行政院國家科學委員會補助專題研究計畫成果報告

※※※※※※※※※※※※※※※※※※※※※※※※※※
※　　　　　　　　　　　　　　　　　　　　　　　※
※　鑑定新的哺乳類 pre-mRNA 剪接因子　※
※　The identification of novel mammalian pre-mRNA　※
※　splicing factors　※
※　　　　　　　　　　　　　　　　　　　　　　　※
※※※※※※※※※※※※※※※※※※※※※※※※※※

計畫類別：■個別型計畫　□整合型計畫

計畫編號：NSC 90－2311－B－040－005－

執行期間：　90 年　8 月　1 日至　91 年　7 月　31 日

計畫主持人：蔡　維　育

本成果報告包括以下應繳交之附件：

□赴國外出差或研習心得報告一份

□赴大陸地區出差或研習心得報告一份

□出席國際學術會議心得報告及發表之論文各一份

□國際合作研究計畫國外研究報告書一份

執行單位：　中山醫學大學醫事技術系

中　華　民　國　91　年　10　月　28　日

一、中文摘要

關鍵詞：哺乳類 pre-mRNA 剪接反應、Prp19p 複合體、Cef1p/Ntc85p、hCDC5p

真核細胞的剪接體(spliceosome)是經過一系列有次序性步驟的過程，組合而成的核醣核酸蛋白複合體(ribonucleoprotein particle)；而 pre-mRNA 剪接反應(splicing reaction)是發生在剪接體上，經過兩步驟催化反應的過程。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 HeLa cDNA 基因庫的篩選，篩選了 3×10^5 個質體，目前我們篩選出 24 個菌落，其中有 5 個菌落有很強的藍色呈色反應，12 個菌落有強的藍色呈色反應，7 個菌落有弱的藍色呈色反應。有很強的藍色呈色反應的 5 個含基因的 pACT2 質體先被 DNA 定序，其中一個基因功能未知，四個基因有已知的功能，但是只有一個基因跟 pre-mRNA 剪接反應有關。這個研究計劃我們希望可以經由新的哺乳類 pre-mRNA 剪接蛋白的鑑定，讓我們對哺乳類 pre-mRNA 剪接反應有更完整的認識。

二、英文摘要

Keywords：mammalian pre-mRNA splicing, Prp19p-associated complex, Cef1p/Ntc85p, hCDC5p

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. 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 HeLa cDNA library. So far, 24 clones were isolated: 5 clones have the strong blue reaction, 12 clones have the middle blue reaction, and 7 clones have the weak blue reaction. 5 strong blue of clones were sequenced: one clone is unknown gene and four clones are known genes. However, only one clone has the known function related to pre-mRNA splicing. Finally, we hope that some novel mammalian splicing factors can be identified and act as useful tools to study the detailed mechanism of mammalian pre-mRNA splicing.

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, and 14).

So far little regulatory splicing factors were identified in mammalian cells with the exception of snRNPs and SR proteins. Recently the functional spliceosome was isolated and the spliceosome-associated proteins were identified (15). In order to discover the molecular mechanism of mammalian pre-mRNA splicing, identification of novel mammalian splicing factor is a useful tool to solve the problem. Therefore, human hCDC5p-associated proteins will be investigated to discover the detailed mechanism of pre-mRNA splicing and yeast two-hybrid system will be utilized to clone the human hCDC5p-associated 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.

四、結果與討論

Yeast two-hybrid assays were carried out to identify novel mammalian splicing factors in the report. The DNA sequence of human hCDC5p can be obtained from data bank, and two oligonucleotides containing the 5' sequence and 3' sequence of hCDC5p ORF were synthesized to isolate the interested clones, hCDC5p, from HeLa cDNA library by PCR. Human hCDC5p has been isolated by PCR, and the DNA fragment containing hCDC5p ORF was sequenced and confirmed. The DNA fragment containing hCDC5p ORF was fused to the GAL4 DNA binding domain in plasmid pAS2-1 and the GAL4 activation domain in plasmid pACT2, and each pair of plasmids was transformed into yeast strain AH109 containing the β-galactosidase reporter gene. Two-hybrid assays were carried out and performed very well because of the self interaction of hCDC5p as the positive control.

Human hCDC5p was used as a useful probe to identify the interacting proteins by yeast two-hybrid assays and HeLa cDNA library was screened twice. 5×10^3 clones were isolated on -Trp, -Leu, -His, -Ade selection plates from 3×10^5 clones and 78 clones were picked up on X-gal plates by blue color. The plasmids, pACT2-cDNA, were isolated from yeast AH109, and electroporated into *E. coli* MC1061, and then confirmed with restriction enzyme digestion. Finally, these plasmids, pACT2-cDNA, were retransformed into AH109 and reconfirmed the specific

interaction with hCDC5p on X-gal plates.

So far, 24 clones were isolated: 5 clones have the strong blue reaction, 12 clones have the middle blue reaction, and 7 clones have the weak blue reaction. 5 strong blue of clones were sequenced and 12 middle blue of clones are going to be sequencing. One clone is an unknown gene and four clones are known genes. However, only one clone has the known function related to pre-mRNA splicing. These specific interactions between these identified clones and hCDC5p will be confirmed by Far Western blotting and co-immunoprecipitation assay, and whether these hCDC5p-associated components function as the pre-mRNA splicing factors in mammalian cell will be examined, too.

五、計畫成果自評

Human hCDC5p was used as a useful probe to identify novel mammalian splicing factors by yeast two-hybrid assays in this report. HeLa cDNA library was screened twice, 24 clones were isolated, 5 strong blue of clones were sequenced, and only one clone has the known function related to pre-mRNA splicing.

During this year some results have been obtained, and these results give the important information to continue the project "The identification of novel mammalian pre-mRNA splicing factors". We hope that these novel mammalian splicing factors can act as useful tools to study the detailed mechanism of mammalian pre-mRNA splicing.

六、參考文獻

1. Sharp, P. A. (1994) Split genes and RNA splicing. *Cell*, **77**, 805-815.
2. Green, M. R. (1986) Pre-mRNA splicing. *Annu. Rev. Genet.*, **20**, 671-708.
3. Ruby, S. W., and Abelson, J. (1991) Pre-mRNA splicing in yeast. *TIG*, **7**, 79-85.
4. Staley, J. P., and Guthrie, C. (1998) Mechanical devices of the spliceosome: motors, clocks, springs, and things. *Cell*, **92**, 315-326.
5. Padgett, R. A., Grabowski, P. J., Konarska, M. M., Seiler, S. R., and Sharp, P. A. (1986) Splicing of messenger RNA precursors. *Annu. Rev. Biochem.*, **55**, 1019-1050.
6. Kaufer, N.F.. and Potashkin J. (2000) Analysis of the splicing machinery in fission yeast: a comparison with budding yeast and mammals. *Nucleic Acids Research,* **28**, 3003-10
7. Tarn, W.-Y., Lee, K.-R., and Cheng, S.-C. (1993a) The yeast PRP19 protein is not tightly associated with small nuclear RNAs, but appears to associate with the spliceosome after binding of U2 to the pre-mRNA and prior to formation of the functional spliceosome. *Mol. Cell. Biol.*, **13**, 1883-1891
8. Tarn, W.-Y., Hsu, C.-H., Huang, K.-T., Chen, H.-R., Kao, H.-Y., Lee, K.-R., and Cheng, S.-C. (1994) Functional association of essential splicing factor(s) with PRP19 in a protein complex. *EMBO J.*, **13**, 2421-2431.

9. Tsai,W.-Y., Chow, Y.-T., Chen, H.-R., Huang, K.-T., Hong, R.-I, Jan, S.-P., Kuo, N.-Y., Tsao, T. Y., Chen, C.-H. and Cheng, S.-C. (1999) Ceflp is a component of the Prp19p-associated complex and essential for pre-mRNA splicing. *J. Biol. Chem.* **274**, 9455-9462.

10. Ohi, R., McCollum, D., Hirani, B., Den Haese, G. J., Zhang, X., Burke, J.D., Turner, K., and Gould, K.L. (1994) The Schizosaccharomyces pombe **cdc5**+ gene encodes an essential protein with homology to c-Myb. *EMBO Journal.* **13**, 471-483

11. Ohi, R., Feoktistova, A., McCann, S., Valentine, V., Look, AT., Lipsick, JS., and Gould, KL. (1998) Myb-related Schizosaccharomyces pombe cdc5p is structurally and functionally conserved in eukaryotes. *Molecular & Cellular Biology.* **18**, 4097-4108

12. Bernstein, H.S., and Coughlin, S.R. (1997) Pombe Cdc5-related protein. A putative human transcription factor implicated in mitogen-activated signaling. *Journal of Biological Chemistry.* 272, 5833-5837

13. Bernstein, HS., and Coughlin, SR. (1998) A mammalian homolog of fission yeast **Cdc5** regulates G2 progression and mitotic entry. *Journal of Biological Chemistry.* **273**, 4666-4671

14. Burns, CG., Ohi, R., Krainer, AR., and Gould, KL. (1999) Evidence that Myb-related CDC5 proteins are required for pre-mRNA splicing. *Proceedings of the National Academy of Sciences of the United States of America.* **96**, 13789-13794

15. Zhou, Z., Licklider, L. J., Gygi, S. P., and Reed, R. (2002) Comprehensive proteomic analysis of the human spliceosome. *Nature*, **418**, 182-185