The Ecdysteroid UDP-Glucosyltransferase Gene Promoter from Autographa californica Multicapsid Nucleopolyhedrovirus

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The ecdysteroid UDP-glucosyltransferase (egt) gene promoter fragments of different lengths were generated from the genomic DNA of the Autographa californica multicapsid nucleopolyhedrovirus (AcMNPV) by PCR. After being purified and enzymatic digestion, they were cloned into the pGEM-3Z vector for construction of reporter plasmids pAcegt542luc, pAcegt309-luc and pAcegt159-luc with the luciferase gene driven by the AcMNPV egt promoter. The results of transient expression in the Spodoptera frugiperda cell line-21 (Sf21) showed that the transcriptional activity of the AcMNPV egt promoter required the transactivation of viral factor(s). The expression of luciferase gene driven by the AcMNPV egt promoter was first detected at 24 h post infection. The egt promoter from the Bombyx mori nucleopolyhedrovirus (BmNPV), closely related to AcMNPV, revealed similar properties to that of the AcMNPV egt promoter. When BmNPV homologous region 3 was subcloned downstream the luciferase gene, the luciferase activity of the reporter plasmid was enhanced by over 1000-fold, but the property of the promoter was not changed. As a substrate of ecdysteroid UDP-glucosyltransferase (EGT), foreign insect ecdysone showed negative effects on egt promoter transcriptional activity. Ecdysone of 1.0–2.0 ug/ml significantly down-regulated the promoter activity. Promoter activities of different lengths showed that an AcMNPV egt promoter fragment of 159 bp has the basal transcriptional activity but it was almost abolished only about 0.2% of that of 309 bp and 542 bp, respectively, and the nucleotide sequence from - 159 to - 309 nt upstream the translation initiation site includes the main cis-acting elements interacting with viral factors.

Key words: Baculovirus, Ecdysteroid UDP-Glucosyltransferase Gene, Promoter