行政院國家科學委員會補助專題研究計畫成果報告

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研究人類 C4/CYP21 基因作中調控基因群具腎上腺專一表現的機制

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計畫主持人：張淑芬

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計畫參與人員：

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- □國際合作研究計畫國外研究報告書一份

執行單位：台北醫學大學 細胞分子生物研究所

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一、中文摘要

人類 *CYP21* 基因在腎上腺皮質產生參與合成 glucocorticoid 及 mineralcorticoid 的酵素。一遺傳疾病、腎上腺皮質增生症 (congenital adrenal hyperplasia)，是因此基因發生突變，使酵素活性缺失而造成。基因體上有一相對於 *CYP21* 的假基因、*CYP21P*，二者和第四血清補體基因 (*C4A/C4B*) 交互並列於第六條染色體 *C4/CYP21* 基因座中，與其他成對的基因組，排列如下 5'-*C4A, ZA, CYP21P, YA, ZA - C4B, ZB, CYP21, YB, XBS, XB*-3'。除 *X* 基因組外，這些基因以相同的方向轉錄。且除由 *C4A/C4B* 產生的第四血清補體、由 *CYP21* 產生的 c21-hydroxylase，及由 *XB* 所製造類似細胞間質蛋白質外，目前有那些蛋白質是由位在這基因座內其它成對基因組所製造的仍不清楚。除了 *C4A/C4B*、*CYP21P* 及 *XB* 基因外，此區域內這些基因群的轉錄產物均只在腎上腺被偵測到，因此本計畫針對基因座內可能存在類似 locus control region (LCR) 的區域與轉錄因子共同調控這些成對基因組在腎上腺呈現專一地表現加以研究。

以腎上腺及非腎上腺細胞株為體外模式，本實驗室已完整地分析並確定於此基因座中可能的腎上腺專一表現調控區域。同時經由明膠上移及蛋白質足跡分析特定 DNA 區域與核蛋白的結合，解讀在該段可能是調節基因座基因表現的調節區域中特定的 DNA 序列的確與腎上腺細胞中特異的核蛋白因子呈現專一的結合。

關鍵詞：腎上腺專一表現、基因座調節區域、人類 *CYP21* 基因

Abstract

Human *CYP21* encodes 21-hydroxylase which mediates the biosynthesis of glucocorticoid and mineralcorticoid in the adrenal cortex. Mutations occurring at the *CYP21* are known to be the major cause for congenital adrenal hyperplasia (CAH), a human inherited disorder. The *CYP21* and its pseudogene, *CYP21P*, locate on chromosome 6, array alternatively with two serum complement genes, the *C4A* and *C4B*, and other duplicated pairs of genes (*XA/XB, XB-S, YA/YB,* and *ZA/ZB*) within the *C4/CYP21* locus. They are all transcribed in the same direction, except the *X* genes. Besides the forth components of the serum complements encoded by the *C4A/C4B* genes, the 21-hydroxylase encoded by the *CYP21*, and the protein homologous to an extracellular matrix encoded by the *XB*, there is no known protein translated from the rest of the duplicated genes within this locus. Surprisingly transcripts from these genes, except those from *C4A/C4B*, *CYP21P* pseudogene, and *XB*, are all expressed in an adrenal-specific manner. Regulation by locus control like regions and possible function in a coordination manner with multiple factors for the adrenal-specific expression of these duplicated genes, are speculated to present within this *C4/CYP21* gene locus.

We have identified several regulatiory sequences farupstream the *CYP21* promoter within this locus to regulate adrenal-specific or steroidogenic-specific expression of the *CYP21* promoter. Gel retardation and footprinting experiments further identified specific DNA sequences interacting with

nuclear proteins in an adrenal-specific or steroidogenic cell-specific manner.

Keywords: adrenal-specific expression, locus control region, human CYP211

二、緣由與目的

The *CYP21* encoding the 21-hydroxylase is located on the short arm of chromosome 6 and is duplicated with its pseudogene, *CYP21P* in tandem with C4 genes (*C4A* and *C4B*) on locus 6p21 (White et al., 1985). Gen alteration from the active *CYP21* sequences to the *CYP21P* sequence accounts for at least 95% of the congenital adrenal hyperplasia (CAH) which is due to the enzymatic deficiency of steroid 21-hydroxylase. Within the human *C4/CYP21* gene locus, several duplicated pairs of genes as well as the *CYP21* genes (*CYP21 and CYP21P*) are found to express in an adrenal-specific manner. It is therefore speculated that an adrenal-specific locus control region within the human *C4/CYP21* locus may be present. We have previously characterized the cis-elements determining the basal promoter of human *CYP21* gene to be within the -166/+1 region upstream from the *CYP21* (Chang and Chung, 1995). This region also confirms the basal promoter activity between the active *CYP21* and the *CYP21P* pseudogene to 8-fold difference, despite the high sequence homology of these two genes within this region. Within the -166/+1 region of the human *CYP21* gene, we have showed that the $^{-104}$G nucleotide is crucial for the expression of its transcription activity, and this may be affected by the interaction with specific nuclear proteins from the adrenal gland (Chin and Chang, 1998). In order to identify the possible LCR within this locus, we have analyzed the influence of DNA fragments upstream the *CYP21P* and *CYP21* genes within the *C4/CYP21* locus on the transcription activity of the *CYP21* basal promoter.

三、成果與討論

Mouse adrenal carcinoma Y1, testicular Leydig MA10 and human hepatocarcinoma HepG2 cell lines are used as the *in vitro* model. We have identified three regions located at 7.5/6.3 kb, 4.6/3.4 kb, and 3.3/2.6 kb upstream the *CYP21* gene to express adrenal cell-specific enhancer activity on *CYP21* basal promoter. Among these regions, DNA fragment at the -4.6/-3.4 kb region showed 4-fold enhanced activity for the *CYP21* basal promoter in adrenal Y1 cells, however, did not change the promoter strength expressed in testicular Leydig MA10 cells, but suppressed the promoter strength in non-steroidogenic HepG2 cells. Furthermore, a small 212 bp fragment upstream the -4.6 kb position which across the promoter of the *ZB* gene expresses inhibitory effect on the *CYP21* promoter activity. From sequence comparison, there is δEF1 transcription factor core binding sequences found within this region, which may interact with another basic helix-loop-helix protein to suppress gene expression.

We have also characterized a 475-bp DNA segment located at about 10 kb upstream the *CYP21P*, spanning from intron 23 to exon 25 region of the *C4A* gene, which exhibited profound adrenal-specific enhancer activity for the basal promoter of both human *CYP21/YB* and *CYP21P/YA* genes. This enhancer activity was further confined to 151-bp region from 10427 to 10275 bp upstream of the *CYP21P*. EMSA experiments indicated that nuclear protein, Sp3, possibly with other factors interacting with the region between the -10346 bp to -10275 bp might be crucial for the expression of this enhancer activity. However, the tissue-specificity, orientation-dependence, and promoter-preference of this enhancer element appeared to be influenced by the presence of neighboring DNA sequences.

四、計畫成果自評

it is rational to speculate that the LCR-like region is located upstream of the pseudogene, *CYP21P* and CYP21 genes. Our study has functionally characterized the regulatory activity of DNA regions within this locus for

the adrenal-specific expression of genes. We have provide some evidences that nuclear proteins from adrenal cells or from steroidogenic cells interacting with specific DNA sequences may be crucial for their regulatory activity. This investigation combined with the previously characterized tissue-specific promoter of this gene may be constructed to a tissue-specific expression vector to allow future gene targeting for gene therapy. However, specific nuclear proteins responsible for this regulatory mechanism need to be further identified and the dynamic chromatin structure within this locus would be another key point to be resolved.

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