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Intracellular trafficking of influenza virus: clinical implications for molecular medicine

Gary R. Whittaker

The trafficking and processing steps that occur in cells that are infected with influenza virus play a crucial role in the outcome of infection. These steps are targets for new and future anti-viral drugs, and can affect the relative virulence of the virus and its ability to cause disease. The virus first binds to its host cell via specific sialic acid residues, which can control the species tropism of the virus. The internalisation of the virus, into the nucleus of the cell, is dependent on a low pH, and this process is therapeutically targeted by the drug amantadine. Following replication, the newly formed viral genomes leave the nucleus and assemble into infectious particles at the plasma membrane. The targeting and processing of the various viral components at this late stage of the infectious cycle can have a major effect on the ability of the virus to spread and cause disease in its host. Finally, the release of viruses is dependent on the enzyme neuraminidase (NA), and this function has recently been targeted by the NA inhibitors, a new generation of drugs against influenza virus.

Like all viruses, influenza has an intimate relationship with its host cell and, during the course of replication, it undergoes many important trafficking steps. A study of such intracellular trafficking has revealed many facets of the virus life cycle, and how the life cycle relates to the pathogenic properties of the virus. Some of these trafficking steps can be targeted by anti-viral drugs, which are used to treat influenza virus infections. The trafficking of influenza virus within its host cell, and how these events are related both to the pathogenic properties of the virus and to therapeutic treatments for viral infection have been discussed in this review. Strategies for vaccination have not been covered; instead, readers are referred to Subbarao (Ref. 1) for more information on this topic.

Aetiology and pathogenesis of influenza viruses

In humans, influenza viruses are common pathogens of the upper respiratory tract, and seasonal epidemics affect 10–20% of the general population. However, the virus can also be deadly. It has been estimated that the now-infamous influenza pandemic of 1918–1919 killed 20–40 million people worldwide (Ref. 2). Influenza viruses infect humans and a wide variety of

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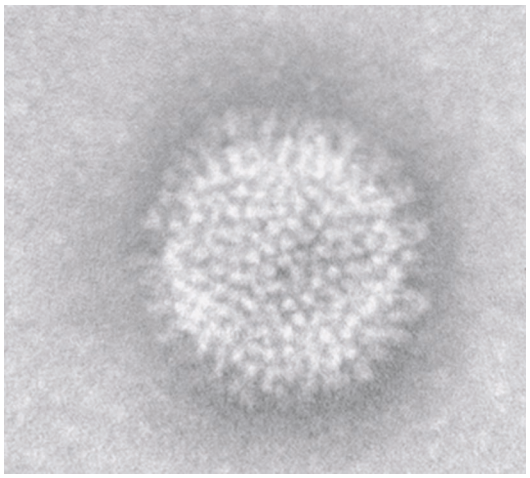
animals (Refs 3, 4). Other mammals that are susceptible to respiratory influenza infection include pigs, horses, mink, seals and whales. The virus also has gastrointestinal tropism in various bird species.

Influenza virus is a member of the *Orthomyxoviridae* family of RNA viruses, and is an enveloped virus (Fig. 1). Its genome consists of individual segments (or genes) of negative-sense RNA. There are three types of influenza viruses: A, B and C. Influenza A viruses are the most widespread and infect many animal species. Influenza B and C viruses were originally thought to cause disease only in humans; however, influenza B virus infection has recently been discovered in seals (Ref. 5). It is currently unclear whether this is a single transmission event or whether it implies a wider distribution of the virus.

Influenza virus subtypes are designated by a nomenclature that is based on their surface glycoproteins, namely haemagglutinin (HA or H) and neuraminidase (NA or N; also known as sialidase). The first human influenza viruses to be isolated during the 1930s were subsequently designated H1N1, based on their serological reaction. This group includes the viruses that are now known to have been present in the pandemic 1918 strain. In 1958, an antigenic shift resulted in the emergence of human H2N2 viruses and, in 1968, a shift to H3N2 viruses occurred in human populations. H3N2 viruses have remained the most prevalent in recent years (Ref. 6), but the re-emergence of H1N1 strains during the 1970s, most probably from a laboratory source, has resulted in co-circulating influenza H1N1 viruses. Influenza B viruses have not been given the same H and N designation and, in recent years, have represented a minor population of circulating viruses in humans. Influenza C viruses generally result in only mild respiratory illness and are much less studied.

The emergence of new influenza strains in the human population occurs via transmission from other animal species, most notably birds. Transmission to humans is most commonly thought to occur through an intermediate such as swine (Ref. 3). Typically, human and avian influenza viruses are quite different and are not infectious for both species. However, pigs can become infected with both types of viruses, and it has been proposed that they act as a 'mixing vessel' for the transmission of avian influenza viruses to humans. Occasionally, direct avian–human transmission can occur with deadly results, as demonstrated by the emergence of the recent H5 'Hong Kong avian flu' during 1997–1998 (Ref. 7). Several instances of the transmission of this virus from domestic chickens to humans proved fatal, but no proven cases of human to human transmission occurred.



Electron micrograph of an influenza virus

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Figure 1. Electron micrograph of an influenza virus. This influenza virus (strain A/WSN/33) was purified from Madin-Darby bovine kidney (MDBK) cells and negative-stained with potassium phosphotungstic acid. (The figure was kindly provided by Elizabeth Wills, Cornell University, Ithaca, NY, USA) **(fig001gwn)**.

Structure of influenza viruses

Influenza viruses are enveloped and are pleiomorphic (i.e. they can vary their size and shape). Viruses isolated from cell culture are typically spherical, with a constant diameter of ~100 nm (see Fig. 1). However, the virus can also be filamentous; particles retain a constant diameter (of 100 nm) but vary in length (up to several micrometers; Ref. 8). Filamentous viruses, such as these, are likely to predominate in clinical situations, and to be important in natural infection by influenza virus (Ref. 9).

Influenza A viruses produce ten proteins from eight RNA segments (Refs 10, 11). The eight negative-sense RNAs are associated with

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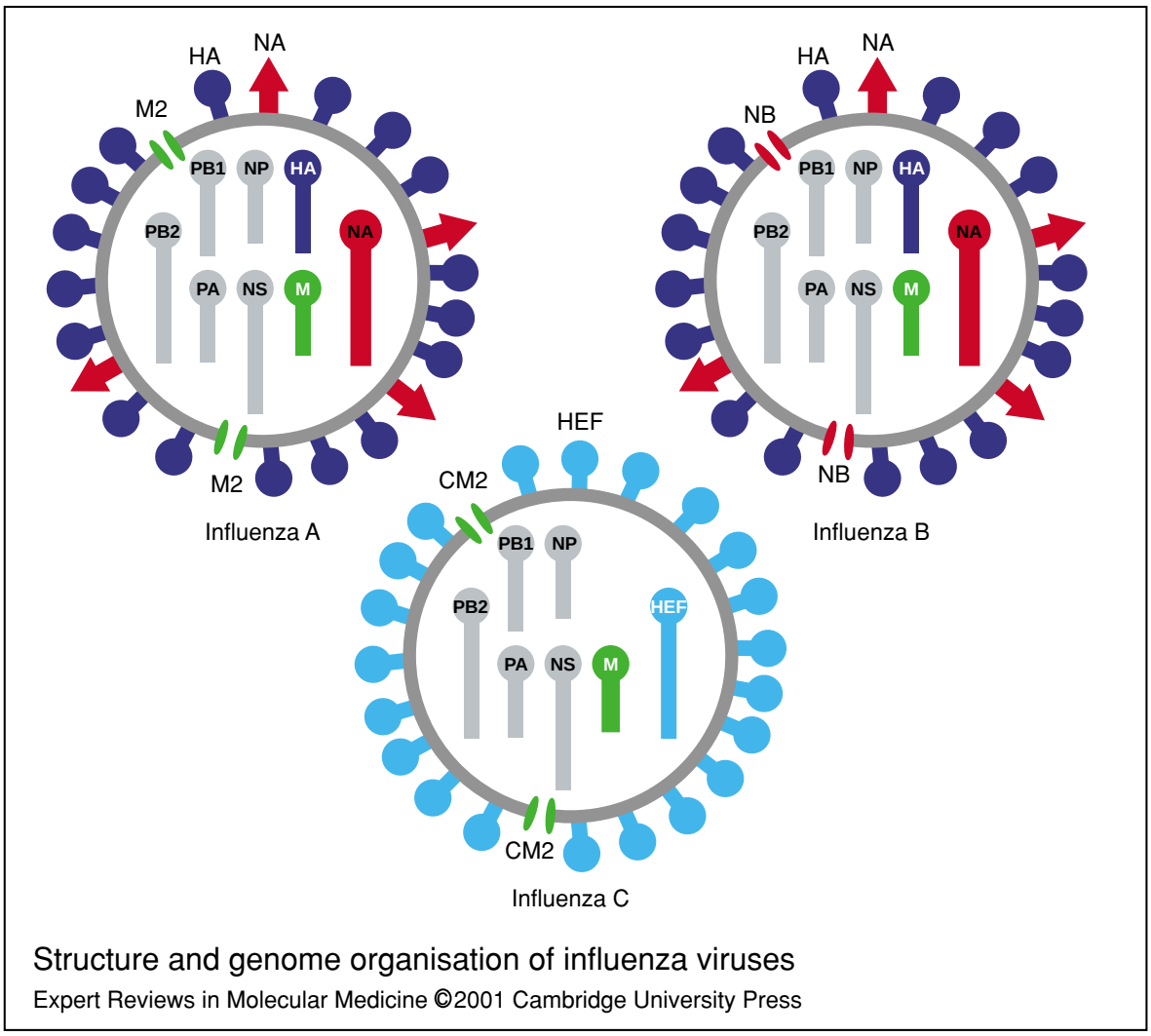

Figure 2. Structure and genome organisation of influenza viruses. The surface proteins of each virus and their respective genes are shown in colour (blue, red and green); other genes are shown in light grey. The interior proteins, namely the matrix protein (M1), the nucleoprotein (NP) and the polymerases are not shown. Influenza A and B viruses contain eight RNA segments (genes), whereas influenza C viruses contain only seven RNA segments. Influenza C viruses contain a single surface glycoprotein (the haemagglutinin-esterase-fusion, or HEF, glycoprotein; shown in light blue), which functionally replaces the two surface glycoproteins that are found in influenza A and B viruses, namely haemagglutinin and neuraminidase [HA (shown in dark blue) and NA (shown in red)]. The envelopes of the three viruses also contain different ion channels, which are encoded by either the M gene (i.e. M2 or CM2, shown as green ovals) or the NA gene (i.e. NB, shown as red ovals) **(fig002gwn)**.

many copies of a nucleoprotein (NP) and a heterotrimeric polymerase, which form the viral ribonucleoproteins (vRNPs). Inside the virus, the vRNPs are surrounded by a shell of the matrix protein (M1). M1 links the vRNPs to the virus envelope, which contains the viral glycoproteins as well as the tetrameric M2 ion channel. Host-cell proteins are typically excluded from the mature virus particles. All influenza viruses have similar internal components, but the constituents of their envelopes can differ markedly (see Fig. 2). Influenza B viruses have an alternative ion channel (NB) that is produced as an overlapping reading frame by alternative initiation from the gene encoding NA. Influenza C viruses have a single glycoprotein (the haemagglutinin-esterase-fusion, or HEF, glycoprotein) that functionally replaces both HA and NA. Influenza C viruses

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also differ in having an alternative ion channel (CM2) and a genome that consists of only seven segments of RNA, rather than eight. Influenza viruses also synthesise two proteins (NS1 and NS2) that were originally considered to be non-structural. However, NS2 is now believed to be part of the virus particle. During virus replication, the genes that encode M and NS are both spliced, accounting for the synthesis of two additional polypeptides.

Cell and tissue tropism of influenza viruses

In vivo, the principal cell types targeted by influenza viruses are the cells in the epithelial lining of the respiratory mucosa, which is a polarised epithelium; that is, it has distinct apical and basolateral surfaces (Refs 12, 13). When an aerosolised virus is inhaled, the virus encounters the apical (or outside) face of columnar epithelial cells. Following replication, virus is also released from the apical face of the cell into the airways of the respiratory tract. The lack of basolateral release generally precludes the systemic spread of influenza viruses in their host. Released viruses can spread from cell to cell, be exhaled and infect a new host. They can also be recognised by cells of the immune system, including alveolar macrophages (which engulf and destroy the virus) and circulating dendritic cells (which migrate out of the lung tissue and present viral antigens to T cells). In the laboratory, viruses are typically studied either in embryonated chicken eggs or in Madin-Darby canine kidney (MDCK) cells, both of which support the multi-cycle growth of influenza viruses (Ref. 14). Owing to the wide distribution of receptors, many other cell types can be infected by the virus, but some only through a single cycle of infection and without the spread of virus from cell to cell.

Virus binding, internalisation, trafficking and export

A single cycle of influenza virus infection in a typical cell is depicted in Figure 3. Briefly, the virus initially binds to its cell-surface receptor and is internalised into endosomes (cytoplasmic vesicles), where the low pH of the environment triggers virus fusion and uncoating. The uncoated vRNPs then enter the nucleus of the host cell for virus replication. Following virus replication, the vRNPs leave the nucleus and move to the plasma membrane, where they associate with viral glycoproteins before final budding and release. Each of these transport events is considered below, in relationship to our current understanding of influenza virus pathogenesis and treatment.

Virus binding

It is well established that human influenza viruses bind to moieties that contain 5-*N*-acetyl neuraminic acid (sialic acid) on the surface of host cells; however, in the pig and horse, *N*-glycolyl neuraminic acids can be used. Binding to sialic acid occurs via a shallow depression near the membrane-distal tip of the HA glycoprotein. It is also well established that some viruses bind preferentially to terminal sialic acids containing α-(2,6) linkages, whereas others favour binding to α-(2,3)-linked sialic acid (Ref. 15). This receptor-binding specificity correlates with a specific amino acid at position 226 of HA. HAs that have leucine at position 226 selectively bind to α-(2,6) sialic acid and occur preferentially in human strains. However, HAs that have glutamine at position 226 are specific for α-(2,3) linkages, and occur mostly in avian and equine strains of the virus. Both α-(2,3)- and α-(2,6)-linked sialic acid occur in the trachea of swine – accounting for the ability of pigs to become infected with both avian and human strains. Thus, receptor binding is one of the initial determinants of pathogenicity, such that the specificity of receptor binding accounts for much of influenza's species tropism (i.e. the predilection of the virus to infect certain animal species and not others).

The cell-surface receptor for influenza viruses can apparently take the form of sialic acid linked to either glycoprotein or glycolipid. In vitro, viruses can bind to, and fuse with, synthetic lipid vesicles that contain only glycolipid (Ref. 16), suggesting that entry does not require a specific cellular protein as a receptor. However, the infection of desialylated cells has been reported recently, suggesting either the presence of sialic-acid-independent receptors or a multi-stage process (Ref. 17). In vivo, other factors may well be important for virus entry. In macrophages, recent evidence suggests that the viruses undergo an additional lectin-like interaction with the mannose receptors of the macrophages, following the initial interaction with sialic acid (Ref. 18). Whether other 'co-receptor-like' activities occur in other cell types remains to be determined.

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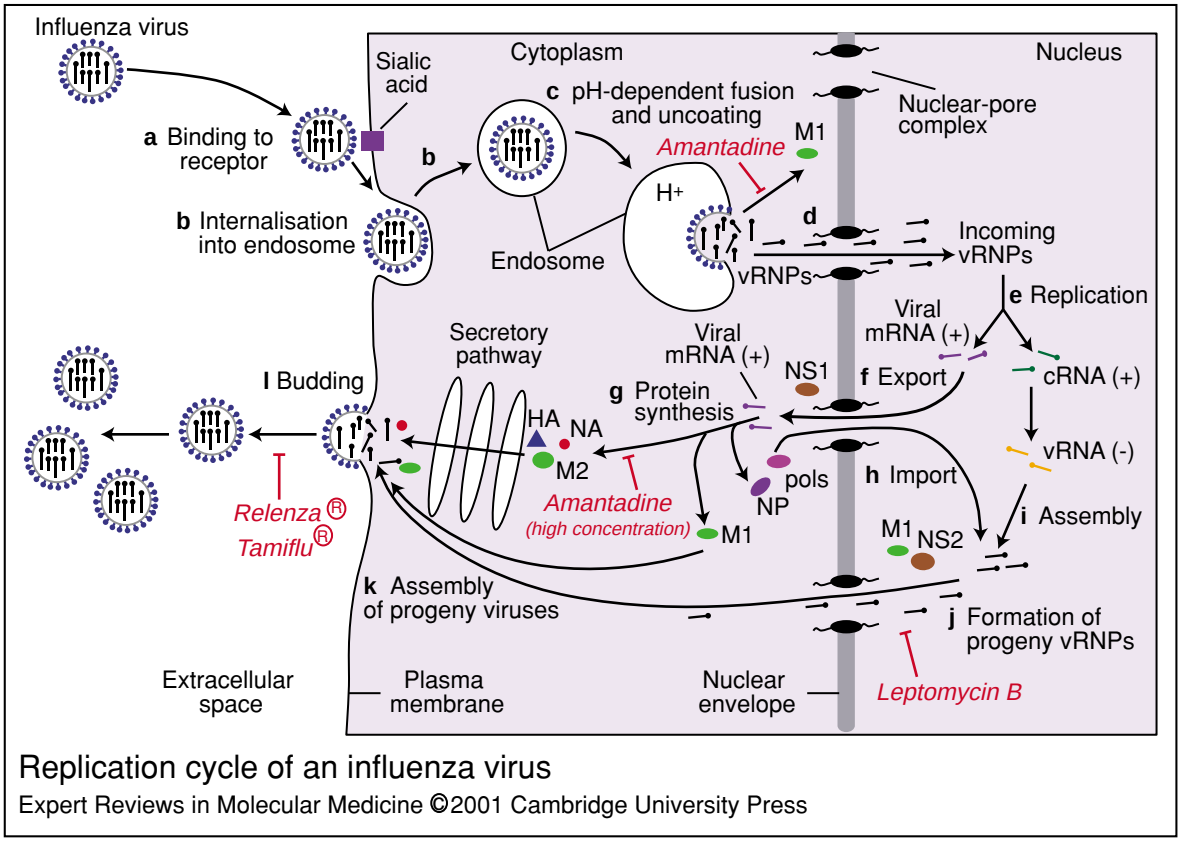

Replication cycle of an influenza virus

Figure 3. Replication cycle of an influenza virus. (a) The virus binds to receptors on the surface of the host cell and (b) is internalised into endosomes. (c) Fusion and uncoating events, which are pH dependent, result in (d) the release of the viral genome (in the form of viral ribonucleoproteins; vRNPs) into the cytoplasm. The vRNPs are then imported into the nucleus for (e) replication. (f) Positive-sense viral messenger RNAs (mRNAs) are exported out of the nucleus into the cytoplasm for (g) protein synthesis. (h) Some of the proteins are imported into the nucleus to assist in viral RNA replication and (i) vRNP assembly, which also occur in the nucleus. (j) Late in infection, the vRNPs form and leave the nucleus, and (k) progeny viruses assemble and (l) bud from the plasma membrane. The sites of action of anti-viral drugs are shown in red, italic text. Abbreviations used: cRNA (+), positive-sense complementary RNA; HA, haemagglutinin; M1, matrix protein; M2, tetrameric ion channel; mRNA (+), positive-sense messenger RNA; NA, neuraminidase; NP, nucleoprotein; NS1, a non-structural protein, NS2, a viral protein; pols, polymerases; vRNA (-), negative-sense genomic RNA **(fig003gwn)**.

Following the interaction of the virus with a receptor at the cell surface, it is rapidly internalised into clathrin-coated pits – a process that is dependent on dynamin, a cellular GTPase (GTP phosphohydrolase; Ref. 19). Viruses are trafficked through the endocytic pathway and ultimately reach a low-pH compartment (where the pH is approximately 5.5; Refs 20, 21). At this pH, the viral fusion machinery is triggered. HA undergoes a conformational change, forming a 'coiled-coil' of α helices and exposing the previously buried hydrophobic fusion peptide, which then inserts into the endosomal membrane (Refs 22, 23). This initiates the fusion event and releases the interior components of the virus (i.e. M1 and vRNPs) into the cytoplasm.

Uncoating and nuclear import

In addition to triggering fusion, endosome acidification has one other crucial function in virus entry. The presence of the M2 ion channel in the envelope of the virus means that the components inside the virus (i.e. M1 and the vRNPs) become exposed to the low pH of the endosome. Exposure to a low pH is necessary both in vitro and in vivo to disrupt M1–vRNP interactions and uncoat the virus (Refs 24, 25). Although the acidification of vRNPs is not required for nuclear import per se

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(Ref. 26), it is required to uncoat completely the M1–vRNP complexes, which are otherwise too big to pass through the size-restricted channel of the nuclear-pore complex. The import of vRNPs occurs through nuclear pores and is mediated by nuclear localisation signals on NPs (Refs 27, 28, 29).

It is now known that two closely related antiviral drugs, namely amantadine (Symmetrel®) and rimantadine (Flumadine®), target the pH-dependent uncoating event. At micromolar concentrations, amantadine inhibits the nuclear import of vRNPs in cell culture (Ref. 30). The target of amantadine is the M2 ion channel (Refs 31, 32). Amantadine blocks the M2 channel and prevents the acidification of the virus interior. Thus, endosome acidification and virus fusion are not compromised, but M1–vRNP dissociation and ultimately nuclear import are prevented. Amantadine therefore inhibits M2-dependent virus uncoating and is specific for influenza A viruses. At micromolar concentrations, it has no effect on either influenza B or C viruses, which have different ion channels. At higher concentrations (e.g. millimolar), amantadine has additional non-specific effects and can act as a weak base, neutralising the acidic pH in both the endosome and the Golgi apparatus of the cell (Refs 31, 33). As discussed below, the Golgi apparatus affects the HA trafficking of certain influenza viruses through the secretory pathway during the later stages of infection.

Although amantadine and rimantadine are useful for certain at-risk populations, they tend not to be heavily prescribed, mainly owing to the rapid emergence of drug-resistant strains (Ref. 34), and their neurological side effects. Drug resistance is caused by mutations occurring in the nucleotides that encode the amino acids lining the interior of the M2 tetramer. Both amantadine and rimantadine appear to act by the same mechanism, although rimantadine is more commonly used because it has fewer side effects.

Virus replication and transcription

Influenza viruses are one of the few RNA viruses to undergo replication and transcription in the nucleus of their host cells (Ref. 35). In the nucleus, the vRNPs serve as templates for the production of two forms of positive-sense RNA: viral messenger RNA (mRNA) and complementary RNA (cRNA; Ref. 36). The synthesis of mRNA is catalysed by the viral RNA-dependent RNA polymerase (comprising the three subunits PA, PB1 and PB2), which is part of the incoming vRNP complex. Viral mRNAs are processed in an analogous fashion to other eukaryotic mRNAs; that is, they are capped (i.e. contain a methylated 5' guanosine residue) and are polyadenylated (i.e. contain a sequence of polyadenylic acid at their 3' end), and are exported from the nucleus for translation by cytoplasmic ribosomes. The nuclear export of viral mRNA utilises the 'machinery' of the host cell, but is selective; export is controlled by the viral non-structural protein NS1 (Ref. 37). Many viral proteins (NP, M1, NS2 and the polymerases) are then imported into the nucleus for the final stages of replication and for vRNP assembly. The viral cRNA is neither capped nor polyadenylated, and remains in the nucleus, where it serves as a template for the production of negative-sense genomic RNA (vRNA).

Nuclear export

Following virus replication in the nucleus, the initial trafficking event for virus assembly is the export of the newly formed vRNPs out of the nucleus. This process appears to be a reversal of the nuclear import process because it occurs through nuclear pores (Ref. 35). Nuclear export is blocked by the antibiotic leptomycin B (Refs 38, 39). Leptomycin B binds to the chromosome region maintenance 1 (CRM1) export receptor, the major receptor in protein-based nuclear export pathways in the cell (Ref. 40). Thus, both the nuclear import and export of vRNPs appear to rely on the protein-based host machinery; viral RNA component(s) appear to have no role. Whereas the signals for nuclear import are found on NPs (see Ref. 29 for a review), the signals for nuclear export are less clearly defined. M1 is a major regulator of nuclear transport (Refs 30, 41); however, it might not form part of a stable nuclear export complex and be exported along with the vRNPs because, under certain circumstances, vRNP nuclear export can occur despite the accumulation of M1 in the nucleus (Refs 42, 43). M1 might act in the nucleus during the final stages of vRNP assembly, and the translocation event might depend on nuclear export signals on the NS2 protein (Refs 44, 45), or even on NP itself.

Glycoprotein processing

Role of HA

HA is the best-characterised envelope glycoprotein and is important for the subsequent pathogenesis

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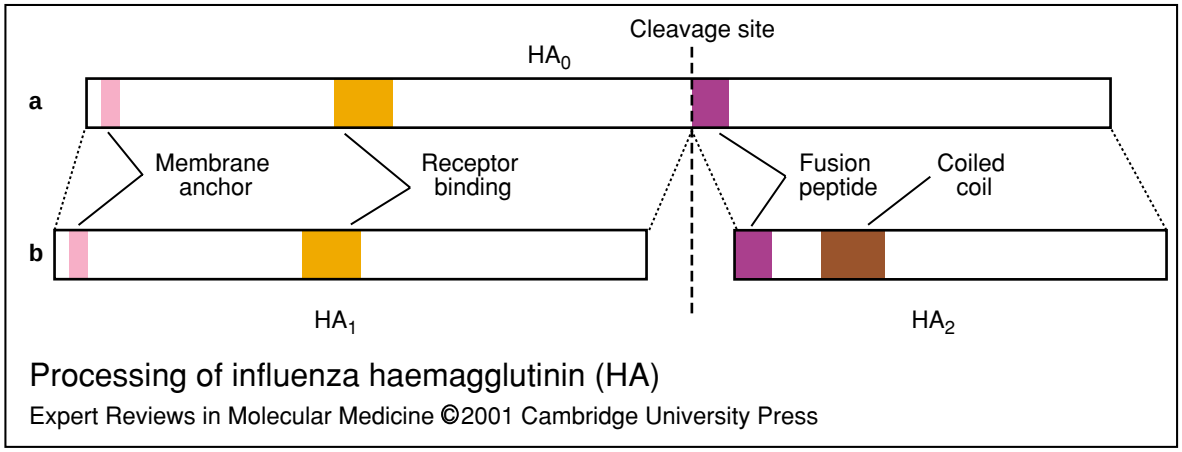

Figure 4. Processing of influenza haemagglutinin (HA). The key regions of HA are shown. (a) The protein is produced as a precursor molecule (HA_0). (b) HA_0 is cleaved by a protease into two active subunits, which are known as HA_1 and HA_2. HA_1 contains the receptor-binding domain, anchors the protein to the membrane and is held together with HA_2 by disulphide bonds. HA_2 contains the fusion peptide that is activated when a coiled coil of α helices is formed, a process that is dependent on a low pH **(fig004gwn)**.

of the virus. Like all glycoproteins, HA is synthesised in the rough endoplasmic reticulum, and is transported to the cell surface via the Golgi apparatus. HA is synthesised as a precursor molecule (HA_0), which undergoes proteolytic processing into two subunits (HA_1 and HA_2), which are held together by disulphide bonds (Fig. 4). This processing is vital for the subsequent infection of new cells (Refs 46, 47). Without proteolysis, the acid-triggered conformational change in HA to expose the fusion peptide cannot occur, and therefore the virus is essentially non-infectious. The HA cleavage site relies on the presence of basic amino acids. In human influenza viruses, there is a single basic amino acid (arginine; R) at the site of cleavage (e.g. HA_1-PSIQV**R**–GL-HA_2). The protease mediating cleavage is thought to be the tryptase Clara, which is released from Clara cells in the epithelial lining of the respiratory tract. The cleavage site is specific and the protease has limited tissue distribution. Both of these features mean that influenza infections are generally limited to the upper respiratory tract. In the laboratory, most cell lines do not support multi-cycle replication unless exogenous trypsin is added. The protease that cleaves HA derives from the host cells, and has not been successfully targeted for therapeutic intervention. By contrast, other viruses, such as human immunodeficiency virus 1 (HIV-1), encode their own proteases, which are excellent targets for anti-viral drugs.

In birds, the situation can be quite different. Whereas most non-virulent or low pathogenicity avian influenza viruses also have a monobasic cleavage site (e.g. HA_1-PEKQT**R**–GL-HA_2), highly pathogenic strains have a polybasic cleavage site (e.g. HA_1-KK**R**E**KR**–GL-HA_2). Thus, they can be cleaved by ubiquitous proteases, such as furin, which is present in the Golgi apparatus of all cells. These HAs also have a high pH optimum of fusion and the M2 ion channel acts to keep the pH of the Golgi above the threshold for fusion. Following the addition of high concentrations of amantadine to these cells, M2 function is blocked and HA undergoes premature pH-mediated activation, and thus infectious progeny viruses are not produced (Ref. 33). Avian influenza subtypes that have polybasic cleavage sites (e.g. fowl plague virus) are not restricted to particular tissues and can cause fatal systemic infections.

One other factor that enhances pathogenicity is bacterial superinfection (Ref. 46). In humans, influenza viruses are normally confined to the upper respiratory tract; however, influenza viruses can invade a patient's lower respiratory tract if it is colonised by bacteria (e.g. in patients suffering from chronic bronchitis or emphysema). Bacteria such as *Staphylococcus aureus*, *Haemophilus influenzae*, *Streptococcus pneumoniae* and various Gram-negative bacilli often produce extracellular proteases that can cleave monobasic cleavage sites on HA, enhancing virus spread. In addition,

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some bacterial products (e.g. lipopolysaccharide) can activate serum plasminogen, as well as inflammatory host proteases such as kallikrein and factor Xa. These host-derived proteases can cleave certain HAs, facilitating the activation of progeny influenza viruses in the lung and the development of deadly influenza pneumonia.

Complementary role of NA

HA cleavage can clearly be a major factor in influenza virus pathogenesis, but the overall process is complex and is affected by both viral and host factors. Importantly, the 1918 pandemic strain was highly virulent, yet contained an unremarkable monobasic HA cleavage site (Ref. 48). However, the recent H5 'Hong Kong avian flu' that infected humans did contain a polybasic cleavage site (Refs 7, 48). A study of the WSN strain of influenza virus has given rise to some clues about the controlling factors for HA-mediated pathogenesis. WSN is an H1N1 virus that was originally isolated from humans in 1933 and has been adapted to, and is neurovirulent in, mice (Ref. 49). It has been known for many years that WSN has a wider than usual cell tropism in the laboratory, and undergoes multi-cycle growth in other cell types besides the canine cell line MDCK, including Madin-Darby bovine kidney (MDBK) cells (Ref. 50). The NA in WSN viruses has a key C-terminal lysine residue (lys 453) and can bind plasminogen and sequester it close to the infected cell surface. Plasmin, the cleaved, active product of plasminogen, can then cleave and activate HA, allowing influenza virus spread (Ref. 51). However, a C-terminal lysine is present in all influenza N1 strains (including the 1918 strain; Ref. 52), and the functional relevance of plasminogen binding is uncertain outside of the laboratory setting.

Virus assembly at the plasma membrane

For virus assembly at the plasma membrane, it is essential that all of the viral components (i.e. HA, NA, M2, M1 and the vRNPs) are trafficked to the correct physical location in the cell and are correctly processed. M1 is central to this interaction. M1 molecules bind to vRNPs, the plasma membrane (possibly via the cytoplasmic tails of the glycoproteins) and also other M1 molecules to form a shell beneath the virus envelope (Fig. 5b). Originally, M1 was thought to bind to membranes via a large, buried hydrophobic surface (Ref. 53), but has recently been shown to act as a peripheral membrane protein and to interact via surface electrostatic interactions in vitro (Ref. 54). It is possible that the binding of M1 to membranes in vivo relies on a combination of both hydrophobic and electrostatic interactions, as well as specific protein interactions with the envelope proteins (Ref. 55). In polarised epithelia, budding of virus particles occurs exclusively from the apical surface (Ref. 56). All of the envelope proteins are localised to the apical surface of polarised cells when expressed individually in heterologous systems, and thus are independently transported to the site of assembly.

Virus budding and release

For the final budding step, it has recently been shown that the viral components coalesce into specific regions of the plasma membrane, which are known as detergent-insoluble glycolipid-enriched domains (DIGs), or lipid rafts. It is likely that these DIGs are specialised sub-compartments of the membrane from which the viruses bud (Refs 57, 58). The formation of viruses at these sites appears to rely on the presence of the cytoplasmic tails of both HA and NA. These glycoproteins, along with M1, M2 and host-cell factors (the actin cytoskeleton and the polarised nature of the cell), appear to control virus morphology, and thus determine the spherical or filamentous nature of the resultant particles (Refs 9, 59, 60). An overall model for virus assembly might involve the initial interaction of M1 with membranes, followed by more-specific interactions of the HA and NA cytoplasmic tails in specialised DIGs.

The final release of viruses from the cell surface relies on the action of the viral NA. NA (sialidase) acts as a receptor-destroying enzyme, by removing sialic acid (the viral receptor) from the surface of host cells (Ref. 61). Without this step, the newly forming virus particles immediately re-bind to their receptor and are not released into the extracellular space. Instead, they remain attached to the cell in large clumps. NA is therefore important for the efficient release of viruses. Indeed, the establishment of a productive infection is dependent on both NA and HA. The carbohydrate residues surrounding the receptor-binding site of HA are known to modulate the affinity of interaction with sialic acid (Ref. 62). Thus, although an increase in the affinity of HA for sialic acid might increase infection, it might counteract the receptor-destroying activity of

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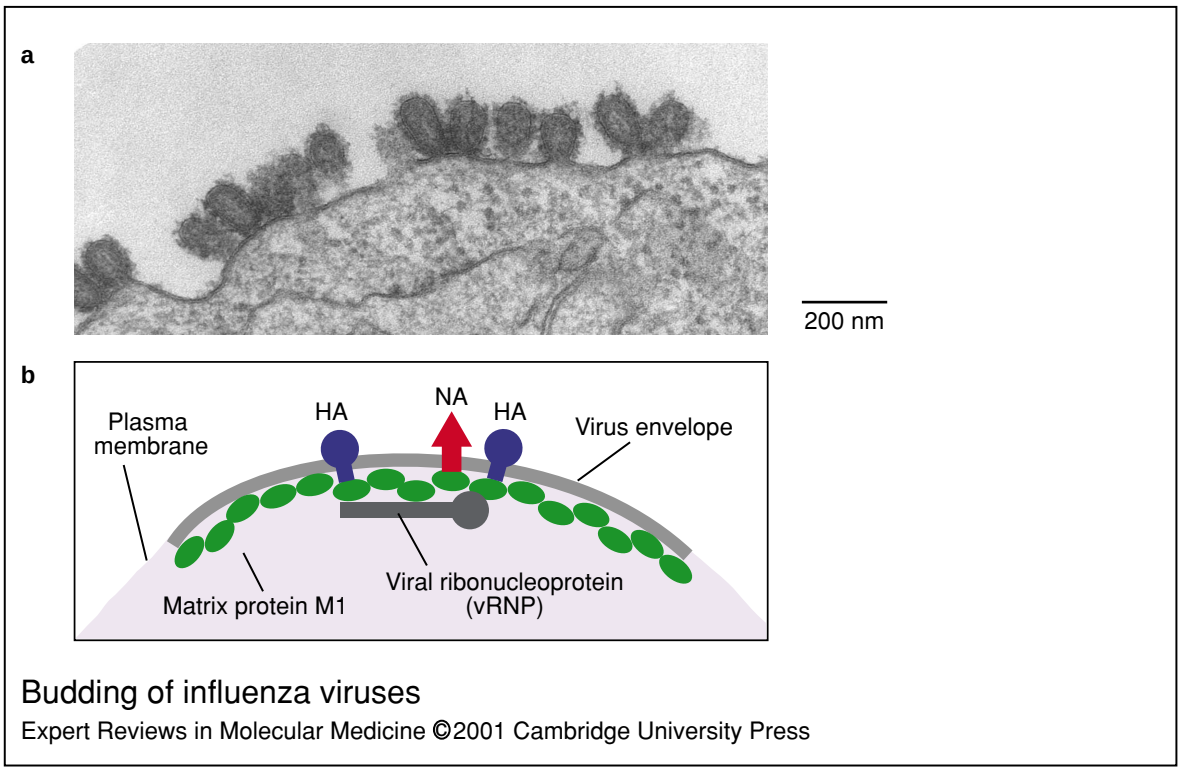

Figure 5. Budding of influenza viruses. (a) This thin-section electron micrograph shows influenza viruses (strain A/WSN/33) budding from the plasma membrane of an infected mouse cell (strain L929). (The micrograph was kindly provided by Melanie Ebersold, Yale University, New Haven, CT, USA). (b) This diagram depicts the molecular interactions that occur when an influenza virus buds from the plasma membrane of a host cell. The matrix protein M1 is central to these interactions. M1 molecules bind to: viral ribonucleoproteins (vRNPs); the plasma membrane, possibly via the cytoplasmic tails of the two surface glycoproteins that are found in influenza A and B viruses, namely haemagglutinin and neuraminidase (HA and NA); and also other M1 molecules to form a shell beneath the virus envelope **(fig005gwn)**.

NA, thereby reducing virus release from the cell surface. Virus release and spread therefore requires a delicate balance between the function of the two glycoproteins.

Recently, the crucial role of NA in the life cycle of the virus has been exploited to great effect for the development of anti-viral drugs. Following the determination of the NA crystal structure (Ref. 63), a concerted effort was made to find small molecules that would bind to, and block, the highly conserved sialic-acid-binding site of NA (Ref. 64). Two analogues of sialic acid, namely Zanamivir (Relenza®) and Oseltmavir (Tamiflu®), have recently been approved for the treatment of influenza (Ref. 65), and other related compounds are currently in development (Ref. 66). These drugs are effective against both influenza A and B viruses. The new inhibitors of influenza NA are significant because they act as anti-viral compounds in a previously unexplored manner – by preventing virus release and spreading from cell to cell. The development of these drugs is a direct consequence of the information provided by X-ray crystallography; thus, along with HIV-1 protease inhibitors, they can be classed as one of the major successes of rational structure-based drug design.

Conclusions

The molecular events occurring throughout the life cycle of influenza viruses have direct implications for the ability of this potentially deadly virus to cause disease in its host and for new viruses to emerge from animal hosts. From initial virus binding, through pH-dependent internalisation and finally to co-ordinated virus assembly and release, an understanding of the fundamental biology of the virus has yielded information that has allowed both the design of anti-viral drugs as well as an understanding of

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the ecology and emergence of the virus. However, many questions still remain regarding the interaction of influenza viruses and their host cells at a molecular level. Although it is well known that sialic acid and a low pH are required for virus entry, it is currently unclear how the virus receptor links to the cellular internalisation machinery for endosomal trafficking. Unlike the case in many other viruses (Ref. 67), the cellular cytoskelton appears to have only a minor role in the intracellular trafficking of incoming influenza viruses. However, the cytoskleton might facilitate the transport of the newly formed vRNPs through the cell and the co-ordination of virus assembly at the plasma membrane. Although a great deal of progress has been made on the viral and cellular determinants of overall pathogenicity, a complete understanding of virulence in vivo appears to be a long way off. In particular, an intensive effort is under way to find out how the pandemic 1918 strain caused such devastating disease. Thus, it appears that this ever-emerging virus will continue to present new challenges with regard to both our understanding of the basic biology of the virus, and how this relates to the development and application of anti-viral strategies.

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Further reading, resources and contacts

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Flint, S.J. et al. (2000) Principles of Virology, ASM Press, Washington DC, USA

The Influenza Sequence Database is a curated database of nucleotide and amino acid sequences, produced by the Los Alamos National Library. It is intended to provide the research community with easy sequence deposit and retrieval capabilities, together with tools tailored, in particular, to the analysis of haemagglutinin and neuraminidase sequences. The website also provides many links to other sites, including Genbank, Medline and the Protein Databank (for retrieval of structural records).

http://www-flu.lanl.gov/

The Influenza Prevention and Control website, produced by the US Centers for Disease Control and Prevention, Atlanta, GA, is a useful information source.

http://www.cdc.gov/ncidod/diseases/flu/fluvirus.htm

All the Virology on the WWW seeks to be the best single site for virology information on the Internet that will be of interest to both the professional virologist and the general public.

http://www.tulane.edu/~dmsander/garryfavweb.html

The Virology at Cornell website has been created by the Departments of Plant Pathology and Microbiology and Immunology at Cornell University, NY, USA (produced by Dr Sondra Lazarowitz). It emphasises molecular aspects of virology, focusing on fundamental principles of virus structure, replication, genetics and virus–host interactions that lead to disease development.

http://ppathw3.cals.cornell.edu/virology/Virology.htm

Features associated with this article

Figures

Figure 1. Electron micrograph of an influenza virus (fig001gwn).
Figure 2. Structure and genome organisation of influenza viruses (fig002gwn).
Figure 3. Replication cycle of an influenza virus (fig003gwn).
Figure 4. Processing of influenza haemagglutinin (HA) (fig004gwn).
Figure 5. Budding of influenza viruses (fig005gwn).

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