The regulation of pacsin3 plasma membrane localization by prenylation on its 471 cysteine residue.

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Running Title: Prenylation of Pacsin3 (pacsin3, a cytoplasmic adapter protein) on its CAAX motif.

Abstract

Pacsin3 (or other name Syndapin3) belongs to the pacsin (or Syndapin) family which are cytoplasmic adapter proteins with an N-terminal FHC, a central coiled coil, and a C-terminal SH3 domain and several potential phosphorylation sites. It has been shown that this protein was mainly expressed in heart and skeletal muscle. The prenylation of protein is one of the modifications of eukaryotic cell proteins on its C-terminal cysteine residue by enzyme-catalyzed attachment of a 15-carbon farnesyl group to cysteine. The C-terminal sequence motif for the prenylation is known as the CAAX (C is cysteine, A is usually but not necessarily an aliphatic residue, and X is typically M, S, or A). With the consensus motif sequence information for prenylation, we noticed it on the rat pacsin3 (471CVGA-COOH). Thus, in this line we determined whether the pacsin3 is modified with the prenylation, and investigated that the functional significance of its prenylation was further substantiated by pull-down assays, immunohistological studies, in the regulatory
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effect of the newly identified modification on the endocytosis of membrane protein, such as the transient receptor potential vanilloid 4.

Introduction

Mouse pacsin 1 (protein kinase C and Casein kinase II substrate in neurons are renamed from syndapin) was first characterized by Plomann et al., who isolated it in a screen for transcripts strongly downregulated in hippocampus after entorhinal cortex lesions (Howard et al., 1999; Modregger et al., 2000). It has been also reported that the pacsin isoforms (1 and 2) also participate in receptor-mediated endocytosis and actin organization (Mori et al., 2003; Schulze & Mann, 2004). At their N-terminus, these proteins present the Fes/CIP4 homology (FCH) domain which is a highly alpha-helical structure also known as the RAEYL motif of the N-terminus of S. pombe Cdc15, a protein involved in mediating the cytoskeletal rearrangements required for cytokinesis (Howard et al., 1999; Mori et al., 2003; Schulze & Mann, 2004). A coiled coil region would follow in the middle part and finally, an SH3 domain is present at the C-terminus. The SH3 motif is present in a wide variety of proteins and interacts with proline-rich regions to mediate protein-protein interactions, Src homology 3 domains; SH3 domains bind to proline-rich ligands with moderate affinity and selectivity, preferentially to PxxP motifs; they play a role in the regulation of enzymes by intramolecular interactions, changing the subcellular localization (Howard et al., 1999; Mori et al., 2003; Schulze & Mann, 2004). Thus, these pacsins represent a subgroup of the larger PCH protein family, which participate in rearrangements of actin networks during vesicle formation and transport. However, they have so far not been associated with microtubular dynamics (Onodera et al., 2005). Recently, several researchers provide evidence that pacsin proteins exist in complexes together with α- and γ-tubulin at centrosomes, suggesting a role in microtubule nucleation (Grimm-Gunter et al., 2008).

The aim of the present study was, therefore, to indentify the modification of pacsin3 (Howard et al., 1999; Modregger et al., 2000; Mori et al., 2003; Onodera et al., 2005; Schulze & Mann, 2004). We describe here that pacsin3 is prenyalted protein on its C-terminal cysteine residue,