- **1913** Virus cultivation in tissue culture (VV) (Steinbault, Lambert)
- **1915** Discovery of bacterial viruses (bacteriophages) (Twort, d’Hérelle)
- **1917** Development of the plaque assay and discovery of the particulate nature of viruses (bacteriophage) (d’Herelle)
- **1919** Virus propagation in embryonated chicken eggs (Woodruff, Goodpasture)
- **1920** First mammalian tumor virus (MMTV) (Little, Bittner)
- **1923** Discovery of human influenza virus (Smith)
- **1929** Discovery of rabbit papillomavirus (Shope)
- **1929** First description of viral mutants (TMV) (Jensen)

#### Meridiovirology (from Latin for midday sequel to dawn)—from the demonstration that tobacco mosaic virus is composed of protein and nucleic acid and its crystallization to the in vitro assembly of infectious TMV from purified RNA and protein
- **1934** Bacteriophage composed of protein and nucleic acids (Schlesinger)
- **1935** TMV crystallized (Stanley)
- **1938** Yellow fever vaccine (Theiler)
- **1939** Electron microscopy of viruses (TMV) (von Borries, Ruska, Ruska)
- **1941** One-step growth cycle (bacteriophage) (Ellis, Delbrück)
- **1942** First virus-associated enzymes (influenza virus) (Hirst)
- **1943** Genetic origins of mutations (bacteriophage) (Luria, Delbruck)
- **1944** Development of influenza vaccine (Francis)
- **1946** Genetic recombination by bacteriophage (Delbruck)
- **1948** Poliovirus replication in non-neuronal cell cultures (Enders, Weller, Robbins)
- **1951** Discovery of eclipse phase of virus infection (bacteriophage) (Doerrmann)
- **1952** Bacteriophage λ discovered (Lederberg)
- **1953** Discovery that lysogenic phage produce diphtheria toxin (Freeman)
- **1955** Discovery that viral genome is nucleic acid (Hershey, Chase)
- **1956** Transduction of genetic information by bacteriophage (Zinder, Lederberg)
- **1957** Discovery of host-controlled restriction and modification (Luria, Bertani, Weigle)
- **1958** Discovery of positive regulatory paradigm (Pardee, Jacob, Monod, Lwoff)
- **1959** Discovery of SV40 (Sweet and Hilleman)
- **1960** Triplet nature of the genetic code demonstrated (bacteriophage) (Crick)
- **1961** Nonsense codons elucidated (bacteriophage) (Crick)
- **1962** Virus particles composed of identical subunits (Watson, Crick)
- **1963** RNA can carry genetic information (TMV) (Schramm, Fraenkel-Conrat, Williams)
- **1964** Discovery of interferon (Isaacs, Lindemann)
- **1965** Discovery of respiratory syncytial virus (Chanock)

#### Janovirology (after the Roman god of endings and beginnings, spanning the interval between classic virology and the beginning of the era dominated by viral sequence information)—encompasses the elucidation of essential features of gene structure, expression, and regulation and the development of essential techniques including cloning and restriction mapping
- **1958** Bacteriophage λ regulation paradigm (Pardee, Jacob, Monod, Lwoff)
- **1960** Discovery of SV40 (Sweet and Hilleman)
- **1967** Triplet nature of the genetic code demonstrated (bacteriophage) (Crick)
- **1969** Nonsense codons elucidated (bacteriophage) (Campbell, Epstein, Bernstein)

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**Abbreviations:** ASV, avocado sunblotch viroid; BMV, brome mosaic virus; HAART, highly active antiretroviral therapy; HIV, human immunodeficiency virus; HPV, human papillomavirus; TBSV, tomato bushy stunt virus; TMV, tobacco mosaic virus; TRSP, tobacco ringspot virus; SV40, simian virus 40; FMDV, foot-and-mouth disease virus; WHO, World Health Organization; MHC, major histocompatibility complex; RSV, Rous sarcoma virus.

**Discoveries recognized by a Nobel Prize are in blue.**
1962 Studies of virus structure (Klug, Caspar)
1964 Demonstration of the colinearity of gene with polypeptide chain (bacteriophage) (Brenner)
First human tumor virus (EBV) (Epstein, Barr, Burkitt)
1965 Experimental transmission of spongiform encephalopathy to primates (kuru) (Gajdusek, Gibbs, Hadlow)
Discovery of hepatitis B virus (Blumberg)
Isolation of bacteriophage λ repressor (Ptashne)
Discovery of viroids (Diener)
First virion-associated polymerase (VV) (McAuslan, Kates)
1970 Discovery of retroviral reverse transcriptase (Temin, Kates)
1971 Discovery of viroids (Diener)
First transgenic mouse (SV40) (Mintz)
1972 Discovery of mRNA capping (Shatkin, Moss)
Neovirology—beginning with the first complete sequencing of viral genomes and atomic resolution structures
1976 First RNA virus genome sequenced (bacteriophage MS2) (Fiers)
Demonstration that retroviral oncogenes are derived from cells (J. M. Bishop, H. Varmus)
First DNA virus genomes sequenced φ X174, SV40) (Sanger, Fiers, Weissman)
RNA splicing discovered (adenovirus) (Roberts, Sharp)
Discovery of tumor suppressor p53 (SV40) (Levine, Crawford)
First virus crystal structure (TBSV) (Harrison)
1979 First human retrovirus (HTLV-1) (Gallo)
1977 First recombinant DNA molecules (phage λ, SV40) (Berg)
Proposal that reassortment of influenza virus segments is the origin of pandemic strains (Webster, Laver)
1980 First infectious molecular clones of an RNA virus (poliovirus) (Baltimore)
Transcriptional enhancers discovered (Chambon, Khouy)
Hepatitis B vaccine
Identification of mammalian transcription factors (MMTV, SV40) (Yamamoto, Tjian)
Insertional activation of cellular oncogenes by retroviruses (Hayward, Astrin)
Identification of polyadenylation signal (Shenk)
Discovery of the Cre/lox recombination system in phage P1 (Sternberg)
1982 Development of antiviral and other drugs (Elion, Hitchings)
Definition of prions (Prusiner)
1983 High-risk human papillomaviruses identified and linked to cervical cancer (zur Hausen)
Discovery of AIDS virus (HIV) (Montagnier, Barre-Sinoussi, Gallo)
1984 Discovery of nuclear localization signals (Smith, Butel)
First infectious, multicomponent virus produced from cloned DNA (BMV) (Ahlquist)
1986 First recombinant viral vaccine (HBV)
Generation of transgenic virus-resistant plants (TMV) (Beachly)
Discovery of hammerhead ribozymes (TRSV, ASV) (Bruening, Symons)
1988 Discovery of Thyroid stimulating hormone (Hunter, Erikson, Eckhart)
First ribozyme with engineered specificity (Haseloff, Gerlach)
Discovery of internal ribosome entry sites (poliovirus) (Wimmer, Sonenberg)
1989 Discovery of Hepatitis C virus (Houghton)
First human gene therapy with a retrovirus vector (Anderson, Blaese)
1990 Discovery of viral anti-apoptotic proteins (baculovirus) (Miller)
1991 Development of HAART treatment for AIDS
1992 Gene silencing by double-stranded RNA, an antiviral response (Fire, Mello)
First ribozyme with engineered specificity (Haseloff, Gerlach)
Discovery of internal ribosome entry sites (poliovirus) (Wimmer, Sonenberg)
1993 Discovery of in vivo RNA silencing by double-stranded RNA, an antiviral response (Fire, Mello)
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that is ravaging our honeybees, threatening natural pollination cycles and thus much of agriculture. During the 1990s, efforts to apply RNA interference to protect crops against infections by highly destructive viruses were halted because of unsubstantiated fears about this kind of genetic modification. With the prospect of worldwide food shortages caused by climate change, decreased investment in genetic technology has serious implications for human health. These and similar concerns provide a rich opportunity for the scientific community to advance ideas in the public domain about new developments and evidence-based risk assessments.

Important Role of Viruses in Basic Biology

Beyond medicine and agriculture, the study of viruses provides great lessons in basic biology. Viral replication is strictly dependent on cell structure, metabolism, and biochemical machinery, and, thus, the roster of important discoveries uncovered by studies of viral replication and transformation is long: the existence of messenger RNA (mRNA) and mRNA processing, including splicing, capping, and polyadenylation; transcriptional control elements and transcription factors; gene silencing mechanisms; cellular oncogenes and tumor suppressor proteins; and signal transduction pathways and tyrosine kinases.

The structural biology revolution was championed by crystallization of tobacco mosaic virus by Wendell Stanley in the 1930s. This line of inquiry produced high-resolution structures of viral proteins and virus particles themselves, the largest biological structures known at the atomic level. Molecular biology emerged from studies of bacterial viruses. Studies of unconventional viruses resulted in discovery of viroids and prions and the concept of protein-folding diseases.

Viral genomes encode products that modulate host defenses, including the immune response that, ideally, clears pathogens with minimum damage to the host. However, much of viral clinical disease is immunopathological in nature, as shown in infections ranging from the common cold to AIDS. Studies of interactions between viruses and cells continue to reveal complex host responses and countermeasures, including histocompatibility antigen functions, intrinsic cell defense mechanisms such as apoptosis, interferons, and RNA interference, and viral countermeasures to evade or antagonize host responses—a discipline called “anti-immunology.”

Virology Intertwined with Technology Development

In the 21st century, we can begin to identify new families of organisms and viruses by high-speed sequencing of RNA and DNA. For example, deep sequencing of mixed populations can reveal novel virus families. Indeed, new polyomaviruses, marine viruses, and bacteriophages have been identified using sequence-based techniques coupled with genomic and metagenomic analyses. Strikingly, some of these viral proteins show little genetic similarity to those from better-known viruses.

In a similar vein, high-throughput sequencing and gene-mapping techniques, the availability of the genome sequence from humans and other organisms, and proteomics and metabolomics will provide us with the ability to study host determinants of viral virulence in ways previously unimagined. Exciting discoveries notwithstanding, identifying new viruses brings a serious challenge. Are these viruses true pathogens or do they have symbiotic relationships with their hosts? For example, perhaps these agents stimulate local and systemic immune responses that protect against or suppress responses that contribute to pathogenesis by more virulent microbes.

Instead of studying one gene or gene product at a time, examining large groups of them using a systems biology approach allows the identification of fundamental biological networks. An important premise of systems biology is that information flows through networks, and disease arises when these networks are perturbed, causing changes in network architecture and dynamics. Technological advances will allow in vitro study of viral infections using conditions that more precisely mimic in vivo environments.

Editor’s Note

This article was adapted from a longer minireview prepared for and published in the Journal of Virology (83:5296–5308).
Viruses will be increasingly viewed not in isolation with their hosts, but in the real world of a microbial ecosystem where a single host is infected with a plethora of microbes, including many viruses. For example, some viral infections are associated with atherosclerosis and obesity. However, whether the associated viral infections are causal, serve as essential cofactors, or are irrelevant is not known. Components of the initial host inflammatory response to an infection, namely cytokines, chemokines, and cells of the innate and adaptive immune systems, can regulate the outcome of infection by a second agent. The degree of susceptibility and response of a virus-infected cell to a secondary infection can be modulated by many cellular factors.

One of the tenets of systems biology is that networks process information and the output can vary depending on the action at key nodes of the network. Therefore, an important use of systems biology is to perturb networks and analyze outcomes. Viral infections provide the opportunity to take a system from state A (uninfected) to state B (infected) with synchrony and technical control. This approach to generate and test hypotheses will be a powerful tool to understand homeostatic control and viral pathogenesis.

Emerging Infections, Natural and Synthetic

We continue to uncover new or previously unrecognized viral infections in plants, animals, and humans, for whom such infections often prove zoonotic, meaning the pathogen moves from wild or domesticated animals to humans. Improved surveillance, more rapid reagent sharing and information transfer, more effective quarantine procedures, and various public health measures will undoubtedly contribute to controlling emerging diseases, but increasing attention and resources are likely to be devoted to expanding the roster of antivirals and vaccines.

One view is that we should accelerate development of new antiviral strategies to protect the public from these emerging infections. To be effective, antiviral drugs must be safe, potent, and administered soon after infection. These requirements constitute substantial impediments to drug discovery, which has limited the number of antivirals in clinical use for acute infections relative to antibiotics. Nonetheless, numerous highly effective antiviral drugs are in widespread use, particularly against HIV. Although we must certainly prepare for future threats, antiviral drug development should not ignore viruses that currently account for a substantial burden of disease.

Meanwhile, vaccines remain among the most cost-effective means of preventing infectious disease morbidity and mortality. However, there are several challenges to developing vaccines. First, we must understand the basic biology of viral evolution and quasispecies. Second, we need to define what constitutes a protective immune response. Third, we have to acknowledge the economics of vaccine development and the risk to the private sector, recognizing that the necessity of immunizing a healthy naive population to prevent a disease will be unacceptable if there are significant vaccine-associated adverse events. Even greater challenges arise in introducing vaccines to the public. Many believe that children already are “overvaccinated” in infancy. In addition, there is a public perception that vaccines cause diseases such as autism or attention deficit disorder despite substantial evidence to the contrary. Successful vaccine efforts will require both sound science and forceful public advocacy.

Highly pathogenic viruses, either in their wild-type state or after genetic manipulation, could be used for terrorism. Although risks of virus-based bioterrorism are considered low, virologists and the scientific community should be vigilant and guard against such misuses of scientific information.

Nature remains dangerous enough, acting through zoonoses such as AIDS, which moved from primates to humans, and the SARS-coronavirus, which was transmitted to humans from bats and civet cats. Heightened concerns about potential viral pandemics and bioterrorism have resulted in the construction of high-containment research facilities and increased scrutiny of the safety of research on viral pathogens, particularly those designated “select agents” by officials of the Centers for Disease Control and Prevention or the U.S. Department of Agriculture. This designation mandates strict regulatory oversight, which should be balanced by consideration of the risks of hindering research. Because many select agents are endemic in some
areas of the world, regulatory decisions about “select agents” should be based on realistic risk assessments.

Training Virologists while Expanding their Portfolios

What constitutes optimum training for virologists? Training the next generation will require more diverse course offerings, enhanced opportunities, especially involving interdisciplinary collaboration and computational approaches, and instruction in teamwork. Systems biology approaches, large-scale genetic screens, metagenomics of host and viral genes from related viruses, and imaging technologies produce enormous amounts of information that can be difficult to integrate into conceptual frameworks. Methods to search, screen, recover, and use this information will require virologists with special expertise in computational methods and information technology.

Continuing research should not only focus on conventional viruses but also enhance our understanding of new classes of subviral infectious agents such as prions. Prions are infectious, misfolded host-derived proteins that can spread disease or phenotypic traits without carrying their own nucleic acid genome. Practical diagnostic tests and treatments must be developed for mammalian prion diseases such as bovine spongiform encephalopathy and Creutzfeldt-Jacob disease. A number of common protein misfolding diseases such as Alzheimer’s disease and other amyloidoses might be transmissible due to prion-like behavior of misfolded proteins. Such possibilities are another important area for investigation.

The general trends likely to drive virology research in the next decade include systems biology of virus-host interactions, viral ecology and the virosphere, evolution of viruses, and improved vaccines and therapeutics. Regardless of the path virology takes in the coming years, history has proved repeatedly that understanding viruses leads to valuable insights into basic biological phenomena.

We anticipate a rich future for viral pathogenesis research. If we could point to one sea change in virology that will affect us all, it would be that we now function in a cross-disciplinary environment. Those in other disciplines who do not master the biology of viruses are likely to provide technical expertise, but they will find it more difficult to share with virologists the joy of understanding fundamental biology, making discoveries, and improving the health and well-being of our planet.