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• 中文關鍵字	輔促進因子; 動力學; 構造; 穩定度
• 英文關鍵字	Coactivator; Dynamics; Structure; Stability
• 中文摘要	人類輔促進子 PC4 由 127 個氨基酸所組成,其 N-端含有一段 Serine-rich 區域,爲常見之增強 Class II genes 轉錄活性之輔促進因子。PC4 能與很多種蛋白質及 DNAs 作用,並在需要促進子之轉錄作用過程中扮演重要的角色。它的活性並受磷酸化作用所調節。爲了進一步了解 PC4 在轉錄活化所扮演的角色、磷酸化調節轉錄之機制及分子間相互辨識及交互作用之結構因子,研究未磷酸化及磷酸化 PC4 之結構、穩定度及可彎性是必需的。而研究結構,首先必需要有純度高且大量的 PC4。在這個計畫的第一個年度,我們致力於 Recombinant PC4 cDNA 之選殖、蛋白質之純化及其特性分析。第二個年度,我們進行化學位移之確認、結構之計算、及穩定度、水合之研究等。明年將繼續完成之。 PC4 cDNA 由 HeLa cDNA library 利用 PCR 放大技術取得。Recombinant PC4 protein 由養殖帶有 pET11a/PC4 plasmid 之 E. coli 細菌,表達及純化得之,或由養殖含 pGEX-2T/GST-PC4 plasmid 之 E. coli,表達 fusion protein,再以 thrombin 切割 GST-PC4 fusion protein,分離及純化得之。所得之 recombinant PC4 protein,再進一步做活性之確認及與它種蛋白質行交互作用之鑑定。Recombinant PC4 像 native PC4 一樣,具有加強 GAL-AH 活化轉錄作用之能力及與 VP16 acidic domain 接合、加強活化作用之特性。Recombinant protein 確實具有與 native protein 相同之活性。 在各種不同 nmr 圖譜分析、確認各殘基之化學位移及取得距離及角度後,使用 distance geometry (如 DGII)等電腦軟體來計算蛋白質之三度空間立體結構,並在不同之溫度及 pH 值及變性劑下,研究蛋白質之穩定度。高解析度三度空間立體結構計算、穩定度、水合之研究,依進度,明年度將繼續完成 (grant (III))。
• 英文摘要	Human positive cofactor (PC4) containing a 127 amino acids with serine-rich regions near the N-terminus, is a general coactivator that enhances transcriptional activation of class II genes. PC4 interacts with a diverse array of proteins and DNAs and plays an important functional role in activator-dependent transcription during DA complex formation. Its coactivation function is medicated by phosphorylation. In order to better understand the

functional role of PC4 in transcription activation and the process regarding the mediation of transcriptional regulation by phosphorylation and the structural basis of molecular recognition and interactions, the structure, stability and flexibility of unphosphorylated and phosphorylated PC4 needed to be studied. For structure studies, the first step is obtaining the pure and enough amount of PC4. In the first year's work of this grant, we were focusing on cloning, purification and characterization of recombinant PC4. In the second year's work, we were focusing on NMR data collection and chemical resonance assignments, three-dimensional structure determination and protein stability, and hydration studies. PC4 cDNA was obtained from amplification by PCR from a HeLa cDNA library. The recombinant PC4 protein was obtained by growing E. coli bacteria harboring pET11a/PC4 in media or obtained by thrombin-cut GST-PC4 fusion protein expressed in E. coli bacteria with a vector (pGEX-2T) encoding a GST-PC4 fusion protein. Several functional and protein-protein interaction assays of recombinant PC4 protein were done in order to confirm that the recombinant proteins retain the function of native protein. These results indicate that recombinant PC4 was functionally equivalent to native PC4, both proteins markedly enhance GAL4-AH-activated transcription and can bind VP16 acidic activation domain to enhance activation. Various NMR data were collected and analyzed. Three-dimensional structures of protein were calculated from structure constraint data using structure-computing program such as distance geometry (DGII) (MSM, Insight II). Protein stability was studying upon a reversible denaturation of protein at extreme conditions of temperature and pH etc. NMR spectroscopy, circular dichroism were used to monitor the denaturation curves. High resolution solution structure determination, stability and hydration studies are continuing to be finished next year (grant (III)).