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Kaposi's sarcoma herpesvirus C-terminal LANA concentrates at pericentromeric and peri-telomeric regions of a subset of mitotic chromosomes

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Abstract

The Kaposi's sarcoma-associated herpesvirus (KSHV) latency-associated nuclear antigen (LANA) tethers KSHV terminal repeat (TR) DNA to mitotic chromosomes to efficiently segregate episomes to progeny nuclei. LANA contains N- and C-terminal chromosome binding regions. We now show that C-terminal LANA preferentially concentrates to paired dots at pericentromeric and peri-telomeric regions of a subset of mitotic chromosomes through residues 996–1139. Deletions within C-terminal LANA abolished both self-association and chromosome binding, consistent with a requirement for self-association to bind chromosomes. A deletion abolishing TR DNA binding did not affect chromosome targeting, indicating LANA's localization is not due to binding its recognition sequence in chromosomal DNA. LANA distributed similarly on human and non-human mitotic chromosomes. These results are consistent with C-terminal LANA interacting with a cell factor that concentrates at pericentromeric and peri-telomeric regions of mitotic chromosomes.

Keywords: KSHV; LANA; Latency; Chromosome; Centromere; Telomere; Herpesvirus; HHV

Introduction

Kaposi's sarcoma (KS)-associated herpesvirus (KSHV) is a ~140 kb gamma-2 herpesvirus (Chang et al., 1994; Russo et al., 1996). KSHV is tightly linked to KS and the lymphoproliferative disorders primary effusion lymphoma (PEL) and multicentric Castleman's disease in patients with and without AIDS (Cesarman et al., 1995a; Moore and Chang, 1995; Soulier et al., 1995). KSHV latently infects tumor cells and virus persists as a multiple copy, covalently closed, circular DNA (episome) (Cesarman et al., 1995b; Decker et al., 1996).

The latency-associated nuclear antigen (LANA) (Kedes et al., 1997; Kellam et al., 1997; Rainbow et al., 1997) mediates KSHV episome persistence (Ballestas et al., 1999; Ballestas and Kaye, 2001; Cotter and Robertson, 1999; Ye et al., 2004). To persist in proliferating cells, episomes must replicate and efficiently segregate to progeny nuclei. LANA self-associates through its C-terminal domain to bind specific sequence within KSHV terminal repeat (TR) DNA and mediate DNA replication (Ballestas and Kaye, 2001; Cotter et al., 2001; Fejer et al., 2003; Garber et al., 2001a, 2002; Grundhoff and Ganem, 2003; Hu et al., 2002; Lim et al., 2002; Schwam et al., 2000). LANA tethers TR DNA to chromosomes during mitosis to efficiently segregate episomes to progeny nuclei. Epstein–Barr virus EBNA1 and bovine papillomavirus E2 also associate with mitotic chromosomes and bind specific viral DNA sequence to mediate episome persistence for their respective viruses (Bastien and McBride, 2000; Hung et al., 2001; Ilves et al., 1999; Lehman and Botchan, 1998; Skiadopoulos and McBride, 1998; Yates et al., 1984, 1985).

LANA contains N- and C-terminal chromosome binding regions (Krithivas et al., 2002; Piolot et al., 2001). LANA N-terminal sequence binds mitotic chromosomes diffusely through the folded regions of histones H2A–H2B and is

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essential for LANA mediated DNA replication and episome maintenance (Barbera et al., 2004, 2006; Lim et al., 2004). The C-terminal LANA chromosome binding region also functions in episome persistence (manuscript in preparation). Here, we characterize the C-terminal LANA chromosome binding domain, including its novel distribution pattern on mitotic chromosomes.

Results

C-terminal LANA concentrates to paired dots on mitotic chromosomes

We investigated the ability of different LANA domains to target mitotic chromosomes because mitotic chromosome attachment is key to LANA's efficient segregation of episomes to progeny nuclei. GFP, GFP LANA, GFP LANA 5–274, GFP LANA 275–777 and GFP LANA 933–1162 (Fig. 1A) were each expressed in BJAB cells (Menezes et al., 1975). GFP does not interfere with LANA chromosome association or its ability to mediate episome persistence (Barbera et al., 2004, 2006; Piolot et al., 2001; Tetsuka et al., 2004). As expected, GFP LANA (green) (Fig. 1B) associated with chromosomes (red) (overlay generates yellow), whereas GFP did not. GFP LANA 5–274 (green) (Fig. 1B) contains LANA N-terminal residues 5–13, which bind core histones H2A–H2B (Barbera et al., 2006), and diffusely painted chromosomes (red) (overlay generates yellow). GFP LANA 275–777 (green) (Fig. 1B) did not bind chromosomes (red), demonstrating that the highly charged and glutamine rich LANA central repeat elements do not target chromosomes. However, as expected (Krithivas et al., 2002), GFP LANA 933–1162 (green) (Fig. 1C, arrow) did bind chromosomes (red) (overlay generates yellow).

The distribution of GFP LANA 933–1162 on chromosomes dramatically differed from that of GFP LANA or GFP LANA 5–274. Rather than broadly distributing over chromosomes, GFP LANA 933–1162 concentrated to paired dots on sister chromatids. Further, in contrast to GFP LANA or GFP LANA 5–274, GFP LANA 933–1162 concentrated on a subset of chromosomes. Approximately five to eight chromosomes were targeted in each mitotic cell. Therefore, GFP LANA 933–1162 concentrates to paired dots on a subset of mitotic chromosomes.

LANA amino acids 996–1139 mediate chromosome association

We localized the chromosome association region within C-terminal LANA. GFP LANA 933–1162, GFP LANA 982–1162, GFP LANA 982–1139 and GFP LANA 996–1151 (Fig. 2A) were each expressed in BJAB cells. GFP LANA 933–1162 (green), GFP LANA 982–1162 (green), GFP LANA 982–1139 (green) and GFP LANA 996–1151 (green) (Fig. 2B) each formed paired dots on a subset of metaphase chromosomes (red) (overlay of green and red generates yellow). GFP LANA 982–1139 differed slightly from GFP LANA 933–1162, GFP LANA 982–1162 and GFP LANA 996–1151 because it was only detected when highly expressed in mitotic cells and as a result also distributed diffusely on chromosomes. Taken together, these data indicate that amino acids 996–1139 of LANA confer binding of C-terminal LANA to mitotic chromosomes.

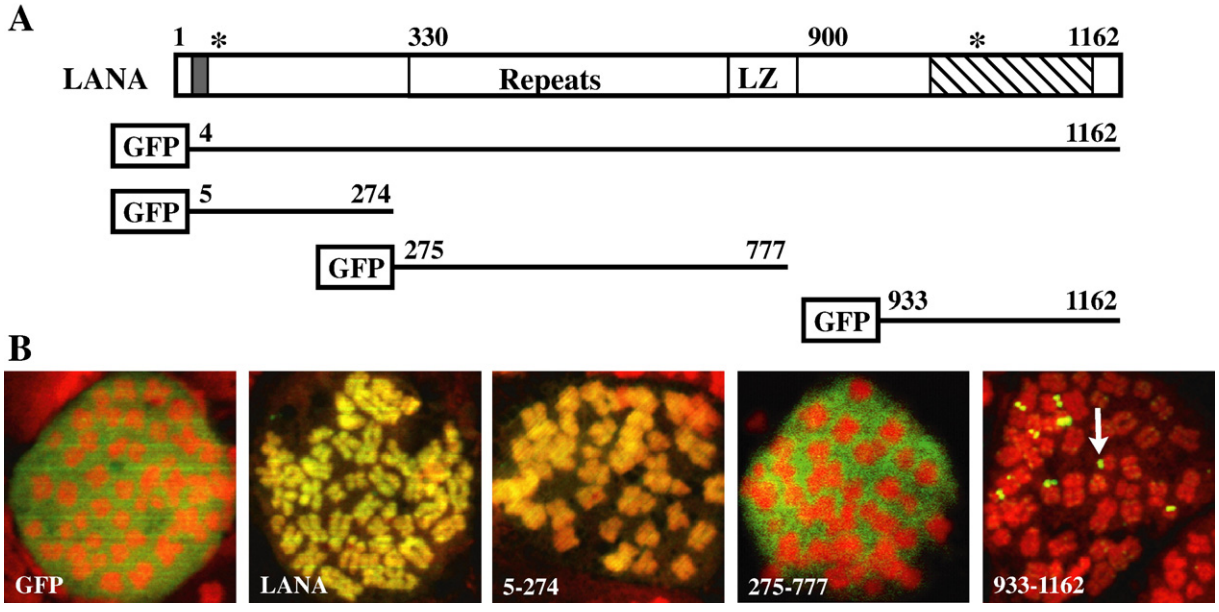

Fig. 1. C-terminal LANA concentrates to paired dots on mitotic chromosomes. (A) Schematic of LANA constructs tested for chromosome association. Indicated are the previously characterized N-terminal chromosome binding region (shaded), N- and C-terminal nuclear localization signals (*), central acidic and glutamine rich repeat region, putative leucine zipper (LZ) and DNA binding domain (hatched). (B) GFP fusion proteins (green) were transiently expressed in BJAB cells and confocal microscopy performed after metaphase arrest with colcemid and counterstaining chromosomes with propidium iodide (red). Overlay of green and red generates yellow. GFP NLS (GFP), GFP LANA (LANA), GFP LANA 5–274 (5–274), GFP LANA 275–777 (275–777) and GFP LANA 933–1162 (933–1162). Arrow indicates paired dots on mitotic chromosomes. Magnification, 630×.