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Post-Transcriptional Gene Regulation by Gamma Herpesviruses

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Abstract The Epstein–Barr virus (EBV) SM protein is a member of a highly conserved family of proteins present in most mammalian herpes viruses. There is a significant amount of functional and sequence divergence among the homologs encoded by the human herpes viruses, including differences in mechanism of action and varying effects on splicing and transcription. Nevertheless, in those cases where it has been studied, these proteins are essential for lytic replication of the virus. The mechanism by which SM regulates gene expression operates at the level of mRNA stability, processing, and export. SM enhances expression of EBV lytic genes and has both positive and negative effects on cellular gene expression. In addition to enhancing accumulation of EBV gene mRNAs, SM has important effects on cellular mRNAs, altering the host cell gene expression profile to facilitate viral replication. This article describes the current state of knowledge regarding the role of EBV SM in cellular and viral gene regulation and summarizes some of the similarities and differences with the ORF57 homolog from Kaposi's sarcoma-associated herpes virus (KSHV/HHV8). J. Cell. Biochem. 95: 698–711, 2005. © 2005 Wiley-Liss, Inc.

Key words: gamma herpes viruses; mRNA processing; viral replication; Epstein–Barr virus

Herpes viruses encode genes that are specifically expressed during the latent or lytic phases of infection. The switch from latent to lytic replication is heralded by the expression of immediate-early genes, which do not require cellular protein synthesis for their transcription. These are then followed by early genes which encode proteins required for replication of viral DNA, followed by expression of late genes, many of which encode structural virion components. In contrast to cellular genes, the overwhelming majority of the genes that are expressed during lytic replication of the human herpes viruses lack introns. Herpes viruses of all three subtypes, alpha, beta, and gamma, from a variety of host species, all express a multifunctional regulatory protein that enhances expression of these intronless viral genes. In addition, these regulatory proteins affect host splicing and cellular gene expression by a variety of mechanisms. This article will review their mechanism and function in infection by Epstein–Barr virus (EBV) and Kaposi's sarcoma associated herpes virus/human herpes virus 8 (KSHV/HHV8).

The EBV member of this family of regulatory proteins, SM, (also referrred to as BMLF1, Mta, and EB2) is a nuclear RNA-binding phosphoprotein that is essential for lytic EBV replication (Fig. 1). The requirement for SM has been demonstrated by the construction of recombinant EBV in which the SM coding region was interrupted [Gruffat et al., 2002]. Such SM-deleted viruses are able to establish latent infection in epithelial cell lines and immortalize and transform primary B lymphocytes, but are incapable of undergoing complete lytic replication and producing progeny virions when the host cells are transfected with the EBV immediate-early transcriptional activator gene Z or treated with TPA, either of which results in productive lytic replication of wild-type EBV. Recombinant herpes simplex viruses (HSV) deleted for the homologous HSV gene *ICP27* are similarly defective in lytic replication [Sacks et al., 1985].

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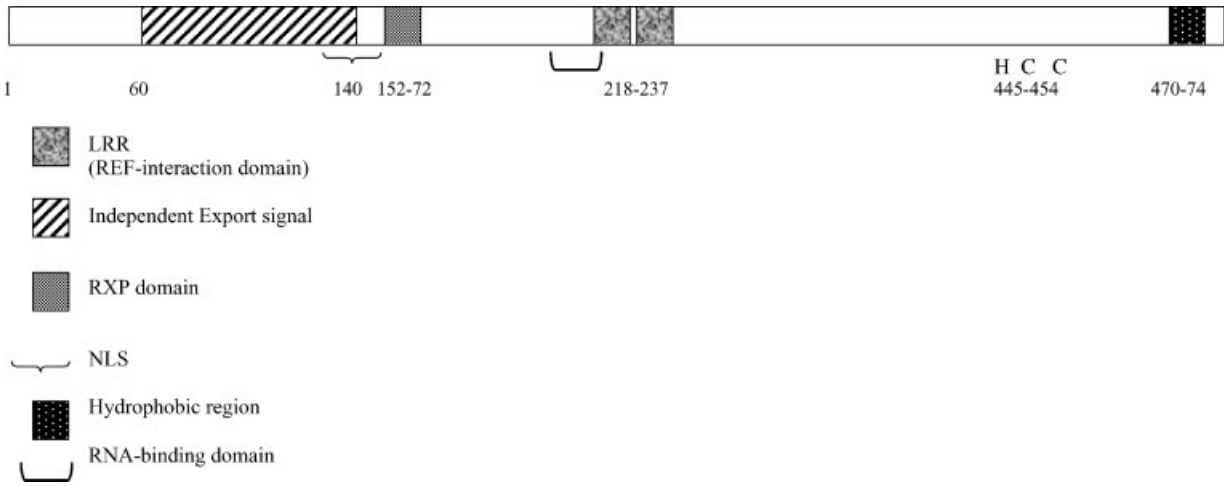

Fig. 1. Structural and functional domains of SM protein. The leucine rich region (LRR) which binds REF/Aly and overlaps with the RNA binding domain is shown containing two potential nuclear export signals (NESs). Another potential NES may be present further upstream. The nuclear localization signal (NLS) lies upstream of the arginine-rich region containing the repeating RXP amino acid motifs. Conserved histidine and two cysteines (HCC) and the hydrophobic region in the carboxy-terminus that are important for proper folding and activity are shown. Amino acid numbers are shown below the diagram.

Interestingly, although the homologs of these genes in HSV, human cytomegalovirus (*hCMV*), *EBV*, and *KSHV* are similar in sequence and behave similarly in transfection assays that measure reporter gene activation, they are different enough in their specific effects that they are quite inefficient in substituting for each other. Thus, transfection of *ICP27* or the *hCMV UL69* genes only minimally rescues replication of SM-deleted EBV, and a recombinant HSV in which SM is substituted for ICP27 replicates several orders of magnitude less efficiently than wild-type HSV [Boyer et al., 2002; Gruffat et al., 2002].

ACTIVATION OF GENE EXPRESSION

The major effect of SM on EBV gene expression is thought to be on activating expression of intronless lytic genes. The lack of introns in many herpesvirus lytic genes poses an intrinsic problem for efficient gene expression. It has long been known that the presence of introns facilitates gene expression and that cDNAs are poorly expressed from vectors in the absence of artificially added introns, although the mechanism underlying this effect was poorly understood [Huang and Gorman, 1990]. Therefore, a common hurdle faced by replicating herpes viruses is the need to express unspliced (intronless) genes efficiently.

SM increases mRNA accumulation of target genes by multiple mechanisms (Fig. 2). Early studies of SM function using reporter plasmids revealed that co-transfection of cloned genomic EBV DNA fragments encoding SM led to increased reporter gene expression [Lieberman et al., 1986; Kenney et al., 1988, 1989a, 1989b; Buisson et al., 1989]. Several studies indicated that gene activation by SM was promoter-independent, and it was initially assumed that the effect was at the level of transcription. Although, one early report suggested that SM did not significantly increase target gene mRNA levels [Kenney et al., 1989a], it is now well established that SM causes an increased accumulation of many mRNAs, including those from reporter genes such as chloramphenicol acetyl transferase (*CAT*) and several early EBV *mRNAs*, [Cook et al., 1994; Gao et al., 1998; Ruvolo et al., 1998]. In the case of some EBV genes, co-transfection of SM leads to more than 50-fold greater expression than that in the absence of SM. Where the effect of SM on transcription has been directly measured using nuclear run-on assays, SM does not increase the rate of transcript initiation [Ruvolo et al., 1998; Nicewonger et al., 2004]. These data clearly established that the major effect of SM is post-transcriptional. SM exerts its effects on target mRNAs by affecting both mRNA stability and nuclear export.

ENHANCED mRNA STABILITY

Due to the difficulty in achieving high efficiency lytic gene expression in most EBV

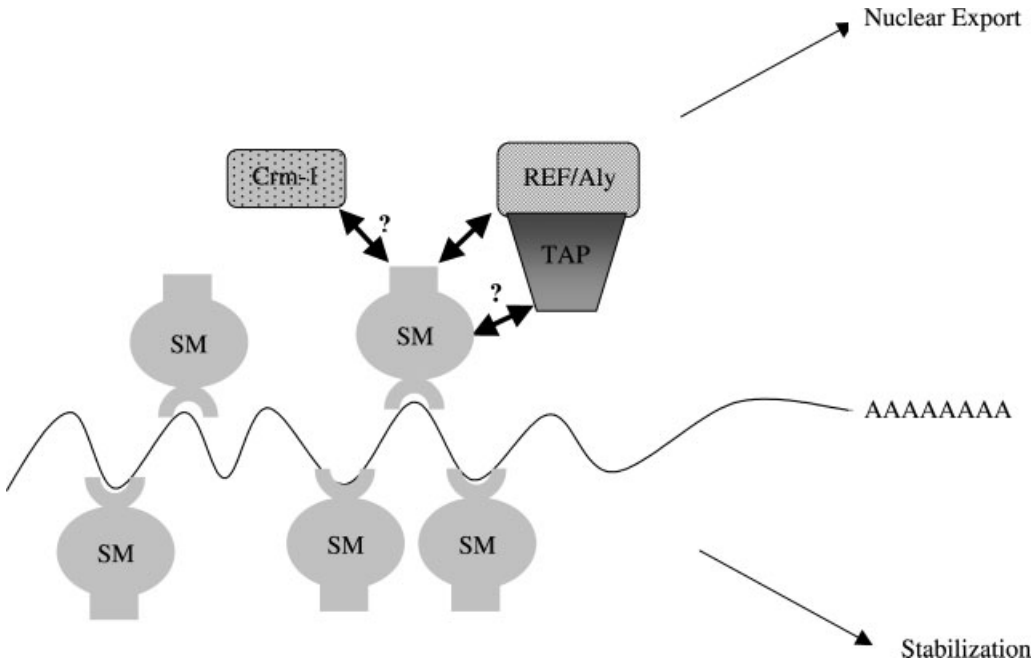

Fig. 2. Enhancement of gene expression by SM. SM is shown binding at multiple sites to an intronless mRNA. SM bound to intronless EBV mRNAs is thought to increase stability of the mRNAs and lead to increased accumulation until the mRNAs are exported to the cytoplasm. Export is facilitated by SM binding to mRNA and interacting with cellular export factors. SM may also exert similar effects on specific cellular mRNAs that undergo splicing.

infected cells in vitro, the majority of evidence regarding SM mechanism derives from transfection experiments. Several targets, including non-EBV genes such as *CAT* and *Renilla luciferase*, as well as EBV genes, have been tested by co-transfection and found to be responsive to SM [Gao et al., 1998; Ruvolo et al., 2001; Nicewonger et al., 2004]. SM leads to increased accumulation of the mRNA transcripts of these genes in the nucleus and the cytoplasm. Increased nuclear stability in the presence of SM appears to be due at least in part, to enhanced 3′ mRNA processing. Exogenous expression of SM leads to increased processing of the EBV DNA polymerase mRNA, which contains a non-canonical poly A signal, and is cleaved and polyadenylated inefficiently [Furnari et al., 1993; Key et al., 1998]. When the 3′ UTR immediately following canonical polyadenylation sequences was replaced with different UTRs from EBV and HSV genes, the effect of SM varied, depending on the specific UTR employed. These data indicate that EBV, like HSV ICP27, may enhance 3′ processing of viral mRNAs [Ruvolo et al., 1998]. Whether SM protects mRNAs from nuclear decay pathways involving deadenylation and nucleolytic degradation remains to be experimentally demonstrated. However, acting in concert with at least one cellular protein, SM increases the half-life of target mRNAs, suggesting that SM protect mRNAs from degradation (see Section on Cellular Effects of SM below).

NUCLEAR mRNA EXPORT

SM, similar to HSV ICP27 and KSHV ORF57 proteins, shuttles between the nucleus and the cytoplasm. When expressed in human cells, SM translocates from human nuclei to mouse nuclei in heterokaryon assays. SM also interacts with components of cellular export pathways and binds RNA. It is therefore likely that SM acts as an export factor facilitating nuclear export of intronless EBV mRNAs that may otherwise be poorly exported to the cytoplasm.

It has recently been shown that during the process of pre-mRNA splicing, mRNA becomes bound by specific cellular proteins at exon–exon junctions which earmark the processed mRNA for export [Luo and Reed, 1999] (for review, see [Dreyfuss et al., 2002]). There are at least six such proteins that comprise a complex, referred to as the exon-junction complex (EJC) that

binds ~20 nt upstream of the exon junction: SRm160, RNPS1, REF/Aly, Y14, magoh, and Upf3 (Fig. 3). The role of these proteins in nuclear export was first suggested by experiments demonstrating that the protein REF/Aly enhanced export of labeled pre-mRNAs which were injected into Xenopus oocyte nuclei [Zhou et al., 2000]. REF/Aly binds to TAP, a central molecule in the nuclear export of vertebrate mRNA [Stutz et al., 2000]. TAP binds to components of the nuclear pore, shuttles from nucleus to cytoplasm and is required for exporting the majority of mRNAs. REF/Aly, magoh, Y14, and Upf3 all can bind TAP and thus may serve as the mediators of TAP recruitment to the mRNP, which enables its export. There are probably other unidentified RNA-binding proteins which can bind to TAP because depletion of the known EJC proteins does not completely block bulk mRNA export whereas TAP is essential [Gatfield and Izaurralde, 2002].

A leucine-rich region (LRR) of SM from 217–236 aa (Fig. 1) has been identified as important in nucleo-cytoplasmic shuttling and SM function. This region has been implicated as involved in interaction with REF/Aly and possibly TAP. GST-REF has been shown to interact with SM synthesized in vitro and this interaction was dependent on the presence of the LRR [Hiriart et al., 2003]. REF and TAP were also co-immunoprecipitated from cells transfected with SM but these interactions were RNA se-sensitive, suggesting that the proteins are part of a ribonucleoprotein complex. The interaction of SM with TAP was not dependent on the presence of the LRR, and the interaction with REF, although reduced, was not completely abolished by deletion of this region, suggesting that association of these proteins with SM is at least partly RNA-dependent.

Another cellular pathway important for 5S RNA and U snRNA export utilizes CRM1

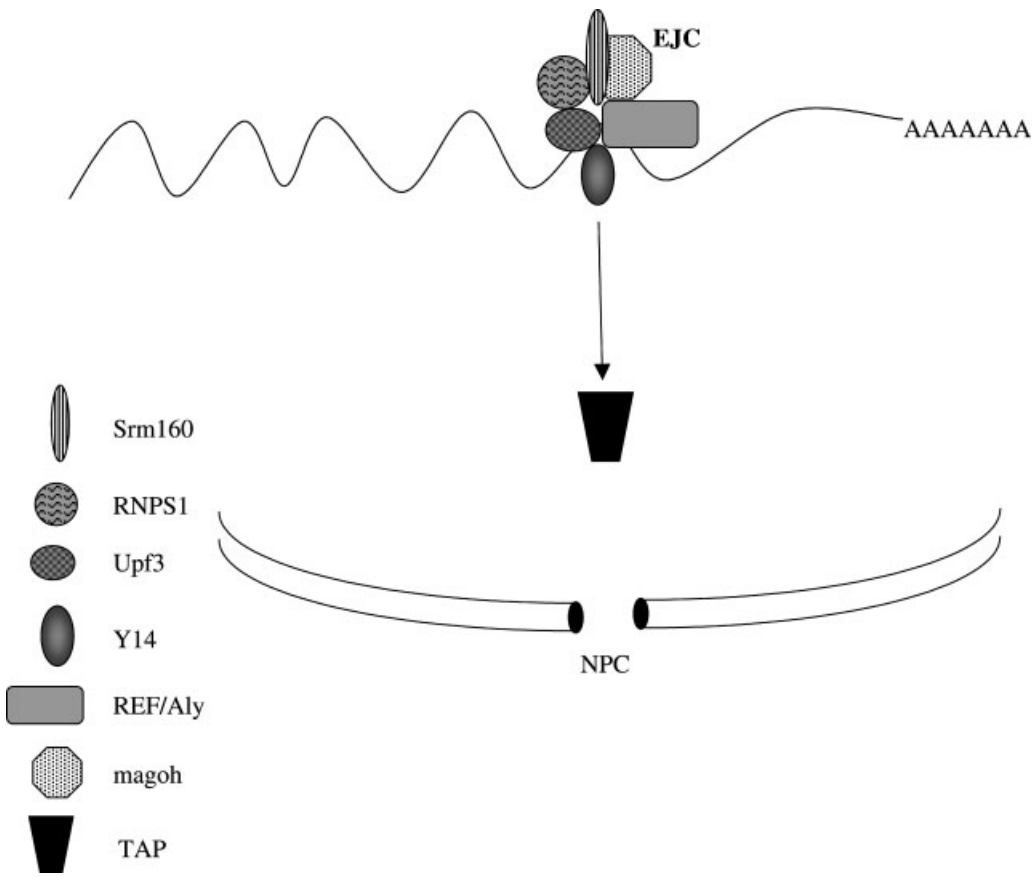

Fig. 3. The exon junction complex and TAP in mRNA export. Six proteins known to associate with mRNA at sites proximal to the exon junction are shown with TAP acting as the essential export factor. One or more of these proteins are likely to be important in recruiting TAP to the spliced mRNA. SM associates with REF and TAP in RNA containing complexes; nuclear pore complex (NPC).

(exportin-1), the first described nuclear export factor [Fornerod et al., 1997; Ossareh-Nazari et al., 1997; Stade et al., 1997]. CRM1 forms a complex with proteins containing a leucine-rich nuclear export signal (NES) and the small GTPase RAN bound to GTP (RAN-GTP) in the nucleus (Fig. 4). CRM1 interacts sequentially with nucleoporins, resulting in translocation through the nuclear pore. In the cytoplasm, RAN-GTP undergoes hydrolysis to yield RAN-GDP, leading to dissociation of the complex, allowing recycling of its components. The prototype of viral hijacking of this pathway is the HIV rev RNA export protein which contains a leucine rich NES, that interacts with CRM1 and allows export of HIV RNAs bound directly to rev (for review, see [Cullen, 2003]).

The interaction of SM with CRM1 is controversial, with two reports suggesting that CRM1 is involved in SM shuttling and RNA export whereas another group failed to confirm such a connection [Boyle et al., 1999b; Farjot et al., 2000; Chen et al., 2001]. The LRR described above contains a motif meeting the general requirements for a CRM1-interacting leucine-rich NES motif ($LX_{2-3}LX_{2-3}LXL$). In our initial studies, overexpression of CRM1 in SM-transfected cells led to a marked translocation of SM to the cytoplasm and SM was co-immunoprecipitated with CRM1. Mutation of

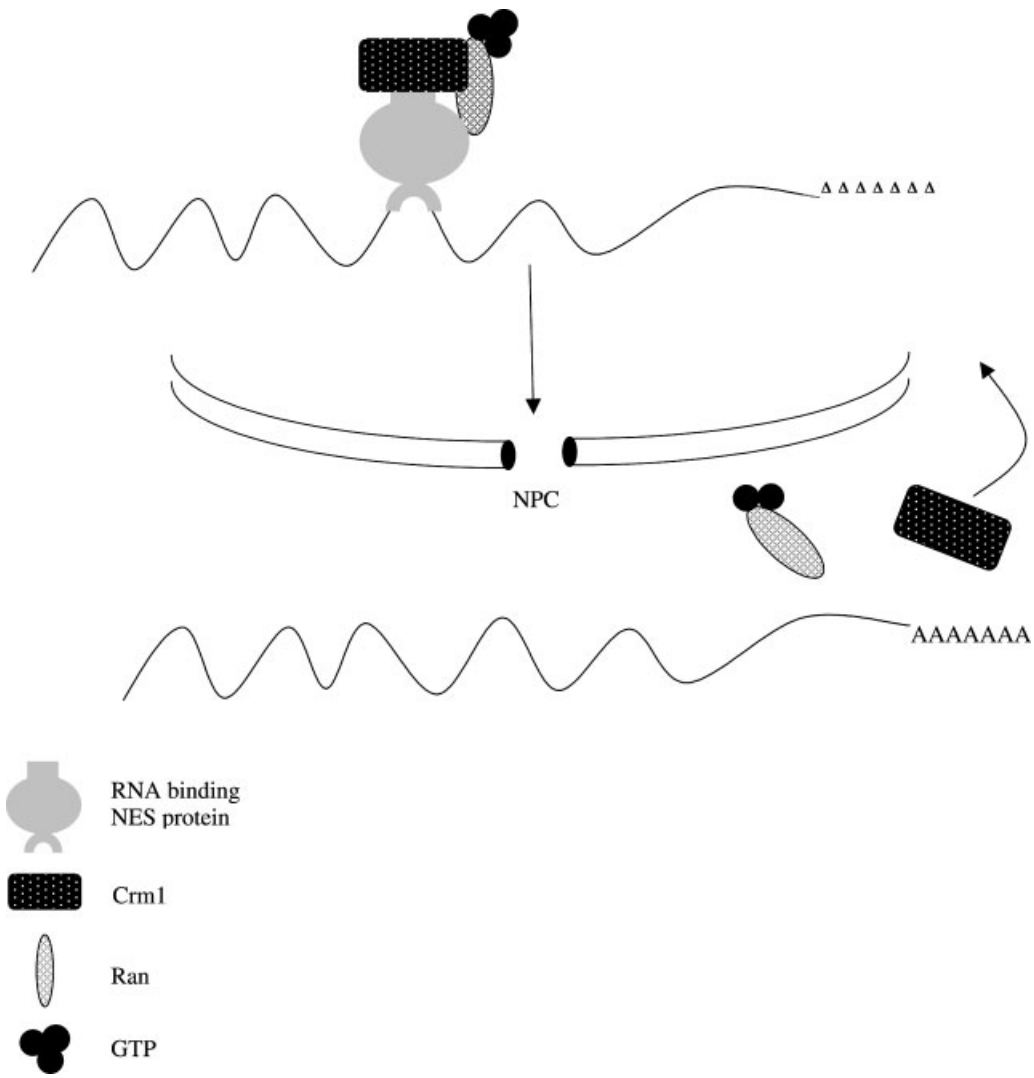

Fig. 4. CRM1 mediated RNA export. CRM1 is shown in association with an RNA binding protein via its nuclear export signal (NES) and RAN-GTP. In the cytoplasm, GTP hydrolysis leads to dissociation of the complex and release of the protein and its bound RNA; NPC.

the core LXL motif or deletion of the entire putative NES led to increased SM accumulation in detergent-insoluble structures, an impaired ability to be translocated to the cytoplasm, and reduced activity in reporter activation assays. Because of the extensive effects of the mutations however, a conclusive role for this domain as a CRM1 interaction region could not be established.

A subsequent study identified an additional region, immediately upstream (218–227 aa), similar to the first potential NES, which may also be involved in nuclear shuttling and mRNA export [Chen et al., 2001]. Mutation of either this upstream sequence (NES2) or deletion of NES1 individually led to dramatic reduction of SM shuttling in a heterokaryon assay and mutation of either sequence also markedly reduced SM-mediated export of EBV BALF2 mRNA. Both sequences were capable of causing cytoplasmic redistribution of a nuclear GFP-tagged reporter protein and the redistribution was sensitive to leptomycin B, a drug that inhibits CRM1 complex formation. These findings are in direct contrast to another study in which almost identical mutations of NES1 did not affect SM shuttling in a heterokaryon assay [Farjot et al., 2000]. Furthermore, the NES1 alone was incapable of substituting for the HIV Rev NES in Rev-mediated RNA export. However, mutations of either of these two motifs did lead to a loss of activity in reporter assays, consistent with the findings of previous studies.

While it is difficult to reconcile some of these contradictory findings, the following picture of SM shuttling and mRNA export emerges. SM is capable of nuclear shuttling and facilitating export of unspliced mRNAs and the region from 218–237 aa is clearly critical for these functions. Whether CRM1 is involved in these functions remain somewhat open to debate although, it is clear that this region does not operate as a classic HIV Rev-type NES. It has been argued that the interpretations of the experiments with LMB are questionable due to the generalized toxic effects of the drug. On the other hand, certain observations suggest an interaction of the CRM1 pathway with SM. Overexpression of CRM1 clearly has effects on intracellular SM localization, CRM1, Ran-GTP, and nup214, a nucleoporin which interacts directly with CRM1, can be co-immunoprecipitated with SM. In addition, dominant-negative fragments of the nucleoporin nup214, which inhibit CRM1 export, also inhibit SM function although other export pathways may also interact with nup214 [Guzik et al., 2001]. One possible explanation for these various findings is that SM may interact with CRM1 indirectly, possibly in specific ribonucleoproteins that include CRM1-binding proteins. Further, a region of SM which appears to directly bind RNA is located immediately adjacent to the LRR, thus raising the possibility that mutations in the LRR may affect RNA binding and thus inhibit the formation of complexes with other RNA-binding proteins.

In the case of HSV ICP27, direct interactions with both REF and TAP have been demonstrated [Chen et al., 2002]. Interestingly, while interaction with TAP appears to be essential for both export and HSV replication, some mutants which have lost the ability to interact with REF are nevertheless viable suggesting that the interaction with TAP may be the most critical. Although it was initially thought that ICP27, which also contains a LRR motif, might interact with CRM1, subsequent studies have not confirmed the association. While export of some HSV mRNAs may be LMB-sensitive, these do not appear to be exported by ICP27 (for review, see [Sandri-Goldin, 2004]).

RNA BINDING

The post-transcriptional effects of SM naturally led to experiments aimed at demonstrating RNA binding by SM protein. Although, an arginine-rich domain consisting of RXP amino acid triplets located in the amino-terminal half of SM does bind RNA in vitro (Fig. 1), it was subsequently demonstrated that deletion of this motif did not affect RNA binding in transfected cells nor did it significantly affect the ability of SM to enhance gene expression or rescue replication of an SM-deleted EBV recombinant [Ruvolo et al., 1998; Semmes et al., 1998; Buisson et al., 1999; Ruvolo et al., 2001; Gruffat et al., 2002]. Another arginine-rich domain, which does not resemble previously defined RNA recognition motifs has been shown to bind RNA in vitro. Deletion of this region impairs the ability of SM to bind RNA and essentially abolishes SM function [Hiriart et al., 2003]. However, no RNA sequence specificity has been demonstrated for either the entire SM protein or the RNA binding region, leaving open the question of how SM might distinguish among various mRNA targets.

GENE-SPECIFIC ACTIVATION

One of the earliest observations and still unexplained aspects of SM function is the specificity of its effect on target mRNAs. SM enhances the expression of certain mRNAs whereas it has little or no effect on other mRNAs [Buisson et al., 1989; Kenney et al., 1989b; Markovitz et al., 1989]. This phenomenon is most clearly obvious with various reporter genes that have been tested in co-transfection experiments with SM. For example, CAT is highly responsive to SM in B lymphocytes, with the levels of CAT protein activity and cytoplasmic mRNA increasing by 10- to 20-fold in the presence of SM. On the other hand, β-galactosidase and firefly luciferase are not responsive to SM in the same lymphocyte cells [Ruvolo et al., 2001]. Similarly, although several of the early EBV genes comprising the DNA polymerase complex, when transfected into EBV-negative cells, are highly dependent on SM for expression, the *BBLF2/3* gene is not [Semmes et al., 1998]. It does not appear that this phenomenon is due to preferential RNA binding since SM associates with both firefly luciferase and CAT mRNAs in vivo despite enhancing expression of CAT but not firefly luciferase [Ruvolo et al., 2001]. No studies to date have identified a specific RNA sequence required to bind SM and most experiments have suggested a relatively non-specific association with mRNA. However, it remains possible that specific mRNAs do, in fact, have a greater affinity for SM in vivo, and non-specific protein RNA interactions have overshadowed the existence of specific associations between SM and RNA as has been shown to occur with other RNA-binding proteins [Schaeffer et al., 2001; Ramos et al., 2003; Darnell et al., 2004]. Recently, HSV RNAs that bind to ICP27 have been identified using a yeast three-hybrid screen [Sokolowski et al., 2003]. The primary sequence of the 31 HSV mRNAs thus identified were quite heterogeneous, suggesting that the element conferring affinity for ICP27 may be a structural motif perhaps similar to the G-quartet identified as the binding site for the FMRP protein [Schaeffer et al., 2001]. A more detailed examination of the binding specifity of SM and related proteins should be highly informative.

Alternative mechanisms to explain gene-specific activation that do not require differential RNA binding are also possible. First, SM by virtue of its effects on stability and export may primarily affect those transcripts that are otherwise inefficiently exported or are unstable. It is also possible that depending on the cellular proteins that decorate a specific mRNA, SM interactions with individual cellular RNA-binding proteins could modulate the expression of cellular genes in a highly specific manner. The relative dependence of various lytic EBV genes on SM for expression remains to be determined but should be simplified with the availability of both SM-deleted EBV recombinants and EBV gene microarrays.

INHIBITION OF SPLICED GENE EXPRESSION

In contrast to its enhancing effect on many unspliced genes, EBV SM inhibits the expression of target genes containing constitutive splicing signals [Ruvolo et al., 1998]. It has been suggested that SM preferentially inhibits splicing directed by weak splicing signals [Buisson et al., 1999]. Introduction of an intron either upstream or downstream of an intronless ORF such as CAT leads to inhibition rather than enhancement of expression by SM [Ruvolo et al., 1998]. Thus, SM has diametrically opposite effects on the same open reading frame depending on the presence of introns. The only cellular gene with its native introns that has been tested in co-transfection assays with SM is the human growth hormone (*hGH*) gene, which contains four introns and five exons within 800 bp. The effect of SM on this gene is dramatic, virtually abrogating hGH expression at the mRNA level [Ruvolo et al., 1998]. In the presence of SM, unspliced hGH mRNA species accumulate in the nucleus [Ruvolo et al., 2004]. These effects of SM on splicing are consistent with reports that SM affects the morphology of speckles containing splicing factor SC35 in transfected cells [Chen et al., 2001]. When measured by microarray analysis, SM expressed in EBV-negative B lymphocytes decreases the amount of the majority of cellular mRNAs [Ruvolo et al., 2003]. While the mechanism of this latter effect has not been proven to be post-transcriptional, it is consistent with SM having a globally repressive effect on cellular splicing, similar to HSV ICP27 [Sandri-Goldin, 1994]. HSV ICP27 alters function of a kinase (SRPK) required for phosphorylation of SR splicing factors that are essential for spliceosome assembly and inhibits splicing during lytic infection prior to the first catalytic step [Bryant et al., 2001; Sciabica et al., 2003].

The mechanisms by which SM affects splicing at the molecular level have yet to be determined. Several mechanisms, which are not mutually exclusive can be envisioned to explain the effect of SM on spliced mRNAs (see Fig. 5). First, SM may bind and inhibit one or more proteins involved in splicing in a manner similar to ICP27. Such a possibility is supported by the finding that specific mutation of C454 to alanine yields an SM mutant relatively unimpaired in the ability to activate reporter genes but does not inhibit splicing of hGH [Ruvolo et al., 2004]. C454 may therefore be important for binding to, and inhibiting the function of a cellular splicing protein. Alternatively, since SM may bind relatively non-specifically to intron-containing as well as intronless species of RNA, SM could inhibit splicing by interacting with unspliced pre-mRNA and preventing access of splicing factors. Whereas binding may protect and enhance export of intronless mRNAs, such binding could have a deleterious effect on the normal pathways of splicing and post-splicing export mediated by the EJC and the TAP pathway.

EFFECT OF SM ON CELLULAR GENE EXPRESSION

Based on these various aspects of SM activity; enhancement of intronless gene expression; a gene-specific mode of action; and an inhibitory effect on splicing of at least some cellular intron-containing genes, a priori prediction of SM effects on specific cellular genes is difficult. The construction of an EBV-negative B lymphoma cell line, BJAB, that expresses a tamoxifen-inducible SM gene has allowed an analysis of the effects of SM on the host cell in the absence of EBV infection [Ruvolo et al., 2003]. SM has a significant growth inhibitory effect on cells within 24 h of expression. The SM-expressing cells do not undergo cell-cycle arrest, however, and do not exhibit any decrease in short-term viability. A comparison of the transcriptional

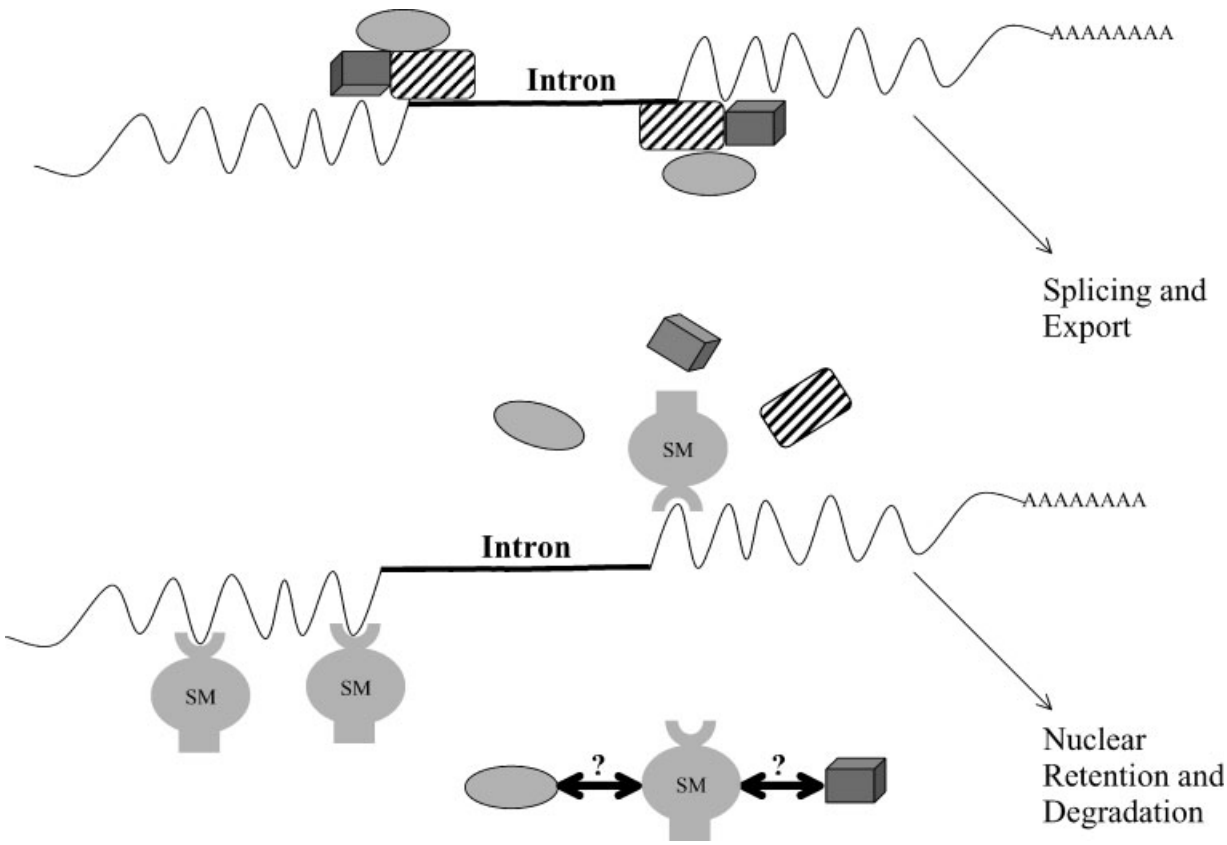

Fig. 5. Splicing inhibition by SM. Normal mRNA splicing leading to nuclear export is diagrammed at top with splicing factors bound to intron–exon junctions. SM is shown binding to pre-mRNA and disrupting formation of splicing complexes, leading to nuclear retention and degradation. SM is also shown possibly binding and sequestering one or more proteins important in splicing.

profile of induced cells expressing SM with cells that were mock induced and not expressing SM protein revealed that expression of the vast majority of cellular genes was either reduced or unchanged.

Surprisingly, of the approximately one dozen genes that were induced by SM, several were known interferon-stimulated genes (ISGs). In addition, STAT 1 mRNA levels were also significantly increased in SM-expressing cells. Since STAT 1 plays a central role in type I IFN signal transduction and *IFN-alfa* and *IFN-beta* are intronless cellular genes, it was possible that SM exerted its effect by increasing type I IFN synthesis. However, IFN-alfa and -beta transcript levels were not detectably increased by SM in microarray analyses, and sensitive ELISAs also did not reveal increased levels of IFN-alfa or -beta in the supernatants of the cells expressing SM. Finally, when SM-expressing cells were co-cultivated with SM-negative cells separated by a semi-permeable membrane, ISGs were not induced in the SM-negative cells, but only in the SM-expressing cells, indicating that a diffusible factor such as IFN does not mediate ISG induction by SM. In fact, the level of ISG induction in the SM-expressing cells exceeded that induced by 1,000 U/ml of type I IFN. While it is possible that small amounts of cell-associated IFNs induced by SM mediate these effects, the most likely explanation is that SM enhances expression of STAT1 mRNA, and thereby leads to induction of ISGs (Fig. 6). Further, the pattern of STAT1 mRNA splicing in the presence of SM differs from that induced by type I IFN treatment. Two isoforms of STAT1 mRNA (STAT1α and STAT1β) are known to be synthesized and are produced by alternative splice-site selection of the final exon [Schindler et al., 1992]. Each of the two isoforms is capable of mediating type I IFN signal transduction when forming an active trimeric complex with STAT2 and IRF9 (p48), but only the STAT1α isoform is thought to be able to form active homodimers that can bind and activate GAS sequences, which mediate IFN-γ signal transduction [Shuai et al., 1992; Muller et al., 1993; Shuai et al., 1993; Shuai et al., 1994]. In SM-expressing lymphoma cells, STAT1β becomes the predominant form of STAT1 expressed, in contrast to the results of IFN treatment. This change in the ratio of STAT1β to STAT1α is likely due to SM altering STAT1 splicing. One potential effect of SM therefore might be to inhibit the ability of the cell to respond to IFN-γ.

While it appears counter-intuitive that induction of an IFN signal transduction pathway by SM would be beneficial for EBV replication, several points are worth noting. First, based on microarray experiments examining the effect of SM on cellular gene expression, it is likely that not every IFN-stimulated gene is upregulated by SM. While several ISGs have been demonstrated to be induced by SM, a comparison of transcriptional profiles of IFN-treated versus SM-expressing cells will be necessary to understand the differences between type I IFN and SM effects on cellular gene expression. Second, the function of many ISGs remain unknown, and it is likely that one or more may be commandeered by incoming viruses to enhance virus replication. Precedent for this hypothesis exists with hCMV, where the viral glycoprotein gB induces ISGs, including viperin (cig 5), a cellular protein which inhibits CMV replication [Zhu et al., 1997; Navarro et al., 1998; Boyle et al., 1999a; Chin and Cresswell, 2001]. However, when induced by hCMV replication viperin is localized differently than when induced by IFN, and it has been proposed that viperin is utilized to facilitate hCMV replication [Chin and Cresswell, 2001]. As described in the following section, SM induces and physically associates with one cellular ISG, which synergizes with SM in enhancing EBV gene expression.

FUNCTIONAL AND PHYSICAL INTERACTIONS OF SM WITH Sp110b

SM has recently been found to physically interact with and induce expression of an interferon-induced protein that is also a component of PML bodies [Nicewonger et al., 2004]. Several ISGs are known to be components of PML bodies, multi-protein nuclear structures usually present at 10–20 per cell (for review, see [Maul et al., 2000]). PML bodies increase in size and number in response to interferon treatment. Infection by several viruses, including HSV, hCMV, and EBV leads to disruption of PML nuclear bodies suggesting that the both viral replication and host defense are linked to the function of the PML nuclear body, which remains poorly understood [Korioth et al., 1996; Everett et al., 1998; Adamson and Kenney, 1999; Bell et al., 2000]. One model of the

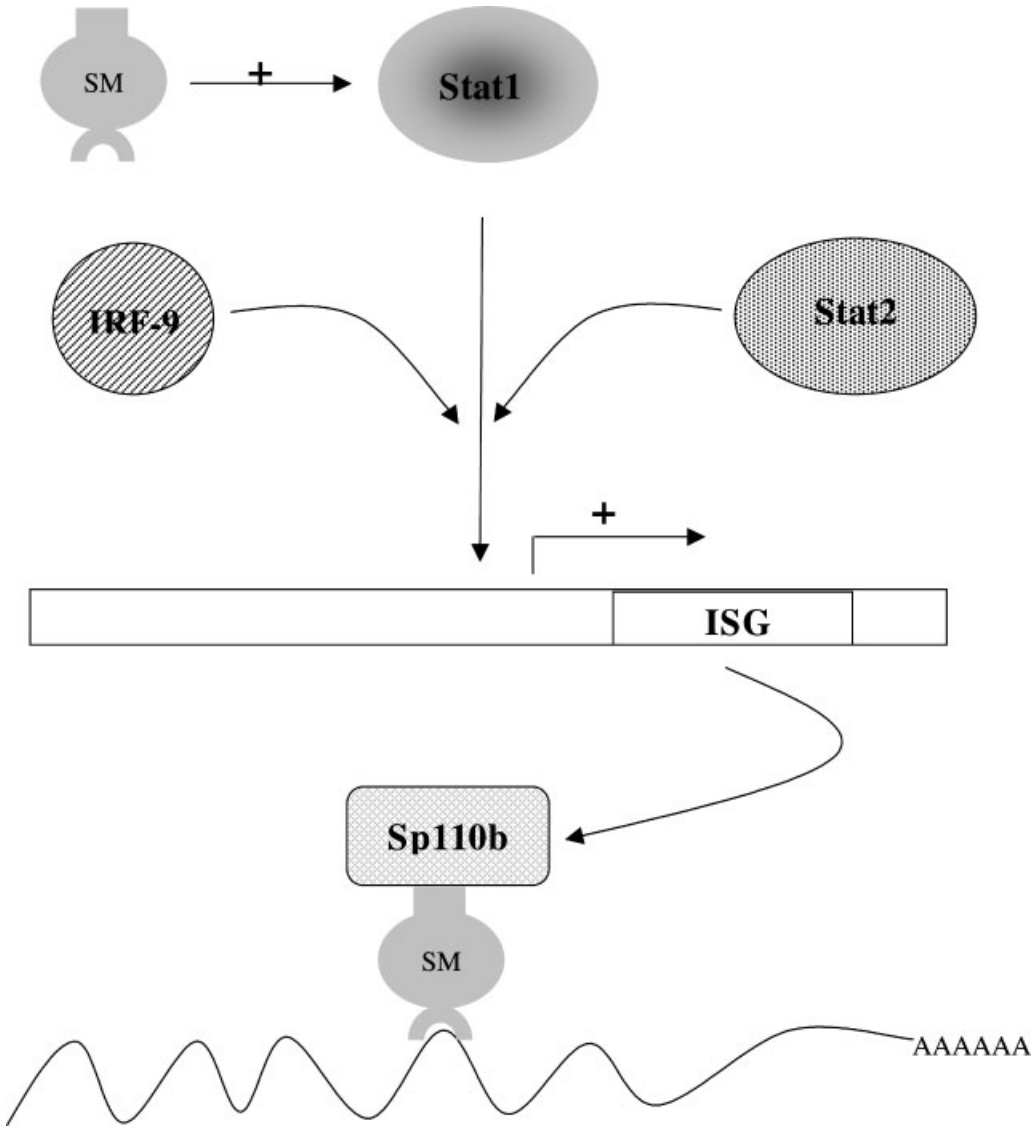

Fig. 6. SM affects cellular gene expression and interacts with cellular gene products. SM is shown increasing cellular STAT1 levels, which leads to transcriptional activation of many interferon-stimulated genes (ISGs) by the trimeric complex composed of STAT1, STAT2, and IRF-9. One such ISG, Sp110b, binds to SM and enhances stability of RNAs bound to SM in the nucleus.

relationship between viral infection and PML bodies is that the PML bodies exert an antiviral function and that their disruption is critical to allow efficient virus replication. A second hypothesis, supported by findings that some protein components of the PML body are retained at sites of virus replication, is that the PML bodies serve as depots of essential factors that the virus must access in order to transcribe or replicate viral DNA.

Sp110b, a cellular protein which is a component of the nuclear body, is induced by interferon and similar to several other ISGs, is also induced by SM [Bloch et al., 2000; Nicewonger et al., 2004] (see Fig. 6). Sp110b is also induced during EBV replication in EBV-infected B lymphoma cells. Importantly, treatment of EBV-negative cells with an agent capable of triggering lytic EBV replication in EBV-positive lymphoma cells, such as anti-IgG, does not lead to Sp110b induction, as it does in EBV-positive cells, indicating that Sp110b is induced by EBV replication itself and not by the induction regimen. Sp110b was initially identified as a SM-binding protein in yeast two-hybrid screens and binds SM in vitro and in vivo. Sp110b binds to SM in an RNA-independent manner via two independent SM-interacting regions.

Sp110b, when co-transfected with SM in reporter assays, synergizes with SM to further enhance expression of *CAT*, *Renilla luciferase*, or *EBV BMRF1* genes although when transfected by itself, it has no effect. This effect is post-transcriptional, and is not dependent on an enhancement of nuclear export, since nuclear as well as cytoplasmic accumulation of the target mRNAs is increased by expression of Sp110b. The synergistic enhancement of SM function by Sp110b has been demonstrated to act at the level of mRNA stability, increasing the half-life of EBV early gene *BMRF1 mRNAs*. Finally, knockdown of Sp110b levels by the use of siRNA, in cells induced to lytically replicate EBV, leads to decreased expression of BMRF1 mRNA, indicating that Sp110b is a functional component of the cellular machinery that SM uses to facilitate EBV lytic gene expression required for EBV replication. These observations, taken together, indicate that Sp110b induction by SM during the course of EBV replication represents one example of an interferon-inducible protein that has been productively co-opted by the incoming virus.

ORF57, SM, AND ICP27 HOMOLOG IN KSHV

The gene homologous to SM in KSHV is known as ORF57, and is similar to SM and ORF57 in H. saimiri, a primate rhadinovirus belonging to the same subfamily as KSHV [Whitehouse et al., 1998; Bello et al., 1999; Goodwin et al., 2000; Gupta et al., 2000; Kirshner et al., 2000]. Much less is known about ORF57, but there are some intriguing differences between SM and KSHV ORF57 which may reflect the different biological behavior and host cell tropism of the two viruses.

KSHV ORF57 has been primarily studied in vitro and it is unknown whether it is strictly required for KSHV replication. ORF57 has been shown to act post-transcriptionally to enhance expression of several KSHV transcripts. ORF57 also shuttles from nucleus to cytoplasm in heterokaryon assays and associates with REF/Aly [Bello et al., 1999; Malik et al., 2004b]. However, it also increases transcription from several KSHV promoters but only in the presence of the DNA binding transcriptional activator ORF50 or Rta, an immediate-early lytic protein critical for initiation of KSHV lytic replication [Kirshner et al., 2000]. A recent study has shown that there may be a direct interaction between ORF57 and ORF50 that leads to enhancement of ORF50 stimulated transcription [Malik et al., 2004a]. The mechanism of this synergistic activity remains to be characterized. Like SM, ORF57 also displays different levels of enhancing activity depending on the target gene and the RNA binding specificity, if any, remains to be determined. The range of KSHV genes which are ORF57-dependent for expression also remains to be determined. In addition, little is known of the effects of ORF57 on cellular gene expression. Although ORF57 does not display potent suppression of host cell splicing or inhibit cell growth in a manner similar to EBV SM, there have been in vitro observations suggesting that ORF57 may enhance cell gene expression [Gupta et al., 2000].

CONCLUSION

Although much has been learned about the function and mechanism of post-transcriptional gene regulation by herpes viruses, many interesting questions still remain to be explored. First, how do proteins such as SM exert gene-specific effects on target genes? Are there structural elements or cis regulatory sequences in certain mRNAs that make them particularly responsive to SM and homologous proteins? Alternatively, the responsiveness of particular genes may reflect the specific complement of cellular proteins that decorate their mRNAs. Thus, specific cellular proteins that interact with SM may confer SM responsiveness to a subset of cellular or viral mRNAs. The answers to these questions may provide opportunities for therapeutic intervention in virus infections and virus-related malignancies.

A second nascent area of investigation is the effect of these viral regulatory proteins on host cell gene expression. While the effect of SM in isolation on some cellular genes has been studied as described above, we still understand little about the role of these proteins in host cell metabolism during viral infection. How important is splicing inhibition in facilitating viral replication? How does alteration of the cell transcriptional profile by SM and its homologs during lytic viral replication affect cellular proliferation and protein synthesis? Comparison of the effects of these proteins on the various cell types that the viruses infect should be very informative, given their gene-specific

mechanism. For example, are there particular endothelium-specific genes whose expression is enhanced by KSHV ORF57 that may be relevant to development of Kaposi's sarcoma? As has been the case for other viral proteins, study of SM, KSHV ORF57 and the other members of this family of regulatory herpes virus proteins has the potential to provide many further insights into the nature of cellular mRNA processing and cellular gene regulation as they relate to oncogenesis.

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