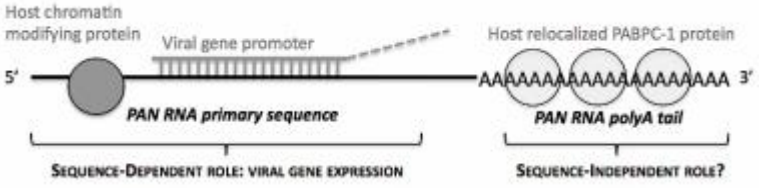
Kaposi's sarcoma-associated herpesvirus (KSHV), a member of the γ-herpesvirus family, is a double-stranded DNA oncovirus that infects B cells to cause multiple lymphomas[1]. Despite the devastating nature of KSHV infection, little is known about the mechanism through which the virus is able to hijack the host cell to promote its own expansive replication during "lytic stage" infection. Specifically, sudden activation of transcription of the viral genome, as well as total shut down of host gene expression and degradation of host mRNAs in a process called "host shut-off," permits cytoplasmic production of new virus particles. My proposal will address the hypothesis that an unusual viral noncoding RNA (ncRNA) may help to orchestrate both viral gene expression and host shut-off through interactions with host proteins. These studies will provide insight ncRNA functions and mechanisms of viral replication.

During KSHV lytic phase onset, a 1.1 kb polyadenylayed nuclear ncRNA (PAN) is hyperexpressed[2]. PAN is expressed at ~500,000 transcripts per nucleus and represents 80% of the total polyadenylated RNA in the infected cell[2]. It has been shown, through generation of a PAN knockout KSHV, that PAN is required for normal levels of viral gene expression during lytic phase[3]. I contributed to the discovery and quantification of decreased viral gene expression upon loss of PAN[4] in Joan Steitz's lab at Yale. PAN interacts with viral gene promoters and binds host chromatin modifying proteins including histone demethylases[4], which loosen genome compaction to provide access to transcription machinery. These data suggest that PAN may concentrate host chromatin modifying proteins near promoters in the viral genome to upregulate viral gene expression. This role for PAN involves specific interactions between its primary sequence and both viral promoter DNA and host proteins, and thus is "sequence-dependent"[4].

Another unusual feature of KSHV lytic phase is that host cytoplasmic polyA binding protein (PABPC-1) relocalizes to the nucleus[5]. PABPC-1 has a highly conserved cytoplasmic role in preventing degradation of mRNAs before translation by binding the polyA tail[6]. PAN and PABPC1 have been estimated to associate stoichiometrically in the nucleus through polyA tail binding.[5] Despite its incredible abundance, the role of this complex in lytic phase is unclear. However, the fact that PABPC-1 relocalization is required for host shut-off during lytic phase[7] suggests a potential function for this nuclear complex of PAN polyA:PABPC-1 in lytic phase, and thus a sequence-independent function for PAN.

A sequence-independent role for PAN bound to PABPC-1 in lytic phase may involve the nuclear export process. PABPC-1 relocalization may disrupt host mRNA export by displacing the export factor nuclear polyA binding protein (PABPN1)[7]. A viral export factor may selectively rescue export of viral transcripts[8]. Through stoichiometric binding, PAN could buffer changes in PABPC-1. Thus, PAN could help to create a balance that favors viral mRNA nuclear export for expression and host mRNA degradation in the nucleus.



I propose to first confirm that the role of PAN in regulating viral gene expression is 1) sequence-dependent, and 2) unaffected by the interaction of PAN with PABPC-1, by testing recombinant PAN variants in *in vivo* functional assays. Additionally, I propose to address the possibility of a role for PAN in nuclear export using cellular fractionation and quantitative measurement of viral and host transcripts in nuclear and cytoplasmic fractions. Use of existing systems[3,4,5,9] for studying lytic phase at the molecular level will allow me to determine definite answers to these questions and provide insight into novel mechanisms of global cellular regulation by viral ncRNAs.

AIM 1: To confirm that the role of PAN RNA in regulating viral gene expression is mediated by the primary sequence of PAN, I will generate a recombinant KSHV vector, "RRV-PAN," in which the PAN RNA sequence is replaced by the nonconserved sequence of a PAN homolog in the related herpesvirus Rhesus rhadinovirus (RRV). I will infect human B cells with wild type (WT) PAN, RRV-PAN, and PAN-KO KSHV and induce lytic phase expression chemically. At various time points after lytic induction I will measure changes in expression of specific viral genes using qRT-PCR. I will determine host chromatin modifying protein interactions with PAN, RRV-PAN, and PAN-KO through cross-linking cells, immunoprecipitating these proteins, and using high-throughput sequencing (HITS-CLIP) to determine whether PAN variants are bound. Any PAN variant missing the primary sequence, which has been implicated in up-regulating viral gene expression, should abrogate interactions between PAN and host proteins and viral promoters, and result in decreased viral gene expression if this is truly a sequence-dependent interaction and function. If the RRV-PAN variant is able to sustain wild type level viral gene expression, there is evidence for a sequence-independent function; sequence-independence could be confirmed by replacing PAN with a completely scrambled sequence.

AIM 2: To determine whether the sequence-independent binding of PABCP-1 to the PAN polyA tail plays a role in regulating viral gene expression, I will use a PAN variant (PANΔA) generated by the Steitz lab with the polyA signal sequence replaced by a cleavage signal at the 3' end of transcript[9]. This will generate a non-polyadenylated PAN and prevent association of PAN with PABPC-1, though PABPC-1 will relocalize to the nucleus normally; this logic should of course be tested in controls. I will measure changes in viral gene expression and assay host protein interactions by qRT-PCR and HITS-CLIP, as above. As deleting the polyA tail may destabilize PAN, I will normalize all relative effects on viral gene expression for each PAN variant and WT PAN to expression levels of PAN determined by qRT-PCR. If the deletion of the PAN polyA tail does not affect the role of PAN in viral gene regulation, this function must independent of PAN-PABCP-1 complex formation and there may be a separate role for this complex in lytic phase.

AIM 3: To address a possible sequence-independent role for PAN in affecting nuclear export through binding PABPC-1, I will infect B cells with no virus, WT PAN, RRV-PAN, PANΔA, and PAN KO KSHV. After inducing lytic phase, I will perform cytoplasmic/nuclear fractionation by centrifugation, isolate mRNA, and quantify relative pools of viral and host transcripts in the nucleus and cytoplasm using unbiased quantitative RNA sequencing. If there is a sequence-independent role for the PAN polyA:PABPC-1 complex in promoting viral mRNA export, WT PAN and RRV-PAN infected cells should have lower Nuclear/Cytoplasmic (N/C) ratios of viral mRNAs than "no virus" controls. Data from PANΔA and PAN KO variants will clarify whether any effects are dependent on the PAN polyA:PABCP-1 complex.

As lytic phase mechanisms of cellular disablement may be conserved between gamma herpesviruses, this work will have broad impact in both elucidating novel sequence-independent ncRNA functions, and aiding translational work to treat evasive and devastating herpesviruses.

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[1] Verma SC, Robertson ES (2003). FEMS Microbiol Lett 222: 155–163.
[2] Sun R, Lin SF, Gradoville L, Miller G (1996). Proc Natl Acad Sci U S A 93: 11883–11888.
[3] Rossetto CC, Pari G (2012). PLoS Pathog 8(5):e1002680.
[4] Borah S, Darricarrère N, Darnell A, Myoung J, Steitz JA (2011). PLoS Pathog 7(10):e1002300.
[5] Lee YJ, Glaunsinger BA (2009). PLoS Biol 7:e1000107.
[6] Gorlach M, Burd CG, Dreyfuss G (1994). Exp Cell Res 211: 400–407.
[7] Mangus DA, Evans MC, Jacobson A (2003). Genome Biol 4: 223.
[8] Boyne JR, Colgan KJ, Whitehouse A (2008). PLoS Pathog 4: e1000194.
[9] Conrad NK, Mili S, Marshall EL, Shu MD, Steitz JA (2006). Mol Cell 28;24(6):943-53.