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IDENTIFICATION OF THE EPSTEIN-BARR VIRUS NUCLEAR ANTIGEN 2 TRANSACTIVATION DOMAIN

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The Epstein-Barr virus nuclear antigen 2 (EBNA2) protein activates the expression of viral and cellular genes. A set of seven yeast GAL4-EBNA2 fusion proteins were constructed in order to identify the transactivation domain of EBNA2. These fusion proteins were tested for their ability to transactivate a target gene in Hela and BJAB cells. This analysis has demonstrated that the EBNA2 polyproline domain is dispensible for transactivation while the acidic carboxy terminus defined by amino acids 337-467 is essential. This result is consistent with the analysis of a variety of viral and cellular eucaryotic transactivators in which an acidic domain of the protein has been shown to be indispensible for function.

The Epstein-Barr virus (EBV) is a human lymphotrophic herpesvirus that causes infectious mononucleosis (1) and is associated with Burkitt's lymphoma and nasopharyngeal carcinoma (2). EBV infection of primate B cells results in a state of sustained cell proliferation or immortalization (3). EBNA2 is necessary for B cell proliferation since the EBV strain P3HR-1, which lacks the EBNA2 gene is unable to immortalize B cells (4). The insertion of a functional EBNA2 gene results in a transformation competent virus (5).

In order to identify those domains of EBNA2 that are required for transactivation, we have constructed a set of seven fusion proteins between EBNA2 and the DNA-binding domain of the yeast transcriptional activator protein GAL4 (6). By linking portions of the EBNA2 protein to the DNA-

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binding domain of GAL4 it is possible to evaluate the ability of various regions of the protein to activate the transcription of a reporter gene placed downstream of a GAL4 DNA-binding site. This type of analysis has been used to identify the transactivation domains of numerous proteins, including adenovirus E1A (7), herpes simplex virus VP16 (8), pseudorabies virus immediate early protein (9), p53 (10) and c-myb (11).

MATERIALS AND METHODS

Cell Lines The cell lines used in this project were the EBV-negative lymphoblastoid cell line BJAB (12) and the human epithelial cell line Hela (13).
Plasmids The plasmid pSG424 (14) (obtained from S. Fields, State University of New York at Stony Brook, NY) was modified by inserting a linker (AATTCGGGGTAACC) containing an ochre termination codon within a BstEII restriction site and renamed pSG424.Bst (Figure 1). The amino acid sequence proline-aspartate-phenylalanine links GAL4 and EBNA2 sequences and the carboxy terminus of the EBNA2 protein fragment contains an additional three amino acids (alanine-asparagine-serine) from the vector polylinker. Plasmids pSG4 and pSGVP served as positive controls (8). All GAL4-EBNA2 fusion constructs were derived from the EBNA2-A allele of the EBV M-ABA strain (15). The GAL4-EBNA2 fusion construct HcA is an HincII-AluI fragment containing amino acids 19-467 of EBNA2-A, HcR is an HincII-RsaI fragment containing amino acids 19-145, and B1 is a BamHI fragment containing amino acids 109-480 of EBNA2-A. Fusion constructs 1, 2, and 4 were created from a set of linker insertion mutants of EBNA2 generated by the method of Stone et al (16). Fusion protein 3 was generated by removing an SphI fragment from the EBNA2 open reading frame (Figure 2).
Transfection Hela cells were transfected by the calcium phosphate technique (17) using 5 μg of effector plasmid, 5 μg of reporter plasmid pGAL4-MMTV-CAT (7), and 5 μg of pCMV-βGAL (18). BJAB cells were transfected by electroporation (19). All transfections were repeated a total of four times for both Hela and BJAB cell lines.
β-Galactosidase and Chloramphenicol Acetyl Transferase Assay Cell lysates were normalized for transfection efficiency by assaying for β-galactosidase activity (18). The chloramphenicol acetyltransferase activity of each cell lysate was determined as described (20).

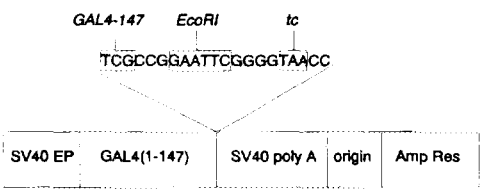

Figure 1. GAL4-EBNA2 Expression Vector pSG424.Bst. SV40 EP - Simian Virus 40 early promoter, GAL4(1-147) - region encoding the first 147 amino acids of GAL4, SV40 poly A - Simian Virus 40 polyadenylation signal, origin - bacterial origin of replication, Amp Res - gene encoding resistance to ampicillin, GAL4-147 - codon 147 of GAL4, EcoRI - restriction enzyme cloning site, tc - termination codon for GAL4-EBNA2 fusion proteins.

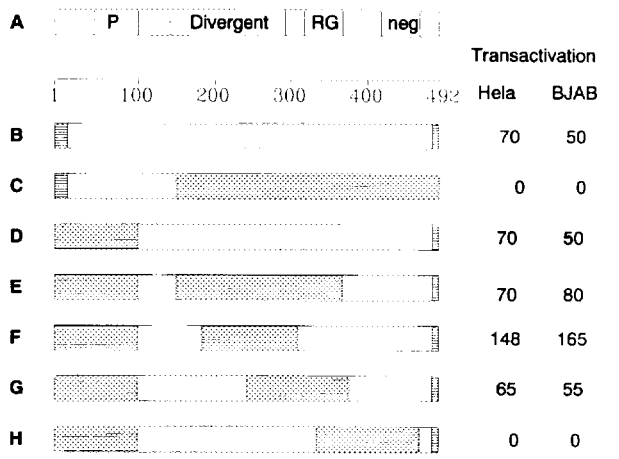

Figure 2. GAL4-EBNA2 Fusion Constructs. (A) Wild-type EBNA2. P - polyproline domain, Divergent - amino acid domain differing between EBNA2 type A and B alleles, RG - arginine-glycine repeat, neg - negatively charged carboxy terminus. The scale indicates amino acid number. GAL4-EBNA2 fusion proteins. (B) HcA, (C) HcR, (D) B1, (E) 1, (F) 2, (G) 3, (H) 4. The rectangles containing horizontal lines indicate regions of EBNA2 that are deleted in the GAL4-EBNA2 fusion proteins. Relative transactivation is normalized to wild-type GAL4 activity which is defined as 100.

RESULTS

The results of the CAT assays for the Hela and BJAB transfections are presented in figures 3 and 4, respectively. From an anaylsis of these results it is evident that Fusion proteins HcA, B1, 1, 2, and 3 increased the expression of the CAT gene in both Hela and BJAB cells. As fusion protein B1 lacks the

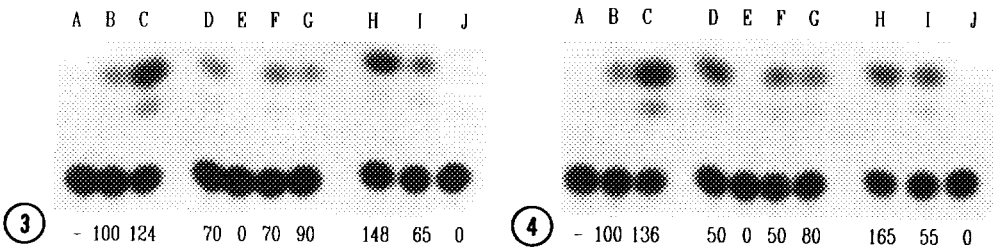

Figure 3. Chloramphenicol acetyltransferase (CAT) assay of transfected Hela cells. In each case the reporter plasmid pGAL4-MMTV-CAT was transfected with effector plasmids : (A) pSG424.Bst, (B) pSG4, (C) pSGVP, (D) HcA, (E) HcR, (F) B1, (G) 1, (H) 2, (I) 3, (J) 4. Relative transactivation is normalized to wild-type GAL4 activity which is defined as 100 (B).

Figure 4. Chloramphenicol acetyltransferase (CAT) assay of transfected BJAB cells. In each case the reporter plasmid pGAL4-MMTV-CAT was transfected with effector plasmids : (A) pSG424.Bst, (B) pSG4, (C) pSGVP, (D) HcA, (E) HcR, (F) B1, (G) 1, (H) 2, (I) 3, (J) 4. Relative transactivation is normalized to wild-type GAL4 activity which is defined as 100 (B).

polyproline region of EBNA2, we conclude that these amino acids are not necessary for transactivation in this system. Furthermore, the divergent region and arginine-glycine repeat are dispensible for transactivation as shown by fusion protein 1. Only fusion proteins HcR and 4 failed to transactivate. These two fusion proteins differ from the other constructs in that HcR and 4 lack the acidic carboxy terminus of EBNA2. This suggests that the acidic domain of EBNA2 as defined by amino acids 337 to 467 is necessary for transactivation.

The relative level of transactivation was comparable for all fusion proteins in both the Hela and BJAB cell backgrounds. Fusion protein 2 produced transactivation levels 2 to 3 times higher than the other GAL4-EBNA2 fusion proteins and GAL4-VP16 which is a very strong transactivator. Fusion protein 2 differs from the other fusion proteins in two respects. Fusion protein 2 possesses a deletion within the divergent region of EBNA2 and retains the arginine-glycine repeat domain. The divergent region may contain an inhibitory domain that prevents the fusion proteins HcA and B1 from achieving full activity. Furthermore the arginine-glycine repeat which has been deleted from fusion proteins 1 and 3 may be necessary for transactivation. The net positive charge of the arginine-glycine repeat may allow the domain to interact with DNA or possibly with a component of the transcription apparatus.

DISCUSSION

Our data are consistent with the interpretation that the acidic carboxy terminus of EBNA2 is necessary for transactivation. This conclusion is drawn from the observation that fusion constructs HcR and 4, both of which lack the acidic carboxy terminus, are unable to transactivate. This result is consistent for both Hela and BJAB cells. Only those fusion proteins that retain the acidic carboxy terminus of EBNA2 are able to transactivate the target promoter. The acidic nature of the EBNA2 transactivation domain is consistant with other transactivator domains that have been identified by the GAL4 assay system. The

acidic carboxy terminus has also been shown to be essential for transformation by intact EBNA2 in recombinant EBV (21).

The data we have presented is similar to the results of a study of EBNA2 transactivation that also employed a set of GAL4-EBNA2 fusion proteins (22). This study analyzed a set of amino terminal deletion mutants of EBNA2 linked to the GAL4 DNA-binding domain whereas in the present study we have examined terminal and internal deletion constructs. In the work by Cohen and Kieff, a 14 amino acid sequence (amino acids 449-462) was found to be the minimal sequence required for transactivation.

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