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Kaposi's sarcoma-associated herpesvirus/human herpesvirus-8 ORF50 gene product contains a potent C-terminal activation domain which activates gene expression via a specific target sequence*

Brief Report

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Summary. The ART (Activator of Replication and Transcription) protein of Kaposi's sarcoma-associated herpesvirus (KSHV), or human herpesvirus-8 (HHV-8), is encoded by the ORF50 gene. It is expressed as an immediate-early gene and plays a crucial role in the transition between latency and productive infection. HHV-8 ART is a transcriptional transactivator which can up-regulate viral gene expression. Transient expression assays showed that ART strongly activated ORF57 and K8 promoter-directed gene expression in both CV-1 and BJAB cells. The ART target site was mapped to a 40-bp region compassing nt 81904 to 81943 on the ORF57 promoter. When linked upstream to a heterologous SV40 promoter, this region by itself was able to confer ART responsiveness. This 40-bp segment contains a 16-bp consensus sequence which is also found in the K8 promoter region located between nt 74769 to 74784. Deletion of the fragment including this 16-bp consensus abrogated the ART responsiveness of the K8 promoter. The role of this 16-bp consensus in ART transactivation was further supported by site-directed mutagenesis. Mutations of the conserved nucleotides within the 16-bp consensus in the ORF57 promoter dramatically impaired its responsiveness to ART. Fusion protein analysis with chimeric proteins containing the DNA binding domain of yeast transactivator Gal4 (residues 1 to 147) and different ART segments defined an acidic C-terminal region (amino acids [aa]

*The GenBank accession number for sequences in this paper is KSU 75698.

527 to 634) as a potent activator. Deletions of this activation domain in the ART protein resulted in a decrease or loss of its ability to activate ORF57 and K8 promoters containing the ART responsive element in transfected cells. How the ART activation domain activates ORF57 and K8 gene expression through the 16-bp consensus sequence remains to be determined.

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Kaposi's sarcoma-associated herpesvirus (KSHV), also known as human herpesvirus-8 (HHV-8), is a newly identified human herpesvirus present in more than 90% of all forms of Kaposi's sarcoma (KS) [3–5, 15]. Infection by this virus is also associated with abnormal lymphoproliferation, including primary effusion lymphoma (PEL) and multicentric Castleman's disease [2, 21]. Nucleotide sequence analysis of HHV-8 has classified it into the gamma-herpesvirus subfamily, whose members include Epstein-Barr virus (EBV) and herpesvirus saimiri (HVS) [19]. Several human B cell lines derived from PEL have been established for in vitro HHV-8 culture. The virus is latent in these cell lines, but can be induced into lytic replication by treatment with phorbol ester or sodium butyrate [1, 14, 18, 26]. In the well-studied EBV latency-lytic cycle switch model, the transactivator Rta (BRLF1), which is activated by the immediate-early (IE) gene product Zta (ZEBRA or EB1), plays an essential role in the induction of viral replication [16, 17, 27]. Rta can activate the expression of many EBV promoters, including that of another transactivator Mta, and lead to activation of viral gene expression. For both EBV and HVS, specific target DNA sequences termed as Rta-responsive elements (RREs) are required for activation by Rta [7, 8, 24, 25]. It has been reported that EBV Rta contains a C-terminal activation domain that consists of three partially overlapping hydrophobic motifs, which is involved in the activation of promoter elements that contain the RRE sequences [9, 13].

The HHV-8 ORF50 encodes a homolog of the EBV lytic transactivator Rta, and its gene product has recently been renamed as the Activator of Replication and Transcription (ART) at the 3rd international workshop on Kaposi sarcoma-associated herpesvirus and related agents. The ORF50 mRNA is expressed as early as 1 h after induction of viral replication and the ART protein is thought to act as the molecular switch controlling viral reactivation by up-regulating the expression of several downstream viral genes [11, 12, 22, 23, 28]. Two of the potential genes that can be activated by ART are ORF57 (homolog of the EBV Mta gene) and K8 (homolog of the EBV Zta gene). Both ORF57 and K8 were demonstrated to express early after induction of viral replication [10, 23, 28], and ART has been reported to activate the promoters of ORF57 and K8 in a dose-dependent manner [12, 20].

We have recently cloned a 453-bp ORF57 promoter fragment (nt 81556 to 82008), including the regulatory elements of the HHV-8 ORF57, to the upstream of the luciferase reporter gene to generate clone p57Pluc1 (Fig. 1A). In the presence of an ART expression plasmid pcDNA50, which contains full-length cDNA of the ORF50, ORF57 promoter-directed luciferase expression was enhanced about 163-fold in CV-1 cells and 64-fold in BJAB cells [6]. We have further

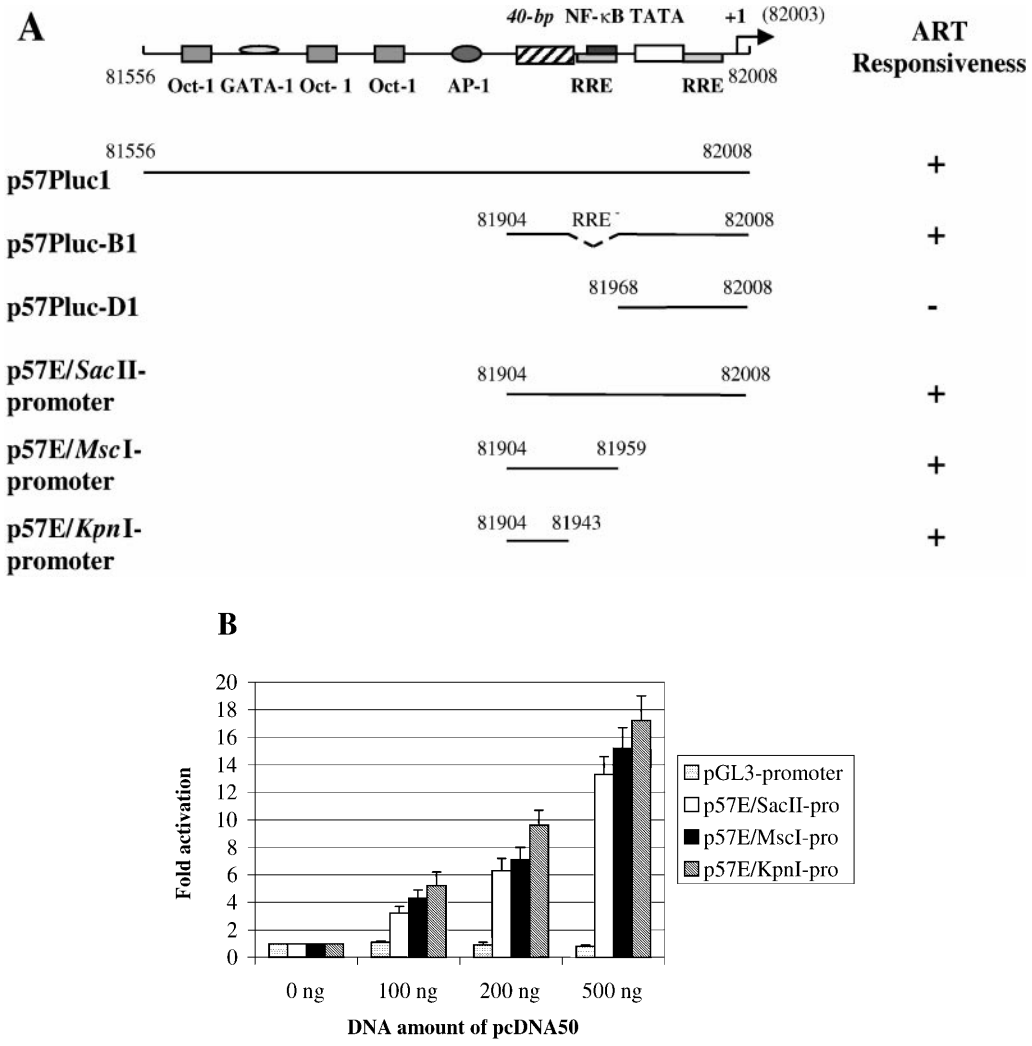
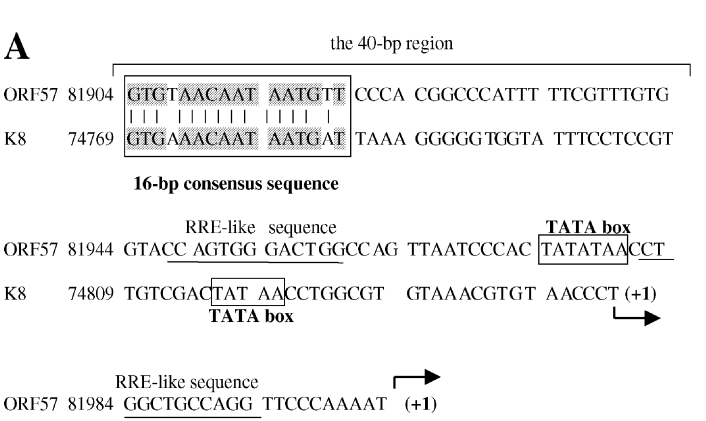
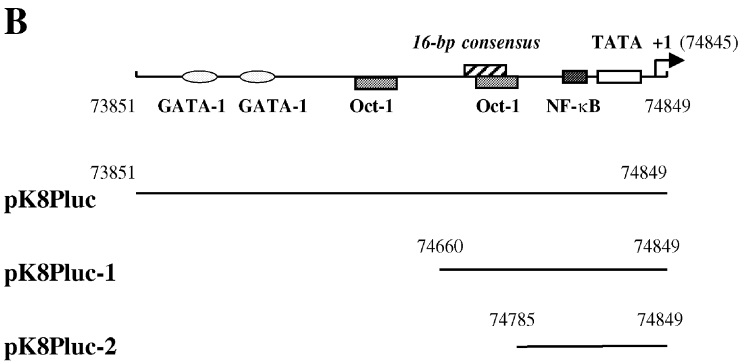
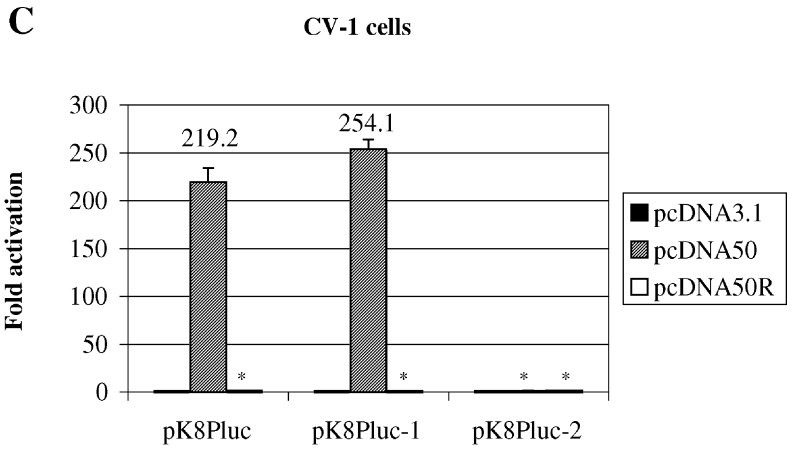
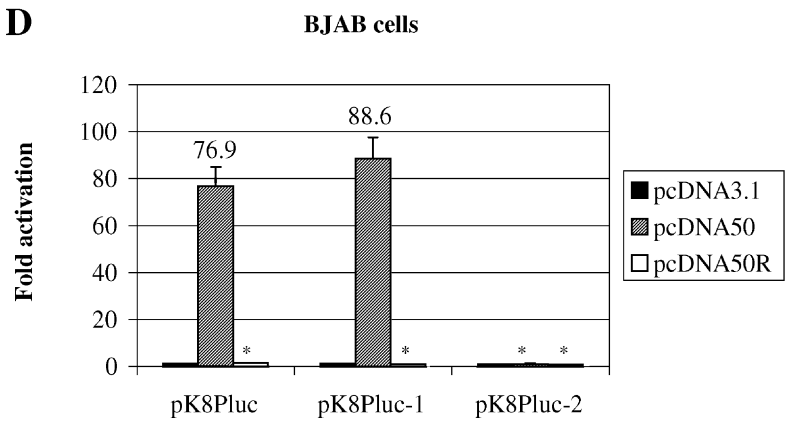


Fig. 1. Characterization of ART responsive element on the ORF57 promoter. **A** Schematic representative of the ORF57 promoter elements and the various clones that were used to map the ART target site. The various potential cellular factor binding sites as well as the 40-bp ART target site are indicated. RRE indicates the Rta responsive element-like sequence; + 1 represents the transcription start site (TSS). Sequences retained in different ORF57 promoter constructs are represented by solid lines. Responsiveness of each construct to ART is indicated by + or −. **B** Activation of the various ORF57-SV40 promoter constructs in the presence of an increasing amount of ART expression plasmid pcDNA50. Activation was detected as an increase in luciferase activity. Each transfection was carried out with 50 ng of reporter plasmid and the indicated amount of pcDNA50 in CV-1 cells using Lipofectamine (Gibco BRL). Total DNA amount used in each transfection was equalized to 800 ng by adding pcDNA3.1(−) vector (Invitrogen). Transfected CV-1 cells were harvested at 48 h post-transfection, and luciferase activities were measured using the Luciferase Assay System (Promega). The data was an average obtained from at least three independent experiments


A
the 40-bp region
ORF57 81904 GTGTAACAAT AATGTT CCCA CGGCCCATTT TTCGTTTGTG
K8 74769 GTGAAACAAT AATGAT TAAA GGGGGTGGTA TTTCCTCCGT
16-bp consensus sequence
RRE-like sequence
TATA box
ORF57 81944 GTACC AGTGG GACTGGCCAG TTAATCCCAC TATATAACCT
K8 74809 TGTCGACTAT AACCTGGCGT GTAAACGTGT AACCCT (+1)
TATA box
RRE-like sequence
ORF57 81984 GGCTGCCAGG TTCCCAAAAT (+1)


B
16-bp consensus
TATA +1 (74845)
73851 GATA-1 GATA-1 Oct-1 Oct-1 NF-κB 74849
73851 74849
pK8Pluc
74660 74849
pK8Pluc-1
74785 74849
pK8Pluc-2


C
CV-1 cells
Fold activation
300
250
200
150
100
50
0
219.2
254.1
*
pK8Pluc
pK8Pluc-1
pK8Pluc-2
pcDNA3.1
pcDNA50
pcDNA50R


D
BJAB cells
Fold activation
120
100
80
60
40
20
0
76.9
88.6
*
pK8Pluc
pK8Pluc-1
pK8Pluc-2
pcDNA3.1
pcDNA50
pcDNA50R

generated several deletion clones including p57Pluc-B1 and p57Pluc-D1 (Fig. 1A), and mapped the ART responsive element to a 40-bp region spanning nt 81904 to 81943 on the ORF57 promoter [6]. A number of transcription factor binding sites such as Oct-1, AP-1, and NF-κB can be identified in this region of the ORF57 promoter. Two RRE consensus sequences, CCN_9GG, which were found to be the target sites of the EBV and HVS ORF50 proteins [7, 8, 24, 25] can also be found in this region. Interestingly, none of these potential elements were necessary for ART responsiveness.

The objective of this study is to further characterize the ART target site and the ART activation domain that is responsible for the activation of its target gene. To confirm the role of this 40-bp sequence to serve as the target site for ART activation, we here examined its effect on a heterologous promoter by inserting this fragment into the 5′ end of an SV40 promoter vector (Fig. 1A). The *Sac*I/*Sac*II fragment spanning nt 81904 to 82008, *Sac*I/*Msc*I fragment spanning nt 81904 to 81959, and the *Sac*I/*Kpn*I fragment spanning nt 81904 to 81943 were inserted into the *Kpn*I site of pGL3-promoter vector (Promega) to generate p57E/*Sac*II-promoter, p57E/*Msc*I-promoter, and p57E/*Kpn*I-promoter. All three clones were activated by the ART expression clone pcDNA50 but not by pcDNA50R which contains the ART cDNA in the opposite orientation, and consistent levels of activation by ART were observed (Fig. 1B). The lower activation levels of the ORF57-SV40 promoters by ART as compared to the native p57Pluc1 promoter construct could be due to the high background luciferase activity of the SV40-based pGL3-promoter vector, even in the absence of ART. This result nevertheless supports our earlier observation that the 40-bp sequence identified in the ORF57 promoter mediates ART responsiveness, and indicates that the presence of this fragment can confer ART responsiveness to a heterologous promoter such as SV40 promoter. The exact mechanism by which ART mediates transactivation via this 40-bp target is not clear; ART might transactivate ORF57 expression by binding directly to the 40-bp target sequence, or it might function indirectly via cellular factors. To determine the possibility of direct binding, gel shift assays

Fig. 2. Analysis of the 16-bp consensus sequence in the K8 promoter. **A** Alignment and comparison of the HHV-8 ORF57 and K8 promoter sequences. Identical nucleotides in the 16-bp consensus sequence (boxed) are shadowed. The TATA boxes and RRE-like sequences are also indicated; + 1 represents the transcription start site. **B** Schematic representation of the various K8 promoter clones. Putative regulatory elements and the 16-bp consensus sequence are indicated. Sequences retained in different K8 promoter constructs are represented by solid lines. **C** ART responsiveness of the various K8 promoter constructs in CV-1 cells. Transfection was carried out as described for Fig. 1B, using 200 ng of reporter plasmids and 500 ng of pcDNA50. Plasmid pcDNA50R that contains ART cDNA in reverse orientation and the pcDNA3.1 vector were used as controls. **D** ART responsiveness of the various K8 promoter constructs in BJAB cells. For each transfection, 1×10^7 of BJAB cells were mixed with 5 μg of reporter plasmids and 10 μg of pcDNA50 or controls, and electroporated at 250 V and 960 μF using GenePulser (BioRad). Luciferase activities were measured at 48 h post-transfection. Asterisks indicate activities at or below the pcDNA3.1 control level, which was normalized to 1

using a ^{32}P-labeled oligonucleotide probe compassing the 40-bp region from nt 81904 to 81943 were performed. The probe was incubated with nuclear extracts from either TPA-treated or untreated BC-3 cells, and from pcDNA50-transfected BJAB cells. However, no specific retarded bands were observed (data not shown). This could either be due to the low abundance of ART in these cells, or it is possible that ART may not be binding directly to this region.

The 40-bp ART responsive region in ORF57 promoter does not exhibit homology with the EBV/HVS RRE or known transcription factor binding sites. However, a more extensive analysis revealed that a 16-bp sequence from nt 74769 to 74784 in the HHV-8 K8 promoter has an 87.5% homology with the first 16 bps of the 40-bp region in the ORF57 promoter. As shown in Fig. 2A, 14 nucleotides within this 16-bp consensus sequence are identical between the ORF57 and K8 promoter segments. This 16-bp consensus conserved in ORF57 and K8 promoters can potentially form a palindrome which may act as the ART responsive element (ARE). Indeed, the K8 promoter was recently shown to be activated in vitro by ART [12, 20]. To further investigate the responsiveness of this 16-bp sequence to ART, the K8 promoter region from nt 73851 to 74849 was PCR-cloned into the pGL3-Basic vector (Promega) to generate the K8 promoter reporter construct pK8Pluc (Fig. 2B). The K8 promoter region also contains several putative transcription factor binding sites, such as GATA-1, Oct-1, and NF-κB, in addition to the 16-bp consensus sequence. Co-transfection of the pK8Pluc and pcDNA50 into CV-1 and BJAB cells showed that the K8 promoter construct was strongly activated by ART (Fig. 2C and 2D). Over 219-fold activation was observed in CV-1 cells and over 76-fold was observed in BJAB cells. The lower activation levels in BJAB cells could be due to the lower transfection efficiencies in these cells. Two 5′-terminal truncated clones were then constructed in order to determine whether the 16-bp consensus sequence or other elements could act as the target site for ART. Clone pK8Pluc-1 contains an insert with deletions of the putative GATA-1 and Oct-1 sites but retains the region from nt 74660 to 74849 which includes the 16-bp sequence. A second clone pK8Pluc-2 has a deletion that includes the 16-bp sequence (Fig. 2B). Co-transfection experiments of these two clones with pcDNA50 indicated that deletion of the upstream transcription factor binding sites, as represented by clone pK8P-luc1, did not interfere ART responsiveness; a 254-fold activation by ART in CV-1 cells and 88-fold in BJAB cells were observed (Fig. 2C and 2D). However, further deletion including the 16-bp sequence, as represented by clone pK8Pluc-2, resulted in a complete loss of the ART responsiveness in both cell types (Fig. 2C and 2D). This result demonstrated that a fragment containing the 16-bp consensus sequence in K8 promoter is also involved in conferring ART responsiveness. The conservation of this 16-bp sequence between ORF57 and K8 promoters and the requirement for this sequence to confer ART responsiveness suggest that this sequence contains the ART responsive element (ARE), and similar mechanism is involved in the activation of the ORF57 and K8 promoters by ART.

To confirm the role of this consensus sequence and the importance of the conserved nucleotides in rendering ART responsiveness, three mutants of the

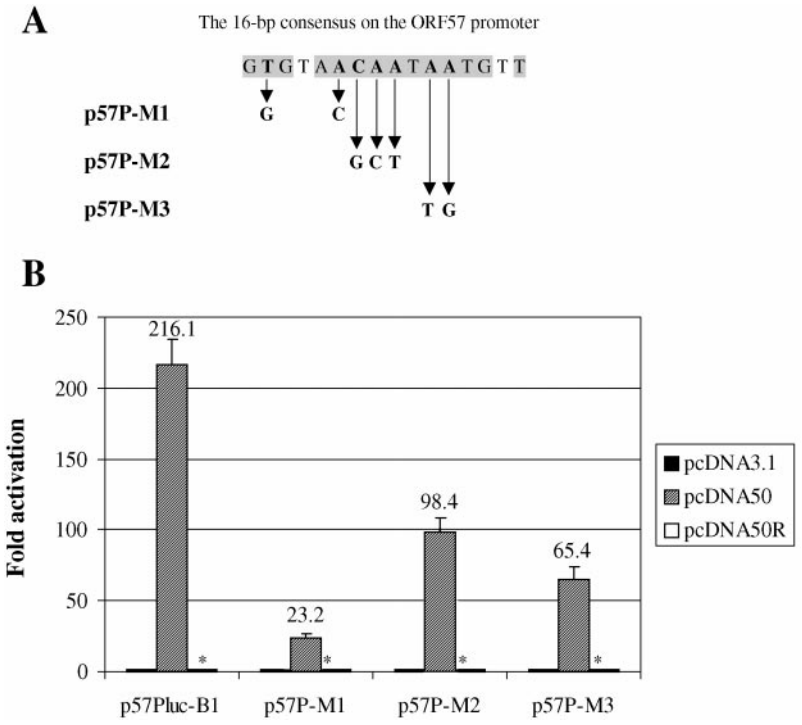


Fig. 3. Mutations in the 16-bp consensus in the ORF57 promoter impaired ART responsiveness. **A** Schematic representation of the mutations introduced into the 16-bp consensus sequence in the ORF57 promoter. Clone p57Pluc-B1 (Fig. 1A) which contains truncated ORF57 promoter was used as the template for two separate PCR reactions. The primers used in the first reaction consisted of an upstream vector-specific primer and a downstream primer containing the desired mutations in the 16-bp sequence. The second reaction was performed with a downstream vector-specific primer and an upstream primer containing the same mutations in the complementary strand. Products of the two PCRs containing the same mutations in their overlaps were used as the template for the second round PCR, with the two vector-specific primers. The second-round PCR product was then inserted into the same restriction enzyme sites in p57Pluc-B1 to replace the wild type ORF57 promoter fragment to generate mutant plasmids p57P-M1, p57P-M2, and p57P-M3. The shaded nucleotides represent those that are conserved between the ORF57 and K8 promoters. The nucleotides that were mutated are bolded. **B** Activation of the wild type and mutant ORF57 promoter constructs by ART. Two hundred ng of each mutant plasmid indicated in **A** was co-transfected into CV-1 cells with 500 ng of pcDNA50 or control plasmids. Luciferase activities were measured at 48 h post-transfection. Asterisks indicate activities at or below the pcDNA3.1 control level, which was normalized to 1

ORF57 promoter reporter plasmid with mutations in the 16-bp consensus were generated by PCR-based site-directed mutagenesis. These three mutants p57P-M1, p57P-M2, and p57P-M3, containing substitutions in the different conserved nucleotides, are shown in Fig. 3A. The ART responsiveness of each mutant was tested by co-transfecting CV-1 cells with the pcDNA50. Mutagenesis of different conserved nucleotides within this 16-bp consensus sequence all dramatically affected ORF57 promoter's responsiveness to ART. Mutations in the p57P-M1

affected ART transactivation most, losing almost 90% of its responsiveness to ART, dropping from 216-fold activation to about 23-fold (Fig. 3B). Mutations in p57P-M2 and p57P-M3 also affected their responsiveness, but not as dramatically as p57P-M1. Activation by ART dropped from 216-fold to about 98-fold for p57P-M2 and to about 65-fold for p57P-M3 (Fig. 3B). These results further confirm the role of this 16-bp consensus sequence in rendering ART responsiveness.

It was reported recently that the activation domain of ART is located in the carboxyl terminus of the protein [12, 20]. To further study the interaction between the ART activation domain and ORF57 and K8 promoter elements that contain the ARE, we decided to further characterized the ART activation domain using the Gal4 fusion protein analysis (Fig. 4A). Different fragments of ART protein were fused downstream to the DNA binding domain of the yeast transactivator Gal4 to generate various Gal4-ART chimeras with overlapping fragments of the ART C-terminal domain (Fig. 4A). The ability of each fusion protein to activate transcription was examined in two strains of *S. cerevisiae*, Y190 and AH109. Y190 contains a LacZ reporter gene under the control of a Gal4-regulated upstream activating sequence (UAS), whereas AH109 contains a Gal4-controlled HIS3 reporter gene. Thus, the ability of the ART segments to function as transcriptional activator was determined by the growth of AH109 in the absence of histidine and by the expression of β-galactosidase in Y190. The full-length ART clone pGBK-ORF50 and the plasmid pCL-1 (Clontech), which expressed the wild-type Gal4 protein, were used as positive controls, and the pGBKT7 vector (Clontech) was used as a negative control. The presence of the full-length ART

Fig. 4. Identification and functional analysis of the activation domain of ART protein. **A** Analysis of transcriptional activation by Gal4-ART fusion proteins in *S. cerevisiae*. Schematic representative of the various plasmids directing the expression of fusion proteins containing ART segments fused to Gal4 DNA binding domain (BD) from aa 1 to 147 are indicated. The hatched boxes represent the segments of the ART that were included in the fusion proteins. The activation of Gal4-controlled reporter gene expression was determined by the growth of yeast strain AH109 in the absence of histidine (His) and the expression of β-galactosidase (β-gal) in strain Y190. β-galactosidase activity was measured by liquid culture assay using ONPG as substrate. One unit of β-galactosidase was defined as the amount needed to hydrolyze 1 μmol of ONPG per min per cell. The β-gal units are the mean of three independent experiments. pCL-1 that encodes the full-length, wild-type Gal4 protein and the pGBKT7 vector were used as positive and negative control, respectively. **B** Schematic representative of the ART protein and the various constructs containing deletions in the ART activation domain. The putative nuclear localization signals (NLS-1, NLS-2), leucine zipper, basic region, as well as the activation domain defined in this paper are indicated. The fragments retained in each construct are indicated by hatched boxes. **C** Activation of different ORF57 promoter constructs by the ART AD deletion clones. Transfection of CV-1 cells was carried out using 200 ng of reporter plasmid and 500 ng of ART constructs or pCMV-Tag2A vector control. **D** Activation of different K8 promoter constructs by the ART AD deletion clones. Transfection was carried out using the same amount of plasmid DNA as described for **C**. Luciferase activities were measured at 48 h post-transfection. Asterisks indicate activities at or below the pCMV-Tag2A control level, which was normalized to 1

A
ART protein
Gal4 BD
527 600 634
691aa
β-gal (units)
His
SacI ApaI BstEII
pGBK-ORF50
14.30±4.77 +
pGBK-527/691
20.27 ±1.62 +
pGBK-527/634
23.38 ±4.54 +
pGBK-527/598
18.87 ±1.14 +
pGBK-600/691
7.75 ± 0.64 +
pGBK-634/691
0.94 ± 0.36 -
pCL-1
69.53 ± 11.90 +
pGBKT7
0.57 ± 0.18 -
B
NLS-1
Leu Zipper
NLS-2
N
C
Basic Region
Activation Domain
1
527
634
691
pCMV-TagAD1
589
pCMV-TagAD2
609
pCMV-TagAD3
626
pCMV-Tag50
691
C
CV-1 cells
Fold activation
200
150
100
50
0
154.5
139.2
3.1
148.3
166.3
7.3
*****
pGL3-Basic
p57Pluc1
p57Pluc-B1
p57Pluc-D1
pCMV-Tag2A
pCMV-TagAD1
pCMV-TagAD2
pCMV-TagAD3
pCMV-Tag50
D
CV-1 cells
Fold activation
166.8
149.2
24.6
155.4
178.9
28.3
*****
pGL3-Basic
pK8Pluc
pK8Pluc-1
pK8Pluc-2
pCMV-Tag2A
pCMV-TagAD1
pCMV-TagAD2
pCMV-TagAD3
pCMV-Tag50

fused to Gal4 (pGBK-ORF50) activated expression of both LacZ and HIS3 in Y190 and AH109 (Fig. 4A). The clone pGBK-527/634 that contained 108 aa of the C-terminus retained the full activation activity, and was consistently found to be more active than the intact pGBK-ORF50 clone. This suggests that deletion of the extreme C-terminal 57-aa segment (aa 635 to 691) has no detrimental effects on the activation function of ART protein. However, further deletion of 36 aa from the C-terminus of clone pGBK-527/634, as represented by clone pGBK-527/598, affected ART function slightly. A drop from 23.38 to 18.87 units of β-galactosidase activities (clone pGBK-527/634 vs. clone pGBK-527/598) was observed. Interestingly, a segment containing the extreme C-terminal region, aa 600 to 691, as represented by clone pGBK-600/691, retained partial activity. These results suggest that more than one sub-domain in the putative activation domain of ART from aa 527 to 634 could be responsible for the transactivation function.

To further confirm that the ART C-terminal domain, responsible for activation in the yeast cells, also mediates the activation of the ORF57 and K8 through the ARE in mammalian cells, several other ART expression clones containing deletions in the C-terminal domain from aa 527 to 634 were generated (Fig. 4B). Clones pCMV-Tag50, pCMV-TagAD1, pCMV-TagAD2 and pCMV-TagAD3 were generated by inserting PCR products spanning ART aa 1–691, 1–589, 1–609 and 1–626 encoding sequences into the *Eco*RV/*Sal*I sites of pCMV-Tag2A vector (Stratagene) respectively. Activation functions of these ART expression clones were then tested with the ORF57 and K8 promoter constructs by transient transfection assays in CV-1 cells (Fig. 4C and 4D). Clone pCMV-TagAD1, which contains aa 1–589 did not activate either the intact or deletion ORF57 and K8 promoters. Clone pCMV-TagAD2 that contains aa 1–609 was found to retain partial activity. As expected, this ART deletion clone was only active with the ORF57 and K8 promoter constructs that contain the 16-bp consensus (p57Pluc1 & p57PlucB1; pK8Pluc & pK8Pluc-1), but not with the constructs where this consensus was deleted (p57PlucD1; pK8Pluc-2). Clone pCMV-TagAD3 that contains aa 1–626 retained almost full activity of the intact ART protein in activating promoter constructs with the 16-bp consensus. These results support our analysis in the yeast system (Fig. 4A), but there are some differences between the activations by ART in yeast and in mammalian cells. The reduction in activation levels by ART C-terminal deletion clones in yeast (Fig. 4A) was not as dramatic as in mammalian cells (Fig. 4C and 4D). The presence of the C-terminal fragment from aa 527 to 598 is sufficient to confer most of the transactivation activity in yeast (as represented by pGBK-527/598 in Fig. 4A). However, the ART C-terminal fragment up to aa 609, as represented by clone pCMV-TagAD2, has very little transactivation activity in CV-1 cells. Its activation function has been reduced by almost 90% (Fig. 4C and 4D). Our transfection experiments thus suggest that in mammalian cells, presence of the activation domain of ART, up to aa 626, may be required for its activation function. The difference in transactivation activities by different regions of ART in yeast and in mammalian cells could be due to the host cell differences or the differential

stability of the expressed proteins in the two cell types. These differences as well as how the activation domain of ART mediates the transactivation of ORF57 and K8 promoters via the 16-bp consensus sequence need to be further characterized.

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References

1. Arvanitakis L, Mesri EA, Nador RG, Said JW, Asch AS, Knowles DM, Cesarman E (1996) Establishment and characterization of a primary effusion (body cavity-based) lymphoma cell line (BC-3) harboring Kaposi's sarcoma-associated herpesvirus (KSHV/HHV-8) in the absence of Epstein-Barr virus. Blood 88: 2648–2654
2. Cesarman E, Chang Y, Moore PS, Said JW, Knowles DW (1995) Kaposi's sarcoma-associated herpesvirus-like DNA sequences in AIDS-related body-cavity-based lymphomas. N Engl J Med 332: 1186–1191
3. Chang Y, Cesarman E, Pessin MS, Lee F, Culpepper J, Knowles DM, Moore PS (1994) Identification of herpesvirus-like DNA sequences in AIDS-associated Kaposi's sarcoma. Science 266: 1865–1869
4. Chang Y, Ziegler J, Wabinga H, Katangole-Mbidda E, Boshoff C, Schulz TF, Whitby D, Maddalena D, Jaffe HW, Weiss RA, Moore PS (1996) Kaposi's sarcoma-associated herpesvirus DNA sequences are present in African endemic and AIDS-associated Kaposi's sarcoma. Arch Int Med 156: 202–204
5. Chang Y, Moore PS (1996) Kaposi's sarcoma (KS)-associated herpesvirus and its role in KS. Infect Agents Dis 5: 215–222
6. Duan W, Wang S, Liu S, Wood C (2001) Characterization of Kaposi's sarcoma-associated herpesvirus/human herpesvirus-8 ORF57 promoter. Arch Virol 146: 403–413
7. Gruffat H, Sergeant A (1994) Characterization of the DNA-binding site repertoire for the Epstein-Barr virus transcription factor R. Nucleic Acids Res 22: 1172–1178
8. Gruffat H, Manet E, Rigolet A, Sergeant A (1990) The enhancer factor R of Epstein-Barr virus (EBV) is a sequence-specific DNA binding protein. Nucleic Acids Res 18: 6835–6843
9. Hardwick JM, Tse L, Applegren N, Nicholas J, Veliuona MA (1992) The Epstein-Barr virus R transactivator (Rta) contains a complex, potent activation domain with properties different from those of VP16. J Virol 66: 5500–5508
10. Lin SF, Robinson DR, Miller G, Kung HJ (1999) Kaposi's sarcoma-associated herpesvirus encodes a bZIP protein with homology to BZLF1 of Epstein-Barr virus. J Virol 73: 1909–1917
11. Lukac DM, Renne R, Kirshner JR, Ganem D (1998) Reactivation of Kaposi's sarcoma-assicoated herpesvirus infection from latency by expression of the ORF50 transactivator, a homolog of the EBV R protein. Virology 252: 304–312
12. Lukac DM, Kirshner JR, Ganem D (1999) Transcriptional activation by the product of open reading grame 50 of Kaposi's sarcoma-assicoated herpesvirus is required for lytic viral reactivation in B cells. J Virol 73: 9348–9361
13. Manet E, Rigolet A, Gruffat H, Giot J, Sergeant A (1991) Domains of the Epstein-Barr virus (EBV) transcription factor R required for dimerization, DNA binding and activation. Nucleic Acids Res 19: 2661–2667
14. Miller G, Heston L, Grogan E, Gradoville L, Rigsby M, Sun R, Shedd D, Kushnaryov VM, Grossberg S, Chang Y (1997) Selective switch between latency and lytic replication

of Kaposi's sarcoma herpesvirus and Epstein-Barr virus in dually infected body cavity lymphoma cells. J Virol 71: 314–324
15. Moore PS, Chang Y (1995) Detection of herpesvirus-like DNA in Kaposi's sarcoma in patients with and without HIV infection. N Engl J Med 332: 1181–1185
16. Quinlivan EB, Holley-Guthrie EA, Norris M, Gutsch D, Bachenheimer S, Kenney S (1993) Direct BRLF1 binding is required for cooperative BZLF1/BRLF1 activation of the Epstein-Barr virus early promoter BMRF1. Nucleic Acids Res 21: 1999–2007
17. Ragoczy T, Miller G (1999) Role of the Epstein-Barr virus Rta protein in activation of distinct classes of viral lytic cycle genes. J Virol 73: 9858–9866
18. Renne R, Zhong W, Herndier B, McGrath M, Abbey N, Kedes D, Ganem D (1996) Lytic growth of Kaposi's sarcoma-associated herpesvirus (human herpesvirus 8) in culture. Nat Med 2: 342–346
19. Russo JJ, Bohenzky RA, Chien MC, Chen J, Yan M, Maddalena D, Parry JP, Peruzzi D, Edelman IS, Chang Y, Moore PS (1996) Nucleotide sequence of the Kaposi sarcoma-associated herpesvirus (HHV8). Proc Natl Acad Sci USA 93: 14862–14867
20. Seaman WT, Ye D, Wang RX, Hale EE, Weisse M, Quinlivan EB (1999) Gene expression from the ORF50/K8 region of Kaposi's sarcoma-associated herpesvirus. Virology 263: 436–449
21. Soulier J, Grollet L, Oksenhendler E, Cacoub P, Cazals-Hatem D, Babinet P, d'Agay MF, Clauvel JP, Raphael M, Degos L (1995) Kaposi's sarcoma-associated herpesvirus-like DNA sequences in multicentric Castleman's disease. Blood 86: 1276–1280
22. Sun R, Lin SF, Gradoville L, Yuan Y, Zhu F, Miller G (1998) A viral gene that activates lytic cycle expression of Kaposi's sarcoma-associated herpesvirus. Proc Natl Acad Sci USA 95: 10866–10871
23. Sun R, Lin SF, Staskus K, Gradoville L, Grogan E, Haase A, Miller G (1999) Kinetics of Kaposi's sarcoma-associated herpesvirus gene expression. J Virol 73: 2232–2242
24. Whitehouse A, Carr IM, Griffiths JC, Meredith DM (1997) The herpesvirus saimiri ORF50 gene, encoding a transcriptional activator homologous to the Epstein-Barr virus R protein, is transcribed from two distinct promoters of different temporal phases. J Virol 71: 2550–2554
25. Whitehouse A, Cooper M, Hall KT, Meredith DM (1998) The open reading frame (ORF) 50a gene product regulates ORF57 gene expression in herpesvirus saimiri. J Virol 72: 1967–1973
26. Yu Y, Black JB, Goldsmith CS, Browning PJ, Bhalla K, Offermann MK (1999) Induction of human herpesvirus-8 DNA replication and transcription by butyrate and TPA in BCBL-1 cells. J Gen Virol 80: 83–90
27. Zalani S, Holley-Guthrie E, Kenney S (1996) Epstein-Barr viral latency is disrupted by the immediate-early BRLF1 protein through a cell-specific mechanism. Proc Natl Acad Sci USA 93: 9194–9199
28. Zhu FX, Cusano T, Yuan Y (1999) Identification of the immediate-early transcripts of Kaposi's sarcoma-associated herpesvirus. J Virol 73: 5556–5567

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