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PDLIM5 mediates PKCε translocation in PMA-induced growth cone collapse

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ABSTRACT

Growth cone collapse is a critical repulsive response to various guidance cues for axon guidance. Protein kinase C epsilon (PKCε) plays important regulation roles in such responses. Translocation of PKCε from cytoplasm to membrane is crucial to archive its regulatory roles in this process. We previously reported that PDLIM5 could selectively recruit PKCε to its specific substrate in neurons. However, the molecular mechanism of PKCε translocation in the neuronal growth cone collapse remains elusive. Here, we demonstrated that PDLIM5 and PKCε co-existed in the nerve growth cones. By interacting with α-actinin, but not β-actin or β-tubulin, PDLIM5 might contribute to regulation of remodeling of the microfilaments in neurons. Meanwhile, PDLIM5 could also bind to PKCε to form PDLIM5-PKCε complexes in growth cones. In the primary cultured neurons, activation of PKCε by PMA resulted in translocation of both PKCε and PDLIM5 from cytoplasm to the membrane. Knockdown of either PDLIM5 or PKCε rescued the neuron from PMA-induced growth cone collapse. Furthermore, in neurons, application of PDLIM5 shRNA or over-expression of PDLIM5 LIM1-3 mutants reduced the amount of PKCε in the membrane. Together, these results suggest that PDLIM5 acts as a scaffold protein by mediated PKCε translocated to the membrane in PMA-induced growth cone collapse.

1. Introduction

During the development of central nervous system (CNS), the extending axons are guided by the extracellular guidance cues to their targets to form precise functional neuronal networks. The correct pathfinding process of an axon is achieved through growth cone, which generates either attractive or repulsive response to different guidance cues. Although numerous intracellular signaling molecules, including protein kinases, phosphatases and calcium ions are associated with the regulation of cytoskeleton dynamics and membrane trafficking in the growth cone development [1–5], questions remain on whether the intracellular signal transduction cascades take place directly beneath or at the growth cone plasma membrane in regulation of neurite outgrowth.

Protein kinase C epsilon (PKCε) is a major PKC isoform in nerve growth cones [6]. The actin-binding site in PKCε and the localization of PKCε are crucial for regulation of neurite outgrowth by PKCε [7–10]. For example, PKCε can be activated by eicosanoid during the thrombin or semaphorin3A-induced growth cone collapse [11]. Translocation of activated PKCε to the cell plasma membrane is necessary for phosphorylation of the myristoylated alanine-rich protein kinase C substrate (MARCKS), and its phosphorylation-triggered release from adhesions causes localized growth cone detachment [12]. Nevertheless, the molecular mechanism of how the PKCε translocate from the cytoplasm to the membrane during this process remains elusive.

PDLIM5 belongs to the family of PDZ-LIM proteins. Previous studies show that PDLIM5 expresses in various brain regions [13]. Genetic linkage studies reveal an association between PDLIM5 and mental disorders such as bipolar disorder and schizophrenia [14,15]. PDZ-LIM proteins have been identified to act as scaffolds, interacting with filamentous actin-associated proteins and allowing these proteins to carry out diverse functions during neuronal development [16–19]. A recent study on the dendritic spine morphology shows that PDLIM5 promotes reduction of the size of the dendritic spines and longer and thin filopodia-like structures, suggesting the contribution of PDLIM5 to the morphological changes of neurons during development [20]. However, the functional role of PDLIM5 in the neural growth cone collapse is still poorly understood.

Our previous work has demonstrated that PDLIM5 selectively binds to both PKCε and N-type Ca^{2+} channels via its C-terminal LIM domain and brings PKCε near its substrate in vivo [21]. In this study, we found that PDLIM5 and PKCε co-existed in the neuron growth cone. Besides binding to α-actinin, PDLIM5 could also interact with PKCε in neurite outgrowth. Knockdown of PDLIM5 or PKCε could protect the neurons from repulsion induced by Phorbol-12-myristate-13-acetate (PMA). During this process, PDLIM5 mediated the translocation of PKCε from cytosol to membrane. Furthermore, overexpression of PDLIM5 LIM

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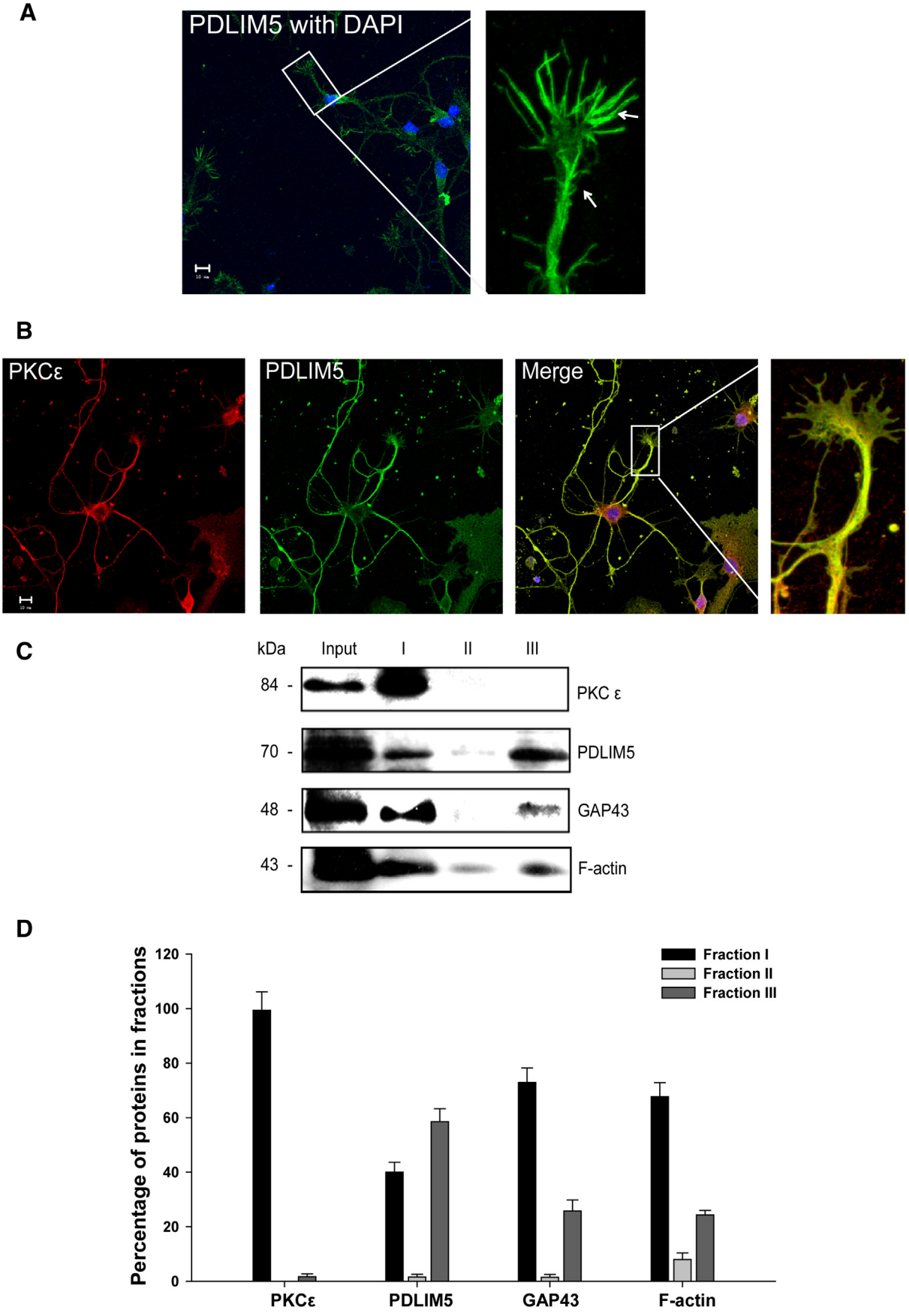
A
PDLIM5 with DAPI
B
PKCε
PDLIM5
Merge
C
kDa
Input
I
II
III
84 -
PKC ε
70 -
PDLIM5
48 -
GAP43
43 -
F-actin
D
Percentage of proteins in fractions
120
100
80
60
40
20
0
Fraction I
Fraction II
Fraction III
PKCε
PDLIM5
GAP43
F-actin