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The interaction between KSHV RTA and cellular RBP-Jκ and their subsequent DNA binding are not sufficient for activation of RBP-Jκ

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Abstract
Kaposi's sarcoma-associated herpesvirus (KSHV) replication and transcription activator (RTA) is necessary and sufficient for the switch from KSHV latency to lytic replication. RTA activates promoters by several mechanisms. RTA can bind to sequences in viral promoters and activate transcription. In addition, RTA interacts with the cellular recombination signal sequence-binding protein-J kappa (RBP-Jκ), a transcriptional repressor, converts the repressor into an activator and activates viral promoters via RBP-Jκ. Because RBP-Jκ is required for RTA to activate lytic replication, it is important to understand how RTA cooperates with RBP-Jκ protein to activate KSHV lytic replication program. Previously, we identified an RTA mutant, RTA-K152E, which has a defect in its direct DNA-binding activity. In this report, the effect of the mutant RTA on KSHV activation via RBP-Jκ protein is examined. We demonstrate that RTA-K152E interacts with RBP-Jκ physically and the mutant RTA and RBP-Jκ complex binds to target DNA properly in vivo and in vitro. However, the complex of RTA-K152E and RBP-Jκ does not activate transcription. Furthermore, the RTA mutant (RTA-K12E) inhibits cellular Notch-mediated RBP-Jκ activation. These data collectively suggest that the complex between KSHV RTA and cellular RBP-Jκ and the subsequent DNA binding by the complex are not sufficient for the activation of RBP-Jκ protein. Other factor(s) whether additional cofactor(s) in the complex or the intrinsic conformation of RTA, are predicted to be required for the activation of RBP-Jκ protein by RTA.

Keywords: KSHV, RTA, RBP-Jκ, Lytic replication, HHV8, PEL

1. Introduction

Kaposi's sarcoma (KS)-associated herpesvirus (KSHV), also known as human herpesvirus 8 (HHV-8), is a gamma herpesvirus. It is believed to be the etiological agent of KS (Chang *et al.*, 1994; Chang and Moore, 1996; Moore and Chang, 2001). KSHV is also implicated in the pathogenesis of AIDS-associated primary effusion lymphoma (PEL, also called body cavity-based lymphoma (BCBL)) and a lymphoproliferative disorder known as multicentric Castleman's disease (Dourmishev *et al.*, 2003; Moore and Chang, 2001; West and Wood, 2003).

As other herpesviruses, KSHV goes through both latency and lytic replication cycles. The expression of the KSHV replication and transcription activator (RTA) is necessary and sufficient for the switch from latency to lytic replication (Dourmishev *et al.*, 2003; West and Wood, 2003). RTA is an immediate early gene (Sarid *et al.*, 1998; Sun *et al.*, 1999; Zhu *et al.*, 1999) and a sequence-specific DNA-binding protein. A number of RTA-responsive elements (RRE) were identified in the transcriptional regulatory regions of different subsequently expressed viral genes (Gradoville *et al.*, 2000; Lukac *et al.*, 1999; Sakakibara *et al.*, 2001; Saveliev *et al.*, 2002; Sun *et al.*, 1998; Sun *et al.*, 1999; Zhu *et al.*, 1999).

In addition to direct DNA binding, the ability of RTA to interact with other factors appears to be necessary for activating its transcriptional potential (Gwack *et al.*, 2001; Gwack *et al.*, 2002; Liao *et al.*, 2003; Wang *et al.*, 2001). One of the interacting factors is the cellular recombination signal sequence-binding protein-J kappa (RBP-Jκ) (also known as CBF-1 and CSL) (Carroll *et al.*, 2006; Liang *et al.*, 2002; Liang and

Ganem, 2003). RBP-Jκ is a sequence-specific DNA-binding protein and a transcriptional repressor that is involved in the Notch signaling pathway.

The Notch signaling pathway regulates cell fate in a variety of organisms (Bray, 2006; He and Pear, 2003). Interaction of Notch receptors (Notch) with their ligands leads to cleavage of the Notch intracellular domain (NICD), which leads to nuclear localization of NICD. In the nucleus, NICD associates with RBP-Jκ and converts RBP-Jκ from a transcriptional repressor to an activator. Furthermore, the NICD-RBP-Jκ complex activates expression of specific target genes (Bray, 2006; He and Pear, 2003).

KSHV RTA can directly bind to RBP-Jκ and the RTA–RBP-Jκ complex binds to DNA and activates transcription of KSHV promoters (Carroll *et al.*, 2006; Liang *et al.*, 2002; Liang and Ganem, 2003). The KSHV genome contains numerous RBP-Jκ elements suggesting that RBP-Jκ plays an important role in viral transcription. The interaction between RTA and RBP-Jκ is essential for the switch from viral latency to lytic replication in rodent cells (Liang and Ganem, 2003). However, it is not clear if interaction between RTA and RBP-Jκ is the only event that is necessary for activating KSHV lytic gene expression.

Previously, we have identified a novel function of RTA, i.e., RTA binds to interferon-stimulated response elements (ISRE) and activate certain cellular interferon-stimulated genes (ISG) (Zhang *et al.*, 2005). In addition, a region in RTA DNA-binding domain has been identified with certain sequence similarity to the DNA-binding domains of interferon regulatory factor (IRF) family. Mutation in one conserved amino acid within this region (RTA-K152E) reduces the ability of RTA to bind to ISRE as well as other RREs. Furthermore, the RTA-K152E fails to activate RTA-responsive promoters and to induce viral lytic gene expression (Zhang *et al.*, 2005).

In this report, we have further characterized the RTA-K152E mutant in terms of activating the cellular RBP-Jκ protein. The mutant RTA is able to interact with RBP-Jκ physically and the RTA-K152E-RBP-Jκ complex is able to bind to DNA in vitro and in vivo. However, the mutant failed to activate RBP-Jκ protein. Thus, the complex between KSHV RTA and cellular RBP-Jκ and their subsequent DNA binding are not sufficient for the activation of RBP-Jκ protein.

2. Materials and methods

2.1. Plasmids and antibodies

Expression plasmids of FLAG-tagged RTA and mutant RTA-K152E were previously described (Zhang *et al.*, 2005). The HA-tagged RBP-Jκ expression plasmid was a gift from Dr. Paul Ling (Baylor College of Medicine). FLAG-tagged RBP-Jκ expression plasmid and the reporter construct containing 3× RBP-Jκ binding site were gifts from Drs. Don Ganem and Yuying Liang (Liang *et al.*, 2002). The constitutively active Notch expression plasmid, pcDNA-3-mNotch-ΔE, was a gift from Dr. Franz Oswald (Oswald *et al.*, 2002). CMV-β-gal and CMV-GFP expression plasmids were from Clontech. Peptide RTA antibody was described (Xu *et al.*, 2007). Tubulin and FLAG-antibodies were purchased from Sigma. HA and RBP-Jκ antibodies were purchased from Santa Cruz.

2.2. Western blot analysis, cell culture, transient transfection and reporter assays

Standard western blot analysis was performed as previously described (Zhang and Pagano, 1997; Zhang and Pagano, 1999; Zhang and Pagano, 2000; Zhang and Pagano, 2001). 293-Bac (a gift from Dr. S.J. Gao) is a human 293 cells derived cell line and containing KSHV genome (Zhou *et al.*, 2002). 293-Bac36 cells were maintained in DMEM plus 10% fetal bovine serum (FBS) plus 0.5 mg/ml hygromycin. 293T cells are a human fibroblast line and were maintained in DMEM plus 10% FBS. Effectene (Qiagen) was used for the transfection of these cells. The luciferase assays were performed using a kit from Promega according to manufacturer's recommendation.

2.3. Preparation of KSHV stocks and detection of virion DNA

293-Bac36 cells were transfected with the designated expression plasmids and culture supernatants were harvested 5 days later. Virions were pelleted by centrifugation at 100,000 × *g* for 1 h. The pellets were subsequently suspended in 1× PBS buffer (1:100 of the volume of the original supernatants). The concentrated viruses were then treated by DNase I at 37 °C for 1 h. DNA was extracted and PCR analyses were carried out with primers targeted K14/vGCR region (BC1 KSHV genome coordinates: 127,649–127,883). The conditions of the PCR assay were established empirically to ensure a linear amplification of template DNA within the amplification conditions.

2.4. Chromatin immunoprecipitation (ChIP)

293-Bac36 cells were transfected with various plasmids. One day later, ChIP assays were performed using the chromatin immunoprecipitation assay kits according to the manufacturer's recommendation (Upstate, Inc.). Anti-FLAG and normal rabbit serum (NRS) was used as designated. The DNA exacted from immunoprecipitates was used as template for PCR analyses with various primers that amplify MTA- and PAN-specific products (Wang *et al.*, 2003a; Wang *et al.*, 2003b). PCR products were resolved in 8% polyacrylamide gel.

2.5. Co-immunoprecipitation (Co-IP)

293T cells grown in 10 cm plates were transfected with the designated plasmids. Cell extracts were prepared using RIPA buffer (1× PBS, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS) supplemented with a protease inhibitor cocktail (Roche) were incubated with either mouse anti-FLAG monoclonal antibody M2 (Sigma) or anti-HA antibody (Santa Cruz) for 1 h on ice. Protein G-sepharose (Pharmacia) was added and the incubation continued at 4 °C overnight with gentle rotation. Beads were washed three times with 1× PBS buffer and boiled in SDS loading buffer and subsequent western blot were essentially the same as described.

2.6. Electrophoretic mobility shift assay (EMSA)

The probes were obtained by first annealing complementary oligonucleotides and then labeling them with (α-^{32}P)dCTP (Amersham) using DNA polymerase Klenow fragment (Fermentas). The RBP-Jκ probe was obtained by annealing two oligonucleotides, 5′-GATCTGGTGTAAACAC GCCGTGGGAAAAAATTTATG-3′ and its complementary stand, with GATC at the 5′ end. DNA-binding reactions were performed essentially as described (Liang *et al.*, 2002; Lukac *et al.*, 2001) with the following modifications: the reaction buffer contained 10 mM Tris–HCl (pH 7.5), 50 mM NaCl, 5.3 mM $MgCl_2$, 1 mM EDTA, 1 μg polydIdC and 4% glycerol and the reactions were carried out at 25 °C for 30 min. If an antibody was used for a super-shift experiment, 1 μl of the target antibody was added into the reaction mixture.

2.7. In vitro transcription and translation

Proteins were synthesized with the TNT coupled transcription and translation kit with rabbit reticulocyte lysate (Promega) according to the manufacturer's instructions. pcDNA3-RTA and RTA-K152E (Zhang *et al.*, 2005) and FLAG-RBP-Jκ were used for in vitro transcription and translation of target proteins. Luc-plasmid was provided by the manufacturer as a positive control for the kit.

3. Results

3.1. RTA-K152E binds to cellular RBP-Jκ

The RTA-K152E mutant does not bind DNA efficiently in vitro and fails to activate several KSHV promoter reporter constructs or endogenous lytic gene expression (Zhang *et al.*, 2005). However, it is not known whether RTA-K152E interacts with RBP-Jκ. To test whether RTA-K152E interacts with RBP-Jκ, plasmids expressing these proteins were transfected into 293T cells and co-immunoprecipitation assays (co-IP) were performed. Expression plasmids containing wtRTA, RTA-K152E and RBP-Jκ were transfected into 293T cells. The transfected RTA and RBP-Jκ were tagged by FLAG or influenza hemagglutinin (HA) epitope, respectively (see Section 2 for details). As shown in Figure 1, both wtRTA and RTA-K152E interacted with RBP-Jκ in co-IP assays. In several experiments, wtRTA and RTA-K152E appeared to interact with RBP-Jκ with similar efficiency. In addition, RTA interacted with endogenous RBP-Jκ in co-IP assays (data not shown). Thus, the RTA-K152E interacted with RBP-Jκ protein in transiently transfected 293T cells.

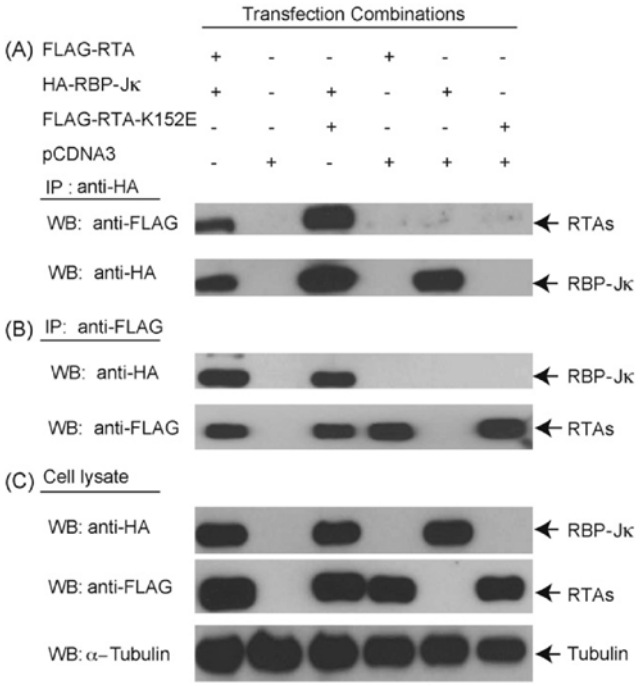

Figure 1. RTA-K152E interacts with RBP-Jκ. 293T cells were transfected with the designated expression plasmids as shown on the top of the figure. Cell extracts from these transfected cells were immunoprecipitated (IP) with either anti-HA (panel A) or anti-FLAG (panel B). The immunoprecipitates were analyzed by western blot (WB) using the indicated antibodies. In panel C, whole cell lysate was used for western blot analyses. The identity of the respective proteins is denoted.

3.2. RTA-K152E binds to RBP-Jκ site indirectly in vitro

To test whether the complex between RTA-K152E and RBP-Jκ interacted with DNA, EMSA was performed with an RBP-Jκ consensus recognition sequence as a probe. The respective proteins were synthesized to similar levels by in vitro transcription and translation (Figure 2B). The expression of RBP-Jκ was also confirmed by western blot with the anti-FLAG antibody (data not shown). Various combinations of in vitro translated proteins were used for EMSA with a RBP-Jκ consensus recognition sequence as probe. As shown in Figure 2, a specific shifted band representing the RBP-Jκ protein binding to the RBP-Jκ probe was observed (lane 3). The shifted band was competed for by incubating with 50× of a cold wild type RBP-Jκ recognition sequence oligonucleotides. In contrast, a mutated RBP-Jκ recognition sequence oligonucleotides did not compete for binding (data not shown). The RTA or RTA-K152E proteins were unable to bind to the RBP-Jκ probe directly, which was expected (lanes 4 and 5). Additional protein–DNA complexes appeared when RTA, or the RTA-K152E mutant, was incubated with RBP-Jκ prior to performing EMSA (lanes 6 and 7). This novel DNA–protein complexes were specific because they disappeared in the presence of a RTA antibody (lanes 8 and 9), but were not affected by normal rabbit serum (NRS) (lanes 10 and 11). Furthermore, the complexes were not present when the RTA antibody was mixed with either RBP-Jκ or RTA individually (lanes 12 and 13). In summary, these results suggested that both wtRTA and the RTA-K152E mutant were able to interact with the RBP-Jκ protein and consequently bind as a complex to a consensus RBP-Jκ recognition site.

3.3. RTA-K152E binds to KSHV DNA in vivo

To test whether a complex between RTA-K152E and RBP-Jκ forms in vivo and binds DNA, ChIP assays were performed.

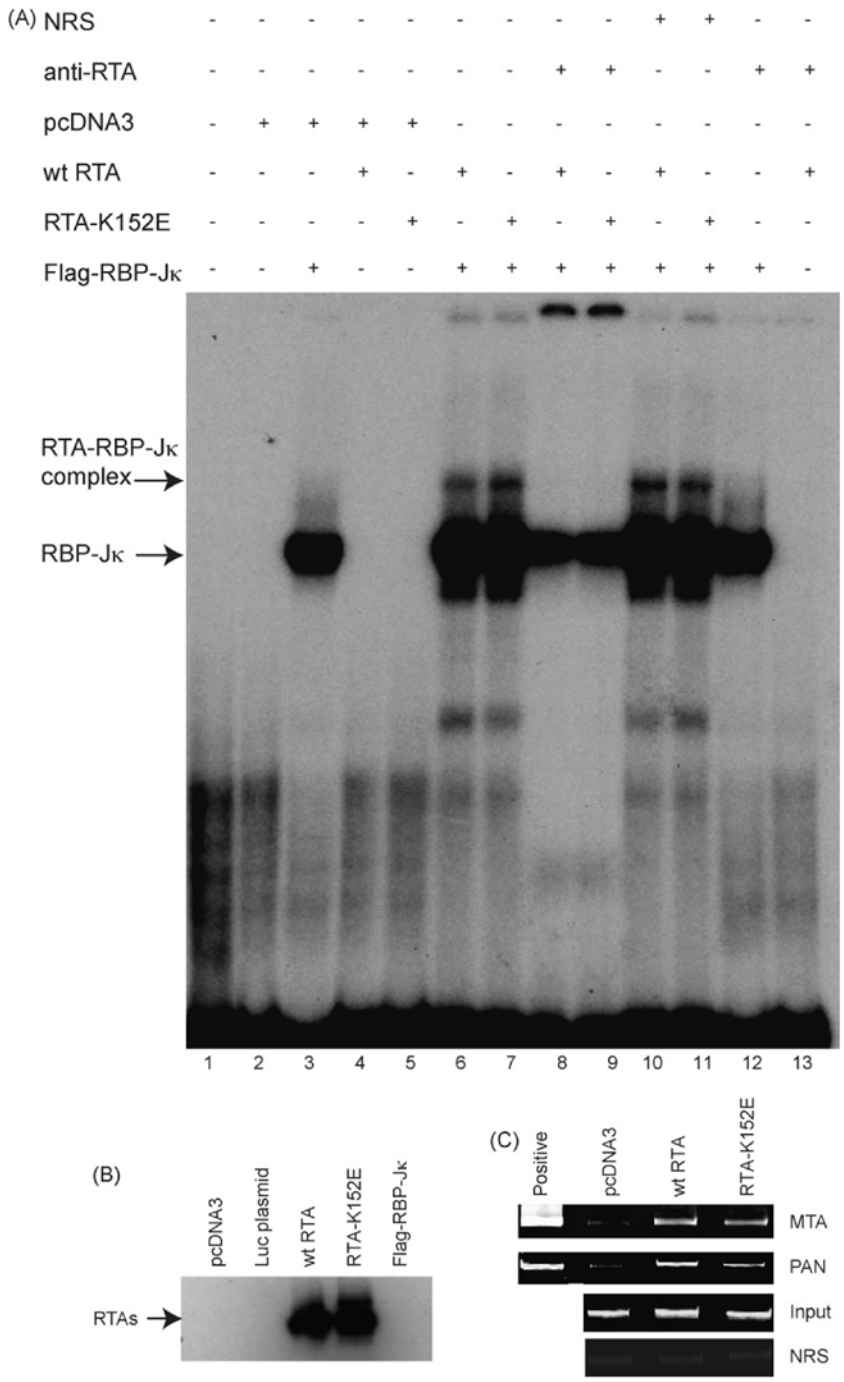

Figure 2. RTA-K152E binds to DNA indirectly via RBP-Jκ. (A) RTA-K152E binds to DNA via RBP-Jκ. The RBP-Jκ probe was labeled with (α-32P)-dCTP. In vitro transcribed and translated proteins were used for EMSA analyses in various combinations as shown on the top of the gel. Normal rabbit serum (NRS) and rabbit polyclonal anti-RTA were used for blocking the complex formations. Specific protein–DNA complexes are as shown. (B) Expression levels of RTA. Equal amounts of in vitro transcribed and translated reticulocyte lysates containing RTA and RTA-K152E proteins were used for western blot analyses. The identity of proteins is as shown. (C) RTA-K152E binds to KSHV genomic DNA in vivo. 293-Bac36 cells were transfected with pcDNA3, RTA or RTA-K152E expression plasmid. Twenty-four hours later, FLAG antibody was used for ChIP analyses to detect in vivo DNA-binding activities of RTA proteins derived from input plasmids. Primers for MTA and Pan promoter regions were used to amplify the DNA from immunoprecipitates. The identity of DNA is as shown. The genomic DNA was used as positive control. Input DNA represents the PCR amplification from same amounts of input lysates without immunoprecipitations. PCR amplification of immunoprecipitates from normal rabbit serum (NRS) is also shown as controls.

The wtRTA or RTA-K152E mutant was transfected into 293-Bac36 cells, a human 293 derived cell line harboring the KSHV genome (Zhou *et al.*, 2002). Expression of the two RTA proteins was similar (data not shown, also Figure 5B). Anti-FLAG antibody was used for ChIP analyses because this approach avoided the endogenous auto-regulation of RTA expression (Deng *et al.*, 2000; Sakakibara *et al.*, 2001). Two KSHV promoter regions, MTA and PAN, were used as tar-

gets for amplifying DNA from the immunoprecipitates as described (Wang *et al.*, 2003a; Wang *et al.*, 2003b). As shown in Figure 2C, both the MTA and Pan promoter regions were amplified from immunoprecipitates regardless of whether wtRTA or RTA-K152E was transfected. However, PCR amplification resulted in the similar amounts of target DNA from the immunoprecipitates by normal rabbit serum (NRS). In addition, input DNA before immunoprecipitations was similar. These results collectively suggested that both wtRTA and RTA-K152E interacted with KSHV genomic DNA in 293T cells with similar efficiencies, which was in agreement with the EMSA data (Figure 2A).

3.4. The RTA-K152E does not activate transcription, in spite of interacting with RBP-Jκ

wtRTA interacts with the RBP-Jκ protein and consequently activates specific viral promoters (Carroll *et al.*, 2006; Liang *et al.*, 2002; Liang and Ganem, 2003). To test whether the RTA-K152E mutant was capable of trans-activating viral transcription, the ability of the RTA-K152E mutant to activate a minimal promoter construct containing multiple RBP-Jκ consensus binding sequences was examined (Liang *et al.*, 2002). The promoter reporter construct and RTA expression plasmids were co-transfected into 293T cells and reporter activities measured. As shown in Figure 3A, wtRTA was able to activate the minimum promoter reporter construct containing RBP-Jκ-binding sites approximately 15-fold in a dose-dependent fashion. In sharp contrast, the RTAK152E failed to activate the reporter construct above basal levels. The mutant RTA was expressed in relatively higher amounts than wtRTA (Figure 3B). Because comparable β-galactosidase activities were observed between wtRTA and the mutant RTA expressing cells, there was no apparent toxicity associated with mutant RTA expressed cells. In addition, same results could be obtained with the similar levels of RTAs by manipulation of transfected plasmid amounts (data not shown). All these results suggested that RTA-K152E was unable to convert RBP-Jκ protein into a transcriptional activator.

3.5. RTA-K152E interferes intracellular Notch signaling

To test the physical interaction between the mutant RTA and RBP-Jκ from another direction, we examined if RTA-K152E affects the Notch signaling pathway. Because RBP-Jκ is involved in the Notch signaling pathway, we suspect that overexpression of the RTA-K152E mutant would alter the Notch signaling pathway by interacting and sequestering the RBP-Jκ protein. A constitutive active mutant of Notch was used for these studies. As shown in Figure 4, the constitutively active Notch mutant activated the RBP-Jκ reporter construct by at least 20-fold in a dose-dependent fashion. The expression of wtRTA-stimulated the RBP-Jκ reporter construct more than 40-fold. The co-expression of wtRTA with activated Notch did not further stimulate the RBP-Jκ reporter construct activity. Although wtRTA binds to RBP-Jκ but because wtRTA itself activates RBP-Jκ, no effect of wtRTA on Notch signaling pathway is anticipated. In sharp contrast, the RTA-K152E mutant interfered with the ability of Notch to activate the RBP-Jκ promoter construct. Due to the fact that mutant RTA interacts with RBP-Jκ, these data suggest that mutant RTA inhibit the Notch signaling and possibly through sequestering RBP-Jκ. These data indirectly support the physical interaction between the mutant RTA and RBP-Jκ in vivo.

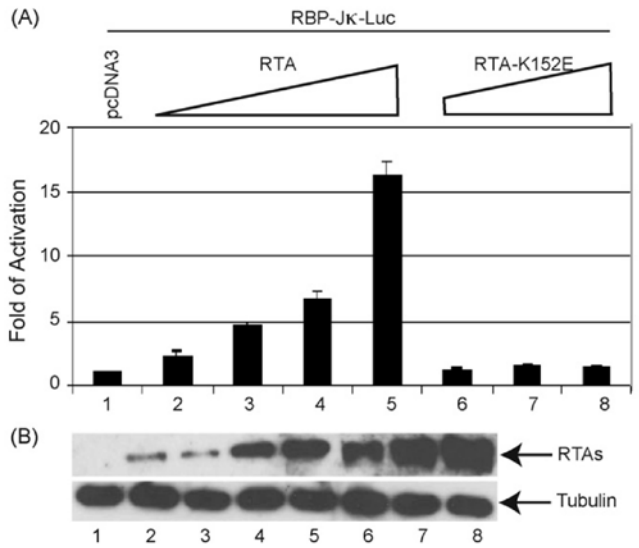

Figure 3. RTA-K152E fails to activate RBP-Jκ protein. (A) RTA-K152E fails to activate the RBP-Jκ protein. 293T cells were transfected with 3× RBP-Jκ-Luc reporter constructs along with CMV-β-gal, RTA or RTA-K152E expression plasmids as shown on the top of the panel. Luciferase activity was normalized by β-galactosidase activity. The fold activation of promoter constructs in response to K-RTA is shown with standard deviations. The results from one representative experiment in triplicates were shown. (B) Expression levels of RTA and its mutant. Western blotting with FLAG antibody was performed. The cell lysate from transfected cells are indicated. The identity of proteins is as shown. Lane numbers denote the same treatment as shown in panel A.

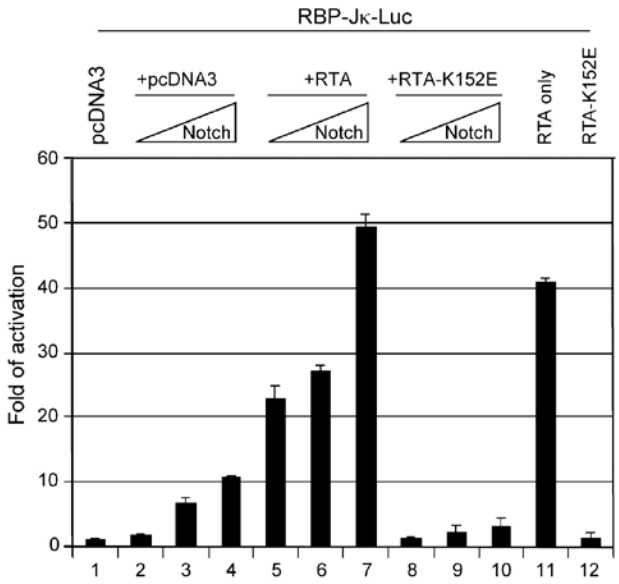

Figure 4. RTA-K152E interferes intracellular Notch signaling. Various amounts of constitutively active Notch were transfected into 293T cells with a fixed amount of the other plasmids as shown on the top of the panel. Luciferase activity was normalized by β-galactosidase activity. The fold activation of promoter constructs was shown with standard deviations. The results from one representative experiment in triplicates were shown.

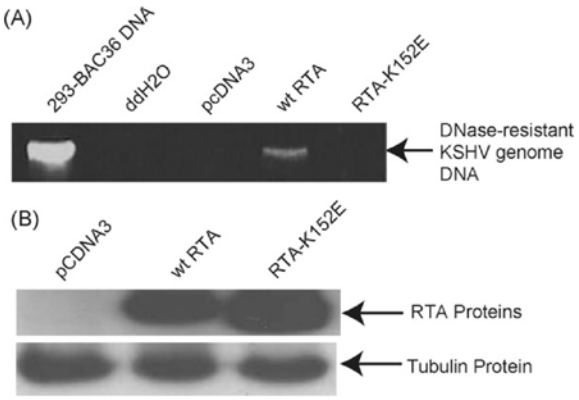

Figure 5. RTA-K152E fails to induce KSHV virion production. (A) RTA-K152E fails to induce DNase-resistant KSHV genomic DNA packaged into virions. The media from pcDNA3, RTA or RTA-K152E transfected 293-Bac36 cells were collected and KSHV viruses were concentrated by centrifugation. After DNase I digestion, PCR analyses were used for detecting KSHV genomic DNA. The genomic DNA isolated from 293-Bac36 was used as positive control. (B) Expression of RTA and its mutant in transfected cells. Lysate from pcDNA3, RTA, or RTA-K152E transfected 293-Bac36 cells were used for western blot analysis with FLAG and tubulin antibodies. The identity of the respective proteins is denoted.

3.6. RTA-K152E fails to induce KSHV virion production

We have previously shown that RTA-K152E fails to activate KSHV lytic genes through direct DNA binding (Zhang *et al.*, 2005). In this report, data in Figure 3 and Figure 4 suggested that RTA-K152E would not activate genes mediated by RTA–RBP-Jκ-complex through an indirect DNA-binding mechanism. Because RTA activates viral promoters through both direct and indirect DNA-binding mechanism, it is thus necessary to directly test if the mutant fails to induce a complete KSHV lytic replication. KSHV virion production is the final step of the lytic replication and we thus measured the production of the virions in response to RTA expression. The expression plasmid of wtRTA or RTA-K152E mutant was transfected into 293-Bac36 cells (Zhou *et al.*, 2002). The expression levels of RTAs were similar in cell lysates (Figure 5B). The media were collected 5 days later and virus particles in the media were concentrated and digested by DNase. Semi-quantitative PCR was subsequently used to detect the DNase-resistant KSHV genomic DNA packaged in virions. The condition of the PCR was established empirically for a linear amplification of template DNA. As expected, wtRTA induced the accumulation of DNase-resistant KSHV genomic DNA (Figure 5A). In contrast, DNase-resistant genomic DNA was not detected following transfection of 293-Bac36 cells with the RTA-K152E mutant. Thus, RTA-K152E mutant was unable to induce KSHV virion production.

4. Discussion

Numerous studies have indicated that RTA activates target promoters by directly binding to DNA and by indirectly binding via interaction with various cellular factors (Dourmishev *et al.*, 2003; West and Wood, 2003). RBP-Jκ plays an essential role in RTA-mediated KSHV lytic replication. In this report, we have addressed whether the RTA-RBP-Jκ interaction is sufficient for the lytic replication of KSHV.

Notch signaling is well conserved in evolution. Many viruses usurp this signaling pathway for their own benefits (Hayward, 2004). However, none of them, including KSHV, can be completely replaced by cellular Notch signaling (Chang *et al.*, 2005). RTA must have its own specific properties to usurp Notch signaling pathway. RTA-K152E has a mutated DNA-binding domain and has a defect in its direct DNA-binding activity as determined by EMSA with the purified protein. Like wtRTA, RTA-K152E interacted with RBP-Jκ physically and the mutant–RBP-Jκ complex was able to bind to DNA (Figure 1 and Figure 2). However unlike the wtRTA, the physical interaction between the RTA mutant and RBP-Jκ failed to activate the RBP-Jκ protein. The mutant further inhibited the function of a constitutively active Notch mutant (Figure 4). Moreover, as an extension to our previous publication, the RTA-K152E failed to induce KSHV virion accumulation (Figure 5). Thus, two apparently contradicting properties of RTA-K152E have clearly emerged from our data: (1) RTA-K152E failed to activate the RBP-Jκ protein; but (2) the mutant had similar capabilities to interact with RBP-Jκ and furthermore to bind to DNA indirectly via the interaction. The two properties collectively suggest that the interaction between RTA and RBP-Jκ and their subsequent DNA binding by the complex were not sufficient for the initiation of lytic replication of KSHV.

It remains unclear how the mutation in the DNA-binding domain of RTA had such a profound effect on RTA functions. Because of the known defect in this mutant, our results might suggest that direct DNA-binding activity of RTA might be required to convert the RBP-Jκ protein from a repressor into an activator. Since RTA interacts with many cellular factors (Carroll *et al.*, 2006; Gwack *et al.*, 2001; Gwack *et al.*, 2002; Liang *et al.*, 2002; Liang and Ganem, 2003; Liao *et al.*, 2003; Wang *et al.*, 2001), it is possible that the K152E mutation affects the binding of an unknown cellular factor. It is also possible that the mutation at the DNA-binding domain (K152E) has changed RTA conformation from a transcriptional activator into a repressor. Based on the structure and function analysis of RTA (Dourmishev *et al.*, 2003; West and Wood, 2003), the activation domain is located at the C-terminal region of the RTA. Thus, it is hard to imagine that a single point mutation at the N-terminus would affect the C-terminal activation domain.

In summary, this report examined the physical interaction between RTA and RBP-Jκ with respect to the activation of the RBP-Jκ protein and the activation of KSHV lytic replication. Our data demonstrated that the physical interaction between KSHV RTA and RBP-Jκ and their subsequent DNA binding by the complex were not sufficient to activate RBP-Jκ and to induce KSHV lytic replication. Other factor(s), whether it is the intrinsic property of RTA or its interaction with other cofactors, are predicted to be required for the activation of RBP-Jκ protein by RTA. These findings have provided a new insight into the structure and functions of RTA.

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