Kaposi’s sarcoma-associated herpesvirus genome replication, partitioning, and maintenance in latency

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INTRODUCTION

Kaposi’s sarcoma (KS)-associated herpesvirus (KSHV) is a gamma-2 herpesvirus discovered from KS specimens in 1994 (Chang et al., 1994). KSHV is closely associated with KS and several non-Hodgkin lymphomas, including primary effusion lymphoma (PEL) and multicentric Castleman’s disease (MCD; Cesaran et al., 1995, 1996; Soulier et al., 1995). While KS is the most common cancer in acquired immune deficiency syndrome patients (Potthoff et al., 2010), KSHV is detected in about 95% of all types of KS lesions by PCR analysis (Dupin et al., 1995; Huang et al., 1995; Moore and Chang, 1995). PEL is a rare B cell lymphoma originated from preterminal B cells, and PEL in AIDS patients is often associated with KSHV as well as EBV. Several KSHV-infected PEL cell lines have been established, and EBV is frequently lost in the course of establishment (Arvanitakis et al., 1996; Gaidano et al., 1996; Renne et al., 1996; Said et al., 1996; Carbone et al., 1997, 1998; Katano et al., 1999). MCD is a plasmacytic lymphadenopathy with polyclonal hyper-immunoglobulinemia and high levels of serum IL-6 (Frizerra et al., 1983; Yoshizaki et al., 1989).

Like all herpesviruses, KSHV has two life cycles: latent and lytic replication phases (for review, see Boshoff and Chang, 2001). Whereas KSHV is usually in latency when it infects KS and PEL cells, in MCD some cells express lytic genes (Katano et al., 2000; Parravicini et al., 2000). On the other hand, it has been reported that KSHV infection itself and/or viral lytic proteins promote cell proliferation and angiogenesis as well as lymphatic reprogramming (Ciufio et al., 2001; Gao et al., 2003; Carroll et al., 2004; Hong et al., 2004; Naranatt et al., 2004; Pan et al., 2004; Wang et al., 2004; Sharma-Walia et al., 2006; Qian et al., 2007, 2008; Sadagopan et al., 2007; Ye et al., 2007).

In latency, the KSHV genome is present as an episome, which is capable of autonomously replicating during 5 phase of the host cell cycle without integration into host chromosomes, and only limited genes are expressed during latency. Therefore, there is no generation of progeny virions. It is very important to elucidate and learn the virus’s survival strategy in order to control infection and to formulate treatment for KSHV-related diseases.

In this review, we would like to focus on studies on the mechanisms underlying viral DNA replication, genome segregation and maintenance, and gene expression regulation in latency, and to discuss these topics in the light of studies on cellular mechanisms.

GENE EXPRESSION CONTROL IN KSHV LATENCY

The KSHV genome is a double-stranded linear DNA in the virion. It is circularized upon infection and is maintained as an episome in the infected nucleus. The complete genome is about 160–170 kbp,
including a 40–50 times repeated sequence called a terminal repeat (TR), which is 801 bp as a unit, at each end of the genome (Figure 1). The viral genome encodes approximately 90 ORFs in the unique region (for reviews, see Moore and Chang, 2001; Dourmishev et al., 2003). In latency, the viral genome is maintained in a cell cycle-dependent manner, and extremely limited viral genes, such as latent gene clusters, are expressed. Although the establishment of latent infection could be this virus’s default infection mode, it is not good for the virus to continue latent infection for a long time, since it will vanish from the earth without progeny virus production. Thus, latency is a state in which the virus waits for an opportunity for the explosive production of progeny virions. Nevertheless, it is very interesting how KSHV establishes latency and is maintained in infected host cells without losing the genomes.

As mentioned above, the limited region within the KSHV genome is transcriptionally active in latency, and this region contains only several genes, including the latency-associated nuclear antigen (Wang and Frappier, 2009), viral cyclin (v-CYC), viral FLICE-inhibitory protein (v-FLIP), kaposin, 17 microRNAs (miRNAs), and viral interferon regulatory factor 3 (v-IRF-3; Chang et al., 1996; Thome et al., 1997; Muralidhar et al., 1998; Lubyova and Pitha, 2000; Gomez-Roman et al., 2001; Rivas et al., 2001; Staudt and Dittmer, 2003; Cai et al., 2005; Pearce et al., 2005; Pfeffer et al., 2005; Samols et al., 2005). Such genes, except for v-IRF-3, are in that limited region, and LANA, v-CYC, and v-FLIP are in one of the unit’s genes. This region forms an active locus for expression including miRNAs and kaposin. It is unclear why this region is active for the expression of genes and is insulated from inactive lytic genes, such as ORF69 and K14 just downstream and upstream, respectively.

Recently, it was reported that CTCF, which is the only insulator protein found in vertebrates, coupled with SMC3, endows this gene insulation. More interestingly, the binding sites are in the genes, i.e., downstream from the LANA transcription start sites, not at the boundary regions (Stedman et al., 2008; Kang and Lieberman, 2009). Transcriptional analysis using the KSHV-BAC system demonstrated that mutations of CTCF binding sites abolished latency-regulated transcription such as K14 and ORF74 during latency (Kang and Lieberman, 2009). CTCF usually binds at the boundary regions between active and inactive loci in mammalian genomes, forming locus control regions (LCRs; Tanimoto et al., 2003). A typical example is an LCR seen in the beta-globin locus. CTCF binds to several DNase I hypersensitivity sites (HS), called HS4 and HS5, and forms boundaries to insulate this locus from the outside locus (Tanimoto et al., 2003; Hou et al., 2008). Thus, latent gene expression in KSHV-infected cells might be regulated differently from the mechanism observed in the beta-globin locus.

Inversely, it is interesting how the viral lytic genes are tightly inactivated in latency. Epigenetic regulation seems to be essential for inactivation as well as activation of latent genes. LANA recruits heterochromatin components to the TR by the interaction between LANA and SUV39H1, which is a key factor that methylates histone H3, which in turn recruits heterochromatin protein 1 (HP1; Sakakibara et al., 2004). Because this mechanism contributes to the propagation and maintenance of heterochromatin, it appears that heterochromatin could spread over the KSHV genome during latency. The propagation of heterochromatin into the active latent gene zone might be blocked by the boundary effect and by the enhancer-blocking activity of an insulator, CTCF which has multiple functions such as gene activation or inactivation, X-chromosome inactivation, and gene imprinting (for review, see Zlatanova and Caiafa, 2009).

Thus, it is thought that not the overall lytic genes region, except for the latent gene clusters, forms heterochromatin during latency, because recent genome-wide analysis using ChIP-on-chip showed that not only latent gene clusters but also several regions of lytic genes are enriched in activating histone marks (acetylated H3 and H3K4me3). However, H3K27me3, which is a bivalent histone marker, is widely distributed through the KSHV genome (Toth et al., 2010), meaning that the genome is poised for reactivation. Furthermore, the treatment of specific histone demethylases of H3K27me3 such as JMJD3 and UTX could induce the lytic reactivation. Immunoprecipitation of methylated DNA assay showed that the KSHV genome was methylated during latency (Gunther and Grundhoff, 2010). Gunther and Grundhoff (2010) suggested that the CpG methylation process could take a long time to prevail over the genome, and thus could not control early latency. There are several reports that DNA methylation of viral genomes is related to the regulation of the gene expression of gammaherpesviruses such as EBV and herpesvirus saimiri (HVS; Minarovits, 2006). Heterochromatin formation on the viral genomes, however, seems to be inconvenient for the rapid induction of lytic replication. Further investigations are needed to clarify how viruses are ready for lytic induction if heterochromatin and/or DNA methylation was formed on the genome.

Viral factors play key roles in maintaining gene expression profiles in latency. Otherwise, modulation by viral and cellular factors maintains viral latency. In addition to the recruitment of

![FIGURE 1 | Terminal repeat (TR) sequences.](image) The KSHV genome has 40–50 units of TR sequences. TR contains LANA-binding site (LBS) and GC-rich sequences termed 32GC. It is thought that one of the LBS-32GC sequence is selected as DNA replication origin (ori-P).
heterochromatin factors on to the genome, LANA itself tends to repress viral lytic gene expression. LANA physically associates with recombination signal sequence-binding protein Jk (RBP-Jk) and represses the replication and transcription activator (RTA) promoter through the RBP-Jk binding site existing within its promoter (Lan et al., 2005a).

Viral FLICE-inhibitory protein, also known as K13, interacts with several NF-κB-related signaling proteins and activates the NF-κB pathways, thus enhancing cell survival (Chaudhary et al., 1999; Field et al., 2003; Matta et al., 2003, 2007; Matta and Chaudhary, 2004). It should be noted that the effect of NF-κB signaling on reactivation depends on cellular context (Grossmann and Ganem, 2008) and seems to be regulated by an intricate balance within the cellular environment. Previous studies, however, demonstrated that v-FLIP repressed the RTA promoter by activating NF-κB binding to the cognate sequence, but in that case it deregulates vIL-6 and hIL-6 expression (Zhao et al., 2007). The reactivation is initiated by RTA, which is a lytic switch protein and a homolog of EBV BRLF1 (Li et al., 2002). The RTA promoter region is highly responsive to 12-O-tetradecanoylphorbol 13-acetate (TPA) or phorbol 12-myristate 13-acetate (PMA), sodium butyrate (NaB), and trichostatin A (TSA), and is associated with several histone deacetylase proteins such as HDAC, which leads to chromatin remodeling of a nucleosome and then regulates KSHV reactivation from latency (Lu et al., 2003). RTA activates various viral genes through direct binding with RTA-responsive elements existing within the K8 and ORF57 promoters (Byun et al., 2002) and also through indirect mechanisms on RTA itself (Liang et al., 2002). RTA activates various viral genes through direct binding with RTA-responsive elements existing within the K8 and ORF57 promoters (Byun et al., 2002) and also through indirect mechanisms on RTA itself (Liang et al., 2002). Although RTA is a strong transactivator and inducer of lytic replication, it also enhances LANA expression and then is involved in the establishment of latency in the early infection phase (Lan et al., 2005b). This feedback mechanism explains the low efficiency of lytic replication and the generation of complete viral particles in KSHV-infected cell lines.

MicroRNAs (miRNAs) are single-stranded and 20- to 23-nucleotide RNA molecules that are involved in gene expression (Bartel, 2004; Bartel and Chen, 2004). Recent studies have highlighted the critical role of viral microRNAs (miRNAs) in the maintenance of KSHV latency (for review, see Ganem and Ziegelbauer, 2008; Boss et al., 2009; Lei et al., 2010). The KSHV genome contains 17 miRNAs that are clustered and located in the intragenic region between kaposin and v-FLIP (Cai et al., 2005; Pearce et al., 2005; Samols et al., 2005; Cai and Cullen, 2006). Surprisingly, a new proteomic approach suggests that a single miRNA can directly lead to the suppression of the synthesis of hundreds of proteins at both mRNA and translation levels, although the level of suppression is mild (Baek et al., 2008; Selbach et al., 2008).

Kaposi’s sarcoma-associated herpesvirus miRNAs are reported to regulate, directly or indirectly, various factors including lytic genes (Murphy et al., 2008; Bellare and Ganem, 2009) and cellular factors such as NF-κB and IkBα, the latter of which is directly regulated by KSHV mir-K1 (Lei et al., 2010b) and Bcl-2 associated factor (BCLAF1) as a target of mir-K5 (Ziegelbauer et al., 2009). mir-K12-7 (Lin et al., 2011) and mir-K9 (Bellare and Ganem, 2009) directly target RTA and contribute to the maintenance of latency. mir-K12-11 shows remarkable homology to cellular miR-155, it inhibits a BACH-1 3’UTR-containing reporter and downregulates the expression of BACH-1, which is a broadly expressed transcriptional repressor that regulates genes involved in the hypoxia response (Gottwein et al., 2007; Skalsky et al., 2007). Thrombospondin 1 (THBS1), an inhibitor of angiogenesis, is targeted by multiple KSHV miRNAs, such as miR-K12-1, miR-K12-3-3p, miR-K12-6-3p, and miR-K12-11 (Samols et al., 2007). mir-K1 represses the expression of p21 via the 3’UTR and attenuates p21-mediated cell cycle arrest during KSHV latency (Gottwein and Cullen, 2010).

**CELLULAR DNA LICENSING AND VIRAL DNA REPLICATION IN LATENCY**

Because eukaryotic DNA replication is strictly regulated by a licensing mechanism, the genome is replicated only once per cell cycle. DNA replication starts at multiple sites on a chromosome; these sites are called the replication origin, whose number is predicted to be 30,000–50,000 (Huberman and Riggs, 1966). The genome size of eukaryotes is about 10^7 to 10^11 bp (fungi to mammals), and the entire DNA must be replicated within a limited time (Wyrick et al., 2001). To achieve this, many proteins participate in DNA replication licensing, including origin recognition complex (ORC), Cdc6, Cdt1, and mini-chromosomal maintenance (MCM) helicase, and so on. First, ORC recognizes and binds to the origins and then recruits a Cdc6 followed by the association of another replication protein, called Cdt1. Finally, the MCM helicase is loaded onto the complex to establish a complete pre-replication complex (pre-RC) (reviews in Nishitani and Lygerou, 2002; DePamphilis, 2003, 2005).

**KSHV ORIGIN OF REPLICATION IN LATENCY**

The features of DNA replication origins have been reported. Although there are no consensus sequences for the replication origin, recent studies showed that CpG islands, promoter regions, DNA topology, and nucleosome positioning are involved in origin selection (Mechali, 2010). *Saccharomyces cerevisiae* (S. cerevisiae) has autonomous replication sequence (ARS) elements that are specific 12 bp consensus sequences and has origin activity (Stinchcomb et al., 1979; Bell and Stillman, 1992). *S. pombe* ARS also has been identified, but it does not share a consensus sequence as in *S. cerevisiae* (Segurado et al., 2003; Dai et al., 2005; Heichinger et al., 2006). Substitution experiments showed that the ARS region could be replaced with a 40-bp poly (dA/dT) fragment (Okuno et al., 1999).

In higher eukaryotes, no consensus sequences are identified, though known origin sequences have been reported (for review, see Aladjem, 2004). It is not yet known why there is no consensus sequence among ORC binding sites of higher eukaryotes, or how they are selected. Replication origins should be determined by different mechanisms, and recent genome-wide analyses show that the origin sequences are closely related with transcriptional regulatory elements and CpG islands but not sequence motifs (Cadoret et al., 2008; Sequeira-Mendes et al., 2009).

The KSHV genome appears to replicate once per cell cycle during latency, as cellular DNA replication. The number of the genome copies is supposed to be 50–100 per KSHV-infected PEL cell and the copy number is kept at the same number, at least
appears to be kept at the same copy number (Cesarman et al., 1995; Ballestas et al., 1999; Ueda et al., 2006). This observation suggests that the KSHV genome uses cellular replication machinery so that viral DNA replication synchronizes with the cell cycle. The KSHV origin of replication in latency, called ori-P, consists of two LANA-binding sites (LBS), in which LBS1 has a higher affinity with LANA (Garber et al., 2002), and the following 32 bp GC-rich segment (termed 32GC in this manuscript) and the ori-P is in the TR region of the KSHV episome (Hu and Renne, 2005; Figure 1). LANA directly binds to LBS and supports viral DNA replication (Garber et al., 2002; Hu et al., 2002; Fejer et al., 2003; Grundhoff and Ganem, 2003). The components of pre-RC, such as ORC, Cdc6, and MCM were recruited to the TR sequences in a LANA-dependent manner (Lim et al., 2002; Ohaski et al., 2004; Stedman et al., 2004; Verma et al., 2006).

In the case of EBV ori-P, the dyad symmetry (DS) and family of repeats (FR) are essential for the ori-P activity (Reisman et al., 1985), though FR rather works for viral genome maintenance. The DS element contains two EBNA1 binding sites and is the functional replicator in the presence of EBNA1 (Wysokenski and Yates, 1989; Harrison et al., 1994). The FR element contains 20 copies of a 30-bp repeat sequence and has an essential role in the long-term maintenance of ori-P-containing plasmid (Krysan et al., 1989; Marchal et al., 1999). It is suggested that cellular replication factors bind to the sequences adjacent to EBNA1 binding sites through the interaction with EBNA1 (Yates et al., 2000; Koons et al., 2001). A chromatin immunoprecipitation assay suggested that the ORC complex and EBNA1 bound to chromatin and ori-P in G0-arrested cells (Ritzi et al., 2003). Nucleosome assembly proteins, such as NAP1 and TAF-I, interact with EBNA1 and are recruited to the ori-P regions. These proteins contribute to the activation of transcription, although TAF-I negatively regulates DNA replication (Wang and Frappier, 2009). The EBNA1 LR1 and LR2 domains are critical for the interaction with ORC and for disrupting this association by binding with G-rich RNA (Norseen et al., 2009). Thus, the latent replication of KSHV and EBV totally depends on cellular DNA replication machinery with the only exception of the requirement of LANA and EBNA1, respectively.

**HOW LANA WORKS**

LANA is a nuclear protein with 1162 amino acids. It shows a functional homology to EBNA1 of EBV and, in part, to E1/E2 of human papillomavirus and to SV40 large T antigen. Especially, the C-terminus of LANA and that of EBNA1 conserve secondary and tertiary structures (Han et al., 2010). The N-terminus of LANA contains a chromosome binding site (CBS) and a nuclear localization signal (NLS), and the C-terminus contains a DNA binding domain and a dimerization domain, called DBD. In the DBD, there appears to be another NLS, but this NLS is rather cryptic and weak, since an N-terminal deleted mutant, which contains 108–1162 aa, is localized in the cytoplasm (Ohaski et al., 2009). The central region is composed of a proline-rich region, an aspartate (D)- and glutamate (E)-rich repetitious region, and a glutamine-rich domain (Garber et al., 2001; Piolot et al., 2001; Figure 2). DBD (923–1162 aa) is necessary and partially sufficient to support ori-P replication compared to the full-length LANA (Hu et al., 2002; Ohaski et al., 2009), and when considering their expression levels in *in vitro* study.

Many studies show that LANA binds to LBS and recruits ORC to the origin as described above. What is the mechanism by which ORC is recruited to ori-P? One possible mechanism is that LANA directly interacts with ORC so that ORC is loaded to the origin (Lim et al., 2002; Stedman et al., 2004; Verma et al., 2006). If LANA could directly interact with ORC and recruit to the origin, we are confronted with this question: why is 32GC required for ori-P activity despite LANA's ability to bind to the ori-P? It could be that the 32GC is required to load ORC and the other pre-RC components to the region next to LBS, although the underlying molecular mechanism is not yet known. Further study is needed to resolve this question.

LANA is highly expressed in KSHV-related malignancies, and plays an essential in episomal maintenance. It interacts with multiple cellular proteins, including tumor suppressors such as p53 (Friborg et al., 1999) and Rb (Radkov et al., 2000), as well as transcription factors such as ATF4/CREB2 (Lim et al., 2000) and STAT3 (Muromoto et al., 2006), chromatin-associated proteins such as HP1 (Lim et al., 2003), histone H2A/B (Barbera et al., 2006b), MeCP2 (Krithivas et al., 2002; Matsumura et al., 2010), and Brd4 (Ottinger et al., 2006), in addition to signal transducers such as GSK-3b (Fujimuro and Hayward, 2003) and so on (Figure 2). Almost all of these proteins interact with the C-terminal domain of LANA, implying the functional importance of this domain. However, careful analysis of protein–protein interaction is required, because only DBD sometimes shows non-specific binding with other proteins and may exhibit different properties from the full-length LANA (our personal observation).

**REGULATION OF KSHV ori-P ACTIVATION**

The origin number varies from species to species. In mammals, it, 30,000–50,000 origins are thought to exist at each cell cycle (Huberman and Riggs, 1966). However, not all of these origins start DNA synthesis at the same time. Some origins are activated early in the S phase, whereas others are activated in the late S phase; that is, the DNA replication timing is controlled (Dimitrova and Gilbert, 1999; Cimbora et al., 2000). How is the timing of DNA replication determined? Some groups have provided possible answers to this question. It was reported that heterochromatin could change the timing of DNA replication by transgene insertion into a mammalian genome (Lin et al., 2003). Sir proteins, which are slencer proteins, can delay replication and correlate with transcriptional silencing (Zappulla et al., 2002). Thus, various studies suggest that heterochromatin modulates replication timing (Goren et al., 2008; Klochkov et al., 2009; Schwaiger et al., 2010) and EBV replication in latency occurs in mid-late S phase (Zhou et al., 2009). In the case of KSHV, the replication timing of the viral genome is not yet known. Considering that ori-P is present in the proximity of heterochromatin because of LANA-dependent accumulation of heterochromatin, DNA replication of the KSHV genome may start at the middle or late S phase.

The frequency of origin usage also differs from origin to origin; some origins are used in every cell cycle, whereas others are used rarely. Various studies including in *Drosophila*, *Xenopus*, and mammals demonstrate how specific origins are selected, but several
The top Figure shows the organization of the LANA protein. LANA has two nuclear localization signals (NLS): one at the N-terminal region and the other at the C-terminal region. The former is a primary NLS and the latter is cryptic. A chromosome binding site (CBS) was reported to be present at the N-terminal end as a histone H1 binding region. The mid-part of LANA consists of a very peculiar amino acid composition called a proline-rich region (P-rich), an extremely aspartate (D)- and glutamate (E)-rich region (DE) and a glutamine (Q)-aspartate (D)-glutamate (E)-rich region (QQQDE/QQQQDE). LEE(D)EQ(E)V looks like a leucine zipper but has not been recognized as a functional determinant. The C-terminal region has roles in both homo-dimerization and DNA binding to LBS. The main interacting proteins with LANA are listed below. The C-terminal region seems to be a region that interacts with many proteins.

questions about the decision mechanism of origins remain unanswered. Considering the genome size of this virus, one origin is enough to complete replication within the S phase; and because of the existence of “origin interference” by the ATR and ATM pathways (Shechter et al., 2004), a single origin must be chosen.

The micrococcal nuclease digestion pattern at TR in G1-arrested cells leads to change, suggesting that the chromatin structure became more accessible to enzymatic digestion (Stedman et al., 2004). Therefore, the chromatin structure may be changed by the recruitment of the replication machinery during the late G1 phase.

A recent study shows that the cellular deubiquitylating enzyme USP7 stimulates EBNA1 binding to its recognition sites so that histone modification at the EBV ori-P is changed by EBNA1 mediating the recruitment of USP7 (Sarkari et al., 2009).

THE ROLES OF NUCLEAR ARCHITECTURES
NUCLEAR MATRIX AS A SCAFFOLD FOR DNA REPLICATION AND TRANSCRIPTION
The nucleus consists of a well-organized structure and is highly complex. The structures of nuclear matrix proteins such as laminas, nuclear mitotic apparatus (NuMA), hnRNPs, and so on, are important for the organization of chromatin, DNA replication, and transcription (Dechat et al., 2008). The nuclear matrix, isolated by Berezney and Coffey (1974), is believed to support the spatial distribution of several nuclear factors, such as DNA replication machinery and transcription factors. The nuclear matrix fraction contains DNase I-resistant and high salt-resistant proteins. Because the nuclear matrix can be visualized only after chromatin extraction, there has been a debate that such a nuclear matrix is an essential component of in vivo nuclear architectures. In previous studies, replication origins have come to the nuclear matrix (van der Velden et al., 1984; Amati and Gasser, 1990; Adom et al., 1992; Brylawski et al., 1993; Fallaux et al., 1996). Eukaryotic DNA is organized into DNA loops generated by the attachment of chromatin to the nuclear matrix via specific regions, referred to as scaffold/matrix attachment regions (Pardoll et al., 1980; Vogelstein et al., 1980; Laemmli et al., 1992; Roberge and Gasser, 1992). DNA loop formation is essential for DNA replication, transcription, and chromosomal packaging (Gasser and Laemmli, 1987; Berezney et al., 1995; Bode et al., 1995; Nickerson et al., 1995; Razin et al., 1995; Jackson, 1997; Volpi et al.,
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2006; Mahy et al., 2002; Sumer et al., 2003; Marenduzzo et al.,
2007).

In EBV, both the latent ori-P and the lytic ori-Lyt are bound to
the nuclear matrix (Jankelevich et al., 1992; Mattia et al.,
1999). EBV nuclear antigen leader protein (EBNA-LP) is a nuclear
matrix-associated protein, and its nuclear matrix-targeting signal
is a 10-aa segment, which also functions as a NLS (Yokoyama
et al., 2001). However, this is not the case for LANA, because
it was reported that C-terminal truncation up to 1128 aa could
not be localized in a high salt-resistant fraction (nuclear matrix
fraction), but the C-terminal region (1129–1143 aa) is high salt
extractable (Viejo-Borbolla et al., 2003). We previously showed
that the N-terminal region up to 107 aa is localized in nucleo-
cytoplasmic and chromatin fractions (Ohsaki et al., 2009). The
localization to the nuclear matrix fraction of LANA might
depend on the conformation of LANA or post-translational
modifications.

On the other hand, a cell fractionation assay has shown that
 cellular pre-RC components, such as ORC2, Cdc6, and Cdt1,
 preferentially localize in the nuclear matrix fraction in a cell cycle-
dependent manner, and LANA itself also can localize in the nuclear
matrix fraction. Accordingly, TR accumulates in the nuclear matrix
fraction during the late G1 phase, suggesting that LANA recruits
the ori-P to the nuclear matrix, so that cellular replication machin-
ery is abundant and available for viral DNA replication during
latency (Ohsaki et al., 2009; Figure 3).

GENOME SEGREGATION MECHANISMS OF KSHV IN
LATENCY

The same copy number of KSHV genomes appears to be main-
tained in daughter cells after every cell division (Ballestas et al.,
1999; Ueda et al., 2006), indicating that a strict genome mainte-
nance mechanism is working. In the KSHV-infected PEL cell lines,
 LANA associates with a condensed mitotic chromatin (Ballestas
et al., 1999; Cotter and Robertson, 1999; Tetsuka et al., 2004). It
was reported that MeCP2, a methyl CpG-binding protein, inter-
acts with the N-terminal of LANA and that DEK protein interacts
with the C-terminal of LANA. These two independent interac-
tions are involved in the tethering of LANA to chromosomes
(Krithivas et al., 2002). Various studies have reported the inter-
action between LANA and multiple cellular proteins associated
with chromatin.

Brd4, which is a member of the BET family that carries two bro-
omodomains and associates with mitotic chromosomes, interacts
with LANA on mitotic chromosomes (You et al., 2006). It has also
been reported that core histones such as H2A and H2B are essen-
tial for LANA N-terminal chromosome binding (Barbera et al.,
2006a,b). Furthermore, LANA interacts with Brd2/Ring3, which is
a member of the BET family of double bromodomain-containing
genes and contains two tandem bromodomains (Viejo-Borbolla
et al., 2005).

Xiao et al. (2010) reported that LANA is associated with
centromeres via the formation of complexes with Cenp-F and
Bub1, which are kinetochore-associated proteins. This suggests
that LANA preferentially interacts with kinetochore-associated
proteins and that its association is critical for segregation into
daughter cells. Though the interaction of LANA with kinetochore
factors might interfere with correct spindle formation, it suggests
that LANA should support viral genome segregation along with
 condensed chromatin.

A NuMA plays a critical role in the nuclear architecture in the
interphase. After nuclear envelope breakdown in mitosis, NuMA
is hyperphosphorylated by p34\textsuperscript{cdk2} and is distributed at spindle
poles, where it remains until the anaphase and plays an essential
role in tethering spindle microtubules to each pole (Merdes et al.,
1996; Gehmlich et al., 2004). Although NuMA drastically alters
the localization and functions in the interphase and M phase, bio-
chemical fractionation analysis shows that NuMA is localized in

![FIGURE 3 | A model for latent DNA replication of KSHV. LANA can associate with the nuclear matrix and can directly bind to the ori-P so that LANA can recruit the ori-P to the nuclear matrix. Cellular DNA replication machinery assembles to the nuclear matrix in a cell cycle-dependent manner, and is therefore available for viral DNA replication.](image-url)
the insoluble nuclear matrix fraction in both phases. A recent study demonstrated that the C-terminus of LANA was co-localized with NuMA during the interphase and that the knockdown of NuMA expression caused the disruption of genome segregation and TR-containing plasmid maintenance (Bhaumik et al., 2008) and thus, KSHV genome segregation is disrupted in the absence of NuMA. Through siRNA and knockdown strategies in mice, NuMA has been shown to be an essential protein for early embryogenesis and cellular proliferation (Harborth et al., 2001; Silk et al., 2009), and it is thus unclear how the interaction with LANA actually works for viral genome segregation.

In either case, LANA has the capability of associating with various cellular proteins so that KSHV can maintain the genome stably if the cells are divided into two daughter cells. Nuclear matrix proteins function as a scaffold of DNA replication, transcription, and repair during the interphase, and also play an essential role in the segregation of condensed chromosomes in mitosis. Condensed chromosomes include, for example, NuMA, which can behave as a component of a spindle pole during mitosis, or Cenp-F, which is a nuclear matrix protein during the interphase and is distributed to kinetochores in mitosis. Taken together, the previous and present results suggest that nuclear architectures such as the nuclear matrix have essential roles not only in DNA replication and transcription but also in genome segregation during mitosis (Figure 4).

CONCLUSION AND PERSPECTIVES FOR FUTURE ANALYSIS

The importance of nuclear architecture is increasingly recognized as important in various nuclear events, such as DNA replication, transcription, and DNA repair. It is well accepted that chromosomes are organized into distinct territories in the interphase. These distributions of chromosomes are closely related to the place for active or inactive transcription, the presence of DNA replication machinery, and the formation of higher-order structures of chromatin loops. DNA looping appears to be mediated by attachment to the nuclear matrix and thus achieves transcriptional control (Ostermeier et al., 2003). Other studies have suggested that the gene-rich chromosomes are frequently located in the nuclear interior. On the other hand, gene-poor chromosomes are located in the nuclear periphery (Tanabe et al., 2002; Reddy et al., 2008). A recent study shows that the transcriptional silencing might be accomplished by the binding of a specific promoter region to lamin type A (Lee et al., 2009). It is reported that replication foci at the middle to late S phase are also preferentially located toward the nuclear periphery, whereas early replication foci are located in the nuclear interior (Grasser et al., 2008) or throughout the nucleus (Izumi et al., 2004).

The spatial and temporal analyses using live-cell imaging revealed that replication forks are generated at the same origin and are closely associated during replication (Kitamura et al., 2006). Interestingly, two replication loci, located at the same distance from the origin, were in closer proximity when DNA replication took place at these loci, after which they moved apart from each other after replication. It is speculated that the replication factory anchors some region and that replicated DNA can move away from a replication factory immediately after DNA synthesis. This anchored region may be a nuclear scaffold such as the nuclear matrix.
The cellular environment is critical for viral survival, for which viruses make full use of the cellular machinery. The analysis of insoluble proteins, including nuclear matrix proteins, is difficult for the investigation of protein–protein and protein–DNA interactions in vitro, because the conditions of in vitro experiments always include soluble fractions. To overcome these problems, recent new approaches, such as genome-wide analysis using chromatin immunoprecipitation or live-cell imaging that reflects the in vivo environment, may be more powerful and accurate.

As for KSHV genome replication and maintenance, it would be first necessary to clarify how LANA is involved in both. Considering the real and actual cellular environments, we need to develop more powerful tools to know what the virus does in cells.

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