USE OF CLONED EPSTEIN-BARR VIRUS DNA TO IDENTIFY GENES THAT DETERMINE THE FATE OF VIRAL INFECTION

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SUMMARY

Transcription of the Epstein-Barr virus (EBV) genome during different cell-virus interactions was analyzed to identify the loci containing genes which may be responsible for determining the fate of an EBV infection. Transcriptionally active regions of the genome were detected by hybridization of $^{32}$P-labeled cDNA (reverse-transcribed from infected-cell RNA) to the different cloned Bam H1 restriction endonuclease fragments of EBV DNA. Analysis of the transcription in restringently infected cells indicated that, in the absence of complete virus replication, transcription of the viral genome is limited to the W-Y-H region of the Bam H1 restriction map. During permissive or productive infection, transcription of approximately 90% of the viral DNA was detected. Analysis of the immediate-early transcription and transcription kinetics during permissive infection indicated that the Bam H1 M region is transcribed first following the onset of productive replication. Transcription of this region was not detected in restringently infected cells or during primary infection of adult human B lymphocytes. This suggests that expression of a gene(s) within the Bam H1 M region is required to initiate the productive cycle of virus replication. Suppression of this gene may therefore be a prerequisite to immortalization of B lymphocytes by EBV.

INTRODUCTION

Human or nonhuman-primate B lymphocytes which have been infected in vitro with Epstein-Barr virus (EBV) acquire the
ability to proliferate indefinitely in cell culture, a process referred to as growth transformation or immortalization (1-3). Most lymphoblastoid-cell lines which have been established following EBV infection are restrictive to virus replication. Cell lines have been established, however, in which 1 to 5% of the cells spontaneously produce EBV particles. These cell lines have been designated as permissively infected, although the majority of the cells remain nonpermissive to virus replication. Cell lines which are completely nonpermissive to EBV replication are referred to as latently or restringently infected.

During the immortalization process, the EBV genome becomes established within the host cell so that a relatively specific number of genomes is maintained within the cells of a given cell line (4-10). The DNA genome within the virion itself is a linear double-stranded molecule approximately $170 \times 10^3$ base pairs (bp) in length, with a molecular weight of about 110 megadaltons (Md) (11-13). However, within the immortalized lymphocyte the genome exists as circular plasmid DNA (14,15). Several reports have suggested that in some instances the EBV genome has integrated into host cell chromosomes (16-18).

Because the complete EBV genome is maintained and propagated within established lymphoblastoid cells, utilization of these cells as a source of virus and virus DNA has greatly benefited studies of genome structure and expression. Two such cell lines which have been extensively employed in these studies are B95-8 and P3HR-1 cells. A small percentage of these immortalized lymphocytes spontaneously produce either the B95-8 or the P3HR-1 strain of EBV, respectively. In addition, the B95-8 strain of EBV has the ability to immortalize human and nonhuman-primate B lymphocytes following infection in vitro, and is therefore considered a prototype strain of EBV. P3HR-1 virus, however, has lost its ability to immortalize lymphocytes. Structural analysis of the genomes of these two strains of EBV has revealed that a deletion of approximately $6.5 \times 10^3$ bp has occurred in the P3HR-1 genome (19,20). A hypothesis has been put forth that the DNA which has been deleted from the P3HR-1 genome encodes a gene product(s) that enables prototype strains of EBV to