應用專一於昆蟲之四環黴素誘導表現系統於生物農藥的發展

Utilization of the insect-specific tetracycline-inducible expression system for the development of biopesticide

計劃編號：NSC 89-2626-B-041-001

執行期限：89 **年** 8 **月** 1 **日至** 90 **年** 7 **月** 31 **日**

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中文摘要

在先前研究裏，將 tet-off **系統應用於昆蟲細胞中獲得** 30 **倍的誘導率，然而在哺乳類細胞中可達到** 1000 **倍的誘導率，因此在本計劃中** tet-off **系統被改造為專一於昆蟲的系統，在此改造中** pTRE **的** mini-CMV **起動子被** HZ-1 **病毒之** pag 1 **基因的起動子取代，經此改造後誘導率可提昇至超過** 1000 **倍，所以此改造之設計相當成功。**

關鍵詞：Tet-off system、pag 1 **基因、**luciferase **基因、**pTRE

Abstract

In previous studies, 30 fold of induction rate could be observed when tet-off system was applied in the sf9 cells. However, over 1000 fold of induction rate can be measured when tet-off system utilized in the mammalian cells. Therefore, in this project tet-off system was modified to be insect cell-specific. The mini-CMV promoter of pTRE of tet-off system was replaced by the promoter of pag 1 gene of HZ-1 virus. Over 1000 fold of induction rate could be observed when the insect-specific tet-off system was used in sf9 cells. The result of this project was very successful.

Key words：Tet-off system、pag 1 gene、luciferase gene、pTRE

Cause and goal

Engineered baculoviruses especially AcMNPV harboring toxic genes have been developed for biopesticide to shorten the long killing time of wild type baculovirus. The recombinant viruses containing the constitutively expressed toxin genes are not suitable to be used as the biopesticides due to the difficulty of mass production. Therefore, the regulatory gene expression systems allowing tight and specific regulation of toxin gene expression are important for the development of engineered biopesticides. Tetracycline inducible expression system (Tet-off system) has been successfully employed in mammalian cell cultures.

Over 1,000 fold of induction can be observed when the tet-off system is employed in the transient-transfected mammalian cells. However, in our previous studies we detected only 30 fold of induction when the tet-off system was employed in the sf21 cells (1). In order to increase the induction fold of tet-off system in the insect cell, the residual basal activity has to be reduced and a strong insect-specific promoter may be used in the tet-off system. Lee *et. al.* (1998) (2) and Chen *et. al.* (2000) (3) demonstrated that the promoter (-90/+29 region) of pag1 gene of insect-specific HZ-1

virus shows the strong activity in the insect cells and can be significantly repressed by the –312/ –90 region of the pag 1 promoter.

In this project, two insect-specific TRE promoters were constructed in which the pag –90/+29 fragment replacing the miniCMV promoter and the pag-312/-90 was upstream of pag promoter. In the presence of tetracycline, tetracycline will prevent the tTA from binding to TRE and the promoter activity of –90/+29 fragment will be inhibited by pag-312/-90. In the absence of tetracycline, multiple tTAs bind to TRE through the tetR domains and may abolish the repression of pag-312/-90 to activate the –90/+29 region of pag1 gene. In this way, the basal activity will be decreased significantly and high expression of the gene of interest is produced by the pag 1 promoter.

Results and Discussion

The construction of insect-specific TRE promoters

Two insect-specific TRE promoters were constructed. The pag –312/-90-TRE-pag –90/+29 promoter contains the pag –312/-90 fragment upstream of TRE and the pag –90/+29 fragment replacing the mini-CMV promoter of pTRELuc (Fig. 1). The TRE-pag –312/+29 promoter contains the pag –312/+29 fragment replacing the mini-CMV promoter (Fig. 2). The pTRELuc contains a luciferase gene under the control of mini-CMV promoter.

In order to replace the mini-CMV promoter of pTRELuc with pag –90/+29, the *Kpn*I/*BamH*I-digested pag –90/+29 PCR fragment was ligated with the *Kpn*I/*BamH*I-linearized pTRELuc to replace the mini-CMV promoter and result in pTRE/pag –90/+29Luc (Fig. 1). To construct pag –312/-90-TRE-pag–90/+29 promoter, the *Xho*I-digested pag –312/-90 PCR fragment amplified from the Hz-1 viral DNA was cloned upstream of TRE to result in the p(pag –312/-90)/TRE/pag –90/+29Luc (Fig. 1). To construct TRE-pag –312/+29 promoter, the pag –312/+29 PCR fragment was ligated with the *BamH*I/*Kpn*I-restricted pTRELuc downstream of TRE to replace the mini-CMV promoter and result in pTRE/pag –312/+29Luc (Fig. 2).

The regulation of pag –312/-90-TRE-pag–90/+29 promoter by tetracycline

In order to assess the control of pag –312/-90-TRE-pag –90/+29 promoter by the tetracycline (Tc), the p(pag –312/-90)/TRE/pag –90/+29Luc was cotransfected into sf9 cells with ptTA in the presence or absence of Tc. As indicated in Fig. 3, the luciferase activity was 183-fold higher in the absence of Tc than in the presence of 1 μg Tc /ml medium, suggesting that the pag –312/-90-TRE-pag–90/+29 promoter could be regulated by Tc. However, in the absence of ptTA and Tc the pag –312/-90-TRE-pag –90/+29 promoter alone could express the comparable luciferase activity (Fig. 3, column C). This result was required to be elucidated. Consistent with the previous results, the luciferase activity of pTRELuc containing mini-CMV promoter was low in the sf9 cells (Fig. 3, columns F, G and H). The above cotransfection experiment was performed three times. The activation folds for three experiments were 183, 276 and 25

fold respectively. It was likely that the sf9 cells were not healthy when the experiment with the lowest activation fold was performed.

The regulation of TRE-pag –312/+29 promoter by tetracycline

In order to assess the control of TRE-pag –312/+29 promoter by Tc, the pTRE/pag –312/+29Luc was cotransfected into sf9 cells with ptTA in the presence or absence of Tc. As demonstrated in Fig. 4, the luciferase activity was 2276-fold higher in the absence of Tc than in the presence of 1 μg Tc /ml medium, suggesting that the TRE-pag–312/+29 promoter could be regulated by Tc. The activity and activation fold of TRE-pag –312/+29 promoter was much higher than those of pag –312/-90 / TRE / pag –90/+29 promoter. Like pag –312 /-90/TRE/pag –90/+29 promoter, in the absence of ptTA and Tc the TRE-pag –312/+29 promoter alone could express the comparable luciferase activity (Fig. 4, column C). The above cotransfection experiment was performed two times. The activation folds for two experiments were 2276 and 867 fold respectively. It was likely that the sf9 cells were not healthy enough when the experiment with the lower activation fold was performed.

Effect of Tc on pag –312/-90-TRE-pag –90

/+29 promoter in the absence of ptTA

In the previous result, the pag –312/-90-TRE-pag –90/+29 promoter alone could express comparable luciferase activity. Maybe some protein(s) in sf9 cells can function like tTA protein to activate the TRE/-90/+29 promoter and can be regulated by Tc. In order to test our hypothesis, the luciferase activity expressed by the pag –312/-90-TRE-pag –90/+29 promoter was measured in the presence of Tc without ptTA present. Results demonstrated that the luciferase activity expressed by the pag –312/-90-TRE-pag –90/+29 promoter alone was reduced 160 fold by Tc (Fig. 5, column A and B), suggesting some protein present in the sf9 cells may act like tTA protein. This phenomenon was required to be elucidated.

Self evaluation

Over 1000 fold of induction rate could be observed when the insect-specific tet-off system was used in sf9 cells. This system is worthwhile to be studied for the application in the industry.

Reference

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GATA elements associated with cellular factors for its regulatory effect. *Journal of General Virology*. 82, 313-320.

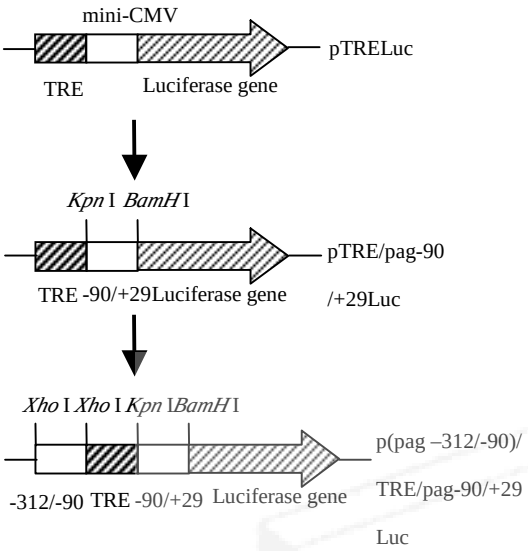

Figure 1. The pag-312/-90-TRE-pag-90/+29

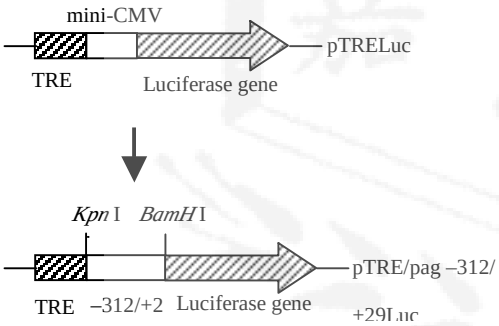

Figure 2. The TRE-pag-312/+29 promoter

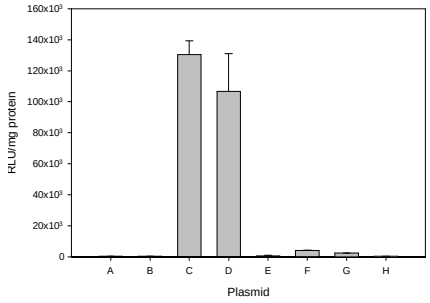

Figure 3. The regulation of pag-312/-90 -TRE pag-90/+29 promoter by Tc

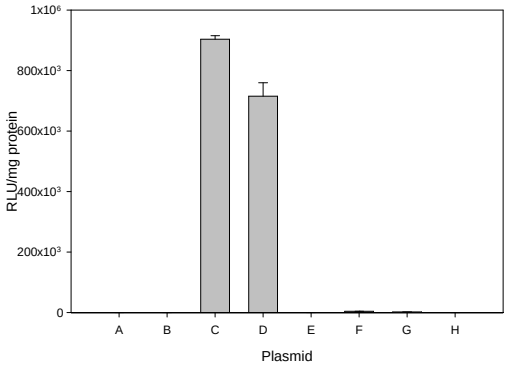

Figure 4. The regulation of TRE-pag-312/ +29 promoter by Tc

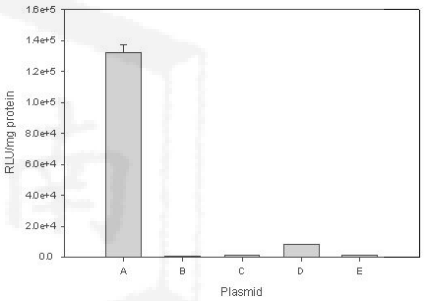

Figure 5. The effect of Tc on the p-312/ -90-TRE-pag-90/+29Promoter without pTtA