The role of cyclase-associated protein in regulating actin filament dynamics – more than a monomer-sequestration factor

Shoichiro Ono
Department of Pathology and Department of Cell Biology, Emory University, Atlanta, GA 30322, USA
sono@emory.edu

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Summary
Dynamic reorganization of the actin cytoskeleton is fundamental to a number of cell biological events. A variety of actin-regulatory proteins modulate polymerization and depolymerization of actin and contribute to actin cytoskeletal reorganization. Cyclase-associated protein (CAP) is a conserved actin-monomer-binding protein that has been studied for over 20 years. Early studies have shown that CAP sequesters actin monomers; recent studies, however, have revealed more active roles of CAP in actin filament dynamics. CAP enhances the recharging of actin monomers with ATP antagonistically to ADF/cofilin, and also promotes the severing of actin filaments in cooperation with ADF/cofilin. Self-oligomerization and binding to other proteins regulate activities and localization of CAP. CAP has crucial roles in cell signaling, development, vesicle trafficking, cell migration and muscle sarcomere assembly. This Commentary discusses the recent advances in our understanding of the functions of CAP and its implications as an important regulator of actin cytoskeletal dynamics, which are involved in various cellular activities.

Key words: Actin turnover, ADF/cofilin, Cyclase-associated protein, Nucleotide exchange, Severing

Introduction
The coordinated regulation of the assembly and disassembly of actin filaments enables spatially and temporally controlled dynamics of actin cytoskeletal structures within cells. The combined functions of a minimal set of actin-regulatory proteins for nucleation, depolymerization and capping can explain the basic mechanism of persistent actin filament turnover, for example, at the leading edge of motile cells or in the comet tails of bacterial pathogens (Bugyi and Carlier, 2010; Pollard and Cooper, 2009). However, the actin-cytoskeletal structures in different types of cell exhibit different patterns of dynamics that involve a number of additional actin-binding proteins. On the one hand, CAP has been described as an actin-monomer-binding protein that can sequester actin monomers and prevent them from polymerization *in vitro* (Hubberstey and Mottillo, 2002). On the other hand, genetic and cell biological studies of CAP in several organisms have suggested that CAP has important roles in the dynamic actin reorganization that cannot be simply explained by its actin-monomer-sequestering function (Balcer et al., 2003; Bertling et al., 2004; Freeman and Field, 2000). Recent studies have revealed new biochemical functions of CAP. It promotes actin filament dynamics (Box 1), closely cooperates with ADF/cofilin (Box 2) *in vitro* and *in vivo* (Moriyama and Yahara, 2002), and self-oligomerization of CAP enhances its activities (Quintero-Monzon et al., 2009). Furthermore, the conservation of CAPs among eukaryotes suggests that CAP is a fundamentally important actin regulator. This article covers biochemical and cell biological functions of CAPs with an emphasis on their roles in regulating the actin cytoskeleton.

Sequence and structure of CAP
Domain organization of CAP
Depending on the species, CAP consists of 450–550 amino acids, and has several distinct motifs and domains (Fig. 1A), which is discussed in this section. Most species have only a single CAP isoform, although vertebrates have two CAP isoforms, CAP1 and CAP2. Parasitic protozoa only have the C-terminal sequence of CAP, the so-called C-CAPs (Hliscs et al., 2010) (Fig. 1A). Isoforms and orthologs of CAP are discussed in the next section.

The most N-terminal amino acids (~40 residues) of CAP are predicted to form a coiled-coil (Fig. 1A); this region of yeast Srv2/CAP is sufficient for binding to adenylyl cyclase, an enzyme that produces cAMP (Nishida et al., 1998) and is required for self-oligomerization of Srv2/CAP (Quintero-Monzon et al., 2009). However, this N-terminal region is unstructured in solution (Mavoungou et al., 2004) and coiled-coil formation has not been structurally demonstrated thus far. Therefore, a coiled-coil might form upon binding to adenylyl cyclase or self-oligomerization of CAP. Most of the N-terminal region is composed of a stable bundle of six antiparallel α-helices (Fig. 1B) that are termed helical folded domain (HFD). Structural analyses show that the HFD forms a laterally associated anti-parallel dimer (Ksiazek et al., 2003; Yusof et al., 2005) (Fig. 1B, bottom). The HFD binds to the ADF/cofilin-G-actin complex (Moriyama and Yahara, 2002; Quintero-Monzon et al., 2009) and also interacts with ADF/cofilin-bound actin filaments to promote their severing (Chaudhry et al., 2013) (Box 2).

In the central region of CAP, there are two proline-rich regions (P1 and P2) (Fig. 1A). P1 is highly conserved in CAPs from different species – with a stretch of ~10 proline residues, whereas P2 is less conserved – with an only scattered appearance

Box 1. Role of adenine nucleotides (ATP and ADP) in actin filament dynamics

One actin molecule binds to one molecule of ATP or ADP, and the nucleotide status of actin strongly affects its properties in both polymerization and depolymerization. ATP-bound monomeric globular (G-) actin (ATP–G-actin) is more competent for polymerization than ADP–G-actin. Polymerized filamentous (F-) actin has two ends with different properties, the pointed (minus) and barbed (plus) ends. ATP–G-actin binds to the barbed end of F-actin with higher affinity than to the pointed end. When a concentration of ATP–G-actin falls below the dissociation constant for ATP–G-actin with the pointed end of F-actin, actin constantly depolymerizes from the pointed end and polymerizes at the barbed end without an apparent change in the ratio of G- and F-actin, which is called treadmilling. However, ADP–G-actin binds to pointed and barbed ends with nearly equal affinity, and therefore it does not undergo treadmilling. Furthermore, exchange of actin-bound nucleotide occurs primarily at G-actin, whereas hydrolysis of ATP into ADP occurs primarily at F-actin. The newly depolymerized actin monomers are predominantly bound by ADP. When free ATP is present, ADP–G-actin can 'recharge' itself by rapidly exchanging ADP for ATP and, thus, maintaining the treadmilling cycle. However, when no free ATP is available, ADP–G-actin cannot recharge itself, which prevents the treadmilling cycle.

of proline residues. Several interaction partners for CAP selectively bind to either P1 or P2. For instance, profilin, an actin-monomer-binding protein, binds to P1 of yeast Srv2/CAP (Bertling et al., 2007) and of mouse CAP1 (Makkonen et al., 2013). The Src homology 3 (SH3) domain of the tyrosine kinase Abl binds to the P1 of human CAP1 (Freeman et al., 1996), that of the yeast actin-binding protein 1 (ABP1) binds to P2 of yeast Srv2/CAP (Freeman et al., 1996; Lila and Drubin, 1997). Also located in the central region of CAP is a Wiscott Aldrich Syndrome protein homology 2 (WH2) domain (Fig. 1A) (Paunola et al., 2002). WH2 of yeast Srv2/CAP binds to G-actin without exhibiting a strong preference for actin-bound nucleotides and has a crucial role in the nucleotide exchange of G-actin, as described below (Chaudhry et al., 2010) (see Box 1 for the significance of nucleotides in actin dynamics).

The C-terminal half of CAP is mostly composed of β-strands, which are arranged into six coils of right-handed β-helixes (Dodatko et al., 2004) (Fig. 1C). The most C-terminal ~35 amino acids contain a dimerization motif, in which β-strands participate in a β-sheet of a second CAP molecule, resulting in a strand-exchanged dimer (Fig. 1C, bottom). In addition, the C-terminal half of CAP contains a sequence motif that is present in a number of proteins, including tubulin-binding cofactor C and X-linked retinitis pigmentosa 2 protein (RP2) (Dodatko et al., 2004); the homologous domains are currently termed CAPs and RP2 (CARP) domains in the Simple Modular Architecture Research Tool (SMART) database (Schultz et al., 1998). The CARP domain of CAP binds to G-actin independently of the WH2 domain (Chaudhry et al., 2010; Makkonen et al., 2013; Mattila et al., 2004; Nomura and Ono, 2013).

CAP isoforms, orthologs and nomenclature

CAPs have been identified in almost all representative eukaryotic species and are most probably present in all eukaryotes (Fig. 2; supplementary material Table S1). The domain structure of CAP is conserved among species. However, C-CAPs in protozoa only have the CARP domain and the dimerization motif (Hliscs et al., 2010) (Fig. 1A). *Leishmania* and *Trypanosoma* C-CAPs have a proline-rich sequence at the N-terminus, but other C-CAPs do not (Hliscs et al., 2010). *Leishmania major* has another long form of a CAP-related protein that contains the CARP domain (Fig. 2, see the atypical CAP in the phylogenetic tree) and contains an N-terminal region that is not homologous to the HFD domain of CAPs (Hliscs et al., 2010). Fungi, protists and many invertebrates only have one CAP gene (Fig. 2). However, multiple CAP isoforms are present in vertebrates, nematodes and green plants (Fig. 2). Vertebrates have two CAP isoforms, CAP1 and CAP2 (Swiston et al., 1995; Yu et al., 1994), which are ~60% identical in their amino acid sequence and exhibit different expression patterns. In mammals, CAP1 is widely expressed – except in skeletal muscle, whereas CAP2 is predominantly expressed in brain, heart, skeletal muscle and testis (Bertling et al., 2004; Swiston et al., 1995). In particular, CAP2 has been shown to be a crucial regulator of sarcomere assembly in striated muscle (heart and skeletal muscle), as discussed below. However, it is currently unknown whether CAP1 and CAP2 have different biochemical properties. Similarly, the nematode *C. elegans* has both a muscle-specific CAP (CAS-1) (Nomura et al., 2012) and a nonmuscle CAP (CAS-2) (Nomura and Ono, 2013). In addition, several CAP isoforms have been identified in several green plants (Fig. 2; supplementary material Table S1), but their functional differences have not been determined. Thus, other than the tissue-specific expression of CAP isoforms, their functional differences are not well understood. Multiple isoforms of other actin regulators, including ADF/cofilin (Ono, 2007; Poukkula et al., 2011) and profilin (Jockusch et al., 2007; Kovar et al., 2000; Polet et al., 2006), are also expressed in these multicellular organisms, suggesting that specific isoforms of these actin regulators cooperate to modulate actin filament dynamics in a tissue-specific manner.

Cyclase-associated protein is the original name for CAP. However, several alternative names have also been used. CAP was originally identified in budding yeast as an adenylyl-cyclase-associated protein (Field et al., 1990) and also as a suppressor of hyperactive RAS2(V19), thus explaining the yeast name Srv2 (Fedor-Chaiken et al., 1990). CAP and Srv2 are identical, and often designated as Srv2/CAP. ASP-56 has been isolated from pig platelets as an actin-monomer-binding protein, and partial peptide sequences have shown that ASP-56 is porcine CAP1 (Gieselmann and Mann, 1992). Rat CAP1 was cloned as a mammalian CAP homolog 1 and originally named MCH1 (Zelicof et al., 1993). A *Drosophila* CAP gene was identified

Box 2. Actin depolymerizing factor (ADF)/cofilin

ADF/cofilin is an actin-regulatory protein that promotes the turnover of actin filaments. It binds to G-actin and F-actin in a molar ratio of 1:1. ADF/cofilin severs F-actin and promotes dissociation of G-actin from filament ends. Because F-actin is stable, enhancement of F-actin disassembly by ADF/cofilin can supply a pool of G-actin for new rounds of actin polymerization and can promote persistent turnover of actin filaments. ADF/cofilin preferentially binds to an actin molecule bound by ADP and inhibits nucleotide exchange of G-actin. Several other actin regulatory proteins including CAP, profilin and AIP1 cooperate with ADF/cofilin in order to regulate the dynamics of actin filaments as shown in Fig. 3.

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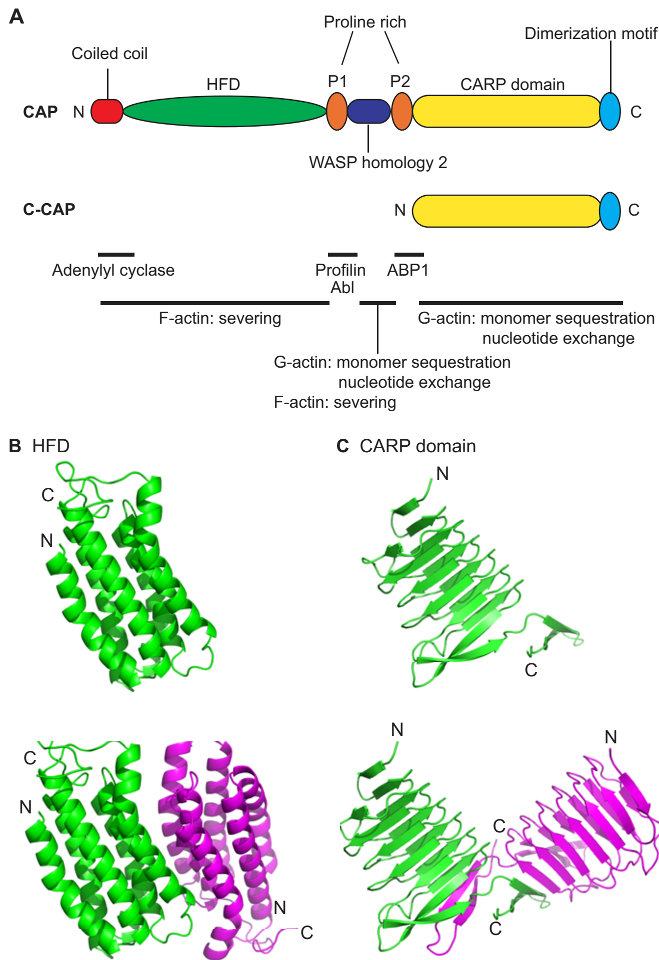

Fig. 1. Structure of CAP. (**A**) Domain organization of CAP and C-CAP (CAP in protozoans). Representative interaction partners and actin-regulatory functions are shown below functional domains. (**B**,**C**) Ribbon diagram of the structures of the helical folded domain (HFD) (Protein Data Bank accession number 1S0P) (B) and β-sheet/β-helix (CARP) domain (Protein Data Bank accession number 1K8F) (C). Structures of monomers (top) and dimers (bottom) are shown. Locations of N- and C-termini are indicated. The molecular graphics were produced with PyMOL (Schrödinger, LLC).

following the screening of mutant flies and is allelic to *capulet* (Baum et al., 2000; Wills et al., 2002) and *act up* (Benlali et al., 2000). *C. elegans* CAP genes were namend *cas-1* and *cas-2* (Nomura et al., 2012), because '*cap*' had already been used for genes that encode subunits of actin capping proteins (Waddle et al., 1993). Apart from these exceptions, cyclase-associated protein or CAP is used as a common name for these proteins in most of the literature.

Actin-regulatory functions of CAPs

Conserved functions of CAPs as regulators of the actin cytoskeleton

Although CAP was originally discovered as a component of the Ras–cAMP signaling pathway, studies of CAPs in various species suggest that this signaling function is specific to only a limited number of organisms; but, instead, their cytoskeletal function appears to be conserved among eukaryotes (Hubberstey and Mottillo, 2002). Two unrelated phenotypes can be detected in Srv2/CAP-deficient yeast cells: defective Ras–cAMP signaling and morphological abnormalities (Fedor-Chaiken et al., 1990; Field et al., 1990). These could be independently suppressed by the expression of an N-terminal half and a C-terminal half of Srv2/CAP, respectively (Gerst et al., 1991). The morphological abnormalities in Srv2/CAP-deficient cells are associated with a lack of polarized actin distribution, the loss of normal actin cables and the formation of abnormal actin bundles (Vojtek et al., 1991). Furthermore, the expression of a heterologous CAP from other species, e.g. fission yeast, in Srv2/CAP-deficient budding yeast can suppress the actin-related phenotype but not the Ras-dependent phenotype, suggesting that the actin-regulatory function is evolutionarily conserved among CAPs (Kawamukai et al., 1992). Similar genetic studies have demonstrated a conserved actin-regulatory function of CAPs from *Lentinus edodes* (shiitake mushroom) (Zhou et al., 1998), rats (Zelicof et al., 1993) and humans (Matviw et al., 1992). Thus, these genetic assays have provided solid evidence that CAPs are, indeed, conserved regulators of the actin cytoskeleton.

Effects of CAP on G-actin – monomer sequestration and enhancement of nucleotide exchange

Early biochemical studies on CAPs suggested that CAPs are actin-monomer sequestering proteins that prevent actin nucleation and elongation from filament ends (Freeman et al., 1995). However, recent studies have revealed that CAPs fulfill more active roles in promoting the dynamics of actin filaments. Therefore, the interpretations of some early genetic and cell biology studies may need to be revised on the basis of these recently identified functions for CAPs in actin dynamics.

CAP binds to G-actin at a molar ratio of 1:1 and inhibits the spontaneous actin polymerization of G-actin. This activity has been demonstrated for CAPs from budding yeast (Freeman et al., 1995), *Dictyostelium* (Gottwald et al., 1996), *Arabidopsis* (Chaudhry et al., 2007), *C. elegans* (CAS-1 and CAS-2) (Nomura et al., 2012; Nomura and Ono, 2013), pig platelets (ASP-56/CAP1) (Gieselmann and Mann, 1992), humans (CAP2) (Peche et al., 2013) and *Cryptosporidium* (C-CAP) (Hliscs et al., 2010). The effect of CAP on actin elongation is somewhat controversial; Freeman et al. (Freeman et al., 1995) reported that yeast Srv2/CAP inhibits the incorporation of G-actin to either pointed or barbed ends of actin filaments, whereas Mattila et al. (Mattila et al., 2004) reported that yeast Srv2/CAP specifically inhibits the incorporation of G-actin to the barbed end. This discrepancy might be owing to different ratios of Srv2/CAP to G-actin used in these studies, because it was shown that human CAP1 accelerates the addition of G-actin to barbed ends when it is present in sub-stoichiometric amounts compared with G-actin, but inhibits monomer addition when CAP1 and G-actin are used at stoichiometric amounts (Moriyama and Yahara, 2002). At the steady state, CAP sequesters G-actin when it is present in a concentration that is equimolar to that of G-actin. As the cellular concentration of CAPs has not been determined, it is unclear whether CAP is present in cells at a concentration that is sufficient to contribute to sequestration of G-actin, which is generally maintained at a high level of 30∼50% of total actin in non-muscle cells (Heacock and Bamburg, 1983). CAPs localize to actin patches in yeast (Lila and Drubin, 1997) and to the leading edges of motile *Dictyostelium* (Gottwald et al., 1996) and mammalian cells (Freeman and Field, 2000; Zelicof et al., 1996). Therefore, the local concentration of CAP could be sufficiently

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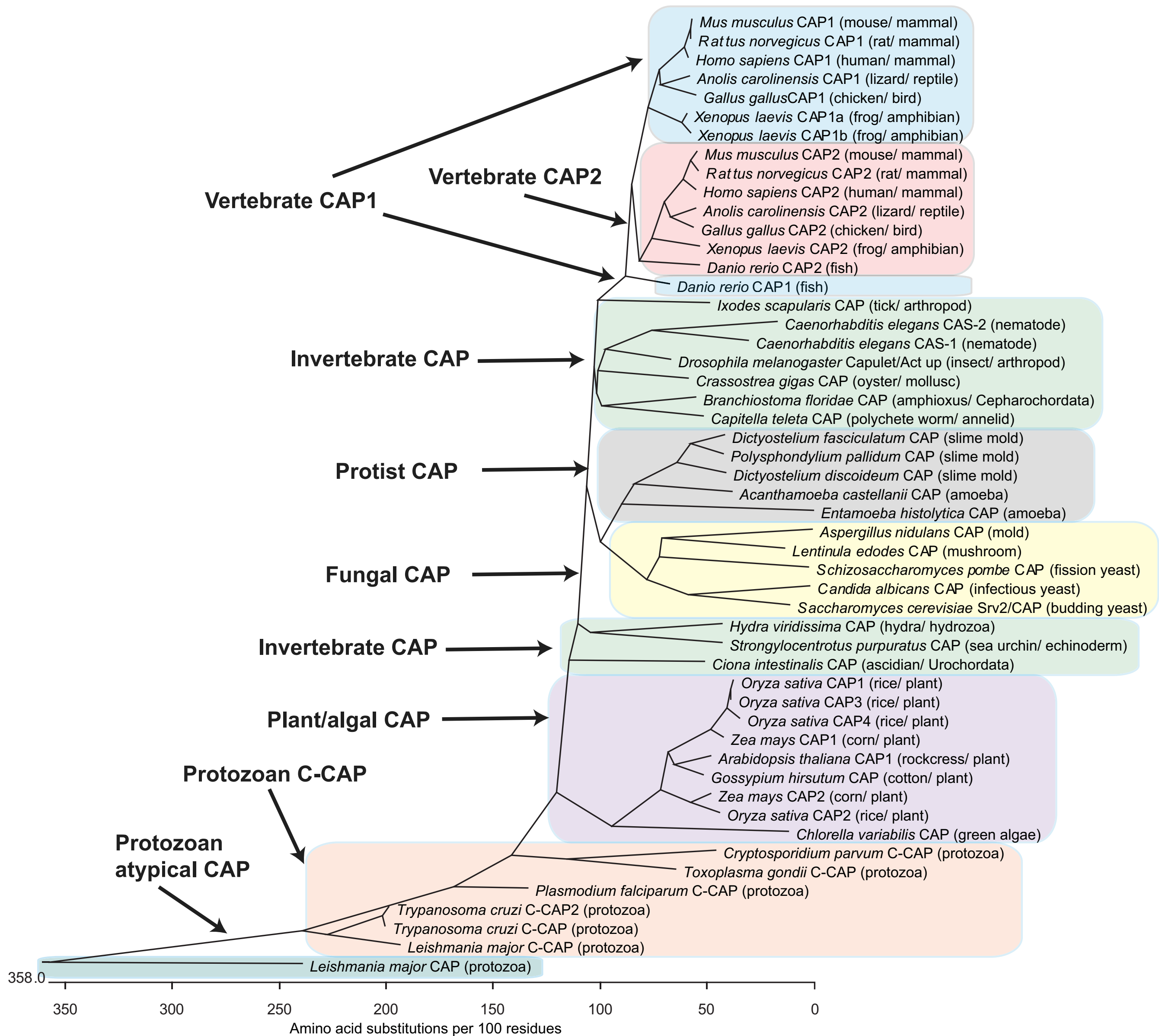

Fig. 2. Phylogenetic tree of CAPs in eukaryotes. Amino acid sequences of CAP-related proteins from representative organisms were analyzed by Clustal W (Thompson et al., 1994) and classified as shown on the left. See supplementary material Table S1 for database accession numbers sequences.

high to sequester actin monomers at these locations. However, it remains unclear whether the actin-monomer sequestering function of CAP is indeed important *in vivo*.

CAP also enhances the rate of exchange of G-actin-bound nucleotides (Fig. 3), which might be its most important actin-regulatory effect. During the 'actin treadmilling' cycle, the constant recharging of G-actin with ATP and ATP hydrolysis by F-actin drive the biased depolymerization of ADP–G-actin from the pointed end of the actin filament and the polymerization of ATP–G-actin from the barbed end (Bugyi and Carlier, 2010; De La Cruz and Pollard, 2001; Kinosian et al., 1993; Pollard, 1986; Pollard et al., 1992) (Box 1). However, several G-actin-binding proteins, including thymosin β4 (Goldschmidt-Clermont et al., 1992) and ADF/cofilin (Nishida, 1985) inhibit this nucleotide exchange and, therefore, prevent this 'recharging' process. Profilin catalytically promotes the nucleotide exchange at G-actin (Goldschmidt-Clermont et al., 1991; Mockrin and Korn, 1980; Nishida, 1985) (Fig. 3) and, therefore, enhances actin turnover in the presence of thymosin β4 (Carlier et al., 1993; Pantaloni and Carlier, 1993) or ADF/cofilin (Blanchoin and Pollard, 1998; Didry et al., 1998). Similarly, CAP is capable of enhancing nucleotide exchange of G-actin and, thus, actin turnover in the presence of ADF/cofilin (Balcer et al., 2003; Chaudhry et al., 2007; Moriyama and Yahara, 2002; Nomura et al., 2012; Quintero-Monzon et al., 2009) (Fig. 3). Such a functional similarity between CAP and profilin was suggested by an early study that showed that the cytoskeletal phenotypes of Srv2/CAP-deficient yeast cells can be suppressed by overexpression of profilin (Vojtek et al., 1991). However, in plants and algae, profilins lack this nucleotide–exchange activity

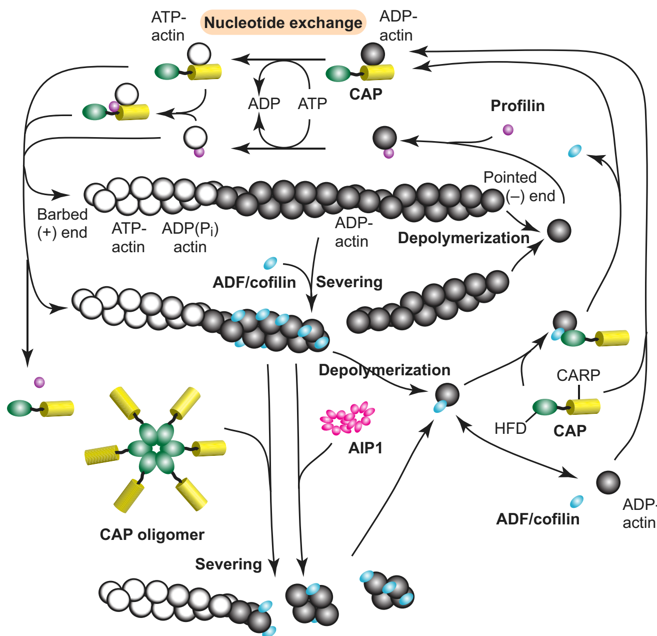

Fig. 3. Regulation of actin filament dynamics by CAP, ADF/cofilin, profilin and AIP1. CAP competes with ADF/cofilin for binding to G-actin (right) and promotes its nucleotide exchange (top). CAP also promotes severing of ADF/cofilin-bound actin filaments (bottom). These activities are similar to those exerted by profilin and AIP1. ADF/cofilin cooperatively binds to actin filaments and severs them at the boundary between ADF/cofilin-bound and the bare segments (Elam et al., 2013). Note that the molecular organization of the CAP oligomer is hypothetical. Also note that CAP binds to actin monomers in an oligomeric form but, for simplicity, only a monomeric CAP is shown as a G-actin-bound form.

(Kovar et al., 2000; Kovar et al., 2001; Perelroizen et al., 1996) and CAP is the only known nucleotide exchanger for G-actin in plants (Chaudhry et al., 2007). However, unlike profilin, which preferentially binds to ATP–G-actin (Pantaloni and Carlier, 1993), CAP binds to ADP–G-actin with a relatively high affinity and effectively competes with ADF/cofilin for binding to ADP–G-actin (Mattila et al., 2004; Moriyama and Yahara, 2002). Yeast profilin has a much weaker nucleotide-exchange activity than mammalian profilin (Eads et al., 1998; Wen et al., 2008) and fails to enhance nucleotide exchange when ADF/cofilin is bound to ADP–G-actin (Chaudhry et al., 2010). Thus, a sequential processing mechanism of actin monomers by CAP (for ADP/ATP exchange) and profilin (for the incorporation of ATP–G-actin to actin barbed-ends) has been proposed in yeast (Mattila et al., 2004). In addition, profilin binds to P1 of Srv2/CAP and collaborates in polarized actin localization in yeast (Bertling et al., 2007) (Fig. 3). However, the functional relationship between CAP and profilin is not well understood in other organisms. For instance, mammalian profilin enhances the nucleotide exchange of G-actin to much faster rates than yeast profilin (Wen et al., 2008), but it is unclear whether profilin and CAP function in a redundant or hierarchical manner to regulate actin turnover in mammalian cells.

In human CAP1 and yeast Srv2/CAP, HFD in the N-terminal half of CAP binds to the ADF/cofilin-actin complex (Fig. 3) and augments the ADF/cofilin-mediated turnover of actin filaments (Moriyama and Yahara, 2002; Quintero-Monzon et al., 2009). These results were originally interpreted as evidence for an active involvement of HFD in the dissociation of the ADF/cofilin-actin complex. However, a recent study shows that HFD is not required for the recycling of ADF/cofilin and G-actin but, instead, has a separate function in actin filament severing (Chaudhry et al., 2013), which is discussed below.

The sites necessary for monomer sequestering and nucleotide exchange activities of CAP reside in its C-terminal half that contains WH2 and CARP (Fig. 1A). The C-terminal half of CAP can bind to one molecule of G-actin (Freeman et al., 1995), and WH2 and CARP bind independently to G-actin (Chaudhry et al., 2010; Makkonen et al., 2013; Mattila et al., 2004; Peche et al., 2013), suggesting that they separately contact G-actin. An N-terminal part of WH2 from other proteins, such as WASP, was found to bind to G-actin at a cleft between its subdomains 1 and 3 (Dominguez, 2007), and WH2 of CAP might bind to G-actin in a similar manner. Although a CARP-binding site on G-actin has not been determined, mutations in its subdomains 2 and 4 – which are the candidate sites for CARP binding – disrupt its interaction with Srv2/CAP in yeast (Amberg et al., 1995). Although the CARP domain is sufficient to promote the nucleotide exchange of ADP–G-actin (Makkonen et al., 2013), WH2 is necessary to facilitate nucleotide exchange when ADF/cofilin is bound to ADP–G-actin (Chaudhry et al., 2010; Nomura and Ono, 2013). Since ADF/cofilin and WH2 share the same binding site on G-actin (Dominguez and Holmes, 2011), WH2 might be needed for CAP to be able to compete with ADF/cofilin for G-actin binding. Nonetheless, the structural basis for the acceleration of the nucleotide exchange of G-actin by CAP is largely unknown.

Effects of CAP on F-actin – enhancement of filament severing

CAP also directly interacts with actin filaments and enhances filament disassembly (Moriyama and Yahara, 2002). Recent microscopic observations have clearly demonstrated that CAP induces the severing of actin filaments (Fig. 3) (Chaudhry et al., 2013; Normoyle and Brieher, 2012). Mammalian CAP1 alone can sever actin filaments at an acidic pH but not at neutral pH (Normoyle and Brieher, 2012), whereas mammalian and avian ADF/cofilins efficiently sever actin filaments at basic pH but not at neutral and acidic pHs (Hawkins et al., 1993; Hayden et al., 1993; Yonezawa et al., 1985). However, when CAP1 and ADF/cofilin are combined, they promote severing of actin filaments within a wide pH range (Normoyle and Brieher, 2012). Thus, either CAP1 or ADF/cofilin alone might function as a pH-dependent F-actin disassembly factor, but CAP1 and ADF/cofilin might act together as pH-independent F-actin disassembly factors. Yeast Srv2/CAP also promotes the severing of ADF/cofilin-bound actin filaments and this activity is mediated by the N-terminal half of Srv2/CAP that contains the coiled-coil region and the HFD (Chaudhry et al., 2013). This activity of CAP resembles that of actin-interacting protein 1 (AIP1), which promotes the severing and disassembly of ADF/cofilin-bound actin filaments (Ono, 2003) (Fig. 3). Indeed, a genetic study in yeast suggests redundant functions of the N-terminal half of Srv2/CAP and AIP1 (Chaudhry et al., 2013). However, the mechanism of how actin filaments are severed by the HFD of CAP still remains unclear. Functionally important surface residues in HFD of yeast Srv2/CAP have been mapped to the surface of the HDF protein, opposite to the location of its N- and C-termini.

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(Quintero-Monzon et al., 2009) (Fig. 1B), but how these residues interact with ADF/cofilin-bound F-actin is unknown. In mammalian CAP2, the WH2 domain is sufficient to sever actin filaments in the absence of ADF/cofilin at a neutral pH (Peche et al., 2013), but its requirement for filament severing in the context of full-length CAP2 still needs to be determined.

Oligomerization of CAP and its effect on actin regulation

Both native and recombinant forms of CAP dimerize or oligomerize, and oligomerization of CAP has been implicated in its function to regulate actin. In yeast cells, Srv2/CAP (55 kDa) and actin (42 kDa) form a high-molecular-mass complex of ~600 kDa (Balcer et al., 2003; Yang et al., 1999) that contains six Srv2/CAP and six actin molecules (Balcer et al., 2003; Quintero-Monzon et al., 2009). Mammalian CAP1 is also always tightly associated with actin monomers in tissues or cells that need to be dissociated using urea when CAP1 is to be purified (Gieselmann and Mann, 1992; Moriyama and Yahara, 2002; Normoyle and Brieher, 2012), which suggests that the formation of a high-molecular-mass CAP-actin complex is a conserved feature. CAP can also dimerize or oligomerize in the absence of actin and, as mentioned above, three separate regions are known to mediate self-association: the N-terminal coiled-coil region (Quintero-Monzon et al., 2009; Yu et al., 1999), the HFD (Ksiazek et al., 2003; Yusof et al., 2005; Yusof et al., 2006) (Fig. 1B) and the C-terminal dimerization motif (Dodatko et al., 2004) (Fig. 1C). Furthermore, a study, in which short CAP fragments where used, suggests that the N-terminal part of CAP can bind to both the N-terminal and C-terminal sections of a second CAP (Hubberstey et al., 1996). A mutational study of *Dictyostelium* CAP suggests that several configurations of a CAP dimer are possible (Yusof et al., 2006). Therefore, a high number of CAP oligomer arrangements are possible, but the actual molecular architecture of the physiological CAP oligomer and CAP-actin complex is currently unknown. Nonetheless, disruption of CAP oligomerization through mutation of its N-terminal coiled-coil region impairs the activity of CAP to promote the disassembly of ADF/cofilin-bound actin filaments (Chaudhry et al., 2013; Quintero-Monzon et al., 2009) and deletion of its C-terminal dimerization motif weakens its binding to actin (Zelicof et al., 1996). Thus, oligomerization of CAP enhances its function to regulate actin to some extent, but the structure-function relationship of CAP oligomers and their activities still remains be elucidated.

The Srv2/CAP-actin complex purified from yeast cells has distinct structural features that suggest a highly ordered assembly of CAP molecules within the complex. Its analysis by electron microscopy shows that there are at least four small lobes in the core and two large peripheral lobes (Balcer et al., 2003), but these structural features could not be correlated with the molecular domains of Srv2 and actin. The purified N-terminal section of yeast Srv2/CAP, which contains the coiled-coil region and the HFD that can sever ADF/cofilin-bound actin filaments, oligomerizes into a hexamer. The latter has a radial pattern composed of six blade-like structures that resemble a wheel-type 'shuriken', a throwing weapon used by Japanese *Ninja* (Chaudhry et al., 2013). However, when the coiled-coil region, which mediates its oligomerization, is absent, HFD alone only has a considerably weaker severing activity, suggesting that CAP oligomerization enhances its activity. Thus, additional structural and functional information for CAP oligomers are required before we fully understand how CAP oligomerization promotes its activities.

Function of CAP as a regulator of the actin cytoskeleton *in vivo*

A link between cellular signaling and the actin cytoskeleton

As noted above, the N- and C-termini of Srv2/CAP independently regulate the Ras–cAMP signalling pathway and the actin cytoskeleton in yeast, respectively, and link these two mechanisms (Freeman et al., 1995; Gerst et al., 1991; Mintzer and Field, 1994). Srv2/CAP mediates the recruitment of Ras and adenylyl cyclase to actin filaments. Interestingly, actin-anchored Ras is an active GTP-bound form and stimulates the production of cAMP, which results in the production of reactive oxygen species and is followed by apoptosis (Gourlay and Ayscough, 2006). The function of CAP as a link between adenylyl cyclase and actin has also been shown in other fungi such as *Candida albicans* (Wang et al., 2010; Zou et al., 2010) and *Magnaporthe oryzae* (a rice pathogen) (Zhou et al., 2012). In *Dictyostelium*, although a direct binding between adenylyl cyclase and CAP has not been detected, CAP also regulates both intracellular cAMP signaling and chemotactic behaviors, and potentially links these two mechanisms (Noegel et al., 2004; Noegel et al., 1999; Sultana et al., 2009; Sultana et al., 2012). However, it is unknown whether CAP is involved in cAMP signaling in animal cells.

As mentioned above, CAP also binds to the SH3 domain of the Abl tyrosine kinase with its proline-rich P1 region (Freeman et al., 1996). In *Drosophila* neuronal development, CAP (Capulet/Act up) and Abl together restrict the midline crossing of axons, suggesting that CAP and Abl are part of a complex that links the growth-cone-repellent signal to changes in the actin filament dynamics (Wills et al., 2002). It has been shown that rat CAP1 is enriched in the neuronal growth cones (Lu et al., 2011; Nozumi et al., 2009), suggesting that CAP is a conserved regulator of neuronal growth. Mutations in *Drosophila* CAP also disturb oocyte polarity (Baum et al., 2000), epithelial cell polarity (Baum and Perrimon, 2001), Hedgehog signaling (Benlali et al., 2000), Hippo signaling (Fernández et al., 2011) and eye development (Benlali et al., 2000; Lin et al., 2012). However, it is not clear whether these phenotypic changes are owing to the direct role of CAP in certain signaling pathways or because of secondary effects of disorganized actin filaments. CAP-mutant *Drosophila* cells accumulate excessive actin filaments that arise owing of impaired actin monomer sequestration (Baum and Perrimon, 2001; Benlali et al., 2000). However, a study in *Drosophila* neuronal dendrites shows that cofilin (Twinstar) localizes to accumulated actin filaments in CAP-null cells (Medina et al., 2008) in a manner similar to that in mammalian cofilin within CAP1-depleted cells (Bertling et al., 2004), suggesting a role of CAP in the correct localization of ADF/cofilin. Therefore, revisiting some of previously characterized CAP-mutant phenotypes in *Drosophila* in the context of actin filament turnover might reveal *in vivo* functions of CAP that have not previously been described.

CAP in cell migration, cytokinesis and cancer

CAP has been implicated in both cell migration and cytokinesis, but its precise role in these processes is not understood. As noted above, CAP is enriched in actin-rich lamellipodia of migrating cells, both in *Dictyostelium* (Gottwald et al., 1996; Noegel et al., 1999) and cultured mammalian cells (Bertling et al., 2004;

Freeman and Field, 2000; Moriyama and Yahara, 2002; Zelicof et al., 1996). The localization of CAP to the leading edge of the cell is mediated through its N-terminal part in an unknown mechanism (Freeman and Field, 2000; Moriyama and Yahara, 2002; Noegel et al., 1999). Knockdown of CAP strongly inhibits the formation of lamellipodia in *Drosophila* S2 cells (Rogers et al., 2003). Knockdown of CAP also reduces the rate of cell migration in *Dictyostelium* (Noegel et al., 1999) and cultured mammalian cells (Bertling et al., 2004; Yamazaki et al., 2009). These reports suggest that CAP enhances lamellipodial protrusion by promoting actin filament dynamics during cell migration. However, CAP also localizes to the posterior cortex of migrating *Dictyostelium* cells (Noegel et al., 1999) and promotes the formation of stress fibers in mammalian fibroblasts (Freeman and Field, 2000), suggesting that it has roles in different aspects of actin dynamics within motile cells; but how exactly it regulates cell migration is still not fully understood.

Reduction of CAP protein levels in *Dictyostelium* leads to an increased number of multinucleated cells, suggestive of a cytokinesis defect (Noegel et al., 1999). However, localization of CAP to cleavage furrows or other cytokinetic structures has not been reported. Deletion of the *CAP* gene in budding yeast or fission yeast causes growth defects at a high temperature or under certain nutrient conditions (Fedor-Chaiken et al., 1990; Field et al., 1990; Kawamukai et al., 1992), but it is unknown whether these are caused by cytokinesis defects.

Cell migration and cytokinesis are aberrantly regulated in cancer cells. Mammalian CAP isoforms are overexpressed in several types of cancer, suggesting a correlation between increased levels of CAP and occurrence of cancer phenotypes. For instance, CAP1 is overexpressed in pancreatic cancer (Yamazaki et al., 2009) and breast cancer (Patsialou et al., 2012), and CAP2 has been found to be overexpressed in hepatocellular carcinoma (Effendi et al., 2013; Shibata et al., 2006). Therefore, if a functional correlation between CAP levels and cancer phenotypes can be established, CAP might serve as a diagnostic marker or therapeutic target for certain types of cancer. In addition, a recent study has shown that CAP1 interacts with the tumor suppressor BRCA1-associated RING domain 1 (BARD1) (Woods et al., 2012), further suggesting an involvement of CAP1 in tumorigenesis. However, the functional significance of this interaction is unknown.

Sarcomere organization in striated muscle

In nematodes and vertebrates, one of the two CAP isoforms is predominantly expressed in striated muscle and has an essential role in the assembly of sarcomeres – the basic contractile units. For example, vertebrate CAP2 is expressed at high levels in certain tissues, including skeletal and cardiac muscles of mammals (Bertling et al., 2004; Peche et al., 2007; Swiston et al., 1995), frogs (*Xenopus*) (Wolanski et al., 2009), and zebrafish (Effendi et al., 2013). Interestingly, the expression of mouse CAP2 is prominently induced during *in vitro* differentiation of embryonic stem cells into cardiomyocytes, indicating that CAP2 is a marker for cardiac development (Christoforou et al., 2008; Terami et al., 2007). In mammalian skeletal and cardiac muscles, CAP2 is localized in the nucleus and diffused in the cytoplasm in skeletal myoblasts and embryonic cardiomyocytes, whereas, in differentiated skeletal myotubes and cardiomyocytes, it localizes to the M-lines, which are at the center of the thick filaments (Peche et al., 2007). CAP2-null mice die earlier than wild-type mice and show a phenotype that is typically associated with dilated cardiomyopathy, including enlarged heart with dilated ventricle and atrium, increased fibrosis, and impaired heart function (Peche et al., 2013; Jeffrey Field, University of Pennsylvania, personal communication). Furthermore, the myofibrils in skeletal and cardiac muscles from CAP2-null mice are disorganized with disturbed M-lines, thin filaments and Z-discs (Peche et al., 2013). Thus, although it is clear that mammalian CAP2 is important for sarcomere organization in striated muscle, the mechanism by which it localizes to the M-line, and its significance, as well as its role in the regulation of sarcomeric actin filaments are not understood. The human CAP2 gene (*CAP2*) is located at the chromosome region 6p22.3, and deletions at this region cause several clinical phenotypes including heart defects (Bremer et al., 2009). Therefore, molecular genetics approaches that target human *CAP2* might reveal specific genetic lesions that are associated with heart diseases.

In the nematode *C. elegans*, the CAP isoform CAS-1 is predominantly expressed in striated muscle and localizes to the M-lines, where it is required for sarcomeric actin organization (Nomura et al., 2012). CAS-1-null worms are homozygous lethal at late embryonic to early larval stages and exhibit severe disorganization of muscle actin filaments with formation of actin aggregates (Nomura et al., 2012). In CAS-1-null worms, the muscle-specific ADF/cofilin UNC-60B is mislocalized to these actin aggregates. UNC-60B is also a crucial regulator of sarcomeric actin organization (Ono et al., 2003; Ono et al., 1999), and its mislocalization in CAS-1-null muscle strongly suggests that CAS-1 is required for correct function of UNC-60B. Thus, the genetic studies in *C. elegans* provide evidence that CAS-1 cooperates with UNC-60B to promote sarcomere organization in striated muscle.

Thus, CAP is a conserved regulator of sarcomere organization. Sarcomeric actin filaments in striated muscle requires a dynamic regulation of actin assembly and disassembly to maintain myofibril integrity (Ono, 2010). The presence of a set of muscle-specific regulators of actin dynamics suggests that the assembly and maintenance of sarcomeres require a specific actin-regulatory mechanism. Mammalian cofilin-2 is a muscle-specific ADF/cofilin (Ono et al., 1994; Vartiainen et al., 2002). Mutations in human cofilin-2 (*CFL2*) cause nemaline myopathy (Agrawal et al., 2007), and cofilin-2-null mice show impaired maintenance of myofibrils in skeletal muscle (Agrawal et al., 2012). Therefore, it will be interesting to determine whether cofilin-2 and CAP2 specifically cooperate to regulate sarcomere assembly in mammalian muscle.

Plant development

In plants, CAP is the only known enhancer of G-actin nucleotide exchange, because plant profilins do not possess this functionality (Chaudhry et al., 2007). Overexpression of *Arabidopsis* CAP1 causes a reduction in the size and number of cells, resulting in smaller leaves and petioles (Barrero et al., 2002). However, overexpression of *Arabidopsis* CAP1 in tobacco plants leads to a reduced organ size that is primarily due to reduced size but not number of cells (Barrero et al., 2003). In addition, CAP1-null mutations in *Arabidopsis* cause a number of developmental defects, including reduced statue and reduced elongation of pollen tubes and root hairs (Deeks et al., 2007), and actin bundle organization is disrupted in root hairs and epidermis. The CAP1-null mutation and mutations in the Arp2/3 complex pathway synergistically enhance morphological defects, suggesting that CAP1 is actively involved in the Arp2/3-dependent actin

regulation, or that CAP1 and Arp2/3 are involved in the same process (Deeks et al., 2007). Plants have multiple CAP isoforms, but their functional differences have not been elucidated.

Other biological functions of CAPs

CAP also has other functions, such as in endocytosis or *Drosophila* development, as discussed in earlier reviews (Hubberstey and Mottillo, 2002; Stevenson and Theurkauf, 2000). More recently, it has been shown that CAP is essential for the correct functioning of the endo-lysosomal system in *Dictyostelium* (Sultana et al., 2005), suggesting that CAP is widely involved in membrane trafficking. In addition, upon induction of apoptosis, mammalian CAP1 translocates to the mitochondria where it further promotes the execution of apoptosis (Wang et al., 2008). It is worth noting that cofilin also translocates to the mitochondria and promotes apoptosis (Chua et al., 2003), suggesting that CAP1 and cofilin are pro-apoptotic proteins, although it remains unclear how exactly they promote apoptosis. Intriguingly, CAP1 has been identified as an autoantigen in patients with systemic lupus erythematosus (Frampton et al., 2000) and rheumatoid arthritis (Kinloch et al., 2005), suggesting that CAP1 also has an extracellular function.

Conclusions

Recent studies have revealed that CAP exerts active roles in promoting actin filament dynamics. The wide distribution of CAPs in different cell types and organisms suggest that CAPs are common regulators of actin dynamics; thus, they are expected to be involved in many diverse processes that require actin reorganization (Fig. 4). Some high-throughput studies have provided hints for these, so far not described, functions of CAPs. For example, upregulation of CAP1 has been reported in the brains of schizophrenic patients (Martins-de-Souza et al., 2010) and in macrophages treated with the anti-tumor agent lapachol (Oliveira et al., 2012), suggesting that CAP1-mediated actin reorganization is induced under these conditions. Despite the fact that several proteins are known to bind to CAP (Fig. 1), the mechanisms, by which CAP activity is regulated, are poorly understood. Mouse CAP1 is phosphorylated (Guo-Lei Zhou, Arkansas State University, and Jeffrey Field, University of Pennsylvania, personal communication), suggesting that activity and localization of CAP can be regulated through kinase signaling pathways. CAP1 is also an intracellular substrate of a matrix metalloproteinase (Cauwe et al., 2008), indicating that the protein level of CAP is controlled by proteolysis. It is also important to better understand the functional relationship between CAP and other actin-regulatory proteins. CAP can participate in both assembly and disassembly of actin filaments, and other actin-regulatory proteins may affect how CAP controls actin dynamics. Thus, further studies on CAPs are most likely to reveal a greater complexity of the regulatory mechanism that governs actin filament dynamics.

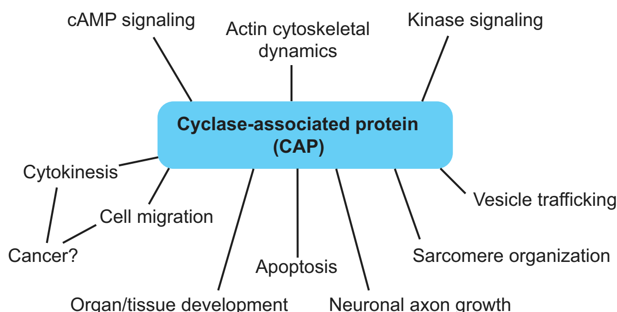

Fig. 4. Biological functions of CAP.

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