The Challenge of Viral snRNPs

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Some gammaherpesviruses encode nuclear noncoding RNAs (ncRNAs) that assemble with host proteins. Their conservation and abundance implies that they serve important functions for the virus. This paper focuses on our studies of three classes of nuclear noncoding herpesvirus RNAs. (1) EBERs 1 and 2 are expressed by Epstein-Barr virus in latent infection of human B lymphocytes. Recent studies revealed three sites on EBER1 that associate with ribosomal protein L22. In addition, heterokaryon assays have definitively shown that both EBERs are confined to the nucleus, arguing that their contribution to viral latency is purely nuclear. (2) HSURs 1–7 are U RNAs encoded by *Herpesvirus saimiri*, which causes aggressive T-cell leukemias and lymphomas. Comparison of monkey T cells transformed with wild-type or mutant virus lacking HSURs 1 and 2 revealed significant changes in host mRNAs implicated in T-cell signaling. (3) PAN is a 1-kb polyadenylated RNA that accumulates in the nucleus of Kaposi's sarcoma-associated herpesvirus lytically infected cells. A novel element, the ENE, is essential for its high accumulation. Recent results indicate that the ENE functions to counteract poly(A)-dependent RNA degradation, which we propose contributes to nuclear surveillance of mRNA transcripts in mammalian cells. Continuing studies of these viral RNAs will provide insights into both cellular and viral gene expression.

Like cellular genomes, some—but not all—viral genomes encode small regulatory RNAs. In addition to recently discovered microRNAs (Cullen 2006), certain mammalian viruses express abundant ncRNAs of approximately 100 to 1000 nucleotides in infected cells. These viral RNAs avidly bind host proteins to become ribonucleoproteins (RNPs). Their cellular abundance and conservation among related viruses suggest important functions. Have they evolved to make the virus a better pathogen, do they serve to help the virus evade some aspect of the host's response to infection, or both?

The small viral RNAs that have been the focus of our research are all nuclear, thereby forming small nuclear RNPs (snRNPs) (Table 1). They are encoded by gammaherpesviruses that infect primates. Gammaherpesviruses are enveloped viruses with large double-stranded DNA genomes. They are lymphotropic, infecting either T or B cells, with their life cycles characterized by both lytic and latent phases. Despite the fact that the viral ncRNAs we are studying were discovered 10–25 years ago, we still lack a molecular understanding of their roles in infected cells. Recently, however, some progress on these challenging problems has been made and is reviewed here.

EBERS

Epstein-Barr virus (EBV) infection of human B lymphocytes often leads to latency (Kieff and Liebowitz 1990), a state in which most viral genes are not expressed. Among the exceptions are those for two abundant (10^6 /cell) small RNAs (~170 nucleotides), EBER1 and 2 (for Epstein Barr-encoded RNA) (Table 1 and Fig. 1A). The EBERs are localized in the nucleoplasm and quantitatively bind the host La protein via the 3' oligo(U) tracts that terminate their transcription by RNA polymerase III (Lerner et al. 1981).

EBER1 also associates with another host protein, large subunit ribosomal protein L22. This interaction results in a relocalization of the L22 protein from the nucleolus to the nucleoplasm in EBV-positive cells (Toczyski et al. 1994). Previous in vitro binding studies suggested that EBER1 stem-loops III and IV serve as binding sites for L22 (Toczyski and Steitz 1993; Dobbelsstein and Shenk 1995). Recently, we carried out experiments using recombinant L22 protein in electrophoretic mobility-shift assays and found that the EBER1 molecule harbors three L22-binding sites (Fok et al. 2006b). These are the above-

Table 1. Gammaherpesviruses Small Nuclear RNAs

RNA	Virus	Abundance (copies/cell)	Length (nt)	5' Terminus	RNA polymerase	Bound proteins
EBER1	EBV	5×10^6	167	pppA	III	La, rpL22, (PKR) ^a
EBER2	EBV	5×10^6	172	pppA	III	La
HSUR 1,2,5	HVS	10^3 – 10^4	114–143	2,2,7m ³ GpppA ^b	II	Sm (HuR, hnRNP D) ^d
HSUR 3,4,6,7	HVS	10^3 – 10^4	75–106	2,2,7m ³ GpppN ^b	II	Sm
PAN	KSHV	2.5×10^5	1077 ^c	7mGpppA ^b	II	?

Modified from Tycowski et al. (2006).

^aIn vivo binding has not been demonstrated.

^bCaps have 5'–5' linkages.

^cThis value excludes the poly(A) tail.

^dShown in vivo only for HSUR 1 (Cook et al. 2004).

Figure 1. EBER1 and EBER2 are restricted to the nucleus. (A) Secondary structures of EBER1 and EBER2. The stem-loops of EBER1 are denoted with roman numerals and EBER-interacting proteins are positioned at their binding sites (Fok et al. 2006b). PKR binding at stem-loop IV has been proposed by Vuyisich et al. (2002) and McKenna et al. (2006). (B) Lack of EBER nucleocytoplasmic shuttling. Heterokaryons were prepared by fusing HKB5c18 cells (Cho et al. 2002) transfected with a plasmid producing hnRNP A1-GFP and mouse NIH-3T3 cells for 6–7 hours in the presence of cycloheximide (procedures in Fok et al. 2006a). Human (H) and mouse (M) nuclei of the heterokaryons are labeled. Heterokaryons were identified by the shuttling of hnRNP A1-GFP (green, panels 2 and 5) into mouse nuclei, the latter distinguished by punctate DAPI staining (panels 1 and 4). Endogenous EBER1 (panel 3) and EBER2 (panel 6) were detected using fluorescent in situ hybridization (yellow).

mentioned stem-loops III and IV, and a previously undescribed site located in stem-loop I (see Fig. 1A). Interestingly, these EBER1 stem-loops do not share significant sequence similarity; hence, constructing a consensus L22-binding site awaits insights from future structural studies. The existence of multiple L22-binding sites on EBER1 inside cells has been confirmed by *in vivo* UV cross-linking data (Fok et al. 2006b). It will be interesting to determine how many of these regions are required to trigger L22 protein redistribution within the nucleus.

Although both EBERs are abundantly expressed in EBV-infected human B cells, they can be deleted from the viral genome with no apparent deleterious effect on the life cycle of EBV or its transformation potency (Swaminathan et al. 1991). On the other hand, the fact that no EBER-negative EBV strain has yet been isolated sug-

gests that EBERs provide an important function(s) for the virus. Indeed, the expression of EBERs alone in EBV-negative cells promotes tumor formation in SCID mice (Komano et al. 1999; Ruf et al. 2000; Yamamoto et al. 2000). EBERs are also sufficient to induce the expression of interleukin (IL)-9 (Yang et al. 2004), IL-10 (Kitagawa et al. 2000), or insulin-like growth factor I (Iwakiri et al. 2003) in EBV-negative cell lines, leading to enhanced cell growth.

The EBERs were initially proposed to function like adenovirus VA1 RNA by inactivating the dsRNA-dependent protein kinase, PKR (Mathews and Shenk 1991), because they partially support lytic growth of a mutant adenovirus deleted for VA1 (Bhat and Thimmappaya 1983) and bind PKR *in vitro* (Sharp et al. 1993; Vuyisich et al. 2002; McKenna et al. 2006). However, since EBERs appear to be nuclear (Howe and Steitz 1986; Barletta et al. 1993) whereas PKR is largely cytoplasmic (Takizawa et al. 2000), it seemed unlikely that EBERs could combat this host antiviral defense by inhibiting PKR if they truly reside in a different cellular compartment.

We addressed the perplexing issue of EBER localization and its relationship to function by analyzing the potential trafficking of EBERs in EBV-transformed cells *in vivo*. We performed heterokaryon assays to ask whether EBERs transiently move from the nuclear to the cytoplasmic compartment (Fig. 1B). Both in this and in oocyte microinjection assays, EBER1 and 2 were confined to the nucleus, arguing that their contribution to viral latency is purely nuclear (Fok et al. 2006a). In contrast, U1 snRNA molecules in the same heterokaryons shuttled as expected from the nucleus to the cytoplasm and back (Fok et al. 2006a).

Recent results from other labs indicate that EBERs do not inhibit PKR activity *in vivo* when cells are challenged with various PKR stimuli (Ruf et al. 2005; Wang et al. 2005). Thus, it now seems unlikely that the physiological function of EBERs is to inhibit PKR, although their molecular activities remain unidentified. Likewise, whether their roles can be attributed to an active function of the EBER particles or to the sequestering of La, ribosomal protein L22, or some other protein partner is unknown. Perhaps a function of the EBERs is to regulate events downstream of the PKR pathway that are important for EBV growth, which may explain their ability to substitute for VA1 function (Mohr and Gluzman 1996; He et al. 1997). Our current efforts are directed at identifying host proteins in addition to La that bind EBER2 and examining changes in B-cell gene expression when EBERs are stably expressed at high levels.

HSURs

Herpesvirus saimiri (HVS) infects new-world primates and produces either lytic infection or malignant transformation of T lymphocytes. In transformed T cells, the most abundant viral transcripts are seven small RNAs (75–143 nucleotides) encoded by a cluster of genes within the viral genome (Lee et al. 1988; Albrecht and Fleckenstein 1992). HSURs (for *Herpesvirus saimiri* U RNAs) are synthesized by RNA polymerase II using the same distinctive promoter and 3'-end signals as cellular U RNAs. Like most

Figure 3. Genomic organization of the HSURs. (A) Scheme depicting the genomes of wild-type (wt) HVS A-11 and mutant (Δ 2a) HVS A-11 used for in vitro transformation of peripheral blood lymphocytes from the common marmoset. The deletion of a 1379-bp region at the leftmost end of the HVS A-11 genome specifically removes the genes that code for HSURs 1 and 2 (Murthy et al. 1989). The genes for STP-A (*saimiri* transformation-associated protein of strain A) and DHFR (dihydrofolate reductase), as well as the genes encoding the other five HSURs, remain intact in the viral genome. (B) Diagram showing the lentiviral vector used to restore the expression of HSURs 1 and 2 in the T-cell line transformed with the mutant HVS A-11 (Cook et al. 2005). The vector (Li et al. 2003) contains the gene coding for enhanced green fluorescent protein (eGFP) under the control of the CMV promoter, which allows the sorting of GFP-expressing cells after transduction.

Chang et al. 2002). At approximately 2×10^5 to 3×10^5 copies per cell, PAN RNA is by far the most abundant lytic phase transcript, comprising as much as 80% of the polyadenylated RNA in lytically reactivated cell lines (Sun et al. 1996; Song et al. 2001).

The remarkable nuclear abundance of PAN RNA is achieved by high transcription rates, as well as through the action of a 79-nucleotide RNA element called the ENE, which acts posttranscriptionally and is located near the 3' end of the transcript (Conrad and Steitz 2005). The ENE not only functions in the context of PAN RNA, but can significantly increase the nuclear abundance of an otherwise inefficiently expressed intronless β -globin transcript (Collis et al. 1990). The nuclear localization of intronless β -globin RNA containing the ENE can be overcome by tethering any of several export factors to the RNA or by inserting an intron (Conrad and Steitz 2005), suggesting that the ENE is either a weak nuclear retention element or that it stabilizes nuclear transcripts that would otherwise be degraded.

Importantly, the ability of the ENE to enhance the nuclear levels of PAN RNA is dependent on the canonical 3'-end formation machinery, arguing that the ENE either inhibits a poly(A)-dependent decay pathway or enhances 3'-end formation of intronless transcripts (Conrad and Steitz 2005). To examine the effects of the ENE on 3' cleavage in vivo, we compared the activity of the ENE to that of the process-

ing enhancer of the mouse histone H2A gene. This 101-nucleotide-long RNA element increases the abundance of intronless transcripts by enhancing 3'-end formation both in vivo and in vitro, in addition to playing a role in mRNA export (Huang and Carmichael 1997; Huang et al. 1999).

We generated several constructs to compare the activities of the ENE and the H2A element. One has the entire PAN transcribed region (Fig. 4A; WT), one deletes the 79-nucleotide ENE (and 36 nucleotides of downstream sequence) replacing it with a BglII restriction site (Δ 115Bgl), and two have the 101-nucleotide H2A element inserted in the forward (H2A-F) or reverse (H2A-R) orientation into the BglII site. We then performed RNase protection assays (RPA) to examine the relative in vivo levels of properly cleaved transcripts and of uncleaved readthrough (RT) transcripts (Fig. 4) (Conrad and Steitz 2005). A representative RPA (Fig. 4B) and quantitation of the results of four independent experiments (Fig. 4C) show that the inclusion of either the ENE or the H2A element increases the abundance of cleaved transcripts approximately 5- and 8-fold, respectively (Fig. 4B, compare "cleaved" lanes 2–5; Fig. 4C, left panel). The H2A element concomitantly decreases the levels of the RT transcripts approximately 9-fold, whereas the levels of the RT transcripts are unaffected by the ENE or the H2A reverse orientation control (Fig. 4B, compare PAN-RT lanes 2–5;

Figure 4. The ENE does not affect 3'-end cleavage efficiency in vivo. (A) Schematic representation of plasmids and PAN probe used for RPA. Boxed regions are the PAN promoter (light gray) and transcribed region (dark gray), whereas the lines represent vector sequence. Boxed letter E indicates the 79-nucleotide ENE in the WT construct. The ENE was replaced with a BglII site in Δ115Bgl, represented by the "V"-shaped line. The 101-nucleotide H2A element (Huang and Carmichael 1997) was inserted into the BglII site in the forward (H2A-F) or reverse (H2A-R) orientation. (B) Representative RPA result. Arrows demark the positions of the undigested PAN or ORF50 probes, the protected ORF50 product (a doublet), as well as the PAN-RT (readthrough) and cleaved products. (C) Quantitation of four independent RPA experiments with standard deviations. The left and middle panels show the levels of cleaved and RT products, respectively, normalized to the amount of WT cleaved product. Note the difference in scale. The right panel shows the cleaved-to-RT ratios for the other three constructs relative to that for WT. For clarity, the y-axis is presented as a broken scale.

Fig. 4C, middle panel). Thus, although both the ENE and the H2A element increase the cleaved-to-RT ratios (Fig. 4C, right panel), they do so by different means. These data are consistent with previous results concluding that the H2A element enhances 3'-end cleavage (Huang and Carmichael 1997; Huang et al. 1999) and that, in contrast, the ENE increases the stability of the cleaved transcript.

To further investigate a role for the ENE in stabilizing polyadenylated transcripts, we examined the effect of the ENE on transcript decay in vivo and in vitro (Conrad et al. 2006). Indeed, in an in vitro decay system (Ford et al. 1999), the ENE inhibits RNA decay by inhibiting deadenylation, the first step in RNA decay. In vivo, PAN RNA produced through a transcriptional pulse (Loflin et al. 1999b) exhibits two-component exponential decay kinetics, with some transcripts being rapidly degraded ($t_{1/2} \sim 15$ min), while others decay more slowly ($t_{1/2} \sim 4$ hr). Presence of the ENE shifts more PAN RNA or intronless β -globin transcripts into the slowly decaying population. Action of the ENE in vivo, as in vitro, is dependent on the poly(A) tail because the decay of non-polyadenylated transcripts (with their 3' ends formed by U7 snRNP-dependent cleavage, which—like those of histone messages—do not undergo polyadenylation) are not affected by the presence of the ENE. These data argue that the PAN ENE inhibits rapid deadenylation-dependent RNA decay in vivo.

How does the ENE protect transcripts from deadenylation? The predicted secondary structure provides a possible answer to this question. The ENE folds into a

predominantly double-stranded RNA with U-rich segments forming a central bulge (11/12 nucleotides are uridines; Fig. 5A). The structure is reminiscent of box H/ACA snoRNAs, which guide site-specific rRNA modification through hybridization with similarly bulged nucleotides in their pseudouridylation pockets (Kiss 2002). We propose that the U-rich regions of the ENE hybridize to the poly(A) tail of PAN *in cis* to protect it from the exonucleolytic attack (Fig. 5B) that initiates the decay process. In fact, we have demonstrated intramolecular interactions between the ENE and poly(A) tail both for naked transcripts and for protein-bound RNAs generated in transfected cells (Conrad et al. 2006). Most strikingly, we find that the interaction of the poly(A) tail with the ENE (Fig. 5B) is sufficient to interfere with the activity of a purified deadenylation on naked RNA in vitro. Surprisingly, this mechanism may not be unique to the ENE. The 3' UTR of the yeast *EDC1* transcript contains a U-rich sequence (36/38 nucleotides are uridines) that allows the mRNA to evade the normal cytoplasmic deadenylation machinery (Muhlrad and Parker 2005). Thus, we predict that some mammalian transcripts will likely use a mechanism similar to that of the ENE for regulation of mRNA stability. Detailed structure/function analyses are under way to define the minimal requirements for ENE activity with the hope of using bioinformatics to predict candidate ENE-like cellular sequences.

Currently, we are solidifying preliminary data that the rapid decay inhibited by the ENE comprises a surveillance

Figure 5. Predicted ENE structure and model for ENE action. (A) Predicted structure of the 79-nucleotide ENE. A second structure of similar free energy is also predicted, but changes are restricted to the upper part of the stem (nucleotides 911–941), while the lower stem and bulged U-rich sequences are maintained as shown. Numbers refer to the nucleotide position relative to the transcription start site as defined in Song et al. (2001). (B) Possible interactions between the U-rich single-stranded regions of the ENE and the poly(A) tail. G-C base pairs in the adjacent stems might engage in additional A-minor interactions (Nissen et al. 2001). Details are described in the text. The number of As depicted in the poly(A) tail is for illustration only and is not a proposal for its true length.

system for mRNA transcripts in the mammalian cell nucleus. Specifically, when transcripts are retained in the nucleus by the VSV M protein (Faria et al. 2005), their decay—whether or not they contain introns—is counteracted by the ENE. Mutation of an intron's 5'- or 3'-splice site leads to transcript decay, which likewise is overcome by the ENE (Conrad et al. 2006). The ENE thus provides a potent probe for elucidating the mechanism of nuclear RNA surveillance in mammalian cells (Moore 2002), whereas other recent advances are in yeast (Bousquet-Antonelli et al. 2000; Hilleren et al. 2001; Libri et al. 2002; Das et al. 2003; Jensen et al. 2004; Kadaba et al. 2004; Fasken and Corbett 2005; Kuai et al. 2005; LaCava et al. 2005; Milligan et al. 2005; Vanacova et al. 2005; Wyers et al. 2005).

A related, but nonoverlapping, challenge is to elucidate how PAN RNA contributes to the lytic reactivation of KSHV. We are tackling this problem using three approaches. First, we are attempting to construct a PAN knockout virus (Zhou et al. 2002). Although successful knockouts have been made for several KSHV genes (Zhou et al. 2002; Luna et al. 2004; Ye et al. 2004; Xu et al. 2005; Han and Swaminathan 2006), deleting PAN is particularly difficult because its gene partially overlaps those of two other viral transcripts. One of these overlapping transcripts encodes the K7 antiapoptotic factor (Wang et al. 2002), whereas the second is a recently identified 6.1-kb RNA called T_{6.1}, containing a 100-amino acid open reading frame of unknown significance (Taylor et al. 2005). The impact of these shared sequences on the interpretation of a PAN knockout phenotype is an important consideration. Second, we are integrating an inducible PAN expression cassette into the genome of a

human B-cell line to compare how host mRNA levels change in response to PAN overexpression. Perhaps PAN RNA, like HSURs 1 and 2, serves to modulate the levels of certain host transcripts in a way advantageous to the virus. Third, we are using affinity selection to capture a tagged version of PAN RNA transfected into human cells to examine which host proteins associate with PAN *in vivo*. Likewise, a tagged version of PAN RNA will be purified from lytically induced B cells to identify any viral proteins that selectively bind PAN. The functional consequences of the RNA–protein interactions observed represent a further challenge for investigation.

CONCLUSIONS

Synthesizing noncoding regulatory RNAs appears to be an especially effective strategy for a virus struggling to thrive in the hostile environment of a host organism. Because the mammalian immune system specializes in protein recognition, a foreign RNA molecule more readily escapes detection. Particularly during latency, when only a few of many viral genes are expressed, it would be disadvantageous to abundantly express a viral protein. Moreover, because an RNA can be designed to bind multiple host proteins to sequester or inactivate them, encoding an RNA multiplies the potential of the limited number of viral genes expressed during latency.

The best-understood small viral RNA is adenovirus VA1, which combats one branch of the interferon pathway by binding and inhibiting PKR. Adenovirus thereby overcomes the PKR-induced phosphorylation of translation initiation factor eIF2 α that would otherwise shut down protein synthesis in infected cells (Mathews and Shenk 1991). Although it seems unlikely that any of the three sets of gammaherpesviruses snRNPs we are studying act exactly in this way, VA1 RNA provides a powerful paradigm for investigating the functions of other viral ncRNAs.

Another striking feature of viral regulatory RNAs is their variety, even among closely related viruses (Table 1). In the three systems we are investigating, both RNA polymerase III and RNA polymerase II are engaged by the virus to synthesize small RNAs, but in the latter case the transcripts mimic either cellular U RNAs (with distinctive caps and no poly(A)) or mRNAs with poly(A) tails. The different classes of RNPs that assemble on these RNAs could play active roles related to those of their cellular counterparts. Recent investigations have further uncovered the fact that gammaherpesviruses also encode microRNAs (Cullen 2006; Nair and Zavolan 2006), but whether these RNAs serve to regulate the translation of viral or host mRNAs is not yet known. Like all studies of viral infection, novel insights into host cell metabolism may be the most significant outcome of the investigation of viral snRNPs.

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REFERENCES

- Albrecht J.C. 2000. Primary structure of the *Herpesvirus ateles* genome. *J. Virol.* **74**: 1033.
- Albrecht J.C. and Fleckenstein B. 1992. Nucleotide sequence of HSUR 6 and HSUR 7, two small RNAs of herpesvirus saimiri. *Nucleic Acids Res.* **20**: 1810.
- Barletta J.M., Kingma D.W., Ling Y., Charache P., Mann R.B., and Ambinder R.F. 1993. Rapid in situ hybridization for the diagnosis of latent Epstein-Barr virus infection. *Mol. Cell. Probes* **7**: 105.
- Bhat R.A. and Thimmappaya B. 1983. Two small RNAs encoded by Epstein-Barr virus can functionally substitute for the virus-associated RNAs in the lytic growth of adenovirus 5. *Proc. Natl. Acad. Sci.* **80**: 4789.
- Bousquet-Antonelli C., Presutti C., and Tollervey D. 2000. Identification of a regulated pathway for nuclear pre-mRNA turnover. *Cell* **102**: 765.
- Chang P.J., Shedd D., Gradoville L., Cho M.S., Chen L.W., Chang J., and Miller G. 2002. Open reading frame 50 protein of Kaposi's sarcoma-associated herpesvirus directly activates the viral PAN and K12 genes by binding to related response elements. *J. Virol.* **76**: 3168.
- Cho M.S., Yee H., and Chan S. 2002. Establishment of a human somatic hybrid cell line for recombinant protein production. *J. Biomed. Sci.* **9**: 631.
- Collis P., Antoniou M., and Grosveld F. 1990. Definition of the minimal requirements within the human beta-globin gene and the dominant control region for high level expression. *EMBO J.* **9**: 233.
- Conrad N.K. and Steitz J.A. 2005. A Kaposi's sarcoma virus RNA element that increases the nuclear abundance of intronless transcripts. *EMBO J.* **24**: 1831.
- Conrad N.K., Mili S., Shu M.-D., Marshall E.L., and Steitz J.A. 2006. Identification of a rapid mammalian deadenylation-dependent decay pathway and its inhibition by a viral RNA element. *Mol. Cell* (in press).
- Cook H.L., Mischo H.E., and Steitz J.A. 2004. The *Herpesvirus saimiri* small nuclear RNAs recruit AU-rich element-binding proteins but do not alter host AU-rich element-containing mRNA levels in virally transformed T cells. *Mol. Cell. Biol.* **24**: 4522.
- Cook H.L., Lytle J.R., Mischo H.E., Li M.J., Rossi J.J., Silva D.P., Desrosiers R.C., and Steitz J.A. 2005. Small nuclear RNAs encoded by *Herpesvirus saimiri* upregulate the expression of genes linked to T cell activation in virally transformed T cells. *Curr. Biol.* **15**: 974.
- Cullen B.R. 2006. Viruses and microRNAs. *Nat. Genet.* (suppl.) **38**: S25.
- Das B., Butler J.S., and Sherman F. 2003. Degradation of normal mRNA in the nucleus of *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **23**: 5502.
- Dobbelstein M. and Shenk T. 1995. *In vitro* selection of RNA ligands for the ribosomal L22 protein associated with Epstein-Barr virus-expressed RNA by using randomized and cDNA-derived RNA libraries. *J. Virol.* **69**: 8027.
- Fan X.C. and Steitz J.A. 1998. Overexpression of HuR, a nuclear-cytoplasmic shuttling protein, increases the *in vivo* stability of ARE-containing mRNAs. *EMBO J.* **17**: 3448.
- Faria P.A., Chakraborty P., Levay A., Barber G.N., Ezelle H.J., Enninga J., Arana C., van Deursen J., and Fontoura B.M. 2005. VSV disrupts the Rael1/mmp41 mRNA nuclear export pathway. *Mol. Cell* **17**: 93.
- Fasken M.B. and Corbett A.H. 2005. Process or perish: Quality control in mRNA biogenesis. *Nat. Struct. Mol. Biol.* **12**: 482.
- Fok V., Friend K., and Steitz J.A. 2006a. Epstein-Barr virus non-coding RNAs are confined to the nucleus, whereas their partner, the human La protein, undergoes nucleocytoplasmic shuttling. *J. Cell Biol.* **173**: 319.
- Fok V., Mitton-Fry R., Grech A., and Steitz J.A. 2006b. Multiple domains of EBER 1, an Epstein-Barr virus non-coding RNA, recruit human ribosomal protein L22. *RNA* **12**: 872.
- Ford L.P., Watson J., Keene J.D., and Wilusz J. 1999. ELAV proteins stabilize deadenylated intermediates in a novel *in vitro* mRNA deadenylation/degradation system. *Genes Dev.* **13**: 188.
- Golembe T.J., Yong J., Battle D.J., Feng W., Wan L., and Dreyfuss G. 2005. Lymphotropic *Herpesvirus saimiri* uses the SMN complex to assemble Sm cores on its small RNAs. *Mol. Cell. Biol.* **25**: 602.
- Goodrich J.A. and Kugel J.F. 2006. Non-coding-RNA regulators of RNA polymerase II transcription. *Nat. Rev. Mol. Cell Biol.* **7**: 12.
- Han Z. and Swaminathan S. 2006. Kaposi's sarcoma-associated herpesvirus lytic gene ORF57 is essential for infectious virion production. *J. Virol.* **80**: 5251.
- He B., Gross M., and Roizman B. 1997. The gamma(1)34.5 protein of herpes simplex virus 1 complexes with protein phosphatase 1alpha to dephosphorylate the alpha subunit of the eukaryotic translation initiation factor 2 and preclude the shut-off of protein synthesis by double-stranded RNA-activated protein kinase. *Proc. Natl. Acad. Sci.* **94**: 843.
- Hilleren P., McCarthy T., Rosbash M., Parker R., and Jensen T.H. 2001. Quality control of mRNA 3'-end processing is linked to the nuclear exosome. *Nature* **413**: 538.
- Howe J.G. and Steitz J.A. 1986. Localization of Epstein-Barr virus-encoded small RNAs by *in situ* hybridization. *Proc. Natl. Acad. Sci.* **83**: 9006.
- Huang Y. and Carmichael G.G. 1997. The mouse histone H2a gene contains a small element that facilitates cytoplasmic accumulation of intronless gene transcripts and of unspliced HIV-1-related mRNAs. *Proc. Natl. Acad. Sci.* **94**: 10104.
- Huang Y., Wimler K.M., and Carmichael G.G. 1999. Intronless mRNA transport elements may affect multiple steps of pre-mRNA processing. *EMBO J.* **18**: 1642.
- Iwakiri D., Eizuru Y., Tokunaga M., and Takada K. 2003. Autocrine growth of Epstein-Barr virus-positive gastric carcinoma cells mediated by an Epstein-Barr virus-encoded small RNA. *Cancer Res.* **63**: 7062.
- Jensen T.H., Boulay J., Olesen J.R., Colin J., Weyler M., and Libri D. 2004. Modulation of transcription affects mRNP quality. *Mol. Cell* **16**: 235.
- Kadaba S., Krueger A., Trice T., Krecic A.M., Hinnebusch A.G., and Anderson J. 2004. Nuclear surveillance and degradation of hypomodified initiator tRNA^{Met} in *S. cerevisiae*. *Genes Dev.* **18**: 1227.
- Kieff E. and Liebowitz D. 1990. Epstein-Barr virus and its replication. In *Fields virology*, 2nd edition (ed. D.M. Knipe et al.), p. 1889. Raven Press, New York.
- Kiss T. 2002. Small nucleolar RNAs: An abundant group of non-coding RNAs with diverse cellular functions. *Cell* **109**: 145.
- Kitagawa N., Goto M., Kurozumi K., Maruo S., Fukayama M., Naoe T., Yasukawa M., Hino K., Suzuki T., Todo S., and Takada K. 2000. Epstein-Barr virus-encoded poly(A)⁺ RNA supports Burkitt's lymphoma growth through interleukin-10 induction. *EMBO J.* **19**: 6742.
- Komano J., Maruo S., Kurozumi K., Oda T., and Takada K. 1999. Oncogenic role of Epstein-Barr virus-encoded RNAs in Burkitt's lymphoma cell line Akata. *J. Virol.* **73**: 9827.
- Kuai L., Das B., and Sherman F. 2005. A nuclear degradation pathway controls the abundance of normal mRNAs in *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci.* **102**: 13962.
- LaCava J., Houseley J., Saveanu C., Petfalski E., Thompson E., Jacquier A., and Tollervey D. 2005. RNA degradation by the exosome is promoted by a nuclear polyadenylation complex. *Cell* **121**: 713.
- Lee S.I., Murthy S.C., Trimble J.J., Desrosiers R.C., and Steitz J.A. 1988. Four novel U RNAs are encoded by a herpesvirus. *Cell* **54**: 599.

- Lerner M.R., Andrews N.C., Miller G., and Steitz J.A. 1981. Two small RNAs encoded by Epstein-Barr virus and complexed with protein are precipitated by antibodies from patients with systemic lupus erythematosus. *Proc. Natl. Acad. Sci.* **78**: 805.
- Li M.J., Bauer G., Michienzi A., Yee J.K., Lee N.S., Kim J., Li S., Castanotto D., Zaia J., and Rossi J.J. 2003. Inhibition of HIV-1 infection by lentiviral vectors expressing Pol III-promoted anti-HIV RNAs. *Mol. Ther.* **8**: 196.
- Libri D., Dower K., Boulay J., Thomsen R., Rosbash M., and Jensen T.H. 2002. Interactions between mRNA export commitment, 3'-end quality control, and nuclear degradation. *Mol. Cell. Biol.* **22**: 8254.
- Loflin P., Chen C.Y., and Shyu A.B. 1999a. Unraveling a cytoplasmic role for hnRNP D in the *in vivo* mRNA destabilization directed by the AU-rich element. *Genes Dev.* **13**: 1884.
- Loflin P.T., Chen C.Y., Xu N., and Shyu A.B. 1999b. Transcriptional pulsing approaches for analysis of mRNA turnover in mammalian cells. *Methods* **17**: 11.
- Luna R.E., Zhou F., Baghian A., Chouljenko V., Forghani B., Gao S.J., and Kousoulas K.G. 2004. Kaposi's sarcoma-associated herpesvirus glycoprotein K8.1 is dispensable for virus entry. *J. Virol.* **78**: 6389.
- Mathews M.B. and Shenk T. 1991. Adenovirus virus-associated RNA and translation control. *J. Virol.* **65**: 5657.
- McKenna S.A., Kim I., Liu C.W., and Puglisi J.D. 2006. Uncoupling of RNA binding and PKR kinase activation by viral inhibitor RNAs. *J. Mol. Biol.* **358**: 1270.
- Milligan L., Torchet C., Allmang C., Shipman T., and Tollervey D. 2005. A nuclear surveillance pathway for mRNAs with defective polyadenylation. *Mol. Cell. Biol.* **25**: 9996.
- Mohr I. and Gluzman Y. 1996. A herpesvirus genetic element which affects translation in the absence of the viral GADD34 function. *EMBO J.* **15**: 4759.
- Moore M.J. 2002. Nuclear RNA turnover. *Cell* **108**: 431.
- Muhrad D. and Parker R. 2005. The yeast EDC1 mRNA undergoes deadenylation-independent decapping stimulated by Not2p, Not4p, and Not5p. *EMBO J.* **24**: 1033.
- Murthy S.C., Trimble J.J., and Desrosiers R.C. 1989. Deletion mutants of herpesvirus saimiri define an open reading frame necessary for transformation. *J. Virol.* **63**: 3307.
- Myer V.E., Lee S.I., and Steitz J.A. 1992. Viral small nuclear ribonucleoproteins bind a protein implicated in messenger RNA destabilization. *Proc. Natl. Acad. Sci.* **89**: 1296.
- Nair V. and Zavolan M. 2006. Virus-encoded microRNAs: Novel regulators of gene expression. *Trends Microbiol.* **14**: 169.
- Nissen P., Ippolito J.A., Ban N., Moore P.B., and Steitz T.A. 2001. RNA tertiary interactions in the large ribosomal subunit: The A-minor motif. *Proc. Natl. Acad. Sci.* **98**: 4899.
- Ruf I.K., Lackey K.A., Warudkar S., and Sample J.T. 2005. Protection from interferon-induced apoptosis by Epstein-Barr virus small RNAs is not mediated by inhibition of PKR. *J. Virol.* **79**: 14562.
- Ruf I.K., Rhyne P.W., Yang C., Cleveland J.L., and Sample J.T. 2000. Epstein-Barr virus small RNAs potentiate tumorigenicity of Burkitt lymphoma cells independently of an effect on apoptosis. *J. Virol.* **74**: 10223.
- Sharp T.V., Schwemmle M., Jeffrey I., Laing K., Mellor H., Proud C.G., Hulse K., and Clemens M.J. 1993. Comparative analysis of the regulation of the interferon-inducible protein kinase PKR by Epstein-Barr virus RNAs EBER-1 and EBER-2 and adenovirus VAI RNA. *Nucleic Acids Res.* **21**: 4483.
- Shaw G. and Kamen R. 1986. A conserved AU sequence from the 3' untranslated region of GM-CSF mRNA mediates selective mRNA degradation. *Cell* **46**: 659.
- Song M.J., Brown H.J., Wu T.T., and Sun R. 2001. Transcription activation of polyadenylated nuclear RNA by rta in human herpesvirus 8/Kaposi's sarcoma-associated herpesvirus. *J. Virol.* **75**: 3129.
- Sun R., Lin S.F., Gradoville L., and Miller G. 1996. Polyadenylated nuclear RNA encoded by Kaposi sarcoma-associated herpesvirus. *Proc. Natl. Acad. Sci.* **93**: 11883.
- Swaminathan S., Tomkinson B., and Kieff E. 1991. Recombinant Epstein-Barr virus with small RNA (EBER) genes deleted transforms lymphocytes and replicates *in vitro*. *Proc. Natl. Acad. Sci.* **88**: 1546.
- Takizawa T., Tatematsu C., Watanabe M., Yoshida M., and Nakajima K. 2000. Three leucine-rich sequences and the N-terminal region of double-stranded RNA-activated protein kinase (PKR) are responsible for its cytoplasmic localization. *J. Biochem.* **128**: 471.
- Taylor J.L., Bennett H.N., Snyder B.A., Moore P.S., and Chang Y. 2005. Transcriptional analysis of latent and inducible Kaposi's sarcoma-associated herpesvirus transcripts in the K4 to K7 region. *J. Virol.* **79**: 15099.
- Toczyski D.P. and Steitz J.A. 1993. The cellular RNA-binding protein EAP recognizes a conserved stem-loop in the Epstein-Barr virus small RNA EBER 1. *Mol. Cell. Biol.* **13**: 703.
- Toczyski D.P., Matera A.G., Ward D.C., and Steitz J.A. 1994. The Epstein-Barr virus (EBV) small RNA EBER1 binds and relocalizes ribosomal protein L22 in EBV-infected human B lymphocytes. *Proc. Natl. Acad. Sci.* **91**: 3463.
- Tycowski K.T., Kolev N.G., Conrad N.K., Fok V., and Steitz J.A. 2006. The ever-growing world of small nuclear ribonucleoproteins. In *The RNA world*, 3rd edition (ed. R.F. Gesteland et al.), p. 327. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.
- Vanacova S., Wolf J., Martin G., Blank D., Dettwiler S., Friedlein A., Langen H., Keith G., and Keller W. 2005. A new yeast poly(A) polymerase complex involved in RNA quality control. *PLoS Biol.* **3**: e189.
- Viejo-Borbolla A., Ottinger M., and Schulz T.F. 2004. Human herpesvirus 8: Biology and role in the pathogenesis of Kaposi's sarcoma and other AIDS-related malignancies. *Curr. HIV/AIDS Rep.* **1**: 5.
- Vuyisich M., Spangord R.J., and Beal P.A. 2002. The binding site of the RNA-dependent protein kinase (PKR) on EBER1 RNA from Epstein-Barr virus. *EMBO Rep.* **3**: 622.
- Wang H.W., Sharp T.V., Koumi A., Koentges G., and Boshoff C. 2002. Characterization of an anti-apoptotic glycoprotein encoded by Kaposi's sarcoma-associated herpesvirus which resembles a spliced variant of human survivin. *EMBO J.* **21**: 2602.
- Wang Y., Xue S.A., Hallden G., Francis J., Yuan M., Griffin B.E., and Lemoine N.R. 2005. Virus-associated RNA I-deleted adenovirus, a potential oncolytic agent targeting EBV-associated tumors. *Cancer Res.* **65**: 1523.
- Wyers F., Rougemaille M., Badis G., Rousselle J.C., Dufour M.E., Boulay J., Regnault B., Devaux F., Namane A., Seraphin B., et al. 2005. Cryptic pol II transcripts are degraded by a nuclear quality control pathway involving a new poly(A) polymerase. *Cell* **121**: 725.
- Xu Y., AuCoin D.P., Huete A.R., Cei S.A., Hanson L.J., and Pari G.S. 2005. A Kaposi's sarcoma-associated herpesvirus/human herpesvirus 8 ORF50 deletion mutant is defective for reactivation of latent virus and DNA replication. *J. Virol.* **79**: 3479.
- Yamamoto N., Takizawa T., Iwanaga Y., Shimizu N., and Yamamoto N. 2000. Malignant transformation of B lymphoma cell line BJAB by Epstein-Barr virus-encoded small RNAs. *FEBS Lett.* **484**: 153.
- Yang L., Aozasa K., Oshimi K., and Takada K. 2004. Epstein-Barr virus (EBV)-encoded RNA promotes growth of EBV-infected T cells through interleukin-9 induction. *Cancer Res.* **64**: 5332.
- Ye F.C., Zhou F.C., Yoo S.M., Xie J.P., Browning P.J., and Gao S.J. 2004. Disruption of Kaposi's sarcoma-associated herpesvirus latent nuclear antigen leads to abortive episome persistence. *J. Virol.* **78**: 11121.
- Zhong W., Wang H., Herndier B., and Ganem D. 1996. Restricted expression of Kaposi sarcoma-associated herpesvirus (human herpesvirus 8) genes in Kaposi sarcoma. *Proc. Natl. Acad. Sci.* **93**: 6641.
- Zhou F.C., Zhang Y.J., Deng J.H., Wang X.P., Pan H.Y., Hettler E., and Gao S.J. 2002. Efficient infection by a recombinant Kaposi's sarcoma-associated herpesvirus cloned in a bacterial artificial chromosome: Application for genetic analysis. *J. Virol.* **76**: 6185.

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