PARP1 AND CTCF INTERACTION GUIDES EBV CHROMATIN REFOLDING TO PROMOTE VIRAL GENE EXPRESSION AND SUSTAIN CELL PROLIFERATION DURING LATENCY

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Epigenetics plays an essential role in regulating EBV gene expression during latency. We and others have previously reported that differences in the histone modifications and DNA methylation levels of the EBV genome exist across different latency types and changes in epigenetic modifications of the viral episome correlate with transcriptional changes of viral genes. Here we show that the viral genome's three-dimensional (3D) structure is an essential epigenetic mechanism that controls EBV latency. Using *in situ* HiC mapping and bioinformatic modeling, we generated the first complete 3D model of the EBV episome in different latency states from B cell lines (LCL and Mutu I cells) and epithelial cell lines (SNU719 and YCCEL-1 cells). We found that the different latent viral episome types displayed vastly distinct 3D viral genome structures, with EBV episome from LCL cells having more intragenomic contacts than episomes from Mutu I and both epithelial cells. By comparing the HiC-3D maps to RNA-seq data for EBV gene expression, we observed that the increases of chromatin loops across the viral genome correspond to more permissive viral gene expression latency programs. Since chromatin loop formation is regulated by the cellular factor CTCF and cohesin complex, we assessed by ChIp-seq the global biding of these factors across the EBV genome in both B and epithelial infected cells. We observed that while differences exist for CTCF and Cohesin binding between EBV episome types from B cells and epithelial cells, these differences alone cannot account for the distinct profiles of the EBV episome found via global HiC analysis. Indeed we found that across the EBV genome, the CTCF-mediated looping is controlled by the activity of PARP1, a NAD+-dependent chromatin-modifying enzyme. By HiC mapping, we observed that PARP inhibition leads to fewer total unique intragenomic interactions within the EBV episome, yet new chromatin loops distinct from the untreated episome are also formed. Our RNA-seq analysis confirmed that PARP1-induced 3D chromatin changes correlated with a deregulated expression of EBV genes. Using ChIP, we determined that CTCF and PARP1 form a complex on EBV chromatin that includes the metabolic enzyme NAMPT, a key enzyme for recycling NAD+ moieties, suggesting a role in NAD+ levels in regulating EBV latency. We determined by ChIP that inhibition of NAMPT affects CTCF and cohesin occupancy across the EBV episome altering viral chromatin composition and EBV gene expression in latently infected cells. Overall, our data demonstrate that the 3D structure of the EBV genome has an essential role in regulating EBV latent gene expression and that changes in EBV 3D chromatin are linked to nuclear metabolism through the CTCF/PARP1/Cohesin complex.