行政院國家科學委員會專題研究計畫　成果報告

剪接因子 hCDC5p 與轉錄因子 C/EBP delta 和 Prox1p 間的功能研究

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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、C/EBPδ、HRAS1

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。

經過兩次 Liver cDNA 基因庫的篩選，篩選了 3×10^5 個質體，目前篩選出 3 個基因是轉錄因子(transcription factor): Prox1、C/EBPδ與 HRAS1。hCDC5p、C/EBPδ與 HRAS1 的 truncated 蛋白被構築，經由酵母菌 two-hybrid 分析，證實 hCDC5p 與 2 個轉錄因子: C/EBPδ與 HRAS1 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，雖然全長的 C/EBPδ與 HARS1 與 hCDC5p 的結合不明顯。這些結果與 cDNA 基因庫的篩選是相似的。而 leucine dimerization domain 有時被歸入 coiled coil domain，初步的結果顯示 hCDC5p 的 N 端與 C 端都有 coiled coil domain，兩個 coiled coil domains 會結合，可能調節 hCDC5p 的活性。同時 hCDC5p 有兩個區域可以活化基因的表現，雖然全長的 hCDC5p 活化基因的表現不明顯， 因此 hCDC5p 似乎經由兩個 coiled coil domains 進行自我抑制的機制；所以 hCDC5p 應該也是一個轉錄因子。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, C/EBPδ, HRAS1

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, three transcription factors, Prox1, C/EBPδ, and HRAS1, were isolated. Different truncated proteins of hCDC5p, C/EBPδ and HRAS1 were constructed and leucine dimerization domain in both proteins has clear interaction with hCDC5p by yeast two-hybrid assay. However, two full lengths of C/EBPδ and HRAS1 have very weak interaction with hCDC5p. These results are similar with those in the liver library screening. Leucine dimerization domain is also called as coiled coil domain, and it seems to interact with another coiled coil domain at the N-terminus of hCDC5p by yeast two-hybrid assay. Although full length of hCDC5p can not activate the gene expression, the transactivation activities in both N-terminus and C-terminus of hCDC5p were observed indicating that hCDC5p probably contains more than one transactivation domain. These results indicate that the interaction between two coiled coil domains of hCDC5p maybe regulates the activity of hCDC5p and behaves a self inhibition for the repression of gene transcription. Therefore, hCDC5p may be a transcription factor and 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 forms 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.

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, three transcription factors, Prox1, C/EBPδ, and HRAS1, were isolated (19, 25, and 29) and these DNA fragments of three transcription factors containing speculated ORFs were isolated from the human liver cDNA library, and confirmed by DNA sequencing. Both genes, Prox1and C/EBPδ, have the same DNA sequences with those in human genome data bank. However, three types of HARS1 cDNAs were isolated.

The specific interaction among hCDC5p and three transcription factors, Prox1, C/EBPδ, and HRAS1, will be investigated by yeast two-hybrid assay. Different truncated proteins of hCDC5p, C/EBPδ, and HRAS1 will be constructed to define the interaction domain in these proteins. If the specific interactions among these proteins are confirmed, these results will imply the important information about the coupling interaction between gene transcription and pre-mRNA splicing.

四、結果與討論

Different truncated proteins of hCDC5p, C/EBPδ and HRAS1 were constructed and leucine dimerization domain in both proteins, C/EBPδ and HRAS1, has clear interaction with hCDC5p by yeast two-hybrid assay. However, full lengths of C/EBP and HRAS1 have very weak interaction with hCDC5p. These results are similar with those in the liver library screening and due to the two possible reasons: the first possibility is that the other regions of these proteins interrupt or block the interaction domains with hCDC5p. The second possibility is that the specific interaction with hCDC5p is regulated by the posttranslational modification of hCDC5p (e.g. phosphorylation).

Although full length of hCDC5p can not activate the gene expression, the transactivation activities in both N-terminus and C-terminus of hCDC5p were observed indicating that hCDC5p probably contains more than one transactivation domain and may be a transcription factor. These results also indicate that hCDC5p maybe contains an inhibition mechanism for the repression of gene transcription.

Recently two coiled coil domains exist at both N and C terminus of hCDC5p, and two domains interact with each other by yeast two-hybrid assay. Leucine dimerization domain sometimes is called as coiled coil domain, and it can interact with coiled coil domain. Therefore, full length of hCDC5p does not activate the gene expression; however, truncated hCDC5p activates the gene expression. These results indicate that both coiled coil domains of hCDC5p interact, form a special conformation, and block the activity of transactivation at gene transcription.

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, and 24). All C/EBPδ family members share a strong homology in the carboxyl-terminus, leucine dimerization domain and basic DNA-binding domain. HRAS1 has the leucine dimerization domain in the carboxyl-terminus, however, its function is unknown (25).

Two transcription factors, 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. Different truncated proteins of hCDC5p, C/EBPδ, and HRAS1 were constructed and leucine dimerization domain in both proteins, C/EBPδ and HRAS1, has clear interaction with hCDC5p. The transactivation activities in both N-terminus and C-terminus of hCDC5p were observed indicating that hCDC5p may be a transcription factor for gene regulation.

Recently two coiled coil domains exist at both N and C terminus of hCDC5p, and two domains interact with each other by yeast two-hybrid assay. Therefore, full length of hCDC5p does not activate the gene expression; however, truncated hCDC5p activates the gene expression. The regulation of transcription activity at hCDC5p maybe is through the intramolecular interaction of two coiled coil domains.

These results give the important information to continue the project “Functional study between splicing factor hCDC5p and transcription factors C/EBP delta and Prox1p”. 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 (26).

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) Cef1p 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

16. Bernstein HS. Coughlin SR. (1997) Pombe Cdc5-related protein. A putative human transcription factor implicated in mitogen-activated signaling. *Journal of Biological Chemistry.* 272, 5833-5837

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

18. Tahirov TH, Sato K, Ichikawa-Iwata E, Sasaki M, Inoue-Bungo T, Shiina M, Kimura K, Takata S, Fujikawa A, Morii H, Kumasaka T, Yamamoto M, Ishii S, Ogata K. (2002) Mechanism of c-Myb-C/EBP beta cooperation from separated sites on a promoter. *Cell*, **108**, 57-70.

19. Kinoshita S, Akira S, Kishimoto T. (1992) A member of the C/EBP family, NF-IL6 beta, forms a heterodimer and transcriptionally synergizes with NF-IL6. *Proceedings of the National Academy of Sciences of the United States of America.* **89**, 1473-1476

20. Parkin SE, Baer M, Copeland TD, Schwartz RC, Johnson PF. (2002) Regulation of CCAAT/enhancer-binding protein (C/EBP) activator proteins by heterodimerization with C/EBPgamma (Ig/EBP). *Journal of Biological Chemistry.* **277**, 23563-72

21. Cortes-Canteli M, Pignatelli M, Santos A, Perez-Castillo A. (2002) CCAAT/enhancer-binding protein beta plays a regulatory role in differentiation and apoptosis of neuroblastoma cells. *Journal of Biological Chemistry.* **277**, 5460-7

22. Rooney JW, Calame KL. (2001) TIF1beta functions as a coactivator for C/EBPbeta and is required for induced differentiation in the myelomonocytic cell line U937. *Genes Dev*, **15**, 3023-38

23. Oelgeschlager M, Kowenz-Leutz E, Schreek S, Leutz A, Luscher B. (2001) Tumorigenic N-terminal deletions of c-Myb modulate DNA binding, transactivation, and cooperativity with C/EBP. *Oncogene*, **20**, 7420-4

24. Charles A, Tang X, Crouch E, Brody JS, Xiao ZX. (2001) Retinoblastoma protein complexes with C/EBP proteins and activates C/EBP-mediated transcription. *J Cell Biochem*, **83**,414-25

25. Weitzel JN, Kasperczyk A, Mohan C, Krontiris TG. (1992) The HRAS1 gene cluster: two upstream regions recognizing transcripts and a third encoding a gene with a leucine zipper domain. *Genomics*, **14**, 309-19

26. Nick, j. P., Andre, F., and Michael, J. D. (2002) Integrating mRNA processing with transcription. *Cell*, **108**, 501-512