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Genome-wide identification of binding sites for Kaposi's sarcoma-associated herpesvirus lytic switch protein, RTA

Jiguo Chen [a,b,1], Fengchun Ye [a,b], Jianping Xie [a,b], Kurt Kuhne [a,c], Shou-Jiang Gao [a,b,c,d,e,*]

[a] Tumor Virology Program, Greehey Children's Cancer Research Institute, The University of Texas Health Science Center at San Antonio, 8403 Floyd Curl Drive, San Antonio, TX 78229, USA
[b] Department of Pediatrics, The University of Texas Health Science Center at San Antonio, 8403 Floyd Curl Drive, San Antonio, TX 78229, USA
[c] Department of Microbiology and Immunology, The University of Texas Health Science Center at San Antonio, 8403 Floyd Curl Drive, San Antonio, TX 78229, USA
[d] Cancer Therapy and Research Center, The University of Texas Health Science Center at San Antonio, 8403 Floyd Curl Drive, San Antonio, TX 78229, USA
[e] Tumor Virology Group, Wuhan Institute of Virology, Chinese Academy of Sciences, 44 Xiaohongshan, Wuhan, China

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ABSTRACT

Kaposi's sarcoma-associated herpesvirus (KSHV) replication and transcription activator (RTA) encoded by ORF50 is a lytic switch protein for viral reactivation from latency. The expression of RTA activates the expression of downstream viral genes, and is necessary for triggering the full viral lytic program. Using chromatin immunoprecipitation assay coupled with a KSHV whole-genome tiling microarray (ChIP-on-chip) approach, we identified a set of 19 RTA binding sites in the KSHV genome in a KSHV-infected cell line BCBL-1. These binding sites are located in the regions of promoters, introns, or exons of KSHV genes including ORF8, ORFK4.1, ORFK5, PAN, ORF16, ORF29, ORF45, ORF50, ORFK8, ORFK10.1, ORF59, ORFK12, ORF71/72, ORFK14/ORF74, and ORFK15, the two origins of lytic replication OriLyt-L and OriLyt-R, and the microRNA cluster. We confirmed these RTA binding sites by ChIP and quantitative real-time PCR. We further mapped the RTA binding site in the first intron of the ORFK15 gene, and determined that it is RTA-responsive. The ORFK15 RTA binding sequence TTCCAGGAA TTCCTGGAA consists of a palindromic structure of two tandem repeats, of which each itself is also an imperfect inverted repeat. Reporter assay and electrophoretic mobility shift assay confirmed the binding of the RTA protein to this sequence *in vitro*. Sequence alignment with other RTA binding sites identified the RTA consensus binding motif as TTCCAGGAT$(N)_{0-16}$TTCCTGGGA. Interestingly, most of the identified RTA binding sites contain only half or part of this RTA binding motif. These results suggest the complexity of RTA binding *in vivo*, and the involvement of other cellular or viral transcription factors during RTA transactivation of target genes.

Introduction

Infection by Kaposi's sarcoma-associated herpesvirus (KSHV), also known as human herpesvirus 8 (HHV-8), is associated with the development of Kaposi's sarcoma (Chang et al., 1994), primary effusion lymphoma (PEL) (Cesarman et al., 1995) and a subset of multicentric Castleman's diseases (Soulier et al., 1995). Like other herpesviruses, the life cycle of KHSV consists of latent and lytic phases that specify expression patterns of four classes of viral genes: latent, immediate-early (IE), early, and late genes (Greene et al., 2007). The IE genes are made immediately after primary infection or upon reactivation from latency, and do not require *de novo* protein synthesis (Deng et al., 2007). IE genes generally encode for regulatory proteins, and are critical for initiating viral transcription (Miller et al., 2007). KSHV replication and transcription activator (RTA) is the gene product of a major IE transcript transcribed from the ORF50 locus (Chen et al., 2000; Lukac et al., 1999; Sun et al., 1998). The switch from KSHV latent to lytic replication can be initiated by specific intracellular signals or extracellular stimuli including chemical inducers such as 12-*O*-tetradecanoyl phorbol-13 acetate (TPA) and sodium butyrate that activate the expression of this key lytic switch protein. The expression of RTA is necessary and sufficient to trigger the full lytic program resulting in the cascade expression of viral proteins, release of viral progeny, and host cell death (Gradoville et al., 2000; Lukac et al., 1998, 1999; Sun et al., 1998; Xu et al., 2005).

RTA has been shown to activate the transcription of several downstream viral genes, including ORFK1 (Bowser et al., 2002, 2006), ORFK2 (Deng et al., 2002; Song et al., 2003), ORFK3 (Rimessi et al., 2001), ORFK5 (Haque et al., 2000), ORFK6 (Chang et al., 2005b), PAN RNAs (Song et al., 2001, 2003, 2002), ORF35 (Masa et al., 2008), ORF49 (Gonzalez et al., 2006), ORF50 itself (Chen et al., 2001; Deng et al., 2000; Sakakibara et al., 2001), ORFK8 (Lukac et al., 2001; Seaman and Quinlivan, 2003; Wang et al., 2004c; Wang and Yuan, 2007),

* Corresponding author. Tumor Virology Program, Greehey Children's Cancer Research Institute, The University of Texas Health Science Center at San Antonio, 8403 Floyd Curl Drive, San Antonio, TX 78229, USA. Fax: +1 210 562 9014.
E-mail address: gaos@uthscsa.edu (S.-J. Gao).
[1] Current affiliation: Department of Biological Sciences, Mississippi State University, Mississippi State, MS39762, USA.

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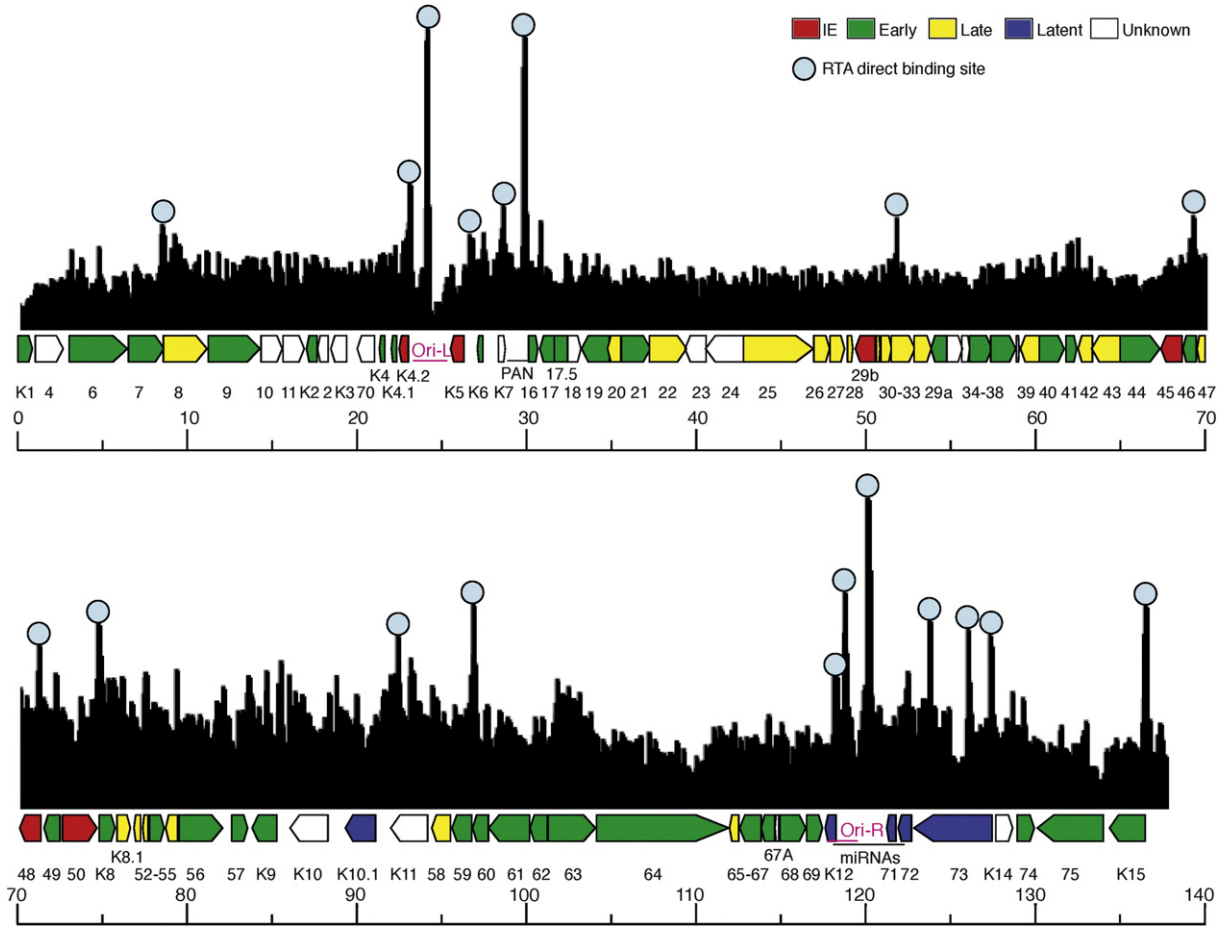

Fig. 1. Summary results of ChIP-on-chip. The peaks represented the relative enrichment of RTA binding signals over non-enriched input signals, along the KSHV genome and ORFs. Circles indicated the RTA direct binding sites identified by the analysis. A total of 19 RTA direct binding sites were identified.

ORF57 (Byun et al., 2002; Song et al., 2003; Wang et al., 2003), ORFK9 (Chen et al., 2000; Ueda et al., 2002), ORF74 (Liang and Ganem, 2004), ORFK12 (Song et al., 2003), and ORFK15 (Wong and Damania, 2006). RTA activates its target genes through at least two mechanisms: direct binding to the RTA-responsive elements (REs), or indirect binding to other cellular factors (Chang et al., 2005b). For example, RTA directly binds to the promoters of PAN RNAs (Song et al., 2002, 2003) and ORFK12 (Chang et al., 2002) but does not bind to the ORF57 and ORFK6 promoters (Chang et al., 2005b). The indirect binding of RTA to its REs may be involved with the interaction of RTA with RBP-Jkappa, a Notch signal pathway transcription factor (Chang et al., 2005a; Liang et al., 2002; Liang and Ganem, 2003, 2004). Other cellular transcriptional factors that are involved in the activation of viral genes include AP-1 and OCT-1 for ORF50 itself (Pan et al., 2006; Sakakibara et al., 2001; Wang et al., 2004a; Xie et al., 2008) and ORFK8 (Carroll et al., 2007), or AP-1 for ORF35 (Masa et al., 2008), AP-1 or SP-1 for ORFK9 (Chen et al., 2000; Ueda et al., 2002), and AP-1 for ORF57 (Byun et al., 2002; Wang et al., 2004a). It has also been shown that RTA recruits CBP, the SWI/SNF chromatin remodeling complex, and the TRAP/mediator co-activator to its target promoters, and that such recruitment is essential for the expression of RTA-dependent viral genes (Gwack et al., 2003).

The RTA protein is mainly encoded by ORF50 but obtains an additional 60 amino acid (aa) to its N-terminal through a splicing event that shifts its start codon across ORF49 (Chen et al., 2000; Sun et al., 1998). The addition of the 60 aa is critical for its function as a viral transactivator because a cloned protein from ORF50 itself neither locates to the nuclei nor has any transactivation capacity (Chen et al., 2000). The full-length functional RTA protein contains 691 aa, and is predicted to have a molecular mass of 73.7 kDa. However, cellular RTA migrated as a series of polypeptides with the major ones at around 119 and 101 kDa, suggesting that RTA is post-translationally modified (Chang and Miller, 2004; Lukac et al., 1999). RTA lacks significant homology with any cellular proteins but is a functional homolog of the RTA proteins from Epstein–Barr virus (EBV), herpesvirus saimiri (HVS) and murine gammaherpesvirus 68 (MHV-68) (Wu et al., 2000). The RTA protein consists of an N-terminal DNA binding domain, a central dimerization domain, a C-terminal acidic activation domain, and two nuclear localization signals (NLSs) (Chen et al., 2000; Lukac et al., 1999). The DNA binding domain of RTA is located at the N-terminus from aa 1 to 530. A deletion mutant of the activation domain sequences, containing only the DNA binding domain (aa 1–530), has been shown to be a dominant-negative mutant of RTA transactivation (Lukac et al., 1999). This mutant maintains its DNA binding activity for RTA REs but no longer activates the expression of downstream lytic genes (Lukac et al., 1999, 2001; Song et al., 2001, 2003). This construct binds to the RTA binding consensus sequence as a tetramer (Bu et al., 2007). Using a purified fusion protein of this mutant GST-50ΔSTAD (aa 1 to 530) expressed in bacteria in an *in vitro* DNA binding assay named systematic evolution of ligands by exponential enrichment assay (SELEX), Ziegelbauer et al. (2006) identified a total of 18 RTA direct binding sites in the KSHV genome including a binding site for the ORF8 promoter; however they did not recover the known high-affinity PAN promoter site, nor the sites for the promoters of ORFK2, ORFK8, ORF57, ORFK12 and ORF74. This may indicate that the bacterially expressed GST-RTA fusion protein and the *in vitro* binding assay might not be the ideal method for delineating the RTA binding sites *in vivo*. To address this problem, we applied an *in vivo* ChIP-on-chip approach coupled with a KSHV genome tiling array and a stable BCBL-1 cell line