Poly(A)-binding protein (PABP): a common viral target

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INTRODUCTION

Viruses are intracellular parasites that depend upon the cells they infect for the production of new virus particles. They are remarkably diverse in structure and in the complexity and organization of their genomes, which can consist of either single- or double-stranded DNA or RNA. Consequently, viruses differ greatly in their coding capacity and in their requirements for reproduction. This is most obviously reflected in the reliance of some viruses on the host cell nucleus for transcription or DNA replication, whereas others are more self-sufficient, encoding their own nucleic acid replication enzymes, and reproduce entirely in the cytoplasm. No known virus encodes all of the components required for the synthesis of viral polypeptides, although mimivirus was recently found to encode several functional aminoacyl-tRNA synthetases [1]. Therefore all viruses share an absolute requirement for host cell ribosomes and most of their associated factors. As a consequence, viruses find themselves in competition with the host for the cellular translation machinery, prompting some viruses to develop strategies to arrest host cell gene expression, collectively referred to as ‘host cell shut-off’, to commandeer the translational apparatus for their own use. Other viruses appear to be able to reproduce successfully in the face of ongoing cellular translation, perhaps employing more subtle means to ensure sufficient synthesis of viral proteins. Many RNA viruses face additional translational challenges as their genome serves as a template for both protein synthesis and RNA replication. RNA polymerases are recruited to the 3′-end of the template and proceed in a 3′→5′ direction, whereas ribosomes bind to the 5′-end and move in the opposite direction. Therefore these processes cannot occur simultaneously and require coordinated control. Regulation of translation is complex and its manipulation by viruses occurs at different levels via mechanisms that are, in many cases, incompletely understood at a molecular level. Nevertheless, the study of viruses has proved fruitful in identifying fundamental aspects of cellular gene expression [2].

In the present review, we focus on recent studies characterizing the interaction of metazoan viruses with cytoplasmic PABP [poly(A)-binding protein], a protein with key roles in cellular translation.

PABP STRUCTURE AND FUNCTIONS

Poly(A)-binding proteins

The majority of work to date on PABPs has focused on the prototypical member of this family, PABP1 (PABP, PABPC1), a multifunctional protein with a variety of roles in mRNA translation and stability [3,4]. However, metazoans often encode multiple cytoplasmic PABPs. In addition to PABP1, vertebrates encode PABP4 [PABP (inducible PABP), PABPC4] and ePABP (embryonic PABP) with tPABP (testis-specific PABP, PABPC2/PABPC3) and the structurally distinct PABP5 (PABPC5) being mammalian-specific (reviewed in detail in [4,5]). Available data suggest that PABP1 and PABP4 are widely expressed, whereas the other family members appear to be more tissue-restricted [5]. Another predominantly cytoplasmic PABP (ePABP2) is found in vertebrates [4]; however, it is more structurally related to nuclear PABP (PABPN1) and is outwith the scope of this review [6]. Since most studies to date have examined the function of PABP1 or have utilized antibodies that do not distinguish between different PABPs, many papers simply collectively refer to PABP, although it is unclear to what extent the functions and expression of these proteins overlap.

PABP1 is composed of four non-identical RRMs (RNA-recognition motifs) and a C-terminus that consists of a proline-rich region and a globular domain (PABC) [3,4] (Figure 1). The RRMs appear to differ in their RNA- and protein-binding specificities [3,4] and the structure of RRMs 1–2 bound to poly(A) has been solved, showing the availability of residues on the face not bound to mRNA to mediate protein–protein interactions [7]. The...
The RRM domains (numbered 1–4) are not functionally equivalent. The C-terminal region consists of a flexible proline-rich linker (wavy line) and a structured C-terminal domain (PABC). Minimal mapped regions for protein binding [8,17,27,39,99,101,117,118] are indicated by horizontal bars. The eIF4B- and PCBP2-binding sites (not depicted) are unclear, but eIF4B binding requires integrity of the PABP C-terminal region, and PCBP2 binds C-terminally of RRM1 and 2 [119,120]. Arrows indicate the order in which the viral protease cleavage sites are located within PABP whose domains are not drawn to scale [34,36,54,61]. The HAV 3C cleavage site (not shown) has not been precisely mapped, but is thought to be at or close to that of PV 3C [37].

C-terminal region of PABP does not bind RNA, but is required for oligomerization, which results in ordered high-affinity binding to poly(A) [4,6]. Both C-terminal regions bind proteins, but the PABC domain, in particular, mediates interactions with proteins that contain a PAM2 motif [8,9]. The mapped regions of interaction of PABP1 protein partners discussed in the present review are shown in Figure 1.

PABP1, a eukaryotic initiation factor

The canonical cap-dependent pathway of initiation has several mRNA-independent and -dependent steps, which are facilitated by eIFs (eukaryotic initiation factors). For a full review of the initiation pathway, see [10]. The mRNA-dependent steps of initiation (Figure 2A) begin with the binding of a protein complex, eIF4F, to the 5′ cap. eIF4F consists of the cap-binding factor, eIF4E, the scaffold protein, eIF4G, and the RNA-dependent helicase, eIF4A. The activity of eIF4A mediates removal of secondary structure within the 5′-UTR (untranslated region) and is aided by the factor eIF4B. Removal of secondary structure permits the binding of the small ribosomal subunit and associated factors (eIFs 1, 1A, 3, 5 and tRNA-Met·eIF2·GTP). Binding of the small ribosomal subunit to the mRNA is also aided by the interaction of its associated factor, eIF3, with eIF4G bound as part of the cap complex. The small ribosomal subunit complex then ‘scans’ through the 5′-UTR in a poorly understood ATP-dependent process to locate an appropriate initiation codon. Initiation codon recognition triggers hydrolysis of eIF2·GTP, reorganization and release of some initiation factors and joining of the large ribosomal subunit to form a translationally competent 80S ribosome, which requires further GTP hydrolysis. Initiation ends with the methionyl-initiator tRNA at the ribosomal P site ready to accept the first elongation factor–tRNA complex.

Although initiation occurs at the 5′-end of the mRNA, it is also stimulated by PABP bound to the 3′-poly(A) tail [3–5]. PABP interacts with several of the factors bound to the 5′-UTR, including...
The Poly(A)-binding protein (PABP) is an initiation factor that plays a crucial role in the translation of mRNAs by recruiting and stabilizing eIF4G, eIF4E and eIF2 subunits for subsequent rounds of initiation. PABP also interacts with the termination factor eRF3 (eukaryotic release factor 3), an interaction that is important in translational termination. PABP has been shown to enhance 60S joining, but may also aid the recycling of ribosomal subunits for subsequent rounds of initiation.

### Other roles of PABP1

Whereas most viral studies have focused on PABP’s function as an initiation factor, PABP has additional roles, which may be the target for viral manipulation. As an initiation factor, PABP plays a global role in the translation of essentially all adenylated mRNAs. However, the translation of some mRNAs is more sensitive to translation initiation factors, including eIF4G, eIF4E and eIF2, are targeted during viral infection, either to subvert the host translation machinery or to evade cellular defence mechanisms, it is now becoming apparent that PABP is also targeted by a wide range of viruses in very diverse manners. However, in most cases, the effects on cellular and viral translation and viral life cycle are only beginning to emerge.

### PROTEOLYSIS OF PABP DURING VIRAL INFECTION

#### Cleavage of PABP by the picornaviral 2A and 3C proteases

A common theme among several classes of RNA viruses is cleavage of PABP by virally encoded proteases. This has been most extensively studied in the Picornaviridae family that comprises the genera Enterovirus [e.g. PV (poliovirus) and CV (Coxsackievirus)], Aphthovirus, Rhinovirus, Hepatovirus [e.g. HAV (hepatitis A virus)], Parechovirus and Cardiovirus. PV, the prototypical Picornavirus, is a lytic virus that contains a small, uncapped, polyadenylated positive-strand RNA genome that is translated as a single polyprotein, which is subsequently processed into individual viral proteins by the viral proteases 2A and 3C. In addition, these proteases have acquired a number of cellular substrates whose proteolysis mediates drastic inhibition of host cell transcription and translation (reviewed in [30,31]).

#### Inhibition of host cell mRNA translation

PV-induced host cell translational shut-off was initially attributed to cleavage of eIF4G by 2A and virally activated cellular proteases, resulting in separation of an eIF4G N-terminal region, which mediates binding to eIF4E and PABP, from the C-terminus, which associates with the 40S ribosome through eIF3 and binds to RNA and eIF4A [32,33]. However, treatments that allow complete eIF4G cleavage result in only partial host shut-off, implying that other processes must also be involved in translational inhibition [31]. Subsequently, PABP was shown to represent an additional target for a number of picornaviral proteases, including PV and CV 2A and 3C and HAV 3C [34–37]. 2A and 3C are cysteine proteases that cleave PABP at several defined sites in the proline-rich linker region between RRM1–RRM4 and CTD (C-terminal domain) [34,36] (Figure 1). Cleavage of PABP in the flexible linker region removes the C-terminal region, abrogating functional interactions between PABP and eIF4B and eIF3. Where investigated, the N-terminal (RRM1–RRM4) cleavage product is able to bind eIF4G [38], but cleavage of PABP may destabilize the PABP–poly(A) association [39] (Figures 1 and 3A).

During infection with PV or the closely related CV, only approx. 30% of PABP is cleaved, whereas host cell translation is completely inhibited [34,35]. However, PABP is preferentially cleaved in ribosome-enriched fractions, indicating the specific inactivation of PABP molecules that are actively engaged in translation [36,40]. 3C, in contrast with 2A, efficiently cleaves poly(A)-bound PABP in vitro and thus appears to be the predominant protease responsible for host shut-off [36,40]. Consistent with this, transient expression of PV 3C in cells inhibits translation of endogenous mRNA and transfected polyadenylated reporter mRNAs [38]. In cell extracts, specific inhibition of poly(A)-dependent translation by recombinant 3C can be largely rescued by the addition of recombinant 3C-resistant PABP [38]. This verifies PABP as a target of 3C, although 3C cleaves other proteins, including the initiation factor eIF5B [41].

### Role of PABP in PV translation and RNA replication

PABP cleavage may also have direct effects on viral translation, which must at times be down-regulated to allow the genomic template RNA to be made available for replication.
PV RNA is polyadenylated, but not capped, and it is translated using an IRES (internal ribosome entry site) via an alternative cap-independent initiation mechanism that requires most of the canonical eIFs with the exception of eIF4E and intact eIF4G. The 2A-cleaved form of eIF4G is functional in IRES-mediated translation [33]. Efficient PV translation also requires a number of cellular ITAFs (IRES trans-activating factors), including La (lupus autoantigen), PTB (polypyrimidine tract-binding protein), PCBP2 [poly r(C)-binding protein 2] and Unr (upstream of N-ras), some of which (La, PTB and PCBP2) are substrates of 3C protease. The functions of ITAFs are not fully delineated, but may remodel the IRES for interactions with the translational machinery (reviewed in [31,42,43]).

In vitro, polyadenylation strongly stimulates translation of PV IRES-driven reporter mRNAs [44], consistent with the sensitivity of such reporters to PABP depletion [45]. Stimulation is dependent upon the PABP–eIF4G interaction as it is greatly reduced or abolished either by 2A protease-mediated eIF4G cleavage or disruption of the PABP–eIF4G or the PABP–poly(A) interactions by a variety of methods [46,47]. However, during PV infection, eIF4G is efficiently cleaved by 2A protease. Perhaps consistent with this, cell-free translation of full-length genomic PV RNA, which expresses 2A, is far less sensitive to PABP depletion than PV IRES reporter mRNA translation in cell extracts with intact eIF4G [45]. Although this seems to diminish the role for PABP in viral translation, the residual PABP-dependence suggests that PABP may stimulate PV translation independently of eIF4G (see also below), in keeping with reports that PABP has multiple effects on initiation and interacts with other eIFs. Moreover, PABP also interacts with Unr [27], raising the possibility that this could contribute to eIF4G-independent effects. In this respect, it is noteworthy that translation of the closely related CV IRES has been reported to be stimulated by PABP via both poly(A)-dependent and -independent mechanisms in vitro [48].

3C protease partly inhibits the translation of a PV IRES reporter mRNA in vitro [49]. This can be largely rescued by addition of 3C-resistant PABP in a strictly poly(A) tail-dependent manner, showing that 3C regulates PABP-mediated IRES stimulation to some extent [49], although cleavage of other translation factors or ITAFs may also be involved [31,41,42]. Importantly, in PV-infected cells expressing 3C-resistant PABP, reporter mRNA translation was prolonged [49], indicating that 3C may normally serve to restrict viral translation and supporting the idea that PABP has a positive role in translation even in the presence of eIF4G cleavage.

PV RNA replication

Picornavirus genomic RNA is replicated to form a complementary strand that serves as a template for the synthesis of new plus strands. Because the same plus strands are also used for translation, both processes are co-ordinated, with translation of the incoming and at least a proportion of newly synthesized PV RNA being a prerequisite for subsequent replication (reviewed in [42,43]). The mechanism governing the transition from translation to RNA synthesis is not yet well understood, but has been proposed to involve modulation of at least two ITAFs [42,43]. 3C-mediated specific proteolysis of PABP molecules that are engaged in translation, as described above, may be a further regulatory factor. Infected cells expressing 3C-resistant PABP show reduced viral RNA replication and virion production. This correlates with continued translation of viral reporters, suggesting that ongoing translation makes the template unavailable for replication, which in turn reduces the number of templates available for further viral translation [49]. Reduced host cell shut-off could have a further impact on viral translation, although this remains to be addressed. Perhaps consistent with observations that not all PABP is cleaved during infection, PABP bound to the PV poly(A) tail appears to play a direct additional role in viral negative-strand synthesis. PABP bound to the PV poly(A) tail has been reported to mediate an alternative closed loop ribonucleoprotein complex with the ITAF protein PCBP2 bound to a 5′ structure (termed ‘cloverleaf’) that is required for RNA replication [50]. However, the overall necessity for PABP in viral replication appears to be relatively low as the titre of infectious particles produced from full-length genomic mRNA was not measurably affected in PABP-depleted extracts [45].
**PABP cleavage by other viral proteases**

**Hepatitis A virus**

HAV is a picornavirus distantly related to PV with a different IRES structure and, unlike most other picornaviruses, encodes no 2A protease homologue. Initiation of HAV translation requires the full set of canonical initiation factors (reviewed in [43]) and, like PV, is stimulated by polyadenylation in a PABP- and eIF4G-dependent manner [44,47]. Recently, it was shown that PABP is cleaved, albeit inefficiently both in vitro and during HAV infection [37]. HAV shows no appreciable host translational shut-off activity [51], but, as described above for PV, must reconcile viral translation with RNA replication. As HAV 3C-mediated PABP cleavage inhibits IRES-dependent translation, a model was proposed in which PABP proteolysis plays a role in template switching [37].

**Caliciviruses**

The family *Caliciviridae* comprises four genera of positive-strand RNA viruses: *Vesivirus* [e.g. FCV (feline calicivirus)], *Lagovirus*, *Norovirus* (e.g. Norwalk virus) and *Sapovirus* [52]. Caliciviruses encode only one known protease, 3CL, which shares sequence similarity with the active site of picornavirus 3C protease [53]. Like PV 3C, recombinant *Norovirus* 3CL preferentially cleaves PABP present in a ribosome-enriched subcellular fraction, and is able to inhibit both endogenous and polyadenylated (but not unadenylated) reporter mRNA translation in HeLa extracts [54]. Studies of FCV-infected feline kidney cells show evidence of host translation shut-off, and a temporal correlation with PABP cleavage suggests that this may underlie host translation inhibition [54], although eIF4G cleavage by an unknown protease has also been reported [55]. Norovirus and FCV 3CL cleave PABP at different positions, albeit within the proline-rich linker region cleaved by picornavirus 2A and 3C [54] (Figure 1). Although calicivirus RNA is polyadenylated, it is not known whether its translation is stimulated by PABP, as this RNA is not physiologically capped, but linked to a 5′-VPg (viral protein genome-linked) protein. However, VPg can interact with cellular initiation factors [56,57], although differences exist among caliciviruses in their functional requirement for components of the eIF4F complex [58]. Thus a potential for PABP complex formation with components of the eIF4F complex exists.

**Lentiviruses**

HIV-1 and -2 are lentiviruses of the *Retroviridae* family. HIV-1 and HIV-2 encode proteases that cleave eIF4G [59,60] and PABP [61]. Both HIV-1 infection and expression of the HIV-1, HIV-2 and related mouse mammary tumour virus proteases lead to PABP proteolysis in cultured cells. *In vitro* analysis of HIV-mediated proteolysis determined that, as with picornavirus and calicivirus proteases, PABP cleavage occurs at several sites within the linker region between RRMs 1–4 and the C-terminal region and, interestingly, also at a site within RRM3 [61] (Figure 1). The significance of these findings is unknown, but, since HIV induces an inhibition of host cell protein synthesis [60,62], PABP and eIF4G degradation provides good candidates for this effect. HIV RNA is capped and polyadenylated; however, it has also been reported to contain IRES sequences, and a dual mode of HIV-1 translation initiation has been proposed [63]. Therefore it would be intriguing to investigate what role, if any, PABP has in these processes.

**Modification of eIF4F–PABP complexes by viruses**

The regulation of the interaction between PABP and the eIF4F complex during viral infection can have both negative consequences for host translation, by exclusion of PABP, or positive effects on viral translation by enhancing its association with eIF4F.

**PABP displacement from eIF4G in rotavirus host translation shut-off**

Rotaviruses, one of 12 genera of the double-stranded RNA *Reoviridae* family, do not cleave PABP or eIF4G [64] and have evolved a different mechanism to shut off cap-dependent protein synthesis. Rotavirus replication occurs entirely in the cytoplasm, where a viral transcriptase synthesizes capped, but non-polyadenylated, mRNAs with a conserved 3′-end that forms a binding site for the rotaviral NSP3 (non-structural protein 3) (reviewed in [65]).

A role for NSP3 in translation emerged when it was shown to associate with eIF4G in infected cells *in vivo* and to bind an N-terminal region of eIF4G directly *in vitro* through the NSP3 C-terminus [64,66]. Interestingly, immunoprecipitation of eIF4G at different time points during rotavirus infection showed a gradual decrease in associated PABP (but not other translation initiation factors) and a concomitant increase in co-purifying NSP3, suggesting that NSP3 replaces PABP in the translation complex. Furthermore, a purified NSP3 C-terminal fragment can displace PABP from eIF4G complexes *in vitro*, demonstrating that this is a direct effect [64]. Consistent with these findings, NSP3 (but not a mutant lacking the C-terminal eIF4G-binding region) can inhibit cellular protein synthesis without other rotavirus factors *in vivo* [67] and silencing of NSP3 during infection prevents viral-mediated repression of polyadenylated reporter gene expression [68]. The exclusion of PABP from eIF4G complexes by NSP3 represents a host shut-off mechanism that is so far unique (Figure 3B), but shares some functional similarity with the cellular protein Paip2 that disrupts the interaction between PABP and eIF4G [69]. An attractive dual-function model was proposed in which NSP3 acts to both repress host translation by evicting PABP and to activate viral translation by functionally replacing PABP in end-to-end complexes, via binding to the 3′-conserved sequence and eIF4G [64,70,71]. However, knockdown of NSP3 does not impair viral synthesis [68], and thus further work appears to be required to prove or refute this activation role during infection.

**Activation of viral translation by recruitment of PABP to the DV (dengue virus) 3′-UTR**

Although some viruses that produce non-adenylated RNA target PABP function to disrupt cellular translation, other non-adenylated viruses employ PABP to promote their own translation. DV belongs to the *Flaviviridae* genus of the *Flaviviridae family* (also encompassing the *Hepacivirus* and *Pestivirus* genera) and has a positive-sense RNA genome with a capped 5′-UTR and a non-adenylated 3′-UTR, which is translated as a single polyprotein and subsequently cleaved. DV does not shut off host cell protein synthesis, but can apparently be translated efficiently under conditions where cap-dependent translation is impaired, suggesting that its translation may not be strictly canonical [72,73]. In the absence of a poly(A) tail, the 3′-UTR of DV appears to promote translation [74–76] in a manner analogous to poly(A) tails [75,77]. A stem–loop near the terminus of the 3′-UTR (3′-SL) accounts for approx. 50% of the translational stimulation provided by the 3′-UTR, a function that does not appear to require viral factors [75,77]. Several cellular factors.

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Promote host shut-off, but replicate exclusively in the cytoplasm, different family of enveloped double-stranded DNA viruses, also dependent translation (reviewed in [2,84]). The Poxviridae herpesviruses have evolved mechanisms to maintain efficient cap-dependent translation [83] and, indeed, not betaherpesvirinae trigger a shut-off of host gene expression, [82]). Although alphaherpesviruses and gammaherpesviruses (but not betaherpesviruses) trigger a shut-off of host gene expression, this does not occur at the level of translation [83] and, indeed, herpesviruses have evolved mechanisms to maintain efficient cap-dependent translation (reviewed in [2,84]). The Poxviridae, a different family of enveloped double-stranded DNA viruses, also promote host shut-off, but replicate exclusively in the cytoplasm, in discrete regions termed replication factories, by virtue of their virally encoded DNA and RNA polymerases. Poxvirus mRNA is capped and polyadenylated in the cytoplasm by viral factors and, like herpesvirus mRNA, is thought to be translated via the normal cellular cap-dependent route (reviewed in [85]).

Increased recruitment of eIF4G to the eIF4F complex is observed during infection of quiescent primary fibroblasts with HSV-1 (herpes simplex virus type 1) (an alphaherpesvirus), HCMV (a betaherpesvirus) or VV (a poxvirus) and reactivation from latency of KSHV (Kaposi’s sarcoma-associated herpesvirus) (a gammaherpesvirus) in primary effusion lymphoma cells [86–90]. With HSV-1, HCMV and VV, this may be due, at least in part, to virus-induced inactivation of a cellular regulatory factor, 4E-BP (eIF4E-binding protein) [91] which binds to and sequesters eIF4E, permitting more efficient eIF4E–eIF4G interaction [86–90]. Stimulation of eIF4F assembly would be expected to lead to an increased efficiency of translation initiation, at least under the experimental conditions [86–90]. HCMV and VV infection, but not HSV-1 infection or KSHV reactivation, additionally result in a dramatic increase in PABP recruitment to the eIF4F complex. With HCMV, this may be a reflection of increased abundance of eIF4G, eIF4E and PABP in infected cells [88–90]. How PABP expression is induced and the reason this is restricted to HCMV infection are not yet known. During VV infection, PABP is relocated to viral replication factories [89] where co-ordinated transcription and translation occur [92], possibly resulting in a high local concentration of PABP, underlying its elevated recruitment to eIF4F. Intriguingly, a subset of VV mRNAs possess 5′-poly(A) sequences which have recently been implicated in an eIF4F-independent initiation mechanism in vitro [93], but it is not known whether these sequences recruit PABP. The role of PABP for VV translation is complicated further by reports suggesting that PABP sequestration by small cytoplasmic non-translated polyadenylated RNAs (POLADs) may result in host translation inhibition [94]. How viral translation would escape this process awaits clarification.

Influenza A virus NS1 (non-structural protein 1) binds PABP

Influenza A viruses are enveloped viruses of the family Orthomyxoviridae that contain a segmented single-stranded negative-sense RNA genome. The multifunctional NS1 is an
RNA-binding protein with roles in viral protein synthesis and RNA replication (for an overview of NS1 functions, see [95]).

NS1 specifically stimulates translation of viral mRNAs by binding to conserved sequences in their 5'-UTR [96,97]. Interestingly, NS1 associates with eIF4G and PABP in infected cells and binds both proteins directly in the absence of RNA [98,99]. Distinct domains of the NS1 protein mediate these interactions. Similarly, the NS1- and PABP-binding domains of eIF4G and the NS1- and eIF4G-binding domains of PABP do not overlap and, in contrast with rotavirus NSP3, the relative amounts of eIF4G and PABP proteins that co-purify with NS1 do not change during infection, indicating that NS1 does not displace PABP from eIF4G [99]. Thus NS1 may specifically stimulate viral translation via formation of a heterotrimeric NS1-eIF4G-PABP complex [99]. As influenza virus mRNA is polyadenylated, this may reinforce the formation of a closed-loop mRNA structure conducive to efficient translation initiation (Figure 4B).

INTRACELLULAR RELOCALIZATION OF PABP DURING VIRAL INFECTION

PABP redistribution by the RV (rubella virus) capsid protein

RV is the sole species of the Rubivirus genus of the Togaviridae family [that also includes the Alphavirus genus (e.g. sindbis virus)]. It is a positive-strand RNA virus containing two open reading frames. The capped and polyadenylated genomic RNA is used to translate the replicase proteins and then serves as a template to produce a genome-length minus-strand RNA, which is used as a template for the plus-strand genomic RNA and a subgenomic RNA, from which the structural proteins, including the capsid protein, are translated [100].

The RV capsid protein directly binds a C-terminal region of PABP both in vivo and in vitro [101]. Interestingly, during RV infection, PABP co-localizes with the RV capsid protein to the perinuclear region of the cell. This is also observed when viral capsid protein is expressed alone and therefore probably reflects direct redistribution of PABP by the capsid protein [101]. The effect of recombinant capsid protein on translation was investigated in extracts programmed with a capped reporter mRNA. Capsid protein inhibited translation which was rescued by the addition of recombinant PABP [101]. However, the relevance of these observations remains obscure as the reporter mRNA was not adenylated, although the observed effects may reflect an in vitro ability of PABP to trans-activate [102].

Strikingly, translation of PABP (but not Paip1, eIF4G or eIF4E) is up-regulated when the capsid protein becomes detectable in the cell [101]. This effect may be specific to RV as it was not observed during infection with Sindbis virus [101]. Increased levels of PABP appear at odds with inactivation of its role in translation. Furthermore, RV RNA is itself capped and polyadenylated, thus it appears to be an open question whether capsid-mediated relocalization of PABP is associated with its recruitment to sites of active (viral) translation such as perinuclear rough endoplasmic reticulum, which accumulates at sites of viral synthesis [100], or serves to sequester PABP to inhibit cellular and/or viral protein synthesis.

Nuclear relocalization of PABP during viral infection

Nuclear export of PABP

PABP is a nucleocytoplasmic shuttling protein, which in the absence of extra-physiological perturbations, shows almost exclusive cytoplasmic steady-state localization [103]. The mechanisms governing nuclear import and export of PABP are poorly understood, and PABP contains no apparent canonical nuclear localization or export signals. However, a TD-NEM (transcription-dependent nuclear export motif) [104] was identified in PABP, and transcriptional inhibition leads to PABP nuclear accumulation [103,104]. Nuclear localization of PABP has also been reported to occur upon overexpression of PABP [103] or inhibition of the nuclear export factor CRM1 (chromosome region maintenance 1) in some [105], but not other [104], studies. Interestingly, nuclear redistribution of PABP has been reported to occur during infection with a number of unrelated viruses.

Rotavirus

During rotavirus infection, PABP is redistributed to the nucleus, and NSP3 is required for this effect [106,107]. However, no effect of NSP3 on cellular transcription has been reported, PABP levels during rotavirus infection are not increased and neither does rotavirus infection cause inhibition of the CRM1 export pathway [107].

NSP3 is sufficient to induce PABP relocalization, and mutational analysis showed that this depends on its interaction with eIF4G and potentially on its ability to evict PABP from initiation complexes [64,107] (see above). In addition, PABP nuclear localization requires the interaction of NSP3 with a cellular protein of unknown function, RoXaN (rotavirus X protein associated with NSP3) [107]. RoXaN is an RNA-binding protein and contains a leucine- and aspartate-rich region that functions as a CRM1-dependent nuclear export signal and is also required for NSP3 interaction [107]. NSP3 mutants that fail to bind RoXaN, but are proficient in binding eIF4G, do not relocalize PABP to the nucleus, suggesting that relocalization requires interactions of NSP3 with both eIF4G and RoXaN [107] (Figure 3B). It is unlikely that NSP3 or RoXaN are directly responsible for the nuclear accumulation of PABP since neither protein interacts with PABP. Furthermore, NSP3 is cytoplasmic and RoXaN is partially depleted from the nucleus during rotavirus infection [107]. It has been suggested that RoXaN may play an indirect role in PABP nuclear export and its sequestration in the cytoplasm by NSP3 is necessary to restrict PABP to the nucleus, reinforcing host translational shut-off by ensuring complete removal of PABP from translation complexes [107].

Gammaherpesviruses

Several studies have shown that PABP accumulates in the nucleus during lytic infection with KSHV, a gammaherpesvirus [90,108,109]. PABP relocalization is mediated by the viral SOX (shut-off and exonuclease) protein [109,110]. KSHV SOX and the closely related muSOX (murine SOX, from murine gammaherpesvirus 68) are present in both the cytoplasm and nucleus and have genetically separable activities in host cell shut-off and in processing and packaging of viral DNA [110–112]. Nuclear relocalization of PABP is temporally correlated with SOX expression, and SOX is sufficient to mediate this effect, which specifically requires its host shut-off activity [109].
SOX-mediated host shut-off acts through the degradation of cellular mRNA [112] in a poorly understood process that is associated with mRNA hyperadenylation in the nucleus [109]. Knockdown of PABP shows that it is required for SOX-mediated RNA turnover [109] and its relocalization may contribute to this process. However, PABP is not required for hyperadenylation and its accumulation in the nucleus does not appear to be a consequence of hyperadenylation [109].

PABP relocalization specifically requires the presence of SOX in the cytoplasm; however, it is unclear how relocalization occurs as SOX does not interact directly with PABP [110]. Therefore, like the RoXaN protein, SOX appears not to accompany PABP to the nucleus, but rather engages an indirect mechanism to achieve PABP redistribution. Whether rotavirus and gammaherpesviruses use distinct pathways to relocate PABP or whether there is interplay between RoXaN and SOX awaits clarification. Another KSHV protein may also be involved, since PABP binds to and co-localizes with the K10/10.1 protein in the nucleus of primary effusion lymphoma cells [108]. However, the function of this protein is unknown and thus the significance of this interaction and its relevance to other PABP relocalization mechanisms remain to be discovered.

Finally, with regard to translation, it is not yet clear whether PABP nuclear sequestration, which becomes apparent before mRNA destruction is maximal [109], has any direct consequences for either host cell or KSHV protein synthesis, which are both mRNA destruction is maximal [109], has any direct consequences for either host cell or KSHV protein synthesis, which are both thought to rely upon cap- and poly(A)-dependent translation.

BUNV (bunyamwera virus)

The Bunyaviridae family comprises five genera of viruses with tripartite negative or ambisense RNA genomes [Orthobunya virus (e.g. BUNV), Hantavirus, Nairovirus, Phlebovirus and Tospovirus]. Bunyavirus mRNAs are capped, but lack a poly(A) tail and are efficiently translated in infected cells, whereas host protein synthesis is repressed. Recently, it was shown that infection with BUNV results in nuclear redistribution of PABP at late times in infection, suggesting a mechanism for host cell shut-off [113]. The BUNV capsid protein, N, binds PABP in vivo and in vitro, but does not accumulate in the nucleus with PABP [113]. The mechanism of nuclear retention is not yet clear, but was shown to be less efficient during infection with a mutant virus lacking the non-structural protein NSs [113]. It may perhaps be of relevance that NSs has been implicated in inhibition of transcription by cellular RNA polymerase II [114], but whether this activity inhibits PABP nuclear export, which has been reported to be sensitive to transcriptional repression [103,104], remains to be established.

SUMMARY AND FUTURE PERSPECTIVE

Now that it is clear that PABP is a common viral target, much exciting work lies ahead in elucidating further the purpose that this serves in the life cycle of very diverse viruses. In some cases, such as PABP eviction from eIF4F by rotavirus, it is clear that this elicits a host shut-off effect, but, in many other cases, such as PABP perinuclear redistribution by RV or PABP nuclear localization by rotavirus, gammaherpesviruses or BUNV, it is not yet evident what advantage is gained by these viruses. Common mechanistic themes and, perhaps, cellular factors may underlie some of these relocalization events and further study is likely to shed light on cellular as well as viral processes. Nevertheless, these viruses have very different life cycles, and it is likely that each virus will benefit in a different way. Indeed, similar alterations of PABP, even in related viruses, can elicit different consequences for infection; PABP cleavage by enteroviruses and calcivirus but contribute to host shut-off, but this does not appear to be a consequence of cleavage during HAV infection. In contrast with the previous examples, recruitment of PABP to initiation complexes by influenza virus, HCMV and VV is likely to promote viral mRNA translation directly, although the level to which this occurs, if at all, during infection remains to be established. An important point in this respect is that of mRNA specificity; is PABP recruited to all viral mRNAs or only to a subset? In the latter event, it may be that the difference in PABP recruitment to the total pool of eIF4F that is observed with HCMV, but not other herpesviruses, depends on the number of affected mRNAs. Further analysis of potential viral mRNA-specific PABP recruitment mechanisms is needed to resolve this.

In addition to its activities in mRNA translation and stability, PABP appears to have a direct function in PV RNA replication, and it would not be surprising if other positive-strand RNA viruses have a similar requirement for PABP. Indeed, PABP stimulates RNA synthesis of a member of the Coronaviridae [115], but it has yet to be determined whether this is a direct effect. Although we have discussed the interaction of viruses with mammalian PABP, PABP targeting appears to be more widespread as a member of the Potyviridae, positive-strand RNA plant viruses, appears to require a PABP homologue, which is redistributed by viral proteins, for efficient replication [116]. It will be of interest to see how these observations relate to the topics discussed in the present review. Moreover, it still remains to be determined whether viruses manipulate other members of the mammalian cytoplasmic PABP family, and whether different PABP homologues have distinct roles during viral infection.

In summary, although these observations are intriguing, many important questions remain with respect to the role of PABP in cellular and viral translation. Key facets of PABP function await clarification and observations that multiple viruses target this protein potentially open new avenues for delineating the fundamental roles and mechanisms of PABP action.

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