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## Review

## Virus-host protein interactions in RNA viruses

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## Abstract

RNA viruses exhibit small-sized genomes that only encode a limited number of viral proteins, but still establish complex networks of interactions with host cell components. Here we summarize recent reports that aim at understanding general features of RNA virus infection networks at the protein level.

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**Keywords:** RNA virus infections; Protein interaction mapping; Host–pathogen interactions

## 1. Introduction

RNA viruses are responsible for numerous diseases in human, animals and plants. They represent a major public health problem since RNA viruses like influenza virus, measles virus, yellow fever virus or hepatitis C virus (HCV) are responsible for tens of thousands of human death every year. However, pathologies induced by this class of viruses are highly variable in terms of symptoms, morbidity and mortality rates, depending on virus species and infected host. By definition, an RNA virus is a virus that has ribonucleic acid (RNA) as its genetic material. The genome of RNA viruses is composed of one or several segments that can be either single-stranded RNA (ssRNA) or double-stranded RNA molecules (dsRNA). Retroviruses also have a single-stranded RNA genome but are generally not considered as RNA viruses because they use DNA intermediates to replicate. ssRNA viruses can be further classified into positive-sense, negative-sense, or ambisense RNA viruses. Whereas positive-sense RNA genomes can be immediately translated by the host cell into viral proteins like cellular mRNA, negative-sense RNA genomes must be transcribed into positive-sense RNA

molecules before translation. Because of their clinical and economical impact, RNA viruses have always been a major field of interest for biomedical research. Thanks to information accumulated over the last decades, we now have access to rather detailed models of RNA virus structures, replication machineries, and pathophysiology. However, to reach a system view of RNA virus replication cycle in host cells and organisms, more functional analyses are necessary, especially in the field of virus-host interactions. Like other viruses, RNA viruses critically need to interact with host factors to hijack the cellular machinery and inhibit host defense mechanisms. However, and in spite of significant efforts from virology laboratories, our understanding of virus-host interactions at the molecular scale remains in its infancy. In VirHostNet, a public database where protein–protein interactions are collected from literature, only 830 virus-host interactions are reported for RNA viruses [1]. This figure is extremely low when considering the 1335 RNA virus species currently referenced in the taxonomy database of the National Center for Biotechnology Information [2]. This suggests that to produce comprehensive virus-host protein interaction maps, high-throughput technologies must be applied like yeast two-hybrid or protein complex analysis by mass spectrometry [3]. Before we summarize recent advances in this field together with future perspectives, specific characteristics of RNA viruses need to be discussed. Indeed, the RNA nature of their genetic material determines some structural features of RNA viruses,

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functional properties of their proteins, and ultimately how they interact with their host.

## 2. Are RNA virus proteins different?

### 2.1. RNA viruses have a high mutation rate

A key feature of RNA viruses is the high mutation rate of their polymerase. Because they don't have proofreading capability, RNA-dependent RNA polymerases have an error rate several logs higher than viral DNA polymerases [4]. For most RNA viruses, overall rates of nucleotide mutations per site per replication fall in the range of  $10^{-3}$  to  $10^{-5}$ . Consequently, any individual virus particle will contain an average of one or more mutations from the consensus wild-type sequence for that virus species [5]. A first consequence is that an RNA virus population is not genetically homogeneous, but represents a swarm of mutants clustered around a consensus sequence often referred as quasispecies. This provides a source of phenotypic variants that can be selected, so that a virus consensus sequence can rapidly change depending on environmental pressure. This genetic flexibility provides a great evolutionary advantage to RNA viruses, but often complicates the analysis of virus-host interactions. Indeed, RNA viruses that are grown *in vitro* can quickly adapt to culture conditions, and eventually develop phenotypic characteristics that are different from primary isolates. As a consequence, viral proteins from laboratory-adapted strains can either lose or gain interactions with cellular factors because selection pressure is different *in vitro* and *in vivo*. A good example is provided by vaccine strains of measles virus that use CD46 receptor to enter the cells whereas most wild-type viruses target CD150/SLAM [6]. This illustrates the critical need to perform experiments with RNA virus strains that have been carefully characterized both genetically and phenotypically. In general, wild-type virus strains corresponding to primary isolates will be preferred to culture-adapted laboratory strains since mutations accumulated *in vitro* can significantly alter virus-host interaction profiles.

### 2.2. RNA viruses need a small number of multifunctional proteins

RNA viruses have to pay the price for this rapid rate of evolution, which is the limited size of their genome. The combination of mutation rate and genome size defines the hypothetical threshold of error catastrophe above which a virus cannot maintain its genomic integrity. As a result, RNA virus genomes are small (<30 Kb) and usually encode for only a dozen of proteins [5]. As a consequence, and in spite of a few noticeable exceptions like HCV, RNA viruses are in general responsible for acute rather than chronic infections. This "bite-and-run" strategy is thought to relate to the fact that, when compared to large DNA viruses associated with chronic infections, RNA virus genomes are too small to encode the large arsenal of virulence factors required to switch their replication cycle into a latent stage and control the host

immune response on the long term. Indeed, strategies developed by RNA and DNA viruses to hijack the host machinery and control the antiviral response are very different. All along their evolution, DNA viruses have captured and integrated in their genome large DNA sequences from their host that encode complex functional domains of eukaryotic origin. This tinkering strategy allows DNA viruses to finely tune the metabolism of infected cells in order to control their own replication. In contrast, RNA virus proteins only have distant or no homologies with their eukaryotic counterparts (although catalytic sites of enzymatic domains can be similar between RNA virus and eukaryotic proteins). This suggests that RNA viruses have evolved a different strategy, and interact with the host by using "homemade" protein-binding motifs.

Since RNA viruses only have a limited set of proteins to disable the host immune system, replicate and spread, their proteins need to be multifunctional. In particular, RNA virus proteins are expected to have on average more binding partners than normally expected for host proteins of the same size. Recently, both the human protein-protein interaction network and the influenza A virus infection network were probed by yeast two-hybrid using the same technological platform [7,8]. Influenza virus proteins were found to have on average twice more cellular partners than host proteins within the human interaction network. This higher connectivity implies that RNA viruses should encode proteins with more but smaller binding interfaces to be able to hit multiple cellular targets. Although statistical arguments are still missing, we will see that empirical observations accumulate in the literature to support this statement. In particular, several RNA virus proteins have been shown to contain multiple linear motifs of a few amino acids to establish interactions with many different cellular partners. Another consequence of size constraints applied on RNA virus genomes is that RNA virus proteins involved in the core replication machinery or the particle itself often perform different tasks in addition to their nominal function. Literature now provides many examples where viral proteins are shown to "moonlight", performing multiple unrelated jobs during virus replication cycle. A good example of such multi-tasking viral proteins is provided by the phosphoprotein P of rabies virus, which is both a key component of the RNA-dependent RNA polymerase complex and a virulence factor essential to block the innate immune response [9]. Thus, RNA virus proteins need both a high tolerance to RNA polymerase-induced mutations and the ability to interact with many different partners. Interestingly, RNA virus proteins have evolved unique biophysical features to fulfill these requirements.

### 2.3. Loose packaging and disordered regions in RNA virus proteins

Recent analyses suggest that viral proteins, and RNA virus proteins in particular, exhibit loosely packed domains [10] and higher rate of intrinsically disordered regions [11]. In loosely packed domains, the energy gap between the native state and the unfolded conformation is very low.

Intrinsically disordered segments correspond to protein regions that exist in a natively unfolded state, and function without a prerequisite to form stably folded structures [12]. Several programs are now available to predict the disordered regions of a protein [13,14]. These two features of RNA virus proteins, i.e. loose packaging and disordered regions, could represent a unique strategy for buffering the deleterious effects of mutations introduced by their low-fidelity RNA-dependent RNA polymerases [10]. Interestingly, intrinsic disorder is also a common feature of highly connected proteins or hubs in protein–protein interaction networks, or interactomes [15]. Indeed, intrinsically disordered regions are much more exposed to their environment than protein segments buried in the core of globular domains, and were found to contain small linear motifs (sometimes referred as MoRFs for Molecular Recognition Features) that play a critical role in protein–protein interactions (Fig. 1). The ELM resource provides a comprehensive database of currently known linear motifs in eukaryotic organisms [16]. In RNA virus proteins, disordered regions were also found to contain linear motifs that contribute to both virus–virus and virus–host protein interactions [17]. These motifs are essential to the dynamic of protein complexes forming the virus replication machinery [18–21]. Viral proteins have evolved specific linear motifs to interact with either viral or host factors, but they can use motif mimicry for hooking specific domains of cellular proteins. For example, proline-rich motifs within disordered regions of viral proteins allow them to interact with a swarm of cellular factors containing an SH3 domain [22]. Because linear motifs undergo a disorder-to-order transition upon binding to a structured domain, they can adopt specific folds

when binding to different partners [15]. In addition and because of their small size, several of these motifs can accumulate in the disordered region of a viral protein. Altogether, this strongly argues that increased structural flexibility provides RNA virus proteins with an effective way to interact with a multiplicity of host cell components and moonlight between different functions. However, this flexibility could translate into a loss of specificity, leading viral proteins to interact with several cellular proteins in addition to their nominal target. These “bystander” interactions might be critical in the development of symptoms and pathological traits associated with a viral infection.

#### 2.4. Estimating the size of an RNA virus infection network

Before we discuss interaction data available in literature, an important question is to estimate the average number of host proteins targeted by an RNA virus during infection. In a recent paper, the 22 500 proteins encoded by the human genome (disregarding upper complexity levels introduced by splice variants) were estimated to form a complex network composed of 130 000 different interactions, suggesting that a human protein has on average 6 binding partners [23]. Of course, each protein will not bind all its partners simultaneously. First, each protein is expressed in multiple copies in a cell, and different subsets of this protein population can ensure interactions with different partners. In addition, interactions can occur sequentially in time when specific conditions are fulfilled (like phosphorylation, ubiquitination or translocation events), and this brings a dynamic dimension to the system. Thus, if we consider that the total number of protein partners is the same for viral and cellular proteins, an RNA virus encoding a dozen of proteins could interact with 60–80 host proteins during infection. However, this is probably an underestimation that does not take into account specific biophysical features of RNA virus proteins that have been discussed above. Although scarce, experimental data seem to corroborate this statement. For example, HCV infection network has been investigated in details using the yeast two-hybrid system [24]. In this publication, de Chassey et al. report 311 novel HCV–host interactions identified with this technology and a literature-curated set of 170 interactions. Interestingly, it was found that NS3, NS5A and CORE are the most connected HCV proteins in this virus–host interactomes with 214, 96 and 76 cellular partners, respectively [24]. In agreement with their high connectivity, they also are the only HCV proteins predicted to contain at least one intrinsically disordered region. Similarly, the ten proteins from influenza A virus were found by Shapira et al. to interact with 87 human proteins through 135 pairwise interactions [8]. These estimations are also corroborated by another publication in which the interactions of rice yellow mottle virus (RYMV) with host proteins were determined by gel exclusion chromatography of virus–host protein complexes, separation on SDS-PAGE, and nano-liquid chromatography combined with tandem mass spectrometry [25]. In contrast to the yeast two-hybrid system that exclusively detects binary

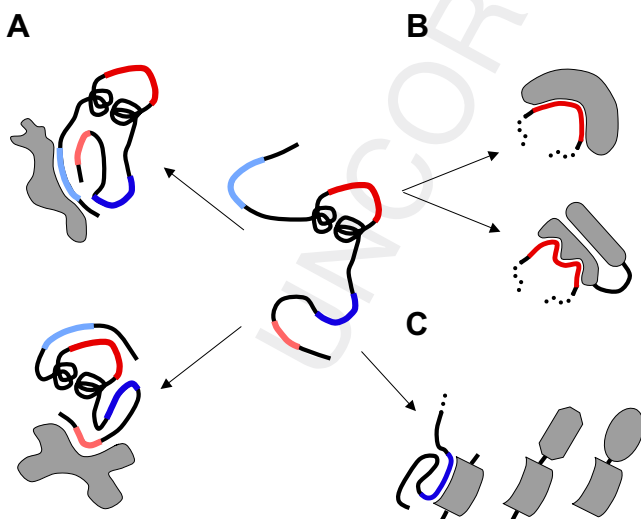


Fig. 1. Intrinsically disordered regions allow viral proteins to bind many partners. Intrinsically disordered proteins can adopt different fold upon binding to different partners (A), and small linear motifs within disordered regions can adopt different fold to interact with different partners (B). Finally, small linear motifs that target a conserved domain allow viral proteins to interact with multiple cellular proteins carrying this domain (C).



interactions, both direct and indirect interactions are detected with this approach. RYMV that encodes for only 5 different proteins was found to interact with 223 different rice proteins. Although this is surprisingly high, all these results suggest that an RNA virus interacts with few dozens to few hundreds of different cellular proteins when infecting cells. Whether this is similar or significantly different from the infection networks of DNA viruses remains to be determined.

### 3. Topological and functional features of host proteins bound by RNA viruses

#### 3.1. Databases of virus-host protein interactions

To perform system level analyses, large amounts of virus-host protein interactions data must be readily accessible. As a first effort to collect this information, some groups have established databases like VirHostNet, VirusMINT or more recently PIG, where virus-host protein interactions retrieved from literature using semi-automated procedures are stored in a standardized format [1,26,27]. Because VirHostNet interface allows users to apply taxonomic filters, we used this functionality to download interactions for RNA viruses but excluding DNA or retroviruses. As mentioned in the Introduction section, only 830 distinct interactions were obtained. VirHostNet database is still a work in progress and some data from literature have not been registered yet, but this small figure essentially highlights our lack of information regarding virus-host protein interactions for RNA viruses. The situation is even more critical when considering that 444 and 200 of these interactions involve HCV and influenza A virus proteins, respectively, whereas the remaining 186 interactions are scattered between 46 different RNA viruses. HCV and influenza A virus represent major public health problem for developed countries, and the strong bias for these two viruses in VirHostNet only reflects the intensity of research efforts dedicated to these pathogens. Thus, the small number of interaction data available in literature and databases on RNA viruses appears to be the main limiting factor to perform system level analyses of virus-host protein interactions. A second limiting factor is the critical lack of information on the dynamic of these interactions in infected cells, and this will require the development of new technologies allowing the *in vivo* imaging of protein interactions. Altogether, this strongly supports the need for global initiatives in order to map virus-host protein interactions in RNA viruses using high-throughput technologies and dynamic imaging systems.

#### 3.2. Topological characteristics of host proteins targeted by RNA viruses

In spite of this major hurdle, it has been possible to extract some general information from currently available datasets. In particular, it has been asked if viral proteins tend to target host proteins with specific properties. Only few publications have tackled these questions for DNA or RNA viruses. In their

seminal work, Calderwood et al. have shown that proteins from Epstein–Barr virus tend to interact preferentially with hub and bottleneck proteins in the human interactome network [28]. Similar results were obtained when performing the analysis on virus-host interaction data retrieved from literature [22,29]. Hubs are defined as proteins with the highest number of direct partners (or degree) within the interactome. In contrast, bottlenecks do not necessarily have a high degree, but correspond to proteins that are central to many shortest paths in the interactome and therefore exhibit a high betweenness centrality coefficient. As a consequence, such proteins tend to connect different subregions of the interactome network. In their study, de Chassey et al. have found that HCV proteins also target host proteins that correspond to hubs and/or bottleneck proteins in the human interactome network [24]. More recently, similar observations were performed on influenza A virus. Interestingly, this work also highlighted the fact that sometimes, several proteins of a virus target the same cellular factor and this may be required for the formation of virus-host multiprotein complexes [8]. Altogether, these results suggest that preferential attachment on essential host proteins is a general hallmark of viral proteins, including RNA virus proteins.

#### 3.3. Biological functions of host proteins targeted by RNA viruses

It is also possible to determine if host proteins targeted by RNA viruses are enriched for specific cellular functions. Using their HCV infection network and KEGG functional annotation, de Chassey et al. have found that the set of human proteins interacting with this virus is highly enriched for signaling pathway components, cell adhesion to the extracellular matrix and cell–cell contacts [24]. Interestingly, HCV proteins and CORE protein in particular were shown to multiply connections with three cellular pathways (insulin, TGF- $\beta$  and Jak-STAT pathways) that are associated with HCV clinical syndromes. It was also found that NS3 and NS5A proteins of HCV interact with cellular proteins involved in focal adhesion and perturb cell adhesion to fibronectin. This mechanism identified by systematic mapping of HCV-host interactions could have consequences both on virus spreading and tumorigenesis. Shapira et al. also analyzed their influenza virus infection network using the same approach, and found an enrichment for 6 signaling pathways: p53-, PML-, TNFR/Fas-mediated apoptosis, NF- $\kappa$ B, WNT/ $\beta$ -catenin, and MAPK [8]. In the future, when more information relative to RNA virus interactions with host proteins will be available, this type of analysis should facilitate the identification of cellular functions that represent prominent targets of this virus class. In particular, it will be interesting to determine if substantial differences can be observed when compared with DNA viruses and retroviruses. Until then, one can determine if specific biological functions are enriched among already known targets of RNA viruses, but it is essential to keep in mind that a lot more information is needed to be more exhaustive and truly conclusive.

## 4. An overview of RNA virus most frequent cellular targets

### 4.1. Interactions with components of the host antiviral response

Fig. 2 is showing a map composed of 169 virus-host protein interactions retrieved from VirHostNet. Only host proteins that are targeted by at least two different RNA viruses are displayed. This figure highlights a set of cellular proteins that represent, on the basis of our current knowledge, most frequent targets of RNA viruses. Interestingly, this set of host proteins is enriched for key components of the innate antiviral response including IFIH1, JAK1, STAT1, STAT2 and Mx1. It is now well established that the innate immune system is initiated by the recognition of danger molecular motifs called Pathogen Associated Molecular Patterns (PAMPs) by different class of Pathogen Recognition Receptors (PRRs). Among well-characterized PAMPs are different molecular motifs that RNA viruses produce during their replication cycle, such as dsRNA or uncapped ssRNA molecules with a 5'-triphosphate [30]. Upon replication of their genome, RNA viruses generate replication intermediates that correspond to dsRNA molecules. Although transient and usually buried inside the virus replication complex, it is usually accepted that minimal amounts of dsRNA molecules become exposed when RNA viruses make mistake during replication. In addition, the synthesis of RNA molecules by RNA polymerases is initiated with a 5'-triphosphate nucleotide, and newly synthesized RNA molecules exhibit this molecular motif at their 5' extremity. Because cellular RNAs are rapidly processed to remove this 5'-triphosphate or hide it into either a cap structure or a ribonucleoprotein complex, exposed 5'-triphosphate ssRNA are rather uncommon in the cytoplasm and represent a “danger signal” for the cell. Indeed, the genome, the antigenome or specific transcripts from RNA viruses often correspond to 5'-triphosphate ssRNA molecules that can be detected by PRRs. Thus, the RNA nature of their genetic material determines the production of molecular structures that will make RNA viruses detectable by the antiviral immune system. PRRs that recognize illegitimate RNA molecules can be classified in two groups: toll-like receptors (TLRs) and RIG-I like receptors (RLRs). TLRs are transmembrane receptors, and only three members of this family have been reported to detect RNA molecules with their extracellular domain: TLR3 that binds dsRNA and TLR7/8 that are activated by G/U rich ssRNA. In contrast, RIG-I and IFIH1 that belong to the RLR family are cytosolic sensors that detect intracellular 5'-triphosphate ssRNA and dsRNA, respectively. Upon activation by their ligands, TLRs and RLRs initiate signaling cascades that converge on three families of transcription factors (NF- $\kappa$ B, IRF3/7, and ATF-2/Jun) to induce type I IFN (IFN- $\alpha/\beta$ ) expression. Secreted IFN- $\alpha/\beta$  binds to their membrane receptor at the surface of both infected cells and neighboring cells. This activates a Jak/STAT signaling cascade, and results in the induction an antiviral gene cluster encoding a large set of proteins and RNA molecules that interfere with viruses at

virtually all steps of their replication cycle. Components of this antiviral system represent preferential targets of viruses in general as recently assessed by statistical analysis of currently available virus-host interaction data [31], and the same conclusion stands for RNA viruses in particular. As shown in Fig. 2, RNA viruses interact with multiple components of this pathway that correspond both to sensors of viral PAMPs (IFIH1, EIF2AK2) and signal amplification mediators (Jak1, STAT1, STAT2). Altogether, this demonstrates that RNA viruses have evolved a complex arsenal of virulence factors to bind and disable the antiviral system within host cells, and a large fraction of virus-host protein interactions in RNA viruses are devoted to this function.

The V protein of measles virus (MV-V) provides a well-characterized example of the molecular strategy developed by RNA viruses to block the IFN- $\alpha/\beta$  pathway. An original editing strategy encodes this essential virulence factor: one non-templated guanine nucleotide is inserted at a specific position of mRNA molecules transcribed from the gene P of this virus. As a consequence, MV-V is similar to the viral phosphoprotein P (MV-P) in its amino-terminal part (AA 1–231; PNT region) but exhibits a specific cysteine-rich C-terminus that folds into a zinc-binding domain (AA 232–299; VCT region). This viral protein interacts with many factors involved in IFN- $\alpha/\beta$  signaling cascade to block the antiviral innate response (Fig. 3). First, the VCT region has been shown to bind IFIH1 and the related protein LGP2, thereby preventing their activation by dsRNA molecules [32]. Interestingly, the same region of MV-V has also been reported to interact directly with IKK- $\alpha$  and IRF7, two signaling molecules activated downstream of TLR7/8 [33]. MV-V was shown to act as a decoy substrate for IKK- $\alpha$ , and this prevents IRF7 phosphorylation and subsequent IFN- $\alpha$  expression in ssRNA-stimulated plasmacytoid dendritic cells. Altogether, these interactions contribute to the inhibition of RNA sensing by PRRs and IFN- $\alpha/\beta$  expression in the infected host. In addition, MV-V was shown to block signaling downstream IFN- $\alpha/\beta$  receptors through interactions with components of the Jak/STAT pathway. The VCT region was reported to bind STAT2, whereas the PNT region interacts both with STAT1 and JAK1 [34–36]. All three interactions contribute synergistically to the inhibition of IFN- $\alpha/\beta$  signaling in MV-V expressing cells. Interestingly, the PNT region that is shared between MV-V and MV-P is intrinsically disordered [19]. This flexibility apparently allows this region to bind STAT1 and JAK1 when part of MV-V, but also to interact with the nucleoprotein N when expressed in the context of MV-P. How the VCT manages to interact with so many different targets will require more investigations, but the presence of loosely packed regions within the zinc-binding fold is probably essential to adapt different partners.

### 4.2. Interaction of RNA virus proteins with other signaling pathways and cellular components

Functional studies have shown that in addition to IFN- $\alpha/\beta$  signaling, RNA viruses hijack many other pathways when

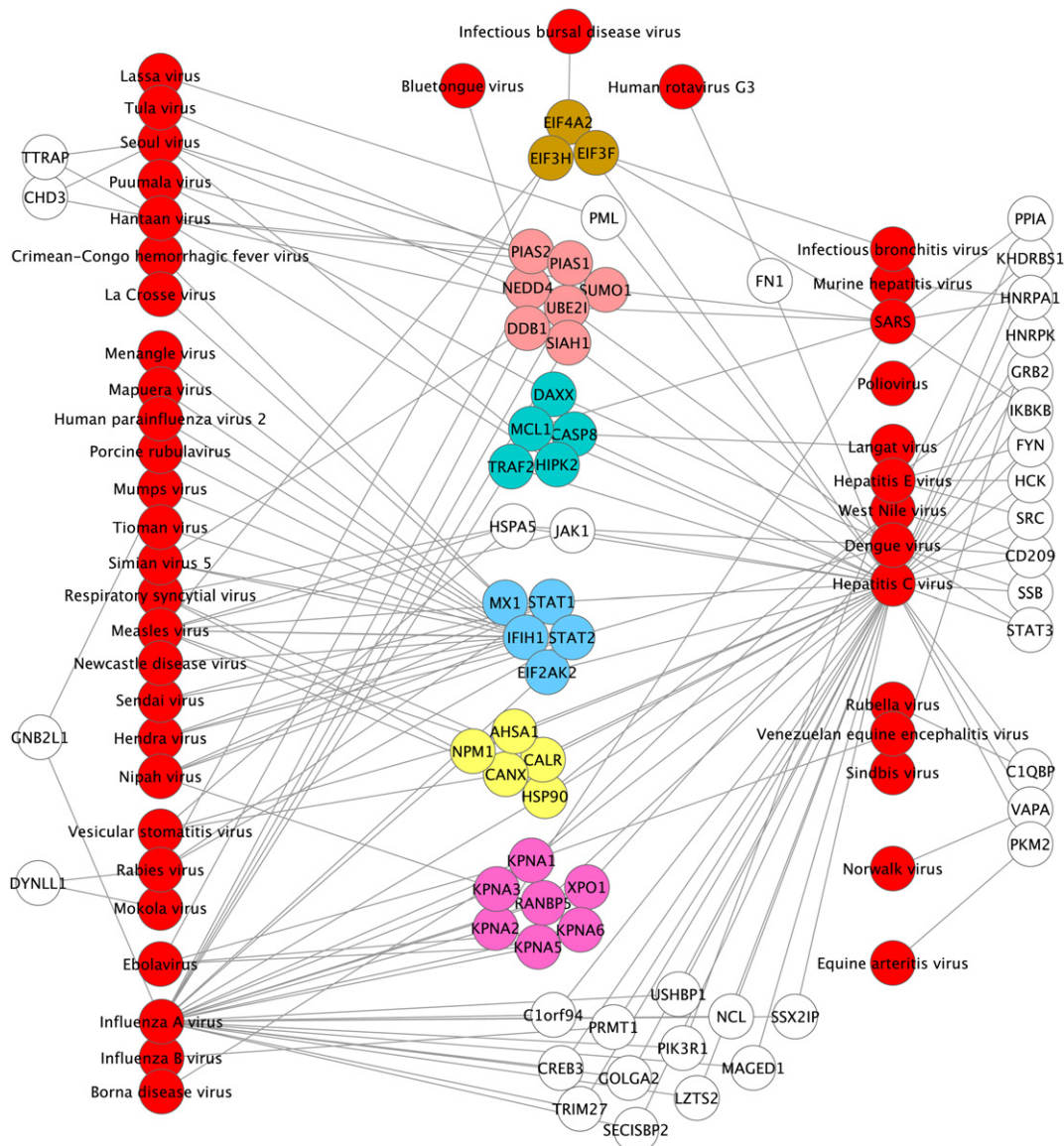


Fig. 2. Host proteins targeted by several RNA viruses. Virus-host protein interactions have been retrieved from VirHostNet using a taxonomic filter to collect all information relative to ssRNA and dsRNA viruses. Negative and positive ssRNA viruses are displayed on the left and right panels, respectively. dsRNA viruses are displayed on top of the figure. Some host proteins have been colored to highlight a specific function: antiviral defense (blue), Ubl conjugation pathway (pink), chaperone (yellow), protein transport (magenta), apoptosis (emerald), initiation factor (light brown). Functional terms correspond to PIR keywords, and were statistically enriched in the interaction map ( $P$ -value  $< 10^{-2}$ ) as determined using DAVID software [59] [For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.].

infecting cells in order to control cell survival, protein translation or cytoplasmic transport for example. At the cellular level, this assessment is usually well supported by an abundant literature. But until now, only a limited set of virus-host protein interactions involved in the control of these pathways has been clearly identified. Several recent reports demonstrate that phosphatidylinositol-3-kinase (PI3K) pathway is an important target of RNA viruses [37]. Both NS5A protein of HCV and NS1 protein of influenza A virus interact with PI3K regulatory subunit p85 (PIK3R1), and their expression alone is sufficient to activate the PI3K/Akt pathway to promote cell survival. Deciphering RNA virus interference with the MEK/ERK signal transduction cascade is also catching the attention

of several research groups [38]. Like the PI3K/Akt pathway, the MEK/ERK pathway promotes cell survival and protein synthesis. Several RNA viruses were found to modulate this pathway by targeting GRB2, an adaptor protein that bridges growth factor receptor tyrosine kinases (RTKs) to MEK/ERK kinases. In our laboratory, we recently found that the C protein of human parainfluenza virus type 3 (hPIV3) interacts with GRB2, and stimulates MEK/ERK signaling [39]. Although enhanced activation of this signaling cascade contributes to hPIV3 replication, this may also increase airway inflammation and can be deleterious for the host. The ORF3 protein of hepatitis E virus needs to interact both with GRB2 and the ERK-specific phosphatase MKP-3 to enhance MEK/ERK



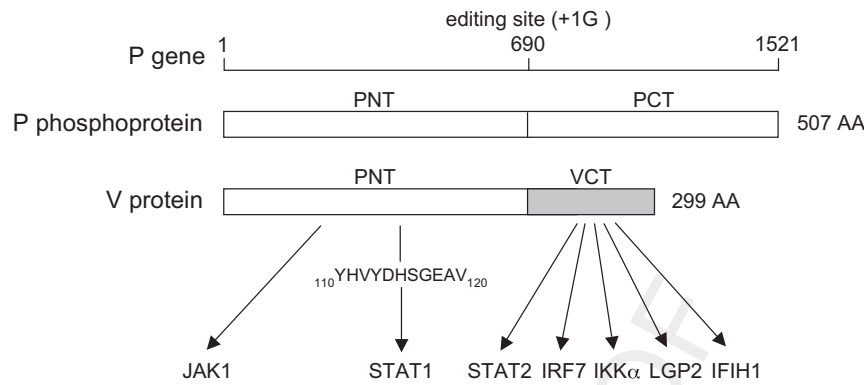


Fig. 3. MV-V, a virulence factor encoded by measles virus, interacts with numerous components of the innate antiviral response. Whereas conventional transcription and translation of the P gene of measles virus leads to the expression of the phosphoprotein P, co-transcriptional insertion of one G residue at the editing site by the virus RNA polymerase allows the expression of MV-V. Thus, MV-V is identical to MV-P in its amino-terminal region (PNT) but exhibits a specific zinger-finger domain in its carboxy-terminal region (VCT). The PNT region interacts with JAK1 but also STAT1, an interaction mediated by a short MV-V peptide of only eleven amino acid residues [34]. The VCT region has also been shown to interact with STAT2, IRF7, IKK- $\alpha$ , LGP2 and IFIH1.

signaling [40,41]. Interestingly, the NS5A protein of HCV was also found to interact with GRB2 and phosphatase 2A, but these interactions both contribute to the inhibition of MEK/ERK signaling in HCV infected cells and may influence viral persistence [42,43]. Activation of MEK/ERK signaling increases the cap-dependent translation of both viral and cellular mRNA, but HCV is cap-independent for its translation and MEK/ERK inhibition was shown to increase its replication [44]. A well-documented example of RNA viruses that directly target the translation machinery of infected cells is provided by picornaviridae, and in particular poliovirus [45]. Two proteases of this virus, 2A and 3C, interact with and cleave eIF4G, a cap-binding protein that is essential to initiate mRNA translation, and the polyadenosine-binding protein (PABP)[46]. Processing of these proteins allows the virus to block host protein expression, whereas translation of viral mRNA, which is cap-independent, remains unaffected.

In addition to virus-host interactions that aim at controlling apoptosis or protein translation in infected cells, RNA viruses have evolved mechanisms to hijack the cytoskeleton and cellular machineries involved in membrane remodeling and vesicular transport. A good example is provided by the large number of RNA viruses that hijack the Endosomal Sorting Complex Required for Transport (ESCRT). After activation, growth factor and cytokine receptors are often internalized and transported into multivesicular endosomes, which are generated by inward budding of intraluminal vesicles. Four protein complexes that are named ESCRT-0, -I, -II and -III are involved in the different steps of this sorting process [47,48]. This membrane-deformation and scission machinery are targeted by numerous RNA viruses for their budding, including Rhabdoviruses, Filoviruses, Arenaviruses and Paramyxoviruses. The matrix proteins of several RNA viruses are able to target the ESCRT machinery since they contain one or several late (L) domains. PT/SAP, YPXL and PPXY are canonical L-domains, and respectively mediate viral protein interactions with ESCRT-associated proteins TSG101, Alix and Nedd4-like E3 ubiquitin ligases. More recently a fourth L-domain (FPIV) was identified

in a paramyxovirus but its binding partner has not been identified yet [49].

Altogether, the few examples presented above provide an overview of cellular pathways that are frequent targets of RNA virus proteins, and illustrate how small linear motifs, like L-domains or the MV-V peptide binding STAT1, are usually sufficient to support these interactions. It also demonstrates that when enough information is available, different RNA viruses are often found to modulate the same pathways, although they can use slightly different mechanisms to reach their target. Our goal with this chapter was not to be exhaustive on virus-host protein interactions in RNA viruses, but to demonstrate the interest of global and integrative approaches to unravel general features of RNA virus biology. In the future, a better understanding of these viruses will require to feed databases with more interaction data, and to compile this information in order to draw more accurate conclusions.

## 5. Future perspectives

### 5.1. High-throughput mapping strategies applied to RNA viruses

Mapping virus-host protein interactions in a systematic way will require the development of different screening systems that can be easily adapted to high-throughput settings. A first step will be to establish large collections of viral ORFs (Open Reading Frames) in a versatile cloning system, a prerequisite to express viral proteins into various screening systems and functional assays [50]. Building such a viral “ORFeome” resource will require a collaborative effort between virology laboratories with the knowledge and access to well-characterized virus strains, both in term of genotype and *in vivo* pathogenicity. This is critical given the propensity of RNA viruses to mutate and quickly evolve upon *in vitro* adaptation, assuming that virus-host interactions can be lost or gained in this process.



To map virus-host protein interactions, high-throughput yeast two-hybrid (HT-Y2H) will be an asset. This screening system is easy to handle, cost efficient and highly sensitive to detect weak or transient protein–protein interactions. Interaction datasets generated with this system are supposedly crippled with artifacts, often referred as false-positive interactions. However, the yeast two-hybrid system has evolved a lot since its inception more than twenty years ago [51]. Recently, it has been well established that interaction datasets generated by HT-Y2H are of high quality with a false-positive rate below 20%, and even more accurate than protein–protein interactions supported by a single publication [23]. Thus, HT-Y2H is suitable to map interactions between RNA virus and host proteomes, and to generate high-quality data.

Nevertheless, a true limitation of the system is that a large fraction of protein–protein interactions is usually missing when performing only one single round of screening [23]. To overcome this limitation, screens can be performed multiple times to reach saturation and against various cDNA libraries to increase coverage of the host proteome. But the most valuable strategy is to combine HT-Y2H with other screening techniques, in particular co-affinity precipitation of virus-host protein complexes followed by mass spectrometry analysis. Tagged viral proteins can be expressed by transient or stable transfection in mammalian cells, and then purified to determine binding partners. Interestingly, reverse genetic systems are available for numerous RNA viruses, and this can be used to generate recombinant viruses carrying tags in fusion with one of their proteins. With such viruses in hands, virus-host protein complexes can be analyzed in infected cells, along virus replication cycle. A proof of concept has been established for influenza A virus, and 37 novel interactors of the viral ribonucleoprotein have been identified by this technique [52]. A major drawback of this approach is that both direct and indirect protein–protein interactions are detected, without any straightforward system to distinguish between the two situations.

The two technologies detailed above are priceless since they allow users to select and identify partners of viral proteins from a pool of cellular proteins. However, it is extremely difficult to reach saturation and obtain a finite list of cellular interactors for a viral protein, even if multiple rounds of HT-Y2H screens are combined with mass spectrometry experiments. As a result, a significant number of protein–protein interactions can be missing in corresponding datasets, and this obviously prevents all attempts to compare the infection networks of different viruses. Thus, when trying to compare two or more viruses for their ability to bind a specific set of cellular preys, interactions must be retested in a one-to-one (or pairwise) setting. This is the only way to demonstrate that a viral protein interacts with a cellular protein, whereas the same viral protein from a different strain or species is unable to do so. The co-affinity purification of two proteins expressed in mammalian cells with distinct tags, followed by a western-blot analysis of the purified complex, has been used for years to test protein–protein interactions. We recently used this approach to demonstrate that the C protein of hPIV3 interacts

with STAT1 and GRB2, whereas the C proteins of Nipah virus and measles virus do not [39]. However, this experimental procedure is labor intensive, and inefficient when testing large matrices of protein–protein pairs. Recently, several methods have been developed to map interactions between protein pairs in a high-throughput setting, including the NAPPA (Nucleic Acid Programmable Protein Array), LUMIER (LUMinescence-based Mammalian IntERactome mapping), MAPPIT (MAMmalian Protein–Protein Interaction Trap), and PCA (Protein Complementation Assay) systems [53]. These technologies can be used to validate interactions identified by HT-Y2H or mass spectrometry in a secondary assay, but also to identify interactions in first instance. Most importantly, interaction maps generated with these technologies that measure interactions in a pairwise setting will be suitable for comparative interactomic approaches where conclusions must be drawn both from interacting and non-interacting virus-host protein pairs (see below). Finally, and because current interactome networks are static, future technologies should aim at monitoring the dynamic of virus-host interactions during virus life cycle.

## 5.2. Comparative interactomics of virus-host protein interactions

Our current arsenal to combat RNA viruses is minimal when compared to the large panel of antibiotics used to fight bacterial infections. A better knowledge of virus-host interactions should greatly help in the design of novel strategies against RNA viruses. For example, antiviral therapies that aim at disrupting specific virus-host protein interactions are being developed against human immunodeficiency virus [54], and the same strategy could be developed against RNA viruses as well. Furthermore, the identification of viral protein regions involved in the binding of host factors could greatly help in the rational design of attenuated virus strains that are defective for these functions, and could be used as potent live vaccines [55]. Although this strategy is still under development, several vaccine strains that have been empirically obtained by *in vitro* passage happened to be defective for some essential virus-host protein interactions. A well-characterized example is measles virus vaccine strain CAM-70 that expresses a mutated form of the V protein that is unable to bind STAT1 and as a consequence, is a poor controller of the innate immune response [56].

Before these therapeutic strategies can be envisioned, a first challenge in the field of RNA virus studies will be to significantly increase our knowledge of virus-host interactions at the molecular level. With such information, it should become possible to define interaction patterns that are either shared or specific among different RNA virus families, genus or species. Such comparisons between virus infection networks, sometimes referred as comparative interactomics [57], should accelerate the identification of signaling pathways, functional modules, and cellular machineries that are essential targets of RNA viruses in general. This will also provide an efficient way to identify sets of interactions that account for specific

symptoms or pathological traits associated with one viral family, one genus, one species or one strain. For example, this approach should be useful to compare the infection networks of wild-type vs. vaccine strains, and could provide molecular basis to their attenuated phenotype. Finally, it would be a great benefit to map the infection network of a virus in different hosts to better understand adaptation phenomena and cross-species transmission. A first example is provided by arboviruses like yellow fever virus or chikungunya virus since they need to replicate both in blood-sucking insects and vertebrates to spread. Are the infection networks of these viruses different in mammalian and insect cells? Comparative interactomic approaches could also be developed to identify virus-host protein interactions that determine the ability of a virus to cross species barriers and adapt to a new host. For example, it has been shown that human parainfluenza virus type 5 (hPIV5), a member of Paramyxoviridae family, can infect several mammalian species but is restricted from efficient replication in mice. The V protein of this virus needs to interact with STAT2 in order to blunt IFN- $\alpha/\beta$  signaling, but fails to do so in mouse cells. Interestingly, transgenic mice that express human STAT2 can be infected with hPIV5 and replicate the virus [58]. This illustrates the role of virus-host protein interactions in host restriction and species barrier phenomenon. As shown here, the compared mapping of virus-host interactions could help to determine the propensity of a virus to infect a new host.

## 6. Concluding remarks

RNA virus infections represent a major public health problem, and this justifies significant efforts to better understand their interactions with the host proteome. This would greatly help in the design of novel therapeutic approaches based on targeting specific virus-host interactions. In addition, their great diversity combined to a relative small size makes of RNA viruses an amazing model to address comparative interactomic questions. Indeed, virus infection networks could be compared at several taxonomic levels, from families to strains, and also between different hosts in order to question virulence, pathophysiology or emergence issues. Finally, this should lead to the identification of viral peptides involved in protein binding, and this could benefit not only to virology but also to functional genomics in general. A complete set of tools is already available to tackle these questions, and needs to be implemented.

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