Interspecies transmission and host restriction of avian H5N1 influenza virus

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Long-term endemicity of avian H5N1 influenza virus in poultry and continuous sporadic human infections in several countries has raised the concern of another potential pandemic influenza. Suspicions of the avian origin of the previous pandemics result in the close investigation of the mechanism of interspecies transmission. Entry and fusion is the first step for the H5N1 influenza virus to get into the host cells affecting the host ranges. Therefore receptor usage study has been a major focus for the last few years. We now know the difference of the sialic acid structures and distributions in different species, even in the different parts of the same host. Many host factors interacting with the influenza virus component proteins have been identified and their role in the host range expansion and interspecies transmission is under detailed scrutiny. Here we review current progress in the receptor usage and host factors.

1 A brief history of influenza virus

The influenza virus is an important human and zoonotic pathogen that spreads around the world for centuries. The two predominant types of influenza viruses that cause human and animal disease are divided into two groups: A and B, in addition to the other type, influenza C. Influenza A viruses can infect almost all mammals and birds. However, how many and which viral subtypes the animal species are able to host are determined by different forms of sialic acid, as virus entry receptor, present on the host cell surface. Birds can host all 16 HA serotypes of the influenza A virus, while humans normally can host only H1, H2, and H3 serotypes. In the 20th century, there are three times of global pandemics of influenza A viruses, including “Spanish Flu” occurring in 1918–1919 which is thought to have killed at least 40 million people, “Asian influenza” occurring in 1957 and “Hong Kong influenza” occurring in 1968[1]. It is believed that the viruses for these cases were originated from avian influenza viruses.

Influenza A virus is a negative stranded RNA virus with 8 segmented genes (Belong to Family Orthomyxoviridae), encoding 11 proteins. Hemagglutinin (HA) and Neuraminidase (NA) are the major surface virus proteins, responsible for the receptor binding, host tropism and virus budding. Based on the HA and NA classification, influenza A virus is named. There are 16 HA types and 9 NA types in total. H1N1 and H3N2 are the common influenza A viruses infecting humans and most of the mammalian species. H5N1 and H9N2 are the major serotypes infecting birds. In this review we focus on the interspecies transmission and host factors involved in human and mammalian infections by H5N1.

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1.1 History of H5N1 epidemic

In the sequence database\(^2\), the history of H5N1 influenza virus can be traced back to year 1959 as the virus sequence of A/chicken/Scotland/1959(H5N1) is deposited though the first H5N1 infection in chickens was recorded in Italy in the 19th century\(^4\). The currently mentioned highly pathogenic H5N1 lineages were evolved from 1996 when pathogens were isolated from sick geese in the Guangdong Province, China (A/Goose/Guangdong/1/1996 (H5N1))\(^5\). This virus strain has its HA gene similar to that of the H5N1 virus which caused 1997 Hong Kong outbreak. The first wave of H5N1 epidemic was from December 2003 to March 2004\(^6\), featuring human fatal cases in Vietnam and felids died in a zoo of Thailand. Meanwhile, outbreaks in poultry were also reported in Korea, Japan and Indonesia. Prior to Wave I, H5N1 viruses were mostly isolated from died chickens and some were from apparently healthy ducks in Southern China in addition to a surprise isolation of H5N1 from a SARS-like patient in Beijing in 2003 during the SARS outbreak\(^7\). The second wave was from June 2004 to November 2004. During this period Malaysia reported poultry cases in villages and China, Indonesia, Thailand and Vietnam reported the recurrence of H5N1 in poultry. In wave III (December 2004 to May 2006), outbreaks were reported in the Qinghai Lake where over 6000 wild migratory birds massacred\(^9\). From then on, poultry and/or migratory bird H5N1 cases have been detected in West Siberia, Kazakhstan, and Mongolia, followed by spread to Europe and Africa due to bird migration\(^12\). After the three waves, H5N1 has spread out worldwide except America and Australia, and expanded its hosts from wild waterfowls to varied animals including humans.

1.2 Human infection of H5N1: breakage of the host range

The first human infection of H5N1 was reported in 1997, as a 3-year-old child was infected and died in Hong Kong SAR (Special Administrative Region)\(^13\), and then the potential that H5N1 was becoming a pandemic virus was raised\(^16\). Since 2003, the cumulative number of human cases has reached 412 cases with 256 fatalities (http://www.who.int/csr/disease/avian_influenza/country/cases_table_2009_03_23a/en/index.html). In China, 38 human cases have been reported, with 25 fatal cases. The recent seven cases (4 fatal) in the spring of 2009 re-pose the threat of H5N1 pandemic. Till now, nearly all the human cases are results of zoonotic infections. Most patients had direct contact with poultry, including plucking and preparing of diseased birds; handling fighting cocks; playing with infected ducks; and consumption of duck’s blood or possibly undercooked poultry. All these make the control of virus circulating in poultry become the essentials. On the other hand, the study of 18 H5N1-infected patients by de Jong and colleagues\(^17\) has revealed that similar and higher virus loads in the nose and pharynx respectively were detected compared to those in H1 or H3 infected patients, and the duration of H5N1 viral shedding is prolonged relative to seasonal influenza. These observations indicate that the treatment to H5N1 patients should not neglect the high viral burden and the resulting inflammation.

Although most human infections are related to the contact with poultry, there are a few incidences that imply the potential (limited) human-to-human transmission\(^18\). The putative transmissions are each limited to one generation rather than a sustained human-to-human transmission. Till now, the only confirmed human-to-human transmission occurred in May of 2006 in the rural northern Sumatra, Indonesia. In this case, seven members of a family were infected with H5N1 virus and six died. The scientists concluded that the virus isolated from the 10-year-old boy was from his aunt and then transmitted to his father, following the analysis of the epidemiologic and genetic sequencing data\(^21\). Slight mutations were also found through the transmission among family members, but no evidence shows the virus acquired the enhanced transmissibility.

The efficient human-to-human transmission is believed to be the last barrier to pandemics, since H5N1 has seemingly overcome the avian-to-human barrier. Whether and when the last barrier will be surmounted is not predictable. To the current knowledge, H5N1 influenza virus has two ways to become a human virus and make pandemic, genetic reassortment and antigenic drift. Genetic reassortment is the approach to gain gene segments from other influenza viruses so as to alter the virulence and host adaptation. Genetic drift is responsible for the changes of the antigenic regions as well as some important mutations.

1.3 Other mammalian infections: expansion of the host range

Besides humans, some other mammals have also been detected with H5N1 infections, including pigs, domestic...
cats, tigers, leopards, dogs and stone martens[22–29] (Figure 1). Except for pigs, all others belong to the order Carnivora. It is generally believed that domestic cats are resistant to influenza A virus infection. However, outbreaks in Vietnam in 2003 featured the infection and death of domestic cats and zoo felids, which means H5N1 virus had overcome another species barrier. One year later, probable tiger-to-tiger transmission was observed at a Thai zoo[27]. Further tests on domestic cats performed by Kuiken et al. showed that cats could be infected by H5N1 either by intra-tracheal inoculation, or by horizontal transmission from other cats, or by feeding on virus-infected birds[24]. The H5N1 infected cats exhibited the significant clinical signs and some died. These findings suggest cats may be another intermediate host to spread the virus from chickens, wild birds to humans, and the infection in cats is able to contribute the adaptation of H5N1 virus to mammals. The first H5N1 isolation in pigs was reported by a Chinese journal in 2004, in which two virus strains were isolated from sera samples from 2001 to 2003[22]. Till then, gene sequences of 11 swine strains, 9 from China and 2 from Indonesia had been deposited in GenBank, though pig infections in China was proved to be sporadic cases via our group[30].

Figure 1 Host range of H5N1 influenza viruses. Hollow-dashed line indicates the possible sporadic transmission and true face needs to be confirmed.

2 Receptor usage and beyond

2.1 Receptors and receptor-binding affinity

The receptors for influenza viruses are sialic acids (SAs), which are usually formed 2,3 or 2,6 configuration linked to the cell-surface glycoproteins and glycolipids. In 1941, George Hirst first reported that chicken erythrocytes could be agglutinated when the cells mixed with influenza viruses, and he thus introduced a novel concept that specific receptors are present on the surface of chicken red cells and bind to hemagglutinin[31]. Gottschalk’s group later discovered the link between the influenza virus and SAs[32,33]. Sialyltransferases are responsible for the linkage of SA onto the terminal sugar of glycoproteins or glycolipids[34], which includes galactose (Gal), N-acetylglucosamine, N-acetylgalactosamine and another SA. Specific for the Gal, SA may bind to the hydroxyl group attached to either carbon-3 or carbon-6 to form a SA 2,3Gal or SA 2,6Gal glycosidic linkage[35].

It is the receptor-binding site (RBS) of the HA1 protein (produced by the cleavage of HA into HA1 and HA2) that recognizes SA 2,3 Gal or SA 2,6 Gal on the host cell surface and consequently initializes the virus attachment and fusion of the viral and cellular membrane. Influenza viruses of different species have varied amino acids constitution at RBS, and those residues attribute to host recognition and the binding affinity. From the determination of the receptor-binding specificity, it is known that human influenza viruses bind SA 2,6Gal and avian viruses bind SA 2,3Gal predominantly. Interestingly, swine viruses bind both SA 2,3Gal and SA 2,6Gal equally or with predominance to SA 2,6Gal[35,36]. On the other hand, experiments have shown that human trachea expresses SA 2,6Gal significantly, duck intestinal mucosa expresses SA 2,3Gal, and pig trachea expresses both SA 2,3 Gal and SA 2,6 Gal linkages[36–39].

In the human airway, there are SA 2,3 Gal oligosaccharides located on non-ciliated cuboidal bronchiolar cells at the junction between the respiratory bronchiole and alveolus, and on cells lining the alveolar wall[40,41].

It is well known that the substitutions from Glutamine to Leucine at position 226 (Gln 226Leu) and Gly228Ser of HA1 of H2 or H3 virus will lead to the change of the binding preference from SA 2,3Gal to SA 2,6Gal[42–44]. For H5N1 virus, the results of glycan microarray analysis revealed that introduction of the human-type residues
at position 226 and 228 (Gln226Leu and Gly228Ser) will reduce the binding affinity to SA 2,3Gal, although no dramatic switch to SA 2,6 Gal binding affinity could be observed\cite{45}. Stevens et al.\cite{45} proposed that the decreased affinity to SA 2,3 Gal would help the H5N1 virus to circumvent the inhibitory effects in respiratory tracts. Although specific substitutions at position 226 and 228 experimentally increased the binding affinity to SA 2,6 Gal, no such human virus was isolated. From the known human infections, Yamada et al.\cite{46} noticed two mutations, Asn182Lys and Gln192Arg (position 186 and 196 respectively in Figure 2) that can confer avian virus the ability to recognize human receptor. From the structural view, the RBS of HA1 comprises three structural elements, one-helix and two loops. Within RBS, a number of conserved residues, including Tyr98, Trp153 and His183, are involved in receptor binding. The residues at positions 186, 196, 226 and 228 have the capability to directly interact with sialic acid\cite{46,47}.

**Figure 2** Structural view of receptor-binding pocket of HA. Structure model used, PDB: 2idx_A.

### 2.2 Are pigs the intermediate hosts?

Pigs are an important host in influenza virus ecology as being involved in genetic reassortment and interspecies transmission. In pig trachea, there exist both SA 2,3 Gal and SA 2,6 Gal, so that HA of both avian and human influenza viruses may find the receptors. The presence of both receptors can also result in the modification of the receptor binding specificities of avian influenza virus from SA 2,3 Gal to SA 2,6 Gal\cite{48}, and hence link the influenza virus from birds to humans. It is thought that human-avian reassortant possesses the capability to adapt the human respiratory system and cause pandemic, as suspected in pathogens in 1957 and 1968\cite{11}. In pig populations H1N1, H3N2 and H1N2 viruses are circulating worldwide and most swine influenza viruses are reassortants originated from human, avian and swine influenza viruses\cite{49}. Given these features, pigs are considered as an intermediate host or “mixing vessel”, for the generation of new strains responsible for pandemic. However, there is no proof for pigs to generate the pandemic viruses.

Could it be true that H5N1 virus obtains genes from human influenza viruses and becomes a pandemic strain via the reassortment events in pigs? No one can answer this question. From the current data, the H5N1 isolations from pigs are very limited\cite{22,23,50}. Sero-epidemiological studies of pigs in Vietnam in 2004 showed 0.25% (8 of 3175) seropositive samples\cite{51}, but no seropositive sera were detected in Korea in 2003\cite{52}. Specifically for Southern China, the so-called influenza epicenter, we performed the serologic surveillance in Fujian Province, China in 2004 and 2007 after the two outbreaks in poultry. The results reveal no evidence of H5N1 infection in pigs\cite{30}, implying that H5N1 infections in pigs are only sporadic cases. Besides, studies of inoculation of domestic piglets with four strains of H5N1 unraveled that pigs had low susceptibility to H5N1 viruses, though pigs could support H5N1 replication. The infected pigs shed H5N1 virus, but the viral loads were lower and time of shedding was shorter compared to swine influenza viruses\cite{53}. The role that pigs played in reassortment of H5N1 virus is arguable, and further studies need to be done to reveal the mechanisms behind.

### 3 Virus adaptations

#### 3.1 Genetic reassortment and H5N1 genotyping

There are two approaches, genetic mutations (antigenic drift) and genetic reassortment (antigenic shift) through which influenza virus adapts to novel hosts. Genetic reassortment occurs when different lineages of influenza A virus infect the same host simultaneously. Diversified virus strains exchange their genes and form new virus strains. Specifically, the exchange of surface proteins of different groups, namely antigenic shift, will cause the changes of virus subtypes. The 1957 Asian influenza virus H2N2 was suspected to be the reassortant of avian
H2N2 and 1918 H1N1 virus\textsuperscript{[54]}. The recent circulating H5N1 viruses all possess the HA and NA genes descendant to those of A/Goose/Guangdong/1/1996, except that viruses isolated from the outbreak in Hong Kong in 1997, where the NA gene is thought related to H6N1 viruses. The internal genes of a single virus strain, on the other hand, are diversified and from varied origins, and consequently formed over 40 genotypes\textsuperscript{[55]}.

Guan and colleagues first proposed genotyping of H5N1\textsuperscript{[56]}, and till 2001 there had been mainly 5 genotypes (A-E). From 2001 to 2004, genotypes Z, Z+, Y, V W and G were evolved, and some genotypes (i.e. C, D and E) disappeared\textsuperscript{[57,58]}. The latest study of H5N1 genotyping by Duan et al. reviewed H5N1 genotype changes from 1996 to 2006\textsuperscript{[55]}. Utilizing the information of 318 H5N1 virus complete genomes, they recognized in total 44 different reassortants, and observed two major genotype replacements (B to Z, 2002; and Z to V, 2005) (Figure 3) (they also redefined the genotype of Clade 2.3.4 from Z to V based on their new analysis). Currently, the dominant genotypes in China are V and Z, as most isolates of Clade 2.3 belong to V and the Qinghai Lake-related viruses belong to Z. Besides, there are also varied genotypes circulating in diversified populations around China.

Genotyping of H5N1 virus tells us that almost all the novel genotypes were found in the isolates from poultry, suggesting that domestic birds played important roles in virus gene reassortment events. It is also mysterious that no new genotype is found first evolved from outside China (i.e. Vietnam, Indonesia)\textsuperscript{[55]}, though the poultry farm and the mode of feeding are similar. From the previous knowledge, we know that the reassortant influenza virus possesses higher adaptation to the novel hosts, and has the ability to form pandemic flu. So clarifying how gene segments from different origins work together within the reassortant virus and how the reassortant H5N1 viruses fit the immune system of hosts are essential to prevent the potentially upcoming pandemics.

3.2 Antigenic drift and positive selection

Antigenic drift is the results of the accumulation of mutations. A virus utilizes this approach to change its antigen and to evade from the host immune system. In H5N1 virus, the antigenic drift usually occurs in its HA gene, because it is the first and most important protein of H5N1 that interacts with the host immune system. Evolutionarily, proteins against the immune system are

![Figure 3: Genotype changes of H5N1 influenza viruses. Only some main genotypes are shown. Different colors represent different origins.](image-url)
likely under natural selection and thus the genetic drifts in the antigenic regions are prone to be fixed. The analyses of phylogeny and positive selection exhibit that H5 HA1 part is under positive selection in a total view of H5N1 evolution\[^{57,59}\]. Using the site and codon models, residues that evolved under different pressures can be estimated. Most of the estimated positively selected sites are located at the global head of HA1 and some are inside the antigenic epitopes identified previously\[^{60,61}\]. The results are similar to that of the evolutionary analysis on H3N2\[^{62}\]. Positively selective pressure is the result of virus adaptation and the fixed residues are likely to contribute to the adaptive features. Epitopes are one of the key features of a virus through which the host immune system can recognize the invaded pathogens. Those under highly selective pressure are possibly responsible for the evasion from the immune system via non-synonymous substitutions. However, the connections between amino acid composition and virus adaptation are far from clarification and further studies need to be done.

3.3 Other genes that affect virus adaptation

Besides HA, other influenza virus genes are also involved in host adaptation and thus may result in pandemic. It is well known PB2 protein is involved in host range restriction of pathogenic avian influenza viruses\[^{63-65}\]. Lysine but not glutamic acid at position 627 was found in mammalian cells but not in avian cells, and reverse genetics also showed that Glu627Lys mutation confers high pathogenicity to mice. This mutation is now related to the human and mammalian cases of H5N1 infections, as those viruses with this mutation appear to overwhelm the host immune response. It is noteworthy that the recently circulating Qinghai Lake-related viruses possess Lys627 in their PB2 proteins\[^{11}\]. Neuraminidase (NA) protein is responsible for the removal of SA from sialyloligosaccharides of HA, NA and the cell surface, thus releasing viruses to reach the surface of the epithelial cells\[^{66}\]. NA is associated with adaptation of H5N1 viruses from waterfowl to poultry, in that almost all viruses isolated from land-based poultry feature a deletion in the stalk of NA. This stalk deletion reduces NA’s functionality\[^{67,68}\], and is thought to balance the reduced affinity of HA to its receptor\[^{69,70}\]. Another protein known to affect host adaptation is NS1, which functions as an interferon-antagonist, allowing virus replication in interferon-competent hosts\[^{71,72}\]. NS1 protein of H5N1 virus can activate the p38 mitogen-activated protein kinase (MAPK) pathway, and may thus cause the cytokine imbalance\[^{14,73,74}\].

3.4 Host factors

It is very important to investigate the molecular basis of interspecies transmission and host range restriction of avian H5N1 virus. Current H5N1 viruses exhibit high virulence, yet are inefficient in transmission in humans, suggesting there are virus-host interactions beyond our current knowledge. Since infection of avian H5N1 influenza virus has been found in human tissues that lack avian-type receptors, the notion of receptor specificities differentiating avian and human influenza viruses may not have accounted for the whole of host restriction. Host factors besides receptor binding may also play important roles in restricting cross species transmission. Better knowledge of determinants of host restriction will allow monitoring of the pandemic potential of avian influenza viruses.

Physical interaction methods have been developed to identify host factors, which interact with viral components. These methods include a yeast two-hybrid system, proteomics analysis, cell-free reconstitution systems and a yeast-based influenza virus replicon system. Till now, many host factors have been identified to interact with influenza virus components and take part in a certain stage of the virus life cycle. The host factors that have been identified to take part in the influenza virus replication according to the viral proteins (NP, PA PB1, PB2, M1) except for the membrane proteins (HA, NA, M2) (Figure 4) are discussed below.

It is shown that PA interacts with human homologue chicken CLE7 cDNA (hCLE)\[^{72}\]. hCLE interacts with the cellular DNA-dependent RNA polymerase II (PolII) and PolII may be involved in the viral mRNA synthesis. RanBP5 which is a Ran-binding protein 5, ErbB3 and Ebp1, an epidermal receptor tyrosine kinase-binding protein were identified to interact with PB1 protein\[^{76,77}\]. RanBP5 helps the nuclear import of the viral polymerase subunits. Ebp1 interacts with PB1 and then inhibits the viral RNA synthesis. NPI-1, NPI-3 and NPI-5 were shown to act as general transport factors (karyopherin a) and nuclear pore-docking proteins to facilitate the transport of the NP and of viral RNA into the nucleus. NPI-5 is identical
with RAF-2p48 and appears to be an essential factor for the promotion of NP-RNA complex formation and stimulation of the viral RNA synthesis. hCRM1 has also been identified to interact with NP and take part in the nuclear export of the vRNP. In addition, Heat shock protein 70 (Hsp70) is related to thermal inhibition of nuclear export of the vRNP.

Recent study of proteomics has suggested a series of cellular proteins that may interact with the viral polymerase and vRNP complexes. The interaction functions and mechanisms between them need further studies. In addition, another study using the proteomic approach suggests that the viral polymerase might be involved in steps of the infection cycle other than RNA replication and transcription.

Influenza A virus matrix protein (M1) is the most abundant and relatively conserved protein in the viral particle. M1 plays a mediator role between the lipid capsule and vRNP and is a multifunctional protein upon the viral life cycle. During the influenza viral RNA synthesis, M1 is shown to inhibit the cell-free viral RNA synthesis and has been identified to have two transcription inhibition domains (TID). M1 is also identified to mediate the nuclear export of newly synthesized vRNP complexes by binding to vRNP and nonstructural viral protein NS2. It is interpreted that M1 is synthesized at a late stage of infection, is imported into the nucleus, binds to vRNP complexes, inhibits the vRNA synthesis, facilitates nuclear export of vRNP complexes, and leads to the incorporation of vRNP complexes into the virions. The viral RNA synthesis occurs at chromatin-related domains in the nucleus. In this context, core histones are identified as M1-interacting proteins. M1 is phosphorylated by extracellular signal-regulated kinase (ERK) downstream of the Ras-activated factor (Raf)/mitogen-activated protein kinase (MEK)/ERK pathway. When cells are treated with a MEK-specific inhibitor after the virus infection, NP and vRNP complexes accumulate in the nucleus. The cellular receptor of activated C kinase (RACK) 1 could interact with M1 to be also involved in M1 phosphorylation. Heat shock cognate (Hsc)70 is identified as an M1-binding protein and vRNP complexes accumulate in the nucleus by heat shock. Intracellular caspase-8 was able to bind M1 and get involved in a caspase-8 mediated apoptosis pathway in influenza virus infected cells. Recently, Cyclophilin A (CypA) has also been identified to interact with M1 protein and impair the early stage of the viral life cycle. The exact mechanism may be that the CypA protein binds to the M1 protein and then hinders the import of the M1 protein during the influenza virus infection. CypA is a member of the immunophilin superfamily that has peptidyl-prolyl cis-trans isomerase activity. CypA is ubiquitously expressed in the cytoplasm of eukaryotic cells. It has been shown CypA regulates the life cycle of several viruses including...
HIV-1, vesicular stomatitis virus (VSV), Vaccinia virus (VV) and so on.

NS1 is proposed to be a multifunctional protein upon influenza virus infection. NS1 binds to NS1-binding protein (NS1-BP), a splicing-related protein, the 30 kDa subunit of cleavage and polyadenylation specificity factor (CPSF), and poly (A)-binding protein-II (PAB-II) [94-96]. NS1 blocks the interaction between CPSF and pre-mRNA, and thereby inhibits poly (A) elongation of cellular mRNA by PAB-II. Furthermore, NS1-I, hStaufen and a subunit of eukaryotic initiation factor 4F (eIF4F) are identified as NS1-binding proteins [95,97,98]. NS2 produced from a spliced form of NS1 mRNA binds to the Ras-related brain (Rab)/human Rab1 interacting protein (hRIP1), a component of nucleoprotein complexes, and may function in the nuclear export of progeny vRNP complexes [96].

Interferons (IFNs) represent the first line of defense against viral infection. Hundreds of genes are regulated by IFN [99], and their products are the mediators of the host antiviral response. Many steps in the viral replication cycle are potential targets of IFN-inducible proteins. Some interferon-inducible proteins have been identified to play important roles in influenza virus replication. Recent study has identified that the expression of viperin inhibits influenza virus replication by perturbing its release from the plasma membrane [100]. Viperin is an evolutionarily conserved protein that is highly inducible by both type I and type II IFNs [101]. In addition, double-stranded RNA (dsRNA) and other viral factors produced during infection initiate a cascade of events resulting in activation of the transcription factors nuclear factor kappa-B (NFκB), ATF2/c-jun, and interferon regulatory factor 3 (IRF-3), which activate expression of the interferon-β gene. IFN-β induces expression of anti-viral Mx proteins and dsRNA-activated kinase (PKR). Mx proteins are key components of the antiviral state induced by interferons in many species. They belong to the class of dynamin-like large guanosine triphosphatases (GTPases). Nuclear Mxα proteins form a complex with influenza virus NP and inhibit the transcription of influenza virus [102]. In addition, activated PKR phosphorylates eukaryotic translation initiation factor eIF2α, arresting translation and hence virus replication. Influenza viruses have evolved ways to block this cellular antiviral response: NS1 sequesters dsRNA, blocking activation of IFN and PKR [103]; the virus also activates cellular p58 protein which inhibits PKR [104].

4 Conclusion and perspectives

Host breakage and expansion of the highly pathogenic H5N1 influenza virus has been seen increasing in the recent years. The molecular mechanism underlying this event is multiple-factorial and rather complicated. Current understanding is mainly based on our observation, or passive approach. We ought to have some hypothesis-driven approach to see the truth and ultimately solve this problem. From the host factor point of view we identified some virus- or viral protein-interacting proteins, but the true relationship is not clear. “-Omics” methods must be widely used in a more systematic biology approach to solve this “networking” interaction. As for the receptor usage we still have much more questions to answer as a lot of observed phenomena cannot be explained using the current entry/fusion model. Are there any new receptors or co-factors for the H5N1 or other influenza viruses to get into the host cells, esp. a new host?

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