AN EBV MUTANT DELETED FOR THE IE LOCUS REVEALS RTA TO BE THE DOMINANT EARLY GENE ACTIVATOR AND ZTA ACTS PRIMARILY AT ORILYT.

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Epstein-Barr Virus (EBV) requires both Rta and Zta to complete its lytic replication cycle. The specific roles played by Rta and Zta is challenging to dissect because they each induce the other's expression. To circumvent this, we constructed an EBV mutant deleted for the Immediate Early (IE) locus encoding Rta and Zta in the Akata strain BACmid (AkataΔRZ). We used RNA-seq to study the dependence of each lytic gene upon Rta and Zta in normal oral keratinocytes infected with AkataΔRZ, a highly physiologic model of EBV epithelial cell infection. As expected, Zta and Rta were both required for late gene expression. Surprisingly, Zta could only activate expression of the OriLyt transcripts (BHLF1 and LF3). In contrast, Rta activated transcription of all EBV early genes. Despite having no effect on its own, Zta could synergize with Rta to further activate some early genes. We observed Rta and Zta binding by ChIP assay at all early promoters examined but the extent of binding and degree of cooperativity did not predict co-activation nor result in increased levels of activating histone marks (H3K9ac and H3K27ac) or RNA polymerase II occupancy. Despite this, we show that Zta co-expression acts to increase transcription and did not appear to alter stability of lytic RNAs. Our results, in contrast those obtained using reporter gene assays, reveal that Rta is the dominant transactivator of early gene expression from the EBV genome. Zta functions primarily as an OriLyt binding protein and to co-activate Rta. It will be important to establish if this hierarchy is unique to epithelial cells or if, in the context of the intact genome, Zta plays a subordinate role to Rta in B lymphocytes as well.