MAPPING OF TRANSCRIPTIONAL REGULATORY DOMAINS OF THE IMMEDIATE-EARLY PROTEIN OF PSEUDORABIES VIRUS

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The immediate-early protein (IE180) of pseudorabies virus (PrV) is a multifunctional protein required for the *trans*-activation of early and late gene transcription and for the autoregulation of its own transcription. To map these functional regions on the IE180 molecule, deletion- and nonsense- mutated genes of the IE gene were constructed and transiently expressed. Transcriptional regulatory domains of the truncated IE180 products expressed from the mutated genes were analyzed by the chloramphenicol acetyltransferase (CAT) assay. Mutated genes were cotransfected with reporter plasmids containing the SV40 early or the PrV IE promoters linked to the CAT gene in Vero cells.

The CAT assays in Vero cells transfected with the SV40–CAT and the PrV IE gene plasmids demonstrated *trans*-activation of the SV40 early promoter by IE180. In the CAT assays using a set of the mutated genes, IE180 and a 3'deletion mutated gene product lacking 23 amino acids increased CAT expression under the control of the SV40 early promoter 3.7-fold and 1.5-fold, respectively. The other did not increase CAT expression. These results indicate that most regions of the IE180 molecule are required for the *trans*–activation to the SV40 early promoter and the 617 amino-terminal amino acids or the 23 carboxy-terminal amino acids in IE180 are critical for the function. In the CAT assays using the PrV IE–CAT plasmid, the IE gene product reduced approximately 70% of the CAT expression under the control of its own promoter. The 3'deletion mutant gene products lacking 260 and 23 amino acids reduced 68% and 50% of the CAT expression, respectively. These results indicate that 1230 amino-terminal amino acids of IE180 are required for the autoregulation of the PrV IE gene.

In the presence of the SV40 early or the PrV IE enhancer, transcriptional regulation of IE180 was examined. The *trans*-activation of IE180 to the SV40 early promoter was not observed in the presence of the SV40 early enhancer. On the other hand, the autoregulation of IE180 to the PrV IE promoter was observed in the presence of the PrV IE enhancer.