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Interaction of the genome maintenance proteins of oncogeneic herpesviruses with cellular chromatin

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ABSTRACT

The Epstein-Barr virus (EBV) nuclear antigen (EBNA)-1 is the only viral protein expressed in all virus-infected cells and EBV-associated malignancies. Similar to the genome maintenance proteins (GMPs) of other gamma-herpesviruses, EBNA1 binds to both viral and cellular DNA and controls the replication and transcription of the viral genome. EBNA1 expression affects cellular transcription but the mechanism and consequences are largely unknown. The work of this thesis aimed to investigate the interaction of EBNA1 with the host cell DNA and to understand how it may impact EBV oncogenesis. EBNA1 is a stable protein due to a Gly-Ala repeat (GAr) domain that acts as a portable inhibitor of proteasomal degradation. We found that, in the absence of the GAr, EBNA1 is rapidly degraded in the cytoplasm but remains long-lived in the nucleus. This correlates with anchoring to cellular chromatin via a bipartite Gly-Arg repeat (GRr) domain that resembles the AT-hook of High Mobility Group-A (HMGA) proteins. Grafting of the GRr to a soluble proteasomal substrate promotes detergent resistant binding to chromatin and inhibits degradation in spite of efficient ubiquitination. Thus the GAr and GRr may cooperatively regulate the stability and functions of EBNA1. This possibility was supported by experiments addressing the transcriptional effects of EBNA1. We found that the GRr is both necessary and sufficient for the capacity of EBNA1 to promote large-scale chromatin decondensation without the recruitment of ATP-dependent remodelers. This correlates with rapid diffusion, measured by fluorescence recovery after photobleaching (FRAP), and with displacement of linker histone H1. Similar to architectural transcription factors, EBNA1 promotes a broad remodelling of transcription involving both up- and down-regulation of a large number of genes. The similarity is further substantiated by the capacity of EBNA1 to regulate the Twist promoter, a known target of HMGA2 architectural factors. Using a set of deletion mutants and GFP-fusion reporters, we found that the two GRr subdomains cooperatively determine the mobility of EBNA1, while mobility is increased by the interposed GAr in a length-dependent manner. The GMPs encoded by herpesviruses belonging to the genera Lymphocryptovirus (LCV) and Rhadinovirus (RHV) share a relatively conserved viral DNA binding domain but differ in their cellular-chromatin targeting module. We found that all GMPs promote chromatin decondensation. However, while the AT-hook containing GMPs of LCVs are highly mobile, the GMPs of RHVs are virtually immobile or show a significantly reduced mobility. Only the RHV GMPs recruit the bromo- and extra terminal domain (BET) proteins BRD2 and BRD4 to the site of chromatin remodelling. Thus, differences in the mode of interaction with cellular chromatin may underlie different strategies for host cell reprogramming during latency. Collectively the findings described in this thesis highlights previously unrecognized properties of the interaction of EBNA1 with cellular chromatin by which the viral protein may reset cellular transcription during infection and prime the infected cells for malignant transformation.