

## Review

## Unique S100 target protein interactions

Atoosa Rezvanpour and Gary S. Shaw

*Department of Biochemistry, The University of Western Ontario, London, Ontario, Canada N6A 5C1*

**Abstract.** Three-dimensional structures of S100B, S100A1, S100A6 and S100A11 have shown that calcium binding to these proteins results in a conformational change allowing them to interact with many biological targets. The structures of some S100 proteins in the presence of peptide targets from Ndr kinase, p53, CapZ, annexins A1 and A2 and the Siah-1 Interacting Protein indicate there are at least three modes of recognition that utilize two distinct surfaces in the S100 proteins. These surfaces have been hypothesized to simultaneously accommodate multiple binding partners. This review focuses on potential multiprotein complexes involving calcium-insensitive S100A10, annexin A2 and several other proteins including AHNAK, dysferlin, NS3, TASK-1 and TRPV5/6.

**Keywords:** Annexin — Multi-protein complex — Calcium-signaling — Membrane repair — Three-dimensional structure

### Introduction

The S100 proteins are a group of proteins comprising at least 25 members in humans including S100B, S100A1, S100A6, S100A10 and S100A11 (Donato 2001; Heizmann et al. 2002). The proteins are dimeric having two “EF-hand” calcium-binding motifs in each subunit. *In vivo* experiments have shown that both homo- and heterodimeric S100 complexes are formed (Deloulme et al. 2000; Propper et al. 1999; Wang et al. 2005). The functions of the S100 proteins are to act as calcium-signaling molecules by converting changes in cellular calcium levels to a variety of biological responses. In this manner, many of the S100 proteins have been shown to modulate enzyme activities, oligomerization of cytoskeletal protein components (tubulin, desmin, glial fibrillary acidic protein), modulate ubiquitination, control membrane vesicle formation and participate in trafficking of proteins to the inner surface of the plasma membrane (Santamaria-Kisiel et al. 2006).

Most S100 proteins bind calcium and undergo a conformational change allowing them to interact with specific target proteins and control a cellular activity (reviewed in Santamaria-Kisiel et al. 2006; Wilder et al. 2006; Zimmer et al. 2003). In general, it has been shown that calcium binding to the first EF-hand (helix I, loop, helix II) is weaker than

binding to the second EF-hand, comprised of helices III and IV. Three-dimensional structures of several S100 proteins in the calcium-free (apo) and calcium-bound states show that the major structural change involves the movement of helix III to expose previously buried residues which create a hydrophobic surface. In one S100 protein, S100A10, substitutions in both its calcium-binding sites have left this protein with the inability to coordinate calcium. Consequently, S100A10 does not undergo a calcium-induced structural change and instead adopts a structure in its calcium-free state that is very similar to the calcium-bound states of other S100 proteins (Rety et al. 1999; Rety et al. 2000). As a result S100A10 has been observed to interact and control the functions of more than a dozen proteins in a calcium-insensitive manner.

Frequent partners for the S100 proteins are members of the annexin protein family. At least 10 different S100-annexin complexes have been characterized (summarized in Santamaria-Kisiel et al. 2006). The annexins are a group of highly helical proteins having twelve members in humans. Each annexin protein has a core domain comprised of four (annexins A1–A5, A7–A11, A13) or eight (annexin A6) structurally conserved repeats, each possessing five  $\alpha$ -helices. Although these proteins bind calcium, a large conformational change analogous to the S100 proteins does not occur. Instead, calcium binding to the annexins has been shown to promote association with phospholipid membranes. In particular, Gerke and Moss (2002) have proposed an elegant hypothesis whereby S100A10 and/or S100A11 can coordi-

Correspondence to: Gary S. Shaw, Department of Biochemistry University of Western Ontario London, Ontario, Canada N6A 5C1  
E-mail: gshaw1@uwo.ca

nate pairs of annexin A1 or A2 proteins allowing them to bridge two membrane surfaces. The mechanism is mediated by a high local calcium concentration that promotes the annexin-membrane interaction and/or conformational change in S100A11. This process promotes membrane fusion required for enlargesome vesicle formation, a requirement in models for the maintenance of membrane lesions, or for vesiculation processes used in endo/exocytosis. In the last few years, several membrane-spanning proteins (TASK-1, dysferlin, NS3) have been found to interact with S100A10 together with annexin A2 as a possible means for trafficking of these proteins to the plasma membrane or assemble multiprotein complexes important in membrane repair processes. In this review we compare the three-dimensional structures of several S100-target protein complexes and use this information to rationalize some newly identified multiprotein S100-annexin complexes.

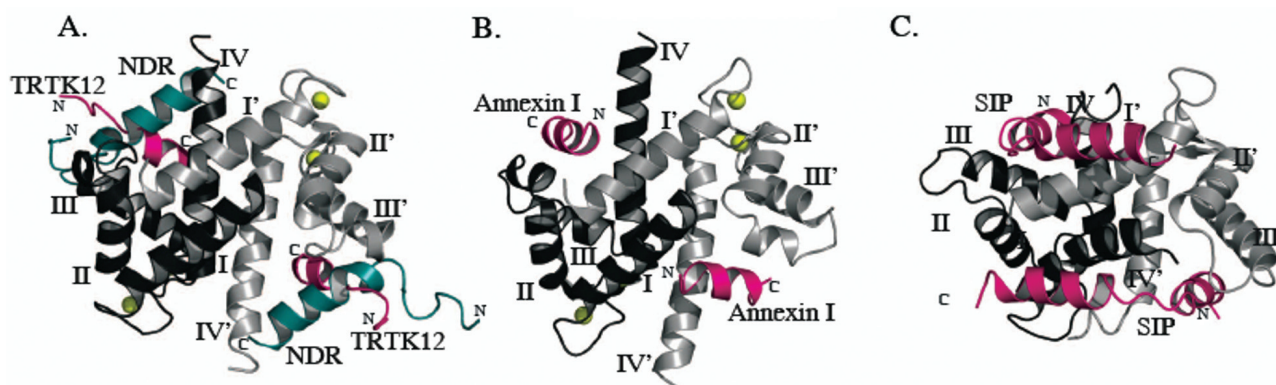
#### Different modes of recognition for S100 target protein complexes

The three-dimensional structures of several S100 proteins have been determined in complex with a variety of peptides derived from their parent proteins. These include calcium-bound S100B (Ca<sup>2+</sup>-S100B) in complex with peptides from the C-terminal region of p53 (Rustandi et al. 2000), the N-terminal regulatory domain from Ndr kinase (Bhattacharya et al. 2003) and the actin-capping protein CapZ (TRTK12) (Inman et al. 2002; McClintock and Shaw 2003); calcium-bound S100A1 (Ca<sup>2+</sup>-S100A1) in complex with peptides from CapZ (Wright et al. 2009) and the cytosolic region of the ryanodine receptor (RyR) (Wright et al. 2008); calcium-bound S100A6 (Ca<sup>2+</sup>-S100A6) in complex with a C-terminal region from the Siah-1 Interacting Protein (SIP) (Lee et al. 2008) and, Ca<sup>2+</sup>-S100A11 in complex with the N-terminus of the phospholipid-binding protein annexin A1 (Rety et al. 2000). In addition, the structure of S100A10, an S100 protein unable to bind calcium due to substitutions in both calcium-binding loops, is available in complex with the N-terminal region from annexin A2 (Rety et al. 1999). In general these structures show there are at least three distinct modes a target protein adopts when binding to an S100 protein (Fig. 1), all showing a symmetric relationship and 1:1 stoichiometry for the target:S100 protomer.

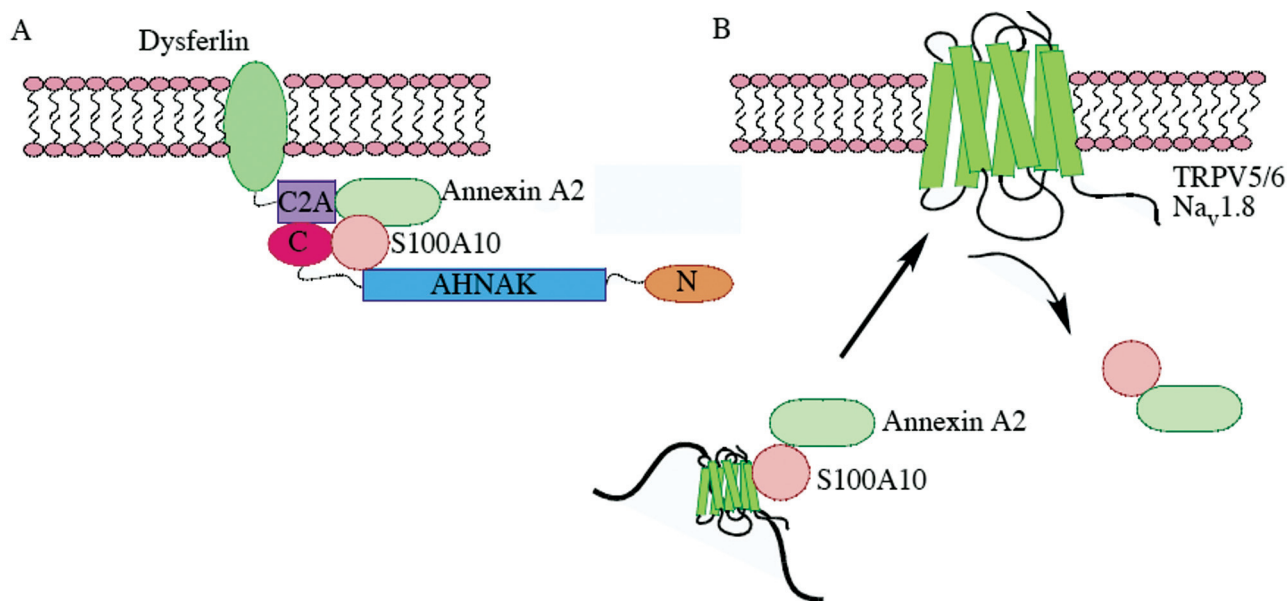
The first mode of binding involves the interaction between the target protein and the hydrophobic surface exposed between helices III and IV due to calcium binding to the S100 protein. In this case, all interactions between the target and the S100 protein occur at the S100 protomer level. This type of interaction is exhibited in the Ca<sup>2+</sup>-S100B structures with TRTK12 (Protein Data Bank accession codes - 1MWN, 1MQ1), Ndr kinase (1PSB, Fig. 1A) and p53 (1DT7), and the

Ca<sup>2+</sup>-S100A1 structures with TRTK12 (2KBM, Fig. 1A) and the ryanodine receptor (2KF2). In the p53 (Rustandi et al. 2000) and Ndr kinase (Bhattacharya et al. 2003) structures, each bound peptide forms a three-turn  $\alpha$ -helix such that its N-terminus lies near the N-terminus of helix III and its C-terminus has key interactions with the C-terminus of helix IV of Ca<sup>2+</sup>-S100B. In contrast, the TRTK12 structures with Ca<sup>2+</sup>-S100A1 (Wright et al. 2009) and Ca<sup>2+</sup>-S100B (Inman et al. 2002) form 1.5 turn  $\alpha$ -helices and are aligned nearly perpendicular to the orientations of the p53 and Ndr peptides such that the C-terminus of TRTK12 is closer to the middle of helix IV (Fig. 1A). Even though the TRTK12 peptide binds within the helix III-IV cleft in both structures the presentation of the peptide is different when bound to Ca<sup>2+</sup>-S100A1 compared to Ca<sup>2+</sup>-S100B. In Ca<sup>2+</sup>-S100A1 the anchoring tryptophan (W7) of TRTK12 interacts with residues towards the C-terminus of helix IV (L81, A84, C85, F88) whereas in Ca<sup>2+</sup>-S100B, this same tryptophan interacts with residues in the linker (I47) and helix III (V53, V56). These differences have the affect of rotating the entire TRTK12 peptide by about 60° and “tipping” the peptide by about 20° towards helix III in the Ca<sup>2+</sup>-S100B structure. Pictorially the binding of a peptide representing the cytosolic region from the ryanodine receptor to Ca<sup>2+</sup>-S100A1 (Wright et al. 2008) appears to be quite similar to that described for TRTK12. However, major differences exist as the RyR peptide forms a three-turn  $\alpha$ -helix and is oriented nearly 180° with respect to TRTK12 such that its C-terminus is proximal to the N-terminus of helix III in Ca<sup>2+</sup>-S100A1. An anchoring tryptophan (W5) in the RyR peptide interacts with residues near the middle of helix IV (L77, A80, L81) but also with I57 in helix III. The diversity of the interactions exhibited by these five S100-target peptide structures can be attributed to the binding surfaces in S100A1 and S100B that are broader and flatter than other calcium-sensor proteins (i.e. calmodulin) and have different distributions of hydrophobic and charged residues (Bhattacharya et al. 2004; Bhattacharya et al. 2003). This observation likely accounts for the broad spectrum of target proteins that interact with the S100 proteins and makes prediction of target binding based on protein sequence somewhat problematical.

The second mode of interaction utilized by the S100 proteins is displayed by structures of Ca<sup>2+</sup>-S100A11 (1QLS, Fig. 1B) and S100A10 (1BT6) with N-terminal peptides from annexins A1 and A2 respectively (Rety et al. 1999; Rety et al. 2000). Unlike the diversity displayed by Ca<sup>2+</sup>-S100A1 and Ca<sup>2+</sup>-S100B, these structures show nearly identical locations and orientations for peptide binding such that < 1 Å rmsd exists between structures. In both structures the annexin peptide bridges the two S100 protomers such that its C-terminus interacts with the linker and helix IV of one protomer while the annexin N-terminus has contacts near the N-terminus of helix I' of the partner protomer. The



**Figure 1.** Different modes for target protein interaction with S100 proteins. (A) Binding to the helix III-IV hydrophobic displayed by TRTK12 (pink) bound to  $\text{Ca}^{2+}$ -S100A1 (2KBM) and NDR kinase (cyan) bound to  $\text{Ca}^{2+}$ -S100B (1PSB). (B) Binding near helix I' at the dimer interface displayed by the N-terminal region of annexin A1 (pink) and  $\text{Ca}^{2+}$ -S100A11 (1QLS). (C) Two-site surface mode displayed by the C-terminal region of SIP bound to  $\text{Ca}^{2+}$ -S100A6 (2JTT). Ribbon diagrams of the calcium-saturated S100 proteins are presented with one of the protomers shaded in black (helices labeled as I-IV) and the other protomer shown in light grey (helices labeled as I'-IV'). Calcium ions are illustrated in yellow spheres.



**Figure 2.** Possible involvement of S100A10 in multiprotein complexes. (A) Proposed interactions in the dysferlin complex based on multiple biochemical experiments. The C-terminus of AHNAK is shown to interact with S100A10 and annexin A2 and the dysferlin C2A domain is near annexin A2 (adapted from Huang et al. 2007). (B) Possible complexes formed by membrane channel proteins such as TRPV5, TRPV6 or Nav1.8 and S100A10-annexin A2 for trafficking to the plasma membrane. Once inserted into the membrane, the S100A10-annexin A2 could dissociate or become associated with phospholipids in the membrane. In all cases, dimeric S100A10 could coordinate two annexin A2 proteins although for simplicity only one is shown.

similarity of these S100-annexin structures is not surprising given the conservation of hydrophobic residues in both annexin peptides. The interacting residues correspond to V3, F6, L7 and L10 in annexin A1 and V3, I6, L7 and L10 in annexin A2 forming a XOOXXOOX interaction motif (X =

hydrophobic residue, O = variable). This level of conservation does not exist between the TRTK12, RyR, p53 and Ndr sequences described earlier in complex with  $\text{Ca}^{2+}$ -S100A1 or  $\text{Ca}^{2+}$ -S100B proteins. Some similarity also exists in the annexin contacting residues in S100A10 and  $\text{Ca}^{2+}$ -S100A11,

especially in helix I' and helix IV. Although it has been shown that N-terminal peptides from annexins A1 and A2 both interact with  $\text{Ca}^{2+}$ -S100A11 (Rintala-Dempsey et al. 2006), it is interesting to note that annexin A1 does not form a tight complex with S100A10 (Streicher et al. 2009). Further, a strong level of specificity has been shown for S100 recognition of annexins A1 and A2. Other than S100A10 and  $\text{Ca}^{2+}$ -S100A11, the only other S100 protein that displays a tight calcium-dependent interaction with annexin A1 is S100A6 (Streicher et al. 2009).

Whereas both the previous modes of target peptide binding with S100 proteins utilized different albeit contiguous surfaces, the recent structure (Lee et al. 2008) of the C-terminal domain from the Siah-1 Interacting Protein (2JTT) shows a hybrid two-site surface (Fig. 1C). In this structure the SIP peptide forms two distinct three-turn  $\alpha$ -helices oriented nearly perpendicular to each other upon binding to  $\text{Ca}^{2+}$ -S100A6. The first helix (Helix A) occupies a position and orientation similar to that observed for the RyR peptide utilizing the hydrophobic groove between helices III and IV. Helix A of SIP lies diagonally across helix III in  $\text{Ca}^{2+}$ -S100A6 with L196 and I199 anchoring the peptide through interactions with residues in helix IV and the C-terminus of helix III respectively. The second helix in SIP (helix B) lies across helix I' of the adjacent protomer, in effect bridging the two S100A6 subunits analogous to the S100-annexin complexes. Whereas the annexin structures only contact the N-terminus of helix I' in either S100A10 or  $\text{Ca}^{2+}$ -S100A11, helix B in SIP has interactions towards the more central portion of helix I' (I9', I13' and K18'). This results in helix B of SIP and helix I' of  $\text{Ca}^{2+}$ -S100A6 running along opposite faces of helix I' forming a near mirror image of each other, albeit helices I and B are oriented in opposite directions. The two binding regions for SIP to  $\text{Ca}^{2+}$ -S100A6 could in part be due to the length of the SIP fragment (189-219; 30 residues) that is significantly longer than any of the other target peptides for which structures are available. Lee and co-workers show that the two helices in SIP are not equivalent in their abilities to coordinate S100A6 with helix A having a much tighter binding, while the less hydrophobic helix B enhances the affinity by about 4-fold.

The three modes of target binding to the S100 proteins utilize two distinct binding regions – the helix III-IV hydrophobic groove and the dimer interface near helix I' on the partner protomer. It has been suggested that the combination of these two regions might represent the full interaction surface for single or multiple protein interactions with the S100 proteins. Otterbein proposed this idea by superimposing the peptide binding regions for annexins A1 or A2, and p53 on the surface of  $\text{Ca}^{2+}$ -S100A6 showing these peptides occupied distinct regions of the surface with no observable steric clashes between the peptides (Otterbein et al. 2002). Subsequently, it was shown for TRTK12 binding to  $\text{Ca}^{2+}$ -

S100B that a region similar to the annexin binding site was left exposed, and that a larger target protein might be accommodated (McClintock and Shaw 2003). The structure of SIP bound to  $\text{Ca}^{2+}$ -S100A6 (~1100 Å) provides the first structural evidence for a target protein that occupies both the helix III-IV groove and helix I' interface, a binding surface that is nearly twice the size of other S100 target protein surfaces (500-700 Å). Amongst the questions raised by this important structure and earlier hypotheses are – is there evidence for other S100 protein complexes that might utilize this larger target protein binding surface or can this surface possibly accommodate simultaneous binding from two distinct target proteins?

### Evidence for multiprotein S100 complexes involving annexin proteins

A variety of S100 protein interactions have been shown to be more complicated than a single target interacting per S100 protomer molecule. For example multiprotein complexes between S100A10 and annexin A2 and either AHNAK (Benaud et al. 2004; De Seranno et al. 2006), TRVP5/6 (Borthwick et al. 2008; van de Graaf et al. 2003) or plasminogen (MacLeod et al. 2003) have been identified from two- and three-hybrid or direct binding experiments. The S100A10-annexin A2 complex is thought to be necessary to recruit an additional target, or allow both targets to bind simultaneously indicating that the binding sites for AHNAK, TRVP5/6 and plasminogen on S100A10 are likely distinct from those of annexin A2. The complexes described here focus on those for S100A10, largely because it functions well in yeast two-hybrid experiments. Other S100 proteins (S100B, S100A1, S100A6) do not respond well in this method due to the calcium-sensitivity of their protein interactions, although homo- and heterodimeric S100 complexes can be identified using two-hybrid experiments (Deloulme et al. 2000; Deloulme et al. 2003).

The interaction between the transient receptor potential cation channel proteins TRPV5 and TRPV6 with S100A10 has been shown using two-hybrid and co-immunoprecipitation experiments (Borthwick et al. 2008; van de Graaf et al. 2003). In this role, the S100A10-annexin A2 complex is thought mediate trafficking of the TRV5 and TRV6 proteins to the plasma membrane where they act as calcium-selective channels. The site of interaction on TRPV5 and TRPV6 for S100A10 has been localized to its intracellular C-terminal tail. Using a series of truncated and substituted proteins this has been refined to contain residues 598-603 (VATTV), a highly conserved region in both channel proteins. Although S100A10 forms a ternary complex with annexin A2 and either TRPV5 or TRPV6, there does not appear to be a direct interaction between the annexin and TRPV

proteins based on negative results from immunoprecipitation experiments. Rather, S100A10 seems to bridge the two proteins indicative of different binding sites for annexin A2 and TRPV5/TRPV6, although this has not been demonstrated yet. Since S100A10 and annexin A2 co-localize with TRPV5/TRPV6 the S100A10-annexin A2 complex has been proposed to traffic these calcium channels to the plasma membrane.

S100A10 has been shown to interact with the potassium channel TASK-1 using two-hybrid, GST pull-down and co-immunoprecipitation experiments and have a role in the trafficking of TASK-1 to the plasma membrane (Girard et al. 2002; Renigunta et al. 2006). TASK-1 is an important regulator of membrane potential that can be affected by a variety of factors including pH, hormone and neurotransmitter binding. Two studies show binding of S100A10 to the C-terminus of TASK-1 albeit to different regions of the channel protein. In one study, S100A10 was observed to interact with the extreme C-terminal sequence (SSV) of TASK-1 previously established as a site for 14-3-3 binding (Girard et al. 2002). In more recent work, the S100A10 site was localized to a more central region in the C-terminus (residues 292-331) of TASK-1 (Renigunta et al. 2006). A portion of this sequence is highly conserved in TASK-1 orthologs, but not in TASK-3, which exhibits little binding to S100A10. Further, a segment of this sequence (FRNVYAEML) bears a strong similarity to the interaction motif (XOOXXOOX) used by annexins A1 and A2 upon binding to S100A11 and S100A10, respectively (Mailliard et al. 1996; Rintala-Dempsey et al. 2008). An interaction between S100A10 and annexin A2 in the presence of TASK-1 can not be shown suggesting the S100A10 binding regions for TASK-1 and annexin A2 at least partly overlap. It has further been shown that the S100A10 interaction promotes retention of TASK-1 in the endoplasmic reticulum due to the presence of a retention signal at the extreme C-terminus of S100A10 (KQKGKK) (Renigunta et al. 2006). Alternatively, binding of S100A10 to TASK-1 has been suggested to mask an endoplasmic retention signal near the C-terminus of TASK-1 (KRR) and promote trafficking of the potassium channel to the plasma membrane (Girard et al. 2002).

S100A10 interacts with the bluetongue virus protein NS3 (Beaton et al. 2002), a membrane spanning protein thought to have a role in the export of virus particles from infected cells. Two-hybrid and affinity experiments show that residues at the N-terminus of NS3 (1-13) are most important for its interaction with S100A10. This region has the potential for  $\alpha$ -helix formation and possesses a sequence (LSGLIQR) corresponding to the annexin binding motif (XOOXXOOX) suggesting these two proteins likely share the same binding region on S100A10 (Fig. 1B). Using synthetic peptides corresponding to the N-terminus of NS3, it was shown that a peptide comprising residues 1-14 could compete with an-

nexin A2 for S100A10 binding. Whereas annexin A2 could displace NS3 from an S100A10-NS3 complex, NS3 could only partially displace annexin A2 from an S100A10-annexin A2 complex indicating annexin A2 has a tighter binding to S100A10 than does NS3. It was suggested that NS3 might play a role in localizing a virus particle to the interior of the plasma membrane by bridging S100A10 and the virus particle providing an efficient route for extrusion from the cell.

The voltage-gated sodium channel  $\text{Na}_v1.8$  has been observed, using two-hybrid experiments, to interact with S100A10 (Okuse et al. 2002; Poon et al. 2004). Further, this interaction facilitates trafficking of the channel protein to the plasma membrane resulting in functional  $\text{Na}^+$  currents. Other voltage-gated channels such as  $\text{Na}_v1.2$ ,  $\text{Na}_v1.5$ ,  $\text{Na}_v1.7$  and  $\text{Na}_v1.9$  have much poorer affinity for S100A10 providing evidence that some degree of specificity for the voltage-gated sodium channel  $\text{Na}_v1.8$  exists. The site of interaction with S100A10 has been localized to the N-terminus of the sodium channel. In particular residues 74-103 of the voltage-gated sodium channel  $\text{Na}_v1.8$  are sufficient to bind to S100A10 in GST pull-down assays. Reciprocal experiments using segments of S100A10 show that a region spanning much of the C-terminal half of the protein is able to interact with the N-terminal region of the voltage-gated sodium channel. This region does not include the extreme N- or C-termini of S100A10, known to interact with the annexin proteins so would appear to be a unique binding surface. However, it has not been shown whether annexin A2 can also bind to S100A10 in the presence of the sodium channel, an event that could facilitate association with the plasma membrane.

A multiprotein complex has been identified between S100A10, annexin A2 and AHNAK (Benaud et al. 2004; De Seranno et al. 2006), a protein found in the lumen of endosome and trafficked to the plasma membrane in response to calcium flux. AHNAK is an important protein for cell membrane differentiation and membrane repair (Kouno et al. 2004), is expressed in epithelial cells and localizes near the plasma membrane. The protein contains three main structural domains; an amino terminus (251 aa), a central region containing twenty-six 128-residue repeats and a C-terminus (1002 aa) (Shtivelman and Bishop 1993). The interaction between S100A10 and annexin A2 utilizes the N-terminus of annexin A2 similar to that observed in the S100A10-annexin A2 tetramer crystal structure (Rety et al. 1999). In the absence of annexin A2, a weak interaction between S100A10 and the C-terminal portion of AHNAK is present. However, the strength of this interaction was increased more than 150-fold in the presence of annexin A2. Further, *in vitro* binding assays showed no detectable interactions between annexin A2 and the C-terminus of AHNAK, in the absence of S100A10, or S100A10 with AHNAK in the absence of annexin A2.

These observations suggest that S100A10-annexin A2 tetramer formation is likely a prerequisite for the interaction with AHNAK. Using a series of GST pull-down assays, a 19-residue region in AHNAK corresponding to residues 5654-5673 was found to be sufficient to bind to S100A10-annexin A2 and can compete for binding to the complex with the entire AHNAK C-terminus having a  $K_d$  of about 30 nM (De Seranno et al. 2006). The AHNAK interaction appears to be specific for annexin A2 since other annexins (A4, A11) are not recovered from immunoprecipitation experiments. However, given the similarity of the binding surfaces and interacting residues for annexins A1 and A2 (Rintala-Dempsey et al. 2008), it is possible that an S100A10-annexin A1 or  $\text{Ca}^{2+}$ -S100A11-annexin A1/A2 may also participate in the AHNAK complex. Interestingly,  $\text{Ca}^{2+}$ -S100B is able to compete with S100A10-annexin A2 for binding to the AHNAK C-terminus. Further, it has been observed that  $\text{Zn}^{2+}$  binding to S100B enhances its association with AHNAK (Gentil et al. 2001). Unlike the S100A10-annexin A2 interaction that utilizes only the C-terminus of AHNAK, several regions in AHNAK are able to interact with  $\text{Ca}^{2+}$ -S100B. These include sequences in the repeat regions in the central portion of AHNAK (820-1330, 2589-3059, 3730-4188) as well as its C-terminus.

S100A10, AHNAK and the annexins have been shown to be constituent proteins in the dysferlin membrane repair complex (Huang et al. 2007). This multiprotein complex is thought to form at the site of muscle, epithelial and auditory cell damage to facilitate wound repair. The mechanism of cell membrane repair involves the aggregation of vesicles containing dysferlin, a type II membrane spanning protein, near the wound. A high calcium concentration (extracellular, via the wound) causes annexin A1 and A2 association with a dysferlin-containing vesicle and damaged plasma membranes. The central protein in this process dysferlin (Doherty and McNally 2003; Glover and Brown 2007; Han and Campbell 2007), has been shown to co-localize and co-immunoprecipitate with annexins A1 and A2 (Lennon et al. 2003). Recent three-hybrid and co-immunoprecipitation experiments have provided some details of the dysferlin repair complex. For example, S100A10 forms a ternary complex with both annexin A2 and AHNAK (Benaud et al. 2004; De Seranno et al. 2006; Huang et al. 2007) as described above. Using a series of truncated AHNAK constructs it has also been shown that the extreme C-terminal domain of AHNAK is responsible for dysferlin binding. Although dysferlin possesses six ~130-residue C2 domains, the AHNAK interaction is localized to only the N-terminal C2A domain of dysferlin, a region that typically coordinates calcium as a prerequisite for phospholipid binding. Calcium binding to the dysferlin C2A domain is not a requirement for its interaction with AHNAK. Although the exact mechanisms whereby AHNAK is recruited for plasma membrane repair

are not clear, annexin A2 and S100A10 co-localize with AHNAK at the plasma membrane along with dysferlin providing some evidence this multiprotein complex functions in the repair process.

## Conclusions

The multiple binding modes used by the S100 proteins to interact with a diverse array of target proteins suggests a great deal of flexibility in the manner an S100 protein recognizes a partner protein. This may prove useful to simultaneously coordinate more than a single target protein by some S100 proteins such as S100A10 that forms a tetrameric complex with annexin A2. Biochemical experiments suggest that S100A10-annexin A2 can further form multiprotein complexes with the cation channel proteins TRPV5 or TRPV6, the voltage-gated sodium channel  $\text{Na}_v1.8$ , or AHNAK and dysferlin. Other multiprotein complexes have been suggested between S100A10-annexin A2 and plasminogen, and  $\text{Ca}^{2+}$ -S100B with TRTK12 and annexin A6. Further, biochemical and structural experiments will be needed to ascertain the general role of S100 proteins in larger multiprotein complexes.

**Acknowledgements.** This work was supported by operating, and resource grants from the Canadian Institutes of Health Research (GSS) and the Canada Research Chairs Program (GSS). The authors have no financial interests related to the material in the manuscript nor to the participation in the 2nd ECS Workshop.

## References

- Beaton A. R., Rodriguez J., Reddy Y. K., Roy P. (2002): The membrane trafficking protein calpactin forms a complex with bluetongue virus protein NS3 and mediates virus release. *Proc. Natl. Acad. Sci. U.S.A.* **99**, 13154–13159
- Benaud C., Gentil B.J., Assard N., Court M., Garin J., Delphin C., Baudier J. (2004): AHNAK interaction with the annexin 2/S100A10 complex regulates cell membrane cytoarchitecture. *J Cell Biol.* **164**, 133–144
- Bhattacharya S., Bunick C. G., Chazin W. J. (2004): Target selectivity in EF-hand calcium binding proteins. *Biochim. Biophys. Acta.* **1742**, 69–79
- Bhattacharya S., Large E., Heizmann C. W., Hemmings B., Chazin W. J. (2003): Structure of the  $\text{Ca}^{2+}$ /S100B/NDR kinase peptide complex: insights into S100 target specificity and activation of the kinase. *Biochemistry* **42**, 14416–14426
- Borthwick L. A., Neal A., Hobson L., Gerke V., Robson L., Muimio R. (2008): The annexin 2-S100A10 complex and its association with TRPV6 is regulated by cAMP/PKA/CnA in airway and gut epithelia. *Cell Calcium* **44**, 147–157
- De Seranno S., Benaud C., Assard N., Khediri S., Gerke V., Baudier J., Delphin C. (2006): Identification of an AHNAK bind-

- ing motif specific for the Annexin2/S100A10 tetramer. *J. Biol. Chem.* **281**, 35030–35038
- Deloulme J. C., Assard N., Mbele G. O., Mangin C., Kuwano R., Baudier J. (2000): S100A6 and S100A11 are specific targets of the calcium- and zinc-binding S100B protein in vivo. *J. Biol. Chem.* **275**, 35302–35310
- Deloulme J. C., Gentil B. J., Baudier J. (2003): Monitoring of S100 homodimerization and heterodimeric interactions by the yeast two-hybrid system. *Microsc. Res. Tech.* **60**, 560–568
- Doherty K. R., McNally E. M. (2003): Repairing the tears: dysferlin in muscle membrane repair. *Trends Mol. Med.* **9**, 327–330
- Donato R. (2001): S100: a multigenic family of calcium-modulated proteins of the EF-hand type with intracellular and extracellular functional roles. *Int. J. Biochem. Cell Biol.* **33**, 637–668
- Gentil B. J., Delphin C., Mbele G. O., Deloulme J. C., Ferro M., Garin J., Baudier J. (2001): The giant protein AHNAK is a specific target for the calcium- and zinc-binding S100B protein: potential implications for  $\text{Ca}^{2+}$  homeostasis regulation by S100B. *J. Biol. Chem.* **276**, 23253–23261
- Gerke V., Moss S. E. (2002): Annexins: from structure to function. *Physiol. Rev.* **82**, 331–371
- Girard C., Tinel N., Terrenoire C., Romey G., Lazdunski M., Borsotto M. (2002): p11, an annexin II subunit, an auxiliary protein associated with the background  $\text{K}^+$  channel, TASK-1. *Embo J.* **21**, 4439–4448
- Glover L., Brown R. H. Jr. (2007): Dysferlin in membrane trafficking and patch repair. *Traffic* **8**, 785–794
- Han R., Campbell K. P. (2007): Dysferlin and muscle membrane repair. *Curr. Opin. Cell Biol.* **19**, 409–416
- Heizmann C. W., Fritz G., Schafer B. W. (2002): S100 proteins: Structure, functions and pathology. *Front. Biosci.* **7**, d1356–1368
- Huang Y., Laval S. H., van Remoortere A., Baudier J., Benaud C., Anderson L. V., Straub V., Deelder A., Frants R. R., den Dunnen J. T., Bushby K., van der Maarel S. M. (2007): AHNAK, a novel component of the dysferlin protein complex, redistributes to the cytoplasm with dysferlin during skeletal muscle regeneration. *Faseb J.* **21**, 732–742
- Inman K. G., Yang R., Rustandi R. R., Miller K. E., Baldisseri D. M., Weber D. J. (2002): Solution NMR structure of S100B bound to the high-affinity target peptide TRTK-12. *J. Mol. Biol.* **324**, 1003–1014
- Kouno M., Kondoh G., Horie K., Komazawa N., Ishii N., Takahashi Y., Takeda J., Hashimoto T. (2004): Ahnak/Desmoyokin is dispensable for proliferation, differentiation, and maintenance of integrity in mouse epidermis. *J. Invest. Dermatol.* **123**, 700–707
- Lee Y. T., Dimitrova Y. N., Schneider G., Ridenour W. B., Bhattacharya S., Soss S. E., Caprioli R. M., Filipek A., Chazin W. J. (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**, 10921–10932
- Lennon N. J., Kho A., Bacskai B. J., Perlmutter S. L., Hyman B. T., Brown R. H. Jr. (2003): Dysferlin interacts with annexins A1 and A2 and mediates sarcolemmal wound-healing. *J. Biol. Chem.* **278**, 50466–50473
- MacLeod T. J., Kwon M., Filipenko N. R., Waisman D. M. (2003): Phospholipid-associated annexin A2-S100A10 heterotetramer and its subunits: characterization of the interaction with tissue plasminogen activator, plasminogen, and plasmin. *J. Biol. Chem.* **278**, 25577–25584
- Mailliard W. S., Haigler H. T., Schlaepfer D. D. (1996): Calcium-dependent binding of S100C to the N-terminal domain of annexin I. *J. Biol. Chem.* **271**, 719–725
- McClintock K. A., Shaw G. S. (2003): A novel S100 target conformation is revealed by the solution structure of the  $\text{Ca}^{2+}$ -S100B-TRTK-12 complex. *J. Biol. Chem.* **278**, 6251–6257
- Okuse K., Malik-Hall M., Baker M. D., Poon W. Y., Kong H., Chao M. V., Wood J. N. (2002): Annexin II light chain regulates sensory neuron-specific sodium channel expression. *Nature* **417**, 653–656
- Otterbein L. R., Kordowska J., Witte-Hoffmann C., Wang C. L., Dominguez R. (2002): Crystal structures of S100A6 in the  $\text{Ca}^{2+}$ -free and  $\text{Ca}^{2+}$ -bound states: the calcium sensor mechanism of S100 proteins revealed at atomic resolution. *Structure* **10**, 557–567
- Poon W. Y., Malik-Hall M., Wood J. N., Okuse K. (2004): Identification of binding domains in the sodium channel  $\text{Na}_v1.8$  intracellular N-terminal region and annexin II light chain p11. *FEBS Lett.* **558**, 114–118
- Propper C., Huang X., Roth J., Sorg C., Nacken W. (1999): Analysis of the MRP8-MRP14 protein-protein interaction by the two-hybrid system suggests a prominent role of the C-terminal domain of S100 proteins in dimer formation. *J. Biol. Chem.* **274**, 183–188
- Renigunta V., Yuan H., Zuzarte M., Rinne S., Koch A., Wischmeyer E., Schlichthorl G., Gao Y., Karschin A., Jacob R., Schwappach B., Daut J., Preisig-Muller R. (2006): The retention factor p11 confers an endoplasmic reticulum-localization signal to the potassium channel TASK-1. *Traffic* **7**, 168–181
- Rety S., Osterloh D., Arie J. P., Tabaries S., Seeman J., Russo-Marie F., Gerke V., Lewit-Bentley A. (2000): Structural basis of the  $\text{Ca}^{2+}$ -dependent association between S100C (S100A11) and its target, the N-terminal part of annexin I. *Structure* **8**, 175–184
- Rety S., Sopkova J., Renouard M., Osterloh D., Gerke V., Tabaries S., Russo-Marie F., Lewit-Bentley A. (1999): The crystal structure of a complex of p11 with the annexin II N-terminal peptide. *Nat. Struct. Biol.* **6**, 89–95
- Rintala-Dempsey A. C., Rezvanpour A., Shaw G. S. (2008): S100-annexin complexes – structural insights. *FEBS J.* **275**, 4956–4966
- Rintala-Dempsey A. C., Santamaria-Kisiel L., Liao Y., Lajoie G., Shaw G. S. (2006): Insights into S100 target specificity examined by a new interaction between S100A11 and annexin A2. *Biochemistry* **45**, 14695–14705
- Rustandi R. R., Baldisseri D. M., Weber D. J. (2000): Structure of the negative regulatory domain of p53 bound to S100B( $\beta\beta$ ). *Nat. Struct. Biol.* **7**, 570–574

- Santamaria-Kisiel L., Rintala-Dempsey A. C., Shaw G. S. (2006): Calcium-dependent and -independent interactions of the S100 protein family. *Biochem. J.* **396**, 201–214
- Shtivelman E., Bishop J. M. (1993): The human gene AHNAK encodes a large phosphoprotein located primarily in the nucleus. *J. Cell Biol.* **120**, 625–630
- Streicher W. W., Lopez M. M., Makhatadze G. I. (2009): Annexin I and annexin II N-terminal peptides binding to S100 protein family members: specificity and thermodynamic characterization. *Biochemistry* **48**, 2788–2798
- van de Graaf S. F., Hoenderop J. G., Gkika D., Lamers D., Prenen J., Rescher U., Gerke V., Staub O., Nilius B., Bindels R. J. (2003): Functional expression of the epithelial Ca<sup>2+</sup> channels (TRPV5 and TRPV6) requires association of the S100A10-annexin 2 complex. *EMBO J.* **22**, 1478–1487
- Wang G., Zhang S., Fernig D. G., Martin-Fernandez M., Rudland P. S., Barraclough R. (2005): Mutually antagonistic actions of S100A4 and S100A1 on normal and metastatic phenotypes. *Oncogene* **24**, 1445–1454
- Wilder P. T., Lin J., Bair C. L., Charpentier T. H., Yang D., Liriano M., Varney K. M., Lee A., Oppenheim A. B., Adhya S., Carrier F., Weber D. J. (2006): Recognition of the tumor suppressor protein p53 and other protein targets by the calcium-binding protein S100B. *Biochim. Biophys. Acta* **1763**, 1284–1297
- Wright N. T., Cannon B. R., Wilder P. T., Morgan M. T., Varney K. M., Zimmer D. B., Weber D. J. (2009): Solution structure of S100A1 bound to the CapZ peptide (TRTK12). *J. Mol. Biol.* **386**, 1265–1277
- Wright N. T., Prosser B. L., Varney K. M., Zimmer D. B., Schneider M. F., Weber D. J. (2008): S100A1 and calmodulin compete for the same binding site on ryanodine receptor. *J. Biol. Chem.* **283**, 26676–26683
- Zimmer D. B., Wright Sadosky P., Weber D. J. (2003): Molecular mechanisms of S100-target protein interactions. *Microsc. Res. Tech.* **60**, 552–559

Received: May 4, 2009

Final version accepted: June 17, 2009