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Review

Nuclear proteins hijacked by mammalian cytoplasmic plus strand RNA viruses

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Abstract

Plus strand RNA viruses that replicate in the cytoplasm face challenges in supporting the numerous biosynthetic functions required for replication and propagation. Most of these viruses are genetically simple and rely heavily on co-opting cellular proteins, particularly cellular RNA-binding proteins, into new roles for support of virus infection at the level of virus-specific translation, and building RNA replication complexes. In the course of infectious cycles many nuclear-cytoplasmic shuttling proteins of mostly nuclear distribution are detained in the cytoplasm by viruses and re-purposed for their own gain. Many mammalian viruses hijack a common group of the same factors. This review summarizes recent gains in our knowledge of how cytoplasmic RNA viruses use these co-opted host nuclear factors in new functional roles supporting virus translation and virus RNA replication and common themes employed between different virus groups.

Keywords:
RNA virus
Enterovirus
Poliovirus
Flavivirus
Norovirus
Hepatitis C virus
Coronavirus
PTB
PCBP2
La protein
UNR
hnRNP C
hnRNP A1
hnRNP K
hnRNP M
RNA helicase A
NSAP
Tia1/TIAR
G3BP1

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Introduction

Viral spread and ultimately pathogenesis require efficient replication in key host cells that aid spread of the virus within hosts and throughout host populations. RNA viruses are typically small, encoding as little as three genes, and thus must rely on many host factors interacting with viral RNAs to assist with essential replication functions, and control many interaction points within host cells to promote replication. This often results in redirecting host metabolism on several levels to support the infection and at the same time suppress innate host defense systems that are triggered. Comparing plus and minus stranded RNA viruses, there are stark differences at the time of uncoating of genomic viral RNA in the cytoplasm. The plus strand RNA virus genome that is released is naked, however the minus strand RNA virus genome is completely enclosed in a functional nucleocapsid with RNA replicase poised ready to produce transcript mRNAs. Thus, the plus strand virus RNA can, and does, interact with many host RNA binding proteins (RBPs), whereas there is a little opportunity for minus strand virus genomic RNA to interact directly with host RBPs. Most RNA-binding proteins are nuclear shuttling proteins and many more nuclear RBPs have been reported to play roles in replication of plus strand RNA viruses than minus strand RNA viruses. Accordingly, this review focuses heavily on plus stranded RNA viruses, particularly mammalian viruses.

RNA viruses interact with a multitude of host factors during the course of infection. Several screening approaches have been employed to identify which of the 15–20,000 proteins that may be expressed in a given cell are host factors required for RNA virus replication. These include genetic screens in yeast that implicated 130 proteins that could affect plant virus replication (tobacco bushy stunt virus) (Jiang et al., 2006) and about 100 genes that affect brome mosaic virus (Kushner et al., 2003; Panavas et al., 2005). RNAi knockdown studies in mammalian cells with Hepatitis C virus (HCV), Dengue virus (DENV) and West Nile virus (WNV) have identified several hundred other genes that affect virus replication. However, many or most of these may function quite indirectly, affecting pathways that produce metabolites or products the virus needs, movement or trafficking of constituents that are directly required, factors that control divergent cation fluxes and ATPase pumps, the stress or innate immune activation levels that counteract general cellular biosynthetic potential, or include general off-target effects from the silencing step. It is likely that the spectrum of factors that directly interact in meaningful ways with virus RNA and proteins will be larger than that known today, but also smaller than the first lists that have emerged from such screenings (Box 1). Recently the novel approach of thioracil cross-linking mass spectroscopy (TUX-MS) was used to more precisely identify host proteins bound to poliovirus RNA during replication. This procedure identified all proteins known to interact with enterovirus RNA, plus 66 additional factors previously unidentified (Lenarcic et al., 2013). Eight of the new proteins were chosen and validated as playing roles in replication, indicating this new method is powerful and should be applied to other virus systems. However, standard molecular biology and biochemical approaches will still be required to tease out the functions and impact of each of these factors on virus replication. Proteins that interact with viral RNA do not present interesting targets for antiviral development unless it is determined that they play critical roles in virus replication.

Plus strand RNA viruses must translate incoming viral genomic RNA as the first biosynthetic step in replication cycles, thus, control of translation becomes the first battleground with the host that involves co-opted nuclear factors. It makes sense for the virus to utilize the host factors it commonly encounters at sites of replication. Thus, translation regulation involves virus co-opting of cellular translation factors. These are mostly cytoplasmic resident proteins since translation is a cytoplasmic process. However, translation does not occur on transcripts that are naked and devoid of RNA-binding proteins, rather, cellular transcripts are continually bound to a host of RNA binding proteins from the instant they emerge from RNA polymerase during their synthesis. In mammalian cells, RNA binding proteins control most aspects of RNA biology and the RNA cycle; from splicing, transport out of the nucleus, cellular function, transcript-specific translation control, and cytoplasmic localization and mRNA half-life. Mammalian cells encode hundreds of RBPs (~860), most with several splice variants (Castello et al., 2012). The cytoplasmic milieu encountered by plus strand RNA virus genomes as they are released from capsids is poised to greet the interloper as any other mRNA, with a ready store of RNA binding proteins ready to interact and impart functions. No wonder viruses have evolved to interact with RBP in...
diverse ways to promote replication. Recent research indicates that interactions of viruses with RBP can both benefit and inhibit virus replication, depending on stages of the replicative cycle. RBP interaction with complexes may afford molecular switching events that promote RNA replication over translation as one example.

The complete virus “interactome” will contain proteins that provide a diverse range of functions during virus replication; including protein and RNA chaperone functions, transport functions, helicases, factors and enzymes that regulate vesicular traffic, membrane functions and lipid metabolism. Notably, many of these are cytoplasmic proteins, thus beyond the purview of this review. Most of the nuclear RBPs are constantly shuttling between the nucleus and cytoplasm, thus, the definition of nuclear factor in this context refers solely to shuttling proteins that are more concentrated in the nucleus under normal physiological conditions.

Nuclear pore disruption: how cytoplasmic viruses get their stuff

An infecting virus may encounter sufficient nuclear shuttling proteins to initiate translation or the first rounds of RNA replication. However, rapidly expanding RNA virus replication requires a growing storehouse of supplies and will quickly expend the limited cytoplasmic supply of many nuclear factors. It was noticed early during studies of poliovirus-infected cells that many nuclear resident proteins showed improper cytoplasmic localization, including PTB, La autoantigen, Sam68, nucleolin and others (Back et al., 2002; McBride et al., 1996; Meerovitch et al., 1993; Waggoner and Sarnow, 1998). In fact, many viruses disrupt trafficking of proteins and mRNPs at the nuclear pore as a mechanism to restock the cytoplasmic storehouse with needed supplies. This dysregulation is usually required for efficient viral replication and can involve increased export of outbound cargo from the nucleus and/or blockage of inbound cargo from the cytoplasm. Detailed descriptions of the mechanisms involved are beyond the scope of this article, but recent reviews cover advances in virus disruption of nuclear pores (Le Sage and Mouland, 2013; Yarbrough et al., 2014). The range of viruses known to disrupt nuclear-cytoplasmic traffic is diverse and includes viruses that replicate in the cytoplasm (enterovirus, coronavirus, rhabdovirus) and viruses that replicate in the nucleus (influenza, HIV-1, Herpes simplex virus 1, Adenovirus). The mechanisms that disrupt nuclear transport are also diverse. Just two examples are enteroviruses that employ viral 2A proteinase to cleave at least three nuclear pore proteins (Nup 62, Nup 98, and Nup 153) (Park et al., 2008, 2010; Watters and Palmenberg, 2011) and coronavirus that employs L protein to induce a phosphorylation cascade that results in hyperphosphorylation of Nup 98, Nup62, Nup153 and Nup 214 as well as dysregulation of the pore complex (Bacot-Davis and Palmenberg, 2013; Basta et al., 2014; Porter and Palmenberg, 2009; Ricour et al., 2009; Watters and Palmenberg, 2011). In addition, L protein directly binds and inhibits the active cellular transport protein RAN GTPase (Bacot-Davis and Palmenberg, 2013). Notably some of the Nup targets are the same for both picornavirus genera, an example of convergent evolution providing two different approaches to accomplish similar functional goals.

Hijacked proteins regulate virus translation in IRESomes

ITAFs function in IRESomes

Picornaviruses such as poliovirus (PV) and encephalomyocarditis virus (EMCV) do not contain m7GTP cap structures on genomic RNA to recruit ribosomes, instead they use an internal cap-independent mode of translation that requires a large folded RNA structure to recruit ribosomes called an internal ribosome entry site (IRES). IRES elements have complex RNA folds and are only active when bound with specific proteins called IRES trans- activating factors (ITAFs) that are thought to provide RNA chaperone functions (Table 1). ITAFs are not canonical translation initiation factors that function in cap-dependent translation but are proteins known to play primary roles in other aspects of RNA biology in the cell. ITAFs are all of cellular, not viral origin, which makes sense for plus strand viruses because the IRES must function before any viral proteins can be synthesized. The IRES plus the required ITAFs and canonical translation factors that make up a functional unit are referred to as IRESomes because they function together as a complex. The functional relationships between ITAFs, canonical translation factors and ribosome recruitment are still unclear but significant strides have been made in understanding them in three virus systems. Although the first ITAFs were discovered in the context of viral IRES translation, many of the same ITAFs are thought to play similar roles promoting cap-independent translation for cellular IRES elements. For example, PTB is an ITAF for BIP, BAG1, Apaf-1, UNR, p53 IRESs; hnRNP A1 is an ITAF for Cyclin D1 and c-myc IRESs; and hnRNP C1/ C2 is an ITAF for XIAP and c-myc IRESs (reviewed in (King et al., 2010)).

Types of viral IRESs

There are distinct classes of virus IRESs that are referred to by a somewhat inconsistent and evolving nomenclature. Picornavirus IRES elements can be classified into at least five types based on structure, limited sequence homology and phylogeny. Type 1 IRESs are large ~450 nucleotide segments that are encoded by poliovirus and other enteroviruses; Type 2 IRESs are similarly large and occur in cardioviruses (EMCV), aphthoviruses (FMDV) and parechoviruses; and Type 3 IRES occurs only in Hepatitis A virus (HAV). Type 4 IRESs (sometimes called Class 2 or Type 3) are smaller ~330 nucleotide segments and occur in non-picornaviruses (hepatitis C virus and pestiviruses) but are also found among the newer picornavirus families Teschovirus, Sapelovirus, Seneovirus, Tremrovirus. Type 5 IRESs are encoded in the newly defined Picornavirus genera Kobuvirus, Salivivirus and Paraturdivirus (Sweeney et al., 2012).

ITAFs of Type 1 and Type 2 IRESs

One of the first host proteins ever shown to interact with picornavirus RNA by UV-crosslinking was polyypyrimidine tract binding protein 1 (PTB1, shortened to PTB) (Hellen et al., 1993). PTB is a shuttling, but mostly nuclear resident protein that associates with pre-mRNAs and plays roles in pre-mRNA processing, alternative splicing, mRNA metabolism and transport (Kafasla et al., 2010; Sawicka et al., 2008). PTB binds polyypyrimidine tracts in pre-mRNA introns to repress exon inclusion but can actually bind quite varied RNA structures. Thus, PTB can also stabilize certain mRNAs against degradation by binding to the 3’ untranslated regions. In virus replication schemes PTB plays roles that support and stimulate cap-independent translation driven by picornavirus and HCV RNA. PTB exists as one of three alternatively spliced isoforms (PTB1, PTB2, PTB4) and contains four-RNA binding domains (RBDs) of the RNP1/RNP2 class (Kafasla et al., 2010; Sawicka et al., 2008).

Though PTB was the first, a wide spectrum of factors, largely nuclear RBPs, have also been proposed as poliovirus ITAFs, including Lupus autoantigen (La) (Meerovitch et al., 1993, 1989), poly(rC)- binding proteins (PCBPs) (Blyth et al., 1996; Gamarnik and Andino, 1997), upstream of N-ras (UNR) (Anderson et al., 2007; Boussadja et al., 2003; Hunt et al., 1998), SRp20 (Bedard et al., 2007) and glycyrrhi- tRNA synthetase (GARS) (Andreev et al., 2012) (Table 1). In order to
make sense of the relative impact of this range of factors on basic translation, Pestova and Hellen have used in vitro translation initiation experiments reconstituted with a complete set of purified factors to define the minimal set of factors absolutely required for IRES translation. In these reconstituted translation systems, the HCV class of IRES minimally requires no ITAFs and only translation factor eIF3 to bind ribosomes (Hellen, 2009; Pestova et al., 1998). For both picornaviruses EMCV and PV, several canonical translation factors and only one IATF is minimally required. EMCV requires only PTB as an IATF and poliovirus requires only PCBP2 as an IATF to initiate translation (Pestova et al., 1996; Sweeney et al., 2014). However, minimal requirements in vitro likely do not reflect the conditions of severe competition among mRNAs for ribosomes in cells, suggesting the other factors proposed as ITAFs may play less fundamental, but still critical roles during infection and can contribute to pathogenesis. In addition, certain ITAFs play crucial roles in regulating the conversion of translation-competent genomic RNAs into replication-competent RNAs.

**PTB regulation of Type1 and Type 2 IRESomes**

PTB was known as a nuclear splicing factor when its role as an ITAF of poliovirus and EMCV was discovered; a classic hijacked nuclear protein pressed into a new role required for the virus. However, PTB is now known to also support cap-independent translation of cellular IRES-containing mRNAs that were discovered after the viral IRESs. These include Apaf-1, Bag-1 and mutant forms of c-myc transcripts associated with more aggressive tumor growth (Cobbold et al., 2010; Sawicka et al., 2008). Thus PTB can interact with a wide range of RNAs to promote cap-independent translation and may be considered a pro-translation, general ITAF.

Continuous investigations of PTB have revealed biochemical details about how it supports IRESome function in virus translation. The RNA binding function of PTB is modular and split between four RNA-binding domains (RBDs) that are distributed along an overall extended and flexible structure. This extended flexible nature of PTB is important for IRES function. The PTB RBDs recognize short pyrimidine-rich sequences but have distinct RNA structural preferences. The two N-terminal RBDs (RBD1 and RBD2) recognize short pyrimidine tracts contained in loops, while the RBD3-4 preferentially bind to larger flexible RNA sequences. These features give PTB the ability to bind to a variety of RNA structures and enables PTB to function as a versatile adapter protein that facilitates formation of many RNA-protein regulatory complexes (Clerte and Hall, 2009).

Recently, EMCV and poliovirus IRES structures have been used most extensively as models to study the ITAF functions of PTB. Mapping studies indicated that two PTB moieties bind the EMCV IRES, one upstream in a non-essential region of the IRES and another in the IRES core. In the core, the orientation of a single PTB binding to IRES was determined by binding of PTB mutants and hydroxyl radical probing. RBD1 and RBD2 bind near the 3' end of the core IRES sequence, and RBD3 binds near the 5' end. Binding of PTB to multiple regions on the IRES simultaneously enables stabilization and constraint of the IRES tertiary structure and is consistent with multiple RBD–RNA interactions proposed in the splicing functions of PTB (Oberstrass et al., 2005; Kafasla et al., 2009). This illuminates how an ITAF mechanistically provides an RNA-chaperone function. PTB is thought to promote RNA looping also, partly because RBDs 3 and 4 bind RNA in anti-parallel directions (Oberstrass et al., 2005; Lamichhane et al., 2010).

Further work established which of the multiple RBD–IRES interactions are critical for ITAF function. Using PTB with point mutations in various RBDs, it was shown that both poliovirus and EMCV IRES RNAs required simultaneous interaction with three of the four PTB RBDs, however the list of most critical RBDs differed
between the viruses (Kafasla et al., 2011). This indicates that the modular and elongated nature of PTB can adapt to disparate RNA structures using different sets of RBDs to provide similar chaperone function.

Besides providing an RNA chaperone function, PTB also provides a second critical function as it helps recruit or position the canonical translation initiation factor eIF4GI on the IRES (Fig. 1A). In the case of PV, both PTB and eIF4G bind the same RNA stem loop structure (stem loop V) comprising much of the IRES core. The orientation of PTB binding is such that RBDs 3 and 4 bind the base of stem loop V, whereas RBDs 1 and 2 bind the stem loop itself in the same region proposed to bind the central domain of eIF4G1. The two proteins do not compete for binding, rather PTB is thought to stimulate IRES activity by ensuring that eIF4G1 binds in the correct orientation and position to function properly for ribosome entry at a nearby AUG (Kafasla et al., 2010).

Other picornaviruses also utilize PTB as an ITAF. Coxsackievirus B3 is another enterovirus closely related to poliovirus with a Type 1 IRES, thus there is no surprise that PTB interacts with CVB3 RNA and promotes translation in a similar fashion as an ITAF. However, PTB also interacts with the CVB3 3′ UTR and this interaction may stimulate IRES translation through long-range looping or genome circularization bridged by PTB (Verma et al., 2010). Foot-and-mouth disease virus IRES is a Type 2 IRES structure distantly related to EMCV, sharing little sequence homology but some secondary structural similarity. However, FMDV requires both PTB and ITAF 45 (also known as Ebp1) to minimally support translation, whereas EMCV requires only PTB. The FMDV IRES also binds three of four PTB RBDs, where RBDs 3 and 4 bind distally located regions of the IRES and can function as a minimal IRES when supplied as a truncated form of PTB (Song et al., 2005). Changes in IRES structure were analyzed to compare effects of ITAF binding on RNA conformation shifts in EMCV and FMDV IRESs. Despite the different ITAF requirements, in both cases when ITAFs interacted with their cognate IRESs, similar conformation changes occurred where two domains were brought into closer compacted proximity (Yu et al., 2011a). Thus, PTB provides the same structural role building the functional IRESome, whether alone or in conjunction with other ITAFs.

### PCBP regulation of Type1 IRESomes

Poly(rC)-binding protein (PCBP) is the only obligate ITAF for enteroviruses using TYPE 1 IRESs (Sweeney et al., 2014). Unlike PTB, that binds widely separated sites on the EMCV IRES, PCBP binds to a restricted area of stem loop IV of the PV IRES (Fig. 1a). This binding region of SLIV is distant from the IRES core in the two dimensional secondary structure (Blyn et al., 1996). But PCBP also binds stem loop B of the cloverleaf structure (CL) at the 5′ terminus of enterovirus RNA (Blyn et al., 2000, 1997). There are four PCBP isoforms, however PCBP1 and PCBP2 are expressed in more abundance and both bind PV IRES. PCBP1/2 isoforms can form dimers or heterodimers and each can interact with PV IRES, but PCBP2 plays a more dominant role. PCBP binds RNA through three KH domains and interacts 6-fold more strongly with the IRES than cloverleaf RNAs in isolation (Gamarnik and Andino, 2000). PCBP2 is the isoform required for both translation initiation and also for RNA replication. KH1 is the primary domain that interacts with PV IRES domain IV (Silvera et al., 1999; Walter et al., 2002). For closely related CVB3, individual KH1 and KH3 domains of PCBP bind to IRES stem loop IV, KH1 interacts with subdomain IV/C RNA, whereas KH3 interacts with subdomain IV/B (Zell et al., 2008a).

Another host factor, Srp20, interacts with PCBP2 through its KH domain and was found to be critical for ITAF function of PCBP2 in cells. In vitro translation in HeLa cell extracts depleted of Srp20 are deficient in supporting poliovirus translation initiation and Srp20 siRNA knockdown in HeLa cells restricted poliovirus translation. (Bedard et al., 2007). Like other ITAFs, Srp20 is strongly relocalized from the nucleus to cytoplasm during poliovirus infection. Srp20 may bind PCBP2 rather than viral RNA directly since deletion of its RRM–RNA interaction motif does not alter its localization during infection, however, this truncated form strongly repressed virus translation, likely via a dominant negative process (Fitzgerald and Semler, 2011).

### Roles of other ITAFs for Type 1 IRESs

Many other factors have been shown to promote virus translation and have considered ITAFs or ITAF ancillary factors, but compared to PCBP and PTB much less is known about their function at the biochemical level in promoting IRES translation. The binding sites of some of these other ITAFs are poorly characterized. La enhances and corrects aberrant translation of PV in reticulocyte lysates that lack other ITAFs (Meerovitch et al., 1993) and 40S ribosome subunit binding to PV IRES is inhibited by a dominant negative La protein (Costa-Mattioli et al., 2004). Nucleolin interacts with poliovirus IRES and enhances IRES-dependent translation (Izumi et al., 2001). UNR has also been reported to interact with IRES elements (PV and rhinovirus) acting as an RNA chaperone and also binds PABP that is bound to poly(A) tails in cellular mRNAs (Anderson et al., 2007; Chang et al., 2004; Hunt et al., 1999). Presumably this interaction may also occur with viral polysomes and can contribute to genome looping or circularization that makes continuous translation more efficient. That UNR is important in vivo was demonstrated by gene knockout of both UNR alleles in mouse cells that reduced translation of PV and human rhinovirus (HRV) IRES’s by 90% and could be rescued by introduction of UNR expression plasmid (Boussadia et al., 2003). RNA affinity pulldown of cellular proteins using EV71 5′ UTR bait revealed PTB, un, PCBP1/2, hnRNP A1 and 10 other proteins. hnRNP A1 interacts with stem-loops II and VI of the EV71 5′ UTR, and is proposed to be another enterovirus ITAF. The roles of this interaction in replication are unclear since knockdown of hnRNP A1 had no effect on viral replication. In contrast, knockdown of both hnRNPs A1 and A2 reduced viral RNA synthesis and virus output, suggesting that hnRNP A2 can substitute for hnRNP A1 (Lin et al., 2009), however the relative importance of hnRNP A1 versus PCBP2 or PTB in EV71 replication was not evaluated. GARS is perhaps the most unusual proposed ITAF; a tRNA synthetase house-keeping enzyme that binds the IRES core in the apical portion of domain V that mimics the anticondon-stem-loop of tRNA<sup>Val</sup> (Andreev et al., 2012).

So what are we to make of all the proposed ITAFs for PV and other closely related enteroviruses? Recently, in vitro reconstitution of translation with purified factors has been accomplished on the PV IRES and other Type 1 IRESs and those experiments indicate that PCBP2 is the only mandatory ITAF required to accomplish 48S ribosome assembly and initiation. PTB showed minor stimulation of translation, and other proposed ITAFs (La, UNR, Srp20, GARS) produced no measurable effect on 43S or 48S translation complex formation in vitro. PCBP2 may also promote recruitment of 43S complexes by direct interaction with elf3 (Sweeney et al., 2014). The weak stimulation of PCBP2-dependent 48S complex formation by PTB in vitro is likely due to its imposed reorientation of elf4G1 binding to the IRES (Kafasla et al., 2010). But many ITAFs promote translation in cells by providing factors that promote efficiency, beyond the minimal requirements for IRES translation initiation. Intracellular conditions encountered by viruses include intense competition among mRNAs for ribosomes in cells, forcing viruses to utilize multiple mechanisms to seize translation control. Thus, the other ITAFs that aid IRES translation efficiency in vivo play less fundamental, but still critical roles during infection and contribute...
to pathogenesis. Future work will discern new molecular details about how this happens.

**ITAFs of Type 3 IRESomes**

Comparatively little is known about factor requirements for the Type 3 IRES of HAV. Unlike most of its picornavirus cousins, this virus replicates very slowly, it does not cause host translation shutoff and is relatively non-cytopathic in tissue culture cells. The IRES is unique in that it requires intact eIF4G1, whereas the PV IRES functions better with only the cleaved central domain of eIF4G1 (Borman and Kean, 1997; Hambridge and Sarnow, 1992). Both PTB and PCBP2 have been implicated as ITAFs for HAV (Yi et al., 2000; Zhang et al., 2007). HAV 3Cpro inhibits HAV IRES-dependent shutoff and is relatively non-cytopathic in tissue culture cells. The virus replicates very slowly, it does not cause host translation

Further, the fact that La protein can be exploited as a therapeutic tool for HCV infection and that La is an ITAF involved in ribosome recruitment has been challenged (Brocard et al., 2007; Domitrovich et al., 2005; Ito and Lai, 1999).

Several lines of evidence indicate that La is an ITAF. La enhances translation in the context of the competitive translation environment during infection in cells with replicons or virus. siRNA depletion of La inhibits HCV translation and a dominant negative form of La inhibits translation (Costa-Mattioli et al., 2004) in vivo. Further, the fact that La protein can be exploited as a therapeutic tool for HCV infection and that La is an ITAF involved in ribosome recruitment has been challenged (Brocard et al., 2007; Domitrovich et al., 2005; Ito and Lai, 1999).

**Box 1—Basic concepts of viral hijacking of nuclear proteins:**

- Many nuclear shuttling host proteins are hijacked by viruses to play roles in replication.
- Viruses increase access to nuclear factors by disrupting regulation of nuclear pore trafficking.
- Proteomic studies have identified many new potential host factors that may interact directly or indirectly with virus RNAs. The impact, role and functions of most of these factors on virus replication are unknown.
- Most nuclear factors hijacked by RNA viruses are RNA-binding proteins and they are hijacked predominately by plus strand RNA viruses.
- A small set of ubiquitous RNA binding proteins are commonly hijacked by a broad range of viruses.
- Production of key virus proteins shifts some host RBP functions from support of translation to support of RNA synthesis.
- Virus sponging or sequestration of host factors may occur, that is different than hijacking a factor for a virus-specific use.
- Some host RBPs that interact with viral RNAs have a negative impact on virus replication.

**Table 1**

| Proviral host factors | Viruses | Functions |
|-----------------------|---------|-----------|
| DHX29                 | Picornavirus, Aichi | ITAF, IRES conformation |
| eEF1a                 | Flavivirus | (-) RNA synthesis, replicate interactions |
| GARS                  | Enterovirus | IRES conformation |
| hnRNP??               | HCV, coronavirus | (+) RNA and (-) RNA synthesis, RNA recruitment? |
| hnRNP C               | Enterovirus | (+) RNA synthesis |
| hnRNP K               | Sindbis | sgrNA promoter transcription |
| hnRNPA1               | HCV, coronavirus, enterovirus 71, alphavirus | genome circularization, RNA promoters, ITAF |
| HuR                   | Alphavirus, HCV | RNA stability |
| ITAF 45 (Ebp1)        | FMDV | ITAF |
| La                    | Enterovirus, HCV, BVDV, CSFV, flavivirus | IRES conformation, translation-replication switching, genome circularization |
| LSM1–7                | HCV | Promote translation |
| NF90/NFAR             | BVDV, HCV | Genome circularization, translation-replication switch |
| NSAP1, hnRNP Q, SYNCRIP | HCV | ITAF, ribosome binding, genome circularization |
| nucleolin             | poliovirus, HCV | ITAF, promote RNA replication, binds replicate |
| PCBP2                 | Enterovirus, HAV | IRES conformation, translation-replication switching, genome circularization |
| PTB                   | Picornavirus, HCV, coronavirus, flavivirus, astrovirus | IRES conformation, eIF4G1 recruitment to IRES, genome circularization |
| RNA Helicase A        | FMDV, HCV, Dengue | Genome circularization protein bridge, promote RNA replication |
| Sarn68                | poliovirus | (+) RNA and (-) RNA synthesis |
| Skp20                 | Poliovirus, CVB3, Rhinovirus | ITAF cofactor |
| TDP2                  | enterovirus | Vpg unl kinase RNA processing |
| Tial, 3TAR            | Flavivirus, WNV | promote (+) strand RNA synthesis |
| Tudor-SN/p100         | Flavivirus, Dengue | promote RNA synthesis |
| tRNA synthetase       | coronavirus | RNA replication |
| Umr                   | Enterovirus | IRES conformation, RNA looping |

| Antiviral host factors | Viruses | Functions |
|-----------------------|---------|-----------|
| Gemin 3               | Poliovirus | SMN complex functions |
| Gemin 5               | FMDV | Competes with PTB binding IRES, SMN complex functions |
| AuF1                  | Enterovirus | Inhibits IRESome function, binds 3’ UTR destabilizing |
| PTB                   | Calicivirus, norovirus | Translation inhibition |
proximity with the IRES (Brocard et al., 2007). PTB is proposed to loop HCV RNA by binding the 3' region containing duplicate dumbbell structures (DB1 and DB2) (light blue) and the 3' CS/SL region (red) containing the conserved 3' SL. All structures from SL-1 through DB-2 participate in pseudoknots (not depicted). To facilitate translation poly(A)-binding protein binds A-rich sequences in the DB region allowing protein-bridge looping to translation factor eIF4G associated with the 5' cap structure. YB-1 binds the 3' SL and represses translation and replication. PTB and La bind 3' UTR may also facilitate translation. (B) Long range interaction between complimentary sequences in 5' and 3' regions (5' CS, 3' CS; 5' UAR, 3' UAR) facilitate genomic looping associated with RNA replication. This looping remodels RNA into new conformations that may promote binding of additional host RBPs not associated with translating DENV RNA such as eEF1, and the dsRNA binding proteins NF90, NF45 and RHA that bind the 3' SL. In HCV, NF90 binds both 5' and 3' UTR of HCV RNA and RHA is involved in bridging 5' and 3' UTRs (not depicted). A similar arrangement may exist in flaviviruses where NF90 and RHA may also bind 5' UTR stem loops and stabilize the looped structure to promote negative strand RNA replication. The NS5 RdRp binds to the 5' SL1 which due to looping helps position the polymerase near the 3' terminus (Bidet and Garcia-Blanco, 2014). NS5 may also be recruited by La, which binds the 5' UTR.

target for HCV suggests a significant role in HCV replication. A cell permeable peptide corresponding to the N-terminal 18 amino acids of La inhibits HCV IRES-mediated translation and also inhibits HCV replication in cells (Fontanes et al., 2009). La-peptide-mediated inhibition of HCV IRES-translation blocked interaction of La and other ITAFs (PTB and PCBP2) with the IRES, but could be rescued with exogenous PTB and PCBP2. This implied that La peptide sequesters PTB and PCBP2 as its mode of action (Fontanes et al., 2009).

Other knockdown experiments implicated several host factors in HCV translation or HCV virus replication in cells, including La, PTB, PCBP2 and proteasome alpha-subunit 7 (PSMA7) (Kruger et al., 2001; Shirasaki et al., 2010). All four factors are induced in Huh-7.5 cells during HCV replication, suggesting the virus has co-opted factors that are naturally up-regulated during infection. shRNA knockdown of La repressed production of HCV core protein 70%, further supporting a role as an ITAF in vivo. La has been reported to be a telomerase component, thus telomerase activity and expression of other telomerase subunits increased coordinately with La induction during chronic HCV infection (Shirasaki et al., 2010). The linkage of HCV-induced La expression to increased telomerase activity in chronically-infected liver may be important in development of hepatocellular carcinoma.

Evidence from in vitro translation experiments in somewhat artificial rabbit reticulocyte lysates suggest that PTB is not a true HCV ITAF that facilitates 40S ribosome subunit binding to the HCV IRES. Rather, PTB may stimulate translation indirectly through bringing other factors bound to the X region of the 3' UTR into proximity with the IRES (Brocard et al., 2007). PTB is proposed to loop HCV RNA by binding the 3' UTR X region and 5' UTR IRES region simultaneously. Another factor, NSAP1 (also called hnRNP Q) stimulates HCV IRES translation by binding an A-rich region downstream of the initiator AUG and facilitating formation of 48S ribosome initiation complex by also binding the 40S ribosomal proteins. This is the first reported interaction of an ITAF directly with a ribosome, thus NSAP1 provides more of a translation-factor role than other canonical ITAFs that play “chaperone-like” roles (Kim et al., 2004; Park et al., 2011).

**ITAFs of Type 5 IRESs**

PTB also supports translation initiation of Aichivirus (AV) that contains a novel type of picornavirus IRES that differs structurally from Type 1 and 2 IRESs. In reconstitution experiments this IRES requires interaction with both eIF4G and PTB. Unlike other IRESs, the AV IRES has a strong requirement for a novel nuclear factor DHX29, that is also a ribosome-associated helicase. DHX29 releases the AV IRES initiation codon from a strong stable hairpin to aid anticodon base pairing by the ribosome (Yu et al., 2011b) (Table 1).

**ITAFs are determinants of tissue and host tropism**

Various ITAFs are either essential for virus translation or play less critical roles that enhance efficiency of translation. High translation efficiency is critical for most viruses to produce sufficient proteases or other factors to control activation of innate immune pathways that would block further replication. Therefore, ITAFs have long been considered host range factors that determine tissue and host tropism and pathogenesis. The attenuated Sabin vaccine strains of poliovirus lost neurovirulence partly through nucleotide changes in the IRES that alter PTB binding (Wimmer et al., 1993). Restricted IRES function will result from a lack of the proper ITAF in certain cells. For instance, a neuronal variant of PTB (nPTB) is required for the...
neurovirulence of the GDVII strain of TMEV (Pilipenko et al., 2001). PTB1 expression patterns have a powerful tropic effect on a chimeric poliovirus containing the HRV2 IRES element (called PV1/RIPO)). This virus has a growth defect in mouse cells because it cannot interact with endogenous murine PTB (Jahan et al., 2013, 2011). This is proposed to interfere with the thermodynamics of folding of the IRESome into a functional structure. The virus also has a severe neuro-attenuation defect in humans but retains highly specific virulence against glioblastoma cells and HeLa cells that express high levels of PTB (Gromeier et al., 2000).

Nuclear factors that antagonize cap-independent viral translation

Modern proteomic approaches are now being applied to identify the full complement of host factors that interact with viral RNAs, and have identified over 30 proteins that interact with FMDV IRES directly or indirectly (Pacheco et al., 2008). Of these Gemin5 was found to bind to the IRES at a domain 5 hairpin flanked by A/U/C-rich sequences via its C-terminal domain. Gemin5 is the RNA-binding component of the survival of motor neuron (SMN) complex that assembles 5 proteins onto spliceosomal snRNAs. Gemin5 binding did not enhance FMDV translation, but rather inhibited it (Table 1) (Box 1). RNA structure analysis using 2-hydroxyl acylation analyzed by primer extension (SHAPE) revealed Gemin5 induced conformational changes that out-competed SHAPE changes induced by PTB. Thus Gemin5 may competitively inhibit PTB IFIT binding (Piñeiro et al., 2013). Gemin5 also interacts with the HCV IRES and may provide similar functions (Piñeiro et al., 2013). Gemin5 is cleaved in FMDV infection by L protease (Piñeiro et al., 2012) similar to Gemin3 cleavage by poliovirus that blocks assembly of the SMN complex (Almstead and Sarnow, 2007). Gemin5 cleavage alleviates translational repression of the IRES. The similarity of Gemin 5 and Gemin 3 cleavages suggests that inactivation of the SMN complex may be required by a wide range of picornaviruses.

RNA decay regulator AUF1 (which has 4 isoforms) is cleaved by poliovirus and rhinovirus 3CD proteinase. AUF1 is a factor that binds AU-rich elements in mRNA 3’ UTRs and promotes rapid mRNA decay and turnover. AUF1 follows the familiar pattern of strong relocalization from nucleus to cytoplasm during poliovirus infection (Rozovics et al., 2012). Surprisingly though, AUF1 does not bind PV 3’ UTR but rather binds the IRES at stem loop IV and negatively regulates rhinovirus and poliovirus infections via translation inhibition. However, discordant findings with closely related CVB3 indicated that AUF1 was bound to the 3’ UTR (Wong et al., 2013). 3Cpro cleavage inactivates RNA binding function of AUF1 and relieves translation restriction (Cathcart et al., 2013). Thus, both AUF1 and Gemin5 act as a “hijacked” restriction factor in translation and must be remedied by inactivation by a viral proteinase. However, AUF1 may not be a restriction factor for all picornaviruses. EMCV infection induces strong cytoplasmic relocalization of AUF1, but was not cleaved by EMCV 3Cpro, even at late times after infection (Cathcart and Semler, 2014).

Caliciviruses such as feline calicivirus and human norovirus share a non-structural protein coding region that is distantly related to picornaviruses, yet translate by an unusual cap-independent mechanism that does not involve an IRES. Rather, translation of norovirus involves interaction of initiation factor elf3 with the viral Vpg protein covalently linked to the 5’ end (Daughenbaugh et al., 2003, 2006). PTB interacts with both 5’ and 3’ ends of feline calicivirus genomic RNA, and also subgenomic RNA. FCV infection induces nuclear to cytoplasmic relocalization of PTB that is coincident with the switch from early translation to late RNA replication. PTB inhibited FCV translation in vitro, thus PTB is proposed to be a negative regulator that may aid the switch from translation to RNA replication (Karakasiliotis et al., 2010).

Nuclear factors may modulate flavivirus translation

Flavivirus RNA is unique in containing a 5’ cap structure, but no poly(A) tail to facilitate typical PABP binding that promotes 5’-3’ looping. Thus, flaviviruses use cap-dependent translation machinery but it is unknown how viral translation is promoted over cellular translation. Dengue virus promotes both cap-dependent translation and a form of non-canonical translation that is not IRES dependent and does not require a functional m7G cap structure (Edgil et al., 2006). Surprisingly, DENV can bind PABP in an A-rich region upstream of the 3’ SL, resulting in promotion of translation (Polacek et al., 2009a, 2009b) likely by binding cap-binding translation factor elf4G and promoting 5’-3’ interactions similar to host mRNA (Fig. 2). DENV RNA is also known to bind several host factors that could play roles in translation or RNA replication, including PTB, La, Y box-binding protein 1 (YB-1), translation elongation factor eEF-1a and p100/Tudor-SN (Table 1) (De Nova-Ocampo et al., 2002; Garcia-Montalvo et al., 2004; Lee et al., 2011; Paranjape and Harris, 2007; Polacek et al., 2009a, 2009b; Yocupicio-Monroy et al., 2007; Yocupicio-Monroy et al., 2003). YB-1 binds the 3’ SL of DENV and represses translation, possibly in a role that regulates the switch from translation to RNA replication discussed below (Paranjape and Harris, 2007), and binding of eEF-1a to the 3’ SL has no effect on translation (Davis et al., 2007) (Fig. 2). La binds to both 5’ and 3’ UTRs (Garcia-Montalvo et al., 2004) and could play a role of stabilizing looped RNA structures via protein bridging and indirectly support translation through ribosome recycling. PTB has been proposed to play positive roles in virus replication but the mechanism(s) remains elusive. PTB relocalizes to the cytoplasm with variable completeness in infections in different cell types. PTB cytoplasmic localization is weak in Huh7 cells, however PTB binds the 3’ stem-loop region of DENV RNA in these infections (Fig. 2) and could play roles in RNA looping to promote translation and RNA replication. Nuclear to cytoplasmic relocalization of PTB was associated with increased DENV translation and replication and siRNA knockdown of PTB inhibited replication and overexpression increased replication (Agis-Juárez et al., 2009). However, in Huh7 cells knockdown studies indicated that PTB did not have an effect on DENV RNA translation, but promoted negative strand RNA synthesis and interacted with viral protein NS4A (Jiang et al., 2009). Thus, additional work is required to pin down the specific mechanisms of PTB-specific stimulation of DENV replication.

Hijacked nuclear proteins regulate viral negative strand RNA replication

In theory, nuclear factors may not be necessary for support of viral RNA synthesis. Each virus synthesizes its own complete RNA-dependent RNA replicase, an enzyme function that is uniquely not present in host cells. Further, in the cytoplasm where virus replication occurs, viruses duplicate functions such as mRNA capping and polyadenylation that are carried out in the nucleus for host transcripts. Thus, one may not expect many dependent interactions between virus RNA replicases and host factors to have evolved to support viral RNA replication. For negative strand viruses that contain RNA covered in nucleo capsids proteins, this may be more expected. However, plus strand RNA viruses make extensive use of host RBPs for important roles in the replicative process, not in RNA polymerase enzymatic activity per se, but in promotion of its temporal and spatial regulation. A key feature for host factors is helping to organize complex 5’–3’ genome interactions in alternate configurations that sequentially promote translation, then RNA synthesis.
Regulated switch from viral translation to RNA replication

All plus strand RNA viruses are faced with the problem that the same genomic template is used for both 5’–3’ transit by ribosomes and 3’–5’ transit by the viral RNA polymerase. Since translation and RNA replication machinery proceed in opposite directions there must be a regulated switch from translation to RNA replication that clears the template of all elongating ribosomes before negative strand RNA synthesis can take place. For plus strand RNA viruses the details of this regulation are emerging. A common theme is that sufficient translation/production of key virus proteins is required before modifications are triggered in host factor structure/protein interactions that shift host factor roles from promoting translation to promoting RNA synthesis. Accumulating evidence indicates that both translation and negative strand RNA replication occur on templates circularized via complex RNA–RNA, protein–RNA and protein–protein interactions. Further, the switch in template use involves complex shifts in these interactions allowing host factors to change roles simultaneously.

PCBP2 helps mediate a switch from poliovirus translation to RNA replication due to changes in its RNA binding properties. PCBP binds both the IRES and 5’ cloverleaf (CL) stem b, and its binding to the cloverleaf stimulates translation early during infection (Garnamnak and Andino, 1998). The PCBP complexed on the 5’ cloverleaf promotes translation in conjunction with the 3’ poly (A) tail in a circularization model based on protein–protein interactions. The C-terminal KH3 domain of PCBP is required to stimulate translation and can be tethered directly to PV RNA and still promote translation. In the current model the 5’CL–PCBP complex interacts with the 3’ poly(A)–PABP complex to form a 5’–3’ circular structure that enhances translation by facilitating ribosome relooding as ribosomes recycle from the stop codon (Fig. 1B) (Ogram et al., 2010). This is consistent with the finding that PCBP2 and 5’ cloverleaf function during de novo assembly of polysomes (Kempf and Barton, 2008). Thus, PCBP strongly promotes translation by interaction with the 5’ CL in the phase before viral proteins accumulate.

But once virus proteins accumulate from sustained translation, PCBP bound to the 5’ CL also promotes binding of viral polymerase precursor 3CD to the adjacent cloverleaf stem loop D (SLD). The recruited 3CD promotes translation inhibition (Garnamnak and Andino, 1998). Further, cleavage of PCBP1 and 2 mediated by 3Cpro occurs during this phase of the replication cycle (Fig. 1B). Cleavage occurs between the KH2 and KH3 domains, resulting in truncated PCBP2 lacking the KH3 domain that cannot support translation but binds the CL and supports RNA replication (Perera et al., 2007).

In fact, several ITAFs for PV are cleaved by 3Cpro in midphase of the replication cycle. In addition to PCBP, PTB is cleaved by 3Cpro in a reaction that was shown to inhibit PV translation (Back et al., 2002) and La is similarly cleaved (Shiroki et al., 1999). Even though cleavage of PCBP2 and PTB inhibits poliovirus translation and contributes to a switch in RNA template usage, for related rhinoviruses things may be different. PCBP2 and PTB are differentially cleaved during infection with three HRV serotypes in Hela cells but are not cleaved in human lung epithelial cells that sustain productive infections. This suggests that some ITAF cleavages may not be required by HRV to mediate a switch in template usage (Chase and Semler, 2014).

Like picornaviruses, HCV must clear genomic RNA templates of ribosomes to allow use by RNA replicase complexes. Several insights have emerged about how HCV regulates the switch and mechanisms involve both host factors and accumulation of nascent HCV proteins. Early studies showed HCV core protein binds to the IRES and linked translation production of HCV core protein to translation repression in a direct feedback loop (Zhang et al., 2002). Expression of HCV NS3 protease also has the effect of blocking IRES translation and increasing replicon replication. NS3 protease and La protein compete for binding the IRES in the same SLIV region, thus NS3 was proposed to directly inhibit ITAF binding to the IRES, thereby reducing translation activity (Ray and Das, 2011). Additional work indicated that La protein binds to a GCCA sequence near and the initiator AUG in SLIV of the IRES, but this enhances RNA replication, not translation, by promoting linkage between 5’ and 3’ UTRs through La (Kumar et al., 2013). Since both La and NS3 have similar dissociation constants for binding HCV IRES RNA, NS3 may not actually evict all the IRES–bound La, but competitively reach a binding equilibrium that promotes replication by recruiting NS5B to a replication complex involving La, NS3 and both ends of the viral RNA (Kumar et al., 2013). Another class of nuclear factors called NF/NFAR proteins NF90/NFAR (nuclear factor associated with dsRNA, also called ILF3) bind to both ends of the genome of HCV and the pestivirus BVDV (Isken et al., 2007, 2003, 2004). NF90/NFAR is a part of group of interacting nuclear factors containing dsRNA-binding motifs and multiple isoforms that include NF45 and RNA helicase A (RHA). NF90/NFAR is associated with HCV translation inhibition and increased RNA replication (Isken et al., 2007, 2004, 2003).

Another group used siRNA approaches to show that efficient HCV translation was dependent upon several members of the cellular 5’–3’ deadenylation-dependent miRNA decay pathway, Rck/p54, LSm1, and PatL1. The requirement of these factors for efficient translation was linked to the 5’ and 3’ UTRs. The 3’ UTR also interacted with LSm1–7 complexes. All of these factors are core components of P-bodies where non-translation silenced mRNAs are stored before undergoing decay. This raises the possibility that the decay factors could play roles in the switch to replication, but the proposed role from experiments for HCV interaction with reconstituted LSm1–7 complexes was support of translation (Scheller et al., 2009). It is possible that co-opting P-body components down-regulates functions of P-bodies that repress HCV translation. HCV infection results in significant reductions in the number of P-bodies in cells both in vitro and also in hepatocytes from patients infected with HCV (Pérèz-Vilaró et al., 2014, 2012).

Host factors in genome circularization

RNA synthesis of viral negative strands initiates replication of genomic templates from the 3’-terminus. Thus, it was a surprise when RNA polymerase complexes for poliovirus were found to bind near the 5’ terminus of the plus strand genome template on the cloverleaf (Andino et al., 1993). This suggested that the template is circularized rather than linear to allow close proximity of the replication complex and 3’ end of the template where the polymerase must initiate. Cellular mRNAs and viral genomes are thought to translate optimally on RNAs circularized by interactions between translation factors elf4G, elf4B and PABP that promote ribosome recycling (Kuyumcu-Martinez et al., 2004). Thus, RNA replication may first begin on templates transitioning from a circular configuration. Indeed, this paradigm seems to be conserved among many plus strand RNA viruses. In viruses, the long-range interactions that circularize templates are mediated by either protein–protein interactions or RNA–RNA interactions or both.

For PV, circularization is also promoted by a complex of PCBP bound to the cloverleaf that interacts with PABP bound to poly (A) tail forming a protein–protein bridge (Herold and Andino, 2001). An analogous arrangement occurs in coronavirus mouse hepatitis virus (MHV) where interaction between 5’ and 3’ ends is promoted by another protein bridge involving PTB bound to 5’ UTR and hnRNP A1 bound to the 3’ UTR (Huang and Lai, 2001). For Dengue virus cyclization occurs via long range RNA–RNA interactions of complementary sequences in the corresponding 5’ and 3’
UTRs (Filomatori et al., 2011; Lodeiro et al., 2009; Polacek et al., 2009a, 2009b; Sztuba-Solinska et al., 2013; Villordo et al., 2010) (Fig. 2). In HCV an RNA sequence in the 5′-SS binds via a kissing-loop to facilitate placement of the polymerase at the 3′-SS (Friebe et al., 2005).

The latter two examples above emphasize RNA–RNA interactions without factors, but it is possible that host factors could promote or stabilize these long-range interactions. For instance, HCV interactions may be aided by hnRNPs A1, which binds 5′-SS and 3′-SS of the genome (Kim et al., 2007). Finally, the host RBP NF90/NFAR-1 circularizes the RNA genome by binding both ends of the genome. These interactions may occur sequentially after kissing loop and hnRNPs A1 interactions form as NF90 binding blocks translation and stimulates RNA replication (Isken et al., 2007, 2004, 2003). RHA can also play a role in the complex with NF90/NFAR-1 as a bridging factor between the 5′ and 3′ UTRs of HCV that promotes genome circularization (Isken et al., 2007).

Several of these factors also bind to DENV2 3′ UTR (NF90, NF45, RHA) and NF90 is critical for replication (Gomila et al., 2011). Together, these findings suggest that conformations of circularized genomic RNA can differ between translation and replication. Accordingly, switching between translation and replication, or looped circularized RNA has not been determined and more precise functional roles of nucleolin have not been identified.

FMDV interacts with RNA helicase A (RHA) and alters its distribution in the cell from mostly nuclear to cytoplasmic. RHA is involved in diverse cellular functions as it enhances gene expression by interacting with CBP/p300 and RNA polymerase II and responds to IFNalpha signaling to increase its activity (Aratani et al., 2001; Fuchsová et al., 2002). During FMDV infection RHA can bind the S domain of the virus 5′ UTR and co-precipitates with FMDV 2C and 3A proteins that function in the replicase complex. Though the precise function of RHA remains undefined, knockdown of RHA reduces virus titers, indicating that RHA plays roles in supporting replication (Lawrence and Rieder, 2009). FMDV-induced movement of RHA out of the nucleus is associated with demethylation or arginine residues at the C terminus and did not require activity of FMDV leader protein. Rather nuclear egress involves demethylation activity provided by Junonji C-domain containing protein 6 (JmJC) that is stimulated by FMDV (Lawrence et al., 2014).

Nuclear factors promote flavivirus negative strand RNA synthesis

Flavivirus 3′ UTRs are complex structures that contain cis-acting elements required for translation, cyclization and replication. Accordingly, this regulatory RNA region binds several host factors that may play roles in RNA synthesis, including La, PTB, YB-1, eEF1A, NF90, RHA, and NF45 (Fig. 2). Flavivirus RNA replication is dependent on many of these co-opted host factors, however not all are thought of as nuclear factors. Translation elongation factor 1A (eEF1A), which is abundant in both the cytoplasm and nucleus, binds to three sites within the 3′ UTR of West Nile virus and other flaviviruses. This interaction supports negative strand RNA synthesis but not translation (Blackwell and Brinton, 1997; Davis et al., 2007). eEF1A immunoprecipitates with the NS5 replicase complex of WNV and colocalizes with replication complexes, further supporting a role for this factor in minus strand RNA synthesis (Davis et al., 2007). Flavivirus RNA shifts to an alternate conformation based on complex long-range binding interactions that form a new panhandle structure. This panhandle structure positions 5′ and 3′ termini close together and promotes RNA replication (Fig. 2). Binding of a complex of double-strand binding factors NF90, RHA and NF45 to the 3′ SL likely occurs in concert with the formation of the panhandle and promotes RNA replication (Gomila et al., 2011). The possibility exists that these factors may also bind the 5′ UTR and stabilize the long-range looped structure similar to their role with HCV RNA (Isken et al., 2007). Furthermore, La binds to both 5′ and 3′ UTRs and could play a role stabilizing this structure as well as recruiting NS5 and NS3 (García-Montalvo et al., 2004). The NS5 RdRp binds to the 5′ SL1 which due to looping helps position the polymerase near the 3′ terminus (Bidet and García-Blanco, 2014).

Similar to picornaviruses and HCV, PTB also binds the 5′ UTR of Dengue virus (DENV) and Japanese Encephalitis virus (JEV) (Anwar et al., 2009; Kim and Jeong, 2006). Although the 5′ UTR binding site suggested a possible role of PTB in flavivirus translation, PTB knockdown inhibits DENV negative strand RNA replication and does not affect virus translation or viral entry. PTB also interacts with NS4A protein, a component of the replicase, further
suggesting that PTB plays a role in viral RNA replication (Anwar et al., 2009; Jiang et al., 2009). Surprisingly, PTB knockdown did not inhibit JEV replication so its requirement is not conserved. PTB also binds 3’ UTRs of both viruses. Though the 5’ UTR of both viruses are conserved, the differential requirement for PTB may stem from the fact that the 3’ UTRs are divergent. In addition, PTB translocates to the cytoplasm in DENV-infected cells and is found colocalized with calnexin endoplasmic reticulum marker, NS1 and NS3 (Agis-Juérez et al., 2009). Further work will be required to elucidate precise functional roles of PTB in flavivirus replication.

**Nuclear factors regulate HCV negative strand RNA synthesis**

For HCV, as discussed above, the switch from translation to RNA replication is stimulated by binding of core and NS3 to the IRES. However, some translation may still occur and be coupled to RNA replication at the same subcellular membrane compartment termed a replicosome. Pulse chase and other analyses in infected cells supported this conclusion since restriction of RNA synthesis with an NS5B polymerase inhibitor reduced translation even when levels of HCV RNA were unaltered (Liu et al., 2012). As with any RNA virus, the overlapping, ongoing replication and translation processes make separation of the roles of host factors very difficult to tease apart.

Even though PTB may not be a bona fide ITAF for HCV, it interacts with the poly(U/C) tract in the HCV 3’ UTR and may promote replication. During infection in HuH7 cells a phosphorylated form of PTB not found in uninfected cells associates with the membrane-bound HCV replication complex (Chang and Luo, 2006).

Synaptotagmin-binding cytoplasmic RNA-interacting protein (SYNCRIP)(NSAP1)(hnRNP Q) is another member of the heterogenous polymerase via its glycine–arginine-rich domain. Transient expression of NS5B recruits or sequesters nucleolin from the nucleus to cytoplasm and may modulate the oligomerization of NS5B that is required for RNR activity (Hirano et al., 2003; Kusakawa et al., 2007).

Finally, the RNA chaperone nucleolin has also been proposed to play a role in HCV replication, as it binds the virus NS5B RNA polymerase via its glycine–arginine-rich domain. Transient expression of NS5B recruits or sequesters nucleolin from the nucleus to cytoplasm and may modulate the oligomerization of NS5B that is required for RNR activity (Hirano et al., 2003; Kusakawa et al., 2007).

**Nuclear factors regulate alphavirus RNA synthesis**

After Sindbis virus (SINV) infection numerous nuclear proteins shift to the cytoplasm including hnRNPK, hnRNPA1, and HuR. hnRNPA1 interacts with the viral 5’ UTR, and with the genomic (G) and subgenomic (SG) RNA promoters. Knockdown of hnRNPA1 resulted in markedly decreased synthesis of G and SG RNA both in infected cells and in vitro (Gui et al., 2010; Lin et al., 2009). A recent report also indicates that SINV infection results in cytoplasmic relocation of PTB and TIA1, but no specific function of these factors in replication is known (Sanz et al., 2015). Several reports indicate that SINV non-structural proteins interact with stress granule nucleating proteins G3BP1 and G3BP2, including nsP4 polymerase (Cristea et al., 2010), nsP3 and nsP2 (Cristea et al., 2006) (Gorchakov et al., 2008). The interaction of G3BP1 with nsP3 has been shown to have the proviral effect of inhibiting stress granule formation (Panas et al., 2012). TIA1 and PTB also enter the cytoplasm during SINV infection but not during ectopic expression of nsP proteins, indicating replicative processes may be required (Sanz et al., 2015). Recently the combined approach of isotope labeling of purified Semiliki Forest virus replicase/modified lysosome complexes with proteomics analysis identified 78 host proteins associated with the functional replicase. Several familiar proteins colocalized with replicase including PCB1, hnRNPM, hnRNPC, and hnRNPK. SILENCING experiments indicated that hnRNPM M and C are antiviral for SFV, Chikungunya and SINV. Differential silencing results with hnRNPK K indicated opposite roles of this RBP in CHIKV and SINV versus SFV. This suggests that interactions of host factors with replicase complexes are not always proviral and that the roles of hnRNPs may be interchangeable or more nuanced in various cells/virus combinations (Varjak et al., 2013).

**Nuclear factors modulate astrovirus and norovirus RNA replication**

PTB also binds the 3’ UTR of astrovirus. Seven host proteins from Caco2 cell lysates could be cross-linked to 3’–UTR RNA probes in vitro and mobility shift assays indicated that two complexes of host factors form on 3’ UTR probes and that PTB is part of one of the complexes. siRNA knockdown of PTB reduced virus replication, suggesting that PTB is required for replication (Espínoa-Hernández et al., 2014). It is unclear if negative strand or plus strand RNA replication is affected by PTB.

Human norovirus was shown to bind La and PTB to the 3’ UTR that contains a small stem-loop structure (Gutiérrez-Escolano et al., 2003), however, since human norovirus cannot be cultivated in vitro, most recent work has focused on murine norovirus that replicates in macrophage cell lines. Murine norovirus RNA contains three stable stem loop structures and a single stranded polypurimidine (pY) tract within the 3’ terminal stem loop. Both PTB and PCBP bind the pY tract, but this is not essential since viruses lacking this region are viable in cells and in mice. However, pY-deleted virus suffer a fitness cost and is less virulent in mice (Bailey et al., 2010). RNA affinity chromatography–mass spectrometry identified over 50 host factors that bound to discreet structures in murine norovirus RNA. Many were common to other RNA viruses discussed above, including PTB, La, DDX3, nucleolin, hnRNPK, PCB1/2, eEF1a and hnRNPA species. siRNA knockdown of PTB, La and DDX3 in RAW264.7 cells resulted in deficient virus replication (Vashist et al., 2012) suggesting these play roles in replication, but much more work will be required to elucidate how these factors function in norovirus infections. A long range 5’–3’ RNA–RNA looping interaction was described in murine norovirus RNA that is stabilized by binding PCB2 and hnRNPA1. Mutations in the RNA–RNA complimentary binding motif reduced binding of both host factors to RNA and also inhibited RNA replication; and PCB2 and hnRNPA1 localized to virus replication complexes in cells. All these results indicate the looping interaction stabilized by the host factors plays an important roles in replication (López-Manríquez et al., 2013).

Feline calicivirus also binds nucleolin on the 3’ UTR together in a complex with viral protein NS6/7 and infection of feline kidney cells results in nuclear-cyttoplasmic rec localization of nucleolin to sites enriched for NS6/7 (Cancio-Lonches et al., 2011). Finally, PTB plays a negative role in calicivirus translation and therefore may promote the switch from translation to replication (Karakasiliotis et al., 2010).

**Nuclear factors in coronavirus RNA replication**

Several host proteins interact with the 3’ end of the transmissible gastroenteritis coronavirus (TGEV) genome. Genomic RNA ends were used as bait in RNA affinity pulldowns to identify host factors. The only protein to bind the 5’ end was PTB, whereas nine other proteins bound the 3’ terminus RNA, including several hnRNPs, PABP and two amino-acyl tRNA synthetases. Knockdown of PABP, hnRNQ and glutamyl-prolyl-tRNA synthetase inhibited
replication of a replicon, indicating that they may play functional roles in replication (Galán et al., 2009). Since hnRNPs oligomerize, they may participate in RNA-protein complexes by bringing transcription-regulating sequences (TRS) TRS-L and TRS-B into close proximity to facilitate coronavirus discontinuous transcription (Sola et al., 2011b).

Further investigation of the role of PTB in TGEV replication indicated that PTB affects virus RNA accumulation and relocates viral RNAs to novel cytoplasmic domains different from replication–transcription sites. Interestingly, siRNA knockdown of PTB in two cell lines increased virus mRNA levels and virus titer, suggesting that PTB interaction inhibits virus replication, the opposite of most other RNA viruses. PTB relocalized from the nucleus to discreet structures that contained TIA1 and TIAR and may be reminiscent of stress granules. These foci also contained viral genomic and subgenomic RNA and PTB could play a role in sequestration of some virus RNA into novel foci involved in posttranscriptional regulation of virus gene expression (Sola et al., 2011a). There is not substantial evidence that the TIA1-foci are bona fide stress granules but may represent replication complexes.

Regulation of plus strand RNA synthesis and RNA processing

Nuclear factors regulate enterovirus plus strand RNA synthesis

Compared to minus strand synthesis, less is known about the synthesis of plus strands from the minus strand anti-genomic template in RNA virus systems such as poliovirus. The template for plus strand synthesis is different, being mostly double-stranded instead of single stranded RNA, and this may have profound influence on the host factors that interact with virus RNA. This double stranded structure is called replicative form (RF) in picornaviruses. hnRNP C supports virus positive strand replication, where replicase builds on the plus strand CL to prime plus strand synthesis but not minus strand synthesis, suggesting the stem-loop structure is functional on the anti-cloverleaf. Second, the complete plus strand version of the CL is required, including binding sites for PCBP and 3CD polymerase precursor. A trans-initiation model was proposed where replicate builds on the plus strand CL to prime plus strand synthesis the same way it does for negative strand RNA synthesis (Vogt and Andino, 2010). However, hnRNP C binding to the anti-CL will also help position the 3' end of the negative strand to allow precise RNA replication initiation by the replicate.

Nuclear factors function in viral RNA processing

After synthesis of PV plus strand transcripts an RNA processing step removes VPg at the 5' end of a portion of transcripts. All PV RNA associated with polysomes lacks VPg, having been removed by a host unlinkase enzyme, whereas all encapsidated RNA retains VPg. Cellular 5' tyrosyl-DNA phosphodiesterase-2 (TDP2) is a hijacked cellular DNA repair enzyme that performs the VPg unlinkase function for enteroviruses. PV infection relocates the bulk of TDP2 from the nucleus to cytoplasm. A hypothesis predicts that VPg is a capsid packaging signal that must be removed so that a pool of nascent transcripts can continue to engage ribosomes and produce additional viral proteins (Virgen-Slane et al., 2012). The effect of preventing VPg unlinking by using click chemistry to form an uncleavable bond for TDP2 was tested and found to not affect translation or replication efficiency, indicating that VPg does not inhibit initial steps in either process (Langereis et al., 2013).

Nuclear factors regulate flavivirus plus strand RNA synthesis

In flaviviruses, plus strand RNA synthesis is initiated by a promoter–protein complex that builds on a conserved stem-loop structure on the 3' terminal 96 nt of minus strand RNA. This structure is specifically bound by four host proteins, one of which is La autoantigen (Yocupicio-Monroy et al., 2003) and another is TIAR. TIAR and its closely related paralog TIA1 are nuclear proteins that shuttle into the cytoplasm during environmental stress and help nucleate formation of RNA stress granules. TIA1 also binds WNV (--) RNA and both proteins bind WNV RNA through their conserved RRM2 motifs (Li et al., 2002). WNV replication was repressed in TIAR knockout cells and mutations of the UAAUU TIAR recognition motif in the 3' SL affected replication but not translation. Several mutant viral RNAs that only weakly bound TIAR rapidly reverted to wild type phenotypes in vivo, suggested that TIAR interaction was not required for low level minus strand replication but allowed efficient high level plus strand RNA synthesis from the minus strand template (Emara et al., 2008).

The ability of WNV and DENV to hijack TIAR and TIA1 from the nucleus and place it into new roles in RNA synthesis provides another advantage; hijacking also sequesters these proteins from their role in forming stress granules (SG) (Emara and Brinton, 2007). Many viruses induce SGs by interrupting cellular pathways and homeostasis, particularly protein synthesis, and by triggering activation of PKR. These virus–derived perturbations drive SG formation through phosphorylation of eIF2α− or other mechanisms (Redersha et al., 2013, 1999; Reineke and Lloyd, 2013). Accumulating evidence suggests that SGs may provide platforms to activate innate immune functions and they are seen as antiviral (Lloyd, 2013). Indeed, many viruses have evolved mechanisms to suppress SG formation, and co-opting key SG nucleating factors such as TIAR and G3BP1 is one mechanism employed by flaviviruses and HCV (Ariumi et al., 2011; Emara and Brinton, 2007; Ruggieri et al., 2012). Further experiments with chimeras of WNV that had different RNA replication efficiencies and different abilities to
induce or control SGs indicated that early viral RNA synthesis cannot exceed the ability to protect product dsRNA with virus-induced membranes. Virus-induced membranes block access of PKR to the dsRNA intermediates. Viruses that replicate too quickly overwhelm the limited virus-induced membranes that protect dsRNA, allowing PKR activation and SGs formation that inhibits replication (Courtney et al., 2012).

Regulation of subgenomic RNA synthesis by host RBPs

Several plus strand viruses have multiple open reading frames and use subgenomic plus strand transcripts (sgRNA) to express structural proteins. The synthesis of subgenomic plus strand RNA is regulated differently than genomic RNA, however is still controlled by transcription elements that function in cis or trans. This provides the opportunity for subgenomic transcription elements to assemble alternate replicase complexes containing different host factors than those modulating genomic RNA replication.

Coronaviruses promote sgRNA transcription using interactions between 5′ terminal leader sequences and intergenic (IG) sequences just upstream of each ORF. One host factor, hnRNP A1 binds to both leader and IG sequences in MHV negative strand template RNA (Li et al., 1997). Overexpression of hnRNP A1 promotes MHV RNA replication while a dominant negative hnRNP A1 reduces replication (Shi et al., 2000) and the interaction of hnRNP A1 with the leader and IG sequences is critical for in vitro sgRNA transcription (Zhang et al., 1999). hnRNP A1 could function by recruiting virus N nucleocapsid protein (Wang and Zhang, 1999). PTB is another prominent nuclear factor that may regulate sgRNA transcription as it binds the plus strand leader sequence and also the 5′ UTR in minus strand RNA in reactions that promote sgRNA replication specifically (Huang and Lai, 1999; Li et al., 1999).

The alphavirus Sindbis virus induces a shift of hnRNP K from the nucleus to cytoplasm where it colocalizes with viral non-structural protein 2 and co-immunoprecipitates with subgenomic but not genomic RNA, nsp1 and nsp2. siRNA knockdown of hnRNP K reduced gene expression from a subgenomic promoter. These findings indicate that hnRNP K has a role, details yet to be determined, in sgRNA synthesis (Burnham et al., 2007).

Host RBPs regulate stability of viral RNA

Viruses must maintain the integrity of their viral RNAs and suppress RNA decay pathways. Alphavirus and coronavirus genomes are capped and poly-adenylated, retaining two modifications that stabilize mRNAs against RNA decay. However, binding of certain host factors to virus RNA can also protect against RNA decay machinery. HuR is associated with prolonged mRNA stability in cells and binds to U-rich elements (UREs) in alphavirus 3′ UTRs (Garneau et al., 2008; Sokoloski et al., 2010). As with many other factors, HuR is selectively relocalized to the cytoplasm during Sindbis virus infection. Two other alphaviruses, Ross River virus and Chikungunya virus, lack the conserved 3′ UREs but still tightly bind HuR via alternative binding elements (Dickson et al., 2012). Knockdown of HuR increased decay of Sindbis virus RNAs and reduced virus replication yields in both human and mosquito cells, indicating that HuR binding to SINV RNA blocks components of the cellular RNA decay pathway (Sokoloski et al., 2010).

Other viruses lack either 5′ m7GTP caps or poly(A) tails and may specifically co-opt or target host factors to protect against decay. Many of these are resident cytoplasmic factors but some are nuclear shuttling proteins. The PV 5′ CL binds PCBP to support RNA replication as discussed above, but this also stabilizes PV plus strand RNA in HeLa lysates. A mutant PCBP protein that cannot bind the CL was associated with increased decay of PV RNA in the HeLa in vitro replication system and it was proposed that PCBP binding blocks Xrn1 exonuclease activity. This was supported by showing that capped PV RNAs bypassed the requirement for PCBP2 in stability assays (Murray et al., 2001). Poliovirus also achieves greater RNA stability through cleavage and proteolytic degradation of RNA decay factors Xrn1, Dcp1a and Pan3 by poliovirus proteinases and virus-activated proteasomal decay (Dougherty et al., 2011). Further, 3Cpro cleavage of the RNA destabilizing factor AUF1 likely contributes to poliovirus RNA stability (Rozovics et al., 2012; Wong et al., 2013).

Flaviviruses generate short non-coding RNAs (sRNA) from the 3′ UTR due to the ability of this structure to stall to Xrn1. DENV2 sRNA accumulates during infection and acts as a sponge and binds host factors G3BP1, G3BP2 and Caprin1. Sequestration of G3BP was linked to dysregulation of translation of several mRNAs of specific interferon stimulated genes, thus interfering in innate immunity (Bidet et al., 2014). Importantly sRNA also sequesters Xrn1, and results in dysregulation of host mRNA stability and accumulation of uncapped mRNA decay intermediates in cells (Moon et al., 2012).

The stem-loops and U-rich tract contained in the 3′ UTR of HCV bind La protein and inhibit decay of HCV RNA in HeLa extracts (Spängberg et al., 2001). Similar to alphaviruses, the HCV 3′ URE can bind HuR and knockdown of HuR expression in cells resulted in reduced HCV translation and RNA replication using replicon models (Korf et al., 2005; Spängberg et al., 2000). The stimulatory effects of HuR on IRES translation were confirmed using dicistronic vectors in rabbit reticulocyte lysates, Xenopus laevis oocytes and HeLa cell extracts but may not involve direct interaction of HuR with the IRES (Rivas-Aravena et al., 2009). Though HuR is associated with RNA stability in many systems, its importance for HCV RNA remains unclear. Proteomics analysis indicates more than 70 host proteins are enriched from cells with HCV 3′ UTR probes and several of these are associated with regulation of RNA stability in cells, including YB-1, N590, hnRNP C and hnRNP D (Harris et al., 2006).

Conclusions

The discussion herein mostly focused on nuclear shuttling RBPs and their roles in regulating mammalian plus strand RNA virus translation and RNA replication. However, cytoplasmic viruses exploit many other types of cytoplasmic factors involved in translation regulation, membrane formation and remodeling, and virus assembly and egress that were not discussed. As research on RNA virus replication has continued, several common themes have emerged. First, the array of host proteins that plays significant roles in replication will continue to grow as the newly expanded list of interacting factors identified from proteomics approaches undergo analysis. Second, although many host proteins are hijacked by viruses, several ubiquitous host proteins, such as PTB, PCBP and hnRNP A1 are used by several classes of viruses, often in different ways. Third, the production of key virus proteins shifts the function of some host factors from support of translation to RNA replication. Fourth, the RNA chaperone functions described for host factors in supporting IRESome activity often provide similar or analogous chaperone functions supporting RNA structures and long range interactions in RNA synthesis. Fifth, the new concept of host factors being sequestered or sponged away from normal host roles may be common, and may be a variant form of hijacking. Sixth, some interactions of host factors with virus RNA inhibit replication or translation and must be opposed by viruses.

Many nuclear host factors discussed are highly conserved, which can enable cross-species infection in the case of alphavirus and flaviviruses, but also restrict virus tissue tropism and control pathogenesis in mammals where gene expression patterns are so
variable. Also, the complete lists of factors used by any one virus are unique evidence that viruses evolve many different ways to take over cell functions. However, many of the diverse host factors that are hijacked perform related functions for the virus in replication.

Our understanding of co- opted factors, though expanding rapidly, is far from complete. We certainly do not have a comprehensive list of factors utilized by any single virus. Future expansion of proteomics research and new approaches such as TUX-MS will fill in the catalog, but substantial traditional biochemical wet bench science will be required to tease apart functions and impact of newly discovered factors, many of which may be novel to science as a whole. Adaptation of in vitro replication systems used for poliovirus to study other virus families will help dissect the roles of factors in viral replication complexes, particularly where the same factor may play roles in sequential replicative processes. Finally, the increased use of super-resolution fluorescence microscopy and cryo-electron microscopy of whole cells will also be important to finely localize factors and virus-induced structures in cells.

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