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Review

Cellular Networks Involved in the Influenza Virus Life Cycle

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Influenza viruses cause epidemics and pandemics. Like all viruses, influenza viruses rely on the host cellular machinery to support their life cycle. Accordingly, identification of the host functions co-opted for viral replication is of interest to understand the mechanisms of the virus life cycle and to find new targets for the development of antiviral compounds. Among the various approaches used to explore host factor involvement in the influenza virus replication cycle, perhaps the most powerful is RNAi-based genome-wide screening, which has shed new light on the search for host factors involved in virus replication. In this review, we examine the cellular genes identified to date as important for influenza virus replication in genome-wide screens, assess pathways that were repeatedly identified in these studies, and discuss how these pathways might be involved in the individual steps of influenza virus replication, ultimately leading to a comprehensive understanding of the virus life cycle.

Introduction

Viruses, which consist of nucleic acid encased in a protein shell, are parasites of their host organisms. Despite their simple structures, viruses employ sophisticated mechanisms to replicate within their hosts. A "host cellular factory" has thousands of machines, which viruses co-opt or subvert for each step of their life cycle. Viruses generally initiate their life cycle by attaching to host cell surface receptors, entering the cells, uncoating the viral nucleic acid, and replicating their genome. After new copies of viral proteins and genes are synthesized, these components assemble into progeny virions, which exit the cell. For each step, viruses need to use many host cellular functions, and identifying these host functions has long been of great interest in virology to further our understanding of the precise mechanisms of the viral life cycle.

Influenza viruses cause annual epidemics and recurring pandemics with potentially severe consequences for public health and the global economy. The first influenza pandemic of the 21st century was caused by the 2009 H1N1 virus and has so far resulted in 42–86 million cases of infection worldwide (http://www.cdc.gov/h1n1flu/estimates_2009_h1n1.htm). In addition, highly pathogenic avian H5N1 influenza A viruses have spread throughout Asia, Europe, and Africa, overcoming host species barriers to infect humans, with fatal outcome in many cases (Webster and Govorkova, 2006; Yen and Webster, 2009). Antiviral drugs, such as oseltamivir, zanamivir (Hayden, 2001), and amantadine/rimantadine (Davies et al., 1964), are available for prophylaxis and treatment of influenza virus infection; however, most human H3N2 and H1N1, including pandemic 2009 H1N1, influenza viruses are resistant to amantadine/rimantadine (Bright et al., 2005, 2006; Dawood et al., 2009). Moreover, the frequency with which H1N1 influenza viruses are becoming oseltamivir resistant is a cause for concern and highlights the urgent need for new antiviral drugs (Nicoll et al., 2008) (http://www.who.int/csr/disease/influenza/h1n1_table/en/index.html).

Influenza A viruses are enveloped negative-strand RNA viruses with eight RNA segments encoding at least 10 viral proteins (reviewed in Palese, 2007). Two additional viral proteins, PB1-F2 and PB1 N40, have been identified, although not all strains encode these proteins (Chen et al., 2001; Wise et al., 2009). The virus particles are enclosed by a lipid envelope, which is derived from the host cellular membrane. Three viral proteins, the surface glycoproteins hemagglutinin (HA) and neuraminidase (NA) and the M2 ion channel protein, are embedded in the lipid bilayer of the viral envelope. The HA protein binds to sialic acid-containing receptors on the host cell surface and mediates fusion of the viral envelope with the endosomal membrane after receptor-mediated endocytosis (Palese, 2007; Skehel and Wiley, 2000). By contrast, the NA protein plays a crucial role late in infection by removing sialic acid from sialyloligosaccharides, thereby releasing newly assembled virions from the cell surface and preventing the self-aggregation of virus particles. Underneath the lipid bilayer lies the matrix protein (M1), a major structural protein. Within the virus shell are eight viral ribonucleoprotein (vRNP) complexes, each composed of viral RNA (vRNA) associated with the nucleoprotein NP and the three components of the viral RNA polymerase complex (PB2, PB1, and PA). The NS1 protein, which counteracts the cellular interferon response, is synthesized from an unspliced mRNA, and a spliced mRNA yields the NS2, or NEP, protein, which mediates nuclear export of vRNP complexes. In addition, the recently identified PB1-F2 protein, which is encoded by the PB1 segment, is thought to play a role in viral pathogenicity (Chen et al., 2001; Conenello et al., 2007; McAuley et al., 2007; Zamarin et al., 2006).

Whereas the functions of the viral proteins have been studied extensively during the last decade, relatively little is known about the cellular factors involved in influenza virus life cycle. Here, we review recent genome-wide screens that aim to close this critical gap in influenza virus research.

Dawn of a New Era: The Application of Genome-wide Screens to Identify Host Factors Involved in Influenza Virus Replication

A number of interactions between viral components and specific host cell gene products have now been identified. Although most host molecules remain elusive, emerging data indicate that their identification and characterization will provide new insights into the mechanisms by which viruses complete their life cycle. Moreover, such knowledge would illuminate potentially useful targets for therapeutic intervention. Indeed, antiviral drugs targeting host cell factors involved in viral replication have been tested for the treatment of human immunodeficiency virus type I (HIV-1) with promising results (Coley et al., 2009). However, this goal would generally take several decades to achieve with conventional genetic screening methods and mammalian cell cultures.

Genome-wide RNAi Screens

Many laboratories have expended great effort in the search for new host factors involved in the virus replication cycle by using various strategies. A powerful approach is systematic, genome-wide RNA interference (RNAi) analysis. RNAi is a regulatory mechanism that uses double-stranded RNA (dsRNA) molecules to direct homology-dependent suppression of gene activity (reviewed in Mello and Conte, 2004). This powerful cellular process has been extensively applied for studies of gene functions and therapeutic approaches for disease treatment. Now, this technology offers a new exciting tool for the exploration of novel host genes involved in virus replication (Brass et al., 2008, 2009; Cherry et al., 2005; Goff, 2008; Hao et al., 2008; König et al., 2008, 2010; Krishnan et al., 2008; Li et al., 2009; Zhou et al., 2008).

The first genome-wide screen for the identification of host factors involved in influenza virus replication was reported by Hao et al. (2008) (Table 1). Because RNAi-based screening was not well established in mammalian cells at that time, the authors used *Drosophila* RNAi technology. Studies in *Drosophila* have made numerous fundamental contributions to our understanding of mammalian cell biology because of the high degree of genetic conservation between *Drosophila* and vertebrates. Hao et al. (2008) first established an influenza virus infection system in *Drosophila* cells by modifying the influenza virus genetically to infect *Drosophila* cells and express a reporter gene product in infected cells. This system supported influenza virus replication from post-entry to the protein expression step in the life cycle (Table 1). The authors tested an RNAi library against 13,071 genes (90% of the *Drosophila* genome) and identified 110 genes whose depletion in *Drosophila* cells significantly affected reporter gene expression from the influenza virus-like RNA (see Table S3 available online for the list of genes). Of those 110 candidates, they validated roles for the host proteins ATP6V0D1 (an ATPase), COX6A1 (a cytochrome c oxidase subunit), and NXF1 (a nuclear RNA export factor) in the replication of H5N1 and H1N1 influenza A viruses in mammalian cells. These studies showed the feasibility and power of genome-wide RNAi screens to identify previously unrecognized host proteins required for influenza virus replication.

The development of mammalian RNAi-based screening now enables the comprehensive analysis of mammalian host cell functions in influenza virus replication. Recently, Brass et al. (2009), König et al. (2010), and Karlas et al. (2010) reported genome-wide RNAi screens in mammalian cells that identified host proteins important for influenza virus replication (Table 1). They tested siRNAs targeting more than 17,000 human genes in human osteosarcoma (Brass et al., 2009) and the human lung cell line A549 (Karlas et al., 2010; König et al., 2010), respectively. Because the readout of the assay was the measurement of a viral protein or a reporter protein encoded by the viral genome, the systems used by Brass et al. (2009) and König et al. (2010) allowed the identification of host gene products important for the early to mid stages of the influenza virus life cycle, including virus entry, uncoating, vRNP nuclear import, genome transcription, and viral protein translation. These systems identified 133 (Brass et al., 2009) and 295 (König et al., 2010) human genes whose depletion affected the efficiency of HA cell surface expression or reporter gene expression, respectively (Table 1; see Table S3 for the list of genes). In contrast, Karlas et al. (2010) studied the entire viral replication cycle, from viral entry to budding, by determining virus infectivity titers in culture supernatants from siRNA-treated, virus-infected A549 cells. This approach identified 287 human genes important for influenza virus replication (Karlas et al., 2010) (see Table S3 for the list of genes).

Shapira et al. (2009) employed a different tactic. They combined the results of yeast two-hybrid analyses, genome-wide transcriptional gene expression profiling, and an RNAi screen (Shapira et al., 2009). First, they built a physical interaction map for viral proteins and human cellular molecules by applying computational analysis to their comprehensive yeast two-hybrid assay. This physical map indicated that the viral proteins of influenza interacted with "a significantly greater number of human proteins than expected from the human interaction network, even when compared to other viruses" (Shapira et al., 2009), suggesting that influenza viruses have to maximize the diversity of functions for each protein (Shapira et al., 2009). They then examined which cellular gene products were differentially expressed in primary human bronchial epithelial cells exposed to influenza virus or viral RNA. Collectively, these approaches identified 1745 potential host factors with roles in the influenza virus life cycle (Shapira et al., 2009). Further validation using siRNAs targeting to 1745 genes narrowed this list to 616 human genes whose products affected influenza virus replication.

Other Approaches

Proteomics approaches and yeast two-hybrid analyses have also been used to identify host molecules that interact with influenza virus components (reviewed in Nagata et al., 2008). Several host factors involved in the steps of viral genome replication and transcription have been identified (Deng et al., 2006; Engelhardt et al., 2005; Huarte et al., 2001; Jorba et al., 2008; Kawaguchi and Nagata, 2007; Momose et al., 2001, 2002; Resa-Infante et al., 2008), as well as NS1 protein-interacting host factors associated with immune responses (reviewed in Hale et al., 2008).