Amino Acids (2011) 41:773–780
DOI 10.1007/s00726-010-0498-2

REVIEW ARTICLE

S100A6 binding protein and Siah-1 interacting protein (CacyBP/SIP): spotlight on properties and cellular function

Gabriela Schneider · Anna Filipek

Received: 15 May 2009 / Accepted: 23 January 2010 / Published online: 25 February 2010

Abstract The CacyBP/SIP protein (S100A6 binding protein and Siah-1 interacting protein) was originally discovered in Ehrlich ascites tumor cells as a S100A6 (calcyclin) target (Filipek and Wojda in Biochem J 320:585–587, 1996; Filipek and Kuźnicki in J Neurochem 70(5):1793–1798, 1998) and later on as a Siah-1 interacting protein (Matsuzawa and Reed in Mol Cell 7(5):915–926, 2001). CacyBP/SIP binds several target proteins such as some calcium binding proteins of the S100 family (Filipek et al. in J Biol Chem 277(32):28848–28852, 2002), Skp1 (Matsuzawa and Reed in Mol Cell 7(5):915–926, 2001), tubulin (Schneider et al. in Biochim Biophys Acta 1773(11):1628–1636, 2007) and ERK1/2 (Kilanczyk et al. in Biochem Biophys Res Commun 380:54–59, 2009). Studies concerning distribution of CacyBP/SIP show that it is present in various tissues and that a particularly high level of CacyBP/SIP is observed in brain (Jastrzebska et al. in J Histochem Cytochem 48(9):1195–1202, 2000). Regarding the function of CacyBP/SIP, there are some reports suggesting its role in cellular processes such as ubiquitination, proliferation, differentiation, tumorigenesis, cytoskeletal rearrangement or regulation of transcription. This review describes the properties of CacyBP/SIP and summarizes all findings concerning its cellular function.

Keywords CacyBP/SIP · S100A6 (calcyclin) · Ubiquitination · Proliferation · Differentiation · Tumorigenesis

G. Schneider · A. Filipek (✉)
Nencki Institute of Experimental Biology,
3 Pasteur Street, 02-093 Warsaw, Poland
e-mail: a.filipek@nencki.gov.pl

Discovery and localization of CacyBP/SIP

The CacyBP/SIP protein (S100A6 binding protein and Siah-1 interacting protein) was originally discovered in Ehrlich ascites tumor cells (Filipek and Wojda 1996) and later found in other cells and tissues of mouse, rat and man. Using different methods such as immunohistochemistry, northern blot and western blot, it was shown that CacyBP/SIP is mainly present in brain and spleen. Moderate level of CacyBP/SIP was detected in stomach, liver and heart and low level in other tissues examined including kidney, lymph node and rectum (Filipek and Wojda 1996; Filipek and Kuźnicki 1998; Zhai et al. 2008). Immunohistochemistry performed on rat brain slices revealed that CacyBP/SIP is present in neurons of the cerebellum, hippocampus and cortex (Jastrzebska et al. 2000). Analysis of CacyBP/SIP mRNA expression during rat brain development showed its highest level in the cerebellum at postnatal day 21, which might suggest the involvement of CacyBP/SIP in development of rat brain.

Immunofluorescence studies performed on mouse neuroblastoma NB2a and human neuroblastoma SH-SY5Y cells showed that CacyBP/SIP is present in the cytoplasm and that it translocates to the perinuclear region or to the nucleus after elevation of intracellular Ca^{2+} (Filipek et al. 2002b; Wu et al. 2003). When the level of intracellular Ca^{2+} was decreased, perinuclear localization was diminished. This Ca^{2+}-dependent translocation of CacyBP/SIP might be due to its post-translational modifications, for instance phosphorylation. Theoretical analysis of the CacyBP/SIP sequence indicated several potential sites that could be phosphorylated by different kinases such as protein kinase C or casein kinase II (Filipek and Kuźnicki 1998). Indeed, it was found that CacyBP/SIP immunoprecipitated from cells with elevated level of Ca^{2+} is

phosphorylated on serine residue(s), while in cells with lower level of intracellular Ca^{2+} no signal from antibodies against phosphorylated serine residue(s) was seen. Thus, phosphorylation of CacyBP/SIP, which occurs in the same Ca^{2+} concentration range that leads to its perinuclear translocation, might contribute to the change of its cellular localization (Filipek et al. 2002b; Wu et al. 2003). Also, a Ca^{2+}-dependent nuclear association was observed for the S100A6 protein which is a target of CacyBP/SIP (Stradal and Gimona 1999). Whether or not S100A6 has any effect on CacyBP/SIP translocation under in vivo conditions requires further studies.

Interaction of CacyBP/SIP with target proteins

Mouse CacyBP/SIP contains 229 amino acids and migrates as a 30 kDa protein in SDS polyacrylamide gel. At the time it was discovered, no homologous protein sequence could be found in databases (SwissProt or GenBank). Nowadays, we know that the amino acid sequence of CacyBP/SIP is partially homologous to that of Sgt1 (suppressor of G(2) allele of Skp1), a protein which plays a role in the kinetochore and the ubiquitin ligase complexes (Kitagawa et al. 1999). This similarity between CacyBP/SIP and Sgt1 is seen in the central and C-terminal parts of both proteins (Nowotny et al. 2003; Bhattacharya et al. 2005). It is, thus, not surprising that CacyBP/SIP and Sgt1 have some common ligands such as S100 proteins and Skp1. However, differences in sequence allow for interactions with ligands which are specific for only one of those proteins. This suggests that despite of sequence homology, Sgt1 and CacyBP/SIP might play a different role in the cell. Studies on the interaction of CacyBP/SIP with its targets allowed for mapping functional domains and provided information regarding its putative function.

Although CacyBP/SIP was discovered as a S100A6 ligand, it appears that it interacts with other members of the S100 family and with proteins such as Siah-1, Skp1, tubulin and ERK1/2 (Fig. 1). The binding of CacyBP/SIP with different S100 proteins was studied by gel overlay and affinity chromatography and the results show that it binds to S100A1, S100A12, S100B and S100P (Filipek et al. 2002a). The binding is specific since other calcium binding proteins such as S100A4, calbindin D9k, calmodulin or parvalbumin do not interact with CacyBP/SIP. The interaction between CacyBP/SIP and S100 proteins is Ca^{2+}-dependent and occurs at low, micromolar concentration of calcium ions indicating that it might occur under physiological conditions (Filipek and Kuźnicki 1998; Filipek et al. 2002a). Detailed studies regarding the CacyBP/SIP–S100A6 complex showed that the C-terminal fragment of CacyBP/SIP is responsible for the interaction with S100A6 (Fig. 1). By means of fluorescence spectroscopy, the dissociation constant of the CacyBP/SIP–S100A6 complex was determined to be 0.96×10^{-6} M (Nowotny et al. 2000). Recent studies pointed out that the S100A6 binding site on CacyBP/SIP consists of 31 amino acids and is located between residues 189 and 219. The structure analysis also revealed that the CacyBP/SIP–S100A6 complex is very unique since the buried surface area at the S100A6–CacyBP/SIP interface is significantly larger than in the case of other known complexes formed between the S100 proteins and their ligands (Lee et al. 2008).

As it was mentioned above, CacyBP/SIP binds the Siah-1 and Skp1 proteins. Siah-1 is a conserved ubiquitin ligase and a homolog of the *Drosophila* seven in absentia gene (Sina) required for degradation of the transcriptional repressor Tramtrack during R7 photoreceptor development (Li et al. 1997). Skp1 (S-phase kinase-associated protein 1) is a component of ubiquitin ligases as well as of the kinetochore complex (Connelly and Hieter 1996; Bai et al. 1996). The binding site of Siah-1 is located in the N-terminal part of CacyBP/SIP and overlaps amino acids 11–80 (Santelli et al. 2005) (Fig. 1). The N-terminal region of CacyBP/SIP also contains a dimerization domain. Detailed structural analysis of the Siah-1–CacyBP/SIP complex showed that CacyBP/SIP contains a Siah-1 binding motif located between residues 60 and 66 and composed of the residues: PAAVVAP (a consensus motif for Siah-1 interacting protein is: PXAXVXP) (Santelli et al. 2005). Mutation of the invariant residues within this motif reduced the binding of CacyBP/SIP to Siah-1 in an in vitro pull-down assay as well as in co-immunoprecipitation and yeast two-hybrid assays. Site-directed mutagenesis and co-immunoprecipitation assay showed that interaction between these two proteins is mediated by ionic contacts involving a negatively charged cluster in the concave surface of the Siah-1 dimer (Matsuzawa et al. 2003). The binding site of Skp1 on CacyBP/SIP was originally described to be located in the C-terminal part of this protein (Matsuzawa and Reed 2001). Detailed analysis showed that the Skp1 binding domain does not overlap with the S100 binding region and that Skp1 binds to the middle part (residues 78–155) of CacyBP/SIP (Filipek et al. 2002a; Bhattacharya et al. 2005). This central part of CacyBP/SIP has high homology to the CS domain, a

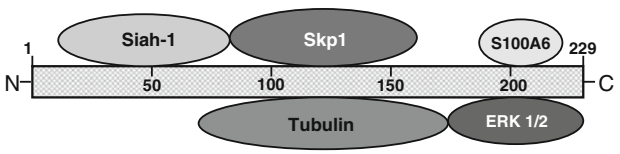

Fig. 1 Schematic representation of CacyBP/SIP indicating the binding sites of its ligands. *Numbers* indicate the position of amino acid residues

domain present in CHORD-containing proteins and in Sgt1 (Sbroggiò et al. 2008).

Another identified ligand of CacyBP/SIP is tubulin. The interaction between these two proteins was shown to be direct and the estimated K_d of CacyBP/SIP–tubulin complex is 1.57×10^{-7} M (Schneider et al. 2007). Using light scattering measurements, it was also found that the tubulin binding domain is located in the middle part of CacyBP/SIP (residues 77–178) (Schneider and Filipek, unpublished results) and does not overlap with the S100A6 binding site (Fig. 1). Thus, this result shows that, at least under in vitro conditions, the S100A6 protein does not influence the CacyBP/SIP–tubulin interaction. It seems also that the tubulin binding site does not overlap with the Siah-1 binding site but it may overlap with the Skp1 binding domain. Immunofluorescence analysis indicates that CacyBP/SIP and tubulin might interact under physiological conditions since both proteins were co-localized in NB2a cells.

Recently, it has been shown that CacyBP/SIP binds to extracellular signal-regulated kinases (ERK1/2) (Kilanczyk et al. 2009) and that the calcium binding protein, S100A6, competes for this interaction (Fig. 1). Moreover, it was found that the E217K mutant of CacyBP/SIP does not bind significantly to ERK1/2, although it retains the ability to interact with S100A6. Molecular modeling showed that the E217K mutation in the 189–219 CacyBP/SIP fragment markedly changes its electrostatic potential, which suggests that the binding with ERK1/2 might have an electrostatic character. In contrast, the interaction between the CacyBP/SIP protein and S100A6 has a hydrophobic nature. Interestingly, it was found that the CacyBP/SIP–ERK1/2 interaction inhibits phosphorylation of the Elk-1 transcription factor by ERK1/2 in vitro and in the nuclear fraction of NB2a cells.

CacyBP/SIP as a component of ubiquitin ligase complex

It has been shown that CacyBP/SIP, together with Siah-1 and Skp1, forms a complex with the TBL1 protein (Matsuzawa and Reed 2001). TBL1, a human homolog of Ebi, is structurally similar to β-TrCP and belongs to the F-box protein family. Since β-TrCP is a component of the SCF (*S*kp1-*c*ullin-*F*-box) ubiquitin ligase responsible for β-catenin ubiquitination, it was proposed that CacyBP/SIP might also be involved in the ubiquitination process. The results showed that a putative ubiquitin ligase complex composed of CacyBP/SIP, Siah-1, Skp1 and TBL1 (called SCF^{TBL1}) ubiquitinates non-phosphorylated β-catenin in contrast to the known $SCF^{\beta TrCP}$ ligase which recognizes and ubiquitinates the phosphorylated protein. Also, the overall scaffold of the SCF^{TBL1} complex is different from the classical $SCF^{\beta TrCP}$ ligase complex. Moreover, it was shown that activity of the SCF^{TBL1} ligase can be regulated by p53 which induces the expression of Siah-1 (Amson et al. 1996; Liu et al. 2001, Matsuzawa et al. 1998). Overexpression or induction of p53 expression in HEK293T or HT1080 cells diminished the level of β-catenin which in consequence caused a decrease in the activity of the Tcf/LEF transcription factor, a binding partner of β-catenin. Transfection of cells with plasmids encoding a dominant-negative mutant of Siah-1, CacyBP/SIP or Ebi prevented the p53-induced decrease in Tcf/LEF activity, which confirms the regulation of β-catenin level by the SCF^{TBL1} complex (Matsuzawa and Reed 2001). Thus, the results indicate that CacyBP/SIP might be a component of the SCF^{TBL1} ligase. Since CacyBP/SIP binds S100 proteins, one may speculate that members of the S100 family can regulate the activity of the SCF^{TBL1} ligase by interaction with CacyBP/SIP. This speculation can be partially supported by a recent work showing that in cells with diminished level of S100A6 the amount of β-catenin is reduced (Lee et al. 2008). These results are in agreement with data regarding the up-regulation of S100 proteins and deficient degradation of β-catenin in tumor and proliferating cells (Lustig and Behrens 2003).

CacyBP/SIP and cell differentiation

Two independent reports showed that the level of CacyBP/SIP is increased upon erythropoietin receptor activation (Xia et al. 2000; Pircher et al. 2001) which suggested that CacyBP/SIP might be involved in a signaling pathway leading to cell differentiation. The up-regulation of CacyBP/SIP is seen in uterus of mice treated with progesterone and 17β-estradiol (Yang et al. 2006). Also, an increased expression of the CacyBP/SIP gene was observed during the decidualization process (Reese et al. 2001). Immunohistochemical analyses performed on decidual tissue during early pregnancy showed that CacyBP/SIP is localized predominantly in stroma and epithelium (Yang et al. 2006). In the decidual stroma, CacyBP/SIP was accumulated around the implanting blastocyst with the progression of decidualization. Antisense oligonucleotides against CacyBP/SIP significantly inhibited apoptosis of endometrial stromal cells induced by UV irradiation. Also, the injection of antisense oligonucleotides into mouse uterine horn impaired the number of implanted blastocysts. These data indicate that CacyBP/SIP might be important for the function of decidual tissue, thus it might play a role in pregnancy establishment. Moreover, it is supposed that the role of CacyBP/SIP in differentiation of cells during decidua formation is through regulation of β-catenin level (Herington et al. 2007).

CacyBP/SIP was also found to be one of the putative differentially expressed genes during neonatal rat heart development (Chim et al. 2000), and moreover it was shown that CacyBP/SIP changes its subcellular localization during differentiation (Wu et al. 2003). The observations regarding the possible involvement of CacyBP/SIP in differentiation were confirmed by Au et al. (2006). These authors showed that mRNA of CacyBP/SIP is up-regulated during rat heart development and that the maximum of its expression is seen at postnatal day 7, a day when cardiomyocytes start to differentiate. Also, it was found that the level of the CacyBP/SIP protein was increased during differentiation of rat H9C2 cardiac myoblasts and, interestingly, up-regulation of this protein was negatively correlated with the level of β-catenin. This confirms the role of CacyBP/SIP in ubiquitination and degradation of β-catenin which was originally showed by Matsuzawa and Reed (2001). Detailed studies of CacyBP/SIP function in differentiation were performed on H9C2 rat cardiac myoblasts. Cells overexpressing the CacyBP/SIP protein, but not the control cells, became multinucleated on the fifth day of differentiation and started to form myotubes. Also, in CacyBP/SIP-overexpressing cells, the mRNA level of myosin light chain 2 and the activity of creatine kinase were significantly higher than in control cells. These results suggest that CayBP/SIP is able to induce differentiation of cardiomyocytes and formation of myotubes. Interestingly, although obvious signs of differentiation were observed in the CacyBP/SIP-overexpressing H9C2 cells, a significant increase in DNA synthesis and in the number of cells entering the S-phase was also seen. Most probably, replication of DNA is needed for the formation of multinucleated cells. The authors of this work also showed that CacyBP/SIP is up-regulated during myocardial infarction. This indicates that CacyBP/SIP might have a protective effect on the cardiomyocytes against hypoxia/reoxygenation but the exact role of CacyBP/SIP in this kind of injury is still unknown. Up-regulation of the CacyBP/SIP protein was also found in neuroblastoma NB2a cells, where it was co-localized with tubulin in cellular processes formed upon differentiation. Moreover, CacyBP/SIP seemed to induce differentiation of these cells, similarly as it was found for cardiomyocytes, since its overexpression caused an increase in GAP-43, a marker of the differentiation process (Schneider et al. 2007).

A recent work shows that the interaction of CacyBP/SIP with ERK1/2 might also be important for cell differentiation. Data obtained from the in vitro kinase assay and luciferase reporter system clearly indicate that the CacyBP/SIP–ERK1/2 interaction inhibits phosphorylation of the Elk-1 transcription factor by ERK1/2. Interestingly, these results are in agreement with data showing that an increased level of CacyBP/SIP, observed in differentiating NB2a cells, inhibits phosphorylation of Elk-1 in the nuclear fraction of these cells. Up to now, many reports have linked the activation of the ERK1/2–Elk-1 pathway with cell proliferation. For instance, it has been shown that phosphorylation/activation of Elk-1 by ERK1/2 is correlated with the activation of immediate early genes such as c-fos (Vickers et al. 2004; Demir and Kurnaz 2008). There are also some reports showing that Elk-1 could be implicated in cell differentiation (Sharrocks 2001; Vanhoutte et al. 2001) but molecular mechanisms leading to this process are poorly understood. Increased level of CacyBP/SIP, observed in the nuclear fraction of differentiating NB2a cells, correlating with decreased level of phosphorylated-Elk-1 and of β-catenin would suggest that CacyBP/SIP might attenuate the proliferative signals and direct cells toward differentiation through inhibition of the Elk-1 phosphorylation/transcriptional activity. Thus, these results suggest that CacyBP/SIP regulates the ERK1/2–Elk-1 pathway and provides a new insight into the molecular events leading to cell differentiation.

Involvement of CacyBP/SIP in cytoskeletal rearrangement

It has been suggested that CacyBP/SIP, via interaction with tubulin, plays a role in reorganization of the cytoskeleton (Schneider et al. 2007). Since CacyBP/SIP and tubulin are highly expressed in brain neurons, interaction between these two proteins might be of physiological relevance. The analysis of CacyBP/SIP localization in brain neurons during aging showed that CacyBP/SIP changes its cellular localization (Filipek et al. 2008). In young animals, this protein was localized in both somata and neuronal processes. Similar localization was observed for tau, a well-known microtubule stabilizing protein. In aged rats, CacyBP/SIP and tau were concentrated in cell bodies, and tubulin staining patterns showed that the microtubule cytoskeleton is impaired. The change in CacyBP/SIP localization did not result from the loss of nerve fibers or from the loss of the CacyBP/SIP protein, but most probably from its phosphorylation or interaction with a phosphorylated form of tau, analogously to the process of MAPs sequestration (Alonso et al. 1997). This observation, together with data on CacyBP/SIP up-regulation in neurons of patients suffering from bipolar disorder (Matigian et al. 2007), point to an important role of this protein in brain neuronal cells.

CacyBP/SIP and tumorigenesis

As it was mentioned above, CacyBP/SIP was shown to be a component of the SCF^{TBL1} ubiquitin ligase regulating the

level of β-catenin. β-Catenin is an oncogene involved in the multi-step process of tumorigenesis including cell proliferation (Sangkhathat et al. 2006), invasion (Lowy et al. 2006) or inhibition of apoptosis (Mezhybovska et al. 2006). Thus, the involvement of CacyBP/SIP in ubiquitination and degradation of β-catenin might point to the role of this protein in tumorigenesis. To check this hypothesis, Sun et al. (2007) studied the expression of the CacyBP/SIP protein in human renal cancer cells and found a decreased level of CacyBP/SIP in cancerous tissues. These observations together with the results showing that overexpression of CacyBP/SIP inhibits proliferation of renal cells suggest that CacyBP/SIP might play a suppressive role in tumorigenesis of renal cell carcinoma. Recently, another group found that CacyBP/SIP might have similar effect on gastric cancer cells (Ning et al. 2007). All these results are in agreement with the findings described by Fukushima et al. (2006) showing that fibroblasts derived from CacyBP/SIP$_{-/-}$ mice had a faster growth rate and enhanced proliferative properties. It has also been reported that diminished expression of CacyBP/SIP in endometrial stroma cells significantly promoted cell viability by inhibition of apoptosis (Yang et al. 2006). Thus, these data suggest that CacyBP/SIP might be a growth-suppressive molecule controlling cell proliferation.

Recent results obtained for a wide range of normal and cancer tissues showed that CacyBP/SIP is expressed in all kinds of tumors investigated including nasopharyngeal carcinoma, osteogenic sarcoma or pancreatic cancer (Zhai et al. 2008). The interesting observation is that in normal tissues such as stomach or colon CacyBP/SIP is weakly or barely detected whereas in gastric or colon tumors this protein seems to be expressed at a high level. Moreover, the level of CacyBP/SIP expression seems to be correlated with metastatic potency (Chen et al. 2008; Zhai et al. 2008). In addition, the CacyBP/SIP ligand, S100A6, was also observed to be up-regulated in many tumors such as colorectal adenocarcinomas (Komatsu et al. 2000), pancreatic cancer (Vimalachandran et al. 2005; Ohuchida et al. 2007) or gastric cancer (Yang et al. 2007). Moreover, S100A6 expression had a positive correlation with metastatic potency of malignant melanoma (Weterman et al. 1992) and was associated with human colorectal adenocarcinoma tumorigenesis and invasion/metastasis (Komatsu et al. 2002). Interestingly, high nuclear level of S100A6 was shown to be associated with poor survival of pancreatic cancer patients (Vimalachandran et al. 2005). In contrast, higher S100A6 level correlates with longer survival of patients with non-small-cell lung cancer (De Petris et al. 2009). These opposite results might be explained by interaction of S100A6 with different ligands such as CacyBP/SIP or p53 (Słomnicki et al. 2009), but the exact role of the CacyBP/SIP–S100A6 interaction in cancer needs further studies.

The effect of CacyBP/SIP on the multidrug resistance phenotype of gastric cancer cells was also studied and the data obtained for cell lines with increased or decreased level of CacyBP/SIP showed that expression of this protein was negatively correlated with sensitivity of gastric cancer cells to vincristine, adriamycin and 5-fluorouracil. These observations suggest an association of CacyBP/SIP with multidrug resistance (Zhao et al. 2002; Shi et al. 2004) and point to CacyBP/SIP as a potential target for the therapy of gastric cancer.

CacyBP/SIP knock-out mice

Some suggestions regarding CacyBP/SIP function come from the knock-out mice. Interestingly, homozygous CacyBP/SIP knock-out mice (CacyBP/SIP$_{-/-}$) were born with normal Mendelian ratio. These animals were fertile and grew normally during 18 months of observation (Fukushima et al. 2006). However, there was no information about the possible differences in the number of offsprings. In 4-week-old animals lacking the CacyBP/SIP protein, thymus and spleen were smaller than in wild-type mice but this difference was not so significant in the case of 8-month-old knock-out mice. This phenotype was similar to that observed for mice expressing stabilized β-catenin in the thymus (Gounari et al. 2001). Also, in the spleen, the size of B and T lymphocyte occupied areas was reduced in CacyBP/SIP$_{-/-}$ mice, suggesting a role of CacyBP/SIP in homeostasis of these cells (Fukushima et al. 2006). Analysis of knock-out animals showed that the T cells at different stages of development had higher level of β-catenin when compared to T cells of wild-type animals. These observations support the hypothesis concerning the regulatory function of CacyBP/SIP in a novel SCF^{TBL1} ubiquitin ligase and in consequence in β-catenin degradation. When mouse embryonic fibroblasts derived from CacyBP/SIP$_{-/-}$ animals were studied, it was found that they had an increased growth rate and higher expression of cyclin D1 and c-myc. Noticeably, the CacyBP/SIP$_{-/-}$ animals did not develop spontaneous tumors or other diseases, although their ability to stop the cell cycle at the G1 phase after DNA damage was changed.

Plant homolog of CacyBP/SIP and its possible function

A protein, named AtCacyBP/SIP, with 32% amino acid sequence identity to human CacyBP/SIP was discovered in *Arabidopsis* (Kim et al. 2006). Analysis of the tissue-specific expression of AtCacyBP/SIP showed that it is highly expressed in flowers, shoot apices and root tips. Studies of subcellular localization of the AtCacyBP/SIP

protein showed its presence in both the nucleus and cytoplasm while mammalian CacyBP/SIP is mainly observed in the cytoplasm (Filipek et al. 2002b). Interestingly, in young spinach leaves, the S100-like proteins were detected and they were also present in the nucleus and cytoplasm (Michetti et al. 1992). This may suggest that in plants the interaction between AtCacyBP/SIP and S100-like proteins can also take place.

In contrast to mammalian CacyBP/SIP, AtCacyBP/SIP does not interact with the SINAT5 protein, an *Arabidopsis* homolog of human Siah-1. The lack of interaction between AtCacyBP/SIP and SINAT5 is most probably due to the lack of a consensus motif PXAXVXP responsible for binding of Siah-1 and indicates that AtCacyBP/SIP might not be a component of a ubiquitin ligase complex in plant cells.

To investigate the function of AtCacyBP/SIP, knock-out and transgenic plants (with overexpression of AtCacyBP/SIP) were generated (Kim et al. 2006). Both types of plants exhibited no phenotypic alterations when grown under normal conditions. Also, no developmental defects or alterations in flowering seasons were observed. To check whether AtCacyBP/SIP plays a role in growth under stress conditions, wild-type plants were exposed to cold, heat, wounding, DNA damaging agent or high salinity. It was found that only high temperature and wounding induce transcription of the AtCacyBP/SIP gene. There was, however, no difference in the sensitivity to high temperature between the knock-out and transgenic plants which suggests that AtCacyBP/SIP does not play a role in response to heat stress. It was also shown that the level of AtCacyBP/SIP was increased in wild-type plants infected with *Pseudomonas syringae* but the exact role of AtCacyBP/SIP in resistance of plants to bacterial infection is not known.

Conclusions

The CacyBP/SIP protein is present in various mammalian tissues and cells. This protein interacts with S100A6 and some other members of the S100 family as well as with Siah-1, Skp1, tubulin and ERK1/2. The binding of CacyBP/SIP with Siah-1 and Skp1 seems to play a role in ubiquitination of β-catenin and the binding with tubulin and ERK1/2 might be important for rearrangements of the cytoskeleton, either in cell differentiation or degeneration. Up to now, the role of the CacyBP/SIP–S100A6 interaction has not been clarified but the competition of S100A6 with ERK1/2 for binding to CacyBP/SIP might suggest involvement of the S100A6-CacyBP/SIP complex in the regulation of Elk-1 transcriptional activity leading to cell differentiation. The possible role of the CacyBP/SIP protein in certain cellular processes is shown in Fig. 2.

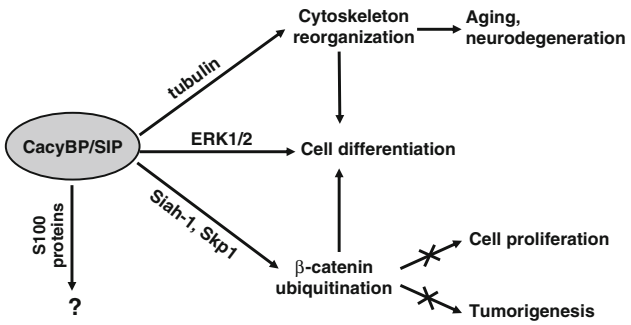

Fig. 2 Involvement of CacyBP/SIP in different cellular processes

Up-regulation of CacyBP/SIP during differentiation and high level of CacyBP/SIP in brain neurons indicate that CacyBP/SIP might be involved in differentiation of neuronal cells. The increased level of CacyBP/SIP in differentiated cells is in agreement with the results obtained for fibroblasts derived from CacyBP/SIP$_{-/-}$ mice which had a faster growth rate and enhanced proliferative properties. Interestingly, overexpression of CacyBP/SIP in renal or gastric cancer cells inhibits their proliferation and the level of CacyBP/SIP is associated with multidrug resistance. Taken together, the CacyBP/SIP protein seems to be involved in many cellular processes under normal and pathological conditions but further studies are needed to clarify its function in cellular signaling pathways.

Acknowledgments We thank Dr. W. Lesniak for helpful discussion and critical reading of the manuscript. This work was supported by a grant to A. Filipek (2 P04A 01030) from the Ministry of Science and Higher Education of Poland and by statutory funds from the Nencki Institute of Experimental Biology.

References

Alonso AD, Grundke-Iqbal I, Barra HS et al (1997) Abnormal phosphorylation of tau and the mechanism of Alzheimer neurofibrillary degeneration: sequestration of microtubule-associated proteins 1 and 2 and the disassembly of microtubules by the abnormal tau. Proc Natl Acad Sci USA 94(1):298–303

Amson RB, Nemani M, Roperch JP et al (1996) Isolation of 10 differentially expressed cDNAs in p53-induced apoptosis: activation of the vertebrate homologue of the drosophila seven in absentia gene. Proc Natl Acad Sci USA 93(9):3953–3957

Au KW, Kou CY, Woo AY et al (2006) Calcyclin binding protein promotes DNA synthesis and differentiation in rat neonatal cardiomyocytes. J Cell Biochem 98(3):555–566

Bai C, Sen P, Hofmann K et al (1996) SKP1 connects cell cycle regulators to the ubiquitin proteolysis machinery through a novel motif, the F-box. Cell 86(2):263–274

Bhattacharya S, Lee YT, Michowski W et al (2005) The modular structure of SIP facilitates its role in stabilizing multiprotein assemblies. Biochemistry 44(27):9462–9471

Chen X, Han G, Zhai H, Zhang F et al (2008) Expression and clinical significance of CacyBP/SIP in pancreatic cancer. Pancreatology 8(4–5):470–477

Chim SS, Cheung SS, Tsui SK (2000) Differential gene expression of rat neonatal heart analyzed by suppression subtractive hybridization and expressed sequence tag sequencing. J Cell Biochem 80(1):24–36

Connelly C, Hieter P (1996) Budding yeast *SKP1* encodes an evolutionarily conserved kinetochore protein required for cell cycle progression. Cell 86(2):275–285

De Petris L, Orre LM, Kanter L et al (2009) Tumor expression of S100A6 correlates with survival of patients with stage I non-small-cell lung cancer. Lung Cancer 63(3):410–417

Demir O, Kurnaz IA (2008) Wildtype Elk-1, but not a SUMOylation mutant, represses egr-1 expression in SH-SY5Y neuroblastomas. Neurosci Lett 437:20–24

Filipek A, Kuźnicki J (1998) Molecular cloning and expression of a mouse brain cDNA encoding a novel protein target of calcyclin. J Neurochem 70(5):1793–1798

Filipek A, Wojda U (1996) p30, a novel protein target of mouse calcyclin (S100A6). Biochem J 320:585–587

Filipek A, Jastrzebska B, Nowotny M et al (2002a) CacyBP/SIP, a calcyclin and Siah-1-interacting protein, binds EF-hand proteins of the S100 family. J Biol Chem 277(32):28848–28852

Filipek A, Jastrzebska B, Nowotny M et al (2002b) Ca2+-dependent translocation of the calcyclin-binding protein in neurons and neuroblastoma NB-2a cells. J Biol Chem 277(23):21103–21109

Filipek A, Schneider G, Mietelska A et al (2008) Age-dependent changes in neuronal distribution of CacyBP/SIP: comparison to tubulin and the tau protein. J Neural Transm 115(9):1257–1264

Fukushima T, Zapata JM, Singha NC et al (2006) Critical function for SIP, a ubiquitin E3 ligase component of the beta-catenin degradation pathway, for thymocyte development and G1 checkpoint. Immunity 24(1):29–39

Gounari F, Aifantis I, Khazaie K et al (2001) Somatic activation of beta-catenin bypasses pre-TCR signaling and TCR selection in thymocyte development. Nat Immunol 2(9):863–869

Herington JL, Bi J, Martin JD et al (2007) Beta-catenin (CTNNB1) in the mouse uterus during decidualization and the potential role of two pathways in regulating its degradation. J Histochem Cytochem 55(9):963–974

Jastrzebska B, Filipek A, Nowicka D et al (2000) Calcyclin (S100A6) binding protein (CacyBP) is highly expressed in brain neurons. J Histochem Cytochem 48(9):1195–1202

Kilanczyk E, Filipek S, Jastrzebska B et al (2009) CacyBP/SIP binds ERK1/2 and affects transcriptional activity of Elk-1. Biochem Biophys Res Commun 380:54–59

Kim YS, Ham BK, Paek KH et al (2006) An Arabidopsis homologue of human seven-in-absentia-interacting protein is involved in pathogen resistance. Moll Cells 21(3):389–394

Kitagawa K, Skowyra D, Elledge SJ et al (1999) SGT1 encodes an essential component of the yeast kinetochore assembly pathway and a novel subunit of the SCF ubiquitin ligase complex. Mol Cell 4(1):21–33

Komatsu K, Kobune-Fujiwara Y, Andoh A et al (2000) Increased expression of S100A6 at the invading fronts of the primary lesion and liver metastasis in patients with colorectal adenocarcinoma. Br J Cancer 83(6):769–774

Komatsu K, Murata K, Kameyama M et al (2002) Expression of S100A6 and S100A4 in matched samples of human colorectal mucosa, primary colorectal adenocarcinomas and liver metastases. Oncology 63(2):192–200

Lee YT, Dimitrova YN, Schneider G et al (2008) Structure of the S100A6 complex with a fragment from the C-terminal domain of Siah-1 interacting protein: a novel mode for S100 protein target recognition. Biochemistry 47(41):10921–10932

Li S, Li Y, Carthew RW et al (1997) Photoreceptor cell differentiation requires regulated proteolysis of the transcriptional repressor Tramtrack. Cell 90(3):469–478

Liu J, Stevens J, Rote CA et al (2001) Siah-1 mediates a novel beta-catenin degradation pathway linking p53 to the adenomatous polyposis coli protein. Mol Cell 7(5):927–936

Lowy AM, Clements WM, Bishop J et al (2006) β-Catenin/Wnt signaling regulates expression of the membrane type 3 matrix metalloproteinase in gastric cancer. Cancer Res 66(9):4734–4741

Lustig B, Behrens J (2003) The Wnt signaling pathway and its role in tumor development. J Cancer Res Clin Oncol 129(4):199–221

Matigian N, Windus L, Smith H et al (2007) Expression profiling in monozygotic twins discordant for bipolar disorder reveals dysregulation of the WNT signalling pathway. Mol Psychiatry 12(9):815–825

Matsuzawa SI, Reed JC (2001) Siah-1, SIP, and Ebi collaborate in a novel pathway for beta-catenin degradation linked to p53 responses. Mol Cell 7(5):915–926

Matsuzawa S, Takayama S, Froesch BA et al (1998) p53-inducible human homologue of Drosophila seven in absentia (Siah) inhibits cell growth: suppression by BAG-1. EMBO J 17(10):2736–2747

Matsuzawa S, Li C, Ni CZ et al (2003) Structural analysis of Siah1 and its interactions with Siah-interacting protein (SIP). J Biol Chem 278(3):1837–1840

Mezhybovska M, Wikström K, Ohd JF et al (2006) The inflammatory mediator leukotriene D4 induces beta-catenin signaling and its association with antiapoptotic Bcl-2 in intestinal epithelial cells. J Biol Chem 281(10):6776–6784

Michetti F, Grilli Caiola M, Botti F et al (1992) Immunochemical and immunohistochemical detection of S-100-like immunoreactivity in spinach tissues. J Histochem Cytochem 40(6):839–843

Ning X, Sun S, Hong L et al (2007) Calcyclin-binding protein inhibits proliferation, tumorigenicity, and invasion of gastric cancer. Mol Cancer Res 5(12):1254–1262

Nowotny M, Bhattacharya S, Filipek A et al (2000) Characterization of the interaction of calcyclin (S100A6) and calcyclin-binding protein. J Biol Chem 275(40):31178–31182

Nowotny M, Spiechowicz M, Jastrzebska B et al (2003) Calcium-regulated interaction of Sgt1 with S100A6 (calcyclin) and other S100 proteins. J Biol Chem 278(29):26923–26928

Ohuchida K, Mizumoto K, Yu J et al (2007) S100A6 is increased in a stepwise manner during pancreatic carcinogenesis: clinical value of expression analysis in 98 pancreatic juice samples. Cancer Epidemiol Biomark Prev 16(4):649–654

Pircher TJ, Geiger JN, Zhang D et al (2001) Integrative signaling by minimal erythropoietin receptor forms and c-Kit. J Biol Chem 276(12):8995–9002

Reese J, Das SK, Paria BC et al (2001) Global gene expression analysis to identify molecular markers of uterine receptivity and embryo implantation. J Biol Chem 276(47):44137–44145

Sangkhathat S, Kusafuka T, Miao J et al (2006) In vitro RNA interference against beta-catenin inhibits the proliferation of pediatric hepatic tumors. Int J Oncol 28(3):715–722

Santelli E, Leone M, Li C et al (2005) Structural analysis of Siah1–Siah-interacting protein interactions and insights into the assembly of an E3 ligase multiprotein complex. J Biol Chem 280(40):34278–34287

Sbroggiò M, Ferretti R, Percivalle E et al (2008) The mammalian CHORD-containing protein melusin is a stress response protein interacting with Hsp90 and Sgt1. FEBS Lett 582(13):1788–1794

Schneider G, Nieznanski K, Kilanczyk E et al (2007) CacyBP/SIP interacts with tubulin in neuroblastoma NB2a cells and induces formation of globular tubulin assemblies. Biochim Biophys Acta 1773(11):1628–1636

Sharrocks AD (2001) The ETS domain transcription factor family. Nat Rev Mol Cell Biol 2:827–837

Shi Y, Hu W, Yin F et al (2004) Regulation of drug sensitivity of gastric cancer cells by human calcyclin-binding protein (CacyBP). Gastric Cancer 7(3):160–166

Słomnicki LP, Nawrot B, Leśniak W (2009) S100A6 binds p53 and affects its activity. Int J Biochem Cell Biol 41(4):784–790

Stradal TB, Gimona M (1999) Ca(2+)-dependent association of S100A6 (Calcyclin) with the plasma membrane and the nuclear envelope. J Biol Chem 274(44):31593–31596

Sun S, Ning X, Liu J et al (2007) Overexpressed CacyBP/SIP leads to the suppression of growth in renal cell carcinoma. Biochem Biophys Res Commun 356(4):864–871

Vanhoutte P, Nissen JL, Brug B, Gaspera BD et al (2001) Opposing role of Elk-1 and its brain-specific isoform, short Elk-1, in nerve growth factor-induced PC12 differentiation. J Biol Chem 276:5189–5196

Vickers ER, Kasza A, Kurnaz IA, Seifert A et al (2004) Ternary complex factor-serum response factor complex-regulated gene activity is required for cellular proliferation and inhibition of apoptotic cell death. Mol Cell Biol 24:10340–10351

Vimalachandran D, Greenhalf W, Thompson C et al (2005) High nuclear S100A6 (Calcyclin) is significantly associated with poor survival in pancreatic cancer patients. Cancer Res 65(8):3218–3225

Weterman MA, Stoopen GM, van Muijen GN et al (1992) Expression of calcyclin in human melanoma cell lines correlates with metastatic behavior in nude mic. Cancer Res 52(5):1291–1296

Wu J, Tan X, Peng X et al (2003) Translocation and phosphorylation of calcyclin binding protein during retinoic acid-induced neuronal differentiation of neuroblastoma SH-SY5Y cells. J Biochem Mol Biol 36(4):354–358

Xia ZB, Dai MS, Magoulas C et al (2000) Differentially expressed genes during in vitro differentiation of murine embryonic stem cells transduced with a human erythropoietin receptor cDNA. J Hematother Stem Cell Res 9(5):651–658

Yang YJ, Liu WM, Zhou JX et al (2006) Expression and hormonal regulation of calcyclin-binding protein (CacyBP) in the mouse uterus during early pregnancy. Life Sci 78(7):753–760

Yang YQ, Zhang LJ, Dong H et al (2007) Upregulated expression of S100A6 in human gastric cancer. J Dig Dis 8(4):186–193

Zhai H, Shi Y, Jin H et al (2008) Expression of calcyclin-binding protein/Siah-1 interacting protein in normal and malignant human tissues: an immunohistochemical survey. J Histochem Cytochem 56(8):765–772

Zhao Y, You H, Liu F et al (2002) Differentially expressed gene profiles between multidrug resistant gastric adenocarcinoma cells and their parental cells. Cancer Lett 185:211–218