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Biologically active milli-calpain associated with caveolae is involved in a spatially compartmentalised signalling involving protein kinase C alpha and myristoylated alanine-rich C-kinase substrate (MARCKS)

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Abstract

We have previously shown that calpain promotes myoblast fusion by acting on protein kinase C-alpha and the cytosolic phosphorylated form of MARCKS. In other cell types, various isoforms of calpain, PKC alpha and MARCKS were found associated with caveolae. These vesicular invaginations of the plasma membrane are essential for myoblast fusion and differentiation. We have isolated caveolae from myoblasts and studied the presence of calpain isoforms and their possible effects on signalling mediated by caveolae-associated PKC. Our results show that milli-calpain co-localizes with myoblast caveolae. Futhermore we provide evidence, using a calcium ionophore and a specific inhibitor of calpains (calpastatin peptide), that milli-calpain reduces the PKC alpha and MARCKS content in these structures. Purified milli-calpain causes the appearance of the active catalytic fragment of PKC alpha (PKM), without having an effect on MARCKS. Addition of phorbol myristate acetate, an activator of PKC, induces tranlocation of PKC alpha towards caveolae and results in a significant reduction of MARCKS associated with caveolae. This phenomenon is not observed when a PKC alpha inhibitor is added at the same time. We conclude that the presence of biologically active milli-calpain within myoblast caveolae induces, in a PKC alpha-dependent manner, MARCKS translocation towards the cytosol. Such a localised signalling event may be essential for myoblast fusion and differentiation.

Keywords: Calpain; Caveolae; Myogenesis; MARCKS; Protein kinase C alpha

Abbreviations: CS peptide, calpastatin peptide; DMEM, Dulbecco's modified Eagle medium; FBS, foetal bovine serum; FITC, fluorescein-isothiocyanate; HS, horse serum; MARCKS, myristoylated alanine rich C kinase substrate; NBT/BCIP, nitroblue tetrazolium/5-bromo-4-chloro-3-indoyl-l-phosphate; PBS, phosphate buffer saline; PKC, protein kinase C; PMA, phorbol myristate acetate; "RAFT", lipid microdomains; TBS, tris buffer saline

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1. Introduction

Calpains are calcium-dependant cysteine-proteases expressed in all mammalian cells. They compose a large family of ubiquitously expressed and tissue specific isoforms. The two best characterised isozymes are the ubiquitously expressed isoforms, μ- and m-calpain. They are heterodimeric proteins comprising a large 80 kDa catalytic subunit and a small 30 kDa regulatory subunit. μ-Calpain requires micromolar levels of Ca^{2+} for activation whereas m-calpain requires millimolar levels, hence their name (Croall & Demartino, 1991). In addition to regulation by calcium, calpain activities are also tightly regulated by a variety of mechanisms, including a specific inhibitor, calpastatin, binding to phospholipids and phosphorylation by ERK (extra-cellular signal regulated kinase), a member of the MAP kinase family (Glading, Uberall, Keyse, Lauffenburger, & Wells, 2001, Glading et al., 2004). The physiological functions of calpains are poorly understood. They appear to be involved in a number of cellular processes, including myoblast fusion (Cottin et al., 2000; Dourdin et al., 1999), PKC-mediated intracellular signalling (Aragon et al., 2002; Touyarot et al., 2000), RhoA- (GTPase) or tyrosine kinase-mediated remodelling of the cytoskeleton and, finally, they play a role in apoptosis (Goll, Thompson, Li, Wei, & Cong, 2003). Alteration of certain members of the calpain family have been identified in a number of human diseases. For instance, the loss of function of calpain 3, causes limb girdle muscular dystrophy type 2A (Richard et al., 1995) and mutations in the gene coding for calpain 10 have been shown to correlate with non-insulin-dependent diabetes (Horikawa et al., 2000).

Caveolae, which are found in the majority of cell types (Couet, Li, Okamoto, Ikezu, & Lisanti, 1997), are specialized lipid rafts able to form 50 to 200 nm vesicular invaginations of the plasma membrane. They were first identified as distinct electrodense structures in electron microscopic studies in the 1950s (Palade, 1955). The lipid composition of caveolae is enriched in glycosphingolipids, cholesterol and phosphatidyl inositol-4, 5-phosphate (PtdInt-P2). They are characterised by the presence of integral membrane proteins called caveolins (21–25 kDa). These are an essential component for the recruitment of proteins and lipids in these structures. Caveolae are involved in different modes of vesicular transport, such as endocytosis, transcytosis or potocytosis (Matveev, Li, Everson, & Smart, 2001) and they are the origin of many signal transduction pathways (Quest, Leyton, & Parraga, 2004). Caveolin-3 is the main structural protein of caveolae membrane domains in muscle cells and it is a key factor in myoblast fusion and myotube formation. We have recently reported that the presence of caveolin-3 is drastically reduced when μ-calpain is overexpressed in myoblasts (Moyen et al., 2004). Under these conditions the cytoskeleton presented an important disorganization at the later stages of myotube differentiation. On the contrary, a loss of expression of caveolin-3 enhances the fusion process (Volonte, Peoples, & Galbiati, 2003). Several mutations in the muscle specific caveolin-3 scaffolding domain lead to a form of autosomal dominant muscular dystrophy, referred as limb girdle muscular dystrophy type 1 C (Minetti et al., 1998). The caveolin scaffolding domain interacts with several signalling molecules, sequestering them in the absence of activating signals (Razani, Schlegel, & Lisanti, 2000).

PKCα, a member of the conventional PKCs family of serine/threonine kinases, is distributed in all tissues (Poole, Pula, Hers, Crosby, & Jones, 2004). Addition of stimuli causes its translocation from the cytosol towards specialized cellular compartments such as the nucleus, focal adhesion contacts or caveolae (Godson, Masliah, Balboa, Ellisman, & Insel, 1996; Orito et al., 2001). The localisation of PKCα with caveolae causes their internalisation (Smart, Ying, & Anderson, 1995; Upla et al., 2004). On the other hand, the scaffolding domain of caveolin is able to inhibit the kinase activity and the autophosphorylation of PKCα (Oka et al., 1997). Thus the activation of PKCα associated with caveolae would be highly regulated.

MARCKS (Myristoylated Alanine Rich C Kinase Substrate), a high affinity cellular substrate for PKCα, also co-localises with specialised raft in neuronal cells (Morash, Byers, & Cook, 2000). MARCKS has recently emerged as a group of PtdIns(4,5)P2 raft-modulating proteins that may regulate actin cytoskeleton dynamics and membrane trafficking (Laux et al., 2000). In myoblasts, PKCα-mediated phosphorylation of MARCKS causes its detachment from membrane and its partial degradation by calpain. These events