應用改造之四環黴素誘導表現系統於生物農藥之開發

Utilization of the modified tetracycline-inducible expression system for the development of biopesticide

計劃編號：NSC 89-2626-B-041-006
執行期限：90 年 8 月 1 日至 91 年 7 月 31 日

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中文摘要

在先前研究裏，將改造過的 tet-off 系統應用於昆蟲細胞中獲得 1,000 倍的誘導率，然而 tet-off 系統在哺乳類細胞中利用永久轉染方法可高達 10,000 倍的誘導率。因此在本計劃中建立 tet-off 系統之永久感染昆蟲細胞株，經過篩選之後，得到三個具有 tTA 活性的細胞株，此三個 Tet-off 系統細胞株僅能分別達到 17、6 及 7 倍的四環黴素誘導率。

關鍵詞：Tet-off system、pag 1 基因、、pTRE

Abstract

In previous studies, 1,000 fold of induction rate could be observed when insect-specific modified tet-off system was applied in the sf9 cells with transient transfection. However, over 10,000 fold of induction rate can be measured when tet-off system utilized in the mammalian cells stable transfection. Therefore, in this project Tet-off stable transfected sf9 cells were established. After G418 and tTA activity screening, three stable clones were found which had high tTA expression. However, these three clones just had 17-, 6- and 7-fold tetracycline induction fold.

Key words : Tet-off system、pag 1 gene、stable transfection

Cause and goal

The elucidation of gene function in the eukaryotic cells is very important for the molecular biology studies. However, it is very difficult to dissect the functions of the individual genes in the complex genetic environment like eukaryotic cells. The systems for mediating the gene under study on/off may facilitate the analysis of gene function. Among the eucaryotic cells, the insect cells, especially sf9 and sf21 cells are usually used as the model system to characterize the gene regulation and more importantly to express the proteins. However, no any inducible systems have been successfully applied in the insect cells. The *Drosaphila* hsp70 promoter system is one of few examples. This system works poorly due to the high basal activity produced without heat shock treatment. In addition, the prolonged heat shock treatment that is necessary for induction give rise to ectopic expression concomitant with the repression of endogenous proteins. Therefore, it is very difficult to distinguish the responses evoked by ectopic expression or by the induced gene. *Drosaphila* metallothionein promoter system is another example. High concentration of metals is required for the induction and the activity of this promoter is relatively low.

The most successful inducible system used in the mammalian cells is the Tet-off

system. In this system, tTA is a fusion protein of VP16 activation domain (AD) of herpes simplex virus and wild type tetracycline repressor (tetR) which recognizes the tet operator (1 and 2). pTet-off is a tTA expression plasmid while pTRE plasmid contains a tetracyline-responsive element (TRE) composed of seven copies of tet operators upstream of human cytomegalovirus immediate early (IE) mini-promoter ($P_{miniCMV}$) which lacks the enhancer region and is required to be activated by the AD domain of tTA. The gene of interest will be cloned under the control of $P_{miniCMV}$. In the absence of tetracycline, multiple tTAs bind to TRE through the tetR domains and using the AD domains activate the transcription of pTRE plasmid. In contrast, tetracycline will prevent the tTA from binding to TRE in the presence of tetracycline because tTA has the higher affinity for tetracycline than for TRE. In this way the gene can be turned on/off by the tetracycline.

In the previous study (NSC 89-2626-B -041-001), we investigated the fold induction of two kinds of insect-specific modified-pTREs by Tc in the sf9 cells by the transient transfection. 276-fold induction of luciferase activity was observed with the modified-pTRE containing the promoter region (pag –90/+29 region) of Hz-1 virus pag 1 gene replacing the mini-CMV promoter and the pag –312/-90 region upstream of TRE (p(pag-312/-90)-TRE-pag-90/+29Luc). 2276 -fold induction of luciferase activity was detected with the modified-pTRE containing the pag –312/+29 region replacing the mini-CMV promoter of pTRE (pTRE-pag –312/+29Luc). However, most studies on the mammalian Tet-off system are performed with the stable transfection system and over 10,000-fold induction can be observed with the double-stable mammalian cell line containing the tTA expression plasmid and Tc-responsive plasmid.

In this project, we established a pTet-off stably transfected sf9 cell line to observe if the fold induction comparable to that of the mammalian cell line.

Results and Discussion

Establishment of pTet-off stably transfected sf9 cell

After G418 screening of pTet-off transiently transfected sf9 cells, 40 G418-resistant clones were established. Then 40 resistant clones were screened with the transient transfection of pTRE-pag –312/+29 Luc for tTA activity. As results shown in Fig.1-5. Compared to sf9 transient transfected with pTRE-pag–312/+29Luc, 4-, 2.1- and 2.1-fold of luciferase activities were observed respectively in clones 32, 33 and 36 (Figures 4 and 5). To determined if the tTA was expressed in the stable clones, tTA cDNA was amplified from total RNA isolated from clones 32, 33 and 36. As results shown in figure 6, clone 32 had the highest tTA expression and tTA also was expressed in clones 33 and 36. The RT-PCR result was consistent with the luciferase activity of transient transfection.

Evaluation of tetracycline-inducible effect in pTet-off stably transfected sf9 cells

To characterize the Tc-inducible effect

in stable clones, the clone 32, 33 and 36 were transient transfected with pTRE-pag–312/ +29Luc in the presence of Tc. The luciferase activities were reduced 17-, 6- and 7- fold in transient transfected clones 32, 33 and 36 respectively in the presence of Tc (Figure 7).

Self evaluation

The Tc induction fold was not expected to be high. It is possible that pag-312/+29 promoter was too strong and therefore was not regulated by tTA. We suggested that the basal promoter region of pag1 gene (pag-69/+29) could be considered to be used in the Tet system.

Reference

1. Gossen, M., and Bujard, H. (1992). Tight control of gene expression of in mammalian cells by tetracycline-responsive promoters. Proc. Natl. Acad. Sci. 89: 5547-5551.
2. Gossen, M., Freundlieb, S., Bender, G., Muller, G., Hillen, W., and Bujard, H. (1995). Transcriptional activation by tetracycline in mammalian cells. Science 268: 1766-1769.

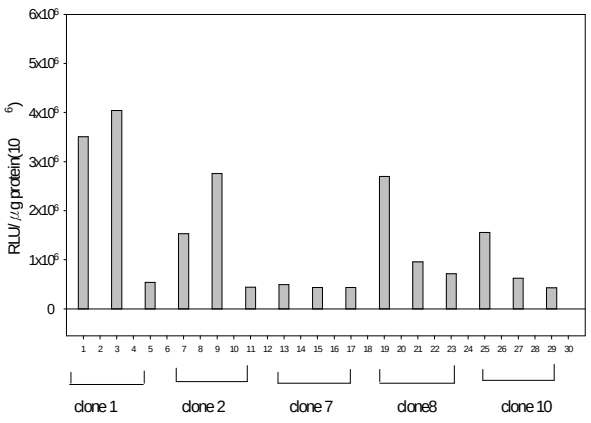

Figure 1. Screening of the pTetoff stable clones. Column 1, clone 1 transfected with pTRE-pag–312/+29Luc; column 3, clone 1/pTRE-pag–312/+29Luc plus 2 μg/ml tetracycline; column 5, clone 1; columns 7, 9 and 11 same as in columns 1, 3 and 5 except that clone 2 was evaluated in the transfection experiment ; columns13, 15 and 17, clone 7 was evaluated; column 19, 21 and 23, clone 8; columns 25, 27 and 29, clone 10.

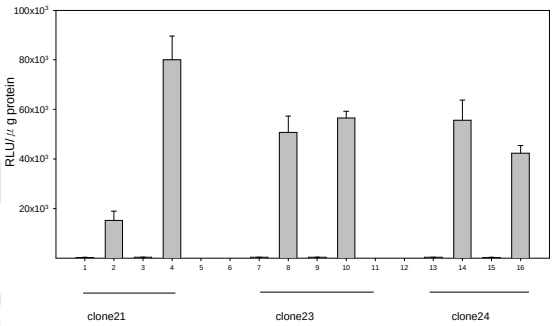

Figure 2. Screening of the pTetoff stable clones. Column 1, sf9; column 2, sf9 transfected with pTRE-pag–312/+29Luc; column 3, clone 21; column 4, clone 21 with pTRE-pag–312/+29Luc; columns 7-10, the legends were same as in columns 1-4 except that clone 23 was investigated ; columns 13-16, clone 24 .

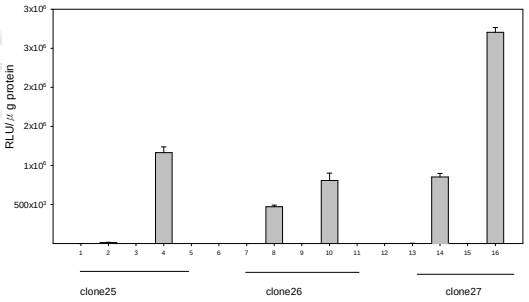

Figure 3. Screening of the pTetoff stable clones. Column 1, clone25; column 2, clone 25 with pTRE-pag–312/+29Luc; column 3, sf9; column 4 transfected with pTRE-pag–312/+29Luc; columns 7-10, the legends were same as in columns 1-4 except that clone 26 was investigated ; columns 13-16, clone 27.

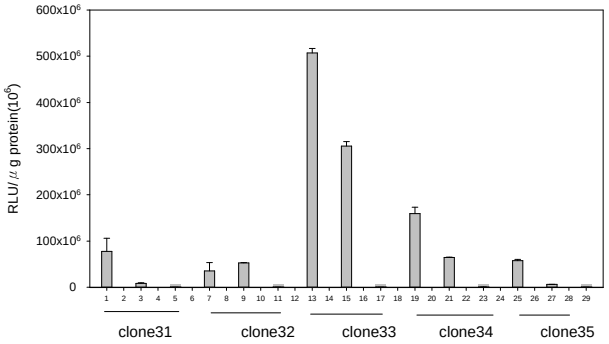

Figure 4. Screening of the pTetoff stable clones. Column 1, clone 31 transfected with pTRE-pag–312/+29Luc; column 3, clone 1 with pTRE-pag–312/+29Luc plus 2 μg/ml Tc; columns 7 and 9, same as in columns 1 and 3 except that clone 32 was evaluated in the transfection experiments ; columns 13 and 15, clone 33 was evaluated; column 19 and 21, clone 34 ; columns 25 and 27, clone 35.

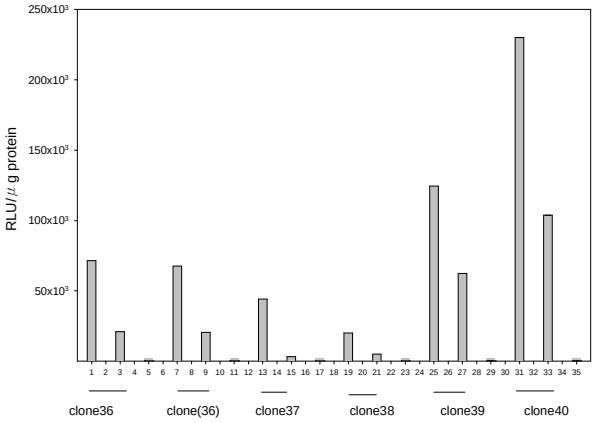

Figure 5. Screening of the pTetoff stable clones. Column 1, clone 36 transfected with pTRE-pag–312/+29Luc; column 3, clone 36/pTRE-pag–312/+29Luc plus 2 μg/ml tetracycline; columns 7 and 9, same as in columns 1 and 3 except that clone (36) was evaluated in the transfection experiments ; columns 13 and 15, clone 37 was evaluated; column 19 and 21, clone 38 ;columns 25 and 27, clone 39; column 31 and 33, clone 40.

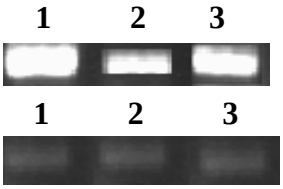

Figure 6. (A) The tTA RT-PCR results of clones 32, 33 and 36. Lane 1, clone 32; lane 2, clone 33; lane 3, clone 36. (B) The actin RT-PCR results of clone 32, 33 and 36. Lane 1, clone 32; lane 2, clone 33; lane 3, clone 36.

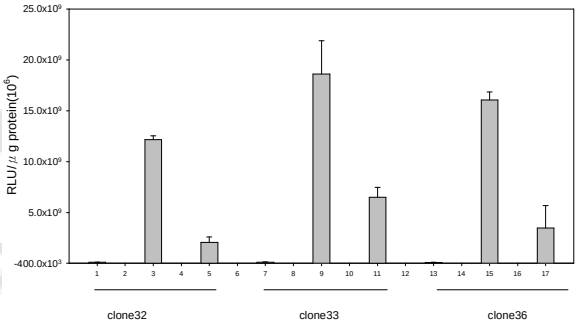

Figure 7. The Tc-inducible effect on pTetoff stable clones. Column 1, clone 32; column 3, clone 32 with pTRE-pag–312/+29Luc; column 5, clone 32 with pTRE-pag–312/+29Luc plus Tc; column 7, 9 and 11, the legends were the same as in columns 1, 3 and 5 except that clone 33 was investigated; columns 13, 15 and 17, clone 36.

A.