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Review

CacyBP/SIP — Structure and variety of functions

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ABSTRACT

Background: CacyBP/SIP (Calcyclin-Binding Protein and Siah-1 Interacting Protein) is a small modular protein implicated in a wide range of cellular processes. It is expressed in different tissues of mammals but homologs are also found in some lower organisms. In mammals, a high level of CacyBP/SIP is present in tumor cells and in neurons. CacyBP/SIP binds several target proteins such as members of the S100 family, components of a ubiquitin ligase complex, and cytoskeletal proteins.
Scope of review: CacyBP/SIP has been shown to be involved in protein de-phosphorylation, ubiquitination, cytoskeletal dynamics, regulation of gene expression, cell proliferation, differentiation, and tumorigenesis. This review focuses on very recent reports on CacyBP/SIP structure and function in these important cellular processes.
Major conclusions: CacyBP/SIP is a multi-domain and multi-functional protein. Altered levels of CacyBP/SIP in several cancers implicate its involvement in the maintenance of cell homeostasis. Changes in CacyBP/SIP subcellular localization in neurons of AD brains suggest that this protein is strongly linked to neurodegenerative diseases. Elucidation of CacyBP/SIP structure and cellular function is leading to greater understanding of its role in normal physiology and disease pathologies.
General significance: The available results suggest that CacyBP/SIP is a key player in multiple biological processes. Detailed characterization of the physical, biochemical and biological properties of CacyBP/SIP will provide better insight into the regulation of its diverse functions *in vivo*, and given the association with specific diseases, will help clarify the potential of therapeutic targeting of this protein.

1. Introduction

CacyBP/SIP (Calcyclin-Binding Protein and Siah-1 Interacting Protein) was originally discovered in mouse Ehrlich ascites tumor cells as an S100A6 (calcyclin) target [1,2] and later found to be a Siah-1 binding partner [3].

CacyBP/SIP is expressed in different mammalian cells and tissues. A high level of this protein is present in the brain and spleen, moderate level in the stomach, liver and heart and low level in kidney, lymph node and rectum [2,4]. More detailed immunohistochemical analyses have shown that in the brain CacyBP/SIP is present in neurons of the cerebellum, hippocampus and cortex, and that mRNA levels change during rat brain development [5]. Studies on mouse and human neuroblastoma cells, NB2a and SH-SY5Y, respectively, have shown that CacyBP/SIP is present in the cytoplasm and translocates to the perinuclear region or to the nucleus upon increase in intracellular Ca^{2+} concentration [6,7] and upon oxidative stress [8].

Analysis of human cDNA libraries revealed the presence of cDNA corresponding to a short, 80 residue CacyBP/SIP form, termed SIP-S. The SIP-S form, which could arise from alternative mRNA splicing, corresponds to the first 80 residues of the full-length 228 amino acid long CacyBP/SIP [3]. Apart from SIP-S, a transcript encoding the C-terminal 185 residues is found in the NCBI database ([3]; NCBI Reference Sequence: NM_001007214.1). Nevertheless, to date, the presence of those shorter isoforms of CacyBP/SIP in cells has not been confirmed experimentally.

CacyBP/SIP is present not only in mammals but also in lower organisms. A CacyBP/SIP gene, assigned as *AccCacyBP*, was cloned from honey bee (*Apis cerana cerana*) [9]. Bioinformatic analysis indicated that *AccCacyBP* is highly conserved and closely related to the CacyBP/SIP gene of other insects. Promoter analysis revealed a number of putative tissue, development and stress-related transcription factor-binding

Abbreviations: AD, Alzheimer's disease; AEDANS, (5-((((2-iodoacetyl)amino)ethyl) amino)-naphthalene-1-sulfonic acid); CacyBP/SIP, Calcyclin-Binding Protein and Siah-1 Interacting Protein; CD, circular dichroism; CHORD, Cysteine and histidine-rich domain; CKII, casein kinase II; CS domain, central domain of CacyBP/SIP; Elk-1, transcription factor; ERK1/2, extracellular signal regulated kinases 1 and 2; HCT116, human colon carcinoma cell line; KIM, kinase interaction motif; NB2a and SH-SY5Y, mouse and human neuroblastoma cell lines; PC12, rat pheochromocytoma; PKC, protein kinase C; SAXS, small-angle X-ray scattering; $SCF^{\beta\text{-}TrCP}$, ubiquitin ligase; SCF^{TBL1}, putative ubiquitin ligase; Sgt1, Suppressor of G2 allele of Skp1; SUMO, Small Ubiquitin related MOdifier.

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sites. Moreover, RT-qPCR demonstrated that in the brain of the honey bee, *AccCacyBP* was developmentally induced and up-regulated by exposure to oxidative stress.

A plant ortholog of CacyBP/SIP, AtSIP, was discovered during sequence analysis of chromosome 1 of *Arabidopsis thaliana* (GenBank: AEE31175.1) [10,11]. This 229 amino acid protein has relatively high homology to mouse CacyBP/SIP. The similarity between the mouse and plant proteins is found throughout the gene and the amino acid sequence identity is approximately 32%. AtSIP expression is tissue-specific, as the protein is present in different anatomical parts of the plant, mainly in flowers and shoot apexes [11]. Determination of the subcellular localization of AtSIP has shown that the protein is present in the cytoplasm and nucleus, while the mammalian CacyBP/SIP is present mainly in the cytoplasm [6].

In the present review we summarize data concerning CacyBP/SIP structure and function in different cellular processes with special attention to recently published reports.

2. CacyBP/SIP structure

2.1. CacyBP/SIP is a multi-domain protein

Mouse CacyBP/SIP protein is composed of 229 residues, while its human ortholog comprises 228 residues. The protein has a molecular mass of 26.5 kDa and migrates in an SDS polyacrylamide gel at the level of ~30 kDa. The sequence identity of mouse and human CacyBP/SIP is ~93% and the main differences (10 out of 17 residues) are found in the N-terminal domain.

CacyBP/SIP consists of three structurally independent domains. Two of them, the N-terminal and central (CS) domains (residues 1–77 and 78–178, respectively) of mouse CacyBP/SIP have a globular character, whereas the C-terminal domain (residues 179–229) is mainly unstructured [12,13]. The N-terminal and CS domains have basic pI, while the C-terminal domain is highly acidic. The calculated pI of the full-length protein is ~7.6. Since full-length CacyBP/SIP seems to be very flexible, attempts to crystallize the entire protein have been unsuccessful [13]. However, the structures of the globular domains were determined using crystallography and NMR for both mouse and human proteins (Fig. 1A).

Analysis of the N-terminal domain (residues 1–77) of mouse CacyBP/SIP using NMR has shown that the first 47 residues form a helical hairpin with a hydrophobic core stabilized by a classic knobs-and-holes arrangement of the side chains (PDB ID: 1YSM) [12]. Results of the crystallographic analysis of the N-terminal domain of human CacyBP/SIP are very similar. In this domain, the hairpin is formed by two helices, α_1 (residues 2–20) and α_2 (residues 24–47), which are connected by a 3-residue long turn (PDB ID: 2A26) [13].

The CS domain of mouse CacyBP/SIP, covering residues 78–178, is composed of a β-sheet sandwich. An X-ray crystal structure for a construct containing residues 61–180 of human CacyBP/SIP shows the same fold (PDB ID: 1X5M). The CS domain exhibits high homology to domains present in CHORD-containing proteins, such as Sgt1, CHP-1, melusin or p23 [14–16]. In some proteins, the CS domain has been shown to take part in Hsp90 recruitment and regulation of formation of protein complexes [17–20], but this has not yet been confirmed for CacyBP/SIP.

The C-terminal domain of mouse CacyBP/SIP consists of residues 179–229. Circular dichroism (CD) spectroscopy revealed that this domain is largely unstructured [21], which was later confirmed by NMR analysis [12]. The percentages of different types of secondary structure in this domain, calculated according to the method of Chen et al. [22], were estimated to be 5% α-helix, 15% β-sheet and 81% random coil. Interestingly, upon binding to S100A6, two helical fragments are formed in the C-terminal domain involving residues 193–200 and 207–216 (PDB ID: 2JTT) [23].

Dimerization of human CacyBP/SIP was reported in 2001 [3]. The N-terminal globular domain (residues 1–47) was defined as the dimerization domain based on the 1.2 Å crystal structure [13]. At that time, CacyBP/SIP dimerization could not be confirmed for mouse ortholog based on indirect NMR data [12]. Recent data obtained using various biochemical and biophysical methods indicate that the recombinant mouse CacyBP/SIP expressed in *Escherichia coli* does exist predominantly in a dimeric form and that the dimer has a non-covalent character [8]. It was found that binding of CacyBP/SIP subunits was driven by a combination of electrostatic (4 surface exposed amino acids: D11, E14, K25, R26) and hydrophobic interactions. Apart from the analyses performed *in vitro*, evidence to support the presence of CacyBP/SIP dimer in cells was obtained using a proximity ligation assay, which suggested that CacyBP/SIP dimer appears mainly in the cytoplasm of neuroblastoma NB2a cells.

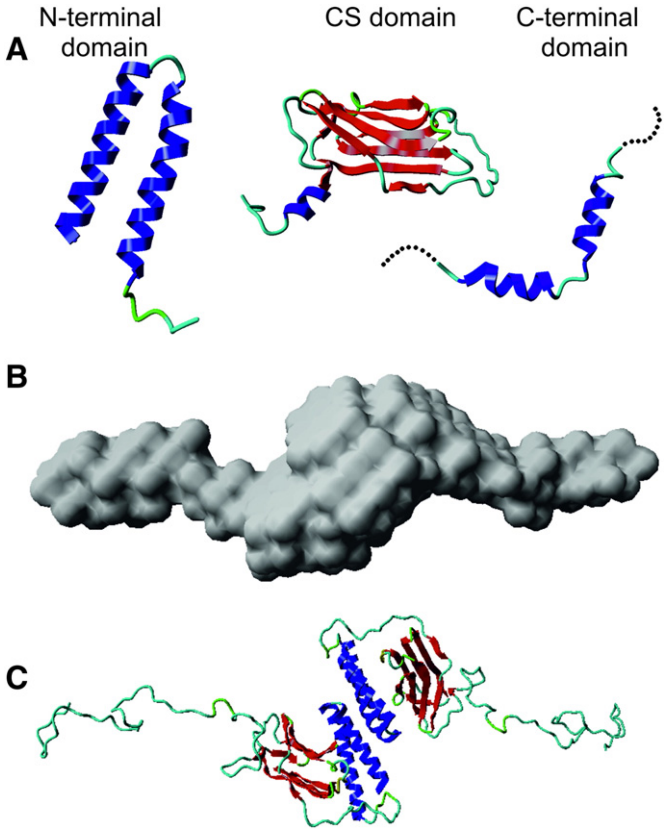

Fig. 1. A – Structures of CacyBP/SIP domains. The NMR structures of the N-terminal domain of mouse protein [12], the CS domain of human protein and the C-terminal domain of mouse CacyBP/SIP upon binding of S100A6 [23]. Black dots represent amino acids within the C-terminal domain for which structural information is lacking. B – Molecular architecture of full-length CacyBP/SIP. Molecular envelope of CacyBP/SIP dimer generated from small angle X-ray scattering data [8]. C – Distribution of domains within the CacyBP/SIP dimer. Representation of the CacyBP/SIP dimer in the same orientation as panel B, showing the likely relative disposition of the three domains.

Given the challenges of crystallizing the full-length, multi-domain CacyBP/SIP protein, the spatial architecture and domain organization were studied using small-angle X-ray scattering (SAXS) [8]. The SAXS results support an anti-parallel orientation of dimer subunits with an extended architecture. In-depth analysis of the data suggests that the globular N-terminal hairpins and CS domains form a relatively compact core and that the flexible C-terminal tails extend away from this core (Fig. 1B and C). The SAXS model of an anti-parallel CacyBP/SIP dimer is consistent with the orientation of the N-terminal domains in the crystal obtained for the human protein [13].

2.2. CacyBP/SIP is a multi-ligand protein

A number of CacyBP/SIP binding partners have been identified. Among them are S100 proteins, components of E3 ubiquitin ligases —