Protein Interactions Targeting the Latency-Associated Nuclear Antigen of Kaposi’s Sarcoma-Associated Herpesvirus to Cell Chromosomes

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Maintenance of Kaposi’s sarcoma-associated herpesvirus (KSHV) latent infection depends on the viral episomes in the nucleus being distributed to daughter cells following cell division. The latency-associated nuclear antigen (LANA) is constitutively expressed in all KSHV-infected cells. LANA binds sequences in the terminal repeat regions of the KSHV genome and tethers the viral episomes to chromosomes. To better understand the mechanism of chromosomal tethering, we performed glutathione S-transferase (GST) affinity and yeast two-hybrid assays to identify LANA-interacting proteins with known chromosomal association. Two of the interactors were the methyl CpG binding protein MeCP2 and the 43-kDa protein DEK. The interactions of MeCP2 and DEK with LANA were confirmed by coimmunoprecipitation. The MeCP2-interacting domain was mapped to the previously described chromatin binding site in the N terminus of LANA, while the DEK-interacting domain mapped to LANA amino acids 986 to 1043 in the C terminus. LANA was unable to associate with mouse chromosomes in chromosome spreads of transfected NIH 3T3 cells. However, LANA was capable of targeting to mouse chromosomes in the presence of human MeCP2 or DEK. The data indicate that LANA is tethered to chromosomes through two independent chromatin binding domains that interact with different protein partners.

The Kaposi’s sarcoma-associated herpesvirus (KSHV), or human herpesvirus 8 (HHV8), is associated with all forms of Kaposi’s sarcoma, primary effusion lymphoma (PEL), and some forms of multicentric Castleman’s disease (6, 9, 15, 16, 48). KSHV infection is predominantly latent, and the genome is maintained as multicopied episomes in the nucleus of the infected cell. The gene coding for latency-associated nuclear antigen (LANA) is one of the few viral latency genes and is expressed from open reading frame 73 (ORF73) as a polycistronic message with the viral FLIP and cyclin homologs (44). LANA is a 222- to 234-kDa nuclear phosphoprotein (26, 41) that consists of amino-terminal and carboxy-terminal domains separated by an acidic internal repeat domain. LANA acts as a transcriptional repressor by interacting with histone deacetylase (HDAC) members (28, 45) and as a transcriptional activator of several promoters, including interleukin-6, telomerase reverse transcriptase, E2F-regulated promoters, and its own promoter (2, 27, 28, 31, 40, 43). LANA interacts with cellular proteins, including Rb, CREB-binding protein (CBP), and p53 (18, 25, 30, 40). Interaction with p53 leads to loss of p53 transcriptional activity and inhibition of apoptosis (18). LANA has oncogenic potential in that it transforms primary rat embryonic fibroblasts in cooperation with H-ras (40).

Viruses such as Epstein-Barr virus (EBV) and KSHV must distribute their episomal genomes to daughter cells during cell division to ensure the continuity of the viral life cycle. LANA’s function has been compared to that of EBV nuclear antigen 1 (EBNA1), which is constitutively expressed in EBV-infected latent cells, binds the EBV genome, and is required for episomal maintenance (23, 56). EBNA1 is essential for EBV DNA association with mitotic chromosomes, and cellular EBP2 has been identified as an EBNA1-interacting protein that can mediate chromosomal tethering (46, 55). LANA can mediate the persistence of extrachromosomal KSHV DNA in uninfected lymphoblasts (4, 10) and colocalizes with viral genomes both in interphase nuclei and on mitotic chromosomes. LANA, specifically its C terminus, binds to two sites within the terminal repeat of the KSHV genome (5, 10, 21, 22). LANA has been shown to accumulate to heterochromatin-associated nuclear bodies and preferentially associates with human chromatin in human-mouse hybrids containing a single fused nucleus (45, 49). LANA associates with human mitotic chromosomes in a random, speckled fashion in infected cells (38, 49), but paints uninfected HeLa cell chromosomes (38). A chromosome binding site (CBS) has been mapped to amino acids (aa) 5 to 22 which mediate the specific interaction of LANA with mitotic chromosomes (38). Interactions with the chromatin-associated proteins Ring3, which localizes to heterochromatin, and histone H1 have also been described previously (11, 32, 39). However, the role of these proteins in LANA-mediated chromosome association is unclear.

We demonstrate here that two independent interactions with cell proteins are involved in LANA tethering to chromosomes. The first is mediated by the N terminus of LANA through the 75-kDa methyl CpG binding protein 2 (MeCP2), and the second is mediated by the C terminus of LANA through the 43-kDa DEK protein.
MATERIALS AND METHODS

Expression plasmids. Glutathione S-transferase (GST)-LANA fusions and yeast Gal4DBD and Gal4ACT constructs were previously described (28). pDY15 expresses GFP-LANA (minus the first 2 aa) in pEGFP-C3 (Clontech). The LANA mt3 fragment was cut from pAK7 with BglII and XhoI to delete LANA aa 1 to 15, blunt ending, and religating. Green fluorescent protein (GFP)-LANA (pDH389) contains LANA codons 1 to 329 cloned in pEGFP-C1 (Clontech) at BglII. GFP-LANA-C (pMW2) contains LANA codons 931 to 1164 cloned in pEGFP-C1 at BglII. LANA m1 (pMF42), LANA m2 (pMF43), and LANA m3 (pMF73) contain LANA codons 1 to 329 fused at an XbaI site to codons 928 to 1108, 928 to 1043, and 928 to 985, respectively. LANA m4 (pMF40) expresses LANA aa 1 to 329, pMF constructs have an SG5-Flag vector background. Myc-DEK (pAK7) was made by using GST-DEK (19) as a template and ligating the PCR product into pJH363 at BglII. The Myc-DEK fragment was cut from pAK7 with EcoRI and BglII and ligated into pEGFP-C2 (Clontech) at EcoRI and BamHI to obtain GFP-Myc-DEK (pAK65). DEK was also cloned into the BglII site of pSG5 (Stratagene) to make pAK6.

GST affinity assay and immunoprecipitation. The GST assay was performed as previously described (28). Briefly, GST and GST fusion proteins were made in bacteria and bound to glutathione Sepharose 4B beads (Amersham) at 4°C overnight. The beads were washed and the amount of protein bound to beads was determined by Coomassie blue staining of proteins separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Equal amounts of each GST protein were used in the affinity assays. HeLa cells were transfected with 10 µg of each of Flag-DeCP2 with calcium phosphate. Cells were harvested 48 h posttransfection, resuspended in lysis buffer, and sonicated. Supernatant from transfected cells was incubated with GST fusion proteins. The beads were washed six times and proteins were run on a SDS-PAGE gel (9% polyacrylamide) and transferred to polyvinylidene fluoride (PVDF) membrane (Bio-Rad). Interacting proteins were detected by mouse anti-Flag (Sigma) antibody (1:1,500) and visualized by using the enhanced chemiluminescence (ECL) reaction (Amersham Life Sciences). Transcription and translation of DEK were done with pAK6 in the TNT Quick Coupled System (Promega). Equal amounts of GST fusion proteins were incubated with 10 µl of the 32P-labeling reaction mixture in 600 µl of lysis buffer (0.2% NP-40, 150 mM NaCl, 1 mg of bovine serum albumin [BSA] per ml). Bound proteins were separated by SDS-PAGE and detected by autoradiography.

For immunoprecipitations, HeLa or Cos1 cells were transfected in 10-cm diameter culture dishes with 10 µg of total DNA by using calcium phosphate (HeLa) or FuGENE6 (Roche) (Cos1) and harvested after 2 days. Cells were washed in 1x phosphate-buffered saline (PBS), lysed in 2 ml of lysis buffer (50 mM Tris [pH 7.9], 100 mM NaCl, 0.5 mM EDTA, 2% glycerol, 0.5 mM phenylmethylsulfonyl fluoride [PMSF], 0.2% NP-40, 2 µg of aprotinin per ml), and sonicated for 10 s. Extracts were precleared with Sephadex G-25 beads (Amersham Life Sciences) and incubated with Flag antibody (Sigma) or control mouse immunoglobulin G (IgG) antibody (Santa Cruz) (5 µg per ml of extract). For direct precipitations, rat anti-LANA antibody (4 µg per 0.5 ml of extract; AbI) was incubated for 2 h. Protein G beads were added for 2 h. Beads were washed six times with lysis buffer, and samples (coimmunoprecipitation, 18 µl; direct precipitation, 5 µl; and input extract, 15 µl) were run on SDS-PAGE (9% polyacrylamide gel). The amount of sample used for direct immunoprecipitations was one-fifth of the amount used for the coimmunoprecipitated sample. Western blot analysis was performed with rat anti-LANA or mouse anti-DEK (BD Transduction Laboratories) monoclonal antibody and peroxidase-conjugated antirat secondary antibody (Amersham) or antimouse IgG secondary antibody. LANA was detected with an anti-Flag or anti-Myc mouse antibody (Sigma) and rhodamine-conjugated donkey anti-mouse IgG secondary antibody (Jackson). LANA was detected with an anti-Flag or anti-Myc mouse antibody (Sigma) and rhodamine-conjugated donkey anti-rat IgG secondary antibody (Jackson). All antibodies were used at a 1:200 dilution in 1% BSA–PBS. Cells were analyzed by confocal microscopy.

RESULTS

Localization of LANA on human chromosomes of KSHV-infected cells. LANA colocalizes with the KSHV genome at discrete spots on chromosomes of KSHV-infected BCBL1 cells (4, 11). To investigate the relationship between the LANA spots and centromere-associated proteins, chromosome spreads were performed on BCBL1 cells (Fig. 1). Centromeres were detected with human serum containing antibodies against centromeric proteins and rhodamine-conjugated donkey anti-human immunoglobulin secondary antibody. LANA was detected with anti-LANA rat monoclonal antibody and FITC-conjugated antirat secondary antibody. Centromeres (red) appeared as discrete doublets on chromosomes. The LANA-staining spots (green) were separate from centromeres.

LANA is targeted to sites of mouse interphase and mitotic heterochromatin by human MeCP2. It has been observed that LANA associates with heterochromatin (11, 38, 45, 49), but the targeting mechanism has not been elucidated. Mouse genomic DNA has pericentromeric heterochromatin (PCH), which consists of transcriptionally inactive DNA regions that contain high concentrations of methylated CpGs. In studies of LANA-mediated transcriptional repression, we found that LANA interacts with the methyl CpG binding protein MeCP2 (unpublished data). We set out to determine if LANA could be...
targeted to heterochromatin via MeCP2. When human MeCP2 and human heterochromatin protein 1 alpha (HP1α), another marker of heterochromatin, are overexpressed in mouse cells, they each localize to PCH and appeared as nuclear spots (29, 54). Immunofluorescence assays were performed with mouse NIH 3T3 cells cotransfected with GFP-LANA and Flag-HP1α. GFP-LANA (green) and Flag-HP1α (red) did not colocalize (Fig. 2A). This indicates that LANA does not localize to mouse heterochromatin when expressed alone, and its localization is not affected by the presence of human HP1α. However, in cells cotransfected with human Flag-MeCP2, GFP-LANA (green) and Flag-MeCP2 (red) colocalized to the nuclear PCH spots (Fig. 2B). Thus, LANA is targeted to mouse PCH by human MeCP2. NIH 3T3 cells were then cotransfected with Flag-MeCP2 and GFP-LANA and then blocked with Colcemid for 6 h, and chromosome spreads were generated (Fig. 2C). Immunofluorescence assays detected Flag-MeCP2 (red) concentrated at the discrete spots of PCH on the mouse chromosome ends. GFP-LANA (green) also localized to the same regions when expressed in the presence of human MeCP2. In the absence of human MeCP2, LANA was never seen associated with NIH 3T3 chromosomes (Fig. 2D). Thus, LANA can be targeted to chromosomes by MeCP2.

**MeCP2 targets LANA to mouse interphase heterochromatin and mitotic chromosomes via LANA aa 1 to 15.** LANA contains a CBS in its N terminus, which is required for LANA’s association with chromosomes (38). We made a LANA mutant (mtLANA), which has had the first 15 aa of LANA deleted, disrupting the CBS while keeping the nuclear localization signal (NLS) intact (Fig. 3A). To test the requirement for the N-terminal CBS for MeCP2 interaction, immunoprecipitation assays were performed with extracts of HeLa cells cotransfected with Flag-MeCP2 and GFP-mtLANA or GFP–wild-type LANA (Fig. 3B). Immunoprecipitated proteins were analyzed by Western blotting, and mtLANA and wild-type LANA were detected with anti-LANA monoclonal antibody. Wild-type LANA (lane 3), but not mtLANA (lane 1), coprecipitated with Flag-MeCP2 in immunoprecipitates generated by using anti-Flag antibodies. Wild-type LANA and mtLANA were present in equal amounts in the transfected-cell extracts, as indicated by direct analysis of extract (lanes 5 and 6) or immunoprecipitation with anti-LANA antibody (lanes 7 and 8).

To determine the effect of the loss of the CBS site on MeCP2-directed chromosome targeting in mouse cells, NIH 3T3 cells were cotransfected with GFP-LANA-N (LANA aa 1 to 329) or GFP–mtLANA and Flag-MeCP2, and immunofluorescence assays were performed. As demonstrated in Fig. 3C, LANA-N (green) was targeted by Flag-MeCP2 (red) to the mouse PCH nuclear spots, although diffuse nuclear staining was also apparent. Full-length LANA may make additional contacts that stabilize the heterochromatin interaction. mtLANA (green) remained nuclear diffuse in the presence of human MeCP2 and was partially excluded from the PCH regions (Fig. 3D). This result indicates that mtLANA was unable to interact with MeCP2 and is not targeted to heterochromatin. Chromosome spreads were made from the same transfection (Fig. 3E). While Flag-MeCP2 (red) again formed discrete spots on mouse chromosome ends, mtLANA was unable to associate with the mouse chromosomes. This result is consistent with mtLANA’s inability to be targeted to mouse PCH by MeCP2. In summary, the previously described essential CBS of LANA is also required for MeCP2 interaction and targeting to chromosomes.

**Localization of LANA C terminus.** It is established that LANA has an N-terminal CBS (38). However, GFP-LANA C-terminus proteins have been expressed and show a nuclear speckled pattern in interphase nuclei (45, 49). Ballestas et al. also recognized that the LANA C terminus can independently associate with human chromosomes (M. E. Ballestas, T. Komatsu, and K. M. Kaye, 4th Int. Workshop KSHV and Related Agents, 2001). There is a cryptic NLS in the C terminus of LANA that is functional when the truncated LANA C terminus is expressed. We first demonstrated that our LANA C-terminus construction could target human chromosomes (Fig. 4). HeLa cells were transfected with GFP-LANA-C and blocked in metaphase by Colcemid for 2 h. Chromosome spreads were made, and chromosomes were viewed by immunofluorescence. LANA-C formed spots on the chromosomes, showing that LANA-C can be targeted to chromosomes in the absence of viral episomes. This confirms that a second LANA CBS and targeting mechanism exist.

**LANA interacts with DEK.** We identified DEK as a LANA-interacting protein in a yeast screen in which Gal4DBD-LANA was cotransformed into yeast with a B-cell cDNA library to seek cellular binding partners for LANA. DEK associates with and paints human chromosomes (24). Interaction between Gal4DBD-LANA and Gal4ACT-DEK is illustrated in Fig. 5A, as measured by induction of β-galactosidase activity in cotransformed yeast. A known LANA-interacting protein, SAP30, was used as a positive control in this assay.

We next examined LANA’s ability to interact in vitro with DEK by using a GST affinity assay. Expression of GST fusion proteins was examined by SDS-PAGE and Coomassie staining, and equal amounts of protein were used in each assay (data not shown). In Fig. 5B, in vitro-transcribed and -translated DEK was labeled with [35S]methionine and incubated with the GST proteins. The 43-kDa DEK protein interacted with the GST fusions expressing LANA(940-1164) (lane 1) and LANA(341-1164) (lane 3). There was a weak and possibly indirect interaction with GST-LANA(1-340) (lane 2). No interaction was observed with control GST-EBNA2(1-58) (GST-E2) (lane 4) or with GST protein (lane 5). Extract (2 μl) was loaded in lane 6. These results mapped the DEK interaction to the C terminus of LANA. To confirm the mapping data, an immunoprecipitation assay was performed with extracts of HeLa cells cotransfected with Flag-LANA or Flag-LANA-C and Myc-DEK (Fig. 5C). Immunoprecipitated proteins were analyzed by Western blotting, and LANA proteins were detected with an anti-Flag mouse antibody. Both Flag-LANA (lane 1) and Flag-LANA-C (lane 3) coprecipitated with Myc-DEK in immunoprecipitates generated with an anti-Myc antibody, but not those generated with a control mouse Ig antibody (lanes 2 and 4).

We further defined the DEK-interacting domain of LANA by using the Flag-tagged LANA deletion mutants shown in Fig. 5D (upper). The in vitro-transcribed and -translated LANA mutants were labeled with [35S]methionine and incubated with GST-DEK or control GST protein (Fig. 5D, lower panel). The LANA mutants m1 and m2 interacted with GST-DEK (lanes 1 and 4). No interaction was observed between LANA mutants
FIG. 2. MeCP2 targets LANA to sites of heterochromatin. NIH 3T3 cells were transfected with GFP-LANA and either Flag-HP1α or Flag-MeCP2. Immunofluorescence assays revealed that (A) GFP-LANA (green) did not localize to sites of mouse heterochromatin marked by Flag-HP1α staining (red). (B) GFP-LANA (green) relocalized to heterochromatin in the presence of Flag-MeCP2 (red). (C) GFP-LANA (green) localized to the Flag-MeCP2 marked pericentromeric regions of mouse NIH 3T3 chromosomes (red spots) in the presence of MeCP2. Inset, higher magnification of the merged image. (D) Chromosome spreads of NIH 3T3 cells transfected with GFP-LANA (green) reveal no association between LANA and mouse chromosomes. DNA was stained with DAPI (blue).
m3 and m4 and GST-DEK (lanes 7 and 10) or between the LANA mutants and the control GST proteins (lanes 2, 5, 8, and 11). Two microliters of extract was loaded for each mutant (lanes 3, 6, 9, and 12). This assay indicated that LANA aa 986 to 1043 are required for interaction with DEK.

Localization of DEK on mouse chromosomes. We investigated DEK’s localization in mouse cells in relation to the localization of human MeCP2. NIH 3T3 cells were transfected with GFP-DEK and Flag-MeCP2, and an immunofluorescence assay was performed. GFP-DEK (green) was nuclear diffuse, in contrast to the Flag-MeCP2 nuclear spots (red) (Fig. 6A). Chromosome spreads of dually transfected cells revealed that GFP-DEK (green) painted mouse chromosomes, while Flag-MeCP2 (red) localized to chromosomes, while mtLANA (green) failed to associate with chromosomes. DNA was stained with DAPI (blue).

FIG. 3. MeCP2 targets LANA to chromosomes via the N-terminal CBS. (A) Schematic of wild-type (wt) LANA N terminus showing the CBS and NLS. The CBS is disrupted in mtLANA. (B) Immunoprecipitation assay with extracts of HeLa cells cotransfected with Flag-MeCP2 and either mtLANA or wild-type LANA. mtLANA did not coprecipitate with Flag-MeCP2 (lane 1), unlike wild-type LANA (lane 3). Neither mtLANA nor wild-type LANA was precipitated by control mouse IgG (lanes 2 and 4). Lanes 5 to 8 show transfected cell extracts (15 μl; lanes 5 and 6) and direct precipitation by anti-LANA antibody (lanes 7 and 8). ms Ab, mouse antibody. (C to E) Immunofluorescence assays performed with NIH 3T3 cells transfected with GFP-LANA-N or GFP-mtLANA and Flag-MeCP2. (C) LANA-N (green) localized to sites of heterochromatin in the presence of MeCP2 (red). (D) mtLANA (green) did not colocalize with heterochromatin in the presence of MeCP2 (red). (E) Chromosome spreads revealed that Flag-MeCP2 (red) localized to chromosomes, while mtLANA (green) failed to associate with chromosomes. DNA was stained with DAPI (blue).

FIG. 4. LANA C-terminus associates independently with chromosomes. Immunofluorescence assay showing chromosome spreads of HeLa cells transfected with GFP-LANA-C (green spots), which associated with human chromosomes. DNA was stained with DAPI (blue). Inset, higher magnification of the merged image.
MeCP2 (red) localized as before to PHC (Fig. 6B). Thus, DEK associates with mouse chromosomes, but is targeted in a different manner from MeCP2.

LANA is targeted to mouse and human chromosomes by DEK. We have shown that the C terminus of LANA can interact with chromosomes and that the C terminus of LANA interacts with DEK. To investigate DEK’s ability to target LANA to chromosomes, NIH 3T3 cells (Fig. 7A) or HeLa cells (Fig. 7B) were cotransfected with Myc-DEK and GFP-mtLANA lacking the N-terminal CBS and then blocked with Colcemid for 6 h, and chromosome spreads were performed. GFP-mtLANA (green) localized to mouse and human chromosomes in the presence of Myc-DEK (red). mtLANA did not associate with chromosomes when expressed alone (Fig. 7C). Taken together, the results indicate that DEK targeting through LANA aa 986 to 1043 provides a second mechanism by which LANA can bind to chromosomes. A model for LANA chromosomal tethering is presented in Fig. 8.

**DISCUSSION**

LANA is a large multifunctional protein capable of interacting with a variety of cellular partners and playing a role in KSHV latency and KSHV-associated tumorigenesis. The colocalization of LANA with KSHV genomes on metaphase chromosomes and the requirement for LANA for episomal maintenance (4, 11) indicate that one of LANA’s key functions in KSHV latency is to tether KSHV genomes to chromosomes during cell division. Szekely et al. (49) showed LANA associated with mouse chromosomes in mouse-PEL hybrids in which the human chromosomes were lost. However, we did not observe any independent LANA binding to mouse chromosomes in NIH 3T3 cells by our means of analysis and were able to use this lack of association as an assay to identify key human proteins that were necessary for chromosome tethering. We demonstrated that LANA is targeted to chromosomes via interactions with two human chromosome-associated cellular proteins, MeCP2 and DEK. Murine homologs of MeCP2 and DEK have been identified with 71 and 67% identities, respectively, to their human counterparts (42, 51). Either the murine MeCP2 and DEK homologs are poorly expressed in NIH 3T3 cells, or the association with LANA is mediated through non-conserved regions of these proteins. Previous studies identified a chromatin binding site in the LANA N terminus (38), and we now also describe a second C-terminal chromatin binding site within LANA aa 986 to 1043.

We found that LANA can be directed to mouse heterochromatin in the presence of human McCP2. Methylation of cytosines at the carbon 5 position of CpG dinucleotides is a
characteristic feature of many eukaryotic genomes. In vertebrates, somatic genomes are globally methylated, with 60 to 90% of all CpGs being methylated. This leaves a small portion of the genome, mostly consisting of CpG islands, in a methyl-free state (3). In the mouse genome, the PCH has the highest concentration of CpG methylation. The rat MeCP2 was the first protein identified to bind a single methylated CpG (29) via its N-terminus methyl binding domain (36). MeCP2 concentrates at mouse PCH while painting mouse chromosome arms at a background level. Human MeCP2 is ubiquitously expressed in adult tissues (12). Quantitative Western blots indicate ~10⁶ MeCP2 molecules per nucleus, while a typical diploid nucleus has ~4 × 10⁷ methyl CpGs, suggesting there are enough MeCP2 binding sites in vertebrate genomic DNA to saturate all MeCP2 molecules. Data suggest that MeCP2 only binds internucleosomal linker DNA by associating with methyl CpGs exposed in the major groove (8). MeCP2 appears literally as a million tiny spots along chromosome arms in mammals such as rats, hamsters, and humans, which have a broad distribution of methylated CpGs (35). LANA targeting to chromosomes via a protein with so many CBSs theoretically ensures that all episomes (estimated to be ~25 to 80 copies per cell) (6, 7, 37) bound to LANA would be tethered to chromosomes and carried to daughter cells after cell division.

DEK was first identified in a chromosomal translocation with the CAN nucleoporin protein in a subset of acute myeloid leukemias (52). Autoantigens to DEK have been associated with several disease states, including systemic lupus erythematosus (13, 14, 53), juvenile rheumatoid arthritis (14, 34, 47, 50), and sarcoidosis (13, 14). Subsequently, DEK was described as a 43-kDa ubiquitously expressed DNA-binding phosphoprotein that recognized peri-ets sites in the human

FIG. 6. DEK interacts with mouse chromosomes. (A) NIH 3T3 cells were transfected with GFP-DEK and Flag-MeCP2. GFP-DEK (green) did not localize to sites of heterochromatin, unlike Flag-MeCP2 (red). (B) Chromosome spreads of NIH 3T3 cells transfected with GFP-DEK and Flag-MeCP2. GFP-DEK (green) painted mouse chromosomes and did not localize to the regions of heterochromatin marked by MeCP2 (red). Inset, higher magnification of the merged image.

FIG. 7. DEK can independently mediate LANA chromosomal tethering. Chromosome spreads of NIH 3T3 cells (A) and HeLa cells (B) transfected with GFP-mtLANA and Myc-DEK. Myc-DEK (red) targeted GFP-mtLANA (green) lacking the N-terminal CBS to mouse and human chromosomes. (C) Chromosome spreads of wild-type (wt) LANA versus mtLANA in HeLa cells. mtLANA lacking the N-terminal CBS does not associate with chromosomes. DNA was stained with DAPI (blue).

FIG. 8. Model of LANA-mediated chromosomal tethering. LANA is targeted to chromosomes through N-terminal interactions with MeCP2, which binds methylated CpG dinucleotides in intranucleosomal linker DNA, and through C-terminal interactions with DEK, which associates with core histone proteins. Chromosomal association mediates episomal tethering through binding of the LANA C terminus to KSHV DNA (5, 10, 22).
immunodeficiency virus type 2 enhancer (17, 19, 20), as a constituent of splicing complexes (33), and as a protein involved in changes of chromatin topology (1). Histones may play a supporting role in LANA tethering to chromosomes. DEK associates with histones H2A and H2B, as well as to a lesser extent, histones H3 and H4 (1). It has been suggested that LANA tethers to chromosomes via histone H1, which is associated with heterochromatin. Interestingly, MeCP2 displaces histone H1 in order to gain access to its binding sites (35). MeCP2 and DEK both have broad distributions on human chromosomes, unlike the punctate localization of the LANA C terminus or of intact LANA on chromosomes of man chromosomes, unlike the punctate localization of the

VOL. 76, 2002 CHROMOSOME TETHERING BY LANA 11603

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