行政院國家科學委員會專題研究計畫　期中進度報告

Tpros 基因在生精過程中功能之探討(1/3)

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一、中文摘要

由 Trops 之表現型態, 我們認為其在青春期開始之造精功能應該扮演很重要之角色, 我們預期基因剔除鼠可能會有生殖功能異常之結果, 但我們目前已篩選了 149 個細胞株, 卻沒有找到正確剔除之胚幹細胞, 我們擔心可能是此質體欲剔除之片斷太大以致重組機率太低, 因此設計另一個剔除質體, 縮短欲刪除之片段, 目前已構築完成, 希望可提高同源重組的成功率。再者, 我們利用各種表現質體試圖純化出 Trops 之融合蛋白, 目前發現用 His-Tag 在 20℃之培養環境下可得到不錯之效果, 正利用此融合蛋白進行 DNA 結合分析, 試圖了解 Trops 較喜歡結合之目標基因。此外, 利用 Trops 之 RNAi 之構築, 我們也已獲得 2 隻 Trops 之 RNAi 基因轉殖鼠, 目前尚在進行大量繁衍, 會再進一步了解 Trops-knock down 之效果及基因轉殖鼠之性狀。

關鍵詞：睪丸、精子熟成、同源箱基因、不孕症

Abstract

From the expression pattern of Tpros, we speculate that Tpros might play an important role in spermatogenesis since puberty. We expect to see reproductive abnormality with the Tpros knock-out mice. However, after screened 149 ES cell clones by PCR analysis, we did not identify any candidate targeted ES clone. We suspect that the low frequency in homologous recombination is from the improper knock-out construct. A modified targeting plasmid was therefore reconstruct to have the deleted Tpros fragment reduced to a compatible size with the replaced Neo cassette. Furthermore, we have tried several different expression constructs to express Tpros-fusion protein. The construct with His-Taq gave a better expression level after a 4-6 hr incubation at 20^{o}C. The identification of DNA binding site as well as the antibody raising are in progress by using the purified Tpros-His fusion protein. Another project trying to characterizing the in vivo function of Tpros is to establish the Tpros RNAi transgenic mice. Two founder line have been obtained and under intensive breeding. The expression level of Tpros and the mouse penotypes with a reduced level of Tpros will be further investigated.

Keywords: Testis; Spermatogenesis; Homeobox gene; Infertility

二、緣由與目的

Male infertility due to defective spermatogenesis affects about 5-7% of all couples trying to conceive. The cause of impaired spermatogenesis in about one third of infertile men is not known. Spermatogenesis is a unique process of continuing differentiation, during which meiosis is followed by extensive changes in cell morphology and intracellular organization. There is no genetic polymorphism other than several mutations on the Y chromosome have been correlated with male infertility. The specific goal of this proposal is molecular and functional characterizations of one novel murine testis-specific homeobox gene that we have recently isolated from degenerate PCR and named *Tpros* (Testis-prospero-like homeobox).

Homeobox genes encode transcription factors which containing a 60-amino acid DNA binding motif, term the homeodomain. The tertiary structure of which conforms to a helix-turn-helix motif conserved in species as evolutionarily distant from yeast to human (1). Homeobox genes are known to be intricately involved in the embryo development (2-3), but less is known about their function in the differentiation processes of the adult organism. As previously stated, spermatogenesis represents such a process, and interestingly, several homeobox genes have been shown to be expressed in murine male reproductive systems, suggesting the possibility that they may regulate developmental events during male gametogenesis (4).

The *in situ* hybridization data showed that *Tpros* is specific expressed in the spermatid stage which indicates that *Tpros* might play an regulatory role during the spermiogenesis. In addition, a highly conserved domain (DCD) downstream of the Tpros homeodomain might play a role in regulaing the functional specificity of Tpros such as the POU or pair domains among other hmoedomain-containing proteins (5-7).

三、結果與討論

To characterized the in vivo function of Tpros, we first tried to establish the Tpros knock-out mice. The targeting vector was construct with 6.5 kb of long arm and 0.6 kb of short arm from which a fragment of more than 10 kb will be deleted in the Tpros genome (Fig. 1). This construct was transfected into the ES cell line by electroporation. Genomic DNAs were isolated and screened by PCR analysis in 149 selected ES clones (Fig. 2). However, no positive clone was identified. We suspected that the deleted region is too big to get a better frequency in homologous recombination. We therefore reduced the deleted region to about 2 kb so a more compatible size to the Neo resistance gene cassette in the targeting plasmid (Fig. 3).

On the other hand, RNAi has been proved to be a very powerful tool in study the gene function. We have tried to injected the Tpros RNAi into the mouse pronuclei to make the transgenic mice. Fortunately, two RNAi transgenic founder have been identified (Fig. 4). These Tpros knockout down mice should be a model to study the effect of Tpros loss of function. Phenotypes from these knock-down mice will imply the n vivo function of Tpros in the spermatogenesis.

The other project we are making progress is that we have purified the Tpros protein. From several E. coli expression constructs (Fig. 5), we found that the Tpros-His fusion protein can be stably expressed from a 4-6 hr incubation at 20oC. We are trying to fish out the Tpros binding site and associate proteins. Some DNA sequences were obtained from the primarily random selection with but no conclusive results yet.

四、計畫成果自評

The major goal of this grand is to understand the molecular function of *Tpros* gene. We have completed a modified version of the Tpros knock-out construct from which should give us a better chance to get the targeted ES cell clone. To characterize the in vivo function of *Tpros* in thespermatogenesis through the mouse model, we have now in the progress to characterize the expression leve and phenotypes of the RNAi transgenic mice. For another specific aims, we make a good progress since we have obtained Tpros protein. Binding site and associate proteins should be able to identified in time.

五、參考文獻

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Figures

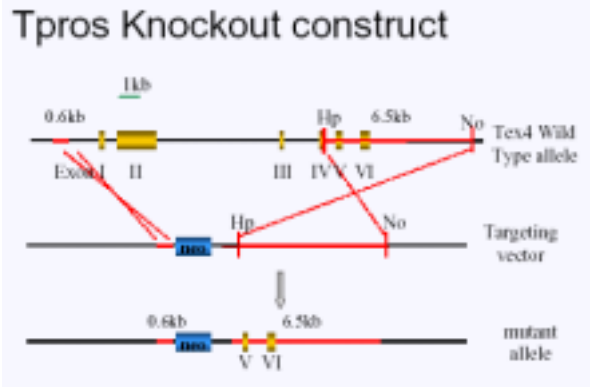


Fig. 1 The first Tpros knock-out construct
The Tpros genomic structure is shown on the top. Two homologous fragments are 6.5 and 0.6 kb individually. The targeted allele is shown in the bottom.

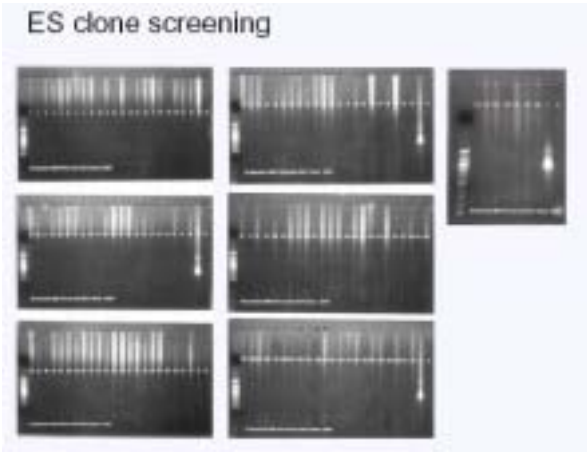


Fig.2 The result from the PCR screening with 149 ES cell DNAs. No positive clone was identified.

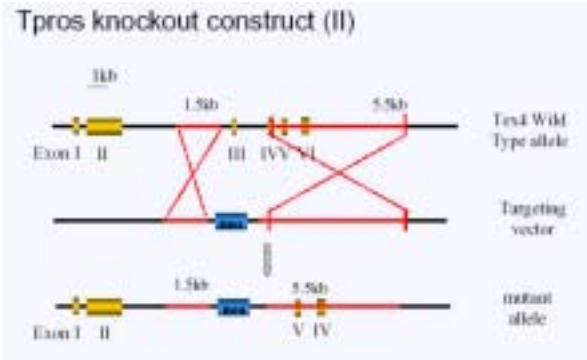
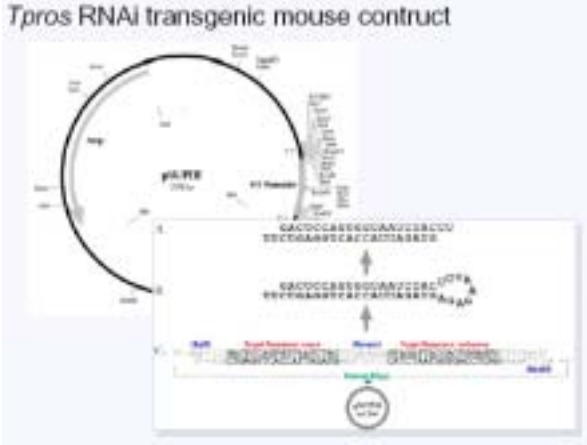


Fig. 3 The modified Tpros knock-out construct. The region intend to be deleted and replaced with Neo cassette was reduced to a size about 1.5 kb. The lon arm and short arm are 5.5 and 1.5 kb individually.

(A)



(B)

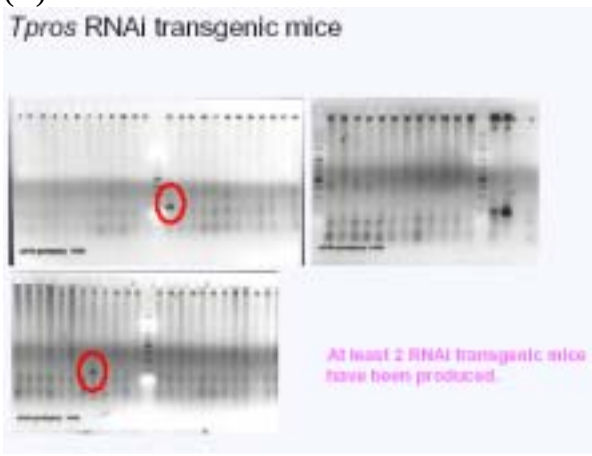
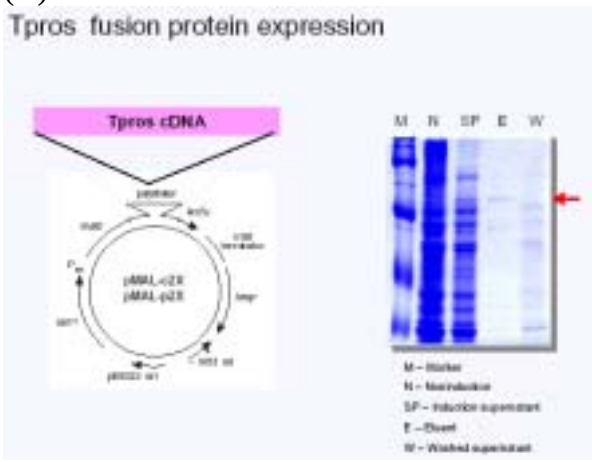


Fig. 4. (A) the Tpros RNAi transgenic mouse contruct. (B) Two transgenic founder mice were identified from PCR screening.

(A)



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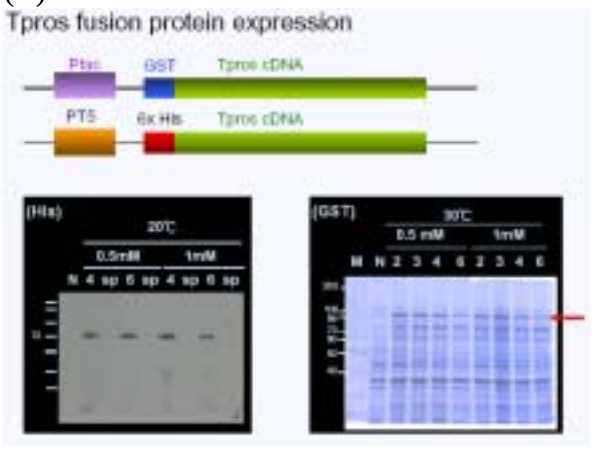

Fig. 5. Tpros fusion protein expression from several different fusion constructs.