行政院國家科學委員會補助專題研究計畫 ■ 成果報告
□期中進度報告

鑑定與研究哺乳類 hCDC5p pre-mRNA 剪接因子的作用蛋白

Identification and study for the interaction proteins of human hCDC5p pre-mRNA splicing factor

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一、中文摘要

關鍵詞：哺乳類 pre-mRNA 剪接反應、hCDC5p、轉錄因子、Prox1、C/EBPδ、HARS1

Cef1p/Ntc85p 是酵母菌 *Saccharomyces cerevisiae* 的一個必需剪接蛋白(splicing factor)，同時會形成一個至少含有 8 個蛋白質的 Prp19p 複合體。人類 hCDC5p 與酵母菌 *Schizosaccharomyces pombe* CDC5p 是酵母菌 *S. cerevisiae* Cef1p/Ntc85p 的功能相似體。所以像 Cef1p/Ntc85p，hCDC5p 與 CDC5p 也都是 pre-mRNA 剪接蛋白；另外 CDC5p 與 hCDC5p 也會形成巨大的蛋白複合體。因此經由酵母菌 two-hybrid 分析，我們利用 hCDC5p 當作探針，可以來尋找 hCDC5p 的結合蛋白。

我們總共進行兩次 Liver cDNA 基因庫的篩選，篩選了 3×10^5 個質體，目前篩選出 12 個菌落，其中並沒有基因跟 pre-mRNA 剪接反應有直接關聯，但是有 3 個基因是轉錄因子(transcription factor): Prox1、C/EBPδ與 HARS1。5 個基因與蛋白運送有關，4 個基因功能未知。經由酵母菌 two-hybrid 分析，證實 hCDC5p 與 3 個轉錄因子: Prox1、C/EBPδ與 HARS1 確實可以特定性結合；同時證實 C/EBPδ 和 HARS1 的 leucine dimerization domain 是負責與 hCDC5p 的結合。這些結果與 cDNA 基因庫的篩選是相似的。所以 hCDC5p 和這些與 hCDC5p 結合的轉錄因子將是研究基因的轉錄與剪接反應彼此如何關聯的有利工具。

二、英文摘要

Keywords: mammalian pre-mRNA splicing, hCDC5p, transcription factor, Prox1, C/EBPδ, HARS1

The Cef1p/Ntc85p protein of the budding yeast *Saccharomyces cerevisiae* is an essential splicing factor and is associated with the Prp19p-associated complex consisting of at least eight protein components. Cef1p/Ntc85p is highly homologous to human hCDC5p and fission yeast *Schizosaccharomyces pombe* CDC5p with 48% identity. Like Cef1p/Ntc85p, human hCDC5p and *S. pombe* Cdc5p are also required for pre-mRNA splicing and both Cef1p/Ntc85p and Cdc5p form the similar large protein complex. Therefore, human hCDC5p will be used as a useful probe to identify novel mammalian splicing factors by yeast two-hybrid assays.

3×10^5 clones were screened from human liver cDNA library. So far, 12 clones were isolated. No clone has the known function related to pre-mRNA splicing. However, three transcription factors, Prox1, C/EBPδ, and HARS1, were isolated, five

clones are related to the function for protein translocation across the nuclear membrane and four clones have the unknown function. Full length of Prox1p interact with hCDC5p, however, full length of C/EBPδ and HARS1 have very weak interaction with hCDC5p by yeast two-hybrid assay. Different truncated proteins of C/EBPδ and HARS1 were constructed and leucine dimerization domain in both proteins has clear interaction with hCDC5p. These results are similar with those in the liver library screening. Therefore, these hCDC5p-interacting transcription factors will be useful tools to study the coupling mechanism between the transcription and the pre-mRNA splicing of genes.

三、緣由與目的

The pre-mRNA splicing reaction takes place in two catalytic steps within the spliceosome, a large multi-protein-snRNA complex that assembles in a stepwise pathway (1, 2, 3, 4, and 5). The comparison of these splicing factors in the two yeast species and humans indicates that most of the splicing factors have been evolutionarily conserved (6). Like Cef1p/Ntc85p, human hCDC5p and *S. pombe* Cdc5p are also required for pre-mRNA splicing (7, 8, and 9). Therefore, recent studies indicate that Cef1p/Ntc85p, PCDC5RP and Cdc5p are functional homolog. Furthermore, both *S. cerevisiae* Cef1p/Ntc85p and *S. pombe* Cdc5p form the similar large protein complex (10, 11, 12, 13, 14 and 15).

Previous studies, indicate that human hCDC5p maybe form the similar large protein complex. Human hCDC5p-associated proteins will be investigated to discover the detailed mechanism of pre-mRNA splicing. Yeast two-hybrid system was utilized to clone the human hCDC5p-associated proteins, and because little regulatory splicing factors were identified in mammalian cells with the exception of snRNPs and SR proteins, we hope that these novel mammalian splicing factors can be identified and act as useful tools to study the detailed mechanism of mammalian pre-mRNA splicing.

Human hCDC5p is required for the pre-mRNA splicing reaction (12, 13, and 14). In addition to the function, it may be a transcription factor for gene regulation and maybe function in cellular mitosis (16 and 17). However, the function of hCDC5p in gene transcription and cell division is still unknown. If the hCDC5p-interacting factors are transcription factors, they will be useful tools to study the coupling mechanism between the transcription and the pre-mRNA splicing of genes.

四、結果與討論

Human hCDC5p was used as a useful probe to identify the interacting proteins by yeast two-hybrid assays and human liver cDNA library was screened twice. 5×10^3 clones were isolated on -Trp, -Leu, -His, -Ade selection plates from 3×10^5 clones. So far, 12 clones were isolated: 2 clones have the strong blue reaction, 6 clones have the middle blue reaction, and 4 clones have the weak blue reaction. These 12 isolated clones were sequenced and identified from human genome data bank.

No clone has the known function related to pre-mRNA splicing. However, three transcription factors, Prox1, C/EBPδ, and HARS1, were isolated (19, 25, and 29), five clones are related to the function for protein translocation across the nuclear membrane and four clones have the unknown function. So far, these DNA fragments of three transcription factors, Prox1, C/EBPδ, and HARS1, containing speculated ORFs were isolated from the human liver cDNA library, and confirmed by DNA sequencing. Both Prox1and C/EBPδ genes have the same DNA sequences with those in human genome data bank. However, Three types of HARS1 cDNAs were isolated, and their properties will be investigated.

Yeast two-hybrid assays found that full length of Prox1p interact with hCDC5p, however, full length of C/EBPδ and HARS1 have very weak interaction with hCDC5p. Different truncated proteins of C/EBPδ and HARS1 were constructed and leucine dimerization domain in both proteins has clear interaction with hCDC5p. These results are similar with those in the human liver cDNA library screening. Different truncated proteins of hCDC5p are being constructed, and the interaction domain of hCDC5p with leucine dimerization domain will be investigated.

Human hCDC5p is required for pre-mRNA splicing (12, 13, and 14). In addition to the function, it may be a transcription factor for gene regulation and maybe function in cellular mitosis (16 and 17). However, the function of hCDC5p in gene transcription and cell division is still unknown. C/EBPδ is a member of the C/EBP family of transcription factors that bind to specific DNA sequences as homo- and heterodimers and regulate the transcription of target genes involved in proliferation and differentiation (18, 19, 20, 21, 22, 23, 24). All C/EBPδ family members share a strong homology in the carboxyl-terminus, leucine dimerization domain and basic DNA-binding domain. The homeobox gene Prox1 was originally cloned by homology to the *Drosophila melanogaster* gene *prospero*. Functional inactivation of the Prox1 gene in mice leads to embryonic lethality and phenotypic alterations of the lens and liver (25), and Prox1 activity is required for both maintenance of the budding of the venous endothelial cells and differentiation toward the lymphatic phenotype (26, and

27). Hepatocyte migration during liver development also requires Prox1 (28). HARS1 has the leucine dimerization domain in the carboxyl-terminus, however, its function is unknown (29).

No clone has the known function related to pre-mRNA splicing in liver cDNA library screening. However, three transcription factors, Prox1, C/EBPδ, and HARS1, were isolated, and they appear the interaction with splicing factor hCDC5p. These results indicate that hCDC5p may have dual functions: a splicing factor for pre-mRNA splicing and a transcription factor for gene regulation.

五、計畫成果自評

During this year some results have been obtained. Human hCDC5p was used as a useful probe to identify novel mammalian splicing factors by yeast two-hybrid assays in this report. Human liver cDNA library was screened twice, 12 clones were isolated and identified from human genome data bank. Full length of DNA fragments for three transcription factors, Prox1, C/EBPδ, and HARS1, were isolated, and they appear the interaction with splicing factor hCDC5p. These results indicate that hCDC5p may be a transcription factor for gene regulation.

These results give the important information to continue the project "The identification and study for the interaction proteins of human hCDC5p pre-mRNA splicing factor". If the hCDC5p and hCDC5p-interacting factors are transcription factors, they will be useful tools to study the coupling mechanism between the transcription and the pre-mRNA splicing of genes (30).

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