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The human cytomegalovirus regulatory protein UL69 and its effect on mRNA export

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1. ABSTRACT

One of the characteristic features of herpesviruses is that most of their genes are intronless. Thus, their replication relies on the selective nuclear export of intronless viral mRNAs, which have to compete with the nuclear export of bulk spliced cellular mRNAs. Therefore, the regulation of nuclear mRNA export is crucial for the replication and pathogenesis of herpesviruses. Besides the thymidine kinase transcript of herpes simplex virus type 1, which contains specific sequences to facilitate the nuclear export of intronless mRNA, other *cis*-acting RNA elements for nuclear mRNA export have not yet been identified in the rest of herpesviral intronless mRNAs. Instead, emerging studies show that herpesviruses encode viral mRNA export factors, which interact with components of the major cellular mRNA export pathway, the RNA polymerase II transcription complex and specific splicing factors to selectively export intronless herpesviral mRNAs to the cytoplasm in infected cells. These herpesviral mRNA export factors are members of a conserved gene family that can be found in all herpesviruses. The human cytomegalovirus transactivator protein UL69, which has been demonstrated to belong to this conserved protein family, shares common features with its herpesviral homologues but also possesses unique properties that will be discussed in this review.

2. INTRODUCTION

In eukaryotes, the nuclear export of mRNAs is a stepwise process, which starts with the recruitment of mRNA export factors to the pre-mRNA during transcription followed by the transport of the matured mRNA in a ribonucleoprotein complex to the cytoplasm through the nuclear pores (Figure 1). In metazoans, the major mRNA export receptor is the essential heterodimeric transport receptor TAP-p15, which shuttles between the nucleus and the cytoplasm and directly interacts with nucleoporins providing a link between mRNA and the nuclear pore (1, 2). However, the interaction of TAP with mRNA is likely to be mediated by adapter proteins, which is supported by the observation that TAP exhibits a low affinity for RNA *in vitro*. Genetic screens in yeast have identified the first adapter protein, called Yra1p (REF or Aly in higher eukaryotes), which also shuttles between the nucleus and the cytoplasm as well as it directly binds Mex67p (yeast ortholog of TAP) and RNA with high affinity (3, 4). Yra1p is a member of the REF protein family that is conserved from yeast to human. In different organisms, REFs are encoded by several genes, and the diversity of the gene products is further increased by the expression of multiple splice variants (3). Recruitment of REFs to mRNAs has been shown to depend on the essential mRNA export factor UAP56 (5, 6). In mammalian cells,

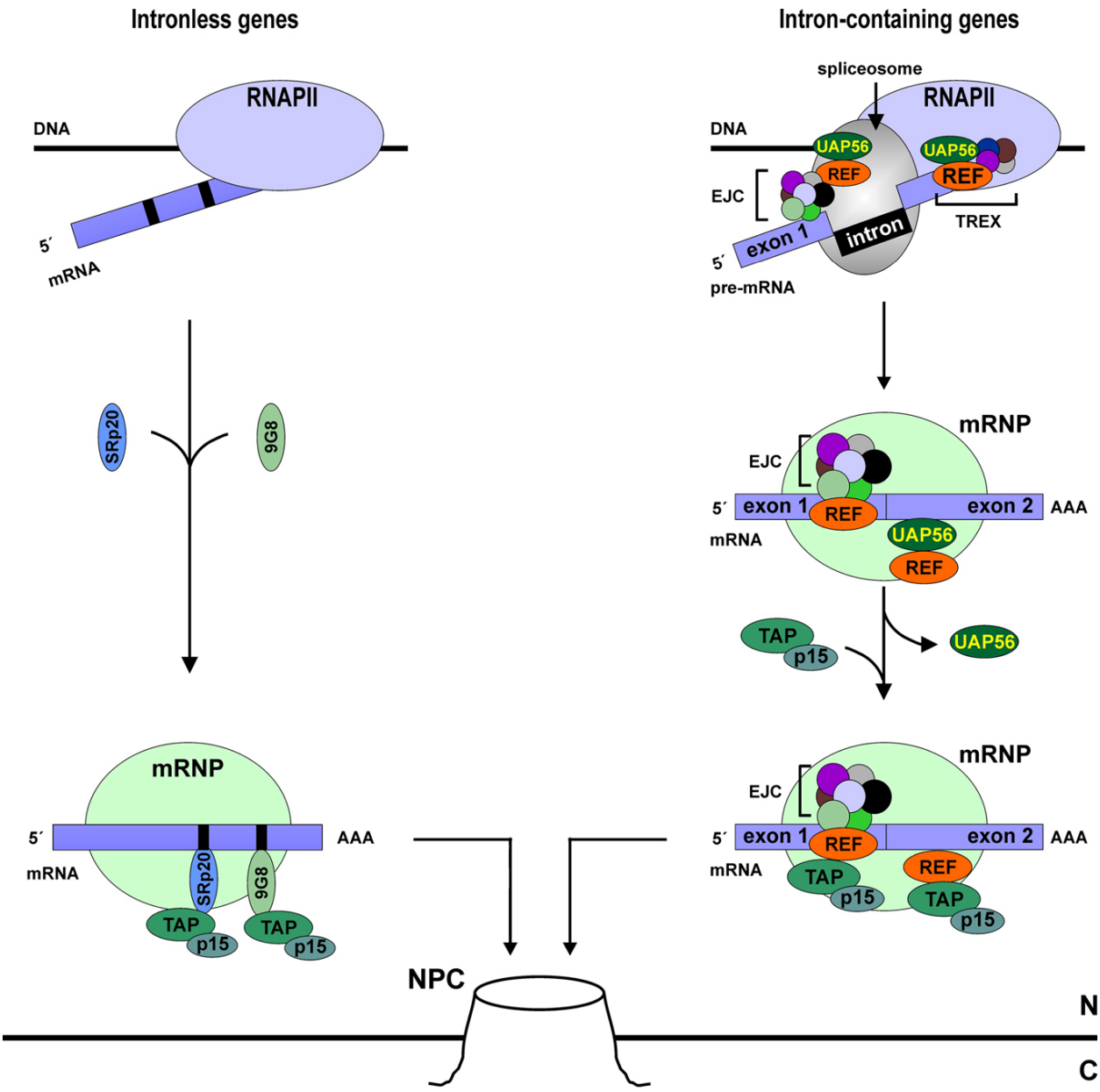

Figure 1. Nuclear export of cellular mRNAs in higher eukaryotes. Transcription generates both intronless and intron-containing pre-mRNAs depending on the structure of the transcribed gene. Subsequently, the properly processed mRNAs are exported to the cytoplasm through the nuclear pores. During splicing of the metazoan pre-mRNA, a protein complex termed the exon junction complex (EJC) is deposited on the spliced mRNAs at a position just upstream of exon junctions. The EJC is believed to serve as a binding platform for mRNA export factors. REF is recruited to the EJC by UAP56, which appears to have roles in splicing and export. After UAP56 leaves the messenger ribonucleoprotein complex (mRNP), REF remains bound to the spliced RNA and interacts with the heterodimeric nuclear export receptor TAP/p15, which mediates the transport of the mRNP to the cytoplasm through the nuclear pores. The mRNA export factors REF and UAP56 can also be recruited to transcripts in a co-transcriptional manner via the so-called transcription and export complex (TREX). In contrast, sequence-specific RNA-binding proteins from the SR protein family seem to mediate the interaction between intronless mRNA and TAP/p15. In the case of histones, the cellular factors SRp20 and 9G8 have been shown to be recruited onto a *cis*-acting RNA sequence of histon mRNAs and directly bind to TAP.

there is another protein, termed URH49, which is >90% identical to UAP56. These proteins seem to have overlapping functions in the processing and nuclear export of mRNAs (7, 8). Interestingly, the binding of UAP56 and TAP to REF appears to be mutually exclusive. Consequently, it was suggested that REF must be released

from UAP56 before interacting with TAP, which then mediates the nuclear export of mRNAs to the cytoplasm (5).

In higher eukaryotes most cellular mRNAs are transcribed from intron-containing genes. Early studies indicated that splicing can enhance the nuclear export of mRNAs (9). Therefore, it was attractive to think that it is splicing, which might recruit the mRNA export factors to most cellular mRNAs. This seems to be supported by the finding that a protein complex is deposited onto the mRNA by the spliceosome near to the exon-exon junction known as the exon-junction complex (EJC), which can serve as a binding platform for export factors (10). EJC proteins also have additional important roles in mRNA processing such as the enhancement of protein translation as well as the destruction of aberrantly processed transcripts (11). Both REF and UAP56 have been shown to interact with components of the splicing machinery and the EJC, thus coupling splicing to mRNA export (see Figure 1). This has been proposed as a mechanism to enhance the nuclear export of RNAs derived from spliced genes (6, 10). However, recent studies suggested that splicing only modestly enhances nuclear mRNA export in mammalian cells but rather, EJC proteins increase the translation of spliced mRNAs (12, 13). Thus, it is still controversial whether or not splicing is required for the nuclear export of spliced mRNAs.

In addition to splicing, recruitment of UAP56 and REF to mRNA was also shown to occur co-transcriptionally (14, 15). Recent studies in yeast revealed an interaction between yeast UAP56 (Sub2p) and the transcription elongation factor Hpr1, which is a component of the transcription elongation complex THO (16). Consequently, it was suggested that THO might participate in the recruitment of UAP56 and REF onto pre-mRNA in yeast. Recently, Mex67p (TAP) has also been shown to be recruited co-transcriptionally by Hpr1 to transcribed genes and might thus be involved in the anchoring of activated genes to nuclear pores (17). THO in conjunction with UAP56 and REF assembles into a multi-protein complex called TREX, which was proposed to couple transcription to mRNA export in yeast. In mammalian cells, a homologous complex to yeast TREX has been found suggesting that this complex is evolutionarily conserved (18). Further studies revealed that, whereas the yeast TREX complex is preferentially recruited to intronless mRNAs via the transcription machinery, the human TREX (hTREX) complex associates with spliced mRNA rather than unspliced transcripts (19, 20). Recruitment of hTREX to intron-containing genes does not appear to require transcription. Consequently, loading of hTREX to spliced mRNA has been suggested to be coupled to transcription indirectly through splicing (19).

Nevertheless, splicing does not appear to be an absolute requirement for the recruitment of mRNA export factors to mRNAs. There are a number of cellular intronless transcripts such as c-jun, some histones and interferons, which are also exported efficiently to the cytoplasm. However, these mRNAs often contain specific sequences, which can recruit export factors independently of splicing. In this case, members of the shuttling SR proteins (e.g., 9G8 and SRp20) serve as adapters between mRNA and TAP (21-23) (Figure 1). Recently, two additional mechanisms have been suggested how the mRNA export factor REF can be recruited to mRNA in a splicing-independent manner: two studies have shown that the cap-binding complex of mRNAs can interact with REF and UAP56 and these interactions seem to be essential for the association of REF and UAP56 with capped intronless mRNAs and the nuclear export of intronless mRNAs (24, 25). Another study reported that binding of the transcription elongation factor Spt6 to RNA polymerase II (RNAII) can provide a co-transcriptional mechanism to recruit REF to mRNAs via the Spt6-interacting protein Iws1 (26).

Viruses replicating in the nucleus often express intronless transcripts, which also have to be transported efficiently to the cytoplasm for translation. For this, several viruses use a similar strategy that requires specific *cis*-acting RNA elements within the intronless transcripts. For example, Hepatitis B virus (HBV) intronless mRNAs contain a sequence, known as posttranscriptional regulatory element (PRE), which facilitates the nuclear export of viral transcripts (27). The majority of genes within herpesviruses are also intronless, but, to date, *cis*-acting RNA elements for mRNA export have only been identified in the thymidine kinase transcript of herpes simplex virus type 1. This RNA sequence, termed pre-mRNA processing enhancer (PPE), enables efficient processing and cytoplasmic accumulation of transcripts derived from a cDNA version of the human beta-globin gene by binding of the cellular protein hnRNP L (28). Recently, hnRNP L was found to associate with REF and TAP suggesting that PPE-containing intronless viral mRNAs also use the major cellular mRNA export pathway (29). Other PPE-like sequences have not yet been found in the remainder of herpesviral intronless mRNAs, which raises the question of how they are committed for nuclear export. Several lines of evidence show that herpesviruses encode a conserved gene family whose protein products function as viral mRNA export factors to selectively transport viral transcripts from the nucleus to the cytoplasm. One of these herpesviral mRNA export factors is the human cytomegalovirus regulatory protein UL69, which will be in the focus of this review.

3. STRUCTURAL PROPERTIES OF THE HUMAN CYTOMEGALOVIRUS PROTEIN UL69 AND ITS HERPESVIRAL HOMOLGUES

The family Herpesviridae is classified into three subfamilies, the Alpha-, Beta- and Gammaherpesvirinae, which is based on their biological characteristics, gene content and sequence similarities. Divergence of these subfamilies might have occured 180-220 million years ago (30). There are several conserved herpesviral genes whose protein products fulfill similar functions in the course of infection. Among these is a family of regulatory factors whose members have been shown to function as post-transcriptional activators that promote the selective nuclear

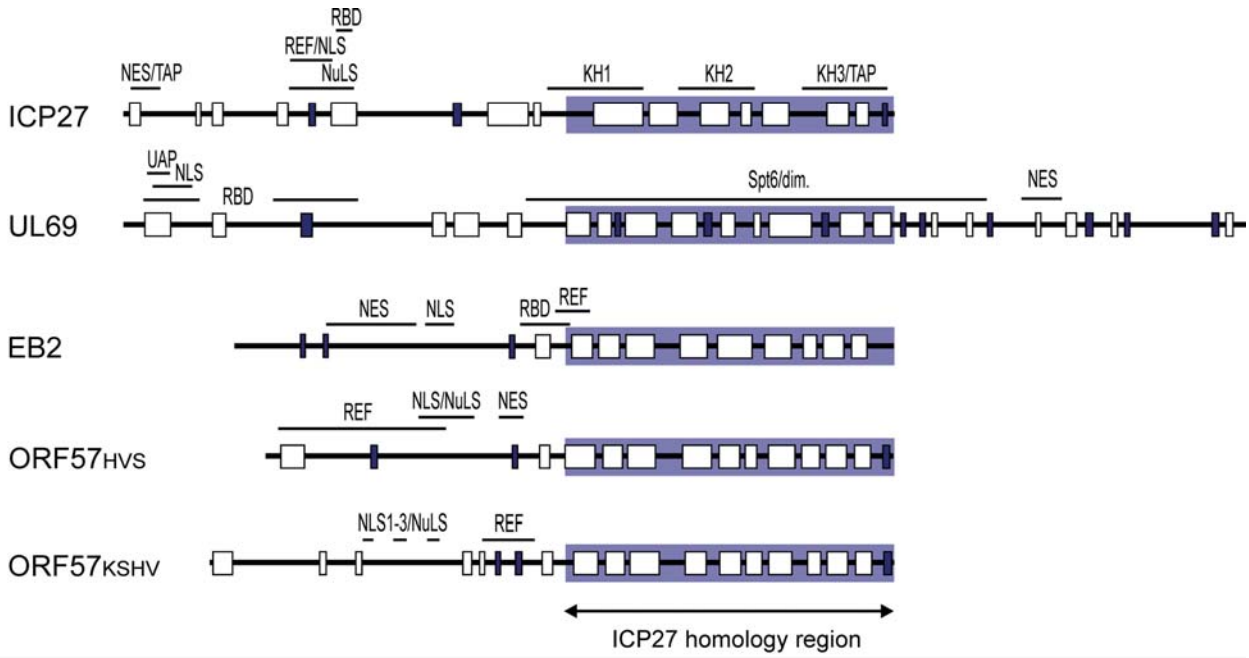

Figure 2. Comparative diagram of the predicted secondary structures and the functional domain organizations of UL69 and its herpesviral homologues. ICP27, viral mRNA export factor of herpes simplex virus type-1 (HSV-1) from the α-herpesvirus subfamily. UL69, RNA export factor of human cytomegalovirus from the β-herpesvirus subfamily; EB2, ORF57HVS, ORF57KSHV, the viral mRNA export factors of Epstein-Barr virus (EBV), herpesvirus saimiri (HVS) and Kaposi´s sarcoma-associated herpesvirus (KSHV), respectively, from the γ–herpesvirus subfamily. The PSIPRED protein structure prediction program (36) was used to create the graphs. The open boxes represent the predicted α helices whereas the shaded boxes stand for the predicted β sheets. The rest of the proteins was predicted to form coiled-coil structures. The ICP27 homology region is indicated by a gray rectangle. NES, nuclear export signal; NLS and NuLS, nuclear and nucleolar localization signal, respectively; RBD, RNA-binding domain; KH1-3, putative KH RNA-binding motifs; dim, dimerization domain; TAP, REF, UAP and Spt6, binding sites of the respective cellular proteins.

export of intronless viral mRNAs in infected cells. This conserved gene family includes the proteins ICP27 of herpes simplex virus type 1 (HSV-1) from the Alphaherpesvirinae, UL69 of human cytomegalovirus (HCMV) from the Betaherpesvirinae, EB2 of Epstein-Barr virus (EBV), ORF57 of Kaposi´s sarcoma-associated herpesvirus (KSHV) and ORF57 of herpes virus saimiri (HVS) from the Gammaherpesvirinae (Figure 2).

Although the amino acid identity among these proteins is low, which ranges from 17% to 36%, they share a conserved homology region having a higher conservation of about 40% sequence identity. This conserved region can be found at the C-terminus of the alpha- and gamma-herpesvirus proteins, whereas it corresponds to the central part of beta-herpesvirus proteins since they have a unique C-terminal region (31). The predicted secondary structure of the herpesviral mRNA export factors shows that the homology region might form a highly structured core domain of the proteins (Figure 2) (32). General features of these viral mRNA export factors are a nucleocytoplasmic shuttling activity, an RNA-binding activity and their propensity to interact with cellular mRNA export factors. Most of these functions can be linked to autonomous sequences or domains of the respective proteins such as a nuclear localization signal (NLS), a nuclear export signal (NES) or an RNA-binding domain (RBD). The proteins consist of 10-15% arginine and lysine residues, which are clustered at the N-terminus. Basic residues are usually a characteristic feature of NLSs and RNA-binding domains, which are often overlapping in viral proteins such as HIV-1 Rev and Tat (33) or HSV-1 ICP27 and HCMV UL69 (Figure 2) (34-37). Protein domains responsible for the interaction with the cellular mRNA export factors (REF, UAP56, URH49 and TAP) can be more extended and often involve several parts of the proteins. For instance, the major TAP-binding region in ICP27 has been mapped to the N-terminus but C-terminal sequences of ICP27 are also needed for the interaction (38). In contrast, the REF-binding domain is restricted to the N-terminus of the proteins and can overlap with other functional domains such as the NLS or the RBD (39, 40). Interestingly, instead of the homology region, the common functional domains essential for mRNA export stimulation of the herpesviral mRNA export factors have been mapped to the N-terminus of the proteins, that shows the lowest amino acid identity and seems to be a poorly folded part of the proteins (Figure 2). Presently, the only common function assigned to the homology region is the self-interaction in case of ICP27 and UL69 (32, 41), whereas unique functions like RNA-binding, mediated by KH-motifs, or splicing inhibition for

ICP27 (42, 43) or Spt6-binding for UL69 (31) can also be coupled to this region (Figure 2).

4. IN AND OUT OF THE NUCLEUS

All members of the ICP27 protein family have been shown to shuttle between the nucleus and the cytoplasm, which is an essential function of viral mRNA export factors. Generally, this transport occurs through interactions between the transport signals NLS and NES of the proteins and import or export receptors that mediate passage through the nuclear pores. In this regard, it was described that UL69 contains an arginine-rich region at the N-terminus, which resembles a bipartite NLS but is different from the identified NLSs of its homologues (44). Indeed, UL69 localizes to the nucleus, but it is excluded from the nucleolus, in contrast to its homologues. Interestingly, in infected cells UL69 appears to accumulate in intranuclear inclusions, which enlarge during the late phase of the replicative cycle of HCMV (45). These intranuclear spots in infected cells are supposed to correspond to viral DNA replication compartments. In agreement with this, UL69 is known to enhance HCMV replication significantly in concert with the major HCMV replication proteins (46). Furthermore, UL69 does not co-localize with intranuclear speckles containing splicing factors such as SC35 and it does not induce the nuclear redistribution of splicing factors, as detected for ICP27. This is consistent with the finding that UL69 has no effect on splicing in contrast to its homologues ICP27 of HSV1 and ORF57 of HVS.

By using a heterokaryon assay, in which HCMV-infected human fibroblasts were fused to murine cells, UL69 has been shown to shuttle between the nucleus and the cytoplasm of infected cells. The nucleocytoplasmic shuttling activity of UL69 can also be detected in the absence of viral infection indicating that this is an intrinsic capability of UL69 (44). Most of the known shuttling proteins use a leucine-rich NES similar to that of HIV-1 Rev, which directly interacts with the CRM1 nuclear export receptor to get transported to the cytoplasm. This transport can be blocked by the antibiotic LMB, which prevents the association of the NES with CRM1 (47). Interestingly, whereas the homologues of UL69, HSV-1 ICP27 and HVS ORF57, possess a leucine-rich NES, which is located within the N-terminal part of these proteins, the 28-amino acid NES of UL69 was mapped within its unique C-terminal region and is not leucine-rich (44, 48, 49). Furthermore, it was shown that the nucleocytoplasmic shuttling activity of UL69 is unaffected by LMB indicating that the nuclear export of UL69 is not mediated by CRM1 (44). Interestingly, despite the presence of leucine-rich NESs within ICP27 of HSV1 and ORF57 of HVS, their nuclear export is not affected by LMB either, suggesting that they also use CRM1-independent nuclear export pathways (40, 50).

Several lines of evidence show that the nucleocytoplasmic shuttling activity of UL69 and its homologues is essential to be able to mediate nuclear mRNA export: (i) transfection experiments showed that nucleocytoplasmic shuttling-deficient UL69 mutants are not capable of stimulating gene expression and cannot facilitate the nuclear export of an unspliced reporter mRNA (44, 51); (ii) in microinjection studies with Xenopus oocytes, ICP27-mediated viral mRNA export could be blocked by an excess of CTE that saturates TAP resulting in retention of ICP27 in the nucleus (52); (iii) similarly, overexpression of a trans-dominant negative form of TAP, which lacks its nucleoporin interaction domain, also retains ICP27 in the nucleus as well as it inhibits the nuclear export activity of HVS ORF57 (40, 50). These results indicate that the UL69 homologues use the major cellular mRNA export receptor to get transported to as well as to carry the target mRNAs to the cytoplasm. Whether this is also true for UL69 remains to be determined since the export pathway used by the unique UL69 NES has not yet been defined.

5. INTERACTION WITH RNA AND CELLULAR mRNA EXPORT FACTORS

Additional features of viral mRNA export factors are their capability to bind to RNA and to interact with cellular proteins involved in mRNA export. Recent studies have shown that UL69 also meets with these requirements (37, 51). For the RNA-binding activity of UL69, three N-terminal arginine-rich motifs R1, R2 and RS are responsible, which show similarity with arginine-rich RNA-binding motifs (ARM) found in a number of viral RNA-binding proteins (37). Interestingly, none of their individual deletions results in a loss of RNA-binding for UL69 but removal of RS in combination with either R1 or R2 abolishes the RNA-binding activity *in vitro* and disrupts the association with RNA *in vivo* (37). Similarly, the EBV viral mRNA export factor EB2 contains an ARM motif to bind RNA (53), whereas ICP27 has an RGG-box RNA-binding domain, which is required for interaction with RNA both *in vivo* and *in vitro* (35, 48). ICP27 includes additional KH-like RNA-binding motifs at its C terminus, which are proposed to be important for association with a subset of mRNAs in HSV-1 infected cells (43). Although there is no evidence for sequence-specific RNA-binding by UL69 and its homologues *in vitro*, several lines of evidence suggest that there must be a mechanism for specific RNA recognition *in vivo*: (i) in reporter assays, UL69 and EB2 display target specificity promoting the nuclear export of heterologous and viral transcripts with various efficiency (37, 54, 55); (ii) to search for ICP27-interacting HSV-1 RNAs, a yeast three-hybrid screen had been performed, which identified 28 transcripts of all kinetic classes. They encode mainly viral proteins required for viral DNA replication and virion maturation (56). (iii) UV-crosslinking experiments using HSV-1 infected cells demonstrated that ICP27 selectively associates with intronless HSV-1 mRNA and not with spliced transcripts (48); (iv) EB2 has also been shown to export a specific set of EBV mRNAs encoding proteins for replication and virion assembly (55, 57); (v) recently, a global analysis of HSV-1 ICP27 and EBV EB2 functions has been performed by DNA microarrays comparing the gene expression pattern of the respective viruses in the presence and absence of these proteins. These studies confirmed and

extended previous findings that only a subset of viral genes is affected by the herpesviral mRNA export factors (58, 59).

Besides containing an RNA-binding domain, the N-terminal end of UL69 mediates the interaction with the cellular mRNA export factor UAP56 and its isoform URH49 (37, 51). Both proteins are essential for cellular mRNA export and are thought to couple mRNA export to transcription and splicing. The interaction between UL69 and UAP56/URH49 was demonstrated both in transfected and infected cells and, importantly, it was excluded that RNA acts as a bridging factor (37). By use of internal deletion mutagenesis the RNA- and UAP56/URH49-binding domains could be separated resulting in either a UAP56- or an RNA-binding deficient UL69 mutant. Subsequently, RNA export assays demonstrated that, whereas the UAP56-binding mutant lost its capability to mediate nuclear export of mRNAs, the RNA-binding mutant retained its mRNA export activity. These results suggest that the binding of UL69 to UAP56 is essential, whereas RNA-binding is dispensable for UL69-mediated mRNA export (37). This finding seems to be contradictory to the assumption that RNA-binding is an essential function for viral mRNA export factors as shown for ICP27 and EB2 (48, 53). In this regard it should be noted that EB2 binds to the cellular mRNA export factors REF and TAP in an RNase sensitive manner suggesting that RNA may be a stabilizing component of the EB2 viral mRNA export complex (39). A similar observation has also been reported for ICP27 and HVS ORF57 (39, 50). In contrast, UL69 forms an RNase insensitive complex with UAP56/URH49, and an RNA-independent interaction is also supported by the observation that an RNA-binding deficient UL69 mutant can still interact with UAP56 and URH49 *in vivo* (37, 51). Furthermore, since the RNA-binding domain overlaps with the UAP56/URH49-binding site within UL69, it might be possible that RNA- and UAP56/URH49-binding to UL69 is mutually exclusive as has been reported for the influenza virus nucleocapsid protein NP. For this protein it was shown that UAP56 promotes the loading of NP onto viral RNAs thus leading to enhanced influenza mRNA synthesis (60). Presently, it is unclear whether RNA-binding of UL69 is needed for the regulation of HCMV gene expression. Alternatively, since UL69 is also a constituent of HCMV virions, RNA-binding by UL69 might be involved in the packaging of RNAs into HCMV viral particles, which incorporate a subset of viral and cellular transcripts in the course of infection (61-63).

In addition to REF-binding, the UL69 homologues have also been reported to interact with TAP either directly or via REF, which has not yet been shown for UL69 (38-40, 50, 64). Consequently, these herpesviral mRNA export factors can directly transport their target mRNAs via the major cellular mRNA export receptor. Early studies using Xenopus oocyte microinjection showed that binding of REF to ICP27 as well as the presence of functional TAP is required for the mRNA export activity of this protein (52). In contrast, controversial results are available for the REF requirement of ICP27-mediated mRNA export in infected cells (38, 40). Since some functional domains such as the REF-binding domain, the NLS and the nucleolar localization signal are overlapping in ICP27, it is difficult to unequivocally conclude from the study of deletion mutants which domain is responsible for the observed defect. Nevertheless, all presently available results point to one general model for the mechanism of the herpesviral mRNA export factors suggesting that they bind to specific intronless herpesviral mRNAs and recruit theses transcripts to the cellular mRNA export pathway via interacting with either REF and TAP or UAP56/URH49 (see Figure 3). The question, however, is still unsolved how the herpesviral mRNA export factors are targeted to viral mRNAs. If specific RNA sequences are not involved, one might speculate that a specific co-transcriptional recruitment during viral transcription takes place.

6. COUPLING BETWEEN VIRAL TRANSCRIPTION AND mRNA EXPORT

Besides the mRNA export function of UAP56/URH49, it is known that UAP56 associates with REF along with the multisubunit THO complex to form the TREX complex, which is involved in the regulation of transcription elongation and mRNA export (18). Although the human TREX complex has been shown to associate with spliced cellular mRNAs rather than unspliced transcripts, HVS ORF57 represents an example for a viral mRNA export factor, which interacts with a modified form of the TREX complex during the nuclear export of intronless HVS mRNAs (Figure 3E) (19, 65). It would be interesting to know whether the TREX complex is directly involved in the transcription regulation of ORF57 target genes and is required for the nuclear export of intronless viral mRNAs, thus coupling viral transcription with the nuclear export of viral mRNAs.

UL69 was shown to transactivate gene expression regulated by various viral and cellular promoters in transient transfection assays (45). To unravel the cellular binding partners that are used by UL69 to activate gene expression, a yeast two-hybrid screen was performed, which then identified Spt6 as an interaction partner of UL69. The interaction between UL69 and Spt6 was both confirmed in transfected and HCMV-infected cells. Importantly, Spt6-binding deficient UL69 mutants lost their transactivation capacity *in vivo*, suggesting that binding of Spt6 to UL69 is essential for the function of UL69 (31).

Spt6 is evolutionarily conserved from yeast to human. Studies in yeast and Drosophila showed that Spt6 co-localizes with elongating RNAPII and is essential for transcription elongation of a subset of genes (66, 67). In addition, Spt6 has been demonstrated to enhance transcription elongation on non-chromatin templates *in vitro* (68). Thus, this protein is considered to be a bona fide transcription elongation factor. Recently, it was reported that Spt6 specifically binds to Ser2-phosphorylated RNAPII and recruits the mRNA export factor REF to target genes via Iws1 that directly interacts with Spt6 (26). Further, blocking Spt6-binding to RNAPII and depletion of Iws1 by RNA interference in cells resulted in a nuclear retention of bulk mRNA (26). Consequently, it was

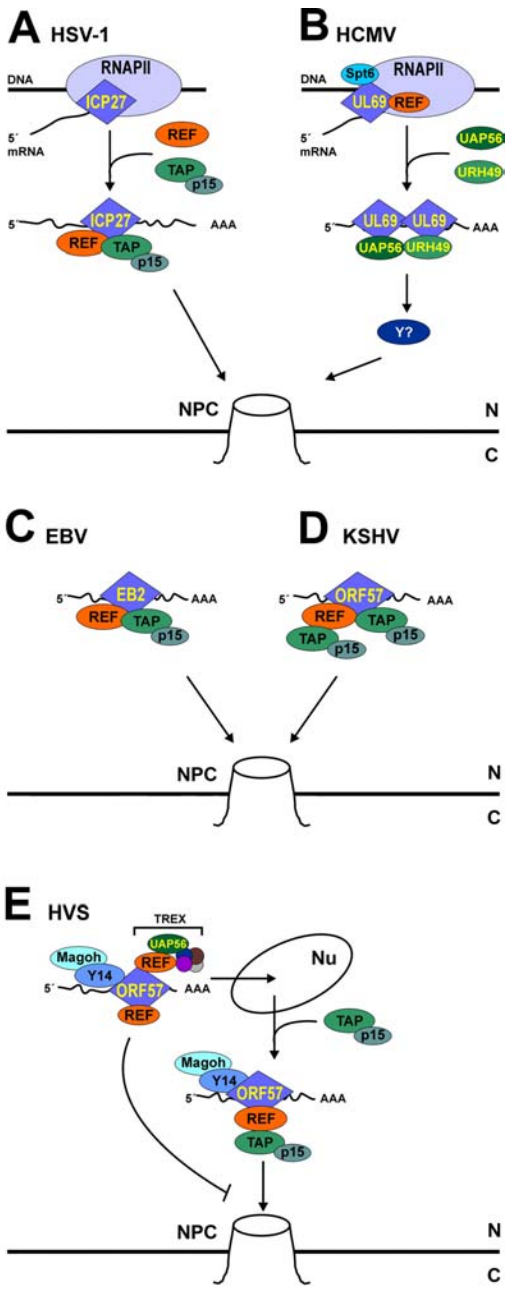

Figure 3. Herpesviral mRNA export complexes. (A) The herpes simplex virus type 1 (HSV-1) mRNA export factor ICP27 mediates the nuclear export of intronless viral mRNAs via interacting with viral mRNAs and the cellular mRNA export factors REF and TAP. (B) The human cytomegalovirus (HCMV) viral mRNA export factor is UL69, which interacts with the cellular mRNA export factors UAP56 and URH49 to promote the nuclear export of inefficiently exported mRNAs. The UL69-mediated viral mRNA export occurs through either TAP/p15 or another nuclear export receptor (Y?) that has not yet been identified. (C) The Epstein-Barr virus mRNA export factor EB2 facilitates the nuclear export of EBV mRNAs via REF and TAP. **(D)** The Kaposi´s sarcoma-associated herpesvirus (KSHV) protein ORF57 also mediates the nuclear export of mRNAs via the cellular mRNA export factors REF and TAP. Although ORF57 can directly bind to TAP, the presence of REF enhances the association of ORF57 with TAP. (E) ORF57 of herpes virus saimiri (HVS) directly binds to REF and indirectly to TAP via REF to facilitate the nuclear export of viral intronless mRNAs. In addition, ORF57 associates with subunits of the exon junction complex such as Magoh and Y14, which might also be required for ORF57-mediated mRNA export. Furthermore, the nucleolar (Nu, nucleolus) trafficking of HVS ORF57 has been shown to be essential for viral mRNA export, which also results in the nucleolar redistribution of components of the TREX complex but their role in HVS mRNA export has not yet been demonstrated.

suggested that Spt6 has a role in mRNA export in such a way that direct binding of Spt6 to RNAPII facilitates the co-transcriptional recruitment of mRNA export factors to transcripts.

Detailed mutagenesis experiments showed that the interaction of UL69 with Spt6 and UAP56/URH49 as well as the binding of Spt6 to RNAPII and UL69 are mediated via separate domains of UL69 and Spt6. Therefore, it would be conceivable that UL69 is co-transcriptionaly recruited to viral genes by binding to Spt6 and then UL69 promotes the loading of UAP56/URH49 onto viral mRNAs thereby targeting them to the cellular mRNA export pathway (Figure 3B). Future studies should address whether Spt6 is required for UL69-mediated mRNA export to support this model. Intriguingly, Spt6 binds to the region of UL69 that is conserved between UL69 homologues, so it would be interesting to investigate whether Spt6 also interacts with other members of this protein family indicating a conserved mechanism by which the herpesviral mRNA export factors could be co-transcriptionally loaded onto viral genes. Besides this scenario, however, there seem to be other ways for co-transcriptional recruitment of herpesviral mRNA export factors. For instance, ICP27 has been demonstrated to interact with RNAPII and facilitates its recruitment to viral transcription sites (Figure 3A) (69, 70), but it is not known whether, once RNAPII is localized to the viral transcription sites, ICP27-binding to RNAPII is required for ICP27-mediated viral mRNA export.

7. CONCLUSIONS

The different herpesviral mRNA export factors cannot or can only partially complement each other in the course of infection indicating that each of these proteins has evolved specific functions that are adapted to the life cycle of the respective herpesvirus (45, 71). In contrast to the nuclear export of retroviral mRNAs, where viral mRNA binds to the nuclear export receptor directly via structured RNA elements or indirectly through a viral adaptor protein, herpesviral mRNA export factors evolved a more elaborated mechanism to facilitate the nuclear export of intronless viral mRNAs. They utilize various constituents of the major cellular mRNA export pathway, of splicing-related complexes such as the EJC and of the RNAPII transcription complex to facilitate the nuclear export of viral mRNAs. Unraveling the mechanism of the herpesviral export factors in viral mRNA transport can also contribute to better understand how the mRNA export pathway is coupled to other mRNA processing events such as transcription and splicing.

In a number of studies it was shown that the herpesviral mRNA export factors are required for efficient replication of the respective herpesviruses, to which their RNA export activity appears to largely contribute. For instance, inactivation of ICP27 or EB2 results in the retention of viral mRNAs that encode essential proteins for viral DNA replication and virion assembly of HSV-1 or EBV, respectively (48, 57, 71, 72). Therefore, understanding the mechanism of how the herpesviral mRNA export factors control the transport of viral mRNAs to the cytoplasm may also advance our knowledge of the pathogenesis of the respective herpesviruses and may reveal novel targets for antiviral therapy.

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