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New insights into the expression and functions of the Kaposi's sarcoma-associated herpesvirus long noncoding PAN RNA

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ABSTRACT

The Kaposi's sarcoma-associated herpesvirus (KSHV) is a clinically relevant pathogen associated with several human diseases that primarily affect immunocompromised individuals. KSHV encodes a noncoding polyadenylated nuclear (PAN) RNA that is essential for viral propagation and viral gene expression. PAN RNA is the most abundant viral transcript produced during lytic replication. The accumulation of PAN RNA depends on high levels of transcription driven by the Rta protein, a KSHV transcription factor necessary and sufficient for latent-to-lytic phase transition. In addition, KSHV uses several posttranscriptional mechanisms to stabilize PAN RNA. A cis-acting element, called the ENE, prevents PAN RNA decay by forming a triple helix with its poly(A) tail. The viral ORF57 and the cellular PABPC1 proteins further contribute to PAN RNA stability during lytic phase. PAN RNA functions are only beginning to be uncovered, but PAN RNA has been proposed to control gene expression by several different mechanisms. PAN RNA associates with the KSHV genome and may regulate gene expression by recruiting chromatin-modifying factors. Moreover, PAN RNA binds the viral latency-associated nuclear antigen (LANA) protein and decreases its repressive activity by sequestering it from the viral genome. Surprisingly, PAN RNA was found to associate with translating ribosomes, so this noncoding RNA may be additionally used to produce viral peptides. In this review, I highlight the mechanisms of PAN RNA accumulation and describe recent insights into potential functions of PAN RNA.

1. Introduction

Herpesviruses infect a wide range of organisms from invertebrates to mammals. Due to millions of years of virus-host co-evolution, herpesviruses generally have low pathogenicity and narrow host range, but they can cause significant disease in humans and present problems for agriculture and aquaculture (Davison, 2002; Davison et al., 2008; McGeoch, 2005; van Beurden and Engelsma, 2012). Mammalian herpesviruses are classified into three subfamilies alpha, beta, and gamma that diverged in a mammalian ancestor ~200 million years ago. Herpesviruses have large (~120–240 kb) double-stranded nuclear genomes, and they use the host cell machinery for transcription and RNA processing. As a result, herpesvirus mRNAs resemble those of the host in that they are RNA polymerase II (Pol II)-transcribed, capped, polyadenylated transcripts, but most herpesvirus genes contain no introns. Given the recent appreciation of the widespread expression of noncoding RNAs (ncRNAs), it is not surprising that herpesviruses also encode ncRNAs. Herpesviruses utilize a wide variety of cellular RNA biogenesis pathways for ncRNA maturation. Specific herpesviruses produce ncRNAs that are stable introns, RNA polymerase III (Pol III) transcripts, long polyadenylated RNAs, nonpolyadenylated Pol II transcripts, antisense RNAs, and tRNA-like RNAs. In addition to these ncRNAs, miRNAs are found in all mammalian herpesviruses (Swaminathan, 2008; Tycowski et al., 2015; Zhu et al., 2013). In most of these cases, little is understood about the functions of herpesviral ncRNAs, but their unique structures and the current literature suggest a broad variety of roles for ncRNAs in herpesvirus replication. Here, I review the literature on one unique herpesvirus ncRNA, the polyadenylated nuclear (PAN) RNA encoded by the Kaposi's sarcoma-associated herpesvirus (KSHV). I further recommend to two recent reviews that provide additional perspectives on this viral RNA (Campbell et al., 2014b; Rossetto and Pari, 2014).

KSHV is a human gammaherpesvirus initially identified based on its close association with Kaposi's sarcoma, a common AIDS-associated malignancy (Chang et al., 1994). KSHV was additionally associated with the lymphoproliferative disorders primary effusion lymphoma (PEL), a subset of multicentric Castleman's disease (MCD), and KSHV inflammatory cytokine syndrome (KICS) (Dittmer and Damania, 2013; Du et al., 2007; Ganem, 2006; Greene et al.,

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2007). Similar to other herpesviruses, the KSHV life cycle includes a latent phase in which the viral DNA is maintained in infected host cells as a circular episome. During latency, no viral replication occurs and only a few viral genes are expressed. However, upon reactivation to the lytic phase, a sophisticated cascade of gene expression leads to the production of infectious virions. PAN RNA accumulates to high levels during the lytic phase of KSHV infection.

2. The discovery of PAN RNA

PAN RNA (also called nut-1 or T1.1) was identified and characterized independently by the laboratories of Drs. Ganem and Miller (Staskus et al., 1997; Sun et al., 1996; Zhong and Ganem, 1997; Zhong et al., 1996). PAN RNA was found in KSHV-infected PEL cell lines as well as in tissue derived from a Kaposi's sarcoma patient lesion. Characterization of the RNA showed that, as the name implies, PAN RNA is a polyadenylated transcript that accumulates in the nucleus of lytically infected cells. PAN RNA is 1077 nucleotides (nt), transcribed by Pol II, and its promoter has typical Pol II promoter elements. Several pieces of data led to its assignment as a noncoding transcript. First, the only open reading frames are short (<65 amino acids) and these have suboptimal translation initiation sequences. Second, PAN RNA is primarily, if not exclusively, nuclear as assessed by both fractionation and in situ hybridization. Third, while PAN RNA can be found in higher molecular weight complexes, the RNA does not co-sediment with polysomes. Together, these data strongly support a nuclear noncoding function for PAN RNA.

Given the current understanding of the number of long ncRNAs (lncRNAs) in the nucleus of mammalian cells (Cech and Steitz, 2014; Rinn and Chang, 2012; Ulitsky and Bartel, 2013), the identification of a herpesviral noncoding polyadenylated nuclear RNA does not seem particularly novel. However, it is worth noting that in 1996, relatively few nuclear polyadenylated RNAs had been characterized (e.g. XIST, H19) (Lee and Jaenisch, 1997; Leighton et al., 1996). Moreover, other gammaherpesviruses were known at that time to encode abundant nuclear RNAs, but these were quite different from PAN RNA. The Epstein-Barr virus (EBV) EBERs are small RNA Pol III-transcribed latent phase RNAs (Rosa et al., 1981). The New World monkey pathogen, herpesvirus saimiri (HVS), encodes HSURs, which are non-polyadenylated Pol II-transcribed RNAs with trimethylguanosine caps similar to spliceosomal small nuclear RNAs (snRNAs) (Lee et al., 1988; Murthy et al., 1986). However, unlike HSURs and snRNAs, PAN RNA is not trimethylguanosine capped nor does it directly associate with the Sm complex (Sun et al., 1996; Zhong and Ganem, 1997). Thus, the description of a lytic phase nuclear, polyadenylated RNA that otherwise resembles an mRNA was quite unexpected.

3. How does PAN RNA accumulate to high levels in lytic phase cells?

PAN RNA is by far the most abundant RNA produced by KSHV during lytic phase. Its expression levels have been estimated to be as high as 5×10^5 copies per cell. Comparatively, the highly expressed housekeeping GAPDH mRNA, is estimated to be only $\sim 10^3$ copies per cell and the abundant U2 snRNA involved in splicing of major class introns is $\sim 5 \times 10^5$. In fact, PAN RNA constitutes as much as 80% of the polyadenylated RNA in a lytically reactivated cell. Granted, this percentage is in the context of lytic infection during which KSHV efficiently degrades the majority host mRNAs through its host shut-off activity (Glaunsinger and Ganem, 2006). Nonetheless, the abundance of PAN RNA is quite remarkable when compared with other polyadenylated RNAs. To achieve such high levels of PAN RNA, the virus uses a robust transcriptional induction coupled with multiple mechanisms of stabilization of the RNA after induction.

3.1. Transcriptional regulation of PAN RNA

PAN RNA is a delayed early transcript that is strongly induced by the KSHV immediate early transcription factor Rta (ORF50) (Lukac, 2012; Miller et al., 2007; Staudt and Dittmer, 2007). Rta is both necessary and sufficient to reactivate latently infected KSHV, and it is the primary driver of latent-to-lytic reactivation in cells. Rta uses at least two modes of transcription activation. Rta directly binds to viral gene promoters and activates their transcription. Alternatively, Rta activates viral gene transcription by interacting with the cellular RBP-Jκ protein (Chang et al., 2005; Liang et al., 2002; Lukac, 2012). PAN RNA promoter falls into the former class as it is directly bound by Rta at an Rta-response element (RRE) approximately 45–75 bp upstream of the PAN RNA transcription start site (TSS) (Fig. 1A, yellow) (Chang et al., 2002; Song et al., 2001, 2002). In fact, sequence specificity required for direct binding by Rta was in large part defined using the PAN RNA promoter. In transfection experiments with PAN RNA driven by its own promoter, expression is undetectable unless Rta expression constructs are co-transfected. However, low levels of PAN RNA have been observed in Rta-null viruses (Rossetto et al., 2013), suggesting that basal transcription or Rta-independent viral transcription programs can induce low levels of PAN RNA.

Additional cellular and viral factors are involved in the regulation of PAN RNA transcription. The PAN RNA promoter is largely RBP-Jκ independent, but an RBP-Jκ binding site is present upstream in the promoter that provides an additional ~2-fold boost in reporter assays in the presence of Rta (Fig. 1A, pink) (Liang et al., 2002). In addition, binding sites for the cellular transcription factors YY1 and Sp1 were identified in the PAN RNA promoter and both YY1 and Sp1 bind to the promoter (Chang et al., 2005).

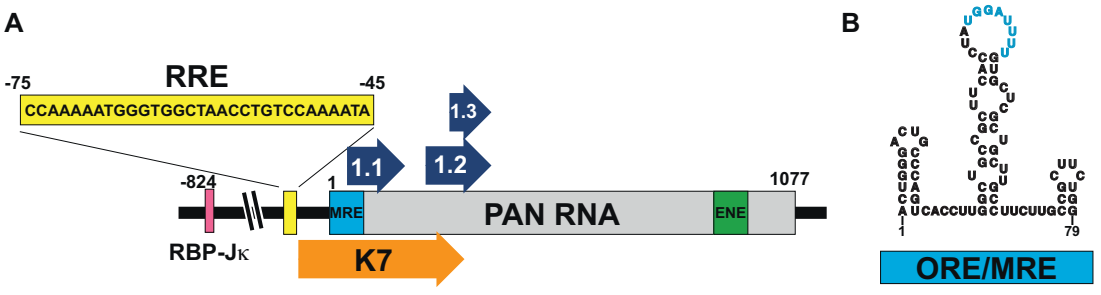

Fig. 1. Cis-acting regulatory elements of PAN RNA. (A) PAN RNA gene with several features highlighted including the Rta-responsive element (yellow), overlapping K7 ORF (orange), putative peptide coding regions (purple), the ORE/MRE (blue), the ENE (green), and an upstream RBP-Jκ site (pink). The diagram is approximately to scale, but the broken lines point out a region where the scale is disrupted. (B) Sequence and predicted secondary structure of the core ORE/MRE element. Mutational analysis supports that the nucleotides shown in blue bind directly to ORF57 (Massimelli et al., 2011; Sei and Conrad, 2011).