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Influenza Virus-Host Interactomes as a Basis for Antiviral Drug Development

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Abstract

Currently, antiviral drugs that target specific viral protein functions are available for the treatment of influenza; however, concern regarding the emergence of drug-resistant viruses is warranted, as is the urgent need for new antiviral targets, including non-viral targets, such as host cellular factors. Viruses rely on host cellular functions to replicate, and therefore a thorough understanding of the roles of virus-host interactions during influenza virus replication is essential to develop novel anti-influenza drugs that target the host factors involved in virus replication. Here, we review recent studies that used several approaches to identify host factors involved in influenza virus replication. These studies have permitted the construction of an interactome map of virus-host interactions in the influenza virus life cycle, clarifying the entire life cycle of this virus and accelerating the development of new antiviral drugs with a low propensity for the development of resistance.

Introduction

Influenza viruses cause annual epidemics and recurring pandemics, such as the Spanish influenza in 1918–1919, the Asian influenza in 1957, the Hong Kong influenza in 1968, and the swine-origin H1N1 2009 pandemic influenza in 2009; these outbreaks have a huge impact on public health and the global economy [1]. In addition, recent sporadic human infections with H5N1 and H7N9 avian influenza viruses have raised concerns regarding the pandemic potential of these viruses [2-5]. Currently, two classes of FDA-approved compounds are available for prophylaxis and treatment of influenza virus infection: M2 ion

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channel inhibitors (amantadine, rimantadine) and neuraminidase (NA) inhibitors (oseltamivir, zanamivir) [6,7]. In addition, several new antiviral compounds have been approved for human use in some countries, including two NA inhibitors (peramivir and laninamivir) [8-11], and a viral polymerase inhibitor [favipiravir (T-705)] [12-14]. However, a major problem concerning anti-influenza drugs that target viral proteins is the frequent emergence of drug-resistant viruses. Indeed, most human H3N2 and H1N1, including pandemic 2009 H1N1, influenza viruses are now resistant to amantadine/rimantadine [15,16], and oseltamivir-resistant H1N1 viruses have emerged in many countries frequently [17]. Therefore, concern regarding the emergence of drug-resistant viruses is clearly warranted, as is the urgent need for new antiviral targets, including non-viral targets, to treat influenza virus infection while minimizing the emergence of drug resistance.

Host factors have been in the spotlight recently as potential antiviral targets to overcome the problems described above. Emergence of resistance could be less frequent when host factors are targeted compared with directly targeting viral proteins. Recently, several drugs targeting host factors identified to be important for virus replication have been developed. For example, the antiretroviral drug maraviroc, which was approved by the FDA in 2007, targets C-C chemokine receptor type 5 (CCR5), a co-receptor for human immunodeficiency virus type 1 (HIV-1), preventing HIV-1 entry [18]. Another example is alisporivir, a cyclophilin inhibitor, which was under clinical evaluation for the treatment of chronic hepatitis C [19,20]; in 2012, however, the FDA halted the clinical trials due to the possible side effect of pancreatitis. Alisporivir inhibits the function of cyclophilin A, which has an essential role in hepatitis C virus (HCV) replication and virus production [21]. In the case of influenza, the sialidase DAS181 (Fludase), which is currently in clinical trials, targets influenza virus receptors, sialic acids, and prevents viral attachment via removal of the sialic acids from the epithelial cells of the respiratory tract (Figure 1) [22-27]. Several other compounds, including protease inhibitors, block cellular proteases, which mediate the HA cleavage that is required for membrane fusion between the viral envelope and the endosomes [28-34], the MEK inhibitor U0126, NF-κB inhibitors such as acetylsalicylic acid (known as aspirin), and agonists of sphingosine-1-phosphate receptors AAL-R, all of which target host cellular functions involved in influenza virus replication, have been studied to explore their potential as new antiviral drugs against influenza (Figure 1) [26,35-41]. In addition, combination therapy with a protease inhibitor and conventional anti-influenza drugs has been recently tested *in vitro* [42].

The advent of the possibility to target host factors involved in virus replication places us at the threshold of a new era of antiviral drug development. However, to explore targets for antiviral therapy, a systematic understanding of the mechanisms of influenza virus replication is essential. Recent efforts to identify host factors involved in influenza virus replication have provided the missing pieces of the virus-host interaction map of the influenza virus life cycle, helping us to move toward the development of host-targeting antiviral drugs. Here, we review the recent progress in the identification of host factors involved in influenza virus replication and the latest efforts to develop antiviral drugs that target host factors.

Strategies to identify host factors involved in influenza virus replication

Recent technological advances have made it possible for researchers to take various approaches to identifying host factors involved in the life cycles of several viruses, including HIV-1, HCV, Dengue virus, and West Nile virus, as well as influenza virus [43-54]. For influenza virus, several strategies have been applied to the search for host factors involved in viral replication, as described below (Figure 2).

Genome-wide screens—The yeast single-gene deletion library consists of almost 5,000 single-gene deletion strains and covers about 80% of the yeast genome. This library has been used extensively in the yeast community for genomic studies. Naito et al. [55] used it to identify host genes involved in influenza virus replication. They used *Saccharomyces cerevisiae* to established a system that supported influenza viral genome replication and transcription and then screened a sub-library that lacked 354 genes encoding putative nucleic acid-binding and related functional proteins. Several host factors that affect influenza viral RNA synthesis, including Tat stimulatory factor 1 (Tat-SF1), were identified in this study[55].

Genome-wide RNAi-based screens also represent an excellent approach to searches for host factors involved in virus replication. In the absence of RNAi-based screening systems in mammalian cells, Hao et al. [48] used *Drosophila* RNAi technology to screen for host factors involved in influenza virus replication. They tested an RNAi library against more than 13,000 genes (approximately 90% of the *Drosophila* genome) and identified 110 *Drosophila* genes that, when depleted, affected influenza virus replication in *Drosophila* cells. Of those 110 candidates, further analysis validated roles for three host proteins—ATP6V0D1 (an ATPase), COX6A1 (a cytochrome C oxidase subunit), and NXF1 (a nuclear RNA export factor)— in influenza virus replication in mammalian cells.

When RNAi-based screening systems in mammalian cells were subsequently established, the roles of mammalian host proteins in influenza virus replication could be comprehensively analyzed. Three key genome-wide RNAi-based screen studies were published in 2009 and 2010 [43,46,47]. Brass et al. [46] identified 133 host factors potentially involved in the influenza life cycle steps of virus entry, uncoating, vRNP nuclear import, genome transcription, and viral protein translation by examining the effects of siRNAs targeting over 17,000 human genes on influenza virus replication in human osteosarcoma. Konig et al. [43] performed a similar study using the human lung cell line A549 and identified 295 host factors that may also be involved in these early to middle steps of the influenza life cycle. In the third key genome-wide RNAi-based screen study, Karlas et al. [47] looked at the entire influenza virus life cycle. By measuring virus titers in the culture supernatant of siRNA-treated, virus-infected cells they identified 287 host factors important for influenza virus replication [47].

Sui et al. [56] applied ‘Random Homozygous Gene Perturbation’ (RHGP) to identify host targets that are required for influenza virus infection. RHGP uses a lentiviral-based genetic element that integrates into the genome leading to abrogation or enhancement of gene expression and an altered phenotype. The authors generated an RHGP library in mammalian cells, which they then infected with influenza virus. When they isolated the cell clones that

survived and sequenced their genomes, they were able to identify 110 host genes that were associated with host cell resistance to influenza virus infection.

Proteomics approaches—Yeast two-hybrid analyses and proteomics approaches have been successfully used to identify host molecules that interact with the influenza viral proteins (reviewed in [57]), and identified several host proteins involved in viral genome replication and transcription [58-65], as have NS1 protein-interacting host factors involved in the immune response to infection [66]. This system also identified 87 human proteins as interaction partners of ten influenza viral proteins and 109 human proteins as interaction partners of three viral polymerase subunits [49,67], making it possible to built interactome maps consisting of influenza viral-host protein interactions.

By using a mass spectrometry approach, Mayer et al. [68] identified 41 host proteins that interact with the influenza viral ribonucleoprotein complex. Of these 41 proteins, they demonstrated a potential role for nucleoplasmin in viral RNA synthesis. Shaw et al. [69] and Hutchinson et al. [70] also used a mass spectrometry approach to identify host cellular proteins that are incorporated into progeny virion particles. They found that influenza virions contain abundant host proteins that make a substantial contribution to the influenza virion architecture. Shaw et al. [69] identified 36 host proteins incorporated in virions, including cytoskeletal proteins, annexins, glycolytic enzymes, and tetraspanin. Hutchinson et al. [70] found that the host proteins that are part of influenza virions produced in virus-infected mammalian cells largely overlapped with those produced in virus-infected chicken eggs, suggesting that these common host proteins are important in the formation of influenza virion particles.

Combining proteomics and RNAi-based screen approaches to construct the interactome map of viral-host protein interactions in influenza virus replication—Shapira et al. [49] used a combination of proteomics and functional genomics to construct an interactome map of the viral-host protein interactions that occur during influenza virus replication. They used a yeast two-hybrid screen to identify host proteins that interact with viral proteins and then built a physical map of these interactions. They also used transcriptional profiling to determine whether the host cell genes were differentially expressed in primary human bronchial epithelial cells upon influenza virus infection (or exposure to influenza viral RNA). By using this approach, they identified 1,745 host factors with potential involvement in influenza virus replication. When they tested siRNAs targeted to these 1,745 human genes, they found 616 host factors with involvement in influenza virus replication.

Watanabe et al. [71] used mass spectrometry and RNAi-based screening approaches to elucidate the physical and functional host-viral interactions during influenza virus replication. They first identified 1,292 host proteins that co-immunoprecipitated with influenza viral proteins that were transiently expressed in human cells. Then, they validated the roles of 324 of these host factors in influenza virus replication by testing the effects of siRNAs targeted to the 1,292 influenza viral host interaction partners on influenza virus production. Further extensive analyses of the 91 top-ranked host factors allowed them to define the steps of the viral life cycle that were affected and to generate an interactome map

of the virus-host protein interactions that are involved in the influenza virus replication cycle.

Exploring antiviral drugs designed to target host factors involved in influenza virus replication

The studies described above have produced a vast amount of data on the host factors that are involved in influenza virus replication. The next step is to select host factors that may be suitable targets for antiviral drugs. Small compounds that inhibit the cellular functions of particular host proteins have been used to validate the requirement of a specific host protein for virus replication (Figure 1). Konig et al. [43] tested the compound KN93, which is a selective inhibitor of CAMK2B (calcium/calmodulin-dependent kinase IIβ) that was shown to be involved in the regulation of viral RNA transcription in their genome-wide screen, and demonstrated that the compound reduced virus titers in influenza virus-infected cells. Karlas et al. [47] showed that treatment with the compound TG003, a small molecule inhibitor of CDC-like kinase 1 (CLK1) identified as a host candidate involved in influenza virus replication in their screen, led to reduced virus production in human cells probably by affecting the splicing of viral RNAs. Brass et al. [46] found that the interferon-inducible transmembrane proteins IFITM1, 2, and 3 hampered the early steps of influenza virus replication *in vitro*, and also suggested that expression of IFITM3 defends host against influenza virus infection *in vivo* [72]. Watanabe et al. [71] tested 11 compounds that inhibit the cellular functions of several host factors identified in their screen. Of these compounds they found that ruxolitinib and gogicide A (which are inhibitors of JAK1 and the Golgi-specific brefeldin A-resistant guanine nucleotide exchange factor GBF1, respectively) markedly decreased virus titers in human cells infected with influenza virus without affecting cell viability.

In addition to approaches targeting host cellular proteins, Morita et al. [73] assessed the possible involvement of lipid products in influenza virus replication. By conducting mediator lipidomics and a bioactive lipid screen, they discovered that the DHA-derived protectin D1 isomer (10S, 17S-dihydroxydocosahexaenoic acid) markedly hampers influenza virus replication *in vitro* by inhibiting the nuclear export of viral mRNA. More importantly, they demonstrated that intravenous administration of protectin D1 protected mice from influenza virus infection, suggesting that protectin D1 has potential as a new therapeutic tool in the treatment and prevention of influenza.

Besides the experimental studies described above, bioinformatics analysis could be an attractive approach to identifying and prioritizing host factors with potential as therapeutic targets (Figure 2). Chassey et al. [74] conducted a meta-analysis using the datasets from the six independent genome-wide screens described above [43,46-49,56] and made a list of 925 host factors important for influenza virus replication (note that this list differs from lists obtained by others [75-77] due to the use of a different data evaluation strategy). By retrieving information from the DrugBank database (http://www.drugbank.ca/), Chassey and colleagues searched for molecules that interacted with these 925 host factors [74]. They found 298 molecules that potentially interact with 100 host factors. Importantly, of the 298

molecules, 204 are already approved by FDA, and therefore, could be highly prioritized candidates for influenza therapy [74].

Matsuoka et al. [78] attempted to apply another bioinfomatics approach to predict and prioritize host factors that could be therapeutic targets by using a comprehensive map of the influenza virus life cycle called 'FluMap' (accessible at http://www.influenza-x.org/flumap/) (Figure 2). FluMap can be used to visualize the entire influenza virus life cycle and was constructed on the basis of information obtained from the literature and publicly available pathway databases. The authors applied a computational network analysis, called a controllability analysis [79,80], to FluMap to identify host molecules that disregulated the virus replication cycle when their functions were disrupted, resulting in inhibition of virus replication. By using the controllability analysis, Matsuoka and colleagues identified 112 critical host molecules [78]. Further *in silico* analysis led them to prioritize several important molecules, such as the nuclear pore complex, Akt, PKC, and the Ran/GTPase complex, which are essential and highly connected within the virus-host network [78]. Accordingly, these molecules can be prioritized as potential new therapeutic targets.

Concluding remarks

Given the high rates of emergence of influenza virus strains that are resistant to the currently available anti-influenza drugs that target viral protein functions, host cellular functions involved in influenza virus replication are attractive targets for antiviral therapy. The host cellular systems are formed by highly complicated and sophisticated networks that consist of interactions between numerous cellular components; viruses hijack these host cellular system, leading to the re-construction of different networks to meet their need to replicate. Recent progress in our understanding of the virus-host interactions during influenza virus replication and of the human interactome network can accelerate the construction of a precise map of the virus-host interactome network during influenza virus replication, leading to a systematic understanding of the mechanisms involved in the influenza virus life cycle. Combining detailed functional approaches (e.g., *in vitro* and *in vivo* analyses) and extensive *in silico* approaches with such a comprehensive map of influenza virus replication could help to identify and prioritize novel host factors with potential as therapeutic targets.

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HIGHLIGHTS

- Host factors are attractive targets for antiviral therapy.
- Recent screens identified host factors involved in influenza virus replication.
- Virus-host interactome screens are powerful tools to identify therapeutic targets.

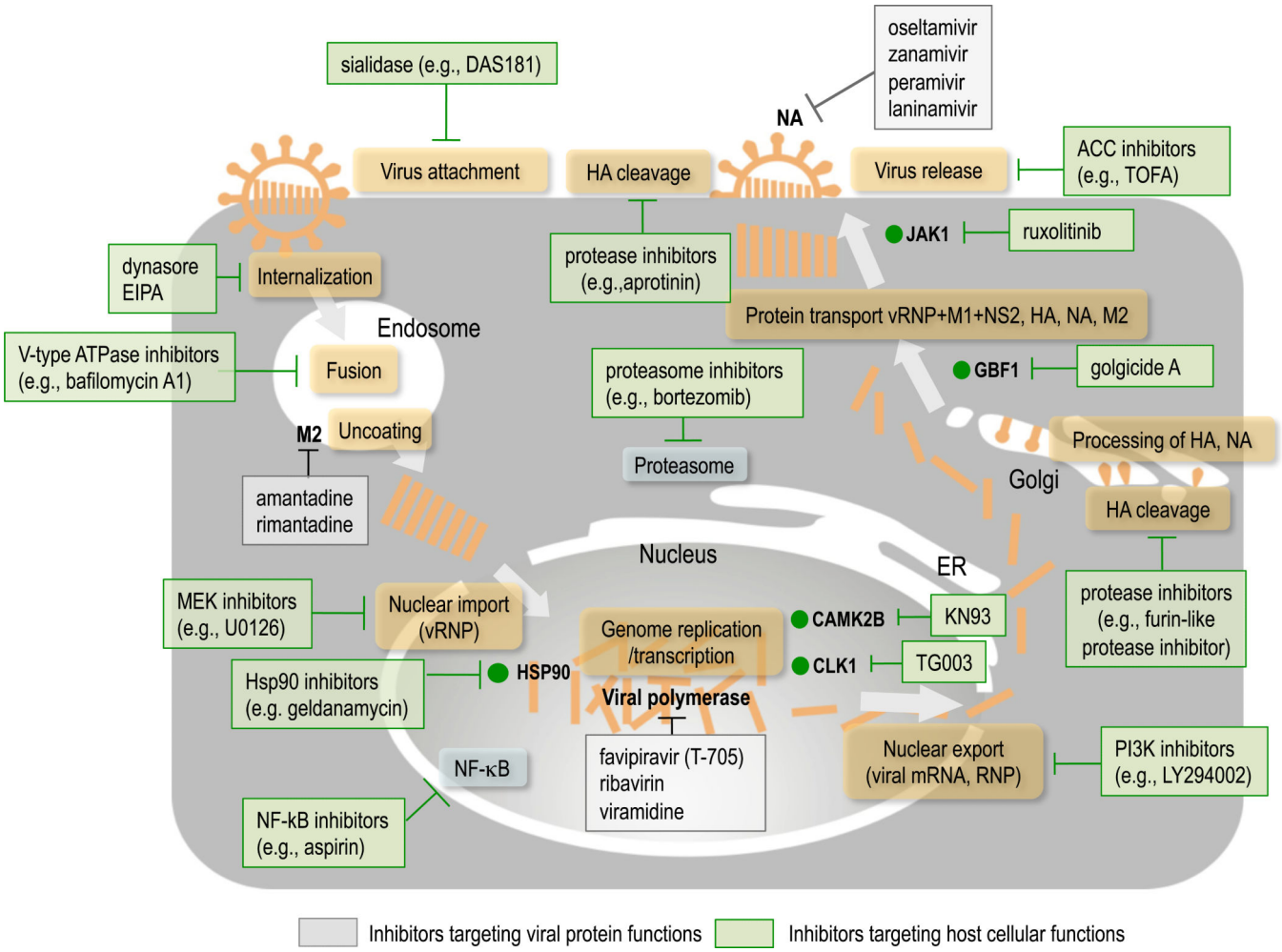

Figure 1.

A schematic diagram of compounds that inhibit influenza virus replication and their known or putative points of action. There are two types of compound: one targets viral protein functions and the other targets host cellular functions. Examples of the former include M2 ion channel inhibitors (e.g., amantadine, rimantadine), NA inhibitors (e.g., oseltamivir, zanamivir, peramivir, and laninamivir) and viral polymerase inhibitors (e.g., favipiravir, rivavirin, and viramidine). Examples of the latter include sialidase (e.g., DAS181) [22-27], dynamin inhibitors (e.g., dynarose) [81], micropinocytosis inhibitors (e.g., EIPA) [81], MEK (MAPK/ERK kinase) inhibitor (e.g., U0126) [35,36], V-type ATPase (vacuolar-type H+ -ATPase) inhibitors (e.g., bafilomycin A) [82], protease inhibitors (e.g., aprotinin [28], which inhibits proteases that cleave cell surface HA proteins that have a single arginine at their cleavage site, and furin-like protease inhibitors, including peptidemimetics derived from decanoylated basic tetrapeptides, such as decRVKR chloromethylketone, which inhibit the HA cleavage of highly pathogenic H5 and H7 viruses [32-34] in the trans-Golgi, resulting in the inhibition of membrane fusion between the viral envelope and the endosomes), Hsp90 inhibitors (e.g., geldamycin) [83], NF-κB inhibitors (e.g., aspirin) [37], PI3K (phosphatidylinositol 3-kinase) inhibitors (e.g., LY294002) [84], ACC (acetyl-CoA carboxylase-α) inhibitors (e.g., TOFA) [85], and proteasome inhibitors (e.g., bortezomib)

[86]. Compounds targeting host factors that have been shown to be involved in influenza virus replication in recent RNAi-based screens are also shown (i.e., KN93, TG003, ruxolitinib, and golgicide A) [43,47,71].

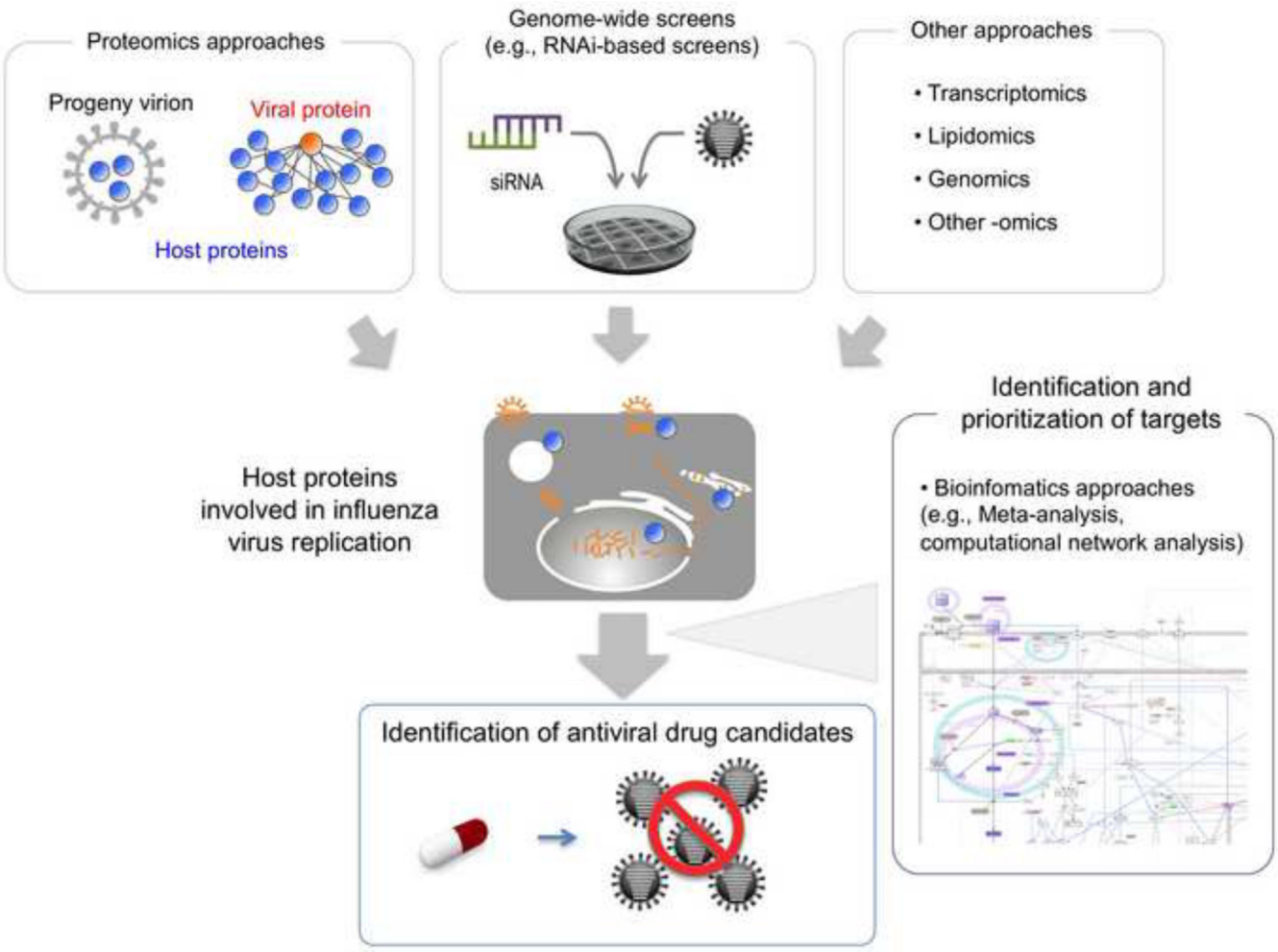

Figure 2.

Overview of the strategies used to explore antiviral drugs targeting host factors involved in influenza virus replication. Various approaches, such as genome-wide screens, proteomics, transcriptomics, and lipidomics have been used to identify host factors involved in the influenza virus life cycle. More detailed experimental studies and bioinformatics analyses will help identify and prioritize host factors with potential as therapeutic targets.