蒐尋對活化因子 pu 具激發作用的桿狀病毒基因

Identification of baculovirus genes that can activate the function of an activator pu

中文摘要

桿狀病毒系統常被用來表現外源蛋白，為了要增加蛋白質的表現量及品質，利用人類巨細胞病毒的 immediate-early 啟動子：CMV 微量啟動子作為產生外源蛋白的啟動子，此 CMV 微量啟動子為一早期啟動子，且在昆蟲細胞中可表現出大量且較不易降解之蛋白質。但是 CMV 微量啟動子的表現需要促進子 pu 序列，且 pu 序列的功能也需要桿狀病毒的共同感染才能發揮作用。而桿狀病毒感染會造成昆蟲細胞的裂解且會改變細胞生理，因此，為了避免此種蛋白質裂解情況，建立一個不需病毒共同感染的蛋白質表現系統是非常有用的。為了建立這個系統，將全部 130 kb 的桿狀病毒基因進行搜尋發現：桿狀病毒基因 orf 147，orf 151，orf 153 及 hr 促進子對 pu 序列是否能有所表現是相當重要的。這結果對建立昆蟲細胞非病毒感染之蛋白質表現系統是相當有利的，所表現出的蛋白質之品質與數量均呈現出不錯的結果。此外，此發現可能也可以提供其他表現系統大量表現外源蛋白之用。

英文摘要

Baculovirus expression vector system is one of the best systems for recombinant protein production. However, the infection of the virus results cell lysis and changes the cellular physiology. This increases the difficulty for protein recovery and decreases the quality of the proteins produced. It would be very useful if a virus free system could be established for the production of foreign proteins. In order to improve the quality of the protein produced, a minimal CMV promoter was previously tested and found could be used for strong foreign protein expression without protein degradation. The expression of minimal CMV promoter requires an activator pu and virus coinfection. In order to establish a virus free system for strong foreign protein expression, viral genes responsible to the activation of pu should be identified. A cosmid library was constructed first to cover the entire 130 kb genome of the baculovirus. These cosmids were used for the primary search of the viral genes responsible for pu stimulation. After several steps of search from 30 kb cosmids to 10 and 5 kb plasmids, the entire viral genome was screened and found that a set of genes containing orf 147, orf 151, orf 153, and hr enhancer are required for the strong stimulation of pu sequence. With the combination of these factors, a virus free system was established for the strong expression of foreign proteins. This virus free system expressed foreign proteins much stronger than that expressed by the recombinant

virus.
Thus, these results were not only very informative for basic research in the understanding of the mode of promoter activation in the baculovirus, it would be very useful to establish a virus free system for high yield and high quality protein expression system in insect cells. This discovery, for the first time, also makes it possible to establish a strong protein expression system in other organisms using an expression cassette from baculovirus.