

## Chapter 2

# Properties of Influenza Viruses

**Abstract** The three genera, viz. *Influenzavirus A*, *Influenzavirus B*, and *Influenzavirus C* are classified as separate genera, out of the five genera in the family *Orthomyxoviridae* (the other two genera being *Thogotovirus* and *Isavirus*). The genome of influenza viruses is made up of single-stranded RNA of negative polarity, containing 7–8 segments. The eight genomic segments of influenza A virus code for nine structural proteins are PB1, PB1-F2, PB2, PA, HA, NA, NP, M1 and M2, and for two non-structural proteins, they are NS1 and NS2. The functions of various proteins of influenza viruses are described. The viruses are pleomorphic and can occur as spherical or filamentous forms, having a size of 80–120 nm in spherical form and >300 nm in filamentous form. The influenza viruses are comparatively unstable in the environment and are susceptible to heat, extremes of pH and dryness, organic solvents and detergents such as sodium deoxycholate (SDC) and sodium dodecyl sulphate (SDS). Although the virus gets inactivated with various concentrations of formalin, binary ethylenimine and beta propiolactone, surprisingly the haemagglutinating and neuraminidase activities are retained. Incomplete influenza virus particles are formed in the cells infected at high multiplicity of infection (M.O.I.). These are also called Defective Interfering (D.I.) particles as their genome is defective, and they interfere with the replication of the complete influenza virus particles.

Of the five genera of family *Orthomyxoviridae* the following three influenza viruses are classified as separate genera: *Influenzavirus A*, *Influenzavirus B*, and *Influenzavirus C*, the other two genera being *Thogotovirus* and *Isavirus*. The two major internal proteins of influenza viruses, nucleoprotein and matrix protein, are responsible for the classification of these influenza viruses into separate genera (Alexander 2001; Palese and Shaw 2007). Type A viruses that affect mammals as well as birds are further classified into subtypes based on their 18 haemagglutinin (H) and 11 neuraminidase (N) proteins (Alexander 2001; Tong et al. 2012, 2013). Except H17, H18 and N10, N11, all other haemagglutinins and neuraminidases in all probable combinations have been detected from birds (OIE 2005).

The treatment of influenza virions with sodium deoxycholate frees various types of nucleocapsids that show heterogeneous sedimentation properties and are associated with different viral RNA. The nucleocapsids from the complete influenza virus were mainly observed at 64 and 56S. The 64S nucleocapsids were not present in incomplete influenza virus. The presence of 18S RNA and 15S RNA in 64S and 56S nucleocapsids, respectively, indicated the association of the sedimentation rates of viral nucleocapsids and RNAs (Kingsbury and Webster 1969). The genome of influenza viruses is segmented single-stranded RNA of negative polarity (To et al. 2012). There are eight RNA segments in influenza A and B viruses while influenza C virus has seven RNA segments. The mRNAs are transcribed from the virion RNA by a virion-associated RNA-dependent-RNA-polymerase. The eight genomic segments of influenza A virus code for nine structural proteins are PB1, PB1-F2, PB2, PA, HA, NA, NP, M1 and M2, and two non-structural proteins are NS1 and NS2 (Chen et al. 2001; Lamb 1983; Lamb and Choppin 1979, 1981; Palese 1977; Swayne and Halvorson 2003; Palese and Shaw 2007). The NS2 has also been reported to be present in purified viral preparations and named as nuclear export protein (NEP) (Richardson and Akkina 1991). A role of NEP has been found in the facilitation of polymerase activity-enhancing conformation (Reuther et al. 2014). The NEP (NS2) protein of influenza B and C virus is responsible for nuclear export activities (Paragas et al. 2001). The genomic segments are enclosed within a capsid made up of helically organised nucleoprotein (NP) (Chenavas et al. 2013). The matrix composed of M1 protein intercepted by ion channels made up of M2 protein lies on the inner side of viral envelope (Schnell and Chou 2008; Stouffer et al. 2008). The functions of the HA is haemagglutination and attachment of the virus to the cells (Mineev et al. 2013). It also produces antibodies in the host after infection and protects further infection. The NA has enzyme activity to aid the release of the new virus from the cell due to its action on the neuraminic acid on the receptors (Alexander 2001). The PB1, PB2 and PA are components of the viral RNA polymerase and are responsible for RNA replication and transcription (Swayne and Halvorson 2003). Viral polymerase activity is regulated by PB1-F2 amino acids (Ueda et al. 2014). Many other functions of the genes and proteins of influenza viruses are discussed in the subsequent chapters.

The gene coding assignments in influenza B virus are similar to influenza A virus except that PB1-F2 is absent and the NA gene segment codes for both NA and NB protein (Shaw et al. 1983). The influenza C virus does not contain PB1-F2 protein, and PA is named as P3 as it lacks the acidic features at neutral pH (Yamashita et al. 1989). The fourth segment of influenza C virus codes for HEF protein, which possess the functions of haemagglutinin, receptor destroying and fusion activities (Herrler et al. 1988). The proteins encoded by various types of influenza viruses are summarized in Table 2.1.

The infection of the cells at high multiplicity of infection (M.O.I.) leads to the production of incomplete influenza virus particles (von Magnus virus) and the phenomenon is called von Magnus phenomenon after its discoverer von Magnus (1954). The genome of these incomplete influenza virus particles is defective,

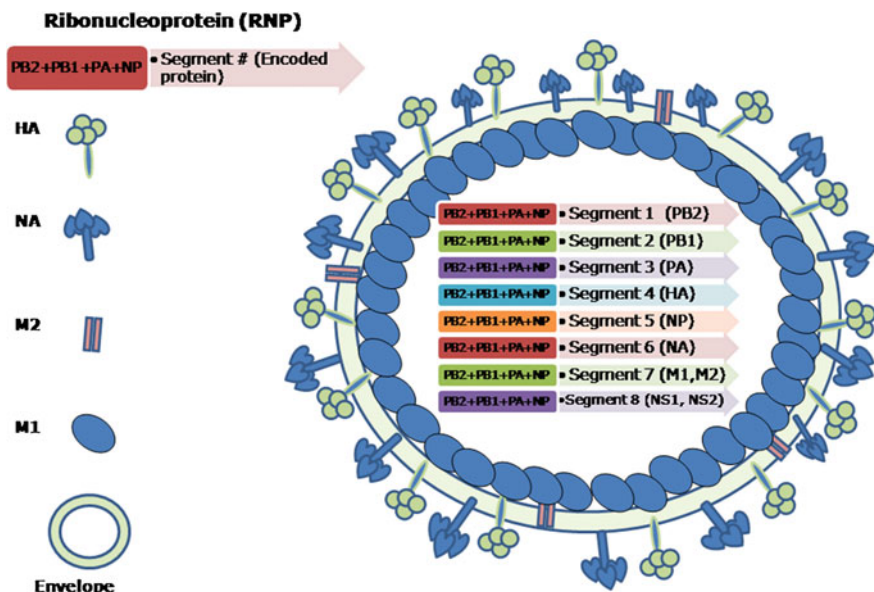
**Table 2.1** Summary of the proteins encoded by various segments in different influenza virus genera

Gene segments	Proteins encoded by various gene segments in		
	Influenza A virus	Influenza B virus	Influenza C <sup>a</sup> virus
1	PB2	PB2	PB2
2	PB1, PB1-F2	PB1	PB1
3	PA	PA	P3
4	HA	HA	HEF
5	NP	NP	NP
6	NA	NA, NB	CM1, CM2
7	M1, M2	M1, BM2	NS1, NS2/NEP
8	NS1, NS2/NEP	NS1, NS2/NEP	

<sup>a</sup> contains seven instead of eight RNA segments

though their proteins and antigenicity is similar to the infectious viruses. The incomplete virus particles are either non-infectious or only partially infectious (Choppin and Pons 1970). These incomplete influenza virus particles are also called Defective Interfering (D.I.) particles as they are defective in their genome, require the help of complete influenza virus particles and interfere with the replication of the complete influenza virus particles (Nayak et al. 1978). The influenza D.I. particles possess small subgenomic RNAs (sgRNAs) derived by internal deletion of standard virus mainly of PB1, PB2, PA segments (Crumpton et al. 1978; Davis et al. 1980; Nakajima et al. 1979; Ueda et al. 1980; Nayak et al. 1982; Nayak 1983; Sivasubramanian and Nayak 1983). The M.O.I.-dependent production of influenza D.I. particles can occur due to defects in the maturation in which case all the newly synthesized set of genomic segments are not assimilated into infectious progeny virions (Lerner and Hodge 1969). The other possibility can be defective synthesis of various RNA segments of the virus within the infected cells (Nayak 1972). The formation of D.I. particles is not limited to influenza A viruses. The production of D.I. influenza B virus by a high multiplicity infection has been reported. These D.I. influenza B virus particles lack RNA segment 7 which codes for M protein and interfere with the replication of wild-type influenza B virus (Tobita et al. 1986).

The influenza A viruses are pleomorphic and can occur as spherical or filamentous forms. The diameter of the spherical virions is in the range of 80–120 nm. The virions isolated from fresh clinical isolates are predominantly filamentous with elongated viral structures >300 nm (Bourmakina and Garcia-Sastre 2003; Burleigh et al. 2005; Elleman and Barclay 2004; Wright et al. 2007). The morphology of influenza B virus is similar to that of influenza A virus. Influenza C viruses exhibit hexagonal reticular structures on the surface (Apostolov and Flewett 1969) and can form long (500 µm) cord-like structures on the surface of infected cells (Muraki et al. 2004; Nishimura et al. 1990, 1994). They are enveloped viruses having 10–12 nm long surface projections or spikes. There are two types of spikes in influenza A and B viruses, (i) rod shaped made up of



**Fig. 2.1** Schematic structure of a typical influenza A virus. The genome of virus consists of eight segments of ssRNA which remain attached to polymerase complex proteins (*PB2*, *PB1*, *PA*) and wrapped in nucleoprotein (*NP*). The matrix protein (*M1*) is located underneath the lipid envelope. The *M2* protein forms the ion channels. The haemagglutinin (*HA*) and neuraminidase (*NA*) form the surface peplomers. The genomic segments 1–6 are monocistronic, while the 7th and the 8th are bicistronic. The *NS1* and *NS2* are considered to be non-structural proteins whereas the other six are structural proteins

homotrimers of haemagglutinin (*HA*) and (ii) mushroom shaped made up of homotetramers of neuraminidase (*NA*) glycoproteins (Cox et al. 2000). A typical schematic structure of influenza A virus is shown in Fig. 2.1. In contrast to influenza A and B viruses, influenza C viruses have only one spike that is made up of the multifunctional haemagglutinin-estrase-fusion (*HEF*) glycoprotein (Nakada et al. 1984, 1985). The envelope of influenza A virus has *HA*, *NA* and matrix 2 (*M2*) proteins whereas influenza B virus has *HA*, *NA*, *NB* and *BM2* while influenza C virus has *HEF* and *CM2* proteins (Betakova et al. 1996; Brassard et al. 1996; Cox et al. 2000; Nakada et al. 1984, 1985; Odagiri et al. 1999; Pekosz and Lamb 1997). Various viral and biological properties that differentiate the influenza A, B, and C viruses are shown in Fig. 2.2.

Influenza viruses are comparatively not very stable and do not survive for long in the environment, and physical factors such as heat, high or low pH and dryness can kill the virus. They are inactivated by organic solvents and detergents such as sodium desoxy cholate (*SDC*) and sodium dodecyl sulphate (*SDS*) (Swayne and Halvorson 2003). In the presence of organic matter, these viruses can be destroyed by chemicals such as formaldehyde, glutaraldehyde, beta propio-lactone and binary ethylenimine. After removal of organic matter, chemical disinfectants such

Characteristics	Influenza A	Influenza B	Influenza C
Number of Genomic Segments	8	8	7
Number of Viral Proteins	10	11	09
Reassortment	Present	Absent	Absent
Species Affected	Humans, swine, equine, avian, Canines, bats marine mammals	Humans,	Humans, swine,
Potential to cause Pandemics	Yes	No	No
Whether present in annual seasonal influenza vaccines	Yes	Yes	No

**Fig. 2.2** Differentiating features of various genera of influenza viruses

as phenolics, quarternary ammonium compounds, 5.25 % sodium hypochlorite, 2 % sodium hydroxide, 4 % sodium carbonate dilute acids and hydroxylamine can destroy these viruses (Franklin and Wecker 1959; King 1991; Swayne and Halvorson 2003). Inactivation with the retention of haemagglutinating and neuraminidase activities can be achieved with various concentrations of formalin, binary ethylenimine and beta propiolactone for laboratory purposes (Laver 1963).

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