BIOPROCESSING OF VIRAL VACCINES

Edited by
Amine Kamen
Laura Cervera

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The concept of this book originates from the delivery of the series of Cell Culture-based Viral Vaccines Course sponsored by the European Society of Animal Cell Technology (ESACT) https://esact.org/cell-culture-based-viral-vaccines-course/ and its content includes some of the material that has been developed and adapted over many years.

The book focuses on cell-culture–produced viral vaccines to meet the needs of the rapidly expanding research and development in academia and industry in the field. This book introduces the basic principles of vaccination and the manufacturing of viral vaccines. *Bioprocessing of Viral Vaccines* will provide an overview of the advanced strategies needed to respond to the challenges of new and established viral infection diseases. The first few chapters cover the basics of virology and immunology as essential concepts to understand the function and design of viral vaccines. The core of the content is dedicated to process development, including upstream processing and cell culture of viral vaccines, downstream processing, and analytical technologies as applied to viral vaccines. Advanced process analytical technologies (PAT) and quality by design (QbD) concepts are also introduced in the context of vaccine manufacturing. The case studies included cover inactivated, attenuated vaccines exemplified by influenza vaccines; sub-unit vaccines exemplified by virus-like particles (VLPs: HPV vaccines) and recombinant protein vaccines (Flublock); vectored vaccines: adenoviruses (AdV) and vesicular stomatitis virus (VSV) vectored vaccines; genomic vaccines (DNA and mRNA), vaccines as developed for COVID-19 response in particular; and a review of all COVID-19 vaccines approved or in advanced clinical trials. Primarily this book is aimed at graduate engineers and professionals in the fields of vaccinology, bioprocessing, and biomanufacturing of viral vaccines.

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Contents

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List of abbreviations

RNA	Ribonucleic acid
DNA	Deoxyribonucleic acid
ss	Single stranded
ds	Double stranded
HIV	Human Immunodeficiency Virus
AIDS	Acquired Immunodeficiency Syndrome
TMV	Tobacco Mosaic Virus
mRNA	messenger RNA
vRNA	viral RNA
DdDp	DNA dependent DNA polymerase
RdRp	RNA dependent RNA polymerase
CME	Clathrin mediated endocytosis
PIP2	Phosphatidylinositol 4,5-bisphosphate
GTP	Guanosine triphosphate
ESCRT	Endosomal sorting complex required for transport
RT	Reverse Transcriptase (enzyme)/ Transcription (process)

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1 Bioprocessing of viral vaccines—Introduction

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CONTENTS

1.1 AN ABBREVIATED HISTORICAL BACKGROUND OF VACCINES

From a broad universal perspective there are clear references in the Chinese and Indian history of ideas that suggest knowledge of vaccination principles [1,2]. However, the *Occidental Modern History of Vaccines* dates back the concept of vaccination to 1796 referring to Edward Jenner. Building on the observation that milk maids who were exposed to infected cows were resistant to smallpox infection, Edward Jenner was the first to demonstrate protection against smallpox infection by exposing the individual's immune system to material from cowpox pustules to provide protection [3].

In the nineteenth century, vaccination became a cause of national prestige, and the first vaccination laws were passed. The leading figure of Louis Pasteur [1822–1895] [4] developed the vaccine against rabies infection and contributed to the global promotion of vaccination through the initiative of the institutes Pasteur network [5]. Some of the most fascinating events over this period were captured in the entertaining book titled: *Plague & Cholera* [6], describing the pioneering humanitarian action of Alexandre Yersin, underlining the dominant role he played in

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the discovery of the plague pathogen *Yersinia pestis* which exemplifies the need of a global perspective for vaccine distribution.

The development of vaccines reached its golden age during the twentieth century with the implementation and widespread use of many successful vaccines. As a result, smallpox has been eradicated (WHO declaration of global eradication by October 1979) and many other infectious diseases that have threatened humanity for centuries have virtually disappeared [7].

Eradication of the polio virus infection was targeted by year 2000 through the WHO Global Eradication Initiative [8]; however, cases persist in war areas not accessible to vaccination.

Accumulated data since 1800 shows a decrease of the global child mortality under the age of 5 years from 43% (1800) to 4.3% (2015) [9]. The decrease of global child mortality has been largely attributed to vaccination. The World Bank estimated that a combination of vaccines, malaria prevention, and improved newborn health care has helped reduce under-5 child mortality globally from 20 million in 1960 to 6.6 million in 2012. Consequently, the Bill and Melinda Gates Foundation is building its strategy to deliver vaccines to low-income countries to achieve in 2035 the goal of reducing the deaths by 1,000 births to 15, a ratio achieved in the United States in 1980.

Overall, these contributions to humanity are major and vaccines are making a great difference in human health, yet they are taken for granted by the public until challenged by a pandemic situation, as illustrated by the current COVID-19 pandemic situation and global exceptional measures implemented.

Table 1.1 shows the vaccines for preventable diseases since their first introduction in the United States in 1798, starting with smallpox. Within the list of vaccines shown here, in white background are microbial types of infections for which vaccine production use microbial fermentations.

Among the vaccines listed, some are for travelers in specific countries where the infectious disease is endemic. For example, yellow fever vaccination is mandatory when traveling to countries in Western Africa where there is a risk of infection. The number of marketed vaccines is increasing, but compared to pharmaceutical drugs, this number remains low.

1.2 ROLE OF PUBLIC HEALTH ORGANIZATIONS AND INDUSTRY

Vaccines are considered commodities and they fall under public health priorities. Governments are engaged globally aiming to implement solutions to address public health emergencies in their countries and globally in cases of pandemic situations. The COVID-19 unfolding pandemic situation since January 2020 is a live demonstration of the needs of these precious commodities to control the global public health situation and reduce the emergence of SARS-CoV-2 variants.

Because of its public health importance, historically, vaccine manufacturing and delivery was managed by public health organizations such as the National Institute of Allergy and Infectious Diseases in the United States; Connaught

TABLE 1.1

List of infectious diseases for which vaccines have been licensed

Disease	Year	Disease	Year
Smallpox	1798*	Bacterial Meningitis	1975&
Rabies	1885*	Pneumonia: polysaccharide	1977&
Typhoid fever	1896*	Adenovirus type 4 and 7	1980&
Cholera	1896*	Hepatitis B	1981&
Plague	1897*	Invasive Hib	1985&
Diphtheria	1923*	Japanese Encephalitis	1992&
Whooping cough: Pertussis	1926*	Hepatitis A	1995&
Lockjaw: Tetanus	1927*	Chickenpox: Varicella	1995&
Tuberculosis	1927*	Lyme disease	1998&
Tick-borne Encephalitis	1937*	Pneumonia: conjugate	2002&
Influenza	1945&	Rotaviral diarrhea	2006&
Yellow Fever	1953&	Shingles: Zoster	2006&
Poliomyelitis	1955&	Papillomavirus	2006&
Measles	1963&	Dengue	2019&
Mumps	1967&	Ebola	2019&
Rubella	1969&	COVID-19	2021&
Anthrax	1970&		

Notes

* Year of first reported use.

& Year of U.S. licensure.

Light gray background: egg-based production of vaccine.

White background: bacterial production of vaccine.

Dark gray background: cell-culture production of vaccine.

The list is Adapted from [10] and updated with data from [11].

Laboratories in Canada; the network of Institutes Pasteur and affiliated organizations; Butantan institute in Brazil; Serum Institute in India; Robert Koch Institute in Germany; Academy of Sciences in China; and many other organizations worldwide involved in the manufacturing, procurement, and distribution of vaccines. The Institutes Pasteur International Network with headquarters in Paris is still operating as a network involving many institutes in the Americas, Asia, and Africa, with a mandate that shifted from the original mission of providing vaccines wherever needed to centers and research organizations responding to broad national priorities.

In recent history and driven by high regulatory manufacturing standards, key industries, such as GlaxoSmithKline (GSK), Sanofi-Pasteur, Merck & Co, Pfizer, and a number of other small and medium size companies have become active in the field of manufacturing and commercialization of vaccines. The current situation of India and China with important governmental and private vaccine manufacturing

capacities is of particular interest in observing the evolution of the vaccine global market. It is very likely that new strategic investments following the COVID-19 pandemic situation will be made by many countries to create sustainable vaccine biomanufacturing capacities, which will probably change the overall global vaccine market shape.

The WHO role has been critical in providing guidance to country national health authorities and regulatory bodies especially in time of pandemic or situation of "global concern," as it has been the case with the Ebola epidemics in 2013–14. Each country relies on a national regulatory authority such as the US-FDA in the United States, Health Canada, EMA in Europe, China-CDC etc. to approve vaccines in each country. Additionally, the WHO has the responsibility to provide "pre-qualification" of vaccine manufacturing capacity to commercialize vaccines in other countries. Details on the mandate of the WHO, organizational structure, and associated centers can be found on the WHO website (WHO, https://www.who.int).

1.3 THE VACCINE MARKET AND ECONOMIC DRIVERS

Vaccine economics is also very specific to the field. Vaccines as commodities are made available at the global scale with the support of public and governmental organizations, charity organizations such as the Bill and Melinda Gates Foundation: https://www.gatesfoundation.org, the Welcome trust: https://wellcome.org, the Rockefeller Foundation: https://www.rockefellerfoundation.org, and many others. Distribution of vaccines at global scale is managed through vaccine alliances such as GAVI: https://www.gavi.org and international bodies like UNICEF: https://www.unicef.org, the World Bank: https://www.worldbank.org/en/home and the World Health Organizations (WHO): https://www.who.int).

The vaccine market, in terms of total value used to be very small corresponding to 2/3 of a percent of the global pharmaceutical market. It is important to note that these figures are pre-COVID-19 pandemic and will be probably significantly revised post-pandemic. However, the importance of vaccines in preventing diseases largely outbalances the cost of the vaccines.

The vaccine market is generally segmented essentially in pediatric vaccines as opposed to adult vaccines, where growth is becoming very sustained with vaccination of aging populations in many countries.

The distribution in the revenue market shares and volume of vaccine sales is monitored by the WHO [12] through the Market Information for Access to Vaccines (MI4A) initiative to enhance vaccine market transparency and understand global vaccine market dynamics. A report of 2019 indicates that a small number of manufacturers dominates the global market with many products in the following order: GSK, Sanofi-Pasteur, Serum Institute of India (SII), Microgen, Merck, Bharat Biotech-Vaccines International Ltd (BBIL), Bio Farma, and Pfizer with five or more licensed vaccines in their portfolio. In perspective, the top five selling vaccines by 2020 are Pfizer's Prevnar 13 for prevention of pneumococcal infection with a sustained sale's growth generating nearly US$6 billion; Pentacel against diphtheria/pertussis/whooping cough/tetanus/polio/Haemophilus influenza type B from Sanofi-

Pasteur; Gardasil against Human papillomavirus (HPV) infection from Merck; Fluzone/Vaxigrip against influenza infection from Sanofi-Pasteur; and Pediatrix for prevention of diphtheria/tetanus/pertussis/whooping cough/hepatitis B/polio from GSK. The sales of the last four vaccines range from US$1.7 to 2.3 billions. Other well-positioned vaccines in the top ten list such as Varivax against varicella, Zostavax against Zoster virus infection, Rotateq against rotavirus infections and Pneumovax 23 for pneumonia infection are all produced by Merck, whereas Twinrix for prevention of Hepatitis A&B is produced by GSK. It is expected that the global vaccine market in 2021 and beyond will be restructured with the approval and sales of new COVID-19 vaccines.

Often referred to as the vaccine market north and south gap, figures collected by the WHO compare the burden of disease (how much diseases are present in population), which is present at 93% in the developing countries and 7% in industrialized country to the vaccine sales as being 82% in industrialized countries and only 18% in developing countries. These figures need to be interpreted taking into consideration the many different vaccine procurement processes put in place by the different countries. For example, high-income countries' (HICs) self-procurement of vaccines account for most of the market value, at over US$12 billion annually representing less than 5% of the global market volume [13].

As a consequence of the 2009 H1N1 influenza pandemic declaration, which raised concerns especially about aging populations, a sense of urgency dominated. The influenza vaccine market was estimated at $2.9 billion in 2011 and increased to $3.8 billion by 2018. Only in the U.S. market, the value increased from $1.6 billion in 2011 to $2.2 billion in 2018. The global market is projected to rise to US $ 58.4 billion by 2024 [14] with more than 120 new products in the development pipeline, among which 60 are of importance for the developing countries.

Clearly, vaccines are becoming an engine for the pharmaceutical industry and new business models are emerging. For example, many companies that did not have vaccines in their portfolio have started making alliances or acquiring smaller companies that could produce vaccines. It is also expected that the global vaccine market will be dramatically reshaped following the COVID-19 pandemic that revealed several challenges additionally to existing ones such as increased competition and narrowed traditional vaccine markets, new vaccine opportunities, and markets and threats driving rapid development of novel technologies within a new regulatory framework. Consequently, the vaccine field has a predicted spectacular growth rate of 10.7% a year for the forecast period 2019–2027 [15].

1.4 SAFETY AND REGULATION OF VACCINES

Safety of vaccines is paramount as they are prophylactic interventions that are delivered to healthy people including infants. Confidence of the population on the safety of vaccines and their efficacy is a priority for the public health regulatory bodies and vaccine manufacturers. It is well understood by the vaccine community that one single failure will impact public faith in the entire product class. Post-marketing surveillance assesses the effectiveness and safety of vaccines and begins

after vaccines are approved for use and includes the monitoring of adverse events following immunization.

Vaccines are regulated by different, specialized branches within the public regulatory bodies. As an example, the process for approval of vaccine in Canada is presented in the following reference [16].

Safety and efficacy are first evaluated in animal studies or preclinical studies. Afterwards, the four phases of the clinical studies are initiated:

> Phase 1 studies a vaccine on a small group of people (usually fewer than 100) for the first time, examining its safety including dosage range and side effects.
>
> Phase 2 studies a vaccine on a larger group of people (usually several hundred or more), to see how effective the vaccine is in preventing a disease, confirming its safety and its optimum dosage.
>
> Phase 3 studies a vaccine on a larger group of people (usually many thousands) to confirm that it is both effective and safe by monitoring its side effects and any adverse reactions.
>
> Phase 4 occurs after the vaccine has been approved for use and is incorporated into immunization surveillance programs. This is also known as post-marketing surveillance. This includes ongoing safety monitoring, assessing vaccine effectiveness in specific population groups, and determining the duration of immunity to inform future decisions on the need for booster doses.

As it is the case for any biologics, any significant change in the vaccine manufacturing process might require clinical demonstrations of safety and eventually efficacy. Manufacturing of vaccines on different sites or countries might require bridging clinical trials. The role of the WHO through "pre-qualification" of vaccine manufacturing sites worldwide is an improved process for facilitating commercialization of vaccines in the different regions of the world.

1.5 BASIC PRINCIPLES OF VIRAL VACCINE DESIGN AND TRADITIONAL PRODUCTION

This textbook focuses only on viral vaccines; therefore, although designed using the same principles to activate immune response, other microbial infections and vaccination strategies will not be discussed. Vaccines might be classified taking into consideration their design and mode of exposure of the dominant antigen to the human immune system.

Figure 1.1 captures the principles of vaccination and provides a simplified view of the possible interaction pathways with key mediators of the immune response. This section will be detailed in Chapter 3 of this textbook by reviewing the basic principles in immunology that are directly applicable to vaccine design and development.

Traditional viral vaccines involve the whole virus as **inactivated** or **live attenuated,** such as influenza vaccines (Chapter 9), or a **sub-unit,** representing **the**

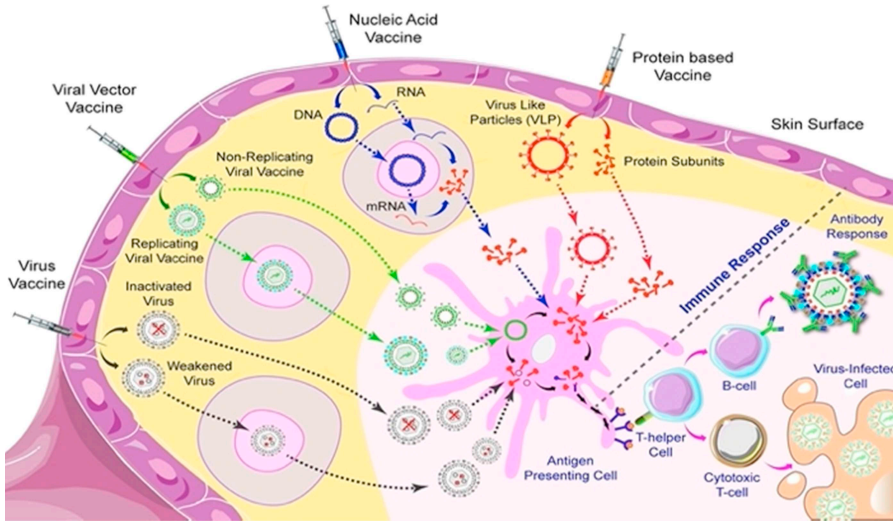

FIGURE 1.1 Description of the different types of vaccines and their possible interaction with the immune system. Credit for the design: Kumar Subramaniam.

dominant antigen extracted from the viral structure, such as Hepatitis B vaccines. Recombinant DNA technology enabled the design of **vectored vaccines** (Chapter 11) such as adeno-vectored vaccines against Ebola, SARS-CoV-2 infections, **virus like-particle vaccines** (Chapter 10) such as human papilloma virus vaccine, and **recombinant protein vaccines** such as recombinant hemagglutinin as the first approved influenza vaccine of its kind. An emerging class of vaccines based on delivery of genomic components of the virus include DNA vaccines and **mRNA vaccines** (Chapter 12). Over the COVID-19 pandemic, a remarkable demonstration has been made on safety, efficacy, and effectiveness of mRNA vaccines against SARS-CoV-2 infection, establishing this vaccine technology platform as a major technological jump in vaccinology. As data collected for phase 4 following the vaccine approval and commercialization of COVID-19 mRNA vaccines are compiled, more insights will be provided on the long-term protection of this class of vaccines. This textbook will detail the design, development, manufacturing of these different classes of vaccines within the core chapters and will illustrate with several case studies each class of these cell-culture produced vaccines.

New approaches based on vaccination using cells as antigen presenter cells (dendritic cells) are evaluated in pre-clinical and clinical trials showing some effectiveness in treatment of cancer, which falls in this case under the umbrella of therapeutic vaccines that is extensively documented in a number of reviews [17], but will not be discussed in this first textbook edition.

Traditionally, virus productions used live animals such as chickens' embryonated eggs to grow the virus in specific egg cavities and collect the virus thereafter in a specific cavity, as illustrated in Figure 1.2.

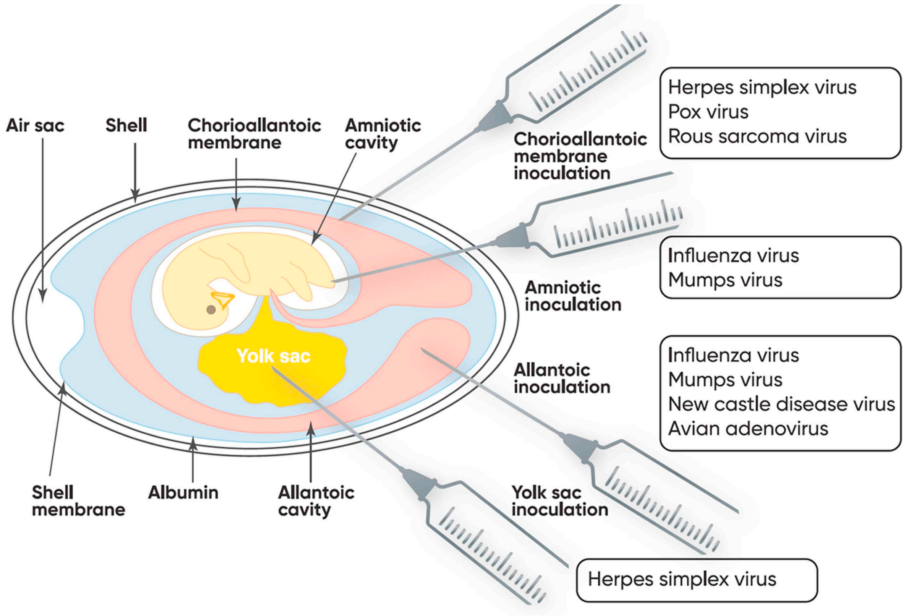

FIGURE 1.2 An embryonated chicken egg showing the different compartments in which viruses may grow. The different routes by which viruses are inoculated into eggs are indicated.

Although 80% of the influenza vaccines are still produced using embryonated egg technology [18], there is a trend to use cell culture technologies in all other cases of traditional viral vaccines including rabies, yellow fever, poliomyelitis, measles, mumps, rubella, adenovirus, Japanese encephalitis, Hepatitis A, varicella, rotavirus, human papilloma virus, dengue, zoster, and Ebola vaccines.

With the exception of mRNA vaccines, all COVID-19 vaccines approved or in advanced clinical trials are cell-culture produced vaccines indicating a solid trend for establishing cell culture advanced technologies for manufacturing viral vaccines.

1.6 CELL-CULTURE PRODUCTION PROCESSES

The cell-culture production process is designed integrating the basic knowledge on virology including the viral structure, its biology, and the viral replication cycle will be extensively described in Chapter 2 as basic virology for process design. Viruses are biological structures with sizes ranging from 20–300 nm, containing DNA or RNA coding for proteins necessary for replication, including non-structural and structural proteins contributing to a "shell" capsid that might be an icosahedron or helical asymmetrical capsid, and eventually an envelope as it is the case for HIV, SARS-CoV, and influenza viruses, for example, or non-enveloped (adenovirus, adeno-associated virus, or human papilloma virus) (Figure 1.3).

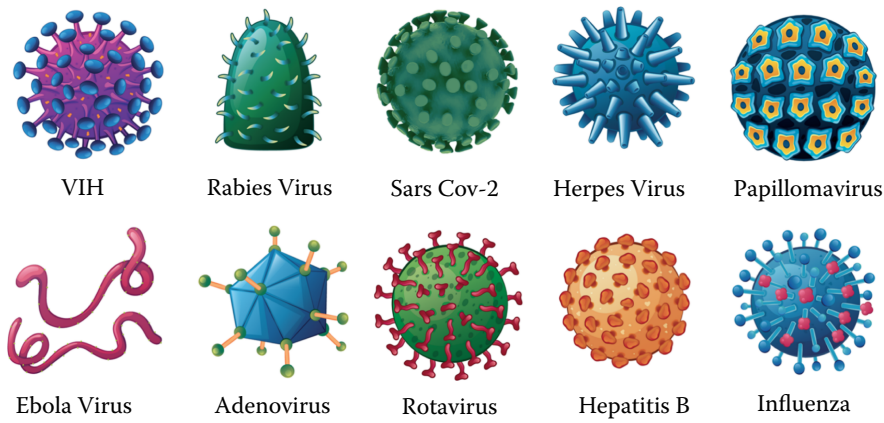

FIGURE 1.3 Example of different types of viruses.

David Baltimore (Nobel Prize in 1975) proposed a virus classification in 1971, focusing on the mode of viral replication. The classification was adopted by the virologists in parallel to standard virus taxonomy, which is based on evolutionary history (Chapter 2).

The general steps involved in virus replication include transport of the virus to the cellular membrane, attachment that might be mediated by specific receptors on the cell membrane, direct penetration, or endosome-mediated entry in the cell cytoplasm and uncoating of the virus structure as early events. Within the cells, the viral genomic coding sequences will determine the early gene transcription-the genome replication-late gene transcription/translation as middle events. Finally, late events include assembly and egress of the virion. Any of these steps and events are viral specifics but remain essential in determining the kinetics of replication in the cell-culture production process.

From a processing standpoint, there are two types of viruses, the ones that end with the lytic cycle (non-enveloped) and the ones that will bud from the cell surface (enveloped). For example, adenovirus has a lytic infection cycle and would infect the cells, replicate, accumulate, and then the cell will lyse and the virus will be released. An example of an enveloped virus is the coronavirus that will bud off the cells at maturation of the virion. The enveloped or non-enveloped nature of the virus has critical effects on the selection of upstream and downstream processing steps of the associated viral vaccines. Therefore, the nature of the infectious viral unit and its interaction with the host cell needs to be well integrated in the design of viral-structure-based vaccines (inactivated, attenuated, and vectored-vaccines) and the overall manufacturing stream. These mechanisms will be described in detail in the virology chapter as well as in the specific case study chapters (Figure 1.4).

Cell culture technology is the most effective mode of production of viral vaccines. Primary cell lines such as chick embryo fibroblasts are used in the production of measles and mumps vaccines, whereas human diploid cell lines

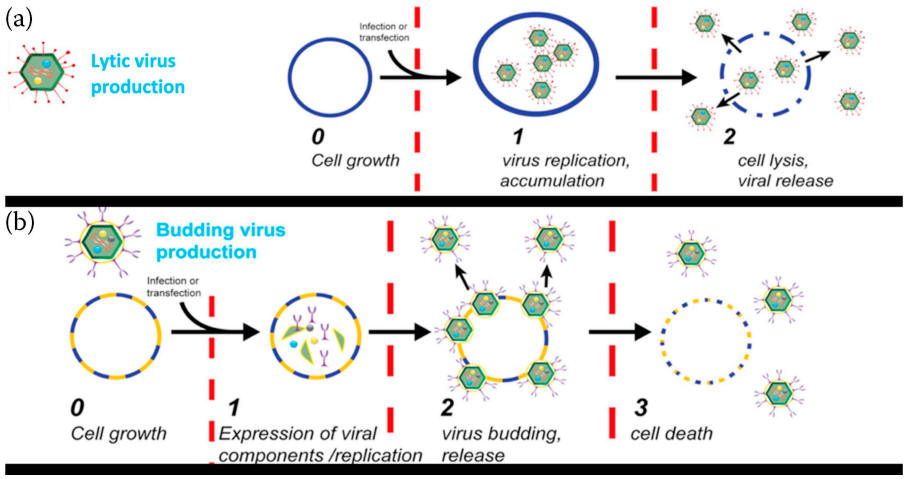

FIGURE 1.4 Non-enveloped lytic virus cycle (A) and enveloped virus cycle (B) replication. Credit to Sven Ansorge for the design of the image.

with limited generations such as Wi-38 and MRC-5 derived human lung fibroblasts are used to produce rubella and varicella vaccines. Vero cell line, a continuous cell line derived from the kidney of the African Green Monkey is used to produce Salk polio, rabies, rotavirus, and influenza vaccines exploiting microcarrier technology to enable industrial scale-up of adherent cell cultures. Other continuous cell lines that have been adapted to suspension cultures include the human embryo kidney cells, HEK293 and the primary embryonic retina cells, PER.C6, two transformed human cell lines, that are used to produce adenovectored vaccines currently licensed for immunization against SARS-CoV-2 infections. Madin-Darby Canine Kidney, MDCK, continuous cell line has been used as a preferred host for replication of influenza strains and has been adapted to suspension culture for industrial manufacturing of influenza vaccines. The development of these different cell culture platforms and the regulatory framework associated with their use for manufacturing viral vaccines is discussed in Chapter 4, dedicated to cell lines for viral vaccines production.

1.7 MANUFACTURING CHALLENGES OF VIRAL VACCINES

The complex structure of viral vaccines is highly associated with the structure and biology of the virus causing the infectious diseases. A primary challenge relates to the size distribution of the viral structures. For example, a measle virus has dimensions ranging from 250 to 400 nm, which is largely beyond the 200 nm sterilizing filter pore size. Consequently, cell-culture production of the measles virus cannot benefit from a final sterilization step, which imposes a complete process under totally sterile conditions. With recombinant vaccines in the class of the most

advanced and characterized vaccines, human papilloma virus (HPV) vaccine, a virus like particle (VLP) vaccine, has a mean size diameter of 40 nm, as compared to an immunoglobin (IgG) with a size of about 10 nm. HPV vaccine manufacturing requires an extensive post-production disassembly/reassembly reprocessing of the capsid structural proteins to achieve a high level of purity of VLPs within a narrow distribution size range.

As detailed in the virology section (Chapter 2), viruses have different structures and properties and analytical methods to monitor their production as vaccines are often under-developed. The potency of the vaccine product that might involve an adjuvant in the final formulation is determined by its interaction with the immune system which is incompletely understood (Chapter 3), contributing to the complexity of viral vaccines design and manufacturing. Viral vaccine production processes are characterized by 1) a lack of platform technologies requiring multiple cell types, mostly operated in adherent cell culture mode; 2) different virus/cell interactions; 3) different scales for production contributing to further complexity in the bioprocessing and biomanufacturing of viral vaccines. This is particularly emphasized when compared to the well-established process for manufacturing monoclonal antibodies using a CHO platform in the broad context of biomanufacturing of biologics.

Manufacturing and releasing a cell-culture produced viral vaccines is lengthy and complex (Figure 1.5). The current good manufacturing process (cGMP) requires establishing and extensively documenting a master cell bank and a master virus seed bank derived from the virus isolates according to regulatory guidelines [19].

In short, the manufacturing process is initiated by cell amplification from a working cell bank, derived from the master cell bank. The cell culture process stream will depend on the cell substrate type that would grow in adherent or suspension cell cultures and would determine the mode of operation and type and scale of the bioreactor for production. In the case of adherent cell lines such as Vero cells, supports such as T-Flasks, roller-bottles, cell factories, or packed-bed bioreactors are required to sustain cell growth. To mitigate surface limitation to produce large quantities of vaccines as is the case for polio vaccines, microcarrier technology as a support might be used to facilitate the scalability up to 3,000 L operational volume. Cell cultures in suspension are generally more amenable to streamlined scale-up to larger volumes up to 10,000 L. As it has been the trend for production of biologics such as recombinant proteins and monoclonal antibodies, single-use equipment is deployed more and more frequently as a rapid response to surge manufacturing of viral vaccines [20].

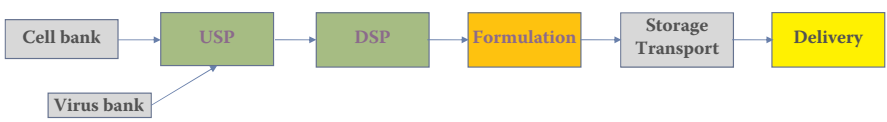

FIGURE 1.5 Typical scheme on cell-based vaccine manufacturing.

Following the cell amplification phase, a viral production phase is initiated. In a first cell culture stream, cells are infected for viral production with a working virus seed stock, derived from the master virus bank according to pre-established process parameters. A second uninfected cell culture stream is processed under similar operating conditions and monitored in parallel to the cell culture viral production stream as a control to demonstrate the absence of non-adventitious agents in the whole cell-culture production process. Depending on the infection/replication kinetics, the *virus bulk* is harvested after days to weeks.

The virus bulk harvest is purified following pre-established downstream process steps optimized and validated during the early process development phase and involve multiple filtration/ultra-filtration or chromatographic steps highly depending on the virus type, its structure, and stability. It is not uncommon to use large-scale ultracentrifugation in the vaccine manufacturing process. For example, hundreds of millions of influenza vaccine doses are produced using large-scale ultracentrifugation.

The upstream, downstream, and analytical protocols are detailed in dedicated core chapters in this textbook (Chapters 5 to 8) and examples are extensively presented and discussed in the case study reports of cell-culture produced vaccines and associated processes (Chapters 9 to 12). A special emphasis is placed on the development and execution of different sets of assays for vaccine lot release. As an example, single-radial immunodiffusion assay (SRID) (which is the only validated assay approved by regulatory agencies for the release of influenza vaccine lots) would require the preparation of a standardized antigen and specific polyclonal antibody. The preparation of the antibody might take weeks to months, and it is produced through immunization of animals, such as mice, rabbits, or sheep, collecting the serum from animals after immunization with the specific antigen. The overall process for preparation of these reagents is very time-consuming, taking weeks to months and might delay the approval of a vaccine for eventual evaluation in humans. A report from the WHO [21] identified the timelines required for the deployment of the SRID assay as one of the many reasons that made the response to the 2009 H1N1 pandemic and the timely availability of vaccine inadequate. Although the vaccine was manufactured, delays due to unavailability of standardized SRID reagents in a timely fashion were observed, raising significant concerns on readiness to respond to pandemic situations. The search for alternative assays to SRID assays fueled significant research in this area underlining the critical role of assays in vaccine development and manufacturing. Ideally, when the mechanism of action of the **vaccine** is known, a **potency assay** that is predictive of the clinical response should be available, serving as a "clinical correlate of protection." The correlation of protection is the minimum immune response that has been demonstrated to provide protection against the infectious disease. It is estimated by experts in the vaccine field that 70% of the time required for vaccine manufacturing is dedicated to quality control and represents several hundreds of analytical tests.

Specific to the vaccine field regulation, virus bulk batches require formal release by agents of the territory competent regulatory agency such as Health Canada, FDA, or

EMA within the premises of the manufacturing facility. The virus bulk is then formulated, filled in product containers, and then packaged in product containers. A second formal release by the regulatory agency agents is conducted prior to shipping. Additional regulatory assessments are conducted by regulatory agencies following shipping and distribution through extensive testing and documentation of release criteria [10].

The formulation of vaccines is an important step in the manufacturing process. This would combine in the bulk vaccine, the antigen product with eventually adjuvants in the case of subunit vaccines and excipients that would contribute to vaccine thermostability enabling delivery to remote areas. This step will also involve a final fill, a sterile filtration, and eventually a freeze-drying in the case of lyophilized vaccines prior to release and storage according to a predetermined cold chain. Adjuvants, as immune potentiators, play a role in the delivery of the antigen as particulates or molecular structures acting as depot/carriers of the antigen to immunostimulants, inducing a broader immune response and providing a better protection while maintaining safety of the vaccine product.

In the case of freeze-dried vaccine formulations, reconstitution of the vaccine prior to injection is required. The principles of freeze-drying to remove the solvent, usually water, from dissolved or dispersed vaccine will be described and exemplified in case studies.

1.8 PANDEMIC PREPAREDNESS AND OUTLOOK

The current COVID-19 pandemic has put the focus on the development process of vaccines and their safety and efficacy. However, this is not the first pandemic that has occurred. By the end of World War I, in 1918, the Spanish Flu (which was an H1N1 influenza-type virus) caused between 30 and 100 million deaths. SARS (Severe Acute Respiratory Syndrome), with a mortality of 10%, appeared in China in 2002. The H1N1 pandemic (2009), called the swine flu, showed that viruses can transmit via animals. All these viruses are RNA viruses, meaning that evolution is possible. MERS, Middle East Respiratory Syndrome, with a mortality of 30%, appeared in Saudi Arabia in 2012. MERS has been thereafter associated with close contact of humans with dromedary camels as the vehicle of disease transmission and has spread to other countries [22].

The field of vaccines, particularly in the context of pandemic preparedness, requires the development of new tools and methods to enable accelerated process and product development and manufacturing. Eventually, all the vaccines that are under development build on prior knowledge on vaccine design and manufacturing technologies with emergence of paradigm shifts such as the messenger RNA technology that led to the rapid development of COVID-19 mRNA vaccines within unprecedented timelines. Importantly, the scale-up potential and the robustness of the manufacturing technology for global delivery remain key drivers in the field of vaccine development, requiring alliances of experts in vaccinology, virology, cell biology, engineering, and medicine.

REFERENCES

[1] "History of Vaccines - A Vaccine History Project of The College of Physicians of Philadelphia | History of Vaccines," https://www.historyofvaccines.org/

[2] A. Boylston, "The origins of inoculation," *J. R. Soc. Med.*, vol. 105, no. 7, p. 309, Jul. 2012, doi: 10.1258/JRSM.2012.12K044

[3] M. R. Hilleman, "Vaccines in historic evolution and perspective: A narrative of vaccine discoveries," *Vaccine*, vol. 18, no. 15, pp. 1436–1447, Feb. 2000, doi: 10.1016/S0264-410X(99)00434-X

[4] G. L. Geison, "The private science of Louis Pasteur," *Priv. Sci. Louis Pasteur*, vol. 306, pp. 22–50, Dec. 1996, doi: 10.1515/9781400864089/HTML

[5] "Pasteur International Network association | Institut Pasteur," https://www.pasteur.fr/en/international/pasteur-international-network-association

[6] P. Deville, *Plague and Cholera,* 2014.

[7] J. B. Ulmer, U. Valley, and R. Rappuoli, "Vaccine manufacturing: Challenges and solutions," *Nat. Biotechnol.*, vol. 24, pp. 1377–1383, 2006, doi: 10.1038/nbt1261

[8] "GPEI – Global Polio Eradication Initiative," https://polioeradication.org/

[9] M. Roser, H. Ritchie, and B. Dadonaite, "Child and Infant Mortality," "https://ourworldindata.org/child-mortality," 2013.

[10] M. C. Flickinger, "Encyclopedia of biotechnology: Bioprocess, bioseparation, and cell technology. Chapter: Viral vaccine production in cell culture (John Aunins)," pp. 7 zv. (XXVII, 5051 str.) TS-WorldCat T4-Biop, 2010.

[11] "Vaccines Licensed for Use in the United States | FDA," https://www.fda.gov/vaccines-blood-biologics/vaccines/vaccines-licensed-use-united-states

[12] "Global Vaccine Market Report Overview of MI4A," 2018, Accessed: Nov. 08, 2021. [Online]. Available: http://who.int/immunization/MI4A

[13] M. Kaddar, "Global Vaccine Market Features and Trends," https://www.who.int/influenza_vaccines_plan/resources/session_10_kaddar.pdf, 2013.

[14] "Vaccines Market | Including & Excluding COVID-19 vaccines | Global Forecast to 2026," https://www.marketsandmarkets.com/Market-Reports/vaccine-technologies-market-1155.html?gclid=CjwKCAiA78aNBhAlEiwA7B76p3fHFVjr3dO5EaXlxFj-m0mxqxkiGyuX5k3QX6pOk14HpuSc4P8KZbxoCBjEQAvD_BwE

[15] "Vaccines Market Size, Share, Growth | Global Industry Report [2027]," https://www.fortunebusinessinsights.com/industry-reports/vaccines-market-101769

[16] P. Health Ontario, "At a Glance: Vaccine regulatory process in Canada 1 AT A GLANCE Vaccine Regulatory Process in Canada."

[17] Y. Gu, X. Zhao, and X. Song, "Ex vivo pulsed dendritic cell vaccination against cancer," *Acta Pharmacol. Sin. 2020 417*, vol. 41, no. 7, pp. 959–969, May 2020, doi: 10.1038/s41401-020-0415-5

[18] E. Sparrow *et al*., "Global production capacity of seasonal and pandemic influenza vaccines in 2019," *Vaccine*, vol. 39, no. 3, pp. 512–520, Jan. 2021, doi: 10.1016/J.VACCINE.2020.12.018

[19] "General Principles for the Development of Vaccines to Protect Against Global Infectious Diseases | FDA," https://www.fda.gov/regulatory-information/search-fda-guidance-documents/general-principles-development-vaccines-protect-against-global-infectious-diseases

[20] E. S. Langer and R. A. Rader, "Biopharmaceutical Manufacturing is Shifting to Single-Use Systems. Are the Dinosaurs, the Large Stainless Steel Facilities, Becoming Extinct? | American Pharmaceutical Review – The Review of American Pharmaceutical Business & Technology," *BioPlan Associates Inc*, 2018. https://www.americanpharmaceuticalreview.com/Featured-Articles/

354820-Biopharmaceutical-Manufacturing-is-Shifting-to-Single-Use-Systems-Are-the-Dinosaurs-the-Large-Stainless-Steel-Facilities-Becoming-Extinct/
[21] World Health Organization (WHO), "PANDEMIC INFLUENZA A (H1N1) Donor Report," https://www.who.int/csr/resources/publications/swineflu/h1n1_donor_032011.pdf, 2011.
[22] "Middle East Respiratory Syndrome Coronavirus (MERS-CoV)." https://www.who.int/news-room/fact-sheets/detail/middle-east-respiratory-syndrome-coronavirus-(mers-cov)

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2 Introduction to virology

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CONTENTS

2.1 INTRODUCTION

2.1.1 What Are Viruses?

Viral infections are known to cause diseases that may or may not require hospitalization; but also, they are the leading cause of a heavy mortality rate, including other health complications. Viral infection proves a greater threat to human health that can be least controlled [1]. Viruses, the causative agents of viral infections, are small, subcellular microorganisms, extremely dependent on host cells and are known as obligate intracellular and parasites. They can infect any form of life (plants, animals, bacteria, fungi). While the first virus discovered was in 1892, known as the TMV

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(Tobacco Mosaic Virus) followed by foot and mouth disease virus in 1898, the first human virus was discovered only in 1901 as the yellow fever virus. But scientists did not see an actual virus until 1930. In 1915, Frederick Twort, a bacteriologist, discovered a bacteriophage, the virus that can infect bacteria, notifying it as a microorganism that would kill bacteria. Hence, it established a unique feature of the virus as their size could vary within a range between 20 nm to 1 micron. They are much smaller than the cells they could infect.

2.1.2 Virus Characteristics

Viruses are among the most symmetrical biological objects. They can be either helical, spherical, icosahedral, or have more complex structure. They could be filamentous with elongated structures. Viruses can be visualized by x-ray crystallography or electron microscopy. Looking at the structure of TMV, it has given the concept that viruses are structurally composed of repeating subunits. The viral structure consists of some key features; namely, the capsid encapsulating the viral genome. The virus may or may not have an envelope layer.

2.1.3 Virus Evolution and Classification

Evolution of viruses has remained very speculative as they do not fossilize. They do not have a common ancestor. There have been different theories of virus evolution. First is the devolution or regressive theory, meaning they could have originated from free-living cells. Second is the escapist or progressive theory, which explained they might have originated from RNA and DNA molecules that escaped from the host cell. The third is the self-replicating theory, explaining a system of self-replication involving evolution alongside the host cell.

Viruses are classified based on their morphology, chemical composition, host organism, or mode of replication. But, since the discovery of viruses, the classification system has been modified to the system that is currently being followed. The first was Holmes' classification, who suggested a first complete taxonomic system. He proposed the order "virales" composing three suborders, namely, Phaginae (virus that infects bacteria), Phytophaginae (virus that infects plants), and Zoophaginae (virus that infects animals). He further created 13 families, 32 genera, and 248 species. Then came the classification system that gained the community support known as the LHT (Lwoff, Horne, and Tournier) system. This system grouped the viruses into one phylum called "vira" with two subphyla defining the genetic material, which is either DNA or RNA. This was further classified into classes based on the symmetry of the viral capsids.

Later, an urgent need to have an official system for taxonomy led to the establishment of the International Committee on Taxonomy of Viruses (ICTV). Thereafter, in 1971, David Baltimore published a classification system that is still in use in parallel. He grouped all viruses into seven groups based on the type of genome. From then until the present day, virus taxonomy has been considered by this committee following the Baltimore classification system (Figure 2.1).

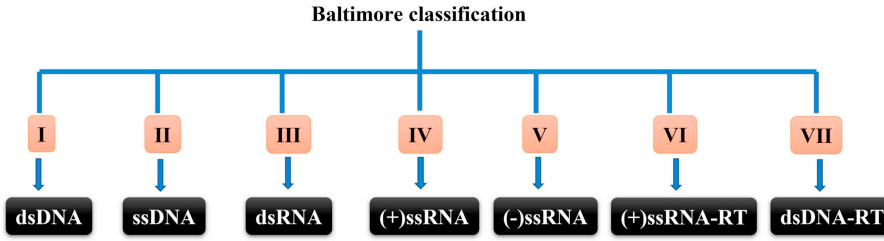

FIGURE 2.1 **Baltimore Classification:** This classification is based on the genome of the virus. **+ve RNA**: similar to mRNA and can be readily translated by host cell; **−ve RNA**: complementary to mRNA and requires conversion to +ve RNA by RNA polymerase prior to translation; **+ve DNA**: also called sense/coding strand. Sequence corresponds to the mRNA transcript ready to be translated; **−ve DNA**: also called anti-sense/template strand. Reverse complementary to the sense strand as well as to the mRNA transcript.

2.2 STRUCTURE AND GENOME

2.2.1 Virus Structure and Function

2.2.1.1 Capsid

A capsid is a protein shell required to protect the viral genome from host nucleases. For some viruses, during infection, the capsid is responsible for attachment to the specific receptors exposed on the host cellular surface. A capsid can be either single- or double-protein shells containing few structural proteins. Hence, multiple copies of the capsid must self-assemble to form the 3D capsid structure, allowing different viruses to have wide ranges of shape and structure.

2.2.1.2 Envelope

Some viral families have an additional protective coat, called the envelope. The envelope is a lipid bilayer which is partly obtained from the host cell membrane. The lipid composition of this envelop closely reflects that of the specific host cell plasma membrane. The exterior of this bilayer exhibits protruding structures known as "spikes," containing virus-coded glycosylated trans-membrane proteins. In enveloped viruses, spikes also assist in the attachment of the virus to the host cell surface.

The viral envelope serves the function of protecting the viral genetic material. It also helps in facilitating the entry of the virus while infecting a host cell along with evading the host immune response. However, due to the envelope's fragile nature, non-enveloped or also known as naked viruses, could be more resistant to parameters such as temperature, pH, and few common chemical disinfectants (Figure 2.2).

2.2.2 Viral Genome

A virus could have either an RNA or DNA genome. This RNA or DNA genome could be again categorized into being either positive or negative sense and single or

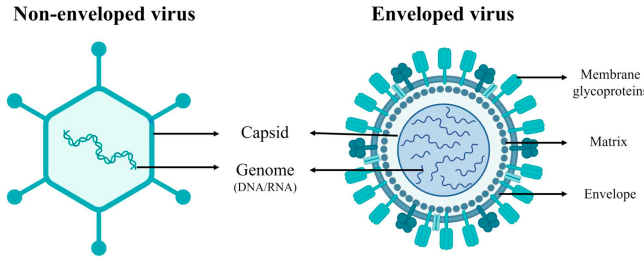

FIGURE 2.2 A non-enveloped virus (example: adenovirus) versus an enveloped virus (example: influenza virus).

double stranded. Because of its limited size, this genome codes for a minimum number of proteins necessary to allow its multiplication by the host cell. A fully assembled infectious virus is called a virion. For example, the influenza A virus consists of eight single-stranded (negative sense) RNA segments encoding for a total of 11 viral proteins.

2.2.3 Viral Genome Transcription Via Intermediates

The seven groups divided by the Baltimore classification explain the virus mechanism to facilitate its genome replication, transcription, and translation using the host cellular machinery. The seven groups have been described in detail in the following (Figure 2.3):

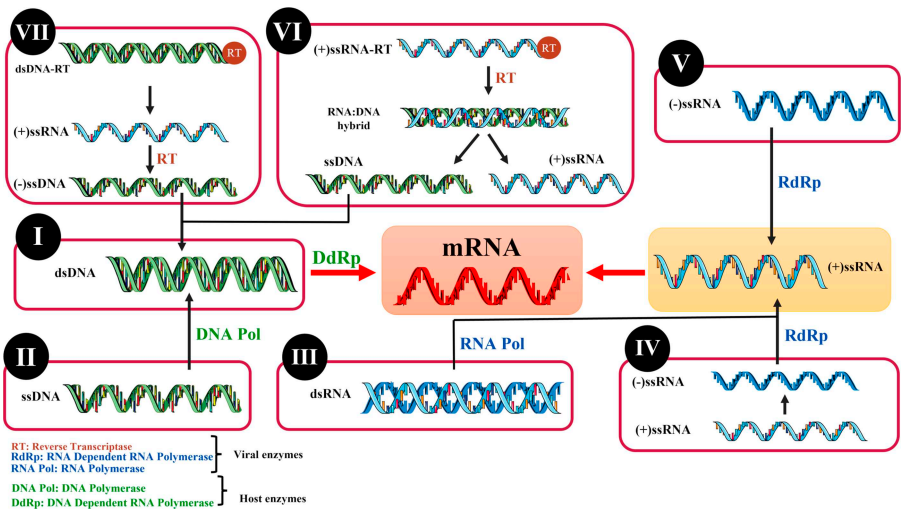

FIGURE 2.3 **Virus transcription via intermediates:** Transcription mechanisms followed by different groups of viruses defined by the Baltimore classification system in a eukaryotic host. The final goal is to produce a functional mRNA to be translated by the host ribosome to synthesize viral proteins.

1. **Group I – dsDNA virus:** The viruses belonging to this group follow the simplest mechanism of transcription. They use DNA-dependent DNA polymerase (DdDp) from the host cell to generate mRNA, which could late translate into functional viral proteins.
   Examples: Adenovirus, Herpesvirus
2. **Group II – ssDNA virus:** This group first needs to create a complementary strand to form a dsDNA. This is achieved by using DNA polymerase from the host to synthesize the strand which is complementary to their ssDNA genome. After the formation of dsDNA, it makes mRNA using the host enzyme DdDp.
   Example: Parvovirus
3. **Group III – dsRNA virus:** This group of viruses contains an RNA polymerase that would transcribe the dsRNA to (+) ssRNA, which would ultimately be the mRNA. This mRNA would serve two purposes. First, it would get translated to the required viral proteins, and second, it would also serve as a template to synthesize (–) ssRNA to form the dsRNA genome for packaging.
   Examples: Reovirus, Rotavirus
4. **Group IV – (+) ssRNA virus:** The genome of this group of viruses is similar to the host mRNA sequence. But the only hurdle is that the host ribosomes do not recognize the viral RNA. Hence, the viral polymerase moves along the (+) strand of the RNA template and elongates the (–) stranded RNA molecule. Now this (–) ssRNA serves as a template for polymerizing new (+) ssRNA, serving as the genome of the virus.
   Examples: Picornavirus, Togavirus, Coronavirus
5. **Group V – ssRNA virus:** This group makes for the largest family of viruses. They carry the RNA-dependent RNA polymerase (RdRp). This enzyme makes two types of (+) RNA strand: 1) a short viral mRNA that would be translated into necessary viral proteins and 2) a full-length RNA that would be replicated to make (–) ssRNA genome for packaging into progeny visions. Here, viral RNA is replicated separately and has a highly organized and regulated packaging process to make sure that one of each distinct RNA has been received by each virion.
   Examples: Orthomyxovirus, Paramyxovirus
6. **Group VI – (+) ssRNA-RT virus:** This group of viruses contains an enzyme called reverse transcriptase (RT). They go opposite or reverse to the normal transcription process. The (+) ssRNA undergoes reverse transcription to make a complementary DNA strand. This DNA strand acts as a template from a dsDNA in the nucleus of the host. This dsDNA is covalently linked to host chromosomal DNA and, therefore, replicates as a host genome. Viruses of this group benefit from the error-prone RT enzyme. It provides them with their ability to evade the immune system by minor changes to their protein capsules.
   Example: Retrovirus
7. **Group VII – dsDNA-RT virus:** This group of viruses replicates through RNA intermediates. The members of this group have a very different genome because of two facts. First, one strand has a protein at the 5' end

and is considered a complete strand. Second, the other strand has a short RNA at the 5' end, making it an incomplete strand. Hence, one strand being complete and the other being incomplete, they have a gapped DNA genome. The mechanism followed by the members of this group must consider the facts that the 1) transcription occurs in the host nucleus, 2) only the RNA part would be exported out of the nucleus, and 3) viral assembly occurs in the host cytoplasm.

Therefore, the gapped genome is first repaired/filled by viral polymerase forming a circular DNA. This DNA is transcribed into RNA by host polymerase. The RNA goes out of the nucleus. Finally, the viral RT enzyme makes DNA from RNA, making it possible to be packaged into virion capsid.

Example: Hepatitis B (Figure 2.3).

2.3 VIRAL INFECTION CYCLE

The viral genome, despite its limited size, will have to follow the general rule of the "Central Dogma" to transfer the information from its genes to the proteins. The viral genome needs to first replicate itself. Second, it must transcribe to form a functional mRNA, and third, this mRNA must undergo translation to generate a functional protein, including all post-translational modifications, producing an infectious virion, and for all these it depends on the host system. An infection cycle needs to follow a series of events ultimately leading to the production of new virions using the host cellular machinery. There are three types of commonly known infection cycles.

2.3.1 Lytic Cycle

A lytic cycle is also known as virulent infection. This type of infection cycle is mostly followed by viruses infecting bacteria (bacteriophages, Figure 2.4). This cycle results

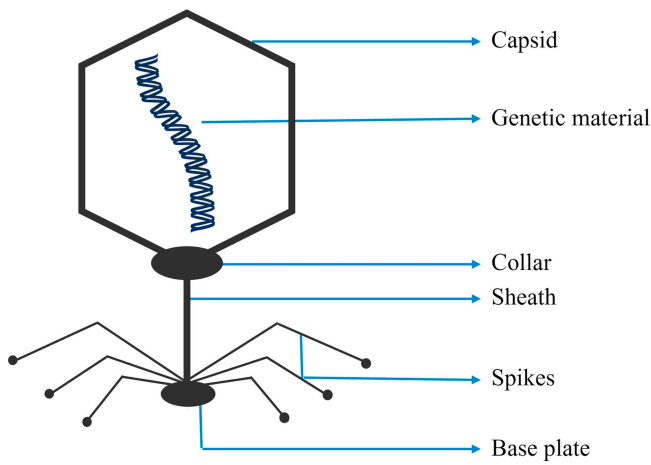

FIGURE 2.4 **Bacteriophage:** A virus that infects bacteria.