行政院國家科學委員會專題研究計畫成果報告

FMR-1 基因調控之下游基因的基因庫建立與選殖

Construction and cloning of cDNA library of *FMR-1* downstream genes

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一、中文摘要

易脆 X 染色體症候群是最常見的遺傳性智能障礙疾病。造成此疾病的主要原因是 *FMR-1* 基因的 5'端不轉譯區的三聯核酸發生擴增突變，導致基因不表現無法做出其基因產物 FMRP 所造成的。FMRP 是一個 RNA 結合蛋白，且其 RNA 的結合是有選擇性的。目前僅知道 *FMR-1* 基因的 mRNA 是其產物的受質，至於細胞內還有那些特定的 mRNA 是其受質則仍不清楚，且 FMRP 結合了 mRNA 之後其作用與目的為何也不甚明瞭。由於易脆 X 染色體症候群主要是細胞中缺乏 FMRP 造成的，因此我們懷疑這些 RNA 受質是否因為缺乏與 FMRP 結合，所以造成疾病？為了瞭解易脆 X 染色體症候群的更深層原因，找出 FMRP 的下游基因是非常重要的！我們在此計劃中以 FMRP 作親合性分析柱，純化出被 FMRP 結合的 mRNA。以這些 mRNA 製造探針來篩選人類胎兒腦部 cDNA 基因庫，初步篩選到 50 多個 clones。

關鍵詞：易脆 X 染色體症候群、*FMR-1*、FMRP

Abstract

The fragile X syndrome is the most common cause of hereditary mental retardation and the second most frequent cause of mental retardation after Down syndrome. The majority of the fragile X syndrome is due to the CGG trinucleotide repeat expansion in the *FMR-1* 5' untranslated region. The expanded CGG repeats will cause methylation of *FMR-1* promoter, leading to suppression of *FMR-1* expression. In general, fragile X syndrome is due to an absence of FMRP production, or the product is dysfunction. FMRP has been demonstrated that it is an RNA binding protein that contains two KH domains and an RGG box domain. It shows selective RNA binding ability but the exact target RNAs (except its mRNA) are unknown. Furthermore, the effect on RNA binding is also unclear. Since the cause of fragile X syndrome is absence of FMRP, we suspect that fragile X syndrome may result from altered translation of transcripts, which normally bind to FMRP. Therefore, it is important to find out the downstream target genes of *FMR-1* to explore the etiology of fragile X syndrome. In this project, we use FMRP-affinity column to purify specific mRNA. By using probe derived from these mRNA, we screened a human fetal brain cDNA library and obtained about 50 candidate clones.

Keywords: Fragile X syndrome, *FMR-1*, FMRP

二、緣由與目的

易脆 X 染色體症候群是造成遺傳性智能不足的主要原因之一，僅次於唐氏症[Gustavson et al., 1986; Webb et al., 1986; Li et al., 1988; Brown et al., 1990]。唐氏症多為偶發性，而易脆 X 染色體症候群為遺傳性。故本症候群之重要性甚至唐氏症。造成易脆 X 染色體症候群的主要原因是位於 *FMR-1* 基因 5'端不轉譯區的 CGG 三聯核酸發生擴增突變，導致 *FMR-1* 基因不表現所致[Fu et al., 1991; Oberlé et al., 1991; Verkerk et al., 1991; Heitz et al., 1991;

Kremer et al., 1991; Wang et al., 1993]。亦有少數原因是 *FMR-1* 基因缺失[Gedeon et al., 1992; Wöhler et al., 1992; Tarleton et al., 1993; Gu et al., 1994; Meijer et al., 1994; Lugenbeel et al., 1995]及點突變[DeBoulle et al., 1993; Wang et al., 1997]。基本上而言，缺乏 *FMR-1* 的產物–FMRP–是造成易脆 X 染色體症候群的主要因素。

目前的文獻顯示，FMRP 是一個 RNA 結合蛋白，它具有兩個 KH domains 及由兩個 RGG box 所組成的 RGG box domain [Ashley et al., 1993; Siomi et al., 1993 and 1994]。FMRP 可與本身、FXR1P、FXR2P 形成 homo-或 heterodimer [Zhang et al., 1995]。在 Ashley 的實驗中，作者們證明了 FMRP 不僅能和 RNA 結構，且其結合是有選擇性的。FMRP 與 mRNA 之結合作用與 mRNA 的轉譯有關[Tamanini et al., 1996; Eberhart et al., 1996; Corbin et al., 1997; Feng et al., 1997]，但真正的目的與機制仍有待研究。我們認為找出這些受 FMRP 調節的下游基因，將是對易脆 X 染色體症候群成因之探討，更向前且深入的邁開大步。本計劃的目的就是以 FMRP 做成親合性分析柱，用以分離特定 mRNA 並建立基因庫，以利將來從此基因庫中選殖出 mRNA 能與 FMRP 結合的特定基因。

三、結果與討論

依據廠商提供的資料，製備 cDNA 基因庫所需的 mRNA 至少要 5μg。以我們的構想從 total RNA 中以 FMRP affinity column 純化出這麼多量的 specific mRNA，在實際操作上有相當的困難存在。固我們改變原來的策略，將 FMRP 抓下來的 RNA 以 oligo-dT 為 primer 合成 cDNA，再以 24-mer 之 random primer (New England BioLab)進行第一次之 PCR 放大(反應體積 30 μl)，然後取 2 μl PCR 產物進行第二次放大，反應同時加入α-^{32}P-dCTP 以標定 DNA。以此標定的 DNA 為探針，去選殖人類胎兒腦部的 cDNA 基因庫 λTriplEx (CLONTECH)。經過兩次的篩檢，我們初步獲得 50 幾個 clones，目前正在進一步確認及分析序列中。

我們雖然改變原有策略，但實務上我們卻能更方便更容易達到原有的目標。整個過程中最不易做的就是 probe 的製備，因為需經過 affinity column 的純化之後，所得到的 mRNA 就已經不多，再加上 reverse transcription 及 random PCR 後，才能得到 probe，所以 probe 可謂得之不易！我們初步獲得的 clones，正進一步分析中。雖然 clone 的數目不是太多，但要一個一個做 *in vitro* transcription (bi-direction)，然後與 FMRP 做 binding assay 來進一步確認，確實也很耗費時間。所幸我們選用的載體在適當的 host cell 內能轉換成質體，省卻了 subclone 的步驟，方便後續實驗的進行。所以只要 FMRP binding assay 確認的 clone，我們便能很快的進行 DNA 定序及蛋白表現。

四、成果自評

本實驗之成果我們還算滿意，至少經過調整策略後避開自己製備 FMRP specific binding cDNA library 這最困難的關卡，進而獲得 candidate clones，也算達成本計劃之基本目標了！

五、參考文獻

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