• 計畫中文名稱	神經生長蛋白 GAP-43 與受體聚合蛋白 Gephyrin 共同調控 GABA-A receptor 活性之研究		
• 計畫英文名稱	Regulation of GABA-A Receptor Activity by Interaction of Growth-Associated Protein 43 and Gephyrin in Cortical Neurons		
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• 中文關鍵字	--		
• 英文關鍵字	--		
• 中文摘要	生長相關蛋白(Growth-associated protein-43; GAP-43)是一表現於囊泡膜上的神經塑性相關蛋白，在神經發育、再生及突觸重整(reorganization)期間會被活化。當細胞內鈣離子濃度增加或神經細胞活化進而活化蛋白激.C (PKC)時， PKC 會將 GAP-43 Ser41 的位置磷酸化，進而使其於神經細胞體的囊泡轉位至生長中神經纖維的細胞膜內面，促使軸突延伸。此外，亦有研究顯示 GAP-43 會調節胞飲作用(endocytosis)，其於 Ser41 去磷酸化時會促進 endocytosis，表示其與調節神經傳導有關。在我們初步的蛋白質體研究中，利用質譜儀（Matrix-assisted laser desorption /ionization time –of flight ; MALDI-TOF）的方法，發現有一種蛋白會結合在 GAP-43 上，稱之爲 Gephyrin。在文獻上，這是第一次發現 GAP-43 與 gephyrin 這兩種蛋白有相關性。Gephyrin 是一種與微小管（microtubule）結合之受體聚合蛋白，能夠聚集位於突觸後膜上的抑制性離子通道型受器，如 glycine receptor 和 γ.aminobutyric acid A receptors (GABAAR) ，這些受器被內部化(internalization)時，也主要與 gephyrin 相結合而共存於突觸後密集區(postsynaptic density)。我們也進一步以免疫沉澱法確認 GAP-43 與 gephyrin 的相互結合，並發現在 PKC 抑制劑 Ro318220 會顯著地增加 GAP-43 與 gephyrin 的結合，及促進 GAP-43 轉移至神經細胞體與 gephyrin 共位的現象。在發育期的大腦皮質神經元細胞實驗結果顯示，Ro318220 會增加 gephyrin 與 GABAAR γ2 subunit (GABAARγ2) 的結合，而測定細胞內鈣濃度的結果顯示，在不降低 GABAAR 表現的情況下，PKC 抑制劑 Ro318220 會使細胞失去對 GABAAR 促進劑誘發增加細胞內鈣濃度的反應。因此，PKC 抑制劑誘導 GABAAR 活性喪失，可能起因於 gephyrin 透過與去磷酸化態的 GAP-43 結合，進而誘導 GABAAR 內部化所造成。由於 GABAAR 對於各腦區的神經活性調節均至爲重要，尤其參與認知功能與動作支配相關的大腦前額葉皮質、基底核，脊髓等區域的功能性神經網路聯結，因此 GABAARr 之活性對神經細胞突觸塑性而言十分重要，所以 GAP-43		

	是否對調控 GABAAR 功能上扮演了一個關鍵性的角色及其作用機制，即成爲重要的課題。的確，我們利用斑馬魚爲動物行爲模式所進行的初步研究結果中顯示，PKC 抑制劑會導致斑馬魚幼魚對有感機械性刺激所產生的反射動作出現過度活動與動作不協調的現象。因此，本計畫研究之中心假說爲「去磷酸化態的 GAP-43 會藉由與 gephyrin 的交互作用，進而導致 GABAAR 的內部化或胞飲作用，造成其活性的降低」。本計畫將以發育期大腦皮質神經元細胞培養爲實驗系統，於計畫的第一年，探討 GAP-43-gephyrin 交互作用與 GABAA receptor 低活性的相關性，利用發展出的突變型 GAP-43，將 Ser41 以 alanine 取代來造成其處於去磷酸化態，探討其是否會提高與 gephyrin 的結合，並研究其會如何影響 GABAAR 的內化或胞飲作用，及對 GABAAR 活性的影響。計畫的第二年，我們將發展另一突變型 GAP-43，將 Ser41 以 asparagine 取代使其成爲類似磷酸化態的 GAP-43，探討其是否會降低與 gephyrin 的結合，並研究其會如何影響 GABAAR 的內化或胞飲作用，及對 GABAAR 活性的影響。計畫的第三年，我們將以上兩年所發展出來的突變型 GAP-43 轉移至斑馬魚胚胎中，研究其對於後腦及脊髓中 GABAAR 活性，及有感機械性刺激誘導之動作協調性的影響。這個計畫的最終目標在於闡明 GAP-43 與 gephyrin 的結合對調控抑制性神經傳導的作用機制。 此外，由此計畫所建立之斑馬魚動物模式，未來將有利於探討與抑制性傳導異常如癲癇與精神分裂症等神經性或精神性疾病相關之研究。
• 英文摘要	Growth-associated protein 43 (GAP-43) is a plasticity-related protein that is associated with vesicular membrane. GAP-43 is activated during neuronal development, regeneration, and synaptic reorganization. It is phosphorylated by PKC at Ser41 upon elevation of intracellular calcium concentration ([Ca2+]i). Phosphorylated GAP-43 is translocated from vesicular compartment of the cell body to the inner membrane of growing neurites to facilitate axonal extension. Translocation in the opposite direction is noted when GAP-43 is dephosphrylated. In addition to its effect on axonal extension, phosphorylated GAP-43 also plays a regulatory role in endocytosis and membrane dynamics that may alter neurotransmitter receptor function. Using MALTI-TOF (Matrix-assisted laser desorption /ionization time–of flight) mass spectrometry, we have identified one of the GAP-43 interacting proteins is gephyrin. To our knowledge, this is the first time that gephyrin has been linked to GAP-43. Gephyrin, a microtubule-associated protein, is important in regulating γ.aminobutyric acid A receptor (GABAAR). This regulatory action is due to its clustering of GABAAR to regulate cell surface dynamic of postsynaptic membranes or GABAAR endocytosis. We found that treatment with Ro318220, a PKC inhibitor which dephosphorylates GAP-43, enhanced association of GAP-43 and gephyrin, and their cytosolic co-localization in neuronal cell bodies. In developing cortical neurons, PKC inhibition by Ro318220 also increased gephyrin interaction with the GABAAR γ2 subunit (GABAARγ2), resulting in the loss of GABAAR response to its agonist. This Ro318220 effect was not accompanied by a reduction in the expression of GABAA receptor. Therefore, Ro318220-induced loss of GABAAR activity may result from dephosphorylation of GAP-43 leading to its translocation from neuites to cell body and bind to gephyrin on the cell body cytoskeleton, followed by internalization or endocytosis of GABAAR from the postsynaptic surface to the GAP-43-associated vesicle. Since GABAAR is the most important inhibitory transmitter receptor in the central nervous system required for the stabilization of neuronal activities for cognitive function and motor control, it is important to elucidate whether GAP-43 plays a key role in regulating the GABAAR function. Indeed, our preliminary study shows that Ro318220 treatment resulted in hyperreactivity and uncoordinated movement in response to mechanosensory stimuli in zebrafish, suggesting its possible effect on GABAAR activity. Our central hypothesis is that dephosphorylation of GAP-43 results in reduction of GABAAR activity by internalization or endocytosis of

	surface GABAAR via increased association of dephophophrylated GAP-43 with gephyrin. In the first year, we will study the causal relationship of dephosphorylated GAP-43-gephyrin interaction in causing GABAAR hypoactivity by generating GAP-43 mutants that stay in dephosphorylated form for increased association with gephyrin. The effect of mutant on the surface GABAAR activity and internalization will be examined. In the second year, we will study whether a GAP-43 mutant with Ser43 replaced by asparagine mimicking the actions in its phosphorylated state reduces its binding to gephyrin to inhibit GABAAR internalization or endocytosis, resulting in enhanced GABAAR activity. In the third year, we will confirm the biological significance of the findings derived in the preceding 2 years to assess the effects of GAP-43 mutants that mimic dephosphorylated or phosphorylated form of GAP-43 on GABAAR activity and the coordination of movement in zebrafish in response to mechanosensory stimuli. The ultimate goal of this project is to delineate the novel role of GAP-43 via association with gephyrin to regulate inhibitory neurotransmission. The zebrafish animal model that expresses movement disorder with GAP-43 mutant derived from this project will be useful for further application onto the studies for GABAA receptor dysfunction-related neurological and psychological disorders, such as epilepsy and schizophrenia.