SARS 冠狀病毒核鞘蛋白之雙聚合區域之結晶結構分析與其包裹病毒 RNA 之機制探討

Structure of the SARS Coronavirus Nucleocapsid protein Dimerization Domain: Implications for Helical Packaging of Viral RNA

中文摘要

嚴重急性呼吸道症候群（Severe Acute Respiratory Syndrome, SARS）爆發於西元 2002 年底，是一種有潛力造成全球風險的傳染性疾病。 造成嚴重急性呼吸道症候群的致病原爲一新品種的冠狀病毒（Coronavirus）。核鞘蛋白（Nucleocapsid）是是 SARS 冠狀病毒中最主要的結構蛋白，具有兩個功能區域： N 端的 RNA 結合區域(RNA-binding domain, RBD)以及 C 端的雙聚合區域(Dimerization domain, DD)，兩功能區域間以一無結構的片段連結。根據本論文的實驗結果，雙聚合區域可與單股的 RNA 以及 DNA 結合，且其結合的親和力強過原本所定義的 RNA 結合區域。在本論文中，我們也解出了雙聚合區域的結晶結構，其解析度可到 2.5 ?。此雙聚合體區域的結晶結構，在每一個晶體的非對稱單元中含有四個雙聚體（dimer），四個雙聚體可環繞成一個八聚體（octamer）的中空環狀結構。從晶體排列的角度來看，接連排列的八聚體可形成一個左手旋的雙股螺旋結構，此雙股螺旋結構具有兩個平行且帶正電的凹槽環繞於其上，爲可能的 RNA 纏繞位置。根據我們解出的結構，可進一步的瞭解核鞘蛋白如何快速且有效的包裹病毒 RNA的機制。更可讓我們對冠狀病毒的ribonucleoprotein的構性有更深入的認識。

英文摘要

The human promyelocytic leukemia HL-60 cells can be induced to differentiation toward macrophage by treatment with PMA. However, very little is known regarding the early events that control differentiation of HL-60 by PMA. In this report, we demonstrated that PMA (10nM) treatment results in cell cycle arrest in G1 phase. Addition of PMA stimulated PKCα translocation in 5 minutes andthe maximum response was seen 30 minutes after PMA was added. Upon activation,PKCα is gradually degraded. The PKCα down regulation was observed within 24hours after PMA treatment. The PKCα translocation is associated withRb underphosphorylation. Rb underphosphorylation was apparent at 5 minutes and gradually disappeared within 24 hours, consistent with the time course of PKCα activation. Because Rb

phosphorylation can be regulated by D type cyclins andCDK4, we also examinedthe expression of D type cyclins, CDK4 and CDK4 inhibitors(p21cip1, p16INK4a) in HL-60 cells after PMA treatment. Cyclin D3 but not cyclinD1 or Cyclin D2 was expressed 10hrs after PMA addition. However, expression of both CDK4 and CDK4 inhibitors(p21cip1, p16INK4a) were not affected by PMAtreatment. Although we can not directly prove that CDK4, p21 cip1 and p16INK4a are regulated by PKCα. According to what Zang et al. (1995)found, PMA inducingHL-60 differentiateinto macrophage, was probably due to PKCα activation, and the induction of CDK4 inhibitor p21cip1, followed by Rb dephosphorylation, and eventually thecell cycle arrested at G1 and committed cell differentiation.