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Is KSHV lytic growth induced by a methylation-sensitive switch?

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Both latent and lytic growth of Kaposi's sarcoma-associated herpesvirus (KSHV or HHV-8) contribute to its pathogenesis. Expression of the immediate-early Lyta/ORF50 gene can single-handedly induce the lytic phase of growth in cells latently infected with KSHV. The recent demonstration that this promoter is regulated by methylation paves the way for further research to understand how the virus makes use of the host's cellular environment to control its life cycle.

Kaposi's sarcoma-associated herpesvirus (KSHV or HHV-8) is a γ-herpesvirus (γ-HV) of the Rhadinovirus family linked to Kaposi's sarcoma (KS), a tumour of endothelial cell origin, and at least two lympho-proliferative disorders, multicentric Castleman's disease (MCD) and primary effusion lymphoma (PEL)[1]. Like all herpesviruses, KSHV has both a latent and a lytic phase during its life cycle. Most KS tumour cells are latently infected and exhibit a restricted pattern of KSHV gene expression[2,3]. Some of these latent viral proteins have been shown to target two known tumour suppressor pathways, pRB/E2F and p53 (Refs 4–6). Although these observations implicate the latent viral proteins in KSHV oncogenesis, it is clear that lytic viral proteins are important for establishing tumours. This is supported by studies showing that antibodies to lytically expressed proteins are significantly elevated in the sera of patients developing KS (Ref. 7), and that treatment with gancyclovir, a drug targeting active herpesvirus replication, markedly reduces the incidence of KS development in HIV-1-infected individuals[8]. Lytic viral growth is obviously also vital for the transmission of the virus. Thus, there are both clinical and biological interests in studying the signals that bring about the switch from latent to lytic growth.

Induction of KSHV lytic phase by acetylation and demethylation

Cells harbouring KSHV can be forced to enter active, lytic replication by treatment with chemical inducers. These include 12-*O*-tetradecanoylphorbol-13-acetate (TPA), which induces histone acetyltransferases, and trichostatin A and sodium butyrate, which inhibit histone deacetylases. Deacetylation of histones in nucleosomes promotes the condensation of chromatin and thus transcriptional repression (Fig. 1a). Conversely, histone acetylation correlates with transcriptional activation[9]. These inducers of KSHV should favour histone acetylation and therefore relieve repression owing to condensed chromatin.

The assembly of repressed chromatin is also associated with the methylation of promoter sequences, and correlates with a decrease in their transcriptional activity. A recent study by Chen and colleagues demonstrated that 5-azacytidine, a DNA methyltransferase inhibitor, also reactivates the lytic programme of KSHV (Ref. 10). This effect is not unique to γ-HVs or other herpesviruses: 5-azacytidine reactivates Epstein–Barr virus (EBV), the closest human γ-HV relative of KSHV, as well as herpes simplex virus 2 (HSV2), an α-HV, from latency[11,12]. However, this observation does raise questions about how methylation influences the entry of KSHV into the lytic cycle.

KSHV and CpG suppression

Whereas acetylation occurs on the lysine residues of histones bound to DNA, methylation is a modification of DNA itself, and can thus have both genetic and epigenetic effects. Owing largely to the action of the methyltransferase Dnmt1, it is estimated that 3–4% of the cytosines in DNA, almost exclusively in the context of CpG dinucleotides, are methylated. Because of the increased likelihood, on an evolutionary timescale, of the deamination of 5′ methyl-cytosine giving rise to thymidine[13,14], the observed frequency of CpGs in the human genome

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is reduced by 80% relative to the expected statistical frequency, a phenomenon known as CpG suppression. This is considered a kind of 'molecular signature' that a genome has been subjected to a high degree of methylation. Within coding sequences, such transition events would clearly be mutagenic; however, methylation within and around promoter sequences can also cause epigenetic changes that lead to transcriptional silencing.

Although vastly under-represented, the CpG dinucleotide can be found in the 3.2 billion base pair human genome in the upstream regions of an estimated 29 000 genes in 'CpG islands'[15]. For transcription to proceed, CpGs in promoter sequences must be kept free of methylation (Fig. 1b). A family of highly conserved proteins, the MBD proteins, have been identified and are involved in the recognition of methyl-CpG and translating it into a functional state. This family includes MeCP2, which can bind a single methylated cytosine[16]. Although the precise mechanisms for preventing methylation at CpG islands are unknown, the involvement of *cis*-acting element(s) (e.g. transcription-factor-binding sites) has been suggested[17].

With approximately 70% of the CpG sites in the human genome methylated, it is clear that the cellular environment is predisposed towards methylation, and a herpesvirus infecting a host cell must contend with this environment. In fact, before the discovery of KSHV in 1994 (Ref. 18) the genomes of the known γ-HVs (including EBV and various murine, bovine and simian family members) were all shown to be CpG suppressed, suggesting they too have been subject to heavy methylation[19]. Surprisingly, KSHV exhibits no obvious genome-wide CpG suppression, a feature it shares with α- and β-herpesviruses[10,20]. However, closer inspection of individual KSHV latent and lytic gene promoters reveals that the upstream region of one immediate-early gene, the ORF50 gene, is CpG suppressed[10]. ORF50/Lyta (lytic transactivator) functions in the induction of lytic growth[21–23] and can single-handedly reactivate the entire lytic programme of KSHV, making its regulation by methylation a question of central importance in KSHV biology[24].

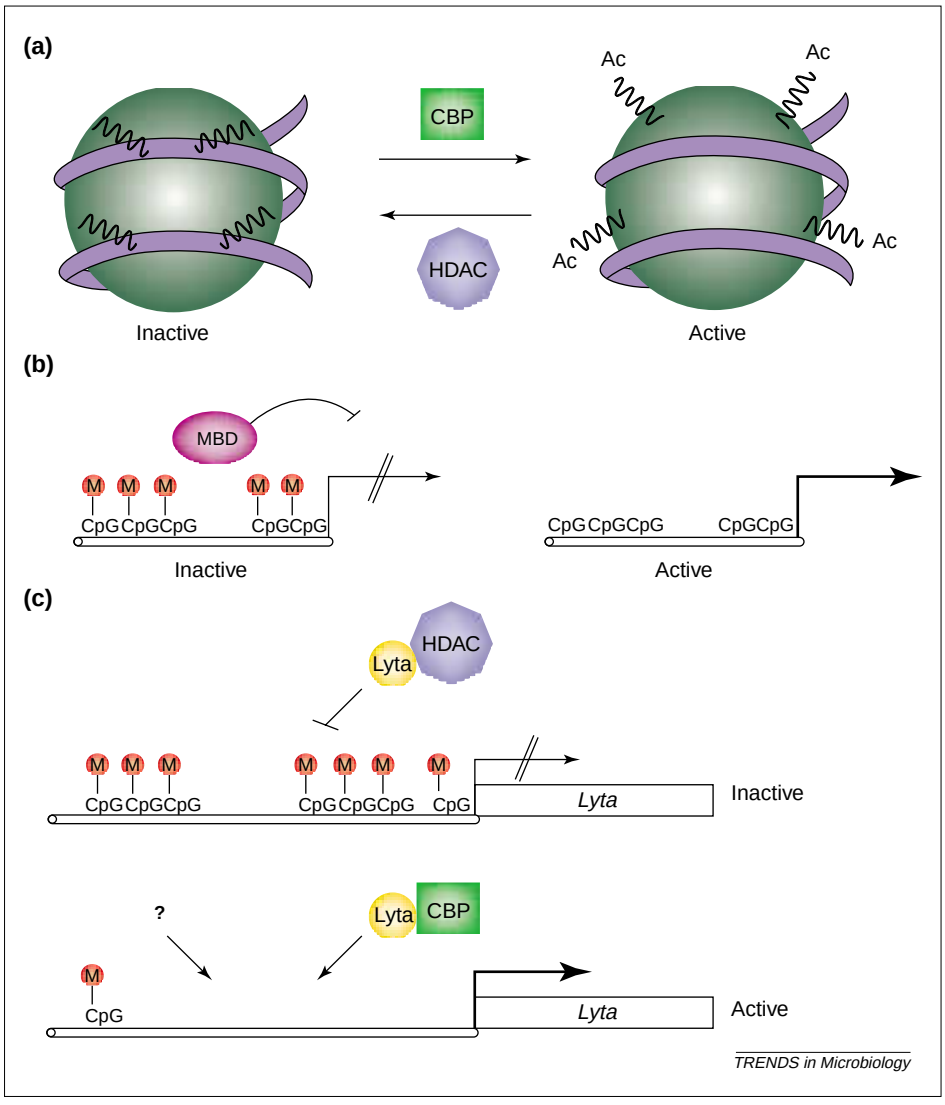

Fig. 1. (a) Acetylation of histones switches nucleosomal conformation to an open, active state, whereas deacetylation promotes a closed, inactive conformation. (b) Methylation on CpG dinucleotides is recognized by proteins with methyl-binding domains, which aid in establishing transcriptionally repressed chromatin. (c) The Lyta promoter is sensitive to methylation and its gene product, which controls the entry of KSHV into lytic growth, interacts with both HDAC and CBP. Abbreviations: CBP, CREB-binding protein; HDAC, histone deacetylase; KSHV, Kaposi's sarcoma-associated herpesvirus; MBD, methyl-binding domain.

The Lyta promoter: methylation

In their study[10], Chen and colleagues found that in BCBL-1, a PEL-derived cell line that is latently infected with KSHV, the Lyta promoter is methylated. This contrasted with the LNA-1 promoter, which was free from methylation, as assessed by Southern blotting and bisulfite genomic sequencing analysis. Treatment with TPA resulted in the progressive reversal of CpG methylation of the Lyta promoter over time. In fact, demethylation of the promoter began as little as one hour after TPA treatment and preceded Lyta transcriptional activity, which is required for its own induction, as well as some early lytic genes and late genes. This effect argues that demethylation of the Lyta promoter is an important first step towards lytic growth.

KSHV-infected biopsies were then analysed for the methylation status of the Lyta promoter and it was found that most CpG sites in the Lyta promoter were demethylated, comparable to the BCBL-1 cells reactivated by TPA treatment, which yielded up to 30% of cells in the lytic phase. This implies that these clinical samples were in the lytic phase owing to Lyta promoter demethylation and subsequent expression. However, KSHV-infected tissues have already been shown to be composed predominantly of latently infected cells, with only a small percentage of cells in the lytic phase[1,25]. Also, the methylation status of the Lyta

promoter and the expression pattern of lytic markers did not strictly correlate with each other. These aspects make these clinical results difficult to interpret and reconcile with previously published reports.

Chen and co-workers then established that the Lyta promoter activity was sensitive to *in vitro* methylation, and this spurred further inspection of the promoter itself. Their results indicated that the Lyta promoter was orientation dependent and that there was an element(s) between –587 and –348 that allowed activation to a high level. Intriguingly, there were two regions, one between –760 and –836 and a second just downstream of this element(s), between –315 and –255, that were hypermethylated and flanked the element(s) responsible for high induction of activity. The promoter also showed autoregulation, implying there might be a positive-feedback loop to enhance entry into the lytic phase.

Recently, the Lyta protein itself has also been shown to interact with both CREB-binding protein (CBP), a transcriptional co-activator with histone acetyltransferase activity, and with HDAC1, a histone deacetylase[26]. Both proteins can modulate its transcriptional activity, suggesting Lyta is sensitive to the level of acetylases and deacetylases in the cell (Fig. 1c). The CpG methylase Dnmt1, has recently been demonstrated to associate with complexes having deacetylase activity[27,28]. Although these findings strongly link methylation and deacetylation, it has proven difficult to determine which activity is responsible for initiating the change to a condensed state of chromatin, that is, methylated CpGs might indirectly recruit deacetylases, which allow condensed chromatin to be assembled or, alternatively, transcriptionally repressed chromatin might recruit methylases that enhance the silenced chromatin conformation.

With the identification of discrete elements, hyper-methylated regions and an acetylation-sensitive factor that controls transcriptional activation of the Lyta promoter, the stage is set for the further elucidation of the interplay among these components and characterization of the signals and mechanisms that control KSHV entry into the lytic phase. Of particular interest will be the characterization of the other immediate-early gene products of unknown function (e.g. ORF45, K8, K8.2 and K4.2) to determine whether they influence methylation and/or transcriptional activity from Lyta or its promoter. The study of viral interactions with, and exploitation of, the cellular machinery has often proved fruitful for understanding eukaryotic biology. In this way, the analysis of the control of KSHV entry into the lytic phase might provide an understanding of epigenetic control of transcription by the processes of methylation and deacetylation, and open up avenues for the development of novel antiviral agents.

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