MINI-REVIEW

Influenza A: Understanding the Viral Life Cycle

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Influenza A virus belongs to the family of Orthomyxoviridae. It is an enveloped virus with a negative sense RNA segmented genome that encodes for 11 viral genes. This virus has evolved a number of mechanisms that enable it to invade host cells and subvert the host cell machinery for its own purpose, that is, for the sole production of more virus. Two of the mechanisms that the virus uses are "cap-snatching" and preventing the host cell from expressing its own genes. This mini-review provides a brief overview as to how the virus is able to invade host cells, replicate itself, and exit the host cell.

INTRODUCTION

Influenza A belongs to the family of Orthomyxoviridae. It is an enveloped virus with a genome made up of negative sense, single-stranded, segmented RNA. The Influenza A viruses have eight segments that encode for the 11 viral genes: hemagglutinin (HA†), neuraminidase (NA), matrix 1 (M1), matrix 2 (M2), nucleoprotein (NP), non-structural protein 1 (NSP1), non-structural protein 2 (NS2; also known as nuclear export protein, NEP), polymerase acidic protein (PA), polymerase basic protein 1 (PB1), polymerase basic protein 2 (PB2) and polymerase basic protein 1 – F2 (PB1-F2) [1].

The influenza virus virions are known to display a number of shapes, with the most abundant one being roughly spherical. The viral envelope is made up of a lipid bilayer that contains three of the viral transmembrane proteins: HA, NA, and M2. This lipid bilayer is derived from the host's plasma membrane and is known to contain both cholesterol-enriched lipid rafts and non-raft lipids [2-4]. HA is the most abundant envelope protein at approximately 80 percent, followed by NA,

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which makes up around 17 percent of the viral envelope proteins. M2 is a very minor component of the envelope, with only 16 to 20 molecules per virion. HA and NA are exclusively associated with the lipid rafts in the viral lipid membrane, whereas M2 is not [4,5]. Sitting just underneath the viral lipid membrane is M1, which forms a matrix holding the viral ribonucleoproteins (vRNPs). These vRNPs are the core of the virus and are made up of the viral negative stranded RNAs, which are wrapped up around NP and very small amounts of NEP. At one end of the vRNPs are the three polymerase (3P) proteins (PB1, PB2 and PA) that make up the viral RNA polymerase complex [1,4,6].

The influenza virus life cycle can be divided into the following stages: entry into the host cell; entry of vRNPs into the nucleus; transcription and replication of the viral genome; export of the vRNPs from the nucleus; and assembly and budding at the host cell plasma membrane. In this review, each stage of the viral life cycle will briefly be described.

ENTRY INTO THE HOST CELL

HA is a homotrimer that forms spikes on the viral lipid membrane. These spikes of HA bind to sialic acid found on the surface of the host cell's membrane [7]. The HA precursor, HA0, is made up of two subunits: HA1, which contains the receptor binding domain, and HA2, which contains the fusion peptide. These subunits are linked by disulphide bonds [8]. Two major linkages are found between sialic acids and the carbohydrates they are bound to in glycoproteins: $\alpha(2,3)$ and $\alpha(2,6)$. These are extremely important for the specificity of the HA molecules in binding to cell surface sialic acid receptors found in different species. Viruses from humans recognize the $\alpha(2,6)$ linkage, whereas those from avians and equines recognize the $\alpha(2,3)$ linkages. Those from swine recognize both [7]. This explains the importance of swine being a good mixing vessel for avian and human influenza

viruses, hence producing dangerous pathogenic viruses.

Upon binding to the host cell's sialic acid residues, receptor-mediated endocytosis occurs and the virus enters the host cell in an endosome. The endosome has a low pH of around 5 to 6, which triggers the fusion of the viral and endosomal membranes. The low pH induces a conformational change in HA0, leading to maintenance of the HA1 receptor-binding domain but exposing the HA2 fusion peptide. This fusion peptide inserts itself into the endosomal membrane, bringing both the viral and endosomal membranes into contact with each other. Several crystal structures of HA in its various conformations, i.e., at neutral and acid pH, have been solved and are reviewed in [7] and [8].

The acidic environment of the endosome is not only important for inducing the conformation in HA0 and, thus, fusion of the viral and endosomal membranes but also opens up the M2 ion channel. M2 is a type III transmembrane protein that forms tetramers, whose transmembrane domains form a channel that acts as a proton-selective ion channel [9,10]. Opening the M2 ion channels acidifies the viral core. This acidic environment in the virion releases the vRNP from M1 such that vRNP is free to enter the host cell's cytoplasm [11].

ENTRY OF vRNPs INTO THE NUCLEUS

Influenza viral transcription and replication occurs in the nucleus; therefore, after being released into the cytoplasm, the vRNP must enter the nucleus. The viral proteins that make up the vRNP are NP, PA, PB1, and PB2. All of these proteins have known nuclear localization signals (NLSs) that can bind to the cellular nuclear import machinery and, thus, enter the nucleus. To date, it is unclear which NLS is the most important for vRNP nuclear entry. The different NLSs present in each of these viral proteins are reviewed in [12]. It is known that import occurs via the Crm1 dependent pathway by binding to the various karyopherins involved in nuclear import, for example, importin α and β.

TRANSCRIPTION AND REPLICATION OF THE VIRAL GENOME

The influenza viral genome is made up of negative sense strands of RNA. In order for the genome to be transcribed, it first must be converted into a positive sense RNA to serve as a template for the production of viral RNAs.

Replication of the genome does not require a primer; instead, the viral RNA dependent RNA polymerase (RdRp) initiates RNA synthesis internally on viral RNA. This is possible, as the extreme 5' and 3' ends of the genome exhibit partial inverse complementarity and, hence, are able to base pair with one another to form various corkscrew configurations. It appears that a great number of di-nucleotide base pairs form, although the full mechanism of viral genome replication is still yet to be understood [13-16].

Given that the influenza A virus only encodes for 11 proteins, it has generated many sophisticated methods of utilizing the host cell's machinery for its own purposes. Through understanding viral transcription, we have learned of a unique mechanism whereby the virus hijacks the host's transcription machinery for its own benefits.

Mature cellular messenger RNAs (mRNAs) have a 5' methylated cap and a poly(A) tail. It is known that the vRNPs have poly(A) tails but no 5' caps. It was confusing when the influenza community discovered that the viral mRNAs did have a 5' methylated cap and a poly(A) tail, but the 5' cap was not found in the viral genome [17,18]. Much study went into this problem, and soon it was determined that the 5' methylated caps of the viral mRNAs actually belonged to the cellular mRNAs. That discovery lead to the formulation of the "cap-snatching" mechanism [19-26]. The viral RdRp is made up of three viral proteins: PB1, PB2, and PA. PB2 has endonuclease activity. It binds to the 5' methylated caps of cellular mRNAs and cleaves the cellular mRNAs' 10 to 15 nucleotides 3' to the cap structure. This cellular capped RNA fragment is used by the viral RdRp to prime viral transcription [27].

Cellular RNA Polymerase II (Pol II) binds to DNA and starts transcription. During transcription initiation, serine 5 on the C-terminal repeat domain (CTD) of Pol II is phosphorylated, leading to the activation of cellular cap synthesis complex. The influenza RdRp has been shown to bind preferentially to this form of Pol II, indicating that this could be the point at which "cap snatching" could occur [28].

Six but two of the viral segments encode for one protein. Segments 7 and 8 encode for two proteins each due to splicing. Segment 7 encodes for M1 and M2; whereas, segment 8 encodes for NS1 and NEP. M2 and NEP are the spliced products and generally are found in much lower abundance than NS1 and M1 [29]. The virus uses the host cell's splicing machinery to express both of these proteins [30]. Despite influenza's need for the cellular splicing machinery, it prevents the host cell from using its own splicing machinery for processing the host cell mRNAs. NS1 binds to U6 small nuclear RNAs (snRNAs) [31,32] and other splicing components, causing them to re-localize to the nucleus of infected cells [33]. In this way, influenza is able to inhibit splicing of cellular mRNAs. It also has been shown to bind to a novel protein called NS1 binding protein (NS1-BP), causing it to re-localize to the nucleus in infected cells. The function of NS1-BP is unknown, although it is predicted to be involved in splicing given its co-localization with SC35, a spliceosome assembly factor [34]. NP also has been shown to interact with UAP56, a splicing factor involved in spliceosomal formation and mRNA nuclear export, although the importance of NP's binding to UAP56 is yet to be established [35].

The mechanism of polyadenylation of viral mRNAs is very unusual. Cellular mRNAs are polyadenylated through cleavage at the polyadenylation signal (AAUAAA) by cleavage and polyadenylation specificity factor (CPSF) and subsequent addition of a poly(A) tail at the 3' end of the mRNA. Viral mRNAs do not contain this sequence; instead, the viral RdRp remains bound to the 5' end of the template

viral RNA, leading to steric blockage at the end of viral RNA synthesis [36,37]. Each viral segment has a stretch of five to seven U residues approximately 17 nucleotides from the 5' end, and this forms the basis of the viral polyadenylation signal [38]. Therefore, polyadenylation of the viral mRNAs occurs due to a stuttering mechanism, whereby the RdRp moves back and forth over this stretch of U residues, leading to the formation of a poly(A) tail [39,40]. Interestingly, NS1 inhibits the nuclear export of cellular mRNAs by preventing cellular mRNAs from being cleaved at the polyadenylation cleavage site [41]. It does this by binding to the CPSF [42] and poly(A) binding protein II (PABPII), which is involved in stimulating poly(A) polymerase to add the poly(A) tail onto newly cleaved mRNAs [43].

EXPORT OF vRNPs FROM THE NUCLEUS

It is known that only negative sense vRNPs are exported from the nucleus [44]. vRNPs appear to be exported out of the nucleus via the CRM1 dependent pathway through the nuclear pores. NP has been shown to interact with CRM1 directly, although no GTP hydrolysis activity could be detected. This indicates an unusual method of export if the binding of NP to CRM1 is critical for export of the vRNPs. M1 is known to interact directly with the vRNPs through the C-terminal end of the protein. Interestingly, the N-terminal portion of the protein is known to have an NLS potentially involved in the import of the vRNPs. It has been shown that the N-terminal portion of M1 can bind to NEP, thus masking the NLS. NEP also has been shown to bind to CRM1 with the accompanying GTP hydrolysis that normally occurs in a CRM1-dependent export pathway. Therefore, it is hypothesised that M1 binds to the negative sense vRNPs, as well as binding to NEP. In turn, NEP binds to CRM1, and through this "daisy-chain" complex, the vRNPs are exported out of the nucleus [12,45,46].

Recently, live imaging has been employed to visualize the movement of vRNPs during the influenza life cycle. It has been shown that NP preferentially localizes to the apical side of infected nuclei, indicating polarized exit of the viral genome [47,48].

ASSEMBLY AND BUDDING AT THE HOST CELL'S PLASMA MEMBRANE

After the vRNPs have left the nucleus, all that is left for the virus to do is form viral particles and leave the cell. Since influenza is an enveloped virus, it uses the host cell's plasma membrane to form the viral particles that leave the cell and go on to infect neighbouring cells. It is possible to create virus particles that do not contain any or only a few vRNPs, but all the viral proteins normally found within the viral lipid bilayer, i.e., HA, NA, and M2, must be present to form a viral particle [4].

Virus particles bud from the apical side of polarized cells [4]. Because of this, HA, NA, and M2 are transported to the apical plasma membrane. It has been shown through deletion and mutational analysis that the tail of M2 is extremely important in the formation of viral particles. Viruses that had the M2 tail deleted or partially mutated produced elongated particles [49]. M1, which is present underneath the lipid bilayer, is important in the final step of closing and budding off of the viral particle [6,50]. Several host factors are involved in the budding off of viruses from plasma membranes, and these are reviewed in [4,51].

There are two models that have been hypothesized to explain the packaging of viral genomic segments into virions: the random packaging model [52,53] and the specific packaging model [54]. The former predicts that viral genomic segments are randomly packaged into virions; whereas, the latter predicts that there are signals present in the viral segments dictating which segments are to be packaged into the virions. Packaging signals have been identified in the 5' and 3' non-coding and coding regions of some of the viral segments [55-59], thus leaning toward the specific packaging model.

One of the most important steps that must occur before the newly made viral particle can leave the plasma membrane is the cleavage of sialic acid residue from glyco-

proteins and glycolipids. NA removes these sialic acids. Without this process, the viral particle would not be released from the plasma membrane [60].

CONCLUSION

This is just a brief overview of the steps in the influenza A viral life cycle from entry into a host cell to exit from the host cell. This is a very complicated process, and there is much still to be learned, such as the complete mechanism of viral genomic RNA replication. Like all viruses, influenza A has evolved to take advantage of host cells by processes such as "cap-snatching" and inhibition of cellular mRNA splicing and export. Influenza A can be a deadly virus causing many pandemics. We hope that by learning how the virus is able to replicate in host cells, we can develop better drugs and vaccines to protect us.

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