中文摘要

Human NADH dehydrogenase (ubiquinone) Fe-S protein 7 (NDUFS7) 為人類粒線體中第一酵素複合體 44 個次單元當中的其中一個次單元。NDUFS7 序列 1-60 的胺基酸被認定為一段有效的 mitochondrial targeting sequences (MTS)，我們也發現此蛋白質 C 端末尾序列含有一段可能的 nuclear localization signal (NLS) 以及一段可能的 nuclear export signal (NES)。NDUFS7 作為粒線體第一酵素複合體中電子傳遞鍊的末端，有一特殊的胺基酸序列(motif): $CCXXE(X)_{60}C(X)_{30}CP$，其在各種物種上具有高度的保留性，此 motif 能夠與帶有 N2 [4Fe-4S] cluster 的鐵硫中心相接，成為重要的氧化還原中心。在先前的研究中我們發現 NDUFS7 為 sumoylation 的受質。Sumoylation 為一種轉譯後修飾，SUMO 蛋白會透過 E1 酵素的活化與 E2 酵素 (UBC9) 相接，再透過 E3 酵素或者獨立將 SUMO 蛋白轉移到受質上。Sumoylation 的相關研究指出，受 sumoylation 修飾的蛋白能參與相當多樣的細胞生物過程，例如:蛋白質的運輸、活性、及對不同壓力的反應等。

在此研究當中，我們讓 NDUFS7 蛋白、SUMO-1 蛋白和 E2 酵素 UBC9 大量表現於 HEK293 細胞中，再透過分離出胞器的方式探討 sumoylation 在細胞當中修飾 NDUFS7 的位置與蛋白運輸之間的關聯，並且施予多種壓力誘導劑 (例如: 氯化鈷、雙氧水、無血清培養液和細胞凋亡誘導試劑) 來探討其影響。從結果中可以發現，被 SUMO-1 修飾的 NDUFS7 能夠在細胞核與細胞質中被觀察到。另外，在施予氯化鈷所引起的缺氧情況下，NDUFS7 的 sumoylation 也顯著增加。此外，我們也建構了 NDUFS7-SUMO-1 的質體並將其用於表現合成蛋白。其結果顯示此合成蛋白無法進入粒線體當中。基於本研究的發現，NDUFS7 的 sumoylation 對於此蛋白質運輸以及其生理意義機制需要更深入的探討。

Abstract

Human NADH dehydrogenase (ubiquinone) Fe-S protein 7 (NDUFS7) is one of 44 subunits in mitochondrial complex I. The N-terminal 1-60 amino acids of NDUFS7 have been defined as a mitochondrial targeting sequence (MTS) and the C-terminus contains a nuclear localization signal (NLS) and a nuclear export signal (NES). The sequence of NDUFS7 is highly conserved in the motif: $CCXXE(X)_{60}C(X)_{30}CP$. It can bind to an iron-sulfur cluster (a [4Fe-4S] cluster) called N2, a redox center in the terminus of complex I electron transfer pathway. In previous study, we identified NDUFS7 as a protein substrate of sumoylation. Posttranslational modifications of proteins by the small ubiquitin-like modifier (SUMO) have been found to be associated with various cellular processes. The SUMO protein can be conjugated to a target protein by sequential actions of enzyme E1 (SAE1/2), enzyme E2 (UBC9) and E3 ligases. The proteins conjugated by SUMO could be involved in subcellular localization, function or responding to stresses.

In this study, NDUFS7, SUMO-1 and UBC9 were co-expressed in HEK293 cells to clarify its subcellular localization by cell fractionation. The result showed that the sumoylated NDUFS7 were present in both the nuclear and cytosol fractions. Then, we established a new construct which can express the NDUFS7-SUMO-1 fusion protein, and found that the fusion protein can block the mitochondrial import. Furthermore, the effect of different stress response on sumoylation of NDUFS7 was explored by treating cells with various stress inducers, such as $CoCl_2$, H_2O_2, starvation and apoptosis reagents. The results suggested that $CoCl_2$-induced hypoxia increases the sumoylation of NDUFS7. The effects of NDUFS7 sumoylation on the subcellular localization of the protein and its physiological consequence would be further explored.