Review

Role of Cbl-associated protein/ponsin in receptor tyrosine kinase signaling and cell adhesion

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Abstract

The Cbl-associated protein/ponsin (CAP) is an adaptor protein that contains a so-called Sorbin homology (SoHo) domain and three Src homology 3 (SH3) domains which are engaged in diverse protein-protein interactions. CAP has been shown to function in the regulation of the actin cytoskeleton and cell adhesion and to be involved in the differentiation of muscle cells and adipocytes. In addition, it participates in signaling pathways through several receptor tyrosine kinases such as insulin and neurotrophin receptors. In the last couple of years, several studies have shed light on the details of these processes and identified novel interaction partners of CAP. In this review, we summarize these recent findings and provide an overview on the function of CAP especially in cell adhesion and membrane receptor signaling.

The SoHo protein family

The Cbl-associated protein (CAP) was first identified as a Src homology 3 (SH3) domain containing protein and thus originally named SH3P12 (Sparks *et al.* 1996). CAP, also known as ponsin, belongs to the sorbin homology (SoHo) adaptor protein family and is encoded by the SORBS1 gene. This protein family includes two other members: Arg-binding protein 2 (ArgBP2), encoded by the *SORBS2* gene, and vinexin, encoded by the *SORBS3* gene, and their corresponding splicing isoforms. On the structural level, these proteins are characterized by an N-terminal SoHo domain and three C-terminal SH3 domains (Reviewed in Kioka *et al.* 2002, Roignot & Soubeyran 2009). The SoHo domain was named after its homology to the soluble peptide sorbin (Pansu *et al.* 1981, Vagne-Descroix *et al.* 1991), and at least human sorbin seems to be generated by splicing of an alternative transcript of the *SORBS2* gene locus (Hand & Eiden 2005). The members of the SoHo protein family are expressed in most tissues and cell types with a quite congruent distribution pattern. Especially in the heart, certain isoforms of vinexin, CAP and ArgBP2 are highly expressed (Kioka *et al.* 1999, Mandai *et al.* 1999, Ribon *et al.* 1998c, Wang *et al.* 1997). In addition to the structural and expressional similarities between the SoHo protein family members, they share many functional properties, such as the participation in the regulation of cell-cell and cell-matrix adhesion, actin cytoskeleton organization and growth factor receptor downstream signaling (Cestra *et al.* 2005, Kioka *et al.* 2002, Roignot & Soubeyran 2009).

The ArgBP2 protein was identified as an interaction partner of the Abl-related gene, Arg (Wang *et al.* 1997), which is a ubiquitously expressed non-receptor tyrosine kinase of the Abl family (Kruh *et al.* 1986, 1990). Various studies have shown that ArgBP2 is a scaffolder protein involved in multiple regulatory pathways converging primarily on the regulation of the cytoskeleton. It can be assumed that the N-terminal part containing the SoHo domain is important for ArgBP2 membrane targeting, since it was shown to interact with α2-spectrin (Cestra *et al.* 2005), a major component of the membrane associated cytoskeleton, and flotillin-1 (flot-1) (Haglund *et al.* 2004), a protein associated with specialized membrane microdomains known as lipid rafts (For a review, see Babuke & Tikkanen 2007, Banning *et al.* 2011)). The SH3 domains of ArgBP2 mediate binding to various proteins that are directly or indirectly involved in the regulation of actin cytoskeletal dynamics, e.g. the GTPases dynamin-1 and -2, components of the WAVE regulating complex (Cestra *et al.* 2005), the actin-binding protein vinculin

(Kawabe *et al.* 1999), the non-receptor tyrosine kinase c-Abl (Wang *et al.* 1997), the E3 ubiquitin ligase Cbl (Soubeyran *et al.* 2003) and the tyrosine kinase Pyk2 (Haglund *et al.* 2004). Knockdown of ArgBP2 and the neuronal nArgBP2 in primary mouse astrocytes resulted in an overall change of the cell morphology with a prominent presence of actin ruffles and a translocation of some focal adhesion (FA) proteins such as paxillin from adhesion sites to the cytoplasm (Cestra *et al.* 2005).

Vinexin was first isolated as a vinculin binding protein (Kioka *et al.* 1999). Three isoforms of vinexin have been described: vinexin α, vinexin β, which lacks the SoHo domain and is thus not a member of the SoHo family (Kioka *et al.* 1999), and vinexin γ (Matsuyama *et al.* 2005). These proteins have been connected to various signaling pathways, e.g. modulation of the epidermal growth factor/ mitogen activated protein kinase (EGFR/MAPK) signaling cascade (Matsuyama *et al.* 2005, Mitsushima *et al.* 2006, Mitsushima *et al.* 2007, Suwa *et al.* 2002) and actin cytoskeleton remodeling processes, such as actin stress fiber formation, cell spreading and migration (Kioka *et al.* 2010, Kioka *et al.* 1999, Mitsushima *et al.* 2006, Mizutani *et al.* 2007). In addition, localization at FAs and adherens junctions due to their interaction with vinculin was demonstrated for vinexin α and vinexin β (Kioka *et al.* 1999, Mizutani *et al.* 2007, Takahashi *et al.* 2005).

Splicing isoforms of CAP

The gene encoding for CAP maps to human chromosome 10q23.3-q24.1. Alternative splicing of this gene results in many different tissue and developmental stage specific isoforms (Figure 1A). With 34 independent exons, 14 of which have been reported so far to be alternatively spliced (Lin *et al.* 2001), human *SORBS1* might be considered as one of the genes with the highest number of splicing variants. Although alternative splicing usually results in a removal or an insertion of protein domains (Cooper 2005), the major domains of CAP, namely SoHo and the three SH3 domains, are present in all identified murine and human CAP isoforms (Lin *et al.* 2001, Zhang *et al.* 2003). However, transcript variation is achieved by the presence of insertion regions encoding for a coiled-coil domain and Pro-rich regions (Gehmlich *et al.* 2010, Matson *et al.* 2005, Zhang *et al.* 2003) that also typically mediate protein-protein interactions (Lupas 1996, Macias *et al.* 2002). Consequently, this is likely to result in a change of CAP interaction networks in different tissues.

The group of A. Saltiel described four alternatively spliced CAP isoforms (Figure 1B) expressed in mouse white adipose tissue (WAT) and ascribed them names CAP1-4 (Zhang *et al.* 2003). CAP2, -3 and -4 were predicted to contain a coiled-coil domain before the N-terminal SH3 domain. Furthermore, CAP4 has been proposed to possess a 70 amino acid-long Pro-rich region N-terminal to the SoHo domain, a smaller part of which is also present in CAP3. All four isoforms have been shown to localize at the plasma membrane and in the cytoplasm of differentiated 3T3-L1 adipocytes. Out of these four isoforms, CAP2 shows a nuclear localization (Zhang *et al.* 2003). A human CAP2 homologue, called R85, was shown to localize in the nucleus and to exhibit a nuclear localization sequence as well (Lebre *et al.* 2001, Nunes *et al.* 2005). Some CAP isoforms were detected in the majority of murine adult tissues examined, the level being the highest in the heart and WAT. The expression of CAP isoforms was induced during adipogenesis where it was shown to be regulated by the transcription factor peroxisome proliferator-activated receptor γ (PPARγ). Moreover, ectopic expression of PPARγ in fibroblasts increased the expression of CAP (Ribon *et al.* 1998b).

The expression of CAP is increased during myogenic differentiation. Very recently, a novel human CAP isoform (human transcript variant 5) was described which is specifically expressed in striated skeletal muscles and localized at certain cell-matrix contacts, the costameres (Gehmlich *et al.* 2010). Human transcript variant 5 was reported to contain a 278 amino acid long insertion between the second and the third SH3 domains of CAP, as a result of the use of two alternatively spliced exons number 30 and 31. The human transcript variant 5 was originally reported to be absent from fetal and adult human hearts. However, Matson *et al.* described a human isoform containing spliced exons 30, 31 and 32, which was expressed not only in the skeletal muscle but also in the heart (Matson *et al.* 2005). Indeed, a murine exon corresponding to the human exon 31 was found to be one of the alternatively spliced exons during mouse heart development (Kalsotra *et al.* 2008), pointing to a possible role that CAP might play also during cardiomyocyte differentiation (see below).

CAP in adhesion and cytoskeletal regulation

The development and maintenance of cell-cell and cell-matrix adhesions are tightly regulated processes that are crucial in a variety of physiological and pathophysiological functions within multicellular organisms. A prominent cell-cell adhesion type is the adherens junction, which is a multi-protein macromolecular assembly characterized by the presence of members of

the cadherin and catenin family. FAs in turn provide an important connection to the extracellular matrix (ECM) via proteins of the integrin family. Both structures are highly dynamic, and in addition to their anchoring function to the actin cytoskeleton, they play important roles in signal transduction (Reviewed in Harris & Tepass 2011, Hartsock & Nelson 2008, Martin *et al.* 2002). CAP has been shown to interact with numerous proteins that are involved in cell adhesion. An overview of these interactions is provided in Figure 2A.

Ribon *et al.* demonstrated for the first time that CAP may play a role in signaling events associated with integrin mediated adhesion by showing that NIH-3T3 cells overexpressing CAP and the insulin receptor (IR) exhibit a strong increase in the number and density of F-actin based stress fibers and an increased number of FAs (Ribon *et al.* 1998a). In *Potorous tridactylis* (kangaroo rat) kidney (PtK2) cells, overexpression of CAP led to significantly larger FAs and formation of prominent stress fibers (Gehmlich *et al.* 2007). However, the increased stress fiber formation seems to be highly dependent on the expression level of CAP (our unpublished results). The observed phenotypes resemble the effect of the small GTPase Rho on actin organization and FAs (Machesky & Hall 1997), suggesting a role of CAP in the Rho dependent pathway. Indeed, in mouse lens epithelial cells, the expression and distribution of CAP appears to be regulated by the Rho kinase mediated actin cytoskeletal organization. This and its localization at fiber cell basal ends and cell-cell junctions suggest a role for

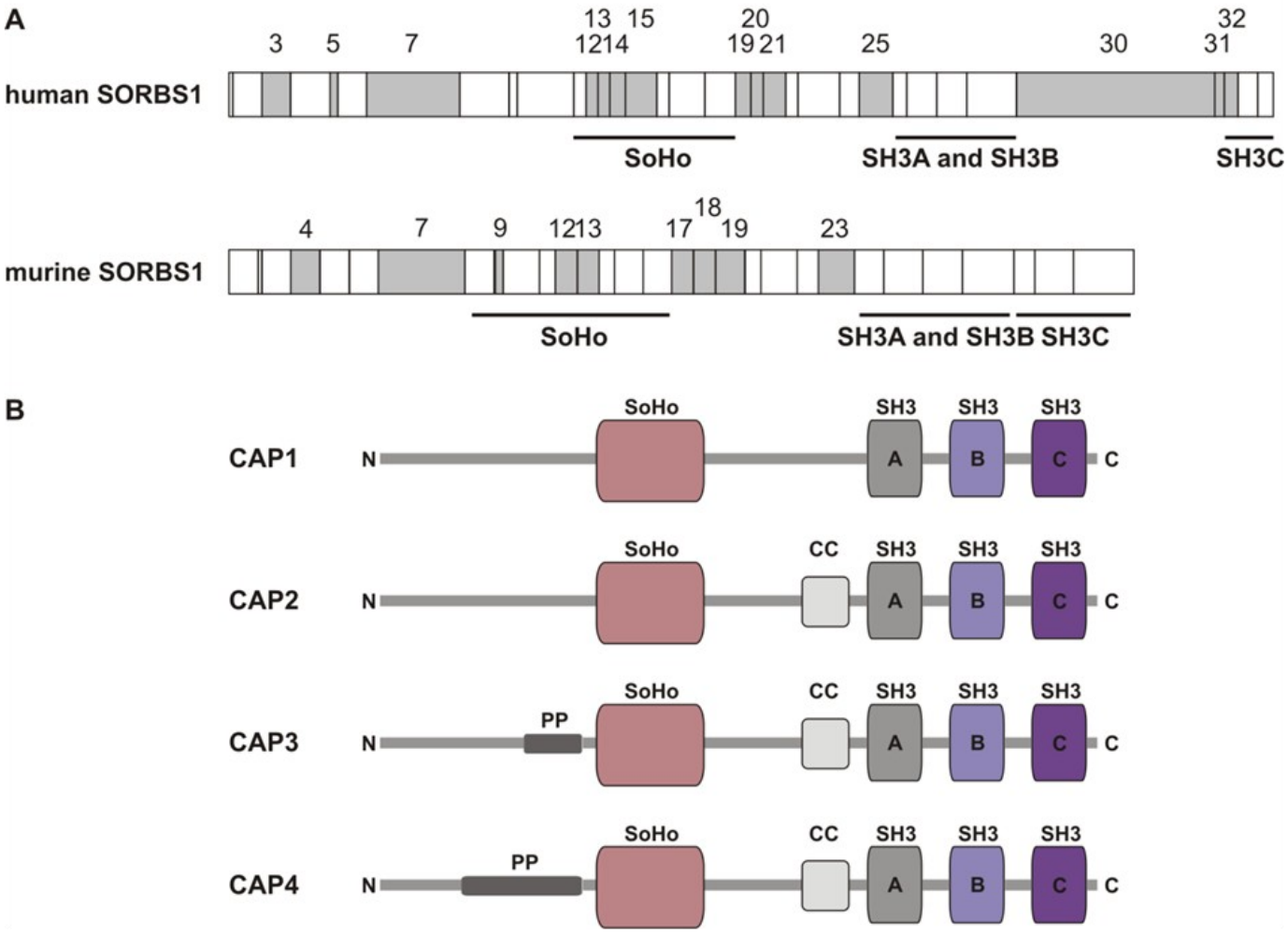

Figure 1. Transcript variants of Cbl-associated protein (CAP). (A) Exon structure of the human and murine *SORBS*1 gene. Alternatively spliced exons are highlighted in grey and the domains encoded by specific exons are pointed out below. The human *SORBS1* gene is composed of 34 independent exons, 14 of which are alternatively spliced. The murine *SORBS1* gene is predicted to be composed of 30 exons, 9 of which have been reported to be alternatively spliced. (B) Published murine CAP isoforms discovered in adipocytes. All four isoforms possess an N-terminal SoHo domain and three C-terminal SH3 domains. Differences are observed in a poly-proline region (PP) N-terminally to the SoHo domain and in the presence of a coiled-coil domain (CC) N-terminally to the SH3A domain. SoHo = Sorbin homology domain; SH3 = Src homology 3 domain.

CAP in lens cytoskeletal organization, fiber cell migration and adhesion (Rao & Maddala 2009).

The presence of CAP in stress fibers and FAs has so far been shown in various cell types (Fernow *et al.* 2009, Mandai *et al.* 1999, Ribon *et al.* 1998a, Zhang *et al.* 2006). We have shown that several Tyr residues in CAP can be phosphorylated by c-Abl and Src kinases (Fernow *et al.* 2009). However, the localization of CAP to stress fibers and FAs is independent of its Tyr phosphorylation. On the other hand, a mutation of one of the major Src phosphorylation sites of CAP, Tyr326, to a phenylalanine, exhibits an inhibitory effect on spreading (Fernow *et al.* 2009). This suggests that the phosphorylation of CAP at Tyr326 may have an impact on FA turnover, which in turn is a prerequisite for proper cell spreading and migration. However, our data show that overexpression of a wild-type CAP-EGFP fusion protein in HeLa cells, which do not express endogenous CAP, has no effect on cell spreading on fibronectin and collagen (Fernow *et al.* 2009). In contrast, the group of A. Saltiel showed that the overexpression of CAP in NIH-3T3 fibroblasts inhibits cell spreading on fibronectin, most likely due to its negative impact on adhesion induced activation of the mitogen activated protein kinase ERK through the PAK/MEK/ERK pathway (Zhang *et al.* 2006). These contradictory results may be due to the analysis using a substantially different cellular background.

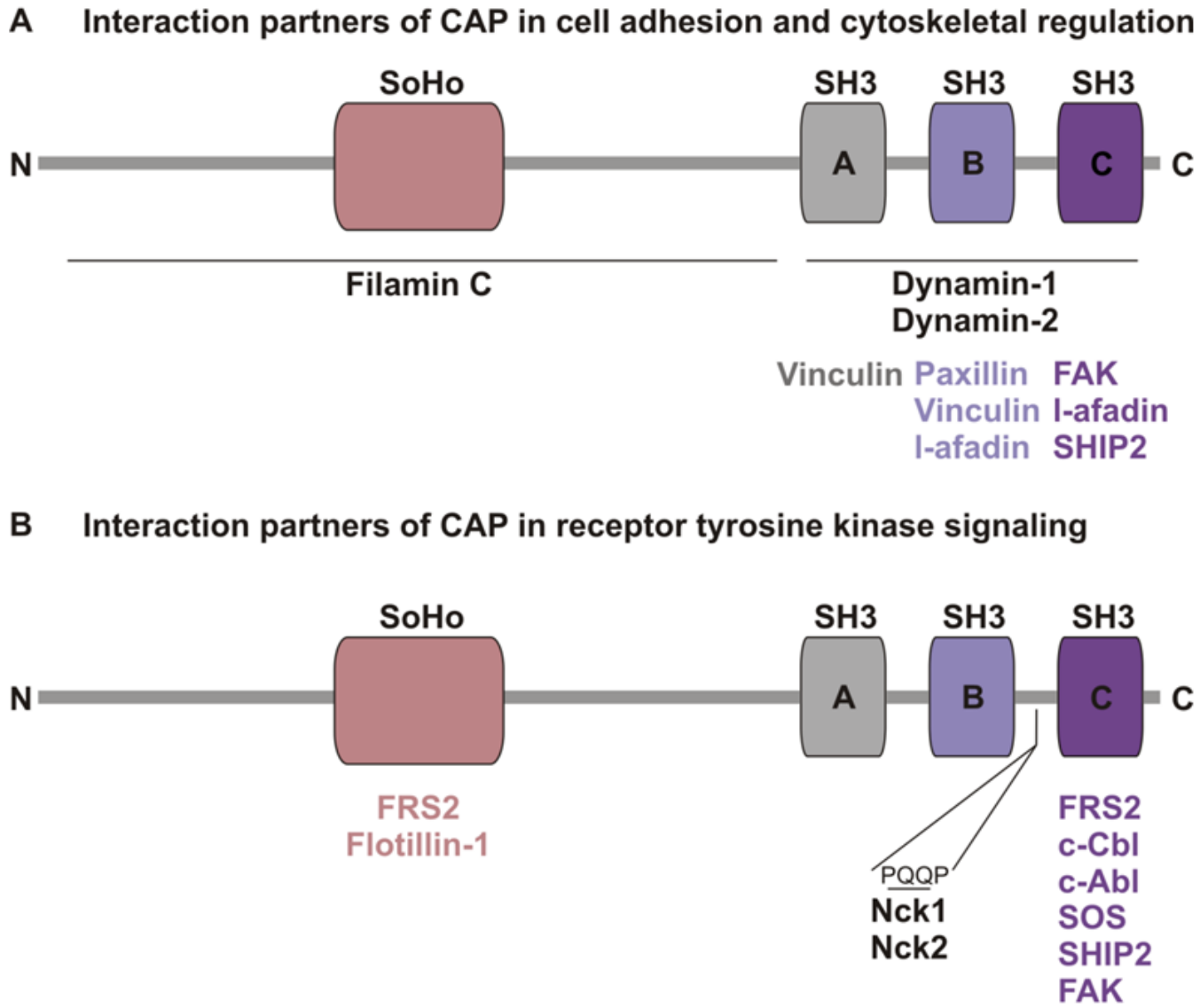

Figure 2. Schematic representation of CAP domains and their interaction partners in cell adhesion, cytoskeletal regulation and receptor tyrosine kinase signaling. CAP is a member of the sorbin homology (SoHo) adaptor protein family, which is characterized by the N-terminal SoHo domain and three C-terminal Src homology 3 (SH3) domains. (A) Interaction partners of CAP in cell adhesion and cytoskeletal regulation. (B) Interaction partners of CAP in receptor tyrosine kinase (RTK) signaling. Color coding shows the respective interaction domain in CAP.

Together, these observations suggest that the functional role of CAP may vary between different cell types.

A direct connection of CAP to FAs is provided by the focal adhesion kinase (FAK), which is a major regulator of FA dynamics. FAK controls cell adhesion and motility and functions as a cytosolic kinase and as a scaffold protein with multiple tyrosine phosphorylation sites (Reviewed in Chacon & Fazzari 2011, Mitra *et al.* 2005). Indirect GST pulldown experiments showed that the third SH3 domain of CAP most efficiently bound to FAK (Ribon *et al.* 1998a). Tyr phosphorylation of proteins involved in cytoskeletal rearrangements and their interactions are critical factors in integrin-mediated signal transduction. Ribon *et al.* were able to show that FAK is less phosphorylated in cells overexpressing CAP (Ribon *et al.* 1998a). In addition to FAK, CAP interacts with the actin-binding and FA associated protein vinculin via the region containing the first and second SH3 domains (Mandai *et al.* 1999). Other SoHo proteins, e.g. vinexin, have also been shown to interact with vinculin (Kioka *et al.* 1999). In vinculin knockout mouse fibroblasts, CAP-GFP exhibits a diffuse and fibrillar distribution, suggesting that vinculin is important for the recruitment of CAP to FAs (Zhang *et al.* 2006). Intriguingly, vinculin is also a component of adherens junctions where it directly interacts with β-catenin (Peng *et al.* 2010) and α-catenin (Watabe-Uchida *et al.* 1998, Weiss *et al.* 1998). Due to its dual localization in FAs and adherens junctions, vinculin is emerging as an important regulator of these cell adhesion types (Chen *et al.* 2005, Peng *et al.* 2012) and provides a functional link from CAP to both cell adhesion structures.

Interestingly, CAP was identified to be an interaction partner of l-afadin, a protein that is also localized at adherens junctions and is capable of directly binding cell adhesion molecules of the nectin immunoglobulin superfamily (Takai *et al.* 2008), the actin cytoskeleton (Mandai *et al.* 1999) and α-catenin (Pokutta *et al.* 2002). CAP binds to the third proline rich region of l-afadin with its second and third SH3 domains (Mandai *et al.* 1999). In accordance with a direct interaction, Mandai *et al.* have also shown that CAP colocalizes with l-afadin and vinculin in adherens junctions. The slightly overlapping interaction domain of l-afadin and vinculin within CAP and the similar localization led to the assumption that l-afadin and vinculin may bind to CAP in a competitive manner (Mandai *et al.* 1999).

Studies by Zhang *et al.* have shown that CAP colocalizes with paxillin at cell matrix adhesion sites (Zhang *et al.* 2006), and it has been identified as a muscle specific interactor of paxillin (Gehmlich *et al.* 2007). Like CAP, paxillin is a FA associated molecule which functions as an adaptor protein recruiting various cytoskeletal and signaling proteins to coordinate downstream signaling by integrin adhesion receptors (For a review, see Schaller 2001). In addition, paxillin was shown to be of importance for costamer formation during muscle differentiation (Quach & Rando 2006). The interaction of CAP with paxillin is mediated by the second SH3 domain of CAP (Gehmlich *et al.* 2007, Zhang *et al.* 2006). Zhang *et al.* showed that transient knockdown of paxillin in REF52 cells had no effect on the localization of CAP, suggesting that paxillin is not important for the recruitment of CAP to FAs. However, overexpression of CAP, which results in coalescence of F-actin in short aggregates, enhanced the association of paxillin and vinculin with actin cytoskeletal structures (Zhang *et al.* 2006). Based on the structural change in cell-matrix adhesions in CAP overexpressing cells, it can again be assumed that CAP is involved in the modulation of FA turnover.

Numerous interaction partners of CAP have been described, most of which bind to the SH3 domains of CAP (Figure 2). To identify new interaction partners that bind to regions other than the SH3 domains of CAP, Zhang *et al.* used an SH3 domain deletion construct of CAP for immunoprecipitation with subsequent mass spectrometry analysis and identified the protein filamin C (FLNc) as a novel interaction partner of CAP (Zhang *et al.* 2007). FLNc is an actin binding protein found in the dystrophin-glycoprotein complex (Thompson *et al.* 2000), which is a specialized multi-component complex of the cardiac and skeletal muscle membrane. This complex provides a strong mechanical and signaling link between the cytoskeleton and the extracellular matrix (Lapidos *et al.* 2004). Overexpression of CAP in L6 myoblasts induced a strong accumulation of endogenous FLNc in actin-rich regions, indicating that CAP acts as an adaptor protein to recruit FLNc to cell-matrix adhesion sites. In addition, in COS-7 cells, ectopic expression of both proteins results in the formation of large actin bundles (Zhang *et al.* 2007), suggesting that CAP enhances the actin crosslinking function of FLNc.

Further interaction partners of CAP that influence the actin cytoskeleton are the phosphatidylinositol 3,4,5-trisphosphate (PtdIns(3,4,5)P3) 5-phosphatase SHIP2 (Vandenbroere *et al.* 2003) and the GTPases dynamin-1 and dynamin-2 (Tosoni & Cestra 2009). SHIP2 contains several motifs capable of mediating protein–protein interactions, including an N-terminal SH2 domain which presumably binds phosphor-Tyr containing motifs, and a pro-rich region. SHIP2 positively regulates cell adhesion and spreading (Prasad *et al.* 2001) and influences the cortical actin cytoskeleton

by modulating the phosphatidylinositol 3-kinase signaling cascade (Dyson *et al.* 2005). Vandenbroere *et al.* identified CAP as a novel interaction partner of SHIP2 in a yeast two-hybrid screen and verified this interaction by immunoprecipitation (Vandenbroere *et al.* 2003). Dynamin-1 and dynamin-2 are key players in the regulation of actin dynamics and endocytosis (Chua *et al.* 2009, Gu *et al.* 2010, Krueger *et al.* 2003). Their interaction with CAP is mediated by one of the SH3 domains of CAP (Figure 2A) and provides an additional link to the actin cytoskeleton (Tosoni & Cestra 2009). Interestingly, overexpression of CAP was shown to result in inhibition of dynamin mediated endocytosis and increased stability of the EGFR (Tosoni & Cestra 2009), suggesting that CAP may also play a role in dynamin dependent endocytosis.

CAP in muscle differentiation

Within the last years, special attention has been paid to functional aspects of CAP during muscle differentiation. During this tightly regulated process, motile and proliferating precursor cells fuse into multinucleated myotubes that mature into contractile muscle fibers. This myogenic program involves complex networks of coordinated changes in gene expression and protein composition accompanied by excessive remodeling of FA-like cell matrix contacts into so called costameres (Ervasti 2003, Le Grand & Rudnicki 2007, Taylor 2002). Mandai *et al.* have shown that CAP is located at costameres in cardiac muscle cells (Mandai *et al.* 1999). In myotubes, costaining of endogenous CAP and vinculin showed that both proteins are concentrated at the costamere region of the sarcolemma membrane (Zhang *et al.* 2007). Expression of CAP is not detectable at the protein level in human skeletal muscle (HSkM) myoblasts (Gehmlich *et al.* 2007) and in rat skeletal muscle L6 myoblasts (Zhang *et al.* 2007). Furthermore, CAP is present only at a very low level in undifferentiated mouse skeletal muscle C2C12 myoblasts (Gehmlich *et al.* 2007). However, with the onset of myogenic differentiation, CAP becomes detectable on protein level (Gehmlich *et al.* 2007, Zhang *et al.* 2007). Immunofluorescent analysis of CAP localization during muscle differentiation showed that at the beginning of the myogenic program, CAP is colocalized with paxillin at FAs. In more terminally differentiated human skeletal muscle cells, both proteins colocalize in pre-costameres. In addition, overexpression of CAP in C2C12 cells results in an increased formation of pre-costameric structures (Gehmlich *et al.* 2007). These data suggest that CAP is capable of modulating the dynamics of FAs and actin cytoskeleton, which are prerequisites for cytoskeletal remodeling during costamere formation.

Nck2/Grb4 is an adaptor protein that participates in ephrin B reverse signaling in neuronal cells, which has been shown to be important for axon pathfinding (Birgbauer *et al.* 2000, Henkemeyer *et al.* 1996). The SH2 domain of Nck2 binds to the phosphotyrosines in the activated ephrin B, and the SH3 domains of Nck2 then recruit CAP by binding to the PQQP motif that is present between the SH3 domains B and C of CAP (Cowan & Henkemeyer 2001). The complex formed between the human CAP transcript variant number 5 and Nck2 was also suggested to play a role in cytoskeletal remodeling during differentiation of skeletal muscle cells. In this isoform of CAP, the insertion of 278 amino acids takes place in the middle of the PQQP motif that binds Nck2 (Gehmlich *et al.* 2010). However, although this binding motif is disrupted in the transcript variant 5, its ability to bind to Nck2 is retained due to the presence of another proline rich motif contained within the insertion in a segment encoded by exon 30 (Gehmlich *et al.* 2010). These data point to the importance of CAP/Nck2 interaction for muscle differentiation, but the exact role of this interaction in this process remains to be clarified.

CAP in TrkA receptor signaling

The Trk receptor tyrosine kinase family consists of three known members, TrkA, TrkB and TrkC, which play important roles in various cellular processes, e.g. proliferation and differentiation, especially in the nervous system. The three receptors show varying affinities to neurotrophins, and Trk A is specifically activated by the nerve growth factor (NGF) (Reviewed in Huang & Reichardt 2003, Kaplan & Miller 2000). In rat pheochromocytoma (PC12) cells, CAP associates with TrkA upon stimulation with NGF (Limpert *et al.* 2007)). This complex formation results in the translocation of TrkA to lipid rafts, which are specialized membrane microdomains enriched in sphingolipids and cholesterol (Lingwood & Simons 2010, Simons & Sampaio 2011). The translocation was shown to be dependent on the interaction of CAP with the lipid raft associated protein flot-1. Deletion of the CAP SoHo domain prevents its interaction with flot-1, abolishes TrkA lipid raft association and inhibits further downstream signaling events such as ERK phosphorylation (Limpert *et al.* 2007). For an overview of the CAP interaction partners during signaling, see Figure 2B.

Recently, we have shown that CAP is not only capable of binding flot-1 via its SoHo domain but also interacts with the fibroblast growth factor receptor substrate 2 (FRS2, Figure 2B). In CAP, the binding to FRS2 is cooperatively mediated by the SoHo domain

and the third SH3 domain (Tomasovic *et al.* 2012). Interestingly, FRS2 has also been shown to directly interact through its phosphotyrosine binding (PTB) domain with TrkA in a phosphotyrosine dependent manner after NGF stimulation (Meakin *et al.* 1999, Ong *et al.* 2000). Furthermore, we demonstrated that flot-1 directly interacts *in vitro* and *in vivo* with FRS2 by binding to its PTB domain. Since both flot-1 and CAP bind to the PTB domain of FRS2, it is likely that CAP and flot-1 compete for the binding to FRS2 to regulate the formation of signaling complexes according to the participating receptor (Tomasovic *et al.* 2012).

CAP in insulin signaling

The reported high expression level of CAP in insulin sensitive tissues, such as adipose tissue and skeletal muscle (Zhang *et al.* 2003), implies that CAP might exert a specific function in these tissues. Indeed, CAP was found to be one of the major players of the insulin signaling cascade (Baumann *et al.* 2000, Kimura *et al.* 2001). Insulin is a very potent anabolic hormone with a plethora of metabolic activities. For example, it stimulates the transport of glucose into adipocytes and muscle cells. By binding to its tyrosine kinase receptor, the IR, insulin initiates a pathway that is composed of two arms that act synergistically (Figure 3). These „signaling arms“ are distinguished by their dependency on the activation of phosphatidylinositol 3-kinase (PI3K), with one being PI3K dependent and the other arm PI3K independent, involving the proteins c-Cbl, CAP and TC10. The activated IR phosphorylates several intracellular substrates, including the insulin receptor substrate (IRS) docking proteins. The phosphorylated Tyr residues of IRS serve as binding sites

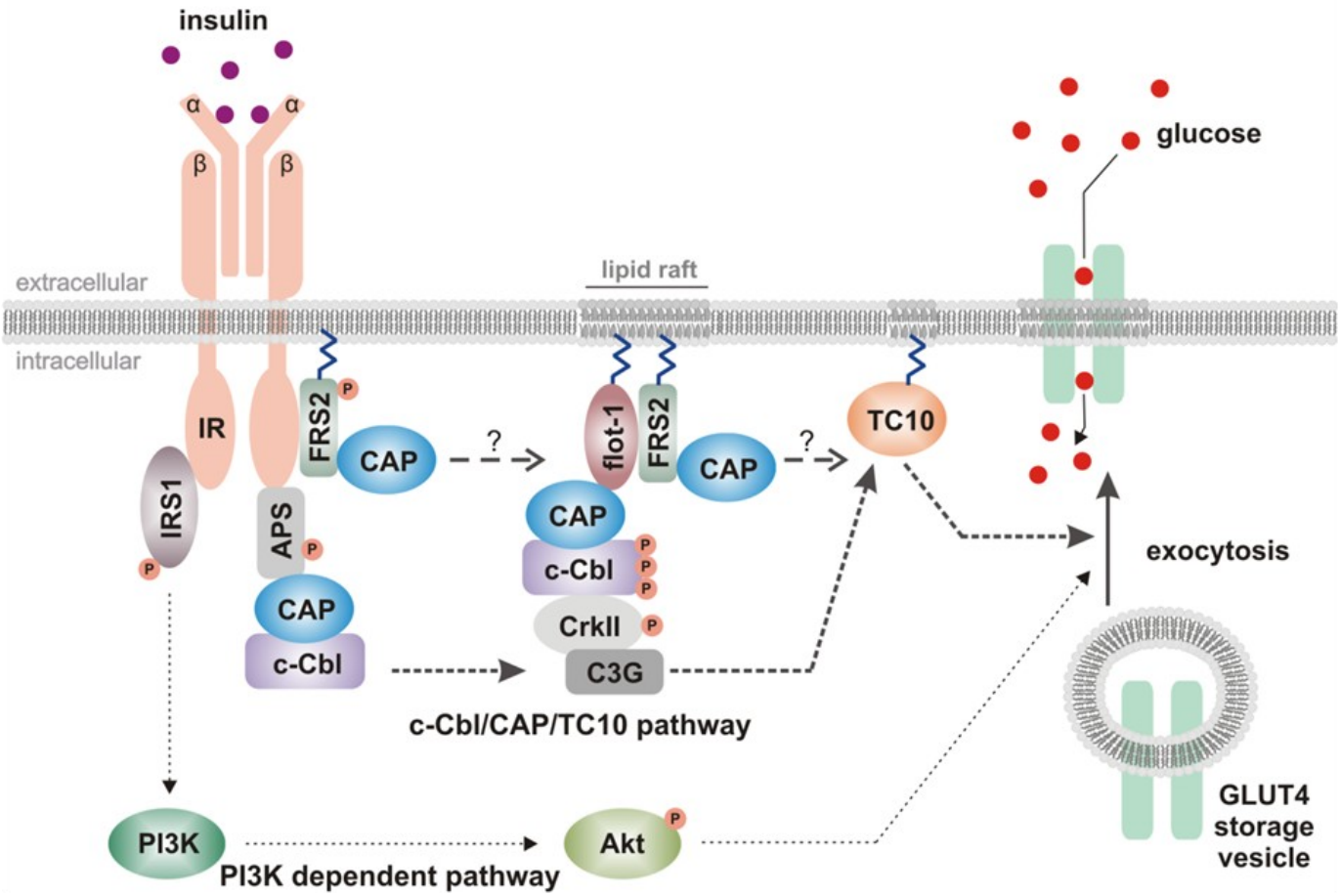

Figure 3. Functional role of CAP in insulin signaling. The insulin signaling pathway can be subdivided into two major branches - the phosphatidyl-inositol 3-kinase (PI3K) dependent and the PI3K independent pathway - both of which induce the recruitment of glucose transporter type 4 (GLUT4) storage vesicles to lipid rafts. CAP was found to be one of the key players in the latter pathway. By binding to flot-1, CAP recruits c-Cbl into lipid rafts upon insulin stimulation. Through this interaction, the c-Cbl/CAP complex is placed in the proper environment for the downstream signaling. FRS2 may provide a further link between CAP and the insulin receptor (IR). FRS2 was found to be enriched in lipid rafts, where it interacts with flot-1 and may assist in scaffolding the components of the c-Cbl/CAP/TC10 dependent pathway.

for SH2 containing signaling molecules such as PI3K. Activation of PI3K results in the production of phosphatidyl-inositol (3,4,5)-trisphosphate (PIP3) that acts as an allosteric regulator of the phosphoinositide dependent kinase (PKD). Active PKD phosphorylates and thereby activates protein kinase B/Akt. Recruitment of the glucose transporter GLUT4 vesicles from internal stores to the plasma membrane takes place, resulting in uptake of glucose into the cell (Cheatham *et al.* 1994, Okada *et al.* 1994, Sharma *et al.* 1998). However, since other growth factors and adhesion molecules that are able to activate PI3K have no effect on glucose transport, a parallel, fine-tuning pathway specific for IR was speculated. Indeed, the PI3K independent arm of insulin signaling pathway was found to be important for the regulated glucose transport to take place. Intriguingly, CAP has been recognized as one of its main mediators. CAP forms a constitutive complex with the ubiquitin ligase c-Cbl (Ribon *et al.* 1998a) and binds to IR upon its activation. The binding to IR was shown to be indirect and mediated by the interaction with the adaptor protein with pleckstrin homology and Src homology 2 domains (APS) (Ahn *et al.* 2004). During this process, c-Cbl is phosphorylated by the IR (Ribon & Saltiel 1997), the CAP/Cbl complex is released from the IR and recruited to lipid rafts (Mastick *et al.* 1998). This recruitment is mediated by the interaction between CAP and flot-1, similarly to the TrkA signaling (Baumann *et al.* 2000).

The SoHo domain of CAP has been shown to bind the first hydrophobic domain of flot-1 (Liu *et al.* 2005), and through this interaction, a ternary complex flot-1/CAP/c-Cbl is formed, placing c-Cbl into the proper environment to propagate the downstream signaling (Baumann *et al.* 2000). CAP mutants that are not able to bind c-Cbl (SH3 deletion mutants) or flot-1 (SoHo deletion mutants) act in an inhibitory manner on the insulin stimulated GLUT4 translocation and glucose uptake (Chiang *et al.* 2001, Kimura *et al.* 2001). Being at "the right place at the right time", phosphorylated c-Cbl can recruit other proteins to lipid rafts, such as the SH2 containing adaptor protein CrkII and GDP-GTP exchange factor C3G (Chiang *et al.* 2001). C3G can then activate the Rho family GTPase TC10, which is targeted to lipid rafts by means of farnesylation and palmitoylation (Watson *et al.* 2001). TC10 then stimulates the movement of GLUT4 towards the plasma membrane. As with TrkA signaling, our recent findings suggest that FRS2, which is able to bind IR, together with CAP and flotillin-1 might be another link between the CAP/Cbl complex and the IR (Tomasovic *et al.* 2012). Interestingly, FRS2 has been shown to become Tyr phosphorylated upon insulin stimulation (Delahaye *et al.* 2000). However, the details of the FRS2 function in insulin signaling need to be clarified in further studies.

Although CAP was ascribed a positive role during insulin signaling, the CAP knockout mouse model exhibiting a genetic deletion of *SORBS1* shows an unexpected insulin sensitivity. These mice are protected against a high-fat diet induced insulin resistance and show a reduced number of lymphocytes and monocytes in the blood and fewer macrophages in the adipose tissue (Lesniewski *et al.* 2007). The observed insulin sensitive phenotype could have at least two explanations. The other members of the SoHo protein family, vinexin α and ArgBP2, might substitute for CAP and overtake its functions. On the other hand, the PI3K dependent arm of the insulin signaling cascade displays an enhanced activity in adipocytes genetically ablated of *SORBS1*. Indeed, Lesniewski *et al.* found an increased phosphorylation of Akt after insulin stimulation in white adipose tissue and skeletal muscle of the CAP knockout mice after high-fat diet (Lesniewski *et al.* 2007). A similar insulin sensitive phenotype was found in both APS and Cbl knockout mice (Minami *et al.* 2003, Molero *et al.* 2004), being in agreement with the proposed enhancement of PI3K dependent signaling in the absence of the PI3K independent one.

Conclusions

Several studies have recently revisited the function of CAP as an adaptor protein that mediates signaling processes and the organization of the actin cytoskeleton. Novel interaction partners have been identified that also play a role in these processes, and studies on knockout mice have revealed surprising aspects on the role of CAP in insulin signaling. In future studies, it will be important to clarify the exact role of the binding partners of CAP in the regulation of its function. Since many of these interaction partners bind to the same domains in CAP, as for example FRS2 and flot-1, both of which interact with the SoHo domain, it is likely that competitive binding and formation of diverse complexes with CAP regulate its function. Another important aspect of CAP that needs to be addressed in more detail is the characterization of the function and expression patterns of the numerous CAP isoforms. Although the SoHo proteins share many interaction partners and a functional redundancy has been suggested, CAP exhibits the highest number of alternatively spliced transcripts within this family. These isoforms may also display different interaction partners, depending on which domains are present as a result of alternative splicing. Thus, it is possible that the individual isoforms of CAP may even show contradictory effects on the same signaling pathway in two

different tissues due to formation of different signaling complexes. This might also explain the unexpected effect on insulin signaling observed in the CAP knock-out mice (Lesniewski *et al.* 2007).

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Conflict of Interest

The authors declare no conflict of interest.

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