行政院國家科學委員會補助專題研究計畫成果報告

The implication of FAK in estrogen-induced signal transduction.

計畫類別：☑個別型計畫　□整合型計畫

計畫編號：NSC 89－2311－B－040－009－

執行期間：　88 年　8 月　1 日 至　89 年 7 月 31 日

計畫主持人：馬 明 琪

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執行單位：私立中山醫學大學生化研究所

中　華　民　國　90 年　10 月　25 日

行政院國家科學委員會專題研究計畫成果報告

Focal adhesion kinase (FAK) 在 estrogen 引發訊息傳遞過程中所扮演的角色

The implication of FAK in estrogen-induced signal transduction

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一、中文摘要

estrogen（雌性荷爾蒙）已知與許多組織的生長、發育相關。而這些作用係透過 estrogen receptor (ER) 來達成。 ER 是一個 nuclear transcription factor；當 estrogen 與其結合時，會誘發一連串反應，進而活化或抑制 target genes 的表達。 固然， estrogen 刺激所產生的生理反應與 ER 被活化成 transcription factor 有相當大的關係；但也不能排除其他 signaling pathways 因受 estrogen 刺激活化、參與其間而有以致之。 惟 estrogen 如何活化 signaling pathways 目前仍不是很清楚。 但先前已有報導指出 estrogen 刺激所造成 DNA 的合成會因 tyrosine kinase inhibitors 的存在而降低；且 estrogen 刺激後細胞內蛋白的 tyrosyl phosphorylation 也會顯著增加。目前已有報導指出 estrogen 刺激會提高 c-Src 活性、 強化 Shc phosphorylation、 活化 Ras/MAPK。 但相較於 c-Src，另一個重要的 tyrosine kinase - focal adhesion kinase (FAK) 其是否受 estrogen 刺激而變化則鮮少有報導。 在探討 estrogen 訊息傳遞的過程中，我們意外發現 estrogen 會刺激 FAK 表達量的增加。 為進一步探討此一現象，我們設計 mammalian DAN expression constructs 來了解 FAK 在 estrogen-induced signaling 中可能扮演的角色。

關鍵詞：雌性激素、tyrosine kinase、FAK

Abstract

Estrogen is known to participate in growth and development in various tissues. And all these cellular functions are mediated through estrogen receptor (ER) that is a nuclear transcription factor. Estrogen binding to the ER triggers a series of molecular events culminating in the activation or repression of target genes. All the physiological functions of estrogen can be attributed to the activated ER that acts as a transcription factor as well as the signaling pathways that are induced or activated by estrogen. However, to date, the effects of estrogen on cellular signaling pathways are still unclear. Previously, it was demonstrated that tyrosine kinase inhibitors could block estrogen-induced mitogenesis. And upon estrogen stimulation, enhanced tyrosyl phosphorylation of total cellular proteins was detected. There were reports indicating the activation of c-Src enzymatic activity, the enhancement of tyrosyl phopsphorylation of Shc and the activation of Ras/MAPK in response to estrogen. However, what happened to the other important tyrosine

kinase, FAK, was still obscure. In the process of investigating the signaling pathways induced by estrogen, we unexpectedly observed that FAK was induced after estrogen treatment. To further study the phenomenon and its implicated significance, we are interested to design mammalian DNA expression constructs and generate cells overexpressing FAK to investigate the participation of FAK in estrogen-induced signaling.

Keywords: estrogen, tyrosine kinase, FAK

二、緣由與目的

Estrogen, an ovarian steroid hormone, regulates the growth and development of normal human mammary tissue and also is involved in the progression of breast tumor (1). Unlike the receptors of peptide growth factors that are receptor tyrosine kinases, the receptor for estrogen is a nuclear transcriptional activator (2). To date, two estrogen receptors (ERs), ER-α (3) and ER-β 1 (4,5) were observed. They were encoded by two different mRNAs. Upon estrogen binding, the ER was subjected to conformational changes that resulted in dimers that recognized the estrogen-resonsive elements (EREs) located upstream of target genes. Through the interaction of the ER with components of the cellular transcription machinery, the transcription of estrogen responsive genes was regulated (6,7).

Tyrosyl phosphorylation is an important post-translational modification. Multiple protein substrates are phosphorylated on tyrosine in response to various cell stimuli, including growth factor activation (8), cell transformation induced by oncogene-encoded tyrosine kinases (9,10), platelet activation (11), agonist-stimulated secretion (12), cell cycle alterations (13,14), and cell migration (15). Although the identities of these protein substrates are not completely known, accumulating evidence indicates that the majority is involved in signal transduction pathways.

Focal adhesion kinase (FAK) was initially identified as a v-Src substrate (16). Later studies indicated that it was also involved in integrin signal transduction (17). Because it was localized at the site of focal contact, it was thus designated as focal adhesion kinase (18). Structural analysis revealed that the kinase domain was localized at the central part of the protein, while the C-terminal region, FAT (focal adhesion targeting sequence), was responsible for its cellular localization (19). In response to the engagement of fibronectin to the integrins, FAK became tyrosyl phosphorylated and activated (20,21). Interestingly, FAK N-terminal portion could interact with the cytoplasmic tail of integrin β1 subunit and overexpression of β subunits (i.e. β1, β2 and β3) could lead to the activation of FAK (22,23). With these findings and other biochemical evidence, FAK has been speculated to involve in cellular growth, cell adhesion, cell mobility and tumor formation.

Proliferation is one of the important functions of estrogen in target tissues. Interestingly, the reduction of estrogen-induced proliferation by tyrosine kinase inhibitors implicating the requirement of functional tyrosine kinase pathways in estrogen action (24). Enhancement of protein tyrosyl phosphorylation was detected in ER-expressing human breast tumor cells, MCF7. And this estrogen-dependent tyrosyl phosphorylation was blocked by antiestrogen (25). Further analysis revealed that c-Src was activated in response to estrogen that could lead to tyrosyl phosphorylation of Shc and triggered Ras/MAPK cascade (26). Based on the activity of FAK could be regulated by c-Src (27,28) and the activity of c-Src was increased in response to estrogen, we would like to study the possible involvement of FAK in estrogen-induced mitogenesis.

三、結果與討論

Enhancement of FAK expression in response to estrogen. To determine whether the expression of FAK will increase in response to estrogen stimulation, MCF7 cells stimulated with 10 nM for different periods of time were analyzed by FAK immunoblotting. As shown in Figure 1, significant increase of FAK expression was

observed after 15 min estrogen stimulation.

Generation of the DNA constructs encoding wt- or mutant FAK. Due to the activity of c-Src was increased in response to estrogen, and Src could mediate FAK phosphorylation, generation of cell lines expressing wt- or mutant FAK is our imminent goal. To accomplish this objective, mammalian DNA expression constructs encoding wt- and mutant FAK need to be made. Since Tyr-397 is the FAK autophosphorylation site whose phosphorylation can provide the binding site for Src, Tyr-576, -577 and -863 are Src-mediated sites whose phosphorylation will influence the enzymatic activity of FAK, therefore, in addition to wt-FAK, we propose to generate the DNA constructs encoding these mutant FAKs. Through RT-PCR, appropriate FAK primers were utilized to obtain the DNA fragments of interest and they were further cloned into the mammalian expression vector, pcDNA. The schematic drawing of the DNA constructs generated is demonstrated in Figure 2 to Figure 5. Establishment of cell lines expressing these various FAK is underway now.

四、 計畫成果自評

本實驗進行順利，除正努力建立細胞株外，相關實驗成果已有一篇投稿送審。

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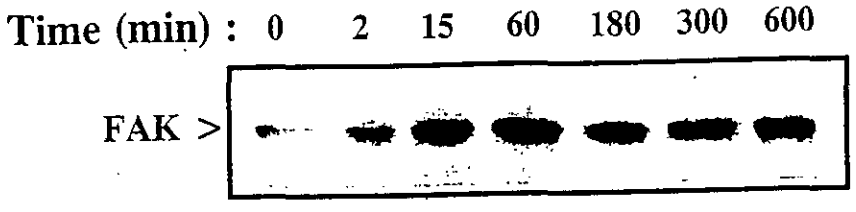

Blot: FAK

Figure 1. Increased FAK expression in response to estrogen.
Total lysates prepared from non-stimulated and estrogen stimulated MCF7 cells are analyzed by western immunoblot with FAK antibody.

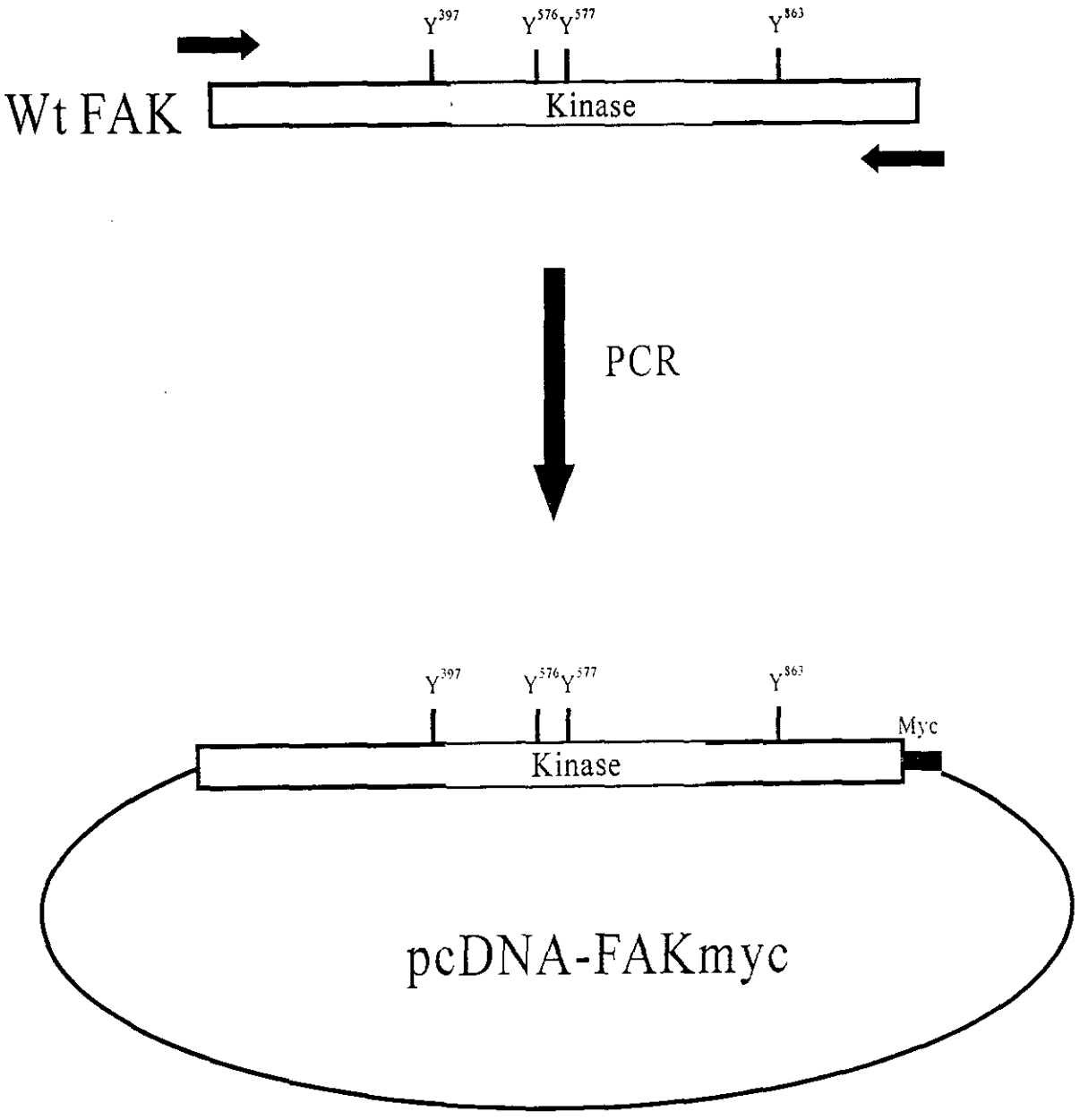

Figure 2. The schematic illustration of the mammalian expression construct encoding wild type FAK.

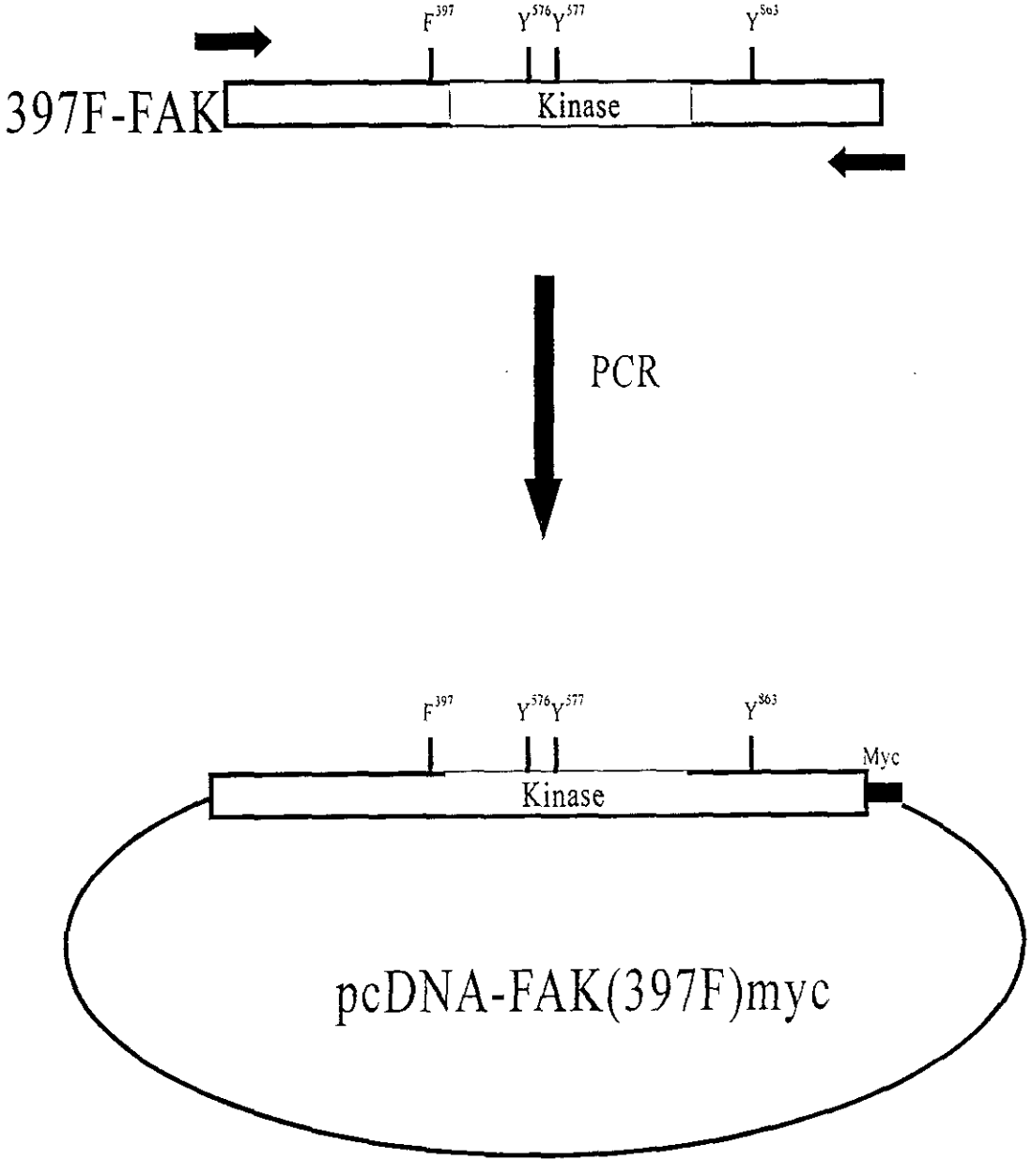

Figure 3. The schematic illustration of the mammalian expression construct encoding 397F-FAK.

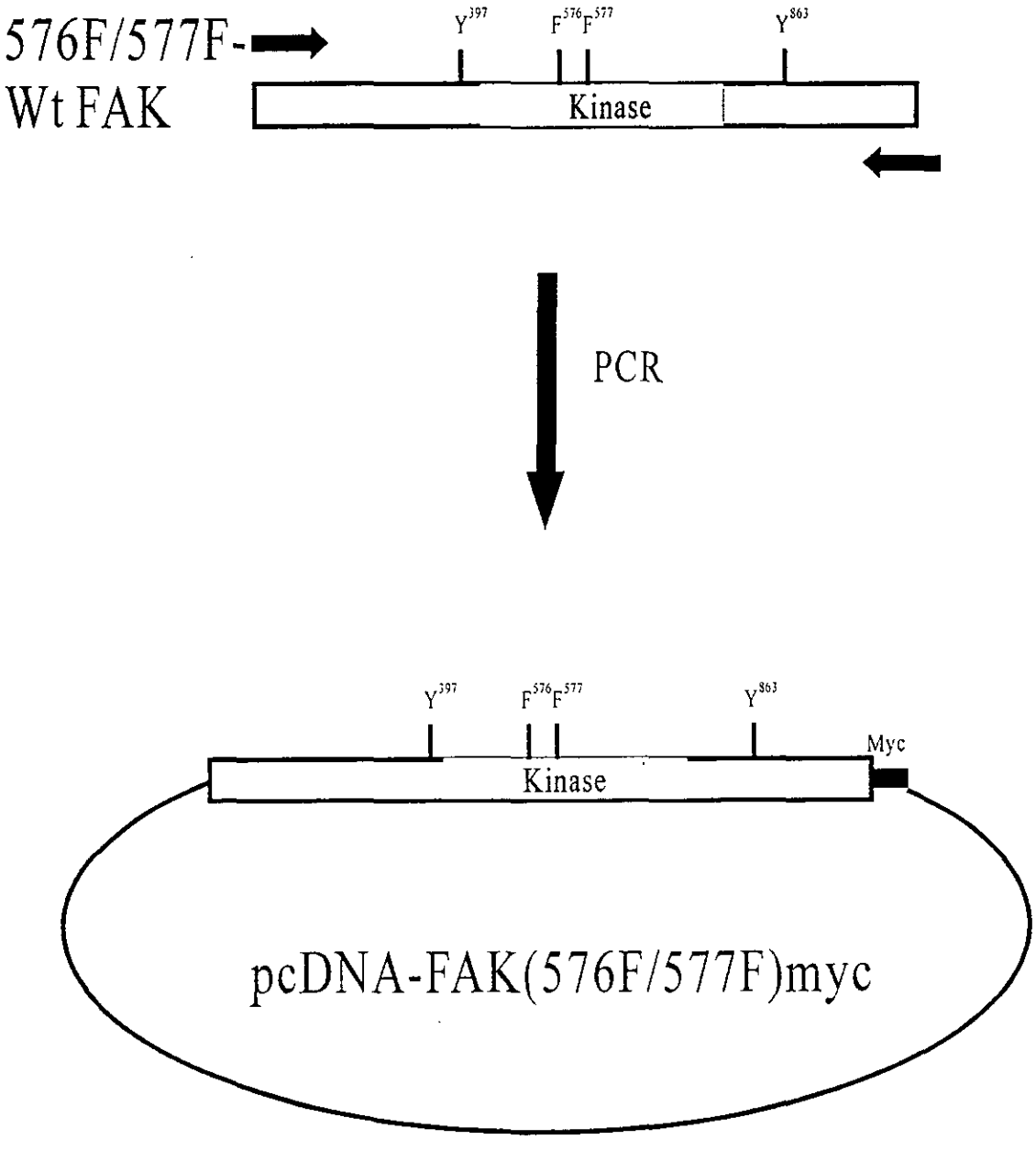

Figure 4. The schematic illustration of the mammalian expression construct Encoding 576F/577F-FAK.

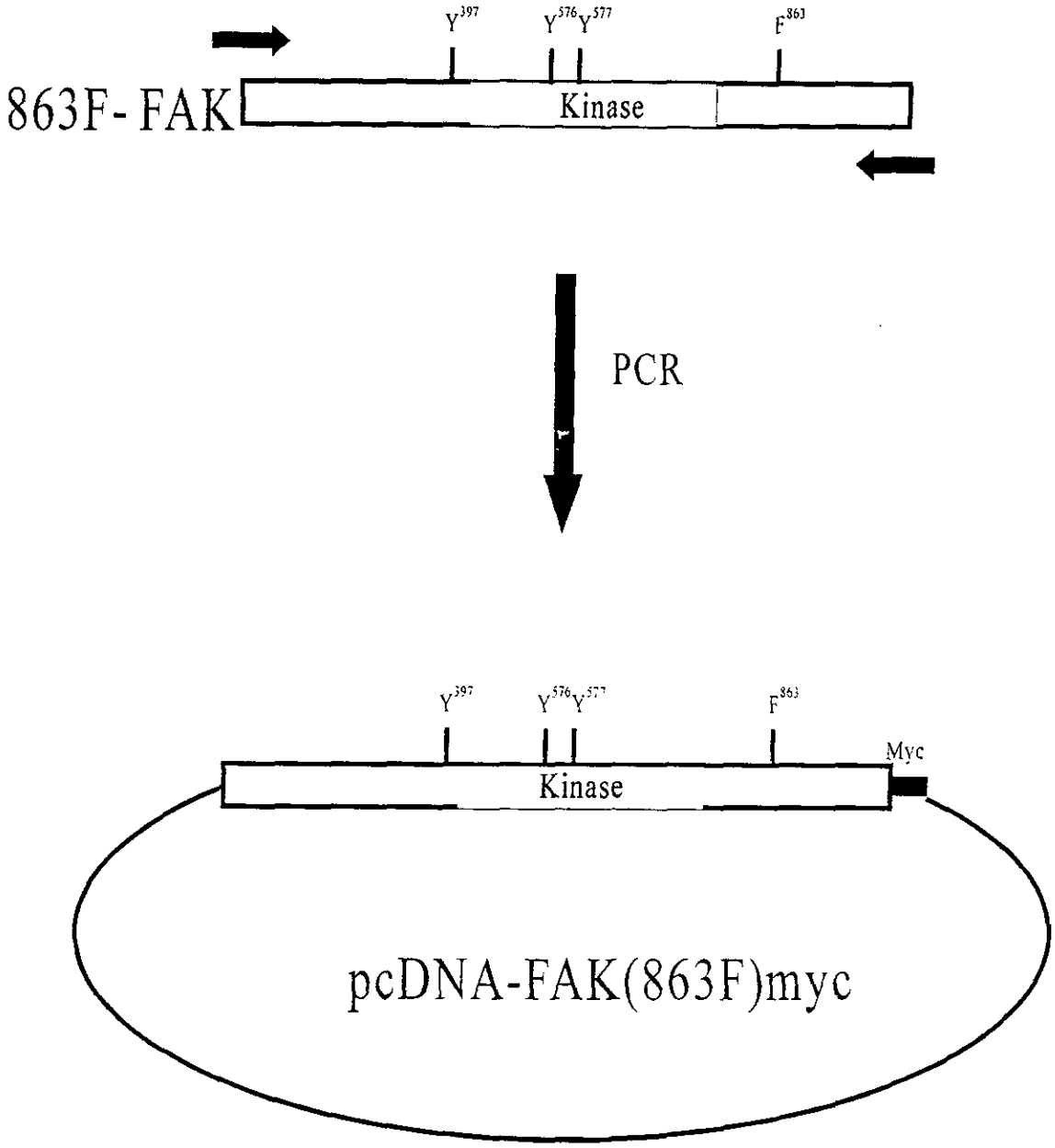

Figure 5. The schematic illustration of the mammalian expression construct encoding 863F-FAK.