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European Journal of Cell Biology 85 (2006) 305–309
European Journal of Cell Biology
www.elsevier.de/ejcb

REVIEW

Caldesmon phosphorylation in actin cytoskeletal remodeling

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Abstract

Caldesmon is an actin-binding protein that is capable of stabilizing actin filaments against actin-severing proteins, inhibiting actomyosin ATPase activity, and inhibiting Arp2/3-mediated actin polymerization in vitro. Caldesmon is a substrate of cdc2 kinase and Erk1/2 MAPK, and phosphorylation by either of these kinases reverses the inhibitory effects of caldesmon. Cdc2-mediated caldesmon phosphorylation and the resulting dissociation of caldesmon from actin filaments are essential for M-phase progression during mitosis. Cells overexpressing the actin-binding carboxyterminal fragment of caldesmon fail to release the fragment completely from actin filaments during mitosis, resulting in a higher frequency of multinucleated cells. PKC-mediated MEK/Erk/caldesmon phosphorylation is an important signaling cascade in the regulation of smooth muscle contraction. Furthermore, PKC activation has been shown to remodel actin stress fibers into F-actin-enriched podosome columns in cultured vascular smooth muscle cells. Podosomes are cytoskeletal adhesion structures associated with the release of metalloproteases and degradation of extracellular matrix during cell invasion. Interestingly, caldesmon is one of the few actin-binding proteins that is associated with podosomes but excluded from focal adhesions. Caldesmon also inhibits the function of gelsolin and Arp2/3 complex that are essential for the formation of podosomes. Thus, caldesmon appears to be well positioned for playing a modulatory role in the formation of podosomes. Defining the roles of actin filament-stabilizing proteins such as caldesmon and tropomyosin in the formation of podosomes should provide a more complete understanding of molecular systems that regulate the remodeling of the actin cytoskeleton in cell transformation and invasion.

Keywords: Podosomes; Caldesmon; Actin filament dynamics

Contents

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doi:10.1016/j.ejcb.2005.08.008

Introduction

Caldesmon is an actin-binding protein that is capable of inhibiting the actomyosin ATPase activity and stabilizing actin filaments against actin-severing proteins (Morgan and Gangopadhyay, 2001; Wang, 2001). Caldesmon is encoded by a single gene that is alternatively spliced to generate two major isoforms: the longer, smooth muscle-specific h-caldesmon, and a shorter, non-muscle l-caldesmon (Dabrowska et al., 2004; Hayashi et al., 1991). The two caldesmon isoforms have almost identical amino- and carboxyterminal domains, but are different in including or excluding a middle single helical region consisting of about 150 amino acid residues. Since the terminal domains contain the major actin- and myosin-binding sites, the two caldesmon isoforms appear to have similar functions in vitro, and are distributed to the same actin stress fibers and membrane ruffles in cells (Yamakita et al., 1990). Recently, Guo and Wang (2005) have specifically knocked out h-caldesmon expression without affecting l-caldesmon expression in mice. Survival of the h-caldesmon knockout mice suggests that l-caldesmon can essentially substitute for h-caldesmon in organ system functions that are critical for survival, although the possibility of quantitative changes in organ functions cannot be excluded.

Caldesmon modulates actin filament dynamics

Caldesmon together with high-molecular-weight tropomyosin has been shown to inhibit the actin filament-severing activity of gelsolin completely by antagonizing the binding of gelsolin to actin (Ishikawa et al., 1989). Furthermore, caldesmon and tropomyosin could anneal gelsolin-severed actin filaments by decreasing the actin-binding affinity of gelsolin, resulting in the dissociation of gelsolin from actin filaments. These findings suggest that caldesmon together with tropomyosin can stabilize actin filaments against both severing and capping activities of gelsolin. Similar results were reported by Dabrowska et al. (1996), except that caldesmon and tropomyosin did not completely protect actin filaments against severing by gelsolin in their study. Recently, Yamakita et al. (2003) reported that caldesmon inhibited Arp2/3-mediated actin polymerization in vitro. Caldesmon has been identified at membrane ruffles, and Arp2/3-mediated actin polymerization is known to be an important mechanism of membrane ruffling (Small et al., 2002). Therefore, the findings of Yamakita et al. (2003) suggest that caldesmon may participate in the regulation of actin dynamics in membrane ruffling. Erk1/2 MAPK-mediated phosphorylation of caldesmon has been shown to reverse the ability of the actin-binding carboxyterminal fragment of caldesmon to stabilize actin filaments against actin-severing proteins (Foster et al., 2004). Similarly, cdc2 kinase and MAPK-mediated phosphorylation of caldesmon have been shown to reverse the inhibitory effect of caldesmon on Arp2/3-mediated actin polymerization (Yamakita et al., 2003). These findings suggest that Erk1/2 MAPK and cdc2 kinase are potential regulators of caldesmon function.

Caldesmon overexpression alters actin filaments in cells

Caldesmon overexpression induced by gene transfection or glucocorticoid treatment has been found to alter actin filaments in various cell types. Overexpression of the actin-binding carboxyterminal fragment of caldesmon has been found to stabilize actin stress fibers and decreased turnover of endogenous tropomyosin in CHO cells (Warren et al., 1994). Furthermore, many cells overexpressing the carboxyterminal fragment of caldesmon failed to release the fragment completely from actin filaments during mitosis, which could be a cause of the higher frequency of multinucleated cells (Warren et al., 1996). Similarly, Surgucheva and Bryan (1995) observed slower than normal growth rates in mouse L cells overexpressing h-caldesmon. Immunofluorescence microscopy studies suggest that caldesmon-overexpressing cells contained more actin filaments than control cells. In human pulmonary arterial endothelial cells, caldesmon overexpression resulted in the formation of a thicker subcortical actin cytoskeletal layer associated with slower speed of cellular migration (Mirzapoiazova et al., 2005). Furthermore, overexpression of tropomyosin together with caldesmon has been shown to reverse ras-transformation of NIH3T3 cells and partially restored the actin cytoskeleton (Shah et al., 2001). Glucocorticoid treatment has been found to induce caldesmon overexpression together with an increase in the amount of actin filaments in A549 cells (Castellino et al., 1995). When caldesmon overexpression was inhibited by antisense oligonucleotide targeting the caldesmon gene in glucocorticoid-treated cells, actin filaments also failed to increase in response to glucocorticoid treatment. These findings suggest that caldesmon is an important regulator in glucocorticoid-induced upregulation of actin filaments. On the contrary, Helfman et al. (1999) reported that caldesmon overexpression in transformed fibroblasts led to decreases in actin stress fibers and focal adhesions. Similarly, Numaguchi et al. (2003) found that caldesmon-GFP overexpression in capillary endothelial cells led to the loss of actin stress fibers and disassembly of focal adhesions. The reasons for the cell type-specific differential effects of caldesmon