Virus-Host Interactions: From Unbiased Genetic Screens to Function

Holly Ramage and Sara Cherry

Department of Microbiology, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104; email: hramage@mail.med.upenn.edu, cherrys@mail.med.upenn.edu

Annu. Rev. Virol. 2015. 2:497–524

First published online as a Review in Advance on August 26, 2015

The *Annual Review of Virology* is online at virology.annualreviews.org

This article's doi: 10.1146/annurev-virology-100114-055238

Keywords

high-throughput screening, RNA interference, haploid genetic screens, gain-of-function screens, pro-viral host factors, antiviral host factors

Abstract

Deciphering the many interactions that occur between a virus and host cell over the course of infection is paramount to understanding mechanisms of pathogenesis and to the future development of antiviral therapies. Over the past decade, researchers have started to understand these complicated relationships through the development of methodologies, including advances in RNA interference, proteomics, and the development of genetic tools such as haploid cell lines, allowing high-throughput screening to identify critical contact points between virus and host. These advances have produced a wealth of data regarding host factors hijacked by viruses to promote infection, as well as antiviral factors responsible for subverting viral infection. This review highlights findings from virus-host screens and discusses our thoughts on the direction of screening strategies moving forward.

INTRODUCTION

In order to propagate, viruses must gain entry into host cells, replicate, produce progeny virus, and promote viral egress to infect new host cells, all while evading host immune defenses. Given that viruses have a limited genome size, it is not possible for them to encode all the proteins required for these processes. Thus, viruses have evolved mechanisms to hijack and subvert host cell machinery to achieve these goals. In response to these invaders, hosts have evolved sophisticated mechanisms to recognize and restrict invading pathogens. Therefore, successful viruses manipulate hosts in a variety of ways, taking advantage of cellular pathways that are beneficial while evading or inactivating factors that are detrimental to viral growth.

The discovery of the cellular factors that impact infection informs mechanisms of viral pathogenesis and host-pathogen relationships. The study of viruses also provides insight into basic cellular functions and molecular mechanisms, because viruses have learned to utilize components for the same or similar roles performed for host cells (e.g., translation machinery). Importantly, identification of host factors that participate in viral restriction can lead to new advances in antiviral therapies, and host-targeted approaches may help to ameliorate the rapid development of resistance against therapies that directly target viruses with high mutation rates.

Recent advances in functional genomics have allowed for the unbiased identification of cellular factors involved in viral infection. Diverse screening techniques have been applied to this arena, including loss-of-function and gain-of-function screens. When applied genome-wide, these techniques allow for the interrogation of cellular requirements for viral infection, generating information on those factors that are most important for viral infection. Integration of other -omics technologies, including proteomics and transcriptomics, can lead to a comprehensive picture of the intricate interactions between viruses and their hosts. These screening platforms primarily rely on cultured cells, and developing technologies have increased the repertoire of cell types that can be used for screening, which is particularly important for those viruses that exhibit narrow cell or species tropism. As more of these large-scale screens are completed, additional comparisons can be made between cell types or between viruses. Comparing and contrasting these host dependencies can inform our understanding of common pathways or factors that are important for infection. This may ultimately allow for the design of broadly acting antiviral agents, which will be useful for combating the viruses that are currently of concern to human health and may allow for a rapid response to the yet-to-be discovered viruses of the future.

TECHNOLOGY: METHODOLOGY

Genetic Screens

Unbiased forward and reverse genetic screens allow for the examination of host cell factors that participate in viral infection and lead to the discovery of novel mechanisms of both host antiviral defenses and the manipulation of host factors required for infection.

Loss-of-function studies using RNA interference. The development of RNA interference (RNAi) technology has allowed for unprecedented interrogation of the basic cellular functions of proteins and protein networks and provides insight into the functions of previously undefined genes (reviewed in 1–3). To date, many studies have utilized RNAi technology to deplete these host factors to determine their significance in the context of virus-host interactions. RNAi involves the sequence-specific knockdown of host cell mRNA, leading to hypomorphic loss-of-function phenotypes. The most commonly used reagent for screening is the direct transfection of small

interfering RNAs (siRNAs), which transiently knock down expression of the gene of interest. Alternatively, short hairpin RNAs (shRNAs) encoded on plasmids can be delivered via transfection or by viral vectors, allowing integration into the host genome for long-term silencing. Both siRNAs and shRNAs can be used singly or in a pooled format. In *Drosophila* cells, RNAi can be very robustly induced by long dsRNAs. *Drosophila* is a highly attractive model for studying viral infection given the conservation between *Drosophila* and mammalian genes and the high degree of genetic tractability in this system (4–8).

Although it is a powerful tool, investigators must be mindful of the limitations of this technology when designing and analyzing RNAi experiments (9–11). The most significant hindrance in gleaning high-quality data from an RNAi screen is the significant potential for false positives due to off-target gene effects. This concern can be addressed by requiring that several distinct siRNAs or shRNAs targeting a single gene cause a similar phenotype to minimize nonspecific effects. Furthermore, the use of independent assays can overcome experimental artifacts. Another concern is false negatives, which can arise from poor knockdown or from cytotoxicity (12). For practical reasons, essential cellular genes are typically eliminated from further study, but they may still be critical for infection by the virus. Factors that are efficiently inhibited by a viral pathogen may not be revealed by loss-of-function approaches.

Concerns about the limitations of this technology were heightened with the publication of several genome-wide screens utilizing a similar technology to identify host factors influencing the same virus. When analyzing the overlap of individual factors discovered in each study, it may at first seem surprising that the majority of genes found are not common to all (or even any two) studies. However, a variety of technical variations can explain some of these discrepancies. Differences in cell type, RNAi reagents, and criteria for hit selection lead to disparate results. Further validation of gene sets by investigators would alleviate many of the false positives, such as including at least two independent reagents in at least two separate assays. A study by Bushman et al. (13) analyzed data from a screen done in duplicate with the same experimental conditions and estimated that comparison of these data sets yields only 50% overlap. However, informatics analysis reveals greater overlap when comparing the enriched pathways and processes. Detailed reviews have discussed the many caveats associated with the generation and interpretation of data from RNAi screening (9, 14, 15).

Another approach that may overcome some of the issues associated with large-scale screening is narrowing the screen to focus on smaller gene sets, which increases the feasibility of performing more replicates with independent reagents and allows higher confidence in the identified candidate genes. Some investigators have focused on particular gene types (e.g., the kinome), whereas others have used -omics technologies, including transcriptomics and proteomics, to identify genes of interest that can be functionally tested using RNAi.

Haploid genetic screens. An alternative to RNAi, which functions at the mRNA level, is to use insertional mutagenesis to generate mutant DNA alleles. This has been an invaluable tool in identifying gene function in haploid organisms, such as bacteria and yeast. However, this approach is challenging in diploid organisms because inactivation of only one gene copy will often have little effect in the context of a second, fully functional copy. To overcome this challenge, a haploid human cell line (16, 17) provides the opportunity to inactivate gene function with a single round of insertional mutagenesis and has been used with great success to examine the host requirements for a number of viral infections. To date, this mutagenesis approach has been performed with either retroviruses or lentiviruses. However, the use of this technique is limited to viruses capable of infecting the only available cell line. Additionally, validation of genes identified should be performed in a distinct cell line, as there are likely significant alterations in a cell line

that is viable with a haploid genome. Of course, the study of genes essential for cell growth or survival is not possible by this approach as cells will not survive in the absence of such genes.

cDNA screens. Loss-of-function screens reveal dependencies of expressed genes. However, many genes require induction or may not be expressed in model cell lines and, thus, would not be identified. A complementary approach is to ectopically express genes to determine the effects of such factors during infection. This method can also strengthen results from loss-of-function studies if ectopic expression reveals the opposite phenotype. However, this would only be apparent if the host factor were limiting. For those factors that are impaired or inactivated by viral infection, loss-of-function approaches may not reveal phenotypes, whereas overexpression may overcome the viral restriction.

Chemical Screens

One goal of genetic screens is to identify potential targets for therapeutics. Another strategy is to directly screen for chemical inhibitors of viral infection that may target host factors. However, the difficulty of target identification makes this approach challenging. This challenge can be overcome by using libraries of small molecules with known targets, revealing important dependencies and potential avenues for treatment.

Screen Design

The major hurdles in performing a high-throughput screen include optimization, miniaturization, and robustness. The initial challenge is to determine the types of genes to be targeted. This determination is followed by the selection of cell type, screen type, and viral assay. The simplest assays often lead to the most robust downstream data, in part because simplicity facilitates performing multiple replicates, increasing the statistical power of the analysis. With all of the above-mentioned methods, there are many considerations that must go into designing an experiment. When undertaking a screen, two important concerns are the desired scope of the study and the establishment of a balance between the magnitude and the stringency of the screen. For instance, the most relevant cell type for infection may not be easily transfectable, necessitating the use of a more genetically tractable cell line. Conducting RNAi studies with relatively few genes allows for rigorous testing of knockdown efficiency while assessing effects on infection, which is not feasible for large-scale RNAi studies. Similarly, when analyzing data, one must carefully consider the establishment of cutoffs for determining significant phenotypes and whether it is more desirable to avoid false positives or false negatives. Valuable information can be gleaned from all of these approaches, but rigorous validation is required, either prior to identifying a phenotype or after identifying a gene set of interest.

Indeed, narrowing a genetic screen to focus on specific types of factors for which a collection of reagents are available can be quite powerful. For example, some screens have focused on a subset of genes that compose the kinome—the complement of kinases in a host cell (18). These enzymes catalyze phosphorylation reactions and are involved in diverse cellular signaling pathways that are likely to be engaged during infection. The so-called druggable genome is a particularly attractive target, as it comprises cellular factors that are thought to be amenable to therapeutic intervention (19). Another strategy is to focus on factors that physically interact with viral proteins. To date, large-scale protein-protein interaction studies have utilized either yeast two-hybrid screening or affinity purification–mass spectrometry (AP-MS). The combination of AP-MS and RNAi is an increasingly popular strategy. AP-MS is not limited to binary interactions and can often be

performed in cell lines that are representative of relevant biological tissues. Although this means that one can enrich for and identify multiple members of a host protein complex, information regarding direct or indirect interactions must be gleaned via other methods in follow-up studies. **Figure 1** illustrates the many options for screen design and assays.

Infection Assays

Virus infection goes through sequential steps: entry, replication, assembly, and egress. Many viruses cause cytopathology upon successful infection. Assays have been established to measure each step in infection or to assess viral spread to neighboring cells. For those viruses that induce cytopathic effects, assays can be designed to select for survival of host cells as a result of inactivation of a pro-viral factor or overexpression of an antiviral factor. Assays monitoring many steps allow for the broadest assessment of host factor dependencies at the cost of specificity. In contrast, screens focusing on a particular step may be easier to interpret. Many screens utilize a modified version of a virus that allows only a single round of infection, thereby focusing on early stages of infection but not assessing viral assembly, egress, or spread. Several flavivirus screens have been conducted using a subgenomic replicon that encodes only the components necessary to carry out viral replication, independent of entry or egress. In some cases, the assay selected is dependent on the type of viral reagents available. For example, pseudotyped viral particles assess only entry and may be the only practical assay available for studying viruses that require high containment, such as Ebola virus. Pseudoviruses are constructed by providing the viral glycoproteins or capsid proteins in *trans* such that the virus no longer encodes these genes, allowing only early steps of the viral infection to proceed. Viral infection or replication can be measured directly by monitoring a viral antigen or through the expression of a reporter gene encoded in the virus or present on a separate construct but dependent on a viral product for expression [e.g., LTR-driven, Tat-dependent reporter gene expression to assess human immunodeficiency virus (HIV) infection]. The reporter gene utilized dictates the method of detection: Fluorescent protein expression can be monitored by flow cytometry, microscopy, or a fluorescent plate reader, whereas luciferase activity is measured by a luminometer. Each of these assays can be automated for high-throughput screening.

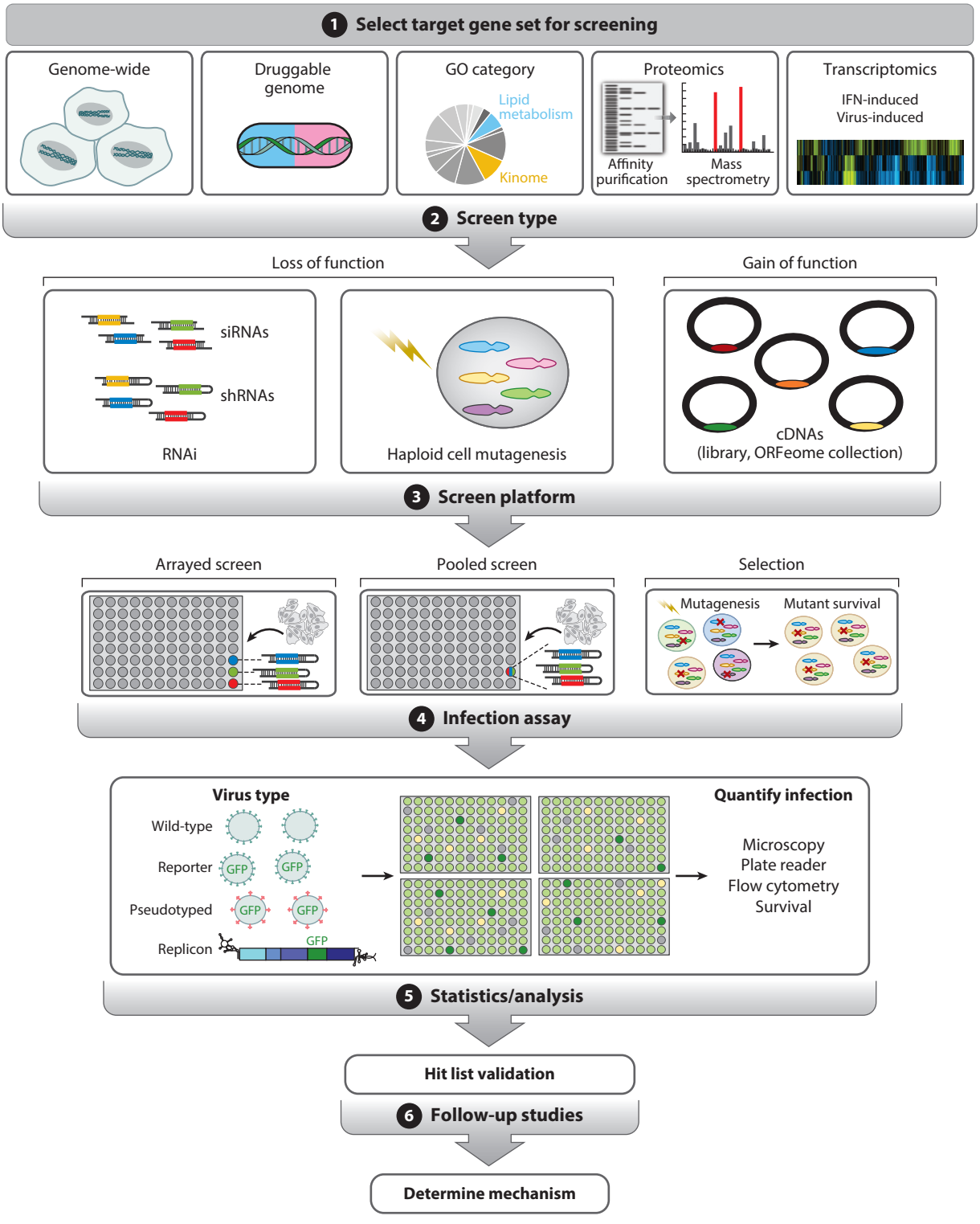
PRO-VIRAL FACTORS: NEW UNDERSTANDINGS AND THERAPEUTIC TARGETS

In this section we discuss the genetic screens that have been performed to identify cellular factors that promote viral replication. We have organized this section by virus family, primarily focusing on the subset of factors identified in a primary screen that were selected for more in-depth studies.

Retroviruses

Retroviruses possess an ssRNA genome that is reverse-transcribed into a DNA intermediate that integrates into the host cell genome. The Retroviridae family is composed of several genera, whose members include viruses that have been modified for use in molecular biology, such as lentiviruses, and the major human pathogen HIV.

Human immunodeficiency virus. Given the enormous global burden of HIV infection, significant research efforts have been devoted to characterizing the mechanisms of infection and to identifying host factors required for infection that may serve as therapeutic targets. To this end, four independent large-scale RNAi screens have been published, providing invaluable insight into


1 Select target gene set for screening
Genome-wide
Druggable genome
GO category
Lipid metabolism
Kinome
Proteomics
Affinity purification
Mass spectrometry
Transcriptomics
IFN-induced
Virus-induced
2 Screen type
Loss of function
Gain of function
siRNAs
shRNAs
RNAi
Haploid cell mutagenesis
cDNAs
(library, ORFeome collection)
3 Screen platform
Arrayed screen
Pooled screen
Selection
Mutagenesis
Mutant survival
4 Infection assay
Virus type
Wild-type
Reporter
Pseudotyped
Replicon
GFP
Quantify infection
Microscopy
Plate reader
Flow cytometry
Survival
5 Statistics/analysis
Hit list validation
6 Follow-up studies
Determine mechanism

host factor requirements. The first three screens were performed with siRNA libraries (20–22), and the fourth utilized shRNAs (23). The siRNA screens have been reviewed previously, including an extensive meta-analysis to analyze the overlap among the studies (13, 14). The outcome of these analyses suggests that variables such as cell type, experimental readout, and hit-picking parameters result in a fairly low overlap in genes identified among the studies. However, common pathways identified include Golgi vesicular transport, the nuclear pore complex (NPC), and the mediator complex, which is required for coupling transcription factors to RNA polymerase II. Other important findings include the requirement for ubiquitin-associated and DNA repair factors. The siRNA screens were performed in transformed fibroblast cell lines that are quite distinct from the natural cell types targeted by HIV in vivo. To address this limitation, a subsequent study used shRNA pools in a Jurkat T cell line (23), selecting for those genes that, when depleted, protected the cells from HIV-dependent cell death. Of those identified, overexpression of three of the genes resulted in increased HIV infection, bolstering confidence in the primary data obtained in the shRNA screen: NRF1 promotes expression of the HIV entry coreceptor CXCR4, NCOA3 promotes HIV-1 transcription, and EXOSC5 promotes proper Gag trafficking.

In a complementary screen, a cDNA library of 15,000 genes was screened to identify factors that impact infection of HIV-IIIb in the HIV-permissive cell type HeLaCD4βgal (24). When ectopically expressed, 315 genes led to a 2-fold change in reporter expression; this group was enriched in genes encoding components involved in microtubule dynamics, cellular metabolism, gene transcription, and inhibition of apoptosis. The serine/threonine kinase mixed lineage kinase 3 (MLK3) enhanced infection by 8-fold, whereas depletion with siRNAs reduced infection. The authors showed that the MLK3 requirement is Tat dependent, leading to the speculation that MLK3 impacts phosphorylation of JNK and activation of the transcription factor AP-1, which may drive Tat-dependent transcription from the HIV LTR.

RNA Viruses

RNA viruses are those viruses whose genomes are composed of RNA, without a DNA intermediate in the viral life cycle. This group comprises a diverse set of human pathogens, including both positive- and negative-sense viruses.

Negative-sense RNA viruses. ssRNA viruses that are negative sense encode RNA genomes that are complementary to the viral mRNA and require the production of a positive-sense RNA strand prior to translation of viral proteins. We first discuss screens performed with segmented negative-sense genomes and then address those with nonsegmented genomes.

Figure 1

Screen design and methodology. The key elements of designing a screen to identify host factors involved in viral infection are depicted: (❶) Selection of the gene set to screen can be large scale (e.g., genome-wide) or more directed (e.g., druggable genome or kinome). (❷) Phenotypic outcomes include loss of function (e.g., RNAi, haploid genetic screens) and gain of function (e.g., cDNA overexpression). (❸) RNAi screening platforms are performed with arrayed reagents (a single RNAi construct in each infection), with pooled reagents (multiple constructs targeting a factor in each infection), or as a selection, in which only a subset of cells survive the screen. (❹) Infection assays are performed with a variety of viral constructs: wild-type virus, virus with an integrated reporter gene, pseudotyped virus, subgenomic viral replicons, etc. (❺) Statistical analysis of data is performed to select the most robust phenotypes, followed by validation with multiple reagents. (❻) Follow-up studies are carried out to decipher mechanisms of individual targets. Abbreviations: GFP, green fluorescent protein; GO, gene ontology; IFN, interferon; ORF, open reading frame; RNAi, RNA interference; shRNA, short hairpin RNA; siRNA, small interfering RNA.

Orthomyxoviruses. The orthomyxoviruses are a group of segmented RNA viruses that consists of six genera. Three of these genera—the influenza A, B, and C viruses—cause influenza in vertebrates. Influenza A viruses are of obvious public health concern as the source of seasonal epidemic and pandemic infections in humans.

Influenza. Multiple genome-wide screens have been undertaken to further elucidate the mechanisms of influenza virus infection and the host cell requirements to support or control infection (25–28). Several reviews have analyzed the commonalities among the findings of these screens (29–33) and revealed overlapping results, including the nuclear RNA export factor NXF1, members of the COPI coatomer complex, and components of the vacuolar ATPase, suggesting that these three categories of proteins are robustly involved in influenza infection.

A more recent study combined AP-MS with siRNA screening to identify host proteins that both interact with influenza A/WSN/33 virus-encoded proteins and are required for viral replication in HEK293 cells (34). Catenin β1 (CTNNB1) and BUB3, which is involved in the mitotic checkpoint, interacted with influenza proteins and were also identified in large-scale RNAi screens (26, 28, 35). Additionally, XPO1 interacted with M2 and was previously found to suppress viral RNA and NP protein export from the nucleus (36, 37). Other factors, including GBF1, JAK1, BRD8, and DDX55, affected the formation of virus-like particles, and known inhibitors of GBF1 and JAK1 bolstered these findings.

The first haploid genetic screen to identify factors involved in infection used influenza virus A (PR/8/34; H1N1) in KBM7 cells (38). In this study, retrovirus-mediated gene-trap vectors were used for mutagenesis. Cells were infected, and because influenza infection is cytolytic, the surviving cells were isolated to determine the sites of disruption. These studies identified two independent insertions disrupting the expression of CMAS, an enzyme required for the sialic acid–containing receptors that are recognized by the influenza HA protein on the surface of influenza-susceptible cells. In addition, insertions in the gene encoding SLC35A2, which transports a glycosyl donor used for glycans that are modified by sialic acids, were also recovered. The authors confirmed that mutants of these two factors are resistant to influenza infection, and they were able to restore that susceptibility by transduction with cDNAs encoding the intact proteins.

Bunyaviruses. Bunyaviruses are a large group of trisegmented, negative-strand RNA viruses, of which four families are arthropod borne. Bunyavirus infection can lead to highly pathogenic diseases, including hemorrhagic fever, encephalitis, and hepatitis. There are no approved human vaccines or therapeutics against this group of important pathogens.

Rift Valley fever virus. Rift Valley fever virus (RVFV) is a mosquito-transmitted human pathogen that causes significant mortality in livestock and humans. Using *Drosophila* cells as a model for the mosquito vector, Hopkins et al. (39) conducted a genome-wide RNAi screen using dsRNAs with the live attenuated MP12 RVFV strain and a microscopy-based assay. They validated 131 genes involved in infection, and within the gene set were 7 genes that promoted infection, including genes involved in entry and the proteasome.

Uukuniemi virus. A genome-wide siRNA screen was also conducted with the nonpathogenic bunyavirus Uukuniemi virus S23 (UUKV) using two distinct siRNA libraries in HeLa cells modified to express the entry receptor CD209 (40). Automated microscopy identified 20 factors that influenced infection upon knockdown in both libraries, including genes involved in endosomal acidification, which is known to be required for entry. One previously unrecognized late endosomal trafficking protein, VAMP3, was found to be required for intracellular trafficking of UUKV to late endosomes during infection.

Andes virus. Andes virus (ANDV) is a bunyavirus in the hantavirus family that is transmitted from rodents to humans. Petersen et al. (41) performed two parallel screens that converged on the identification of the sterol regulatory pathway as required for infection. Haploid HAP1 cells were mutagenized with a lentivirally delivered gene-trap construct and infected with a vesicular stomatitis virus (VSV) recombinant carrying the ANDV envelope. VSV is a lytic virus; thus, disruption of the genes required for either ANDV entry or VSV replication allows cell survival. Surviving cells were pooled and sequenced, and the four most highly targeted genes were in the sterol regulatory element–binding pathway (*SREBF2*, *SCAP*, *S1P*, and *S2P*). In parallel, an siRNA screen of the druggable genome in HEK293T cells was performed using a replication-incompetent VSV expressing the ANDV glycoprotein and a luciferase reporter. Nine genes were validated with independent siRNAs and were specific for ANDV entry, having no impact on VSV replication. Among these was *SREBF2*, which was also found in the haploid screen, providing independent identification of sterol regulatory element–binding pathway proteins. Further genetic and chemical studies confirmed that the sterol pathway is essential for ANDV entry, likely at the step of internalization. It remains to be seen whether this pathway is important for infection by additional hantaviruses.

Arenaviruses. Arenaviruses are negative-strand, bisegmented viruses that are categorized as Old World or New World and infect rodents as a reservoir (42). Several arenaviruses cause disease in humans, including lymphocytic choriomeningitis virus (LCMV), Lassa virus, and Junín virus.

Lassa virus. Lassa virus is an Old World arenavirus that is endemic to West Africa; it infects 300,000–500,000 people annually (43). Entry of Lassa virus is dependent on the heavily glycosylated α-dystroglycan (α-DG). Defects in the glycosylation of α-DG lead to resistance, as do congenital disorders such as Walker-Warburg syndrome (WWS) that impair the ability of α-DG to interact with endogenous substrates such as laminin. To determine additional factors that may be required for α-DG glycosylation and Lassa virus entry, Jae et al. (44) performed a haploid genetic screen that identified factors required for infection using VSV pseudotyped with the Lassa virus envelope. They isolated mutants in the gene encoding α-DG, as well as in other genes. To focus on those genes encoding factors involved in α-DG glycosylation, the authors independently enriched for cells deficient in surface α-DG, thereby identifying all known Walker-Warburg syndrome genes and confirming the link between this disorder and Lassa virus entry. Further studies found additional factors, including TMEM5 and SGK196, required for α-DG laminin binding and Lassa virus entry. Additional factors found to be required for Lassa virus entry included LAMP1, as an essential intracellular receptor, and factors responsible for *N*-glycosylation and sialylation (e.g., ST3GAL4) (45). The authors suggested a model in which the Lassa virus envelope binds α-DG at the cell surface and, upon internalization into a low-pH compartment, engages LAMP1; this engagement is dependent on sialyation by ST3GAL4. This requirement for LAMP1 explains some species tropisms of Lassa virus.

Junín virus. In 2013, Lavanya et al. (46) conducted an siRNA screen of the druggable genome to find host cell factors affecting infection of human U2OS cells with Junín virus, a New World arenavirus, using a murine leukemia virus pseudotyped with the Junín virus glycoprotein (Parodi strain) and encoding β-galactosidase as a reporter to monitor infection by microscopy. The authors validated 26 factors with at least two independent siRNAs, of which 10 impacted Junín virus, but not VSV, infection. Among the Junín virus–specific factors was CACNA2D2, a component of a voltage-gated calcium channel, which was specifically required for viral entry. Given that drugs against this target are in use, it may be a viable candidate for antiviral treatment.

Nonsegmented negative-sense RNA viruses. The nonsegmented negative-strand RNA viruses have genomes that consist of a single RNA. The viruses discussed below are all members of the order Mononegavirales.

Vesicular stomatitis virus. There have been two genome-wide siRNA screens to identify host factors required for VSV infection in HeLa cells using green fluorescent protein (GFP)-expressing VSV (47, 48). Panda et al. (47) validated 72 genes and tested them for roles in infection with human parainfluenza virus type 3 (HPIV3), a nonsegmented, negative-strand RNA virus in the family Paramyxoviridae, and LCMV, a segmented, negative-strand RNA virus in the family Arenaviridae. The authors found that 25 genes impacted these other viral infections, including the genes encoding the COPI coatomer complex, which was required for viral gene expression. GBF and the COPI complex were also identified as factors required for influenza, HPIV3, hepatitis C virus (HCV), West Nile virus (WNV), Sindbis virus, and LCMV infection (5, 26, 47, 49, 50). SLC46A1, a previously uncharacterized proton-coupled folate transporter, was specifically required for VSV infection and likely facilitates viral entry. Whereas the screen by Panda et al. (47) was performed under multicycle conditions, the screen by Lee et al. (48) was limited to the analysis of early stages of infection. Those authors identified a large number of genes encoding proteins involved in translation, and discovered that RpL40, along with a subset of cellular proteins, is specifically required for translation of VSV and other members of Mononegavirales (51). In addition, they found 3 genes required for entry (*GPR149*, *PSCA*, and *LSM5*) and an additional 20 genes that impact replication. An analysis of both studies revealed an overlap of 8 host factors, including 4 members of the COPI coatomer complex.

Borna disease virus. Borna disease virus (BDV) is an enveloped, negative-strand RNA neurotropic virus that mainly infects horses and other warm-blooded animals and is also thought to infect humans. Clemente et al. (52) performed an siRNA screen against the druggable genome using a recombinant VSV expressing the BDV surface glycoprotein in a human oligodendroglial cell line (O1). They validated 24 genes that resulted in reduced levels of bona fide BDV infection and used specific inhibitors to demonstrate the requirement for 3 of these genes (*FURIN*, *CTSL*, and *ADAM17*) in BDV entry.

Ebola virus. In 2011, Carette et al. (17) used haploid HAP1 cells mutagenized with a retroviral gene-trap vector to screen for factors required for Ebola virus entry using a recombinant VSV expressing the Ebola virus glycoprotein. This infection is cytolytic, so surviving cells were selected because they were likely protected from infection. This screen enriched for cells with mutations in cathepsin B, which was previously found to be important for Ebola virus entry, and NPC1, an endosomal/lysosomal cholesterol transporter that also functions in endosome/lysosome fusion and fission. It was demonstrated that NPC1 acts as an intracellular receptor for filoviruses (53). In a complementary study, a small-molecule screen identified NPC1 as the target of an inhibitor of Ebola virus entry (54). Additionally, NPC1 was found to be essential for entry of Ebola virus but was dispensable for a number of other viruses, suggesting an Ebola virus–specific role. Together, these two studies demonstrate the power of these unbiased screens to identify new receptors.

Positive-strand RNA viruses. ssRNA viruses that are positive sense encode RNA genomes that can be directly translated by host cell machinery.

Flaviviruses. The Flaviviridae family of viruses consists of four genera of enveloped viruses with positive-strand ssRNA virus genomes. These are translated as one long polyprotein that is processed into individual viral proteins by host and viral proteases. The *Hepacivirus* genus has only

one member, HCV, with several distinct genotypes. The *Flavivirus* genus, for which the family is named, largely consists of arthropod-borne viruses, including dengue virus (DENV), yellow fever virus (YFV), and WNV.

Hepatitis C virus. HCV is a hepatotropic virus that infects nearly 170 million people worldwide and can lead to the development of liver failure and hepatocellular carcinoma. Research efforts to better understand the virus were hampered due to the lack of an in vitro system for virus replication, but the development of cell culture systems (55, 56) over the past several years has led to significant advances in our understanding of the virus.

Two studies have employed genome-wide siRNA screens to assess cellular factors affecting HCV infection: Li et al. (57) used the full-length JFH-1 genotype 2a virus in Huh7.5.1 cells, whereas Tai et al. (49) used a subgenomic HCV genotype 1b replicon reporter construct. Both screens revealed an enrichment in Golgi vesicle binding and organization proteins, including members of the COPI complex. Li et al. also found 30 factors that were previously identified to influence HCV or WNV infection, including DDX3X and Raf1/MAPK3, which were found in two additional HCV screens (58, 59). In a follow-up study, Li et al. further delineated the stage of infection influenced by identified factors (60). In an siRNA screen of 62 host factors that physically interacted with HCV or belonged to pathways implicated in HCV replication, Randall et al. (58) found that RNAi machinery components, such as Dicer, promote HCV replication, consistent with the requirement for the endogenous microRNA miR-122 in HCV replication (61).

Two studies have targeted the druggable genome (62, 63). Ng et al. (63) used an HCV genotype 1b replicon system and an siRNA library and identified nine genes, including *TBXA2R*, which encodes a G protein–coupled receptor; two genes encoding transcription factors (*RelA* and *NFκB2*); and two genes encoding related transporter proteins (*SLC12A4* and *SLC12A5*). Vaillancourt et al. (62) used an adenoviral-based shRNA library and identified PI4KA as the most robust hit, which promoted replication of the genotype 1b replicon as well as those of two additional HCV genotypes. Because of the requirements for membrane rearrangements during HCV infection, Berger et al. (64) screened a library of 140 membrane trafficking genes for their roles in the production of infectious JC1 genotype 2a virus. They validated 7 factors, including PI4KA and the membrane trafficking proteins Rab5A and Rab7L1.

Three siRNA screens have targeted the host cell kinome (59, 65, 66). In the first, the authors used a replicon derived from HCV genotype 1b, expressing a luciferase reporter gene to monitor replication, and confirmed the requirement for three kinases: carboxy-terminal Src kinase (Csk), Janus kinase 1 (Jak1), and vaccinia-related kinase 1 (Vrk1) (65). A full-length JC1 genotype 2a reporter virus was used to identify kinases impacting entry and replication of HCV (59). Validation experiments confirmed factors belonging to the ErbB and the MAPK signaling pathways, and the most robust infection phenotypes were observed when targeting choline kinase α (CHKA) and phosphatidylinositol 4-kinase α (PI4KA). Lupberger et al. (66) specifically identified kinases that are required for HCV entry into host cells. These include epidermal growth factor receptor (EGFR), ephrin receptor A2 (EphA2), and cell division cycle 2 kinase (CDC2), which were required for cell entry for all of the major HCV genotypes in many different cell types.

Many of these screens have identified a dependence on PI4KA, which is likely required for the formation of the membranous structures that serve as the site of viral replication (49, 57, 59, 60, 62, 64). The involvement of PI4KA was further characterized by colocalization experiments, which showed that PI4KA and HCV NS5A are closely associated in infected cells, and NS5A was shown to enhance PI4KA enzymatic activity (67).

HCV exhibits highly specific cell tropism; in an elegant screen to identify factors required for entry of host cells by HCV, the Rice laboratory (68) generated a cDNA library from the

Huh7.5 cell line. This was transfected into a nonpermissive mouse cell line, NIH3T3, ectopically expressing human CD81, SR-B1, and CLDN1, which are thought to be critical for HCV entry, to identify additional factors that could increase susceptibility (68). The screen utilized lentiviral particles pseudotyped with the HCV glycoproteins E1 and E2. This led to the discovery that occludin, a protein present in the tight junction complex of polarized epithelial cells, promotes HCV entry. This, along with previous studies, shows the utility of screening methods to identify novel entry factors (69, 70).

Two recent studies combined proteomics and RNAi to identify host factors that physically interact with HCV proteins to influence infection (71, 72). Germain et al. (72) performed AP-MS to generate an HCV-host protein-protein interactome with 7 HCV proteins ectopically expressed in HEK293T cells. The authors screened 234 interacting factors by RNAi using a genotype 1b replicon and a full-length genotype 2a virus and found that 32 genes were required for HCV replication. Ramage et al. (71) also used AP-MS, performing parallel studies in HEK293T and Huh7 hepatoma cells with all 10 HCV proteins. Using shRNAs to knock down a subset of selected interactors and a full-length JC1 genotype 2a reporter virus, they identified 75 host proteins influencing infection. Through integration of the proteomics and RNAi studies, an HCV-host interactome was developed that consisted of 134 host proteins, revealing an enrichment in factors associated with mitochondrial function, Golgi vesicle transport, and RNA processing. Further characterization of WIBG, an RNA-binding protein associated with nonsense-mediated decay (NMD), revealed an important role for NMD in HCV infection. Indeed, HCV infection leads to an accumulation of NMD substrates, indicating an inhibition of NMD. NMD likely has broader roles in virus infection, because recent studies found that this pathway is antiviral against Sindbis virus and potato virus X (73, 74).

West Nile virus. A genome-wide siRNA screen using HeLa cells to identify host factors involved in WNV (strain 2741) infection employed an image-based assay to detect the expression of the viral envelope protein (75). The authors identified 20 ubiquitination factors, including members of the endoplasmic reticulum–associated degradation (ERAD) pathway, that promoted infection. Substrates targeted by the ERAD pathway are ultimately degraded by the proteasome, and the authors showed that proteasomal inhibition also attenuated WNV infection. Notably, these factors were also assessed for an effect on DENV infection, and many components of the ERAD pathway were important for infection with both of these viruses. Gilfoy et al. (76) used a druggable genome siRNA library and replication-incompetent luciferase-expressing WNV replicon particles in Huh7 cells and identified 50 genes that were queried across multiple cell lines and assays, validating two subunits of the proteasome, PSMA1 and PSMA2, consistent with findings by Krishnan et al. (75). In a genome-wide RNAi screen in insect cells using a microscopy-based assay with WNV (strain NY2000), 96 genes promoted infection (4). Of these, 30% encoded factors involved in clathrin-mediated endocytosis and acidification, the known entry pathway of WNV. This screen also validated the role of the signal-recognition particle in viral infection, which is likely required for translation of the polyprotein.

Dengue virus. DENV infects approximately 300 million people annually and is transmitted by mosquitoes to humans (77). Sessions et al. (6) performed a genome-wide RNAi screen in *Drosophila* cells by monitoring viral gene expression via image analysis using a *Drosophila*-adapted DENV, DENV2; 118 host genes promoted infection, and 82 conserved genes were tested for their roles in human Huh7 cells. When depleted, 42 of these factors impacted DENV2 infection in human cells. Further study suggested that FLJ20254 (TMEM214), TAZ, EXDL2, and CNOT2 are required for early events, whereas NPR2 and SEC61B are required later. FLJ20254 (TMEM214), which is involved in responses to endoplasmic reticulum stress, is also required for gene expression of

other RNA viruses, including YFV and coxsackievirus B3 (CVB3), suggesting that this pathway is regulated during infection by multiple viruses (78).

Yellow fever virus. A genome-wide siRNA screen in Huh7 cells using the YFV vaccine strain 17D (YFV-17D) was analyzed by microscopy (79). GRK2 promoted infection in both Huh7 cells and mouse embryonic fibroblasts. Furthermore, GRK2 promoted DENV and HCV replication, indicating that it is involved in the replication of diverse flaviviruses.

Alphaviruses. Alphaviruses are enveloped, positive-strand ssRNA viruses that are transmitted from mosquitoes, including the prototypical Sindbis virus and the emerging chikungunya virus (CHKV) (80).

Sindbis virus. Sindbis virus is the prototypical alphavirus and has been used in two genome-wide RNAi screens: one in insect cells (5, 81) and the other in mammalian cells (50). The screen in *Drosophila* cells used Sindbis virus encoding a GFP reporter coupled with a high-content assay to validate 57 genes required for infection and 37 genes that restrict infection (5). Furthermore, ~40% of the genes required for infection were involved in entry, including one encoding a cell surface receptor, NRAMP, that is used as a receptor in both insect and human cells (81). The authors identified additional genes, including *VCP* and *SEC61A*, that encode proteins that regulate trafficking of this receptor (5). The genome-wide RNAi screen in human U2OS cells validated 62 genes that restricted infection and 56 genes that enhanced infection when targeted by multiple siRNAs (50). Further study found that Fuz and TSPAN9 were required for virus internalization and fusion, respectively. Both screens identified the vATPase and the COPI coatomer as required for infection.

Picornaviruses and picorna-like viruses. The picornaviruses are a family of nonenveloped, positive-strand ssRNA viruses that includes many important human pathogens, such as rhinovirus, poliovirus, and CVB (82).

Drosophila C virus. The first genome-wide RNAi screen to identify cellular factors required for infection was performed in *Drosophila* cells using the picorna-like Drosophila C virus (DCV) (83); 112 genes were validated, including 68 genes encoding ribosomal proteins and other translation factors (e.g., RACK1) that result in reduced DCV infection when depleted, as determined by antibody staining. DCV and other picornaviruses use internal ribosome entry sites for translation, and the authors found that internal ribosome entry site–dependent translation is more sensitive to depletion of ribosomes than is canonical cap-dependent translation in insect and vertebrate cells for both DCV and poliovirus. Recent studies confirmed these results and found that HCV is also sensitive to the levels of the ribosomal protein machinery, with a specific dependency on RACK1 (84, 85), which was also identified in the genome-wide screen (83). The *Drosophila* screen was the first to identify the COPI coatomer complex as required for infection by both DCV and poliovirus in insect and human cells, respectively (83). Many subsequent screens have also identified dependencies on the COPI coatomer complex for viral replication (5, 25–28, 47, 49, 50, 57), making this an essential host factor across disparate viruses, although the requirements in different viruses may be mechanistically distinct.

Coxsackievirus B and poliovirus. Enteroviruses can cross the blood-brain barrier and cause encephalitis. To identify factors that may play roles in this process, Coyne et al. (86) performed an siRNA screen against the druggable genome in polarized human brain microvascular cells (HBMEC) for genes that impact poliovirus and CVB using a microscopy-based assay. There were 63 factors that impacted both viruses, whereas 23 genes were specific for poliovirus and 31 for CVB. Members of the adenylate cyclase family (ADCY1, ADCY4, ADCY6, and ADCY7)

that catalyze the conversion of ATP to cAMP, along with many factors that are dependent on cAMP levels for activation, were important for both viruses. These included both cAMP protein kinase (PKA) and the cAMP-response element (CRE)-binding protein (CREB), a transcription factor. Interestingly, the authors found that the small GTPase, Rab17, specifically regulated CVB but not poliovirus infection, whereas a distinct Rab, Rab34, regulated poliovirus but not CVB infection, suggesting that different vesicular trafficking proteins regulate specific enterovirus infections. Tyrosine kinases were also identified as regulating enterovirus infection. However, whereas CVB infection was influenced by the Yes tyrosine kinase, poliovirus infection was affected by the Lyn tyrosine kinase. These comparisons demonstrate that similar viruses can hijack different members of the same class of protein for replication.

DNA Viruses

Poxviruses. Poxviruses (of the family Poxviridae) are large dsDNA viruses that include several pathogenic members, including smallpox virus.

Vaccinia virus. The prototypical poxvirus is vaccinia virus (VACV), which has been extensively studied using screening. One of the established routes for cell entry of VACV is through macropinocytosis, a process that requires the activation of kinases (87). Moser et al. (7) screened kinases and phosphatases for entry factors using VACV WRN expressing β-galactosidase via an image-based screen in *Drosophila* cells and identified a requirement for all three components of the AMP-activated protein kinase (AMPK). Further study found that AMPK is required for macropinocytosis and VACV entry in insect and vertebrate cells. However, because *Drosophila* cells abort VACV infection before genome replication (88), the screen provided information only on very early events. Mercer et al. (89) performed an siRNA screen of the druggable genome in human HeLa cells using a mature virion form of VACV expressing GFP. Upon depletion, 188 genes, including the gene encoding AMPK, inhibited VACV gene expression (90). They further characterized a large group of genes encoding factors involved in ubiquitylation and proteasomal degradation and found that the proteasome is required for viral uncoating and genome release. Moreover, Cullin-3-dependent ubiquitylation and proteasome function were required for DNA replication.

Sivan et al. (91) used VACV IHD-J/GFP derived from a virus strain that expresses GFP and has a point mutation that accelerates the release of progeny from the cell surface (92). Genome-wide RNAi screening in HeLa cells identified roles for many factors, including the translation machinery as well as the ubiquitin-proteasome pathway. In particular, the authors identified a role for nuclear pore (Nup) proteins in virion morphogenesis, providing insight into a previous report that suggested a role for the nucleus in VACV replication, which occurs in the cytoplasm (93).

A genome-wide pooled shRNA screen in A549 cells used a modified VACV expressing the core protein A4L fused to a fluorescent reporter (94). Infected cells were fluorescence-activated cell sorted from reporter-negative cells to enrich for cells in which a host protein essential for VACV replication, but not essential for host cell survival, had been suppressed. The top 172 genes (targeted by at least two shRNAs) were rescreened, 34 genes were validated, and 7 genes, including *HSF1*, were found to be positive in all replicates. HSF1 is a transcription factor, and analysis of the VACV-induced host genes revealed an enrichment in genes encoding HSF1-binding sites. The authors showed that HSF1 is activated (through an unknown mechanism) and localizes to the nucleus during VACV infection, and that loss of HSF1 results in decreased VACV gene expression.

It is also possible to use RNAi to target the viral genome. Whereas RNA viruses have only a small number of gene products, some DNA viruses have a much larger gene product repertoire, with many of unknown function. To identify the roles for these genes, Kilcher et al. (95) performed

an RNAi screen targeting 80 VACV genes that are conserved in other poxviruses to discover virally encoded factors required for intermediate gene expression. They found 15 VACV genes that impacted intermediate expression; four of these genes affected the prereplication complex and one gene, D5, was required for core disassembly. This approach may be widely adopted to query open reading frames in large viruses.

Papillomaviruses. Papillomaviruses are nonenveloped DNA viruses that are of great significance to human health, as they include human papillomavirus (HPV), the causative agent of the majority of cervical cancers (96).

Human papillomavirus 16. To evaluate cellular factors required for HPV16 entry, two screens have been conducted in HeLa cells using an HPV16 that encodes a modified pseudogenome expressing GFP (97, 98). This allows the identification and image-based quantification of cells in which the pseudogenome has been successfully uncoated, imported into the nucleus, transcribed, and translated. Lipovsky et al. (98) screened in HeLa S3 cells using a pooled, genome-wide library and validated 92 genes with two or more siRNAs. Analysis revealed an enrichment in Golgi stack and *trans*-Golgi network factors. Genes encoding proteins involved in retrograde transport were confirmed through the use of small-molecule inhibition. Furthermore, a component of the retromer, VPS26, which was a robust hit in this screen, was shown to interact with the HPV16 protein L2. The authors proposed a model in which HPV16 hijacks the retromer for escape from the endosome to another vesicle, perhaps avoiding host antiviral mechanisms. An siRNA screen of the druggable genome validated 162 genes and further explored the role of mitosis regulators to find that HPV16 requires nuclear envelope breakdown during mitosis for the viral DNA to access the nucleoplasm (97).

Overview of Pro-Viral Factors

A subset of established and novel pro-viral contact points are depicted in the cellular diagram in **Figure 2**. While this is by no means a comprehensive picture of all pro-viral factors identified in screens, we highlight several factors that have been found in multiple screens, with multiple viruses, as well as new findings that are of particular interest or for which follow-up or mechanistic studies were performed.

ANTIVIRAL FACTORS: INTERFERON-STIMULATED GENES AND BEYOND

There has been an explosion of studies aimed at identifying cellular factors that restrict infection. Many of these have focused on interferon-stimulated genes (ISGs), because many known antiviral factors are transcriptionally induced following interferon production in response to viral infection. Some studies have ectopically expressed ISGs to determine those that are sufficient to restrict viral infection, whereas other studies have performed loss-of-function screens to identify genes that are required for interferon-dependent restriction.

Interferon-Stimulated Genes and Interferon Effector Genes

Viral infection is sensed by pattern-recognition receptors, which leads to the induction of type I interferons. These are released by cells and bind to their receptors on both infected cells and bystander cells to transcriptionally induce hundreds of genes, collectively known as ISGs, which

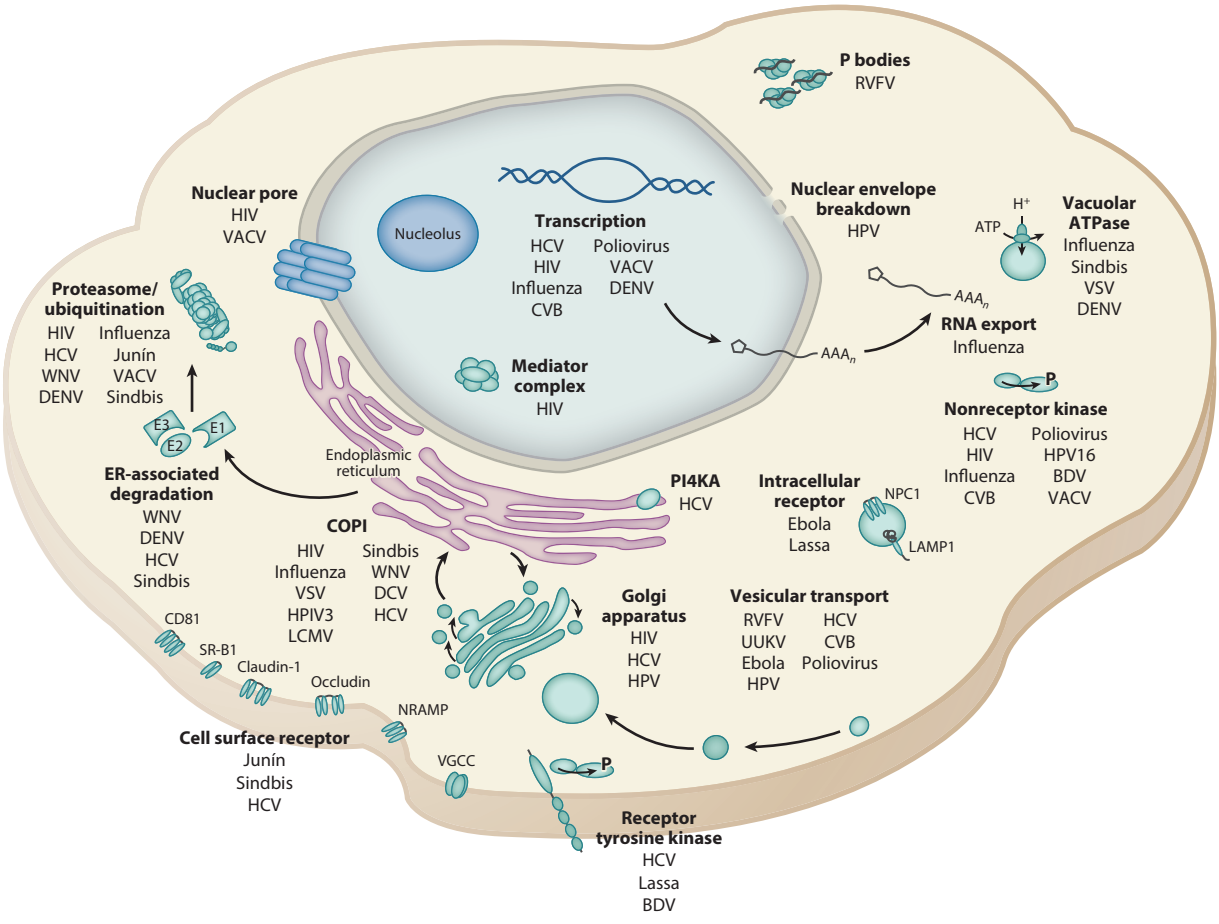


Figure 2

Pro-viral factors identified in screens. An illustration of select cellular pro-viral factors identified in screens, with the viruses targeting each component indicated. This figure is not comprehensive; rather, it depicts several factors that have been found in multiple screens, with multiple viruses, as well as new findings that are of particular interest or for which follow-up or mechanistic studies were performed. Abbreviations: BDV, Borna disease virus; CVB, coxsackievirus B; DCV, Drosophila C virus; DENV, dengue virus; ER, endoplasmic reticulum; HCV, hepatitis C virus; HIV, human immunodeficiency virus; HPIV3, human parainfluenza virus type 3; HPV, human papillomavirus; LCMV, lymphocytic choriomeningitis virus; RVFV, Rift Valley fever virus; UUKV, Uukuniemi virus; VACV, vaccinia virus; VSV, vesicular stomatitis virus; WNV, West Nile virus. Figure adapted with permission from Reference 99.

induce an antiviral state (100). Although some of these genes encode known antiviral effectors, the activities of the products of most of these genes are largely unknown. Given the potential antiviral roles of ISG proteins, several groups have conducted screens in which they specifically inhibit (101–105) or overexpress ISG proteins (101, 106–115) to directly test their function in restricting viral infection, and the majority of these screens have been previously reviewed (100, 116–123). Additional screens have been developed that test the role of ISG proteins in antiviral signaling pathways. Here we discuss recent studies that identify upstream modulators of interferon signaling and ISG transcription and highlight non-ISG antiviral host factors found in screens.

Transcriptional Modulators of Antiviral Signaling

Toll-like receptors (TLRs) are pattern-recognition receptors that can respond to viral infection, some of which recognize viral nucleic acids (124, 125). Transcriptional profiling of TLR responses to a number of stimuli, including poly(I:C) treatment, identified 280 genes (126, 127), and the 17 most highly induced factors were assessed for their requirement in TLR-dependent gene expression using shRNAs. The tyrosine kinase adapter Crkl was phosphorylated during stimulation of TLR signaling and was required for full induction of a subset of TLR-responsive genes. Functional studies also revealed a role for polo-like kinases (PLKs) in TLR signaling. Indeed, inhibition of PLKs blocked the nuclear translocation of IRF3, an important antiviral transcription factor, and inhibited the transcription of TLR-dependent antiviral genes in response to several viruses.

Several studies have utilized RNAi to identify novel regulators of ISG expression. In one study, a combination of bioinformatics, transcriptomics, and proteomics was used to develop a set of genes with potential roles in DNA sensing (128). In total, 809 candidate genes were identified based on this and previous studies (128–131). These were screened by RNAi in mouse embryonic fibroblasts for their role in dsDNA-dependent gene induction by monitoring expression of the ISG *CLCX10*. Further study found 4 factors that enhanced and 10 factors that abrogated innate immune responses to retroviral infection upon depletion. A factor required for signaling, ABCF1, was found to interact with two putative DNA-sensing factors, HMGB2 and IFI204. In another study, 245 ISGs were targeted by shRNAs to determine an effect on WNV infection in interferon-β-treated HeLa cells (102). The authors discovered one ISG protein, activating signal cointegrating complex 3 (ASCC3), that was a negative regulator of interferon signaling. Depletion of ASCC3 resulted in the upregulation of several ISGs and inhibited infection by WNV, CHKV, and encephalomyocarditis virus (EMCV). Varble et al. (104) used a pooled library of Sindbis viruses carrying miRNA mimics against the cellular genome. They serially passaged the viruses to enrich for those encoding miRNA mimics that targeted restriction factors and identified two transcription factors, Zfx and Mga, that induced the expression of ISGs.

Two recent overexpression studies also found regulators of ISG expression. Schoggins et al. (112) overexpressed 350 ISGs to determine activity against 14 different viruses in a variety of cell types; they identified 47 genes whose products restrict infection of at least one virus and 25 genes whose products have pro-viral functions. Among the restriction factors was the cytosolic DNA sensor cyclic GMP-AMP synthase (cGAS), which inhibited several RNA viruses. In another study, a genome-wide, high-throughput cDNA overexpression screen was conducted using luciferase expression driven by the IFIT1 promoter as a reporter for ISG induction in Huh7 cells (113). The authors identified 15 genes encoding proteins that induced ISG expression, including known regulators, such as MAVS and IRF1, along with tyrosine kinase nonreceptor 1 (TNK1), which they found to act through the JAK-STAT pathway by promoting phosphorylation of STAT1. Another cDNA screen identified 9 genes that potentiated STAT1 signaling including TLX, an orphan nuclear hormone receptor (132).

Other Antiviral Factors

Emerging data from many screens suggest that antiviral factors can be broadly active against diverse viruses. In the mammalian WNV RNAi screen, Krishnan et al. (75) found that all 22 genes that were antiviral were also active against DENV, whereas only 36% of the genes that promoted infection were active against both WNV and DENV. Further, they found that SLC16A4, a plasma membrane–associated transporter of monocarboxylic acids, restricted infection by delaying viral replication.

A screen with WNV in *Drosophila* cells, optimized to identify restriction factors, validated 50 genes that restrict infection (4). Further analysis found that many of these were antiviral against additional viruses, including 7 genes that were antiviral against a second strain of WNV as well as against DENV, Sindbis virus, RVFV, and VSV. These included the gene encoding RuvBL1, which the authors found to work through the Tip60 chromatin-remodeling complex. Another broadly antiviral gene encodes XPO1, a nuclear export protein required for the mRNA export of several factors, including aldolase A (ALDOA), also shown to be a viral restriction factor through an unknown mechanism. Other complexes found to be potently antiviral, including the mediator complex, were found in other screens (20–22).

Comparative screening with poliovirus and CVB found that 77% of genes that restricted infection impacted both viruses, including the genes for TLR8 and IRAK1, which are involved in type I interferon signaling that has been previously implicated in enteroviral infection, along with the genes for Akt and MAPKs, which had not previously been identified as restriction factors (86, 133). These results were bolstered through the use of chemical inhibition and overexpression of dominant-negative mutants, which upregulated and downregulated infection, respectively.

RNA Biology

Viruses present diverse RNAs to cells and must perform sophisticated gymnastics to replicate their genomes. Therefore, it is not surprising that many antiviral factors are involved in RNA biology.

A small-scale screen using dsRNAs targeting 100 genes in *Drosophila* identified Ars2 as a host factor restricting VSV infection (134). Analysis of a panel of RNA viruses found that Ars2 was broadly antiviral at the level of RNA replication and stability. In insects, RNAi is antiviral, and Ars2 is required for siRNA- and miRNA-mediated silencing, physically interacting with the cap-binding complex (CBP20 and CBP80) as well as with the upstream components of the silencing machinery (134, 135).

Hopkins et al. (39) performed a genome-wide RNAi screen in *Drosophila* cells to identify genes involved in RVFV infection using the MP12 strain and microscopy. They found that 124 validated genes restricted infection and 7 genes promoted infection. Of the antiviral genes, 3 encode components found in P bodies, foci in the cytoplasm that consist of enzymes involved in mRNA turnover, including Dcp2, me31b, and LSM7, which are also implicated in mRNA decapping. Colocalization experiments suggested that the RVFV N protein localizes to P bodies and that Dcp2 restricts infection in adult flies and in mosquito cells. Moreover, the authors found that RVFV cap-snatches primers for transcription from host mRNAs destined for degradation by the decapping enzyme Dcp2. Recent studies also found that decapping is antiviral against RVFV in human cells (136).

Many DEAD box helicase proteins, including RIG-I and MDA5, act as sensors for viral infection (137–139). In a screen of conserved DEAD box helicases that influence RVFV infection in *Drosophila* cells (140), me31B, CG10333, and Rm62 were found to be antiviral factors. me31b was also identified in the genome-wide screen and thus validated the assay (39). Further study was performed on Rm62, which was found to act as a restriction factor against bunyaviral infection in flies but had no effect on several other viruses tested. In human U2OS cells, DDX17 restricted bunyaviral infection, and further studies showed that DDX17 directly binds to RVFV RNA to restrict viral infection, highlighting a new pattern-recognition receptor.

The avoidance of RNA decay and associated machinery is clearly important for RNA viruses. In 2014, Balistreri et al. (73) conducted a genome-wide siRNA screen of HeLa cells infected with Semliki Forest virus (SFV). Among the most significant phenotypes observed was an increase in SFV infection upon depletion of UPF1, a central component of the NMD pathway, and this

restriction occurred at the level of viral RNA. Additional factors involved in NMD were also restrictive, suggesting that NMD functions in the direct degradation of viral RNA. Taken together with other recent reports of the engagement of NMD in viral infection, this finding identifies the NMD pathway as an exciting new area of research (71, 74).

A recent study utilized an RNAi screening approach, coupled with a meta-analysis of data sets from previous studies including RNAi, proteomics, yeast two-hybrid screening, and transcriptomics analyses, to develop a set of 204 host factors that may be important for HCV infection (141). Further screening validated 40 genes that facilitate HCV replication and 16 genes that restrict infection. Among the antiviral factors was heterogeneous nuclear ribonucleoprotein K (HNRNPK), which interacts with the HCV core and NS3 proteins as well as HCV RNA and acts to suppress HCV particle production. The authors speculated that HNRNPK acts to sequester HCV RNA, preventing the formation of virions. Interestingly, the role of HNRNPK seems to be specific for HCV, as HNRNPK did not impact DENV.

THE FUTURE OF SCREENING

Although we have gleaned valuable information about viruses and their relationships with host cells from the screens described above, the future holds even more promise. The generation of tailored gene sets, improved bioinformatics analysis, and increased validation of data will facilitate new discoveries. Furthermore, improvements in existing technologies and the emergence of new methodologies promise to reveal additional insight into virus-host interactions. In this section, we highlight the improvements and the new technologies that we anticipate will be critical to viral screening strategies in the future.

Improved Validation of Gene Sets

Some of the more recent studies we highlight in this review utilize multiple lines of evidence to converge on significant pathways in viral infection. The most common strategy is the incorporation of data from gene expression and protein-protein interaction studies. Moving forward, establishing parameters for standardization in screens will allow for more direct comparison of data and may eliminate some of the variability inherent in multiple, large-scale screens. Databases established for comparisons between RNAi studies, such as Minimum Information About an RNAi Experiment (MIARE; **http://miare.sourceforge.net**) and GenomeRNAi (142), will certainly aid in these efforts. Two very recent studies developed unique statistical methods to rigorously identify the most important targets. In the first study, the authors used a method termed the Parallel Mixed Model (PMM), which incorporates both the degree of knockdown and a false discovery rate for each factor tested (143). This method was applied to a screen using a single-kinome RNAi library to screen eight distinct pathogens: five bacterial and three viral (adenovirus, rhinovirus, and VACV). Using 11 siRNAs obtained from three different vendors and a common protocol for all pathogens, the authors were able to identify both generic and specific factors influencing infection. In the second study, Zhu et al. (144) employed a screen using multiple orthologous RNAi reagents and integrated results from previous screens, gene expression data, and a statistical method [RNAi gene enrichment ranking (145)] to determine factors affecting HIV infection. This approach confirmed many of the shared targets established from previous screens, as well as identifying some novel genes.

New Reagents

We can expect that emerging technologies, as well as advances in existing technologies, will provide a number of new tools that can be added to the repertoire of high-throughput screening reagents.

Gene-trapped KBM7 cell library. The utility of haploid genetic screens for the analysis of factors required for infection has been demonstrated in a number of screens discussed in this review (17, 41, 45). Recently, Bürckstümmer et al. (146) developed a reversible gene-trap collection that can be used to screen for a phenotype of interest. This collection is available to the scientific community and can be used to determine the effect of specific disruptions on viral infection (**http://clones.haplogen.org**).

CRISPR-Cas9. The advent of DNA-editing technologies has transformed our ability to perform genetic studies in a variety of systems, including the alteration of mammalian cells. This technology has been developed from RNA-guided endonucleases termed Cas9 from the microbial adaptive immune system known as the clustered regularly interspaced short palindromic repeat (CRISPR) system. It allows the targeted editing of any part of the genome using a short RNA guide and has been successful in the modification of traditionally intractable systems (147–149), allowing for studies of viruses exhibiting strong cell tropism in difficult-to-transfect cell types. Recently, Shalem et al. (150) published a study in which a genome-wide CRISPR-Cas9 library was generated and used for screening purposes. As this revolutionary technology continues to develop, these library reagents will be applied for the study of viral infections.

cDNA libraries. Several gain-of-function studies have been analyzed in the context of viral infection through the use of overexpression libraries (24, 68, 107, 108). The availability of a collection of overexpression constructs also facilitates confirmation of the specificity of phenotypes identified in RNAi screens. As with any technology, cDNA libraries continue to improve in recent iterations (e.g., fully sequenced, various isoforms). As such, the generation of an increasingly robust genome-scale cDNA expression collection available in a lentiviral vector format will enable both targeted experiments and high-throughput screens in diverse cell types (151).

Integration of Other Data

The integration of multiple types of experiments allows for many different perspectives on the changes that occur in host cells during infection, as well as providing multiple lines of evidence for the involvement of a particular factor or pathway.

Transcriptomics. Transcriptomics data provide a window into the myriad changes in gene expression that are triggered by viral infection. Monitoring the transcriptional responses to viral infection can provide a more integrated view of the manipulation of host cells and can also be used to define a gene set for subsequent screening. To date, this approach has primarily been used to identify interferon-stimulated antiviral host factors, but it can be applied more broadly in the future. Whole-transcriptome changes have been traditionally monitored by microarray, but the development of RNA sequencing technology gives a more in-depth analysis of RNA profiles, including splice forms and noncoding RNAs, which have been difficult to capture with microarrays (152).

Proteomics. Proteomics technologies have been improving in sensitivity and accuracy. There are many additional proteomics techniques that can be used to monitor host changes in virally infected cells. Protein abundance studies provide information regarding global changes in host protein expression or degradation and can allow these changes to be assessed in a temporal manner during infection. Another critical parameter in monitoring the host cell environment is changes in posttranslational modifications, including phosphorylation, ubiquitylation, and methylation. These modifications can inform both the changes initiated by the cell to combat viral infection and the host pathways and proteins that are manipulated by the virus. Several of the studies highlighted

here have incorporated the use of proteomics as a complementary approach to screening. The screens discussed here have specifically utilized protein-protein interaction studies to focus RNAi screening on factors that function in virus-host protein-protein complexes (34, 71, 72). This technique allows targeted identification of host proteins that both physically interact with viruses and have an effect on infection. A recent review detailed the contributions of proteomics studies to our understanding of virus-host interactions and highlighted the research potential in continuing to employ these techniques (153).

Metabolomics. In addition to other approaches, untargeted metabolomics using liquid chromatography–mass spectrometry (LC-MS)-based methods can identify metabolite alterations in extracts from virally infected as compared with naive cells. LC-MS metabolomics is extremely sensitive, allows for the detection of thousands of molecules from complex mixtures, and can be used to analyze both hydrophobic and hydrophilic metabolites (154). Additionally, this approach can potentially identify structurally novel metabolites that are produced during viral infection (154). This technique can be used to examine the changing physiology of a cell during viral infection, providing insights into how host cells are manipulated.

Human genetics. The integration of information obtained from human genetics studies has provided insight into host factor dependencies for viral infection, such as human CCR5 variants associated with HIV resistance (155). In the future, data from these genetic techniques can be utilized to compile candidate gene sets for virus-host interaction studies.

Genome-wide association studies involve the comprehensive identification of single-nucleotide polymorphisms in the genome across a population to determine variations that are associated with susceptibility or resistance to a particular disease. As the experimental costs are reduced, the sequencing of a genome or an exome (the subset of the human genome that is protein-coding) is becoming routine and will likely be incorporated into screening data sets in the future, leading to new discoveries in virus-host interactions (156).

CONCLUDING REMARKS

Thus far, screening techniques have provided a wealth of information regarding host-pathogen interactions during viral infection. The growing number of these studies across viral families promotes the comparison of data sets and the identification of common factors across viral infections. However, it is becoming clear that guidelines for data analysis and hit selection would increase the likelihood that genes identified play bona fide roles in infection. We hope that the combination of improving technologies, better validation of gene sets, and standardization in data analyses will promote a deeper understanding of virus-host interactions to aid in the treatment of humans and animals during viral infection.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

We thank Mike Shales for graphical support. This work was supported by grants from the National Institutes of Health (R01AI074951, U54AI057168, and R01AI095500) to S.C. S.C. is a recipient of the Burroughs Wellcome Investigators in the Pathogenesis of Infectious Disease award.

LITERATURE CITED

1. Hannon GJ, Rossi JJ. 2004. Unlocking the potential of the human genome with RNA interference. *Nature* 431:371–78
2. Moffat J, Sabatini DM. 2006. Building mammalian signalling pathways with RNAi screens. *Nat. Rev. Mol. Cell Biol.* 7:177–87
3. Falschlehner C, Steinbrink S, Erdmann G, Boutros M. 2010. High-throughput RNAi screening to dissect cellular pathways: a how-to guide. *Biotechnol. J.* 5:368–76
4. Yasunaga A, Hanna SL, Li J, Cho H, Rose PP, et al. 2014. Genome-wide RNAi screen identifies broadly-acting host factors that inhibit arbovirus infection. *PLOS Pathog.* 10:e1003914
5. Panda D, Rose PP, Hanna SL, Gold B, Hopkins KC, et al. 2013. Genome-wide RNAi screen identifies SEC61A and VCP as conserved regulators of Sindbis virus entry. *Cell Rep.* 5:1737–48
6. Sessions OM, Barrows NJ, Souza-Neto JA, Robinson TJ, Hershey CL, et al. 2009. Discovery of insect and human dengue virus host factors. *Nature* 458:1047–50
7. Moser TS, Jones RG, Thompson CB, Coyne CB, Cherry S. 2010. A kinome RNAi screen identified AMPK as promoting poxvirus entry through the control of actin dynamics. *PLOS Pathog.* 6:e1000954
8. Panda D, Pascual-Garcia P, Dunagin M, Tudor M, Hopkins KC, et al. 2014. Nup98 promotes antiviral gene expression to restrict RNA viral infection in *Drosophila*. *PNAS* 111:E3890–99
9. Mohr S, Bakal C, Perrimon N. 2010. Genomic screening with RNAi: results and challenges. *Annu. Rev. Biochem.* 79:37–64
10. Campeau E, Gobeil S. 2011. RNA interference in mammals: behind the screen. *Brief. Funct. Genomics* 10:215–26
11. Boutros M, Ahringer J. 2008. The art and design of genetic screens: RNA interference. *Nat. Rev. Genet.* 9:554–66
12. Hao L, He Q, Wang Z, Craven M, Newton MA, Ahlquist P. 2013. Limited agreement of independent RNAi screens for virus-required host genes owes more to false-negative than false-positive factors. *PLOS Comput. Biol.* 9:e1003235
13. Bushman FD, Malani N, Fernandes J, D'Orso I, Cagney G, Diamond TL, et al. 2009. Host cell factors in HIV replication: meta-analysis of genome-wide studies. *PLOS Pathog.* 5:e1000437
14. Cherry S. 2009. What have RNAi screens taught us about viral-host interactions? *Curr. Opin. Microbiol.* 12:446–52
15. Panda D, Cherry S. 2012. Cell-based genomic screening: elucidating virus-host interactions. *Curr. Opin. Virol.* 2:784–92
16. Kotecki M, Reddy PS, Cochran BH. 1999. Isolation and characterization of a near-haploid human cell line. *Exp. Cell Res.* 252:273–80
17. Carette JE, Raaben M, Wong AC, Herbert AS, Obernosterer G, et al. 2011. Ebola virus entry requires the cholesterol transporter Niemann-Pick C1. *Nature* 477:340–43
18. Johnson SA, Hunter T. 2005. Kinomics: methods for deciphering the kinome. *Nat. Methods* 2:17–25
19. Hopkins AL, Groom CR. 2002. The druggable genome. *Nat. Rev. Drug Discov.* 1:727–30
20. Brass AL, Dykxhoorn DM, Benita Y, Yan N, Engelman A, et al. 2008. Identification of host proteins required for HIV infection through a functional genomic screen. *Science* 319:921–26
21. Konig R, Zhou Y, Elleder D, Diamond TL, Bonamy GM, et al. 2008. Global analysis of host-pathogen interactions that regulate early-stage HIV-1 replication. *Cell* 135:49–60
22. Zhou H, Xu M, Huang Q, Gates AT, Zhang XD, et al. 2008. Genome-scale RNAi screen for host factors required for HIV replication. *Cell Host Microbe* 4:495–504
23. Yeung ML, Houzet L, Yedavalli VS, Jeang KT. 2009. A genome-wide short hairpin RNA screening of Jurkat T-cells for human proteins contributing to productive HIV-1 replication. *J. Biol. Chem.* 284:19463–73
24. Nguyen DG, Yin H, Zhou Y, Wolff KC, Kuhen KL, Caldwell JS. 2007. Identification of novel therapeutic targets for HIV infection through functional genomic cDNA screening. *Virology* 362:16–25
25. Hao L, Sakurai A, Watanabe T, Sorensen E, Nidom CA, et al. 2008. Drosophila RNAi screen identifies host genes important for influenza virus replication. *Nature* 454:890–93

26. Brass AL, Huang IC, Benita Y, John SP, Krishnan MN, et al. 2009. The IFITM proteins mediate cellular resistance to influenza A H1N1 virus, West Nile virus, and dengue virus. *Cell* 139:1243–54
27. Konig R, Stertz S, Zhou Y, Inoue A, Hoffmann HH, et al. 2010. Human host factors required for influenza virus replication. *Nature* 463:813–17
28. Karlas A, Machuy N, Shin Y, Pleissner KP, Artarini A, et al. 2010. Genome-wide RNAi screen identifies human host factors crucial for influenza virus replication. *Nature* 463:818–22
29. Chin CR, Brass AL. 2013. A genome wide RNA interference screening method to identify host factors that modulate influenza A virus replication. *Methods* 59:217–24
30. Stertz S, Shaw ML. 2011. Uncovering the global host cell requirements for influenza virus replication via RNAi screening. *Microbes Infect.* 13:516–25
31. Mehle A, Doudna JA. 2010. A host of factors regulating influenza virus replication. *Viruses* 2:566–73
32. Min JY, Subbarao K. 2010. Cellular targets for influenza drugs. *Nat. Biotechnol.* 28:239–40
33. Watanabe T, Watanabe S, Kawaoka Y. 2010. Cellular networks involved in the influenza virus life cycle. *Cell Host Microbe* 7:427–39
34. Watanabe T, Kawakami E, Shoemaker JE, Lopes TJ, Matsuoka Y, et al. 2014. Influenza virus-host interactome screen as a platform for antiviral drug development. *Cell Host Microbe* 16:795–805
35. Shapira SD, Gat-Viks I, Shum BO, Dricot A, de Grace MM, et al. 2009. A physical and regulatory map of host-influenza interactions reveals pathways in H1N1 infection. *Cell* 139:1255–67
36. Elton D, Simpson-Holley M, Archer K, Medcalf L, Hallam R, et al. 2001. Interaction of the influenza virus nucleoprotein with the cellular CRM1-mediated nuclear export pathway. *J. Virol.* 75:408–19
37. Neumann G, Hughes MT, Kawaoka Y. 2000. Influenza A virus NS2 protein mediates vRNP nuclear export through NES-independent interaction with hCRM1. *EMBO J.* 19:6751–58
38. Carette JE, Guimaraes CP, Varadarajan M, Park AS, Wuethrich I, et al. 2009. Haploid genetic screens in human cells identify host factors used by pathogens. *Science* 326:1231–35
39. Hopkins KC, McLane LM, Maqbool T, Panda D, Gordesky-Gold B, Cherry S. 2013. A genome-wide RNAi screen reveals that mRNA decapping restricts bunyaviral replication by limiting the pools of Dcp2-accessible targets for cap-snatching. *Genes Dev.* 27:1511–25
40. Meier R, Franceschini A, Horvath P, Tetard M, Mancini R, et al. 2014. Genome-wide small interfering RNA screens reveal VAMP3 as a novel host factor required for Uukuniemi virus late penetration. *J. Virol.* 88:8565–78
41. Petersen J, Drake MJ, Bruce EA, Riblett AM, Didigu CA, et al. 2014. The major cellular sterol regulatory pathway is required for Andes virus infection. *PLOS Pathog.* 10:e1003911
42. Emonet SF, de la Torre JC, Domingo E, Sevilla N. 2009. Arenavirus genetic diversity and its biological implications. *Infect. Genet. Evol.* 9:417–29
43. Ogbu O, Ajuluchukwu E, Uneke CJ. 2007. Lassa fever in West African sub-region: an overview. *J. Vector Borne Dis.* 44:1–11
44. Jae LT, Raaben M, Riemersma M, van Beusekom E, Blomen VA, et al. 2013. Deciphering the glycosylome of dystroglycanopathies using haploid screens for Lassa virus entry. *Science* 340:479–83
45. Jae LT, Raaben M, Herbert AS, Kuehne AI, Wirchnianski AS, et al. 2014. Lassa virus entry requires a trigger-induced receptor switch. *Science* 344:1506–10
46. Lavanya M, Cuevas CD, Thomas M, Cherry S, Ross SR. 2013. siRNA screen for genes that affect Junín virus entry uncovers voltage-gated calcium channels as a therapeutic target. *Sci. Transl. Med.* 5:204ra131
47. Panda D, Das A, Dinh PX, Subramaniam S, Nayak D, et al. 2011. RNAi screening reveals requirement for host cell secretory pathway in infection by diverse families of negative-strand RNA viruses. *PNAS* 108:19036–41
48. Lee AS, Burdeinick-Kerr R, Whelan SP. 2014. A genome-wide small interfering RNA screen identifies host factors required for vesicular stomatitis virus infection. *J. Virol.* 88:8355–60
49. Tai AW, Benita Y, Peng LF, Kim SS, Sakamoto N, et al. 2009. A functional genomic screen identifies cellular cofactors of hepatitis C virus replication. *Cell Host Microbe* 5:298–307
50. Ooi YS, Stiles KM, Liu CY, Taylor GM, Kielian M. 2013. Genome-wide RNAi screen identifies novel host proteins required for alphavirus entry. *PLOS Pathog.* 9:e1003835
51. Lee AS, Burdeinick-Kerr R, Whelan SP. 2013. A ribosome-specialized translation initiation pathway is required for cap-dependent translation of vesicular stomatitis virus mRNAs. *PNAS* 110:324–29

52. Clemente R, Sisman E, Aza-Blanc P, de la Torre JC. 2010. Identification of host factors involved in Borna disease virus cell entry through a small interfering RNA functional genetic screen. *J. Virol.* 84:3562–75
53. Miller EH, Obernosterer G, Raaben M, Herbert AS, Deffieu MS, et al. 2012. Ebola virus entry requires the host-programmed recognition of an intracellular receptor. *EMBO J.* 31:1947–60
54. Cote M, Misasi J, Ren T, Bruchez A, Lee K, et al. 2011. Small molecule inhibitors reveal Niemann-Pick C1 is essential for Ebola virus infection. *Nature* 477:344–48
55. Wakita T, Pietschmann T, Kato T, Date T, Miyamoto M, et al. 2005. Production of infectious hepatitis C virus in tissue culture from a cloned viral genome. *Nat. Med.* 11:791–96
56. Zhong J, Gastaminza P, Cheng G, Kapadia S, Kato T, et al. 2005. Robust hepatitis C virus infection in vitro. *PNAS* 102:9294–99
57. Li Q, Brass AL, Ng A, Hu Z, Xavier RJ, et al. 2009. A genome-wide genetic screen for host factors required for hepatitis C virus propagation. *PNAS* 106:16410–15
58. Randall G, Panis M, Cooper JD, Tellinghuisen TL, Sukhodolets KE, et al. 2007. Cellular cofactors affecting hepatitis C virus infection and replication. *PNAS* 104:12884–89
59. Reiss S, Rebhan I, Backes P, Romero-Brey I, Erfle H, et al. 2011. Recruitment and activation of a lipid kinase by hepatitis C virus NS5A is essential for integrity of the membranous replication compartment. *Cell Host Microbe* 9:32–45
60. Li Q, Zhang YY, Chiu S, Hu Z, Lan KH, et al. 2014. Integrative functional genomics of hepatitis C virus infection identifies host dependencies in complete viral replication cycle. *PLOS Pathog.* 10:e1004163
61. Jopling CL, Yi M, Lancaster AM, Lemon SM, Sarnow P. 2005. Modulation of hepatitis C virus RNA abundance by a liver-specific microRNA. *Science* 309:1577–81
62. Vaillancourt FH, Pilote L, Cartier M, Lippens J, Liuzzi M, et al. 2009. Identification of a lipid kinase as a host factor involved in hepatitis C virus RNA replication. *Virology* 387:5–10
63. Ng TI, Mo H, Pilot-Matias T, He Y, Koev G, et al. 2007. Identification of host genes involved in hepatitis C virus replication by small interfering RNA technology. *Hepatology* 45:1413–21
64. Berger KL, Cooper JD, Heaton NS, Yoon R, Oakland TE, et al. 2009. Roles for endocytic trafficking and phosphatidylinositol 4-kinase III alpha in hepatitis C virus replication. *PNAS* 106:7577–82
65. Supekova L, Supek F, Lee J, Chen S, Gray N, et al. 2008. Identification of human kinases involved in hepatitis C virus replication by small interference RNA library screening. *J. Biol. Chem.* 283:29–36
66. Lupberger J, Zeisel MB, Xiao F, Thumann C, Fofana I, et al. 2011. EGFR and EphA2 are host factors for hepatitis C virus entry and possible targets for antiviral therapy. *Nat. Med.* 17:589–95
67. Berger KL, Kelly SM, Jordan TX, Tartell MA, Randall G. 2011. Hepatitis C virus stimulates the phosphatidylinositol 4-kinase III alpha-dependent phosphatidylinositol 4-phosphate production that is essential for its replication. *J. Virol.* 85:8870–83
68. Ploss A, Evans MJ, Gaysinskaya VA, Panis M, You H, et al. 2009. Human occludin is a hepatitis C virus entry factor required for infection of mouse cells. *Nature* 457:882–86
69. Evans MJ, von Hahn T, Tscherne DM, Syder AJ, Panis M, et al. 2007. Claudin-1 is a hepatitis C virus co-receptor required for a late step in entry. *Nature* 446:801–5
70. Pileri P, Uematsu Y, Campagnoli S, Galli G, Falugi F, et al. 1998. Binding of hepatitis C virus to CD81. *Science* 282:938–41
71. Ramage HR, Kumar GR, Verschueren E, Johnson JR, Von Dollen J, et al. 2015. A combined proteomics/genomics approach links hepatitis C virus infection with nonsense-mediated mRNA decay. *Mol. Cell* 57:329–40
72. Germain MA, Chatel-Chaix L, Gagne B, Bonneil E, Thibault P, et al. 2014. Elucidating novel hepatitis C virus-host interactions using combined mass spectrometry and functional genomics approaches. *Mol. Cell. Proteomics* 13:184–203
73. Balistreri G, Horvath P, Schweingruber C, Zund D, McInerney G, et al. 2014. The host nonsense-mediated mRNA decay pathway restricts mammalian RNA virus replication. *Cell Host Microbe* 16:403–11
74. Garcia D, Garcia S, Voinnet O. 2014. Nonsense-mediated decay serves as a general viral restriction mechanism in plants. *Cell Host Microbe* 16:391–402
75. Krishnan MN, Ng A, Sukumaran B, Gilfoy FD, Uchil PD, et al. 2008. RNA interference screen for human genes associated with West Nile virus infection. *Nature* 455:242–45

76. Gilfoy F, Fayzulin R, Mason PW. 2009. West Nile virus genome amplification requires the functional activities of the proteasome. *Virology* 385:74–84
77. Bhatt S, Gething PW, Brady OJ, Messina JP, Farlow AW, et al. 2013. The global distribution and burden of dengue. *Nature* 496:504–7
78. Li C, Wei J, Li Y, He X, Zhou Q, et al. 2013. Transmembrane protein 214 (TMEM214) mediates endoplasmic reticulum stress-induced caspase 4 enzyme activation and apoptosis. *J. Biol. Chem.* 288:17908–17
79. Le Sommer C, Barrows NJ, Bradrick SS, Pearson JL, Garcia-Blanco MA. 2012. G protein-coupled receptor kinase 2 promotes *Flaviviridae* entry and replication. *PLOS Negl. Trop. Dis.* 6:e1820
80. Schwartz O, Albert ML. 2010. Biology and pathogenesis of chikungunya virus. *Nat. Rev. Microbiol.* 8:491–500
81. Rose PP, Hanna SL, Spiridigliozzi A, Wannissorn N, Beiting DP, et al. 2011. Natural resistance-associated macrophage protein is a cellular receptor for Sindbis virus in both insect and mammalian hosts. *Cell Host Microbe* 10:97–104
82. Whitton JL, Cornell CT, Feuer R. 2005. Host and virus determinants of picornavirus pathogenesis and tropism. *Nat. Rev. Microbiol.* 3:765–76
83. Cherry S, Doukas T, Armknecht S, Whelan S, Wang H, et al. 2005. Genome-wide RNAi screen reveals a specific sensitivity of IRES-containing RNA viruses to host translation inhibition. *Genes Dev.* 19:445–52
84. Huang JY, Su WC, Jeng KS, Chang TH, Lai MM. 2012. Attenuation of 40S ribosomal subunit abundance differentially affects host and HCV translation and suppresses HCV replication. *PLOS Pathog.* 8:e1002766
85. Majzoub K, Hafirassou ML, Meignin C, Goto A, Marzi S, et al. 2014. RACK1 controls IRES-mediated translation of viruses. *Cell* 159:1086–95
86. Coyne CB, Bozym R, Morosky SA, Hanna SL, Mukherjee A, et al. 2011. Comparative RNAi screening reveals host factors involved in enterovirus infection of polarized endothelial monolayers. *Cell Host Microbe* 9:70–82
87. Swanson JA. 2008. Shaping cups into phagosomes and macropinosomes. *Nat. Rev. Mol. Cell Biol.* 9:639–49
88. Bengali Z, Satheshkumar PS, Yang Z, Weisberg AS, Paran N, Moss B. 2011. Drosophila S2 cells are non-permissive for vaccinia virus DNA replication following entry via low pH-dependent endocytosis and early transcription. *PLOS ONE* 6:e17248
89. Mercer J, Helenius A. 2008. Vaccinia virus uses macropinocytosis and apoptotic mimicry to enter host cells. *Science* 320:531–35
90. Mercer J, Snijder B, Sacher R, Burkard C, Bleck CK, et al. 2012. RNAi screening reveals proteasome- and Cullin3-dependent stages in vaccinia virus infection. *Cell Rep.* 2:1036–47
91. Sivan G, Martin SE, Myers TG, Buehler E, Szymczyk KH, et al. 2013. Human genome-wide RNAi screen reveals a role for nuclear pore proteins in poxvirus morphogenesis. *PNAS* 110:3519–24
92. Blasco R, Sisler JR, Moss B. 1993. Dissociation of progeny vaccinia virus from the cell membrane is regulated by a viral envelope glycoprotein: effect of a point mutation in the lectin homology domain of the A34R gene. *J. Virol.* 67:3319–25
93. Hruby DE, Guarino LA, Kates JR. 1979. Vaccinia virus replication. I. Requirement for the host-cell nucleus. *J. Virol.* 29:705–15
94. Filone CM, Caballero IS, Dower K, Mendillo ML, Cowley GS, et al. 2014. The master regulator of the cellular stress response (HSF1) is critical for orthopoxvirus infection. *PLOS Pathog.* 10:e1003904
95. Kilcher S, Schmidt FI, Schneider C, Kopf M, Helenius A, Mercer J. 2014. siRNA screen of early poxvirus genes identifies the AAA+ ATPase D5 as the virus genome-uncoating factor. *Cell Host Microbe* 15:103–12
96. Moody CA, Laimins LA. 2010. Human papillomavirus oncoproteins: pathways to transformation. *Nat. Rev. Cancer* 10:550–60
97. Aydin I, Weber S, Snijder B, Samperio Ventayol P, Kuhbacher A, et al. 2014. Large scale RNAi reveals the requirement of nuclear envelope breakdown for nuclear import of human papillomaviruses. *PLOS Pathog.* 10:e1004162
98. Lipovsky A, Popa A, Pimienta G, Wyler M, Bhan A, et al. 2013. Genome-wide siRNA screen identifies the retromer as a cellular entry factor for human papillomavirus. *PNAS* 110:7452–57
99. Davis ZH, Verschueren E, Jang GM, Kleffman K, Johnson JR, et al. 2015. Global mapping of herpesvirus-host protein complexes reveals a transcription strategy for late genes. *Mol. Cell* 57:349–60

100. Schneider WM, Chevillotte MD, Rice CM. 2014. Interferon-stimulated genes: a complex web of host defenses. *Annu. Rev. Immunol.* 32:513–45
101. Zhang Y, Burke CW, Ryman KD, Klimstra WB. 2007. Identification and characterization of interferon-induced proteins that inhibit alphavirus replication. *J. Virol.* 81:11246–55
102. Li J, Ding SC, Cho H, Chung BC, Gale M Jr, et al. 2013. A short hairpin RNA screen of interferon-stimulated genes identifies a novel negative regulator of the cellular antiviral response. *mBio* 4:e00385-13
103. Zhao H, Lin W, Kumthip K, Cheng D, Fusco DN, et al. 2012. A functional genomic screen reveals novel host genes that mediate interferon-alpha's effects against hepatitis C virus. *J. Hepatol.* 56:326–33
104. Varble A, Benitez AA, Schmid S, Sachs D, Shim JV, et al. 2013. An in vivo RNAi screening approach to identify host determinants of virus replication. *Cell Host Microbe* 14:346–56
105. Metz P, Dazert E, Ruggieri A, Mazur J, Kaderali L, et al. 2012. Identification of type I and type II interferon-induced effectors controlling hepatitis C virus replication. *Hepatology* 56:2082–93
106. Lenschow DJ, Giannakopoulos NV, Gunn LJ, Johnston C, O'Guin AK, et al. 2005. Identification of interferon-stimulated gene 15 as an antiviral molecule during Sindbis virus infection in vivo. *J. Virol.* 79:13974–83
107. Jiang D, Guo H, Xu C, Chang J, Gu B, et al. 2008. Identification of three interferon-inducible cellular enzymes that inhibit the replication of hepatitis C virus. *J. Virol.* 82:1665–78
108. Jiang D, Weidner JM, Qing M, Pan XB, Guo H, et al. 2010. Identification of five interferon-induced cellular proteins that inhibit West Nile virus and dengue virus infections. *J. Virol.* 84:8332–41
109. Itsui Y, Sakamoto N, Kurosaki M, Kanazawa N, Tanabe Y, et al. 2006. Expressional screening of interferon-stimulated genes for antiviral activity against hepatitis C virus replication. *J. Viral Hepat.* 13:690–700
110. Schoggins JW, Wilson SJ, Panis M, Murphy MY, Jones CT, et al. 2011. A diverse range of gene products are effectors of the type I interferon antiviral response. *Nature* 472:481–85
111. Liu SY, Sanchez DJ, Aliyari R, Lu S, Cheng G. 2012. Systematic identification of type I and type II interferon-induced antiviral factors. *PNAS* 109:4239–44
112. Schoggins JW, MacDuff DA, Imanaka N, Gainey MD, Shrestha B, et al. 2014. Pan-viral specificity of IFN-induced genes reveals new roles for cGAS in innate immunity. *Nature* 505:691–95
113. Ooi EL, Chan ST, Cho NE, Wilkins C, Woodward J, et al. 2014. Novel antiviral host factor, TNK1, regulates IFN signaling through serine phosphorylation of STAT1. *PNAS* 111:1909–14
114. Schoggins JW, Dorner M, Feulner M, Imanaka N, Murphy MY, et al. 2012. Dengue reporter viruses reveal viral dynamics in interferon receptor-deficient mice and sensitivity to interferon effectors in vitro. *PNAS* 109:14610–15
115. Karki S, Li MM, Schoggins JW, Tian S, Rice CM, MacDonald MR. 2012. Multiple interferon stimulated genes synergize with the zinc finger antiviral protein to mediate anti-alphavirus activity. *PLOS ONE* 7:e37398
116. Sen GC, Sarkar SN. 2007. The interferon-stimulated genes: targets of direct signaling by interferons, double-stranded RNA, and viruses. *Curr. Top. Microbiol. Immunol.* 316:233–50
117. Schoggins JW. 2014. Interferon-stimulated genes: roles in viral pathogenesis. *Curr. Opin. Virol.* 6:40–46
118. Randall RE, Goodbourn S. 2008. Interferons and viruses: an interplay between induction, signalling, antiviral responses and virus countermeasures. *J. Gen. Virol.* 89:1–47
119. Schoggins JW, Rice CM. 2011. Interferon-stimulated genes and their antiviral effector functions. *Curr. Opin. Virol.* 1:519–25
120. Iwasaki A, Pillai PS. 2014. Innate immunity to influenza virus infection. *Nat. Rev. Immunol.* 14:315–28
121. Horner SM, Gale M Jr. 2013. Regulation of hepatic innate immunity by hepatitis C virus. *Nat. Med.* 19:879–88
122. Metz P, Reuter A, Bender S, Bartenschlager R. 2013. Interferon-stimulated genes and their role in controlling hepatitis C virus. *J. Hepatol.* 59:1331–41
123. Suthar MS, Aguirre S, Fernandez-Sesma A. 2013. Innate immune sensing of flaviviruses. *PLOS Pathog.* 9:e1003541
124. Rathinam VA, Fitzgerald KA. 2011. Innate immune sensing of DNA viruses. *Virology* 411:153–62
125. Sharma S, Fitzgerald KA. 2011. Innate immune sensing of DNA. *PLOS Pathog.* 7:e1001310

126. Chevrier N, Mertins P, Artyomov MN, Shalek AK, Iannacone M, et al. 2011. Systematic discovery of TLR signaling components delineates viral-sensing circuits. *Cell* 147:853–67
127. Amit I, Garber M, Chevrier N, Leite AP, Donner Y, et al. 2009. Unbiased reconstruction of a mammalian transcriptional network mediating pathogen responses. *Science* 326:257–63
128. Lee MN, Roy M, Ong SE, Mertins P, Villani AC, et al. 2013. Identification of regulators of the innate immune response to cytosolic DNA and retroviral infection by an integrative approach. *Nat. Immunol.* 14:179–85
129. Bürckstümmer T, Baumann C, Bluml S, Dixit E, Dürnberger G, et al. 2009. An orthogonal proteomic-genomic screen identifies AIM2 as a cytoplasmic DNA sensor for the inflammasome. *Nat. Immunol.* 10:266–72
130. Tanaka Y, Chen ZJ. 2012. STING specifies IRF3 phosphorylation by TBK1 in the cytosolic DNA signaling pathway. *Sci. Signal.* 5:ra20
131. Tsuchida T, Zou J, Saitoh T, Kumar H, Abe T, et al. 2010. The ubiquitin ligase TRIM56 regulates innate immune responses to intracellular double-stranded DNA. *Immunity* 33:765–76
132. Beiting DP, Hidano S, Baggs JE, Geskes JM, Fang Q, et al. 2015. The orphan nuclear receptor TLX is an enhancer of STAT1-mediated transcription and immunity to *Toxoplasma gondii*. *PLOS Biol.* 13:e1002200
133. Lancaster KZ, Pfeiffer JK. 2010. Limited trafficking of a neurotropic virus through inefficient retrograde axonal transport and the type I interferon response. *PLOS Pathog.* 6:e1000791
134. Sabin LR, Zhou R, Gruber JJ, Lukinova N, Bambina S, et al. 2009. Ars2 regulates both miRNA- and siRNA-dependent silencing and suppresses RNA virus infection in *Drosophila*. *Cell* 138:340–51
135. Gruber JJ, Zatechka DS, Sabin LR, Yong J, Lum JJ, et al. 2009. Ars2 links the nuclear cap-binding complex to RNA interference and cell proliferation. *Cell* 138:328–39
136. Hopkins KC, Tartell MA, Herrmann C, Hackett BA, Taschuk F, et al. 2015. Virus-induced translational arrest through 4EBP1/2-dependent decay of 5′-TOP mRNAs restricts viral infection. *PNAS* 112:E2920–29
137. Zhang Z, Yuan B, Bao M, Lu N, Kim T, Liu YJ. 2011. The helicase DDX41 senses intracellular DNA mediated by the adaptor STING in dendritic cells. *Nat. Immunol.* 12:959–65
138. Zhang Z, Kim T, Bao M, Facchinetti V, Jung SY, et al. 2011. DDX1, DDX21, and DHX36 helicases form a complex with the adaptor molecule TRIF to sense dsRNA in dendritic cells. *Immunity* 34:866–78
139. Miyashita M, Oshiumi H, Matsumoto M, Seya T. 2011. DDX60, a DEXD/H box helicase, is a novel antiviral factor promoting RIG-I-like receptor-mediated signaling. *Mol. Cell. Biol.* 31:3802–19
140. Moy RH, Cole BS, Yasunaga A, Gold B, Shankarling G, et al. 2014. Stem-loop recognition by DDX17 facilitates miRNA processing and antiviral defense. *Cell* 158:764–77
141. Poenisch M, Metz P, Blankenburg H, Ruggieri A, Lee JY, et al. 2015. Identification of HNRNPK as regulator of hepatitis C virus particle production. *PLOS Pathog.* 11:e1004573
142. Gilsdorf M, Horn T, Arziman Z, Pelz O, Kiner E, Boutros M. 2010. GenomeRNAi: a database for cell-based RNAi phenotypes. 2009 update. *Nucleic Acids Res.* 38:D448–52
143. Ramo P, Drewek A, Arrieumerlou C, Beerenwinkel N, Ben-Tekaya H, et al. 2014. Simultaneous analysis of large-scale RNAi screens for pathogen entry. *BMC Genomics* 15:1162
144. Zhu J, Davoli T, Perriera JM, Chin CR, Gaiha GD, et al. 2014. Comprehensive identification of host modulators of HIV-1 replication using multiple orthologous RNAi reagents. *Cell Rep.* 9:752–66
145. Luo B, Cheung HW, Subramanian A, Sharifnia T, Okamoto M, et al. 2008. Highly parallel identification of essential genes in cancer cells. *PNAS* 105:20380–85
146. Bürckstümmer T, Banning C, Hainzl P, Schobesberger R, Kerzendorfer C, et al. 2013. A reversible gene trap collection empowers haploid genetics in human cells. *Nat. Methods* 10:965–71
147. Hsu PD, Lander ES, Zhang F. 2014. Development and applications of CRISPR-Cas9 for genome engineering. *Cell* 157:1262–78
148. Sander JD, Joung JK. 2014. CRISPR-Cas systems for editing, regulating and targeting genomes. *Nat. Biotechnol.* 32:347–55
149. Doudna JA, Charpentier E. 2014. The new frontier of genome engineering with CRISPR-Cas9. *Science* 346:1258096
150. Shalem O, Sanjana NE, Hartenian E, Shi X, Scott DA, et al. 2014. Genome-scale CRISPR-Cas9 knockout screening in human cells. *Science* 343:84–87

151. Yang X, Boehm JS, Yang X, Salehi-Ashtiani K, Hao T, et al. 2011. A public genome-scale lentiviral expression library of human ORFs. *Nat. Methods* 8:659–61
152. Wang Z, Gerstein M, Snyder M. 2009. RNA-Seq: a revolutionary tool for transcriptomics. *Nat. Rev. Genet.* 10:57–63
153. Greco TM, Diner BA, Cristea IM. 2014. The impact of mass spectrometry–based proteomics on fundamental discoveries in virology. *Annu. Rev. Virol.* 1:581–604
154. Vinayavekhin N, Saghatelian A. 2010. Untargeted metabolomics. *Curr. Protoc. Mol. Biol.* 90:30.1.1–30.1.24
155. Davila S, Hibberd ML. 2009. Genome-wide association studies are coming for human infectious diseases. *Genome Med.* 1:19
156. Bamshad MJ, Ng SB, Bigham AW, Tabor HK, Emond MJ, et al. 2011. Exome sequencing as a tool for Mendelian disease gene discovery. *Nat. Rev. Genet.* 12:745–55