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Split genes and their expression in Kaposi's sarcoma-associated herpesvirus

Zhi-Ming Zheng

HIV and AIDS Malignancy Branch, Center for Cancer Research, National Cancer Institute, Bethesda, MD 20892

SUMMARY

A split or interrupted gene is defined as a gene consisting of introns and exons. Removal (splicing) of the intron (s) from a primary transcript (pre-mRNA) is an essential process to create a mRNA. Initial assignment of a potential protein coding region in KSHV genome was based on initiation codon context and predicted protein size larger than 100 amino acids, but the gene discontinuity was disregarded. Experimental investigation of the assigned ORFs has demonstrated that there are up to 25 split genes, more than one fourth of the total KSHV genes described in KSHV genome. This includes the genes involved in all phases (latent, immediate early, early and late) of KSHV infection. The complexity of a split gene expression depends upon the availability of a proximal promoter and polyadenylation (pA) signal. Sharing a single promoter or a single pA signal by two or three genes is not uncommon in the expression of KSHV split genes and the resulting transcripts are usually polycistronic. Among those of KSHV split genes, 15 genes express a bicistronic or tricistronic RNA and 10 genes express a monocistronic RNA. Alternative RNA splicing could happen in a particular pre-mRNA due to intron or exon inclusion or skipping or the presence of an alternative 5′ ss or 3′ ss. This may, respectively, result in at least 8 species of K8 and 14 species of K15 transcripts. This appears to be related to cell differentiation and stages of the virus infection, presumably involving in viral *cis* elements and *trans* splicing factors.

INTRODUCTION

Kaposi's sarcoma (KS)-associated herpesvirus (KSHV), also called human herpesvirus 8 (HHV8), is a human gammaherpesvirus from KS tissues of AIDS patients that was recently identified using representational difference analysis [1]. Subsequent epidemiological investigations have demonstrated that infection with the newly described KSHV is a etiologically implicated in development of KS, multicentric Castleman's disease (MCD) and primary effusion lymphoma (PEL), also known as body cavity-based B-cell lymphoma [1–4]. KS is a tumour of endothelial cell origin and MCD is a B-cell lymphoproliferative disorder. Recent reports further suggest that KSHV infection might play a role indirectly in the pathogenesis of multiple myeloma [5–7]. Like other gammaherpesvirus such as human B lymphocyte-tropic Epstein–Barr virus (EBV), monkey T lymphocyte-tropic herpesvirus saimiri and murine B lymphocyte-tropic herpesvirus 68, KSHV is another human

Mailing address: HIV and AIDS Malignancy Branch, 10 Center Dr. Rm. 10 S255 MSC 1868, Bethesda, MD 20892-1868, Tel.: 301-594-1382 (O), 301-402-1153 (L), Fax: 301-480-8250, zhengt@exchange.nih.gov.

lymphocyte-tropic virus and usually infects B lymphocytes and establishes latency in the lymphocytes following primary infection. B-cell lines derived from PEL regularly harbour KSHV DNA [3,4]. However, endothelial cells are susceptible to KSHV infection [8–10] and can be converted by KSHV infection into spindle cells seen in KS [9].

KSHV has a genome size of ~165 kb that can encode up to 90 viral proteins [11,12]. Of the 81 open reading frames (ORFs) initially assigned within KSHV's long unique region, 66 are homologous to ORFs in herpesvirus saimiri (HVS) [11], and 63 are homologous to ORFs in murine herpesvirus 68 [13]; only 15 (K1–K15) are unique to KSHV. Recently, several other ORFs unique to KSHV but not described in the initial sequence report on the KSHV genome [11] have been designated with a decimal K number, including K4.1, K4.2, K8.1, K10.1, K10.5, K10.7, K11.1 (vIRF-2) and K14.1 [12,14–16]. Some of the KSHV-expressed gene products are homologues of cellular proteins, including Bcl-2 [17,18], cyclin D [19,20], interleukin 6 [21], MIP-1 [21], G-protein coupled receptor (GPCR) [20], interferon regulatory factor (IRF) [22] and DNA synthetic enzymes including thymidylate synthase, dihydrofolate reductase, DNA polymerase, thymidine kinase and ribonucleotide reductases [23]. Through structural and functional analysis of individual viral genes, recent studies have demonstrated that KSHV, like many other herpesviruses, expresses its genes in a sophisticated way. This includes using both the viral DNA strands for gene expression, having two or three different genes share one promoter or one single polyadenylation (pA) signal, and having the resulting polycistronic pre-mRNAs (RNAs overlapping the coding regions of other RNAs) undergo extensive alternative splicing. The present review summarizes the progress being made in KSHV split-gene studies and emphasizes the regulation of split-gene expression at the post-transcriptional level.

HOW MANY GENES IN THE KSHV GENOME ARE SPLIT GENES?

A split or interrupted gene is defined as a gene consisting of introns (intervening sequences between exons) and exons (segments of an interrupted gene that are represented in the mRNA). Thus, a simple split gene has at least two exons and one intron. Removal (splicing) of the intron(s) from a primary transcript (pre-mRNA) is an essential process for the creation of an mRNA. Thus, defining the exon–intron boundary is the first step to involve the accurate recognition of a 5′ splice site (5′ ss; the junction between the 5′ end of the intron and the 3′ end of the exon) and a 3′ splice site (3′ ss; the junction between the 3′ end of the intron and the 5′ end of the exon) by cellular splicing machinery. Most viral and eukaryotic RNA introns are GU-AG introns (see Figure 4) presumably containing consensus sequences of 5′ ss GURAGU and 3′ ss YNYURAC-Py-AG (R, purine; Y, pyrimidine; N, any nucleotide; Py, polypyrimidine tract).

The initial assignment of a potential protein coding region in the KSHV genome was based on the initiation codon context and the predicted protein in size larger than 100 amino acids [11,12]. Although the studies were milestones in KSHV research, their difficulties in taking account of gene discontinuity based on the inital KSHV genome sequence constituted a weak point in their ORF assignments and underestimated the potential complexity of the gene structure. Experimental investigation of the assigned ORFs has identified up to 25 genes in the KSHV genome as split genes (Table 1). The number of split genes reported so

far is more than one-fourth of the total numbers of KSHV genes described, and includes the genes involved in all phases of KSHV infection (latent, immediate early, early, and late). More KSHV split genes are expected to be found as more careful studies are completed. Among the KSHV split genes, the most complex is K15, which has eight exons and seven introns [24,25].

It remains unclear why there are so many split genes in the KSHV genome. The virus might have been evolved to have many split genes to provide more coding capacity in a compact genome. Alternatively, a genome containing split genes might have more efficient expression [26,27]. In addition, a virus containing more split genes could be more versatile in diversifying its gene expression in response to cell activation and differentiation, which could make it a better pirate for taking over cellular machinery. It has been known for some time that lymphocyte-tropic human herpesviruses including EBV [28,29], HHV6 [30–32] and CMV [33–35], usually have more split genes than dermatotropic herpesviruses (HSV-1, HSV-2 and VZV). With more split genes in their genome, the lymphocyte-tropic viruses, which use lymphocytes as their latent sites, put their gene expression under the regulation by lymphocyte activation and differentiation. KSHV is a B lymphocyte-tropic virus and expression of its genes in B cells resembles that of other lymphocyte-tropic herpesviruses, especially EBV.

EXPRESSION OF KSHV SPLIT GENES BY ALTERNATIVE TRANSCRIPTION INITIATION AND ALTERNATIVE POLYADENYLATION

The molecular events involved in the expression of KSHV split genes are just beginning to be understood. Considerable insight has been gained from studies of similar issues in the related virus EBV. The complexity of split gene expression depends upon the availability of a proximal promoter and pA signal, and upon how each gene is organized in the genome. In general, each gene has a promoter upstream and a pA signal downstream of its ORF. In KSHV, however, not every ORF has an available promoter or pA site immediately upstream or downstream. Expression of these ORFs requires the use of another gene's promoter or pA site far upstream or downstream. The resulting transcription initiation or RNA polyadenylation often produces a bicistronic or tricistronic RNA.

Expression of ORF 50, K8 and K8.1 in lytic infection involves alternative transcription initiation (Figure 1). The KSHV ORF50 (RTA), K8 (K-bZIP) and K8.1 locus encompasses about 5400 nts and is located at map position 0.43–0.46 of the KSHV genome. Although the three genes are positioned in the virus genome side by side, they belong to three different categories of genes, immediate early (ORF50), early (K8) and late (K8.1), and thus are expressed at different stages of the viral lytic cycle by using individual promoters for each gene: promoter P71560 for ORF 50 [36], promoter P74845 for K8 [37] and promoter P75901 for K8.1 [38]. ORF50 (110 kDa) [36] functions as a transactivator of lytic induction, K8α (38 kDa) [39] is a bZIP protein that might relate to viral DNA replication, and K8.1A (35–37 kDa) is a viral envelope glycoprotein responsible for cell attachment [40,41]. Interestingly, the ORF50, K8 and K8.1 transcripts all share a single pA signal at nt 76 714 for their RNA processing (Figure 1) [36–38,42]. Thus, expression of the ORF50 and K8 produces, respectively, a tricistronic and a bicistronic pre-mRNA.

Expression of ORF 73/72/K13 in viral latent phase involves alternative RNA splicing and alternative polyadenylation [43–47]. Three ORFs initiate their transcription from the opposite strand at the same promoter P127880 and produce three transcript isoforms of 5.4, 3.3 and 1.7 kb in size, derived from alternative RNA splicing and alternative polyadenylation. Due to the nature of sharing a single promoter for their transcription, all three RNAs have the same leader exon (Figure 2). The leader exon alternatively splices to a coding exon at nt 127313 30 ss or at nt 123773 3′ ss, producing the 5.4 (from usage of the nt 127313 3′ ss) or the 1.7 kb transcript (from usage of the nt 123773 3′ss). The mechanism by which the alternative splice sites are chosen is unknown. Both transcripts share the same pA signal at nt 122094 [43–45] and co-exist during the latent phase of KSHV infection. A minor form of the 3.3 kb transcript that uses the nt 127313 3′ ss is derived from alternative usage of a non-canonical pA signal (AGUAAA) at nt 124061 (Figure 2) (Canham M and Talbot SJ, personal communication). How this non-canonical pA signal is selected in latent KSHV infection remains to be investigated.

Due to the nature of such gene structures, regulation of both transcription and RNA processing are common scenarios for expression of these genes and such regulation is closely related to activation and differentiation of the host cells. As discussed above, not all exons in a split KSHV gene have coding potential. Some are leader exons preceding a coding exon, as can be found in the ORF 73/72/K13 [43–46] and K5 transcripts [48]. Others are terminal exons that make the transcripts accessible to a proximal pA signal for RNA processing. The latter can be found in the transcripts of ORF50 and K8 [38,49].

EXPRESSION OF KSHV SPLIT GENES BY ALTERNATIVE RNA SPLICING

In addition to alternative promoter and pA site usage, alternative RNA splicing is another common way to control expression of KSHV split genes. Alternative RNA splicing in the expression of KSHV split genes occurs in all phases of the virus life cycle, as was seen with ORF50, K8 and K8.1 transcripts. Alternative splicing of those transcripts may result in at least 19 species of spliced products (Figure 1). As discussed above, the KSHV ORF 50, K8 and K8.1 genes all share a common pA signal at nt 76714 downstream of the K8.1 coding region, but each gene has its own promoter (Figure 1) [36–39]. As a result, a transcribed ORF 50 pre-mRNA transverses the K8 and K8.1 coding regions and is tricistronic with five exons and four introns (RNA D), whereas a K8 pre-mRNA transcript overlaps the K8.1 coding region and thus is bicistronic with four exons and three introns (RNA L) (Figure 1). Interestingly, intron 3 of the ORF50 transcript (RNA D) or intron 2 of the K8 transcripts (RNA L) could be either spliced out or retained as part of exon 3 of ORF 50 (RNA C) or exon 2 of K8 (RNA K). Exclusion of this intron produces an α form of the mRNAs and inclusion of the intron creates a β form. However, the α forms of the K8 mRNAs are dominant in chemical-activated KSHV-positive cells and encode a large amount of the K-bZIP protein. Recent studies on splicing of the K8 pre-mRNAs showed that the β form of the message might be the precursor of the α message, since splicing of intron 2 of K8 requires removal of intron 3 [38]. In other words, selection of the nt 75838 5′ ss activates splicing of the intron immediately upstream. More importantly, exon 3 of the K8 has three 5′ ss, at nt 75838, nt 76155 and nt 76338. Selection of the nt 75838 5′ ss dictates production of K8 α mRNAs and overwhelms the K8 pre-mRNA processing. However, alternative

selection of the other two 5′ ss downstream of the nt 75838 5′ ss is feasible and leads to production of two additional bicistronic mRNAs: K8/K8.1α and β. The selection of these alternative 5′ ss in combination with the inclusion or exclusion of intron 2 results in the production of eight different K8 RNA species. Since all primary ORF50 transcripts transverse the K8 region, theoretically, at least eight other species of ORF50 RNA might exist. However, exon 2 of the ORF50 pre-mRNA has a size of 2751 nts. As an internal exon, it is defined by cross-talk of a 3′ ss and a 5′ ss over the exon [50]. According to current exon definition, an oversized internal exon (>500 nts) will be difficult for the cellular splicing machinery to recognize [51].

KSHV K8.1 transcription does not initiate from its promoter, P75901, until the late stage of the virus life cycle. The transcribed pre-mRNA shares its 3′ terminal exon with ORF50 and K8, but uses a 3′ terminal intron of ORF50 and K8 as its own exon 1. Compared with the ORF50 and K8 transcripts, the K8.1 pre-mRNA is much simpler and has only two exons and one intron. However, its exon 1 has two 5′ ss positioned at nt 76115 and nt 76338 that can be alternatively selected for the pre-mRNA splicing (Figure 1). Selection of two alternative 5′ ss produces two spliced mRNAs: K8.1α and K8.1β Interestingly, data published from different laboratories [52,53] show that the nt 76338 5′ ss is preferentially selected for production of the K8.1β RNA. Why the K8.1 pre-mRNAs prefer the 3′ ss-proximal nt 76338 5′ ss over the distal nt 76155 5′ss remains unknown.

The KSHV K15 gene (either P [prototype] or M [minor] type) is a split gene containing eight exons and seven introns and is located at the right end of the KSHV genome (Figure 3). The K15 RNA structure and the predicted K15 protein sequence resemble those of EBV LMP2A [24,25,54] and LMP1 [25]. The finding that K15 inhibits B-cell receptor-mediated signalling [54] and interacts with TNF receptor-associated factors (TRAFs) [25] as well as with HS1 associated protein X-1 (HAX-1) [55] suggests that K15 plays a role in maintaining viral latency in vivo and possibly has some growth promotion potential. The full-length K15 cDNA with eight exons encodes a protein of approximately 50 kDa in an in vitro transcription and translation assay [55], but the 50 kDa K15 protein is usually cleaved as 35- and 23-kDa forms in transiently transfected cells, and predominantly as the 23 kDa form in PEL cells, including BC3 and JSC-1 cells [55]. Expression of the K15 gene in latent and activated KSHV-positive PEL cells features extensive alternative RNA splicing and thus greatly increased mRNA diversity. There are at least 14 different spliced forms of the K15 RNAs that have been discovered in PEL cells [24,25,54]. Studies in several laboratories have suggested that these RNA isoforms are created by two major splicing mechanisms: alternative 5′ ss usage and intron/exon skipping (Figure 3). However, there is not much known about how this alternative splicing is controlled, nor the coding capacity of each spliced product. The full-length K15 RNA transcript has eight exons with sizes of 7–7.5 and 10 kb, but the spliced isoforms have fewer exons (usually missing 50-half of the K15 exons) with a variable size of 3, 4–4.5 and 5.5 kb. These isoforms are usually weakly expressed in latently infected PEL cells, but are inducible by TPA [24,25,54]. However, a probe comprising the 5′-half of K15 might not detect the spliced isoforms in response to chemical treatment [24,25,54] because of alternative splicing in this region. Nevertheless, the TPA increased expression of K15 RNAs [24,25,54] does not seem to correlate with expression of a K15-specific 23 kDa protein in PEL cells [55].

MECHANISMS POSSIBLY INVOLVED IN THE REGULATION OF KSHV RNA SPLICING

Pre-mRNA splicing involves five small U RNAs (U1, U2, U4, U5 and U6) and many cellular splicing factors, including 10 SR proteins (serine/arginine-rich proteins, such as ASF/SF2, SC35, SRp40) and 12 SR-related proteins (such as U1-70K, $U2AF^{65}$, $U2AF^{35}$). Recognition of a 5′ ss by U1 and a 3′ ss branch point by U2 requires an interconnection of many of those splicing factors [56,57]. Depending on the features of the splice sites and presence or absence of cis-acting elements in the pre-mRNAs, the recruitment of additional trans-splicing factors to the splice sites may strengthen the recognition (Figure 4). The presence of an alternative 5′ ss or 3′ ss in a pre-mRNA complicates the recognition and the switch to alternative splice sites usually correlates with cell differentiation and stages of the virus life cycle. Although the expression and phosphorylation status of SR proteins also correspond with tissue development and cell differentiation, the mechanisms that regulate RNA alternative splicing remain largely unknown.

Expression of KSHV ORF73/72/K13 tricistronic and bicistronic RNAs appears to be KS stage- and cell-specific. As discussed previously, KSHV ORFs 73, 72 and K13 are transcribed as a tricistron from the same promoter, P127880 in KSHV-infected cells. Since this tricistron has one 5′ ss, but two alternative 3′ ss (Figure 2), splicing of an intron of 499 nts from the tricistron leads to the selection of a 5′ ss proximal 3′ ss and results in production of the spliced tricistronic transcript (ORF73/72/K13) predominately responsible for LANA 1 translation [47,58,59]. However, the majority of the tricistrons splice over to the distal 3′ ss by removing a 4 kb intron, and consequently produce the bicistronic transcript (ORF72/K13) responsible for v-cyclin and v-FLIP translation. Translation of v-FLIP is carried out on this bicistronic RNA mainly via an internal ribosome entry site residing in the v-cyclin coding region [45,60,61]. Selection of the distal over the proximal 3′ ss appears to occur at a constant ratio, but can be enhanced by chemical stimulation of latently infected B cells [47,62]. Interestingly, the transcripts from the ORF 73/72/K13 locus are spliced differentially in KS lesions at different stages of development. The distal 3′ ss are preferentially selected in late-stage nodular KS lesions, but not in early lesions. Thus, the bicistronic RNAs are highly expressed and dominate in almost 70% of spindle cells. In contrast, tricistronic ORF73/72/K13 is expressed abundantly in KSHV-infected lymph node cells [58]. The observations in KS lesions of the choice of a distal over a proximal 3′ ss or vice versa strongly imply that the stage- or cell differentiation-related splicing events are the consequence of the regulation by viral cis elements or cellular splicing factors. It will be highly important to identify and characterize the viral cis elements and cellular splicing factors involved in the alternative splicing of KSHV RNAs.

Two KSHV ORFs, ORF 57 and PAN (T1.1 or nut-1), might be involved in the regulation of RNA splicing (Figure 4), including KSHV, but direct evidence is lacking. PAN RNA is a polyadenylated viral early gene transcript having no coding function [63–65]. It is abundant in nuclei of KSHV-infected B cells, has persistent expression into the late viral cycle, and bears stretches of U1 [63] and a region complementary to U12 RNAs [64,65], implying that it may modulate the recognition of splice sites in spliceosome-mediated RNA splicing

(Figure 4). KSHV ORF57 is a split gene (Table 1), and during lytic replication encodes a nuclear-protein that is a homologue of HSV ICP27 [66,67], HVS ORF57 [68–70], EBV SM protein [71,72], human CMV UL69 [73] and VZV ORF4 [74]. Among these, ICP27 is a prototype protein that inhibits RNA splicing [75–77] and mediates viral intronless RNA export [78]. Similar to its homologues, KSHV ORF57 has been demonstrated to be a posttranscriptional regulator that increases the accumulation of target mRNAs, particularly in the cytoplasm, but that has no global inhibitory effects on the expression of intron-containing genes [79,80]. Although HSV ICP27 [78], EBV SM [81,82] and HVS ORF57 [69,83] all bind viral RNAs and mediate viral RNA export (possibly via the CRM-1 export pathway), no cytoplasmic translocation of KSHV ORF57 has been observed in transiently transfected cells [79,80,84]. This suggests that the interaction of KSHV ORF57 with cellular export factors differs from that of its homologues in other herpesviruses. Nevertheless, KSHV ORF57 co-localizes with the cellular splicing factor SC35 [84]. The finding that HVS ORF57, like HSV ICP27[67], is capable of redistributing the cellular SR proteins SC35 and snRNP U2 [70], active components of the spliceosome, suggests that its homologue KSHV ORF57 may also potentially function in RNA splicing.

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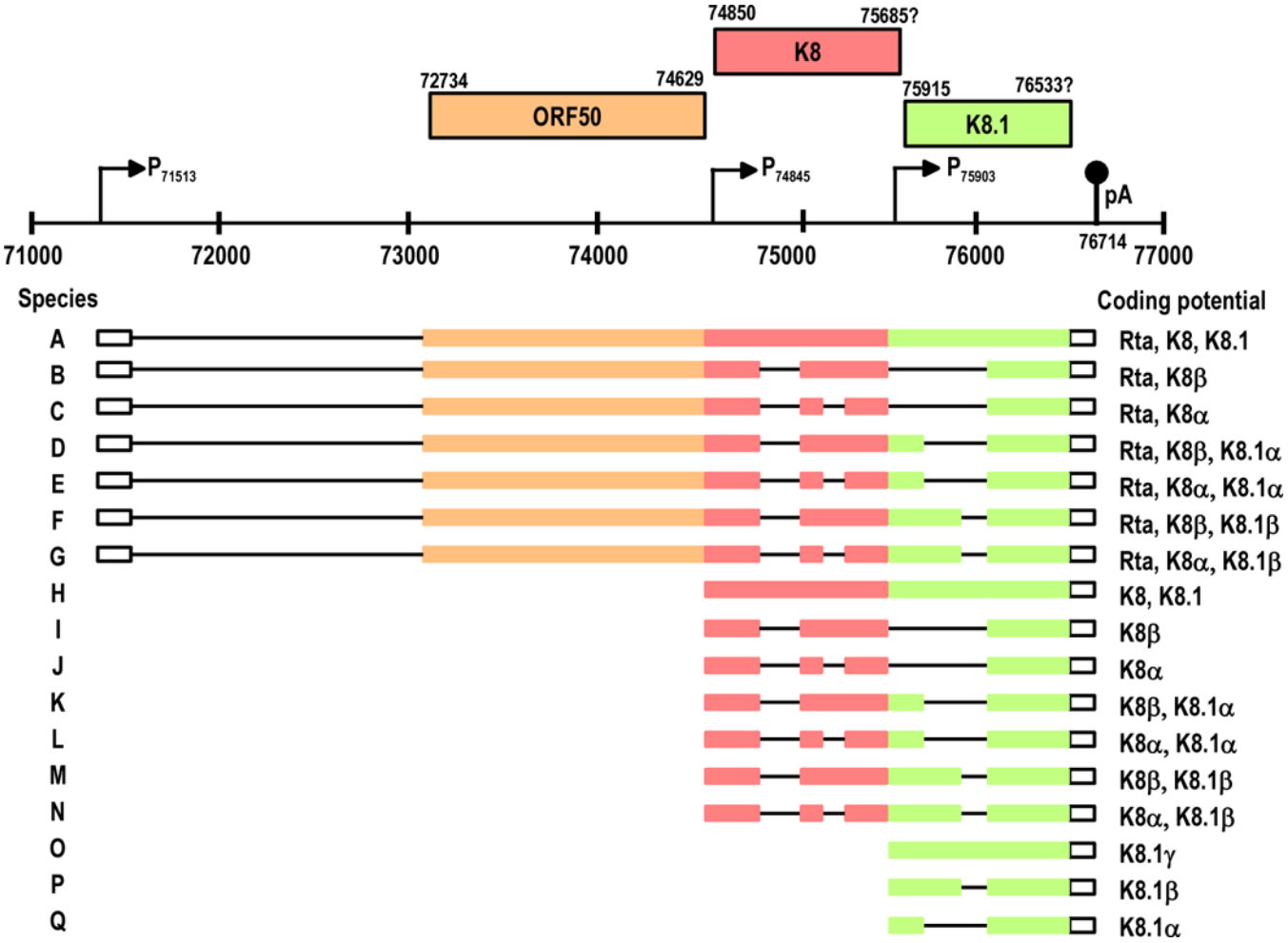

FIG. 1.

The ORF50 (immediately early), K8 (early), and K8.1 (late) transcripts are initiated from alternative promoters but share a common pA site at nt 76714, and produce an array of spliced and unspliced transcripts (36–38, 49). Because of alternative splicing, a total of approximately 19 RNA species (A to S) are predicted from three pre-mRNAs: tricistronic ORF50/K8/K8.1, bicistronic K8/K8.1, and monocistronic K8.1. Splicing of these pre-mRNAs often results in inclusion of the intron at nt 75563 to nt 75645 (RNAs C, E, G, K, M and O) due to suboptimal features of the splice sites. Among those transcripts, RNA D encodes for Rta, RNA L encodes for K-bZip and RNA R encodes for K8.1A in lytic infection.

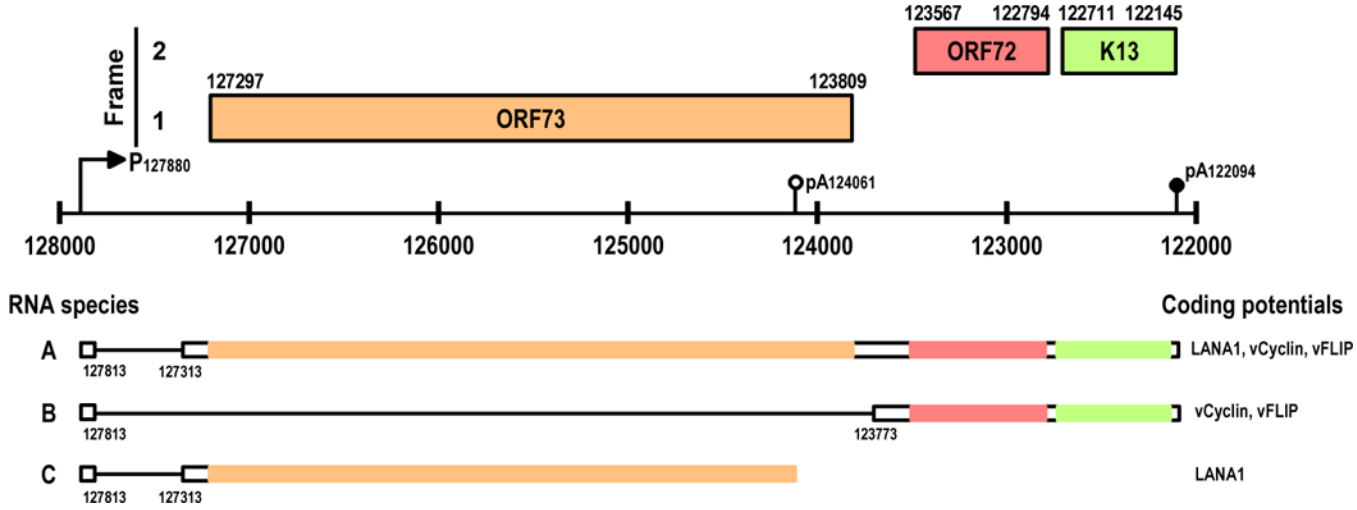

FIG. 2.

Alternative splicing and polyadenylation of ORF 73/72/K13 transcripts initiated from a single promoter, P127880 (44, 46). The coding direction of these genes is backward because they are transcribed from the opposite strand. The presence of an alternative 3′ ss in exon 2 and an alternative non-canonical pA signal (AGUAAA) at nt 124061 leads to the production of a 1.7 kb bicistronic RNA (RNA B, alternative 3′ ss selection) and a 3.3 kb monocistronic RNA (RNA C, alternative polyadenylation) (Canham, M & Talbot, SJ., personal communication). Tricistronic RNA A (ORF73/72/K13) is mainly expressed in lymph node cells and bicistronic RNA B (ORF72/K13) is most common in late-stage KS lesions (70% spindle cells) (58).

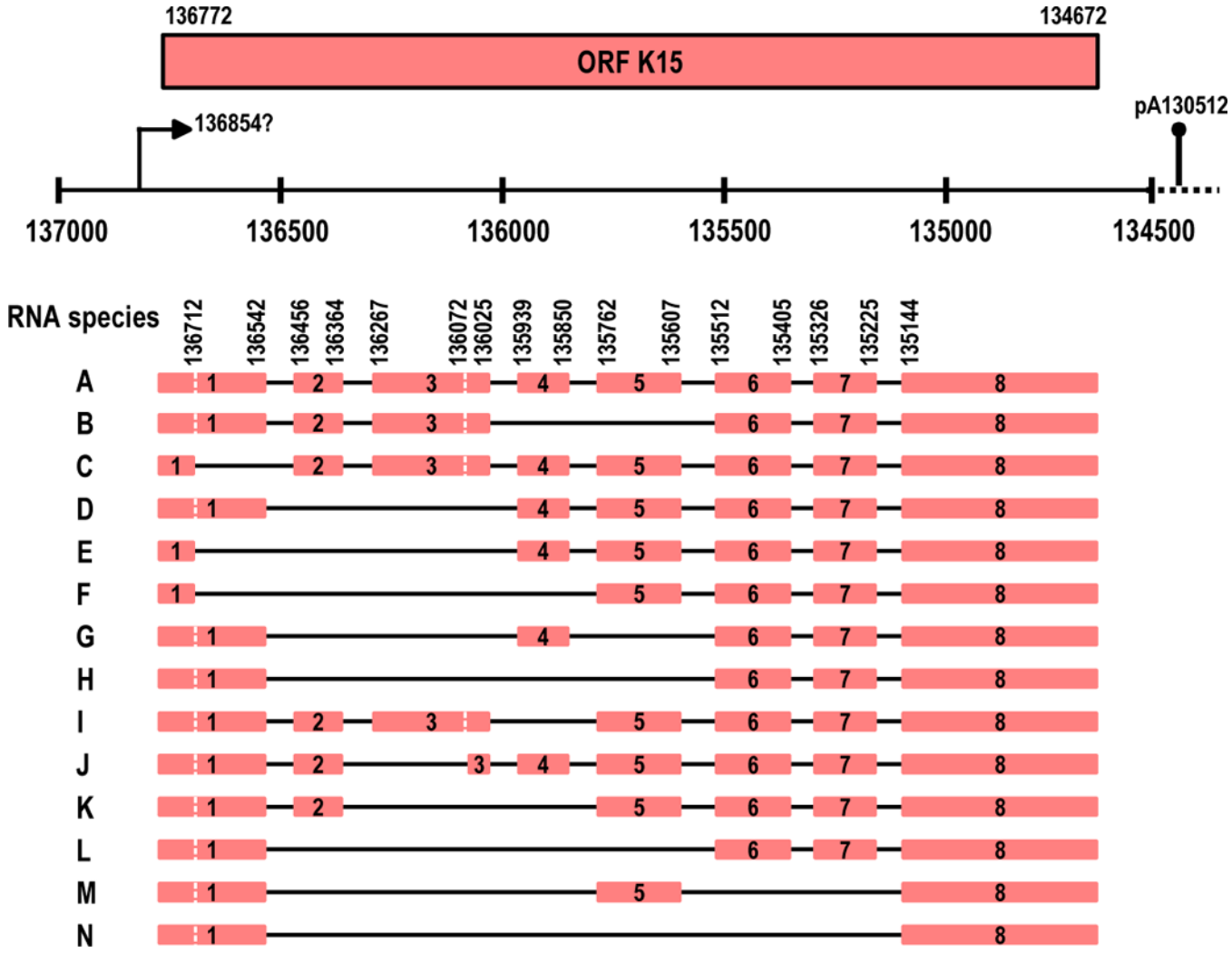

FIG. 3.

Extensive exon and intron skipping and alternative splicing of K15 pre-mRNA (24,25). A dashed vertical line on exon 1 and 3 of each RNA species represents a possible alternative 5′ ss. The depiction also shows a potential K15 promoter P_{136854} (TATATAA) and a putative K15 pA site at nt 130512 downstream of ORF75 (43). K15 is latently expressed in KSHV-positive PEL cell lines and plasmablasts in MCD (55).

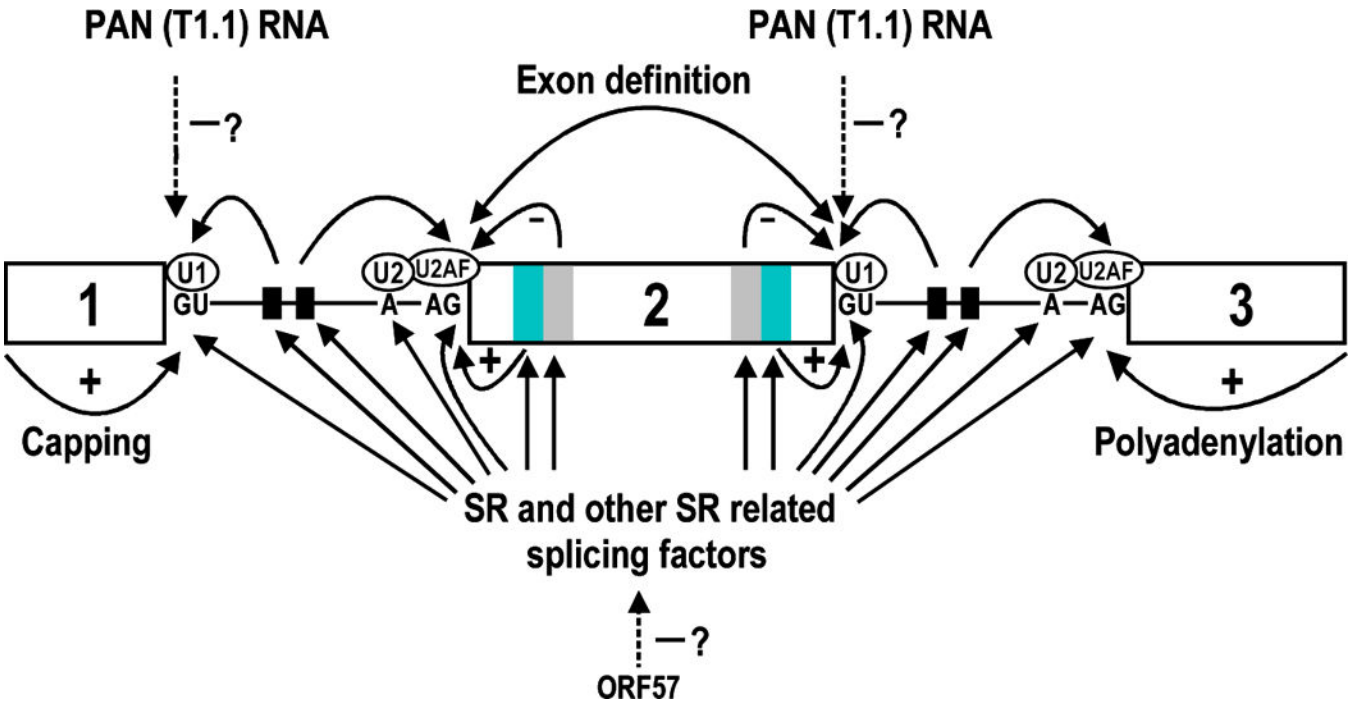

FIG. 4.

Regulation of splice site selection by *cis* elements and *trans* splicing factors and possible involvement of KSHV gene products. Diagram shows a pre-mRNA containing three exons (open boxes) and two introns (lines). Two categories of splicing regulatory elements, splicing enhancers and splicing suppressors (silencers), have been described recently in the exons (grey boxes) or introns (small black boxes) of several viral and mammalian pre-mRNA substrates (94–99). The heavy- and light-grey boxes in exon 2 are, respectively, exonic splicing enhancers (ESE) and exonic splicing suppressor (ESS) which are often juxtaposed. The small black boxes in the introns are either intronic splicing enhancers (ISE) or intronic splicing suppressors (ISS). Through interacting with SR and other SR related splicing factors, these regulatory elements positively (+) or negatively (−) control the selection of a splice site upstream or downstream. An internal exon is also usually defined as less than 500 nts in size (51). RNA 5′ capping (100, 101) and 3′ polyadenylation (102, 103) couple with RNA splicing to promote recognition of terminal splice sites. Negative effects of KSHV ORF57 and PAN RNA on RNA splicing are hypothetical.

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Table 1

Split genes in the KSHV genome[a]

Name	cell homolog	Start[b]	Stop[b]	# of exons	# of introns	Message[c]	Function	References
ORF4	RCA	1142	2794	2	1	M	Complement regulator	85
ORF29		54676	49362	2	1	M	Virion package	86,87
ORF29B		50417	49362	2	1	B		87
ORF40		60368	61681	2	1	B	Primase	88,89
ORF41		61827	62444	2	1	M	Primase	88,89
ORF48		71381	70173	2	1	B		87
ORF50		71596	74629	5	4	T	Transactivator	36,49
ORF57		82717	83544	2	1	M	ICP27-like	79,80,84
ORF72	Cyclin D	123567	122793	2	1	B,T		44,47
ORF73		127297	123808	2	1	T	LANA 1	44,47
ORF74	GPCR	129372	130399	2	1	B	vIL8 receptor	47,59,90
K3		19609	18608	3	2	B		91
K5		26483	25713	2	1	M		48
K8		74850	75791	4	3	B	K-bZIP	37,38,49
K8.1		75915	76695	2	1	M	Glycoprotein	38,52,53
K10	IRF	88478	86074	3	2	M		14
K10.1	IRF	88910	86074	2	1	B		14
K10.5	IRF	91394	89600	2	1	M	LANA 2	15,92
K10.7	IRF	91394	90936	2	1	B		14
K11	IRF	93367	91964	2	1	B		14
K11.1	IRF	94127	93636	2	1	B		14,16
K12		118101	117912	2	1	M	Kaposin A	93
K13	FLIP	122711	122145	2	1	B,T		44
K14	OX-2	127884	128929	2	1	B		47,59
K15		136772	134672	8	7	M	LMP2A	24,25,54

[a] RCA, regulator of complement activation; GPCR, G-protein-coupled receptor; IRF, interferon regulatory factor; FLIP, Fas-ligand interleukin converting enzyme (flice)-like caspase inhibitory protein; K-bZIP, K8-encoded basic-leucine zipper protein; LANA 1, latency-associated nuclear antigen 1; LANA 2, latency-associated nuclear antigen 2; LMP2A, latent membrane protein 2A; OX-2, glycoprotein CD200; vIL8 receptor, viral interleukin-8 like receptor.

[b] Nucleotide positions of the coding region in the KSHV (BC-1) genome (GenBank accession number U75698) based on experimental data.

[c] B, bicistronic; M, monocistronic; T, tricistronic.