製藥與生物技術國家型計畫

89 年度第二次徵求獲通過計畫－第二年成果報告

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計畫中文名稱　建構 Ecdysone 誘導式基因表現系以研究 DNA 微矩陣鑑識之基因的策略

計畫英文名稱　A strategy of generating ecdysone inducible expression system for studying the function of genes identified by DNA microarray

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90年度專題研究計畫成果報告內容

一、計畫緣起。　二, 計畫目的。　三, 執行進度：1. 研究成果。

Introduction, Backgrounds, and Summary of Accomplished Works

A major challenge for biomedical researchers in the post-genomic era is to identify which genes, among thousands of candidates found by methods like library substraction, differential display, and DNA microarray, are truly important in disease pathogenesis and genuine molecular targets for drug development. Although the above molecular genetic tools are powerful to pick up potential candidates in term of their differential expression levels under different physiological or pathological conditions, whether they are directly linked to pathogenesis usually awaits further functional assays in a cellular or organismal context. One of the most powerful ways of studying gene functions is to analyze the phenotypic changes when the activity of the gene of interest is varied in a precisely controled manner. Fine-tuning of gene activity is of particular importance for metabolic pathway or developmental switching when quantitative parameters usually define ultimately different outcomes. Furthermore, if the genes of interest are potentially toxic while overexpressed, such as those involved in the cell death signaling, there would be tremendous difficulties in generating useful stable cell clones. Regulated gene expression system is thus an appealing solution to these problems. However, the earlier version of regulatory expression systems, such as those exploiting heat shock, heavy metal, and steroid hormone promoters, are flawed because of the leakiness of these promoter elements in controling the gene expression and also because the inducers (heat, heavy metals, and steroid hormone) by themselves would activate endogenous genes to confound the experimental results[1]. Therefore, extensive application of these systems was limited. Recently, several newer version of regulatory expression systems became available, such as tetracycline repressible system, ecdysone inducible system, and Lac operon inducible system [2,3,4] (please refer also to **appendix 1 and 2**). These expression systems share in common the regulatability of the expression of exogenous genes, and offer several benefits over the traditional expression system based on constitutively active promoter: (1) Inducible expression of exogenous genes, and therefore minimize the possibility of selecting secondary mutation during the

course of selecting stable clones. (2) Tightly regulated expression of exogenous genes allows physiological range of protein expression which prevents cellular toxicity potentially elicited by uncontroled overexpression of genes with pleiotropic effects. (3) A perfectly matched control cell population when the exogenous gene is either not induced or suppressed. Although regulatory expression systems are ideal tools for examining the effects of expressing the genes on cell behaviors, it is not straightforward to create inducible expression systems in every cell line scientists usually use.

In order to exercise the powerfulness of these gene expression systems, one must first transplant the regulatory units which function well in either prokaryotic or invertebral system into higher animal cells. This process usually involves intensive cloning, cell culturing, testing procedures, and is very time-consuming. Because of the effort required for establishing the regulatory expression system, large-scale screening could not be easily performed and this makes a successful outcome could not be surely guaranteed. Although it is desirable to use inducible gene expression system for studying gene functions, it takes lengthy effort to generate inducible gene expression system by conventional strategy. We had established a simple and efficient scheme to generate inducible gene expression system without going through the tedious single clone selection procedure.

The initial step, transfecting cell lines with the plasmids expressing the regulatory proteins (tTA or rtTA, in tetracycline repressible system, and heterodimeric ecdysone receptor in ecdysone inducible system), is not different between the traditional way and the new strategy described here (please refer to **appendix 3**). A plasmid encoding the regulatory protein (pTet-ON or pTet-OFF, for tetracycline inducible system, and pVgRXR for ecdysone inducible system) is stably transfected into the cell lines. These regulatory plasmids are driven by constitutively active viral promoter to ensure adequate expression of the regulatory proteins. After transfection, only cells uptaking the plasmids would express the drug selectable markers (G418 resistance for pTet-ON/OFF in tetracycline repressible systemor, and zeocin resistance for pVgRXR in

case of ecdysone inducible system) and survive the drug selection. Only a few of those cells uptaking the plasmids would eventually integrate the exogenous DNA into their chromosomes; most of the uptaken plasmids are in episomal forms, and would therefore be cleared out of the cells during subsequent cell division. Finally, a population of candidate clones is enriched during the selection process, and only a certain portion of these stable clones could express the regulatory proteins in a desirable way. **In the conventional way**, the candidate clones are individually expanded and tested for their inducibility, usually by luciferase reporter assay under inducible and non-inducible condition, so as to further pick up the clones possessing the optimal regulated characteristics; namely, maximal reporter activity under induced condition while minimal reporter activity under non-induced condition (please refer to **appendix 4A**). This is the critical step in generating a regulatory expression system which demands most time and energy.

In contrast to the traditional way of selecting optimal responder using individual cloning and luciferase reporter assay, **we had proposed a new strategy**. Instead of isolating and maintaining the surviving candidate clones separately, we simply trypsinize those surviving candidates after drug selection (G418 for tetracycline regulatory system, and zeocin ecdysone inducible system) and pool them all together awaiting further selection by FACS. A reporter plasmid, pIND-GFP is constructed by cloning GFP under the control of tetracycline or ecdysone responsive element. Then, this plasmid is transfected into the population of candidate clones, which are pooled during the drug selection procedure. The responsive clones could be distinguished from the non-responsive clones simply by the brightness of GFP fluorescence under inducible condition (please refer to **appendix 4B**), and could readily be sorted by FACS (**Positive Selection**). After several rounds of positive selection, the candidate population of cells would again be transfected with the same GFP reporter plasmid, but this time those cells with leaky expression characteristic would be sorted out by FACS under non-inducible condition (**Negative Selection**) (please refer to **appendix 5**).

While we had success in using this strategy to set ecdysone inducible expression system in MDCK and HEK 293 cell lines(please refer to **appendix 6 and appendix 7**), our initial attempt to apply similar strategy set up tetracycline regulatory system was hampered. We found the TRE promoter, which is composed by a minimal CMV promoter and seven tandem repeats of Tet operator sequence, is very active when transiently transfected into cell line. Even without the presence of regulatory proteins (tTA in Tet-OFF system or rtTA in Tet-ON system), this promoter could be activated very efficiently. The explanation for such a lack of regulated property is because the promoter, when transiently transfected into cells, is in an episomal status, and there is no so-called chromosome inhibitory effect to govern the regulatability of the promoter. Therefore, the minimal CMV promoter in the TRE promoter could then attract transcription machinery to install gene expression even when tTA or rtTA is not binding to the promoter. To circumvent this problem, we decide to complement the Tet-ON system with a tetracycline regulated gene silencing system, as suggested by the inventor of Tet system, Dr. Hermann Bujard. $tTS^{Kid\text{-}1}$ is a fusion protein constructed by fusing TetR (tetracycline regulated repressor), nuclear localization signal (NLS) of SV40 Tag, and a 61-amino acid KRAB domain of human kidney protein Kid-1.

This fusion protein could be combined with rtTA (regulatory protein for Tet-ON system) to provide ideal inducibility of TRE promoter for gene expression[5] (please refer to **appendix 8**). Our preliminary data showed it indeed worked nicely (please refer to **appendix 9**). In order to ensure both rtTA and tTS expressed at adequate level simultaneously in the same cell line, we make use of two different IRESs (internal ribosomal entry sites) to construct a plasmid expressing a tri-cistronic transcript.

The logical basis behind the design of this construct is as follows:
The two IRESs allow cap-independent translation initiation from downstream AUG start codon. The second IRES is weaker than the first IRES in term of initiating protein translation which makes puromycin selection favorable for a higher expression of genes placed upstream of this attenuated IRES. Therefore, clones surviving puromycin selection would very likely express high level of both tTS and rtTA. The expression of rtTA is coupled to the expression of tTS by a competent IRES, and this helps reduce quite a lot of the background expression of the reporter under non-inducible condition, while maintains the highest possible activation level after induction **(appendix 10)**. We then sequentially transfected this pVgRXR and tricistronic construct into HEK293 cells, and selected stable clones expressing both regulators for Tet-ON and ecdysone inducible systems by the GFP-based strategy I just mentioned. We named this particular clone as **HEK293.3** which is capable of both tetracycline and ecdysone inducible expression. We would refer to this stable line later in this proposal.

While we were able to make both Ecdysone and Tetracycline inducible systems work independently, we also tried to explore the possibility of combining tetracycline regulatory expressiong system and ecdysone inducible system for studying complicated biological events. We are interested in a special form of detachment-induced apoptosis, anoikis[6]. Epithelial cells, endothelial cells, muscle cells, and perhaps many other cell types undergo programed cell death when they are deprived of the contact with extracellular matrix, which presumably interact through integrin complex to provide important cues for cellular survival and differentiation. Anoikis is regulated by many factors, including cell-substratum, cell-cell interaction, and cytoskeleton organization. Rho family small GTPases (RhoA/Rac1/Cdc42) are major players controling all these processes[7]. Thus, it is very likely Rho family proteins regulate detachment-induced anoikis. In order to demonstrate how a dual-regulatory cell line could be used for studying anoikis, we took advantage of an MDCK cell line already expressing dominant negative Rac1 (Rac1N17) under tetracycline repressible system[8-12], and superimposed

this cell line with ecdysone inducible system to express constitutively active Cdc42 (Cdc42V12). We demonstrated in this pilot study that the ecdysone inducible system could be combined with tetracycline regulated system to study the interplay of two closely related small GTPases, Cdc42 and Rac1, and their effects on detachment induced apoptosis. In this dual regulated expression system, mutant Cdc42 and Rac1 genes were inducibly expressed under the control of ecdysone and tetracycline respectively **(appendix 11A)**. Their effects on anoikis could then be compared directly in one single cell line in an experimentally defined way **(appendix 11B)**.

In summary, we are now able to exploit an efficient FACS-based scheme in setting up ecdysone and tetracycline inducible expression systems in MDCK and HEK293 cells **(appendix 12)**. We are confident similar success could be expected when the strategy is applied to other cell lines. In the meantime, we have also generated a cell line expressing two closely related small GTPases independently under ecdsone inducible and tetracycline repressible systems, and demonstrated indeed a dual regulatory cell expression system could be used in dissecting the signal controling detachment induced apoptosis. We wish in the future this system could be used in conjunction with DNA microarray technique to explore the functions of genes identified by DNA microarray method. and further used to pick up potentially interacting genes.

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Mol. Biol. Cell, 1999, 10: 4369-4384.

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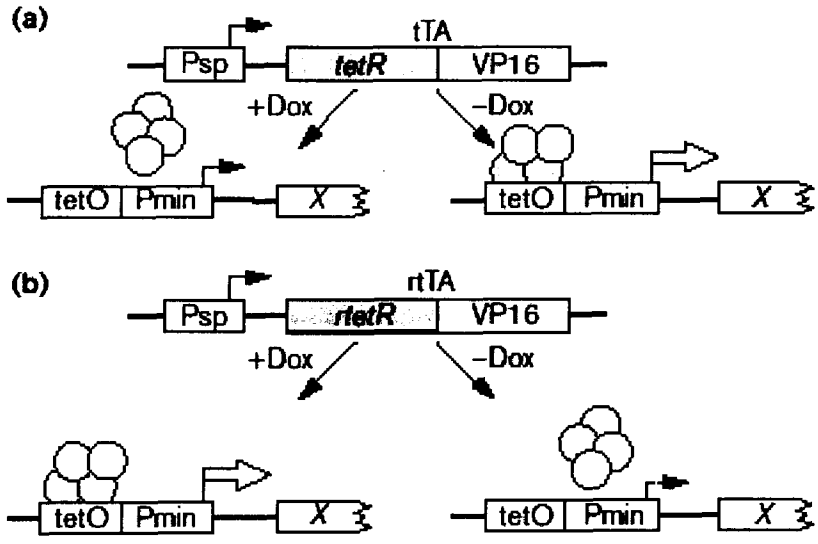
How Tetracyline repressible system works

The Tet Expression System is based on two regulatory elements derived from the tetracycline-resistance operon of the E. coli Tn10 transposon—the tetracycline repressor protein (TetR) and the tetracycline operator sequence (tetO) to which TetR binds. The gene to be expressed is cloned into the pTRE "response" plasmid which features a compound promoter consisting of seven copies of tetO (TRE), and a minimal CMV promoter.

The "response" plasmid is controled by another "regulator" plasmid which is a fusion of the wild-type TetR to the VP16 activation domain (AD) of herpes simplex virus; so called tTA standing for tetracycline regulated transactivator. tTA binds the tetO sequences which brings the VP16 activation domain into close proximity with the TRE and thereby activates transcription in the absence of Tc. Thus, as Tc is added to the culture medium, transcription is turned off in a dose-dependent manner.

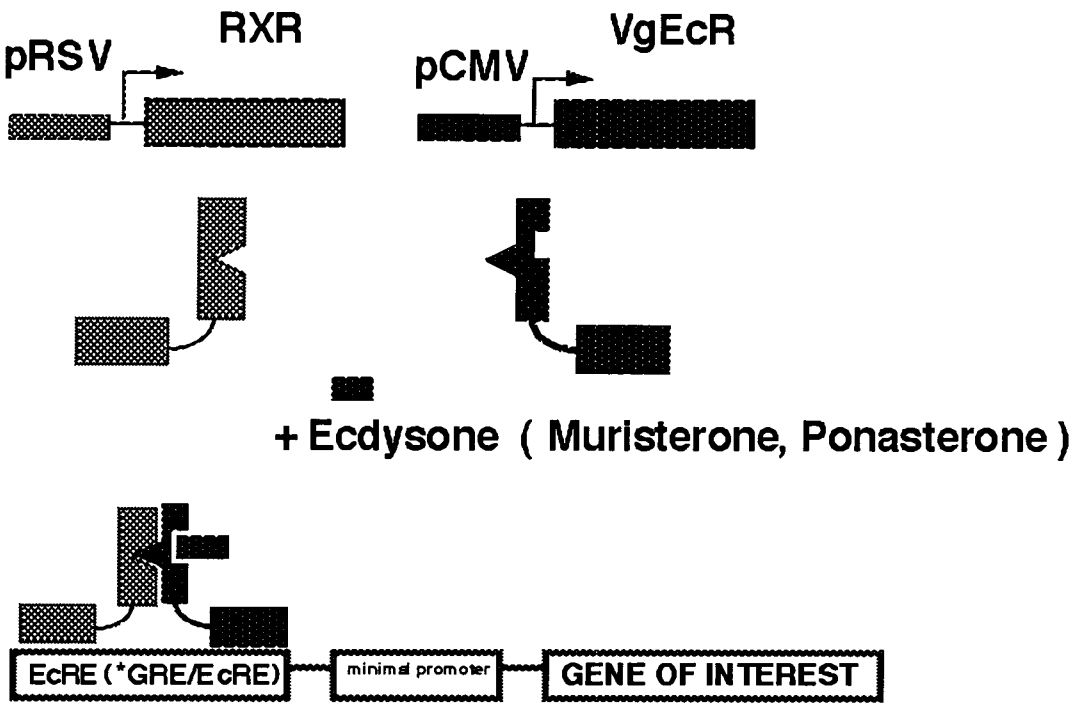
The Tet-On System is based on the "reverse" TetR (rTetR), which differs from TetR by four amino acid changes. When fused to the VP16 AD, rTetR creates a "reverse" tTA (rtTA) that activates transcription in the presence of Dox.



Appendix 1

How Ecdysone inducible system works

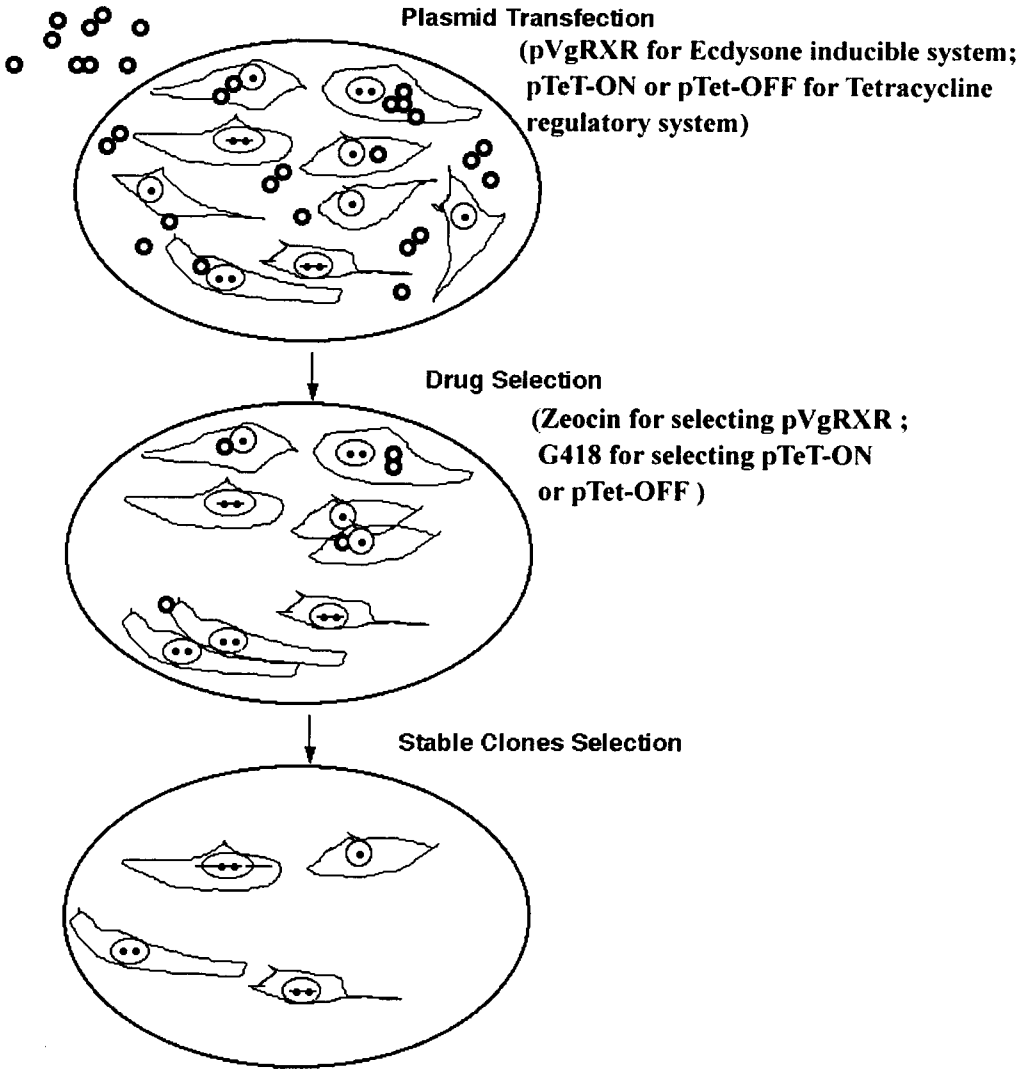
Ecdysone regulates Drosophila metamorphosis by binding to its receptor and transactivate genes which are responsible for the molting process. Ecdysone inducible expression system takes advantage of the fact that ecdysone heterodimeric receptor would dimerize, translocate from cytosol into nucleus, and transactivate genes located downstream to ecdysone responsive element only in the presence of its cognate ligand, ecdysone. Therefore, a mammalian cell line is firstly made to express ecdysone receptor (commercial vector expressing the heterodimeric receptor, RXR and VgEcR under RSV and CMV promoters is available). Then, your gene of interest is cloned into an expression plasmid (called pIND by the manufacturer) and placed downstream to the ecdysone responsive promoter. Finally, once the plasmid carrying the gene of interest is transfected into the cell line already expressing the heterodimeric ecdysone receptor, the expression of the particular gene would be tightly regulated by the presence or absence of ecdysone in the culture medium.



Appendix 2

Transfecting plasmids expressing regulatory proteins into cell lines

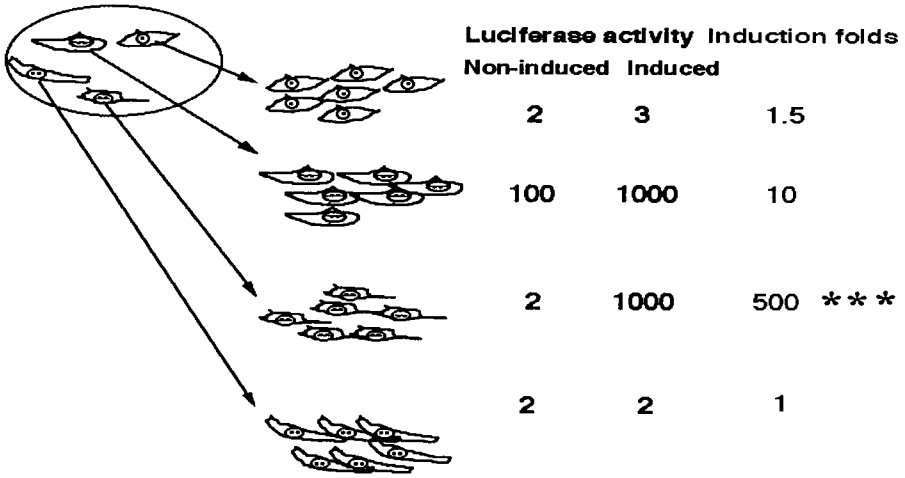
During the first step of establishing cell lines possessing regulatory expression characteristics, plasmids expressing regulatory proteins (tetracycline-regulated trans-activator, tTA, in tetracycline repressible system, and heterodimeric ecdysone receptor in ecdysone inducible system) are transfected into the cell lines. Because the plasmids also carry drug selectable markers, the cells uptaking the plasmids could be selected in the presence of cytotoxic drugs.



Appendix 3

Tradition Way of Making Ecdysone Inducible Cell Line

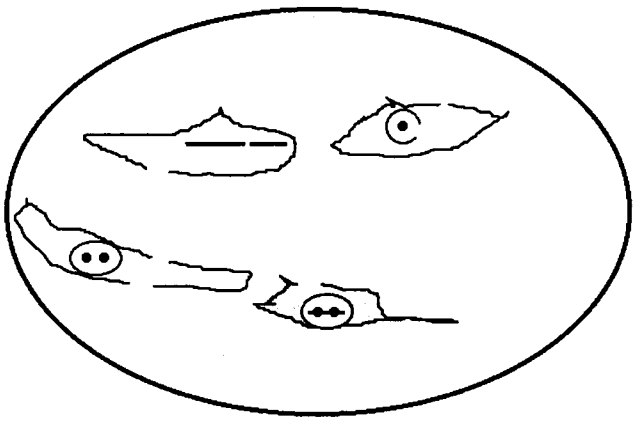
After selecting the candidate clones by drug resistance, the individual clones are expanded and tested for their inducibility, usually by luciferase reporter assay under inducible and non-inducible condition.



Appendix 4A

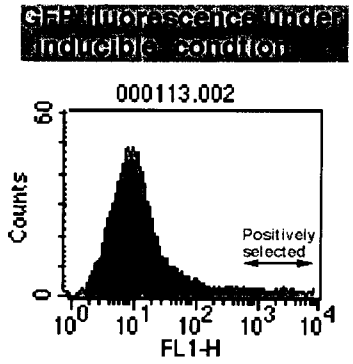
Selecting Ecdysone Inducible Cell Line by Mass Cell Culture and GFP Reporter Plasmid

A reporter plasmid is constructed by cloning GFP under the control of ecdysone responsive element. Then, this plasmid is transfected into the pooled population of candidate clones. The responsive clones could be distinguished from the non-responsive clones by the brightness of GFP fluorescence under inducible condition, and could be sorted by FACS (**Positive Selection**).

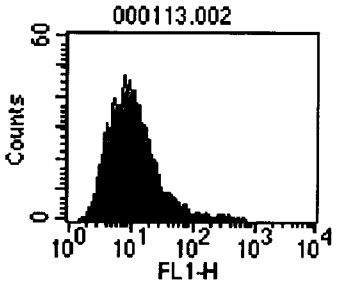


Appendix 4B

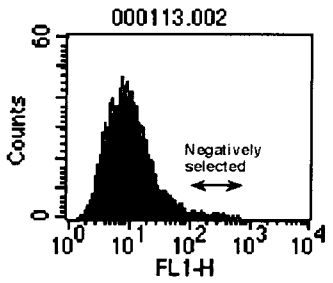
How the positive and negative FACS selection work



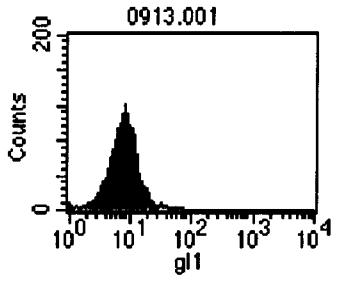
GFP fluorescence under non-inducible condtion



GFP fluorescence under non-inducible condtion



GFP fluorescence under untransfected condtion



For each round of **positive selection**, a GFP reporter plasmid is transiently transfected into the candidate population pool, and the transfected cell population was cultured in the absence or presence of ecdysone analoque, Ponasterone. Since the GFP reporter is constructed right after an ecdysone regulatory promoter, the brightness of GFP fluorescence could be used as an indication of the inducibility of the cell clones.

After substracting the clones which either do not express the GFP reporter activity or express suboptimal reporter activity in a leaky way under non-inducible condition, the true responders would be collected by FACSorting.

After several rounds of positive selection, the candidate population of cells would again be transiently transfected with the same GFP reporter plasmid.

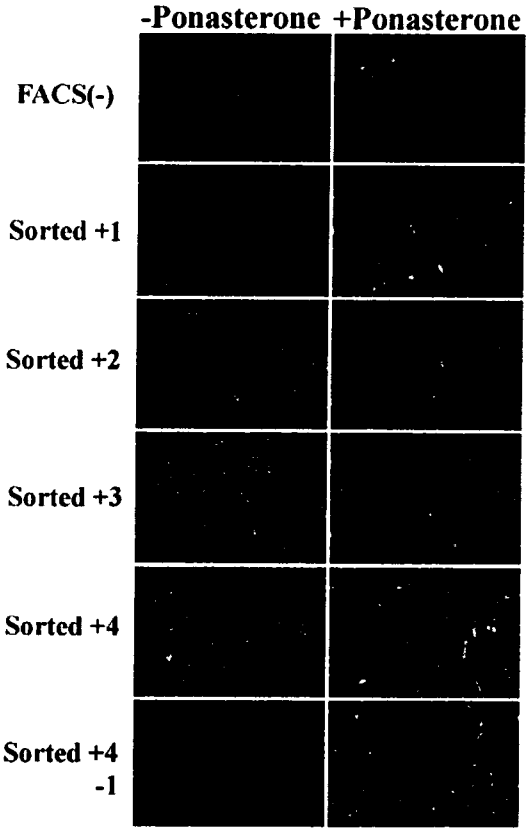
Those cells with leaky expression characteristic would be sorted out by FACS under non-inducible condition (**Negative Selection**), after comparing the fluorescence profile of non-transfected parental cells.

After repeating positive and negative selections, cells possessing maximal inducibility and minimal leakiness could be enriched.

Appendix 5

Compositive images showing the fluorescence of transiently transfected GFP reporter plasmid in sequentially sorted population of MDCK cells

MDCK cells were stably transfected with pVgRXR (Invitrogen Co.) which expressed heterodimeric ecdysone receptors (**FACS(-)**). This population of MDCK cells were then sequentially transfected and positively selected by the green fluorescence expressed by a reporter plasmid construct (pIND-EGFP). pIND-EGFP expresses EGFP under ecdysone regulated promoter element. After being selected under inducible condition by FACSorting, cells were left in the absence of inducer; i.e., ecdysone or its analogue, ponasterone so that the fluoresence would disappear as the transiently transfected pIND-EGFP plasmids were purged from the cells. After the cells ceased emitting green fluorescence, they were sent for another round of positive selection after being transfected again with the same reporter plasmid, pIND-EGFP, in the presence of ponasterone.

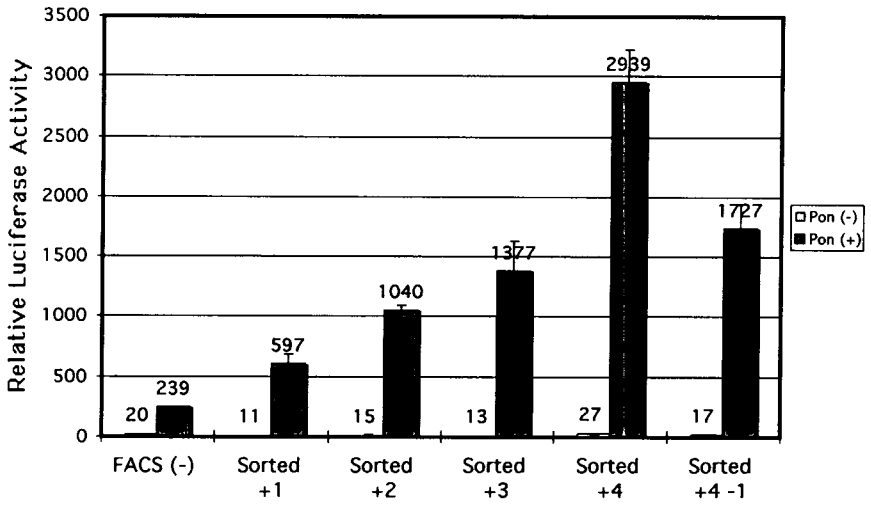


Totally, there were four such rounds of positive selection. Cells were saved after each round and named according to the number of positive selection they had gone through (**Sorted +1, +2, +3,** and **+4**). As shown in the picture, the ecdysone analogue ponasterone could increasingly induce green fluorescence in pIND-EGFP transfected cells after sequential enrichment; however, there were also increasing population of green fluorescent cells under non-inducible condition. Therefore, a round of negative selection was performed by transfecting the Sorted + 4 cells with pIND-EGFP again and sorting out those leakily expressing cells under non-inducible condition. As shown in that pair of images at the bottom (**Sorted +4-1**), one round of negative selection could clean out the leakily expressing cells. Please note the total cell number in each image was revealed by Hoechst 33342 nuclear staining. The above composite image was made by transiently transfecting each collection of cells after every selection procedure at the same time with pIND-EGFP and splitting the transfected cells into two halves; one in the absence, and the other in the presence of inducer. This composite simulates the condition of the cells before and after each round of selection.

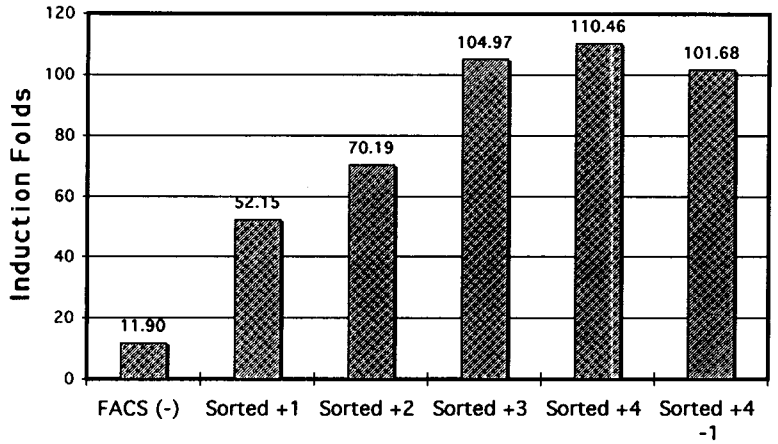
Appendix 5

Quantifying ecdysone inducibility in MDCK cells

The above populations of MDCK cells were examined by luciferase activity assay for quantitative analysis of the inducibility of each collection of cells after positive and negative selection. Cells were transiently transfected with a reporter plasmid expressing luciferase under the control of ecdysone responsive element (pIND-Luc). Compatible with the previous fluorescence images, positive selection increases the expressed luciferase activity of selected cells.



Although negative selection seemed to decrease the absolute luciferase assay value under inducible condition, the induction fold (the luciferase activity under inducible condition divided by the luciferase activity under non-inducible condition) did not further increase.

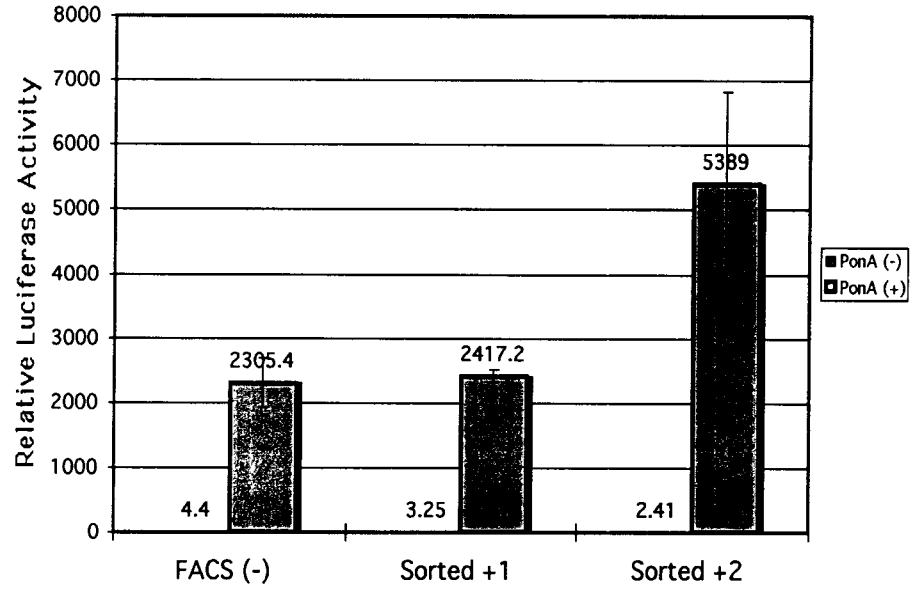


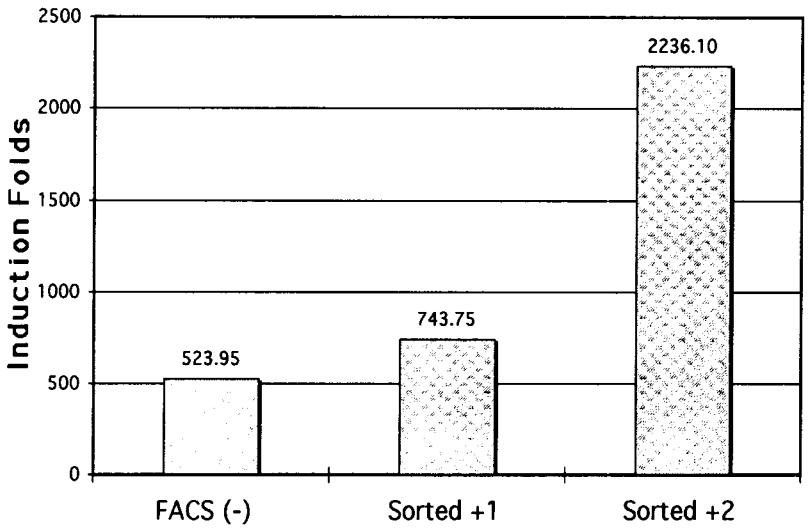
We reason this is due to concomitant decrease of the luciferase activity under both induced and non-induced condition, which reflects elimination of cells which possess leaky expression characteristics.

Appendix 6

Quantifying ecdysone inducibility in HEK293 cells

We applied similar GFP reporter and FACSorting based strategy in HEK293 cells and had been able to establish a population of HEK293 cells responsive of ecdysone regulation. We examined the inducibility of the cells by transiently transfecting them with pIND-Luc under induced and non-induced conditions. The responsiveness is even more impressive than MDCK cells after just two rounds of positive selection. Due to the low background value under non-inducible condition, we did not perform any round of negative selection to decrease the leakiness.



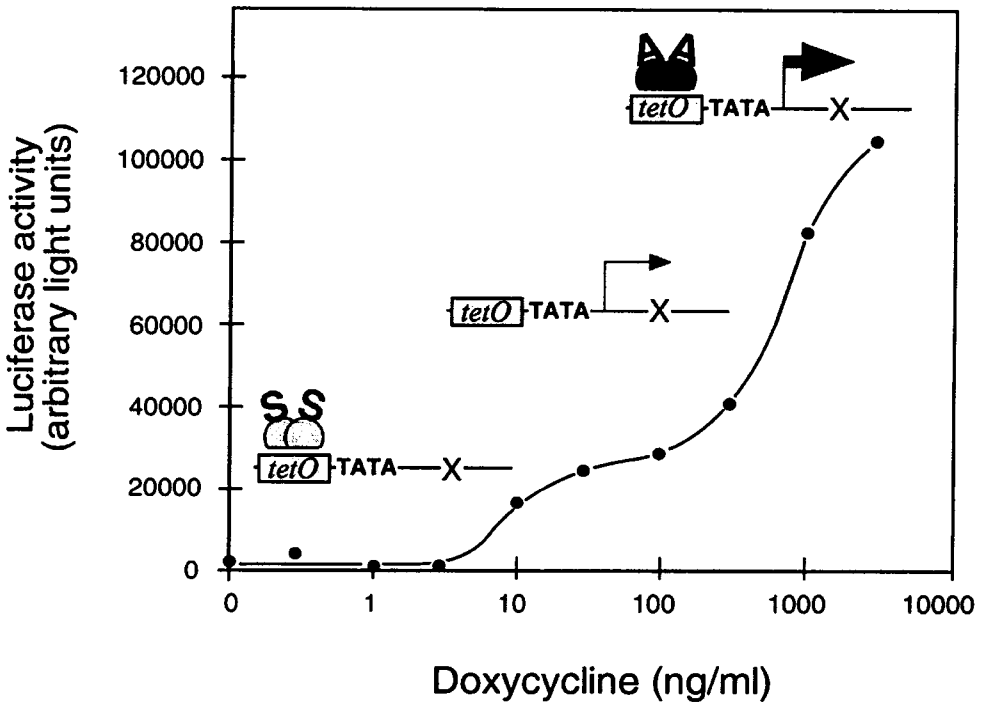


Appendix 7

Tet-ON system could be complemented with tetracycline regulated transcriptional silencer (tTS) to minimize leakiness of tetracycline responsive promoter

Basically, there are three players involved in making this system work:
(1) rtTA (reverse type tetracycline regulated trans-activator, which binds to Tet operator sequence, *tetO*, in the presence of tetracycline or its analoque doxycycline)
(2) tTS (classical Tet repressor fused to the transcriptional silencer, KRAB domain of Kid-1 protein, which binds to Tet operator sequence, *tetO,* in the absence of tetracycline or its analoque doxycycline)
(3) Target plasmid with a mininal CMV promoter plus seven tandem repeat of Tet operator sequence, *tetO,* followed by the reporter gene or gene of interest

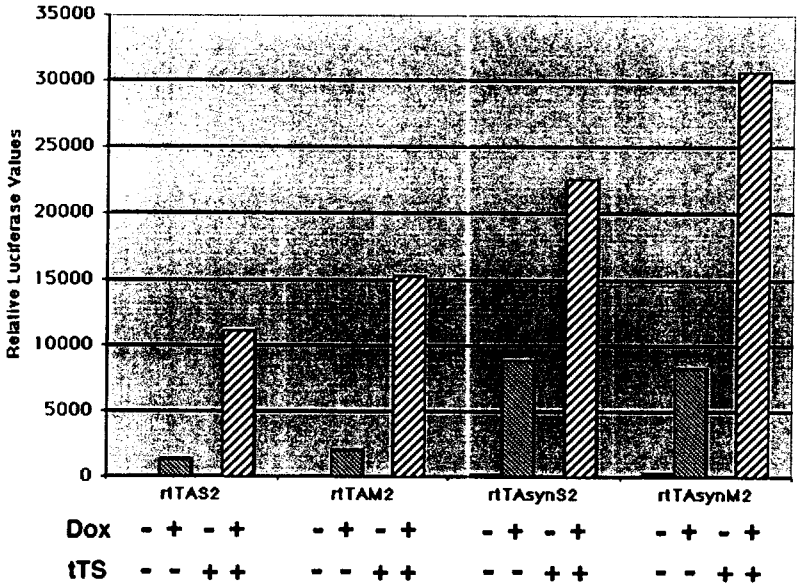
By changing the concentratioin of doxycycline, the experimenter could manipulate the association status of either tTS or rtTA with *tetO*, and therefore decide whether the gene (or reporter) activity is totally shut-down (when tTS binds to *tetO* and shield it from any endogenous transcriptional machinery), or minimally expressed (when neither tTS nor rtTA binds to *tetO*, and only the endogenous transcriptional machinery acts through the minimal CMV promoter), or fully turned on (when rtTA binds to *tetO* and activate RNA transcription).

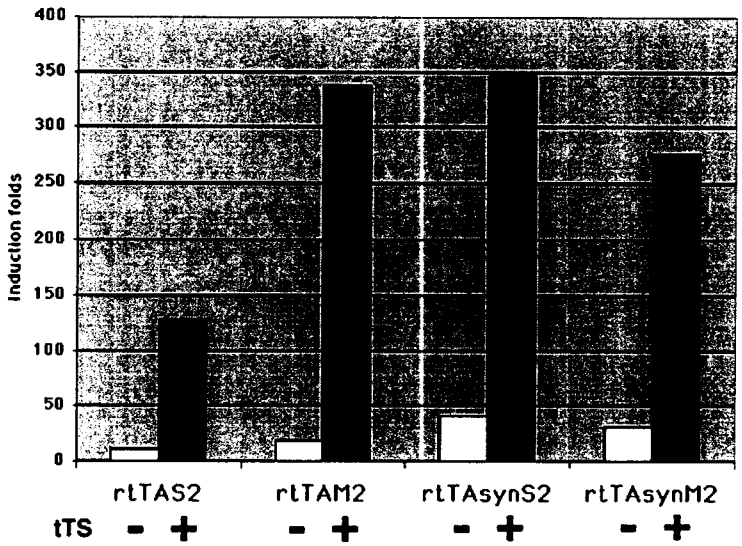


Appendix 8

Tetracycline regulated transcriptional silencer (tTS) helps suppress unwanted leaky expression under non-inducible condition

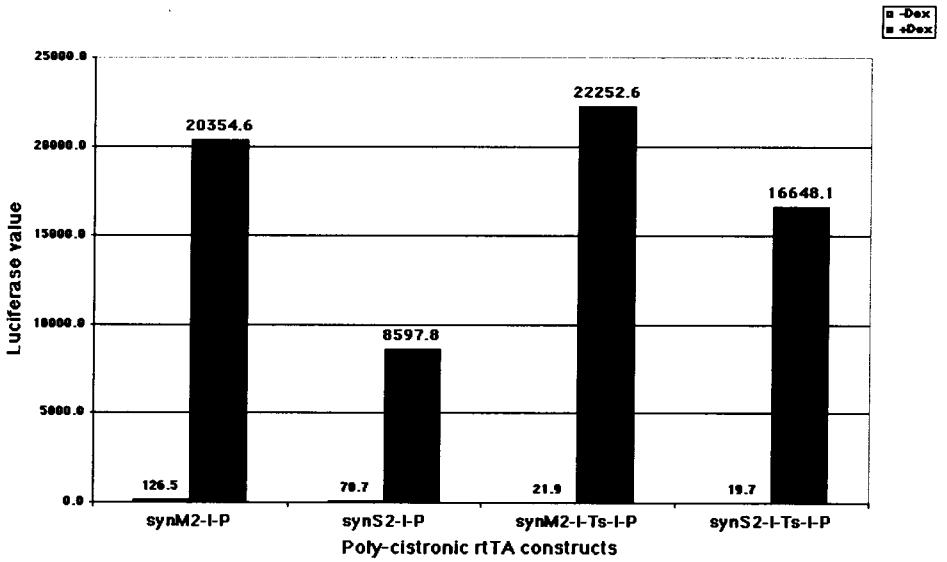
When conjugating with tetracycline regulated transcriptional silencer (tTS), all the pTet-ON type rtTA mutants (S2, M2, synS2, and synM2) we had tested so far showed decreased activating ability when the inducer (doxycycline, Dox) was absent while the activating ability was increased when the inducer was present, compared to the condition when there was no tTS in the transfection mixture. This resulted in a tremendous increase of overall induction folds for all the tested rtTA mutants.

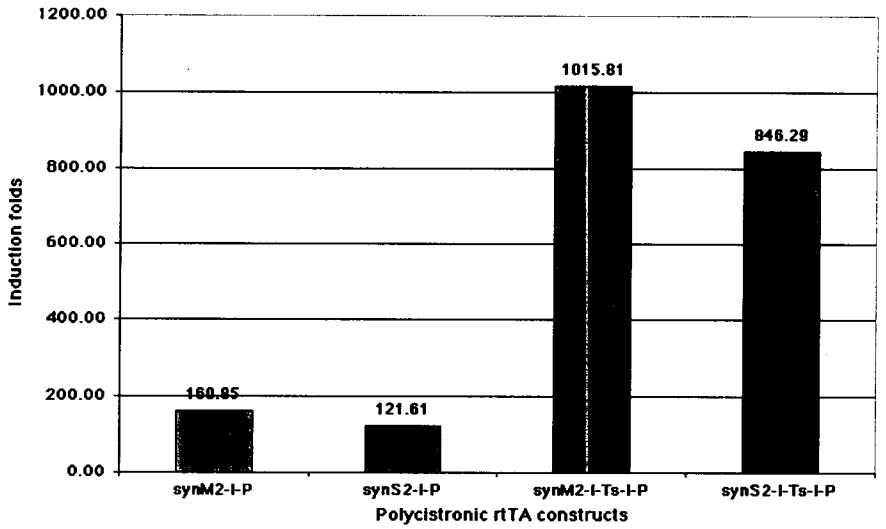




Appendix 9

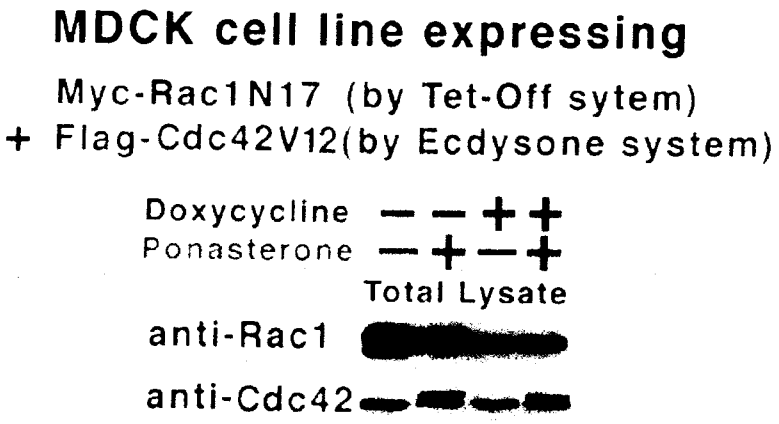
Tricistronic construct conjugating rtTA and Tetracycline regulated transcriptional silencer (tTS) helps suppress unwanted leaky expression under non-inducible condition



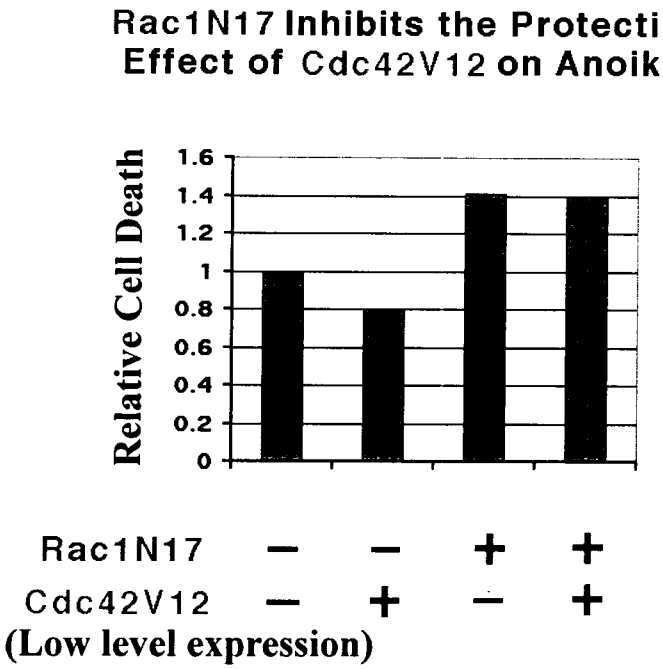


Please note, without the presence of tTS in the polycistronic constructs, synM2-I-P shows inducibility comparable to synM2-I-Ts-I-P when stimulated by Doxycyline; however, the addition of tTS into the polycistronic constructs help decrease the promoter activity under non-inducible condition. Therefore, the overall induction fold of activation (luciferase activity under inducible promoter activity devided by the luciferase activity under non-inducible condition) increases by one order of magnitude by the addition of tTS.

Appendix 10



Appendix 11A



Appendix 11B

Representative Examples of the Plasmids and Cell Lines Generated in the Past

Inventory of the Plasmids:

pIND-GFP and **pIND-RFP: green** and **red** fluorescence protein expression vector under ecdysone inducible promoter

pTRE-GFP and **pTRE- RFP: green** and **red** fluorescence protein expression vector under tetracycline inducible promoter

synM2-I-P: M2 version of rtTA mutant linked to puromycin selection marker by IRES (internal ribosome entry site)

synS2-I-P: S2 version of rtTA mutant linked to puromycin selection marker by IRES (internal ribosome entry site)

synM2-I-Ts-I-P: M2 version of rtTA mutant linked to tetracycline regulated transcriptional silencer, by one IRES, which is in turn linked to puromycin selection marker by another IRES

synS2-I-Ts-I-P: S2 version of rtTA mutant linked to tetracycline regulated transcriptional silencer, by one IRES, which is in turn linked to puromycin selection marker by another IRES

Inventory of dual regulatory cell lines:

(1) HEK293.3
An HEK293 cells capable of double regulation after being transfected with Ecdysone receptor expressing plasmid, pVgRXR (Invitrogen) and rtTA/tTS conjugated plasmids.

(2) T23T.1
This is a subclone of MDCK cells capable of both ecdysone and tetracycline regulated expression.

appendix 12

(3) Rac1V12.1 **(Rac1V12: constitutively active Rac1V12 mutant under tetracycline inducible system)**
This is a subclone of Rac1V12 capable of expressing another gene under ecdysone regulated expression.

(3a) Rac1V12.1 **+ pIF-RhoAV14**
This is a subclone of Rac1V12.1 which expresses myc-tagged Rac1V12 under tetracycline repressible system, and expresses Flag-tagged **RhoAV14** under ecdysone inducible expression.
(3b) Rac1V12.1 **+ pIF-Cdc42V12**
This is a subclone of Rac1V12.1 which expresses myc-tagged Rac1V12 under tetracycline repressible system, and expresses Flag-tagged **Cdc42V12** under ecdysone inducible expression.
(3c) Rac1V12.1 **+ pIF-Cdc42N17**
This is a subclone of Rac1V12.1 which expresses myc-tagged Rac1V12 under tetracycline repressible system, and expresses Flag-tagged Cdc42N17 under ecdysone inducible expression.

(4) Rac1N17.1 **(Rac1N17: constitutively active Rac1N17 mutant under tetracycline inducible system)**
This is a subclone of Rac1N17 capable of capable of expressing another gene under ecdysone regulated expression.

(4a) Rac1N17.1 **+ pIF-RhoAV14**
This is a subclone of Rac1N17.1 which expresses myc-tagged Rac1N17 under tetracycline repressible system, and expresses Flag-tagged **RhoAV14** under ecdysone inducible expression.
(4b) Rac1N17.1 **+ pIF-Cdc42V12**
This is a subclone of Rac1N17.1 which expresses myc-tagged Rac1N17 under tetracycline repressible system, and expresses Flag-tagged **Cdc42V12** under ecdysone inducible expression.
(4c) Rac1N17.1 **+ pIF-Cdc42N17**
This is a subclone of Rac1N17.1 which expresses myc-tagged Rac1N17 under tetracycline repressible system, and expresses Flag-tagged Cdc42N17 under ecdysone inducible expression.

appendix 12

出席國際學術會議
參加第四十一屆美國細胞生物學會心得報告

經費補助: 國家科學委員會專題研究計畫

計畫名稱: 建構 Ecdysone 誘導式基因表現系統以研究
DNA 微矩陣鑑識之基因的策略

計畫編號: NSC 90-2323-B-002-009

執行期限: 民國 90 年 8 月 1 日起至 91 年 7 月 31 日

周祖述 助理教授

台大醫學院 臨床醫學研究所

Meeting report for THE AMERICAN SOCIETY FOR CELL BIOLOGY 41ST ANNUAL MEETING December 8-12, 2001 Washington, DC

On December 8, I went to the Special Interest Subgroup Meetings on Urothelial Cell Biology in Room 11 (The organizers of this meeting were Ricardo Saban, Oklahoma University Health Science Center, and Timothy G. Hammond, Tulane Medical School.)

The urothelium, the epithelium lining the surface of the urinary bladder, is a unique cell type with high plasticity and a variety of cell functions. Urothelium represents the first line of bladder defense and an interface between pathogens and defense mechanisms. Functions of the urothelium include control of permeability and immune responses. Cell-cell communication seems to play a major role on urothelial cell response to injury and infection. This response is modulated by Nerve and EGF-like Growth Factors. In addition, despite the fact that urothelium is a frequent site of cancer formation, few experimental model systems are currently available or well characterized for studying urothelial cancer. The identification of uroplakin genes that are specifically expressed in the urothelium has made it possible to target unique oncogenic events to the in vivo urothelium and to evaluate their biological potential in inducing urothelial transformation and tumorigenesis.

At 6:00 PM, I went to the Keynote Symposium in Hall C. The topics and speakers were
Genomics, Stem Cells and Functional Approaches to Cell Biology of the New Century
Former U.S. Representative The Hon. John Porter
Shirley Tilghman, Princeton University
Irving Weissman, Stanford University
Craig Venter, Celera Genomics

After the Keynote Symposium, I went to the reception for dinner.

At 8-9:30AM on December 9, I went to Symposium I (Hall C) for the topic about Genotype/Phenotype Plasticity in Differentiation and Cancer. The speaker s were
Mina Bissell, Lawrence Berkeley National Laboratories
Craig Thompson, University of Pennsylvania
Kenneth Kinzler, The Johns Hopkins University

At 10:30-12PM on December 9, I went to Symposium II (Hall C) for the topic about Cellular Processes That Regulate Membrane Trafficking. The speaker s were
Frances Brodsky, University of California, San Francisco
Ira Mellman, Yale University
Randy Schekman, University of California, Berkeley

At 3:30-5:45PM on December 9, I went to Minisymposium 8 (Room 20) for the topic about ECM Proteins and their Receptors: Structure, Function and Modulation
(The organizers of this minisymposium were Timothy Springer, and Mary Zutter)

At 8-9:30AM on December 10, I went to Symposium III (Hall C) for the topic about The Cell Biology of Sensation. The speaker s were
David Corey, Massachusetts General Hospital
Catherine Dulac, Harvard University
Charles Zuker, University of California, San Diego

At 10:30-12PM on December 10, I went to Symposium IV (Hall C) for the topic about Cellular Regulation of Chromosome Functions. The speaker s were
Tatsuya Hirano, Cold Spring Harbor Laboratory
Barbara Meyer, University of California, Berkeley
Carl Wu, NIH, The National Cancer Institute

At 3:30-5:45PM on December 10, I went to Minisymposium 13 (Room 31) for the topic about
Receptor Signaling Pathways: Structure, Function and Modulation
(The organizers of this minisymposium were Dafna Bar-Sagi, and Kunxin Luo)

At 8-9:30AM on December 11, I went to Symposium V(Hall C) for the topic about The Molecular Basis of Diseases. The speaker s were
Stanley Falkow, Stanford University
Jonathan Seidman, Harvard Medical School
Bruce Spiegelman, Dana Farber Cancer Institute

At 10:30-12PM on December 11, I went to Symposium VI (Hall C) for the topic about Cellular Cytoskeletal Mechanisms and the Cell Cycle. The speaker s were
Anthony Hyman, Max-Planck Institute
Rong Li, Harvard Medical School
Edward Salmon, University of North Carolina

At 3:30-5:45PM on December 11, I went to Minisymposium 17 (Room 40) Cytoskeletal Dynamics in Living Cells. (The organizers of this minisymposium were Patricia Wadsworth, Clare Waterman-Storer).

At 8-9:30AM on December 12, I went to Symposium VII(Hall C) for the topic about The Understanding Signaling Networks. The speaker s were
Henry Bourne, University of California, San Francisco
Joanne Chory, The Salk Institute
Michael Dustin, New York University

At 10:30-12PM on December 12, I went to Symposium VIII (Hall C) for the topic about Cell Polarity and Development. The speaker s were
Chris Doe, University of Oregon
Kenneth Kemphues, Cornell University
Matthias Peter, Swiss Institute for Experimental Cancer Research

At 3:30-5:45PM on December 12, I went to Minisymposium 27 (Room 38) Establishment and Maintenance of Cell Polarity. (The organizers of this minisymposium were Karl Matter, Keith Mostov).