Corrections

BIOCHEMISTRY

The authors note that Fig. 2 and its corresponding legend appeared incorrectly. The corrected figure and its corrected legend appear below. In addition, the authors note that on page 1797, right column, last paragraph, Fig. 2C should appear as Fig. 2B.

Fig. 2. The WD40 repeat domain of Dos1 is essential but not sufficient for heterochromatin formation at the S. pombe centromere. (A) Schematic diagram of S. pombe centromere 1. The position of the centromeric otr1R::ura4 reporter insertion used in this study is indicated. Comparative growth assay of the serially diluted dos1 null strain with the centromeric otr1R::ura4 reporter expressing the indicated Dos1 fragments from a plasmid. Strains were examined for growth on pombe glutamate media (PMG) lacking leucine and supplemented with 1 g/L 5-FOA (+FOA –Leu), PMG media lacking uracil and leucine (–Ura –Leu), and PMG media lacking leucine (–Leu). Cells were always grown on a PMG medium lacking leucine to select for Dos1 expressing plasmid. (B) OSS-Rik1AC was coexpressed with FLAG-Dos1 truncations and pulled down with Strep-Tactin beads to detect whether the interactions are still preserved in Dos1 truncations.

NEUROSCIENCE

The authors note that in all experiments, the concentration for MnCl2 should be 0.4 mmole/kg body weight instead of 40 mmole/kg body weight. The incorrect text appears on page E2493, Fig. 2 legend, lines 1, 2, and 5; on page E2494, Fig. 4 legend, line 3; on page E2494, left column, first full paragraph, line 10; and on page E2498, right column, fourth full paragraph, lines 3 and 4. This error does not affect the conclusions of the article.

NEUROSCIENCE

The authors note that the following statement should be added as a new Acknowledgments section: “We thank Jennifer Frascino and Erin Light for assistance. This work was supported by the Medical Research Service of the Department of Veteran Affairs and National Institute of Mental Health Grant MH24600.”
Correction for “Kaposi’s sarcoma-associated herpesvirus LANA recruits the DNA polymerase clamp loader to mediate efficient replication and virus persistence,” by Qiming Sun, Toshiki Tsurimoto, Franceline Juillard, Lin Li, Shijun Li, Erika De León Vázquez, She Chen, and Kenneth Kaye, which appeared in issue 32, August 12, 2014, of Proc Natl Acad Sci USA (111:11816–11821; first published July 28, 2014; 10.1073/pnas. 1404219111).

The authors note that Fig. 3 appeared incorrectly. The corrected figure and its legend appear below.

Fig. 3. LANA interaction with RFC is critical for LANA-mediated episome persistence. (A) BJAB or BJAB/LANA outgrowth in microtiter plates after seeding at 1,000, 100, or 10 cells per well in the presence or absence of RFC1 knockdown (KD). Averages of three experiments are shown. Error bars indicate SD. (B) G418-resistant outgrowth of BJAB or BJAB/LANA cells after p8TR transfection with or without RFC1 knockdown. Averages of three experiments, with SD, are shown. (C) Gardella gel analysis (27) assessing the presence of episomal DNA in BJAB or BJAB/LANA cells with or without RFC1 KD after 20 d of G418 selection. Numbers refer to independently derived G418-resistant cell lines expanded from individual microtiter wells. The two leftmost lanes have increasing amounts of naked p8TR plasmid. O, gel origin. (D) Western blot analysis for LANA, RFC1, or Tub in cell lines used for Gardella gel analysis (27) in C. The asterisk indicates nonspecific bands. (E) LANA immunostaining in the indicated cell lines from C with or without RFC1 KD: Cell lines 1, 5, and 6 (WT, Upper) or cell lines 9, 1, and 3 (RFC1 KD, Lower) contain successively lower levels of episomal DNA as observed in C. Broad nuclear LANA staining indicates episome loss (arrowheads), whereas LANA dots (circled cells) indicate sites of episomes. (Magnification: 630×.) (F) Quantification of average percentage of cells containing LANA dots. Averages of three experiments, with SD, are shown.

www.pnas.org/cgi/doi/10.1073/pnas.1416630111
Kaposi’s sarcoma-associated herpesvirus LANA recruits the DNA polymerase clamp loader to mediate efficient replication and virus persistence

Qiming Sun, Toshiki Tsurimoto, Franceline Juillard, Lin Li, Shijun Li, Erika De León Vázquez, She Chen, and Kenneth Kaye

Kaposi’s sarcoma-associated herpesvirus (KSHV) latently infects tumor cells and persists as a multiple-copy, extrachromosomal, circular episome. To persist, the viral genome must replicate with each cell cycle. The KSHV latency-associated nuclear antigen (LANA) mediates viral DNA replication and persistence, but little is known regarding the underlying mechanisms. We find that LANA recruits replication factor C (RFC), the DNA polymerase clamp loader, to drive DNA replication efficiently. Mutated LANA lacking RFC interaction had reduced viral episome replication and persistence. RFC depletion had a negative impact on LANA’s ability to replicate and maintain viral DNA in cells containing artificial KSHV episomes or in infected cells, leading to loss of virus. LANA substantially increased PCNA loading onto DNA in vitro and recruited RFC and PCNA to KSHV DNA in cells. These findings suggest that PCNA loading is a rate-limiting step in DNA replication that is incompatible with viral survival. LANA enhancement of PCNA loading permits efficient virus replication and persistence, revealing a previously unidentified mechanism for KSHV latency.

To gain insight into the molecular mechanism underlying these effects, we sought to identify host cell protein(s) interacting with this internal sequence. We generated stable cell lines capable of doxycycline-inducible expression of LANA or LANA deletion mutants containing N-terminal ZZ and C-terminal FLAG tags (Fig. 1A). LANA expression was adjusted to levels similar to that of an infected tumor cell line (Fig. S1A). To ensure the N- and C-tags did not disrupt LANA function, we assessed LANA episome maintenance activity. Cells expressing ZZ-LANA-FLAG or control FLAG-ZZ fusion proteins were transfected with p8TR-P, which contains eight TR copies, and cells placed under puromycin selection, for which resistance is encoded on p8TR-P. Puromycin-resistant colonies were expanded and assessed for episomes by Gardella gels (27). LANA-expressing cell lines had episomal DNA in all lanes, whereas cells expressing ZZ-FLAG never contained episomes (Fig. S1B), indicating preservation of LANA function.

Next, LANA- or LANA mutant-associated complexes were purified from cell extracts by sequential tandem affinity purification (TAP), and the final FLAG-peptide eluate was resolved by 4–12% gradient SDS/PAGE and visualized by silver staining (Fig. 1B). Excision of bands and analysis by MS identified known LANA-associated proteins, including core histones. In addition, we identified the RFC complex, which consists of five subunits:RFC, p140, p116, p40, and p20.

**Significance**

Kaposi’s sarcoma herpesvirus (KSHV) latently infects tumor cells and viral episomal DNA replicates once each cell cycle. KSHV does not express DNA replication proteins during latency. Instead, KSHV latency-associated nuclear antigen (LANA) recruits host cell DNA replication machinery to the replication origin. However, the mechanism by which LANA mediates replication is uncertain. Here, we show LANA recruits replication factor C, the DNA polymerase clamp loader, in a critical step for viral DNA replication. Our findings suggest that PCNA loading is a rate-limiting step in DNA replication that is incompatible with KSHV persistence. LANA-enhanced PCNA loading is necessary for virus replication and persistent infection. These data reveal a therapeutic target for inhibition of KSHV persistence in malignant cells.

Author contributions: Q.S. and K.K. designed research; Q.S., T.T., S.C., and K.K. performed research; E.D.L.V. contributed new reagents/analytic tools; Q.S., T.T., S.C., and K.K. analyzed data; and Q.S. and K.K. wrote the paper.

The authors declare no conflict of interest.

This Direct Submission article had a prearranged editor.

*This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1404219111/-/DCSupplemental.*
RFC1, RFC2, RFC3, RFC4, and RFC5. Notably, RFC was only detected in the LANA and LANA mutants with ZZ and FLAG tags. Deleted residues are indicated to the left. The N-terminal vertical bar indicates nuclear localization signal. C, C-terminal region; DE, aspartate and glutamate region; L2, predicted leucine zipper; P, proline-rich region; Q, glutamine-rich region. (B) Silver-stained gel after affinity purification with ZZ–FLAG control, LANA, or each LANA mutant. Arrowheads indicate bait proteins. MW, molecular weight (molecular mass). (C) Western blots for RFC1 or LANA after immunoprecipitation (IP) with anti-LANA antibody from the indicated cell lines. (D) Immunoblots of LANA TAP eluants for LANA (anti-FLAG) or RFC1. (E) Western blot. (F) Western blots for LANA or RFC1 after immunoprecipitation of LANA from cell lines stably expressing LANA or LANA mutants. Tub, tubulin.

**Interaction with RFC Is Crucial for LANA-Mediated DNA Replication.** We hypothesized that LANA may recruit the RFC complex to facilitate viral DNA replication. We recently showed that LANA residues 262–320 are critical for efficient replication of TR DNA in B-cell lines (25). To test this requirement in epithelial cells (which KSHV also infects), we performed a LANA-mediated DNA replication assay with LANA or LANA deletion mutants. A plasmid containing eight copies of the KSHV TR was purified from doxycycline-methyleas (Dam) positive bacteria and transfected into 293T cells expressing LANA or LANA mutants. DpnI requires Dam methylation for digestion. As expected, LANA had robust DNA replication activity (~25-fold over control) (Fig. 2A), whereas replication was greatly diminished in the cells with LANA mutants (Δ264–929 and Δ262–320) that lack RFC interaction. In contrast, DNA replication was largely preserved in cells expressing LANAΔ332–929 or Δ288–320, which both retain RFC binding. The modest reductions in LANAΔ332–929 and LANAΔ288–320 replication are consistent with their less efficient RFC interaction compared with LANA (Fig. 1D and E). The reduced replication activities were not due to reduced LANA expression in these mutants (Fig. 2B).

To assess RFC’s role in LANA-mediated DNA replication further, we constructed BJAB or BJAB/LANA cell lines with inducible knockdown for RFC1 expression and assessed LANA DNA replication in the presence or absence of ~75% RFC1 knockdown (Fig. 2C). LANA replication was robust in the absence of RFC knockdown but was substantially reduced after RFC knockdown (Fig. 2D). Together, these results indicate that LANA’s interaction with RFC1 is critical for efficient DNA replication.
RFC Knockdown Results in Highly Deficient LANA-Mediated Episome Persistence. We assessed the role of LANA’s interaction with RFC in episome persistence. Because we recently showed that LANAΔ262–320, which cannot interact with RFC (Fig. 1E), is substantially deficient for episome persistence (25), we addressed the impact of RFC1 knockdown on LANA function. Importantly, RFC1 knockdown had little or no effect on cell proliferation as assessed by cell outgrowth after limiting dilution (Fig. 3A) or by cell cycle analysis (Fig. S3A and B). Because RFC1 is essential for cell growth in yeast (28), this finding indicates the knockdown did not exceed a threshold necessary for normal or near-normal cell growth. After transfection of p8STR DNA (encoding G418 resistance) into BJAB or BJAB/LANA cells and seeding cells at 1,000, 100, or 10 cells per well in microtiter plates, outgrowth of G418-resistant cell lines was much more robust in BJAB/LANA cells compared with BJAB cells (Fig. 3B). This result is due to the much higher efficiency of LANA episome persistence compared with integration, which occurs infrequently, and is required for p8STR persistence in the absence of LANA. However, after induction of RFC1 knockdown, LANA cell outgrowth was substantially reduced (Fig. 3B). Further, Gardella gel analysis (27) of G418-resistant cell lines demonstrated markedly reduced levels of episomal DNA in the presence of RFC1 knockdown (Fig. 3C). This reduction was not due to reduced LANA expression (Fig. 3D). We also detected LANA by immunostaining in G418-resistant cell lines. LANA reorganizes from broad nuclear localization (Fig. 3E, arrowheads) in the absence of TR DNA to bright dots (Fig. 3E, circled cells) at sites of TR episomes (7). In RFC1 knockdown cells, the percentage of cells containing LANA dots was greatly reduced, and directly correlated with the amount of reduction in episomal DNA signal observed by Gardella gel analysis (27) (Fig. 3E and F). For instance, RFC1 knockdown in cell line 3 had only ~20% of cells with LANA dots (Fig. 3F) and had only a small amount of episomal DNA by Gardella gel analysis (27) (Fig. 3C). Therefore, RFC1 knockdown severely impairs LANA-mediated episome persistence.

RFC1 Is Critical for Persistence of KSHV Infection. Because the prior experiments used artificial episomes containing TR DNA, we also assessed the effect of RFC1 knockdown on BJAB lymphoma cells latently infected with KSHV expressing GFP. Latent infection requires puromycin selection (encoded by the recombinant KSHV) for stable persistence. In the absence of selection, there is gradual loss of KSHV episomes because BJAB cells are not dependent on KSHV for viability. RFC1 knockdown (Fig. S4) accelerated GFP loss from infected cells (Fig. 4A). Immunostaining at day 9 after removal of selection demonstrated loss of infection as evidenced by absence of LANA staining (Fig. 4B, arrowhead indicates no LANA staining; compare with presence of red LANA dots in circled cells) in ~45% of cells with RFC1 knockdown compared with only ~13% without knockdown (Fig. 4B and C). Therefore, RFC deficiency accelerates loss of KSHV infection from BJAB cells.

Fig. 3. LANA interaction with RFC is critical for LANA-mediated episome persistence. (A) BJAB or BJAB/LANA outgrowth in microtiter plates after seeding at 1,000, 100, or 10 cells per well in the presence or absence of RFC1 knockdown (KD). Averages of three experiments are shown. Error bars indicate SD. (B) G418-resistant outgrowth of BJAB or BJAB/LANA cells after p8STR transfection with or without RFC1 knockdown. Averages of three experiments, with SD, are shown. (C) Gardella gel analysis (27) assessing the presence of episomal DNA in BJAB or BJAB/LANA cells with or without RFC1 KD after 20 d of G418 selection. Numbers refer to independently derived G418-resistant cell lines expanded from individual microtiter wells. The two leftmost lanes have increasing amounts of naked p8STR plasmid. (D) Gel origin. (E) Western blot analysis for LANA, RFC1, or Tub in cell lines used for Gardella gel analysis (27) in C. The asterisk indicates nonspecific bands. (F) LANA immunostaining in the indicated cell lines from C with or without RFC1 KD. Cell lines 1, 5, and 6 (WT, Upper) or cell lines 9, 1, and 3 (RFC1 KD, Lower) contain successively lower levels of episomal DNA as observed in C. Broad nuclear LANA staining indicates episome loss (arrowheads), whereas LANA dots (circled cells) indicate sites of episomes. (Magnification: 630×.) (F) Quantification of average percentage of cells containing LANA dots. Averages of three experiments, with SD, are shown.
We also assessed the effect of RFC1 knockdown in BCBL-1 PEL cells, which are naturally infected with KSHV. BCBL-1 cell outgrowth was reduced in cell line 3 with RFC1 knockdown (Fig. 4D) after seeding at 1,000, 100, or 10 cells per well in microtiter plates (Fig. 4E), consistent with loss of viral episomes, because BCBL-1 cells are dependent on latent KSHV infection for viability and proliferation. Further, Gardella gel analysis (27) demonstrated loss of both episomal and linear (from lytic replication) DNA only in cell lines 2 and 3 with RFC1 knockdown (Fig. 4D and F). This observation was further verified by real-time PCR of KSHV DNA (Fig. 4G). Taken together, these results demonstrate that diminished RFC levels substantially compromise the persistence of KSHV infection.

LANA Promotes PCNA Loading onto DNA. Because RFC loads PCNA onto DNA, we wished to investigate the effect of LANA on PCNA loading. Purified LANA (Fig. S5) or PCNA was incubated with RFC bound to beads. As expected, RFC precipitated PCNA (Fig. 5A, lane 6). RFC also efficiently precipitated LANA (Fig. 5A, lane 7).

Fig. 4. RFC deficiency results in loss of KSHV infection. (A) Loss of KSHV GFP expression from BJAB/KSHV cells was monitored in the absence or presence of RFC knockdown. Averages of three experiments at each time point, with error bars indicating SD, are shown. (B) LANA immunostaining (red) of BJAB/KSHV cells with or without doxycycline induction of RFC1 knockdown. DAPI (blue) stains DNA in cell nuclei. Dashed circles indicate examples of cells with LANA dots, and the arrowhead indicates a cell lacking LANA. (Magnification: 630×.) (C) Quantification of cells containing LANA dots. (D) Immunoblot for RFC1 or tubulin in BCBL-1 cells with or without doxycycline induction of RFC1 knockdown. (E) BCBL-1 outgrowth in microtiter plates after seeding at 1,000, 100, or 10 cells per well in the presence or absence of RFC1 knockdown. Averages of three experiments, with error bars indicating SD, are shown. (F) Gardella gel analysis (27) of BCBL-1 cells in the presence or absence of RFC knockdown. E, episomal KSHV; L, linear KSHV DNA due to lytic replication. (G) Real-time PCR for KSHV DNA in BCBL-1 cells with or without RFC knockdown. Averages of three experiments are shown, with error bars indicating SD.

Fig. 5. LANA enhances RFC loading of PCNA onto DNA. (A) Assessment of direct RFC binding to LANA or PCNA after incubation with anti-GFP beads with or without bound GFP-RFC. PCNA is loaded in lanes 2, 4, and 6 and migrates at ~35 kDa. The double asterisk indicates heavy chain (lanes 4–7) and degraded GFP-RFC1 (lanes 6 and 7); the single asterisk indicates a light chain. Ctrl, control. (B) PCNA loading onto DNA in the presence or absence of LANA. (C) Signal of PCNA bound to DNA from B was quantified and plotted against RFC input. A value of 1 corresponds to ~1 ng. (D) ChIP assay for LANA, RFC1, or PCNA bound to TR DNA in BCBL-1 cells. (E) ChIP assay for RFC1 or PCNA binding to TR DNA after transfection of p8TR into BJAB, BJAB/LANA, or BJAB/LANAΔ262–320 cells. Averages of three experiments, with SDs, are shown in D and E.
RFC1 and PCNA were clamp-loading ATPase, consists of five subunits (39), raising the possibility that these proteins may function in specialized pathways, other than RFC (26, 29, 30). In alternative RFC complexes, other subunits substitute for RFC1 to function in downstream, rate-limiting steps. Additional, non-TR replication origins also occur in latent KSHV (34). The observed virus loss with RFC deficiency suggests that these sites may be similarly sensitive to RFC deficiency, perhaps through LANA effects. Other episomal tumor viruses, including EBV, papillomaviruses, and polyomaviruses, may face similar needs to accelerate replication (40–43). Whereas EBV nuclear antigen 1 interacts with ORCs and other licensing factors (44), Merkel cell polyomavirus (MCV) large T antigen recruits RFC to sites of MCV replication (45), suggesting that acceleration of PCNA loading may also occur with MCV.

This work implicates LANA recruitment of RFC as an attractive target for disruption. LANA’s enhancement of PCNA loading is critical for efficient viral replication and persistence. Therefore, strategies that inhibit LANA’s interaction with RFC may be effective for virus eradication.

Materials and Methods

Cell Lines. Cell lines were maintained under standard conditions. The generation of LANA-expressing cell lines is described in Supplemental Materials and Methods.

TAP and MS. TAP of LANA complexes from human cells was performed using cell lines stably expressing ZZ-LANA-FLAG or ZZ-LANA-FLAG mutants. MS identified coprecipitating proteins.

Fluorescence Microscopy. LANA was detected by immunofluorescent microscopy using a Zeiss microscope and magnification of 630×.

DNA Replication Assay. KSHV DNA replication assays were performed as described (46), with minor modifications described in Supplemental Materials and Methods.

Episome Maintenance Assays. Episome maintenance was assessed in the presence or absence of RFC1 knockdown. Gardella gels (27) were used to assess the presence of episomal DNA. Loss of infection from BJAB/KSHV cells (47) was assessed by monitoring loss of GFP expression from the recombinant KSHV.

PCNA Loading Assay. RFC loading of PCNA onto nicked DNA containing the KSHV TR was assessed in the presence or absence of purified LANA as described in Supplemental Materials and Methods.

ChiP Assays. Formaldehyde cross-linking and ChiP assays were performed as described (48, 49), with some modifications as described in Supplemental Materials and Methods.

ACKNOWLEDGMENTS. We thank Chantal Beauchemin for helpful advice, Rolf Renne for TR DNA deleted for the LBS, and Michael Lagunoff for BJAB/KSHV cells. This work was supported by National Institutes of Health National Cancer Institute Grant CA082036 (to K.K.) and Japan Society for the Promotion of Science KAKENHI Grants 25440011 and 2513171 (to Y.T.).

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Supporting Information

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SI Materials and Methods

Cell Lines. BJAB cells were maintained in RPMI medium containing 10% bovine growth serum (BGS, HyClone) and 15 μg/mL gentamicin. Kaposi’s sarcoma-associated herpesvirus (KSHV)-infected body cavity-based B-cell lymphoma-1 (BCBL-1) cells were maintained in RPMI medium containing 20% BGS and 15 μg/mL gentamicin. 293T cells were maintained in DMEM containing 10% Tet-proof BGS (Clontech) and 15 μg/mL gentamicin. Flp-In 293 T-Rex (also referred to as 293/FRT; Invitrogen) cells were maintained in DMEM containing 10% Tet-proof BGS and 15 μg/mL gentamicin.

Plasmids. To construct the vectors expressing WT latency-associated nuclear antigen (LANA) or LANA mutants, the NruI site of plasmid cdDNA5-ZZ-FLAG (pcDNA5-ZZ-FLAG) (1) was destroyed, and the resultant plasmid was designated pcDNA5-ZZ-FLAG-mNru. The coding sequences for LANAΔ33–929 and LANAΔ33–888 (2) were PCR-amplified, inserted into pZZ-FLAG-mNru, and termed pZZ-Δ33–929-FLAG and pZZ-Δ33–888-FLAG, respectively. pSG5-T7LANAΔ264–929, pSG5-T7LANAΔ332–929, and pSG5-T7LANA (3) were digested with Ascl and NruI, and the smaller fragments were inserted into pZZ-Δ33–929-FLAG between the Ascl and NruI sites to generate pZZ-Δ264–929-FLAG, pZZ-Δ332–929-FLAG, and pZZ-full-length (FL)-LANA-FLAG. These constructs include the initial LANA codons beginning with ATG. Human replication factor C (RFC) 1, RFC2, RFC3, RFC4, and RFC5 were PCR-amplified from insect cell expression vectors (4) and inserted into pcDNA4-FLAG (1) to generate pcDNA4-RFC1, pcDNA4-RFC2, pcDNA4-RFC3, pcDNA4-RFC4, and pcDNA4-RFC5, respectively.

For expression of FL-LANA for purification, pcDNAEF monomeric Azami-Green (mAG), a derivative of pcDNA3.1hygro (Invitrogen) carrying the HindIII/XbaI fragment of CSII-EF-MCS (Invitrogen) was inserted into pcDNAEFmAGLANAC1, which was provided by Ambion (with guanine and cytosine percent stoichiometry, resulting in a conformational change in the repressor, rendering it unable to bind to the Tet operon).

Plasmids containing a single WT TR copy or a single TR copy that contains 20-bp deletions of the two adjacent LANA binding sites were transfected with 5 μg of pTripz-RFC1 (catalog no. RHS4696-264), into 10 million cells. Twenty-four hours posttransfection, single colonies were picked and screened for the inducible expression of ZZ-FLAG-tagged proteins upon doxycycline (DOX) treatment.

Generation of Stable Cell Lines. Stable 293 cell lines were obtained according to the protocol provided by the manufacturer (Invitrogen). Briefly, pOG44 (Invitrogen) was cotransfected into the Flp-In 293 T-Rex cell (Invitrogen) with pZZ-FLAG-mNru, pZZ-Δ33–929-FLAG and pZZ-Δ33–888-FLAG, pZZ-Δ264–929-FLAG, pZZ-Δ264–929-FLAG, or pZZ-FL-LANA-FLAG. Transfections were performed in a six-well dish at 75% cell confluence using 1.5 μg of DNA in a ratio of pOG44 to LANA vectors of 1 μg to 0.5 μg. Selection was performed with DMEM supplemented with 10% TET-proof BGS, 0.5 μg/mL blasticidin (selection for the Tet-repressor expression vector, pCDNA6-TR, integrated in the Flp-In 293 T-Rex cells), 50 μg/mL hygromycin (selection for pC8N5 vector-encoding target sequence in the flp-in site), and 15 μg/mL gentamicin. Single colonies were picked and screened for the inducible expression of ZZ-FLAG-tagged proteins upon doxycycline (DOX) treatment. Clones that exhibited the best inducible gene expression were used for large-scale cultures and subsequent protein purification. Drug selection was removed for large-scale cultures.

To establish BJAB stable cell lines with inducible RFC1 knockdown, BJAB cells or BJAB cells stably expressing T7-LANA were transfected with 5 μg of pTRipz-RFC1 (catalog no. RHS4696-100897382, clone ID V3THS_390989; Thermo Fisher Scientific) by nucleofection, using Amaxa nucleofector II (solution V, program C-009), into 10 million cells. Twenty-four hours posttransfection, cells were placed under selection using 1 μg/mL puromycin, for
which resistance is encoded by the vector. Clonal cell lines were then generated and analyzed for inducible RFC1 knockdown.

To establish BCBL-1 stable cell lines with inducible RFC1 knockdown, 10 million cells were transfected with 5 μg of pTRipz-RFC1 (Thermo Fisher Scientific) using Amaxa nucleofector II (solution V, program T-001). Twenty-four hours post-transfection, cells were placed under selection with 0.75 μg/mL puromycin after seeding into 96-well plates at a concentration of 10 cells per milliliter to obtain ∼1.5 cells per well. Clonal stable cell lines were selected and analyzed for inducible knockdown of RFC1 by Western blot analysis.

Tandem Affinity Purification and MS. Tandem affinity purification (TAP) of LANAs complexes from human cells was performed. Stable cell lines capable of expressing ZZ-LANA-FLAG upon DOX induction were used. Doses of DOX were used to induce LANA expression close to physiological levels, similar to the naturally KSHV-infected primary effusion lymphoma cell line BCBL-1. Briefly, 8 × 10⁶ cells were collected and thoroughly washed with PBS. To isolate nuclei, cells were resuspended in hypotonic buffer [10 mM Tris-HCl, 1.5 mM MgCl₂, 10 mM KCl, 1 mM DTT, 1 mM NaF, 1 mM Na₃VO₄, protease mixture (pH 7.9)], and swollen cells were treated by douncing. After centrifugation at 4,000 × g for 20 min, the pellet was harvested and resuspended in hypotonic buffer [20 mM Tris-HCl, 25% glycerol, 0.42 M NaCl, 1.5 mM MgCl₂, 1 mM NaF, 1 mM Na₃VO₄, 0.5% Nonidet P-40 (American Bioanalytical), 0.2 mM EDTA, 1 mM DTT, protease mixture (pH 7.9)]. After vigorous vortexing, the nuclear extract was harvested after centrifugation at 15,000 × g for 20 min at 4 °C. Next, 0.4 mL of packed IgG beads (GE Healthcare) (and ethidium bromide at 10 μg/mL in Fig. 1D) was added to the supernatant, followed by gentle rotation overnight at 4 °C for 12 h. The protein-bound IgG beads were washed three times using a buffer composed of the aforementioned hypotonic and hypertonic buffers at a ratio of 2:1. Bound proteins were eluted after incubation with tobacco etch virus (TEV) protease (Invitrogen) at 4 °C for 8 h. The TEV protease cleaves between the ZZ-tag and LANA or between the LANA mutants, leaving the ZZ-tag on the beads. Next, proteins were bound to anti-FLAG antibody–conjugated beads (Sigma). Beads were then incubated with 4 U/mL DNasel (Sigma) at 4 °C for ~12 h to eliminate potential protein associations caused by any “bridging” DNA bound to LANA. After incubation, beads were pelleted and washed twice for 5 min each time using washing buffer [20 mM Hepes (pH 7.5), 150 mM NaCl, 1 mM EDTA]. Anti-FLAG bead-bound proteins were then eluted using 2 × 70 μL of elution buffer [20 mM Hepes (pH 7.5), 150 mM NaCl, 1 mM EDTA, 0.2 μg/μL 3× FLAG peptide], resolved by SDS/PAGE in a 4–12% gradient gel, and visualized by silver staining. Another gel run in 1× TAP hypotonic and hypertonic buffers at a ratio of 2:1 three times between the ZZ-tag and LANA or between the LANA mutants, protease (Invitrogen) at 4 °C for 8 h. The TEV protease cleaves at 4 °C. The beads were next incubated with 4 U/mL DNaseI (Sigma) at 4 °C for 12 h. The protein-bound IgG beads were washed again and subjected to MS analysis. The titrated expression and purification were performed in three separate experiments for each cell line; 100 cells were assessed for the presence of dots in each experiment, and the SD was determined. Images were captured using confocal microscopy at a magnification of 630× using a Zeiss AxioPlan 2 microscope.

DNA Replication Assay. DNA replication assays were performed as described previously (8), with minor modifications. For the replication assay in 293T cells (Fig. 2A), cells in 15-cm dishes at 50% confluence were first transfected with GenEscort (Wisegen) using polyethylenimine (PEI) solution (20 μg of PEI/10 μg of DNA) with amounts of vector DNA expressing LANA or LANA mutants that resulted in similar LANA expression levels (8 μg of empty vector, 4 μg of pSG5-T7LANAΔ286–929, 4 μg of pSG5-T7LANAΔ332–929, 8 μg of pSG5-T7LANA262–320, 32 μg of pSG5-T7LANAΔ288–320, and 10 μg of pSG5-T7LANA per 15-cm dish). Twenty-four hours later, p8TR-gB (8) (4 μg of DNA per 15-cm dish) was transfected into each dish. For transfection, the frozen 1 mg/mL PEI stock solution in PBS was thawed in a 37 °C water bath for 5 min to avoid any precipitation. DNA was added to 500 μL of DMEM (without serum), and PEI was separately added to 500 μL of DMEM (without serum). After 10 min at RT, the two solutions were mixed, incubated for 15–20 min at RT, and gently mixed by tapping, and the solution was added to a 15-cm dish containing cells. Twenty-four hours after p8TR-gB was transfected, the cells were trypsinized. One third of the cells were collected for normalization of transfection efficiency, and the rest of the cells were further cultured in 15-cm dishes for an additional 48 h. Cells were subsequently collected and analyzed for DNA replication. The results represent the average of four experiments.

For the DNA replication assay performed in BJAB cells, cells carrying RFC1 shRNA were induced with 1 μg/mL DOX for 3 d. Ten million uninduced control cells or RFC1 control cells were transfected by nucleofection with 2 μg of p8STR using Amaxa nucleofector program O-17 and with solution V using 2 μg of DNA. After transfection, cells were seeded into 25-cm² flasks in 5 mL of medium. Twenty-four hours after transfection, half of the cells were collected and used to normalize transfection efficiency and the other half of the cells were further cultured. Seventy-two hours posttransfection, low-molecular weight DNA was harvested from cells by the Hirt method (9). Hirt DNAs (9) were assayed for replication using real-time PCR as previously described. The results represent the average of four experiments.

Episome Maintenance Assays. BJAB or BJAB/LANA cells were induced for RFC1 knockdown with 1 μg/mL DOX for 3 d. Ten million BJAB or BJAB/LANA cells with or without RFC1 knockdown were then transfected by nucleofection with 2 μg of p8STR using Amaxa nucleofector program O-17 and solution V. Twenty-four hours posttransfection, cells were seeded into microtiter plates at 1,000, 100, or 10 cells per well in medium containing G418 at 0.6 mg/mL or G418 at 0.6 mg/mL and

Fluorescence Microscopy. A total of 2 × 10⁵ cells were collected in a microfuge at 450 × g for 5 min. After aspiration of the supernatant, 1 mL of hypotonic buffer (1% Na citrate, 1 mM MgCl₂, 1 mM CaCl₂) was added. After incubation for 5 min at room temperature (RT), cells were spread onto slides using a cytospin (Thermoshandon) and fixed in 4% paraformaldehyde in PBS for 10 min at RT. After three washes with PBS, cells were permeabilized using 0.5% Triton X-100 in PBS for 5 min at 4 °C. To detect LANA, primary monoclonal antibody IA-2-12 (a gift from Mary Ballestas, University of Alabama School of Medicine, Birmingham, AL) at a ratio of 1:2,000 was incubated with cells for 2 h at RT. Cells were then incubated with secondary anti-mouse Alexa488 or Alexa568 antibody (Abcam). Cells were counterstained with DAPI (Invitrogen), and coverslips were applied with Aqua-Poly/Mount (Polysciences). Quantification of LANA dots was performed in three separate experiments for each cell line; 100 cells were assessed for the presence of dots in each experiment, and the SD was determined. Images were captured using confocal microscopy at a magnification of 630× using a Zeiss AxioPlan 2 microscope.

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0.5 μg/mL DOX. Macroscopic colony formation was assessed after 16 d. G418-resistant cell lines were also expanded. Gardella gel analysis (10) was performed on G418-resistant cells using cell lines expanded from plates initially seeded at 100 cells per well. Cells were loaded into loading gel wells made of agarose containing DNase-free protease (Sigma) and SDS, which results in in situ lysis of cells, followed by electrophoresis in Tris-borate-EDTA buffer. DNA was transferred to a nylon membrane, and KSHV DNA was detected using a 32P-labeled TR probe.

The BCBL-1 stable cell lines clone 2, clone 3, and clone 4 were induced with DOX (500 ng/mL) for 3 d. The knockdown of RFC1 was confirmed by Western blot analysis. On day 0, 2 million uninduced or DOX-induced cells were seeded in 30 mL (6.6 × 10^3 cells per milliliter) of RPMI 1640 medium containing 10% FBS (Clontech) in a T75 flask with or without DOX (day 0) and continuously expanded. Cells were sampled at the indicated time points and analyzed by real-time PCR or Gardella gel assay (10) using the standard protocol described above. Each PCR was performed in triplicate, and the data shown are representative of three experiments. For the microtiter outgrowth assay, clone 3 and clone 4 were induced with 1 μg/mL DOX for 3 d and then were seeded into 96-well microtiter plates at 1,000, 100, or 10 cells per well in medium containing 0.5 μg/mL DOX. Colony formation was monitored both macroscopically and microscopically, and was recorded 12 d after seeding. Wells containing at least 20 cells were scored as positive.

**GFP Decay Assay.** BJAB cells infected with recombinant KSHV containing a GFP expression cassette (11) were cultured in RPMI medium containing 10% BCS, 15 μg/mL gentamicin, and 10 μg/mL puromycin. To generate BJAB cells infected with KSHV with RFC-inducible knockdown or with an inducible scrambled RFC1 negative control sequence, 2 μg of pCDNA6-TR-RFC1 or 2 μg of pCDNA6-TR-RFC1-scrambled was cotransfected with 4 μg of pHygro-BABE into 10 million cells by nucleofection using Amaxa nucleofactor program O-17 and solution V. Ten minutes after transfection, cells were seeded into T75 culture flasks and puromycin selection continued. At 24 h posttransfection, hygromycin was added into the culture at a concentration of 100 μg/mL and puromycin selection continued. After 7 d of selection, the cells were treated with 1 μg/mL DOX and tested for RFC1 knockdown by Western blot analysis by using anti-FLAG antibody (M2; Sigma). The RFC knockdown efficiency by Western blot analysis was then kept in continuous culture under hygromycin selection. BJAB/KSHV cells expressing RFC shRNA or scrambled shRNA and Tet repressor were treated with DOX for 3 d either to induce RFC1 knockdown or as a negative control (with the scrambled sequence); puromycin and hygromycin were then removed from the culture medium to permit episome loss, which was monitored by detection of GFP expression using flow cytometry. Each time point was assessed in triplicate, and the result shown in Fig. 4d is representative of two experiments.

**Preparation of Purified FL-LANA.** To generate FL-LANA, pcDNA-mAG-LANA-FL expressing double-tagged LANA was transiently transfected into 3 × 10^5 293T cells in three 150-mm plates in DMEM containing 10% FCS at about 50% confluence as described by Uno et al. (5). Cells were collected 48 h after transfection, washed once with 25 mM Tris-HCI (pH 7.4), 0.136 M NaCl, 6.7 mM KCl, 0.2 mM NaHPO₄, and suspended in 2.4 mL of buffer B [50 mM Tris-HCl (pH 8.0), 0.15 M NaCl, 1 mM EDTA, 10% glycerol, 0.1 mM PMSF, 20 μg/mL leupeptin]. Cells were lysed by addition of Nonidet P-40 and NaCl to a final concentration of 0.5% and 0.5 M, respectively, at 4 °C for 30 min. The cell lysate, obtained by centrifugation at 130,000 × g for 30 min, was diluted by the addition of 1.5× buffer H lacking NaCl and then loaded on Q-Sepharose (5 mL; GE Healthcare) at 0.2 M NaCl in buffer H [25 mM Hepes (pH 8.0), 1 mM EDTA, 0.01% Nonidet P-40, 0.1 mM PMSF, 20 μg/mL leupeptin]. The column was subsequently washed with 0.5 M NaCl, and mAG-LANA was eluted behind the bulk protein peak. The mAG-LANA-enriched fractions were pooled and loaded onto Ni-NTA-Sepharose HP (1 mL, GE Healthcare). The mAG-LANA, eluted at 20 mM imidazole, was further loaded onto an anti-Flag-agarose column (1 mL; Sigma) and eluted with 100 μg/mL FLAG peptide (Sigma). The eluted mAG-LANA was concentrated with Resource-O (0.15 mL; GE Healthcare) using a 1.5-mL gradient of NaCl from 0.2 to 0.8 M. A total of 160 μg/mL purified mAG-LANA at its peak fraction was obtained. Purified protein was analyzed by SDS/PAGE and Coomassie blue staining.

**In Vitro Binding of LANA to RFC.** To prepare RFC-prebound beads, 2 μL of anti-GFP beads was incubated with 7 μL of High 5 insect cell lysate that was either uninfected or infected with baculoviruses for expression of GFP-RFC1 and RFC2–RFC5 as described (4). Baculovirus for expression of GFP-RFC1 was constructed by insertion of the PCR-amplified GFP coding sequence from pEGFP1 (Invitrogen) at the N-terminal end of human RFC1. The beads were then washed and suspended with buffer H containing 0.1 M NaCl. Anti-GFP beads (anti-GFP monoclonal antibody agarose; MBL) prebound with or without GFP-RFC1 were mixed with 100 ng of purified proliferating cell nuclear antigen (PCNA) (12) or 320 ng of mAG-LANA in 30 μL of buffer H with 0.1 M NaCl and incubated at 4 °C for 1 h. The beads were collected by centrifugation, washed three times with buffer H containing 0.1 M NaCl, and suspended in 20 μL of 1× SDS loading buffer (13). Five microliters of each sample was used for electrophoresis, and proteins were visualized by silver staining or immunoblotting.

For the binding assay in the presence of nuclease, the washing and incubation buffer was changed to mHBSG [10 mM Heps (pH 7.5), 0.01% Tween 20, 10 mM MgCl₂, 0.2 mM EDTA, 150 mM NaCl, 10% glycerol]. The RFC prebound beads were incubated with 320 ng of mAG-LANA or 250 ng of control His-tagged human minichromosome maintenance complex component 2 (MCM2) with or without 1.25 units of turbo nuclease (Amaxa). Bound proteins were visualized by silver staining or immunoblotting.

**PCNA Loading Assay.** LANA was incubated with DNA in a binding reaction performed in 20 μL of DNA binding reaction mixture [50 mM Tris-HCl (pH 7.5), 50 mM NaCl, 10 mM MgCl₂, 1 mM EDTA, 0.1 mM DTT, 10% glycerol] containing 1 μg/mL polyd/ DC (GE Healthcare). DNA was biotin-labeled after digestion with Cfr10I (pUC19GAP1) or BglII (pTAGAPTR1-3), and the ends were filled using biotinylated dCTP with Klenow polymerase. To determine the optimal concentration of LANA for the in vitro PCNA loading assay, magnetic-SA beads (15 μg, Dynabeads M-280 Streptavidin; Invitrogen) bound with equimolar amounts of pUC19GAP1 (~60 ng) or pTAGAPTR1-3 (~72 ng) or without DNA were incubated at RT for 30 min after mixing with 130 ng, 260 ng, or 390 ng of LANA. The DNA bead-bound fraction was recovered in 16 μL of 1× SDS loading buffer, and 8-μL aliquots were resolved by electrophoresis in a 10% SDS polyacrylamide gel, followed by immunoblotting with anti-FLAG antibody (M2; Sigma).

Magnetic-SA beads (15 μg) bound with pTAGAPTR1-3 (~72 ng) were prebound with or without 260 ng of LANA in 20 μL of DNA binding reaction mixture with 1 μg/mL polyd/DC (per reaction) at RT for 30 min. The LANA prebound or unbound beads were suspended in loading reaction mixture [10 mM Heps (pH 7.5), 0.01% Tween 20, 10 mM MgCl₂, 0.2 mM EDTA, 0.15 M NaCl, 50 mM creatine phosphate, 25 μg/mL creatine phosphokinase, 0.4 mM DTT, 2 mM ATP] containing 480 ng of PCNA and 0, 3, 6, 9, 12, or 15 ng of RFC, and were incubated at 32 °C for 30 min after mixing with RFC. The DNA bead-bound fraction was recovered in 16 μL of 1× SDS loading buffer, and
8-μL aliquots were resolved by electrophoresis in a 10% SDS polyacrylamide gel followed by immunoblotting with anti-FLAG and anti-PCNA antibody, respectively.

**ChIP Assays.** Formaldehyde cross-linking and ChIP assays of cells were performed as described (14, 15), with some modifications. Twenty million BJAB or BCBL-1 cells in a T175 flask at exponential growth stage were cross-linked by the addition of formaldehyde to a final concentration of 1% directly into the medium and were gently shaken at a speed of 80 rpm for 5 min at RT. Cross-linking was stopped with the addition of glycine at a final concentration of 125 mM, and cells were shaken at RT for another 10 min. Cells were collected by centrifugation (2,000 × g for 4 min) and washed three times with ice-cold 1× PBS. Cell pellets were resuspended in cell lysis buffer [5 mM piperazine-N,N′-bis(2-ethanesulfonic acid) (KOH) (pH 8.0), 85 mM KCl, 0.5% Nonidet P-40] containing protease mixture (Roche). After incubation for 10 min on ice, samples were dounced several times on ice with a type B homogenizer. Nuclei were collected by centrifugation (2,000 × g for 15 min). Nuclei were resuspended in nuclear lysis buffer [50 mM Tris (pH 8.1), 10 mM EDTA, 1% SDS] containing protease mixture (Roche) and incubated on ice for 10 min. Chromatin was then sonicated to an average length of ~600 bp (which was later confirmed on a 1.5% agarose gel), keeping samples on ice. Debris was cleared by centrifugation at 13,000 × g for 10 min at 4 °C. Supernatant was transferred to a new tube and diluted fivefold in ChIP dilution buffer [0.01% SDS, 1.1% Triton X-100, 1.2 mM EDTA, 16.7 mM Tris (pH 8.1), 167 mM NaCl plus protease inhibitors]. To reduce nonspecific background, samples were preclarified with 80 μL of a salmon sperm DNA/protein A agarose slurry for 30 min at 4 °C with agitation. Beads were collected by brief centrifugation at 1,000 × g for 5 min at 4 °C, and supernatant fraction was saved. Twenty percent of the total supernatant was used for total input control, and the rest of the supernatant was divided into two fractions: one for incubation with 5 μg of species-matched IgG control and the second for incubation with 5 μg of specific antibody [affinity-purified anti-LANA antibody, anti-RFC1 rabbit polyclonal antibody (Bethyl), or anti-PCNA rabbit polyclonal antibody PC10 (Cell Signaling)]. Incubations were performed overnight at 4 °C with rotation. Immune complexes were collected using 60 μL of salmon sperm DNA/protein A agarose slurry for 1 h at 4 °C with rotation. Beads were then washed at RT consecutively for 3–5 min on a rotating platform with 1 mL of each of the following solutions: (i) once in low-salt wash buffer [0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris (pH 8.1), 150 mM NaCl], (ii) once in high-salt wash buffer [0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris (pH 8.1), 500 mM NaCl], (iii) once in LiCl wash buffer [0.25 M LiCl, 1% Nonidet P-40, 1% deoxycholate, 1 mM EDTA, 10 mM Tris (pH 8.0)], and (iv) twice in 1× Tris EDTA buffer. Complexes were eluted by adding 250 μL of elution buffer [1% SDS, 0.1 M NaHCO₃] to the pelleted beads. After centrifugation at 13,000 × g for 3 min at RT, supernatants were transferred to new tubes. Complexes were then eluted a second time, and the two elutions were combined. The formaldehyde cross-linking was then reversed with the addition of 1 μL of 10 mg/mL RNase; 5 M NaCl was added to adjust the final concentration to 0.3 M NaCl and incubated at 65 °C for 4–5 h. Two and one-half volumes of 100% ethanol was then added, and DNA was precipitated overnight at ~20 °C. DNA was collected by centrifugation, and the pellet was resuspended in 100 μL of water. Next, 2 μL of 0.5 M EDTA, 4 μL of 1 M Tris (pH 6.5), and 1 μL of 20 mg/mL proteinase K were added and incubated for 1–2 h at 45 °C. DNA was purified using QiaQuick spin columns (Qiagen) and eluted in 50 μL of 10 mM Tris (pH 8.0). Two microliters of DNA was used in quantitative PCR reactions (forward primer 5′-GGGGGAACCCCGGCGACGGCGAG-3′ and reverse primer 5′-232GGGCTCCCCAAACACGCTCA-3′ flanking KSHV TR nucleotides 677–777). PCR was performed after melting at 95 °C for 10 min, followed by 40 cycles of 95 °C for 5 s and 60 °C for 1 min. For quantification, 5%, 1%, or 0.2% of input DNA was used to generate a standard curve. For measuring the LANA-directed TR loading of RFC and PCNA by ChIP assay, 10 million BJAB, BJAB/LANA, or BJAB/LANAΔ262–320 cells were transfected with 5 μg of p8TR using the conditions described above. Thirty-six hours posttransfection, ChIP was performed using RFC1 and PCNA antibodies. For assessing the role of the LBS in LANA-directed TR loading of RFC and PCNA, 10 million BJAB or BJAB/LANA cells were transfected with 5 μg of plasmid containing a copy of TR DNA or a copy of TR DNA deleted for the LBS using the conditions above. Twenty-four hours posttransfection, ChIP was performed using affinity-purified anti-LANA, RFC1, or PCNA antibody.

Fig. S1. ZZ-LANA-FLAG maintains episomes. (A) LANA Western blot of 293FRT/ZZ-LANA-FLAG cells before or after induction with increasing amounts of DOX. A similar number of BCBL-1 primary effusion lymphoma cells are shown in lane 1. (B) Episome maintenance was assessed in 293FRT/ZZ-LANA-FLAG or 293FRT/ZZ-FLAG cells. Cells were transfected with p8TR plasmid containing a puromycin resistance cassette and subjected to puromycin selection. After 20 d, a Gardella gel assay (10) was performed on puromycin-resistant cells. BCBL-1 cells are shown in lane 1. Lanes 2–8 contain cells from independently derived, puromycin-resistant ZZ-FLAG 293FRT or ZZ-LANA-FLAG 293FRT cell lines.

Fig. S2. ZZ-LANA-FLAG interacts with RFC. RFC peptides identified by MS following LANA TAP are indicated (underlined).

Fig. S3. RFC1 knockdown did not alter the cell cycle of BJAB or BJAB/LANA cells. Cell cycle analyses of BJAB cells (A) or BJAB/LANA cells (B) with or without induction of RFC1 knockdown are shown.
Fig. S4. Western blot for RFC1 or tubulin in BJAB/KSHV cells with or without incubation with DOX.

Fig. S5. Purification of LANA. Purification of recombinant FL-LANA from 293T cells. Successive steps in LANA purification are shown: lane 1, Q-Sepharose; lane 2, nickel-nitritriacetic acid (Ni-NTA) following imidazole; lane 3, anti-FLAG elution; and lanes 4 and 5, concentration using ResourceQ and NaCl gradient elution.

Fig. S6. Nuclease treatment does not disrupt LANA interaction with RFC. Assessment of RFC binding to LANA or to negative control (Ctrl) minichromosome maintenance complex component 2 (MCM2) after incubation with anti-GFP beads with or without bound GFP-RFC. Incubations were performed in the presence or absence of nuclease. A total of 30 ng or 320 ng of LANA was used for input or incubations with beads, respectively. A total of 20 ng or 250 ng of MCM2 was used for input or incubations with beads, respectively. The double asterisk indicates heavy chain (lanes 3–8) and degraded GFP-RFC1 (lanes 6–8).
Fig. S7. Purified LANA specifically binds GAP1-TR DNA. Magnetic beads or beads bound with pUC19GAP1 or pTAGAPTR1-3 were incubated with LANA, and binding was assessed by immunoblotting with anti-FLAG antibody. The asterisk indicates degradation product.

Fig. S8. Enrichment of LANA, RFC, and PCNA at TR DNA is dependent on the presence of the LBS. (A) ChIP assay for LANA binding to TR DNA after transfection of TR DNA or TR DNA deleted for the LBS into BJAB/LANA cells. (B) ChIP assay for RFC1 or PCNA binding to TR DNA after transfection of TR DNA or TR DNA deleted for the LBS into BJAB (LANA-negative) or BJAB/LANA cells. Averages of three experiments, with SDs, are shown.