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Review

Functional roles of S100 proteins, calcium-binding proteins of the EF-hand type

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Abstract

A multigenic family of Ca^{2+}-binding proteins of the EF-hand type known as S100 comprises 19 members that are differentially expressed in a large number of cell types. Members of this protein family have been implicated in the Ca^{2+}-dependent (and, in some cases, Zn^{2+}- or Cu^{2+}-dependent) regulation of a variety of intracellular activities such as protein phosphorylation, enzyme activities, cell proliferation (including neoplastic transformation) and differentiation, the dynamics of cytoskeleton constituents, the structural organization of membranes, intracellular Ca^{2+} homeostasis, inflammation, and in protection from oxidative cell damage. Some S100 members are released or secreted into the extracellular space and exert trophic or toxic effects depending on their concentration, act as chemoattractants for leukocytes, modulate cell proliferation, or regulate macrophage activation. Structural data suggest that many S100 members exist within cells as dimers in which the two monomers are related by a two-fold axis of rotation and that Ca^{2+} binding induces in individual monomers the exposure of a binding surface with which S100 dimers are believed to interact with their target proteins. Thus, any S100 dimer is suggested to expose two binding surfaces on opposite sides, which renders homodimeric S100 proteins ideal for crossbridging two homologous or heterologous target proteins. Although in some cases different S100 proteins share their target proteins, in most cases a high degree of target specificity has been described, suggesting that individual S100 members might be implicated in the regulation of specific activities. On the other hand, the relatively large number of target proteins identified for a single S100 protein might depend on the specific role played by the individual regions that in an S100 molecule contribute to the formation of the binding surface. The pleiotropic roles played by S100 members, the identification of S100 target proteins, the analysis of functional correlates of S100-target protein interactions, and the elucidation of the three-dimensional structure of some S100 members have greatly increased the interest in S100 proteins and our knowledge of S100 protein biology in the last few years. S100 proteins probably are an example of calcium-modulated, regulatory proteins that intervene in the fine tuning of a relatively large number of specific intracellular and (in the case of some members) extracellular activities. Systems, including knock-out animal models, should be now used with the aim of defining the correspondence between the in vitro regulatory role(s) attributed to individual members of this protein family and the in vivo function(s) of each S100 protein.

Keywords: S100; Calcium; Structure; Target protein; Signalling

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1. Introduction

S100 is a multigenic family of low molecular weight (M_r between 9 and 13 kDa) Ca^{2+}-binding proteins of the EF-hand (i.e. helix-loop-helix) type comprising 19 members as listed in Table 1 (for reviews see [1–3]). S100 was discovered in 1965 as a brain protein fraction [4] which was shown several years later to consist of two closely related molecules, S100α (S100A1) and S100β (S100B) [5,6]. Because of its abundance in the nervous system and owing to the limited sensitivity of the immunochemical methods available at that time, S100 was regarded as a brain-specific protein, restricted to glial cells. In 1981 S100 protein was shown not to be restricted to the nervous tissue [7]. Since then, hundreds of papers have described the cellular and subcellular localization of classical S100 proteins (S100A1 and S100B) in a large number of cell types in vertebrates (reviewed in [8]). Yet, neither the two above proteins nor any other member of this family discovered thus far appear to be ubiquitous [1–3]. In mammals, S100B is most abundant in glial cells of the central and peripheral nervous systems, and in melanocytes, chondrocytes and adipocytes, whereas S100A1 is most abundant in cardiomyocytes, slow-twitch skeletal muscle cells, salivary epithelial cells and renal cells [1–3,8]. S100A1 has also been detected in hippocampal neurons [9], and recent observations indicate that S100B is expressed in subpopulations of neurons [10–15]. The other members of the S100 family have a more restricted tissue and/or cellular distribution, e.g. lung and kidney (S100A2), fibroblasts (S100A4, S100A6, S100A10), myoepithelial cells (S100A4), tumor cells (S100B, S100A4, S100A6), epithelial cells (S100A7, S100A8, S100A9, S100A10), granulocytes and monocytes (S100A8, S100A9, S100A12, and the S100A8-like protein CP-10), smooth and heart muscle cells (S100A2, S100A4, S100A6, S100A11), placenta (S100P), granular cells of the epidermis (profilaggrin, trychohyalin, repetin) (reviewed in [3]). Taken together, these data suggest that individual S100 proteins might have specific functional roles in the cell types where they are expressed. Because of their non-ubiquity and their up-regulation in specific tumor cells several S100 proteins are being used in diagnostic pathology [8,16–23].

Interestingly, three epidermal proteins, profilaggrin, trichohyalin, and repetin (Rptn) with ability to bind keratin intermediate filaments (IFs) and implicated in the regulation of keratin IF lateral association, contain EF-hand Ca^{2+}-binding domains within a sequence exhibiting high identity with other members of the S100 family [24–27]. These three proteins are thought to represent fused genes of the cornified cell envelope precursor protein genes and

have been regarded as a subgroup of the S100 protein family [3].

Preceded by the observation that Ca^{2+} was capable of inducing conformational changes in classical brain S100 protein (mixture of S100A1 and S100B) and that Ca^{2+} and K^+ had antagonizing effects on S100 conformation [28], between 1978 and 1981 came the findings that brain S100 actually is a mixture of two closely related protein species, S100A1 and S100B (formerly known as S100α and S100β, respectively); that S100A1 and S100B form the homodimers $S100A1_2$ and $S100B_2$ (formerly termed $S100a_o$ and S100b, respectively) as well as the S100A1/S100B heterodimer (formerly termed S100a); and that each monomer contains a canonical Ca^{2+}-binding site of the EF-hand type in its C-terminal half and a non-conventional (longer and rearranged) EF-hand Ca^{2+}-binding site in its N-terminal half [5,6].

The Ca^{2+}-binding properties of individual S100 proteins have been documented by a number of experimental approaches, including intrinsic fluorescence spectroscopy, equilibrium dialysis with $^{45}Ca^{2+}$, gel overlay with $^{45}Ca^{2+}$, circular dichroism, and fluorescence spectroscopy with derivatized proteins [1–3]. There is consensus that the Ca^{2+}-binding affinity of classical S100 proteins in solution is low under physiologic ionic conditions, and that this affinity increases by several orders of magnitude in the presence of S100 targets. Actually, in many cases the interaction of an S100 protein with its target protein(s) and regulatory effects of an S100 protein on cellular activities were observed to occur at free Ca^{2+} concentrations significantly lower than those at which half-maximal binding of Ca^{2+} to an S100 protein in solution was detected [1–3]. All these observations point to the occurrence of large conformational changes and/or a variety of conformational states in S100 proteins in the presence of Ca^{2+} and/or S100 target proteins [1–3,29,30]. As an exception to the rule that S100 proteins undergo conformational changes in the presence of Ca^{2+}, S100A10 (formerly known as p11 or calpactin I light chain) [31–33] does not bind Ca^{2+} and does not undergo Ca^{2+}-induced conformational changes [31–33]. This protein, which is considered to be in a permanent 'Ca^{2+}-on state', exists as a homodimer that functionally crosslinks two copies of annexin II (a member of a multigenic family of Ca^{2+}-dependent phospholipid-, membrane- and cytoskeleton-binding proteins [34,35]) to form the annexin II_2-$S100A10_2$ heterotetramer (also known as calpactin I) irrespective of the presence or absence of Ca^{2+} [31–33,36]. Mutations and deletions in an ancestral S100 gene resulted in locking S100A10 in an active, Ca^{2+}-insensitive conformation. However, S100B also may exist in a Ca^{2+}-insensitive state [37,38] and Ca^{2+}-independent binding of S100A1 with certain target proteins has been documented [39]. In general, however, S100 proteins are suggested to function as Ca^{2+} sensor proteins that, with a few exceptions (see Section 2.2), undergo Ca^{2+}-dependent conformational changes. These latter result in the exposure of a binding surface through which S100 proteins interact with target proteins, as is the case with other Ca^{2+} sensor proteins (e.g. calmodulin and troponin C). Thus, those S100 proteins that undergo Ca^{2+}-induced conformational changes and interact with target proteins in a Ca^{2+}-dependent manner reasonably play a role in Ca^{2+} signal transduction. S100A1 and S100B also bind Zn^{2+} with a rather high affinity [1–3]. Ca^{2+} binding to Zn^{2+}-loaded S100A1 and S100B occurs with a significantly higher affinity [1–3]. Zn^{2+} binding was also reported for S100A3 (S100E), S100A6 (calcyclin), S100A8, the S100A8-like protein CP-10, S100A9 (MRP14) and S100A12 (calgranulin C) [1–3,40,41]. Finally, recent evidence indicates that S100B also binds copper [42].

Among the various second messengers Ca^{2+} probably is the most ancient and energetically inexpensive [43]. The rise in cytoplasmic Ca^{2+} is a universal signal that controls a variety of processes in eukaryotic cells (e.g. cell growth and differentiation, cell motility, muscle contraction, gene expression, secretion, nerve impulse transmission, apoptosis). Cells have evolved a battery of intracellular Ca^{2+}-binding proteins, among which are S100 proteins, that act to transduce the Ca^{2+} signal [44,45]. S100 proteins have been identified in and purified from vertebrate tissues [1–3]. Although an S100B-like protein has been immunologically detected in some invertebrates and in spinach leaves [1,8,46], there is no definite evidence that members of the S100 protein family are expressed in invertebrates and/or in plants. The available genomic databases for plants, yeast, the nematode *Caenorhabditis elegans*, and *Drosophila*

demonstrate that S100 proteins are not expressed in these organisms.

Several reviews have appeared on S100 proteins in the last decade dealing with the tissue, cellular and subcellular distribution of these proteins, their functional properties, the structure and chromosomal localizations of their genes, and implications of some S100 proteins in the pathophysiology of several diseases [1–3,8,47–49]. Some of these reviews also have discussed an emerging aspect of the S100 protein biology, i.e. the release of some S100 members by definite cell types and the ability of released S100 proteins to modulate certain activities in target cells. In the present review functional aspects of S100 proteins will be commented on also in light of recent observations on novel regulatory effects of some S100 proteins and the tertiary and quaternary structure of S100A6, S100B, S100A7 (psoriasin) and S100A10. The aim of the present review is twofold, describing the state-of-the-art of the S100 protein story and pointing to those regulatory functions of S100 proteins that are likely to occur in vivo. Concerning the latter point, the following criteria have been used in this review to establish intracellular and/or extracellular functions of S100 proteins: (i) identification of the S100-binding site on a target protein and/or the binding site for a target protein on an S100 protein; (ii) functional correlates of S100 protein-target protein interactions; (iii) identification of regulators of S100 protein-target protein interactions; (iv) cellular and subcellular colocalization of an S100 protein and its target protein(s); (v) co-immunoprecipitation of S100 protein-target protein complexes; (vi) effects of inhibition of S100 protein synthesis or of overexpression of S100 protein; (vii) identification of the mechanism of S100 protein release to the extracellular space; (viii) identification of the S100-binding site(s) on S100 protein target cells; and (ix) identification of the intracellular signalling pathways activated upon interaction of an S100 protein with its target cell(s).

2. S100 proteins form dimers: functional implications

2.1. Disulfide crosslinked S100 dimers

Most of S100 proteins have a strong tendency to dimerize. In particular, under non-reducing conditions, like those found in the extracellular space, S100B can form disulfide crosslinked homodimers [50,51], an event that appears to be facilitated by the presence of high Ca^{2+} concentrations and/or lipids [52]. S100A10, S100A2 (S100L) and S100A6 also form disulfide crosslinked dimers [53–56]. The presence of two cysteines at positions 68 and 84, that is typical of S100B [5,51], is essential to the formation and the biological activities of disulfide crosslinked $S100B_2$ dimers [51]. Although the presence of S100B in the extracellular space in mature tissues (brain) has been documented in but one report [57], secretion of this protein by cultured astrocytes has been reported as has been secretion of S100A2, S100A7, S100A8 (MRP8), the S100A8-like protein CP-10, and S100A9 (MRP14) by specific cell types [58–61] (see Section 3.2).

2.2. S100 proteins form dimers within cells

It is now established that S100A1, S100B, S100A6, S100A7 S100A10 and S100A11 (S100C) form homodimers and that S100A1/S100B and S100A8/S100A9 heterodimers can form even under reducing conditions, i.e. within cells, and that in at least S100 homodimers the two subunits are held together by non-covalent bonds [62–67]. $S100A8_2$/S100A9 trimers and $S100A8_2$/$S100A9_2$ tetramers also form in vivo [62]. Ca^{2+} appears to strongly stimulate the formation of the S100A8/S100A9 heterodimer [62]. Probably, most of S100 proteins can form homodimers (with the exception of calbindin D_{9k} and, presumably, profilaggrin, trichohyalin, and repetin) [63–67]. Recent observations on the structure of S100A6, S100B and S100A7 in the absence of Ca^{2+}, as investigated by NMR spectroscopy, X-ray crystallography, or multiple anomalous wavelength dispersion, indicate that S100A6, S100B and S100A7 homodimers, and, by analogy, most of the members of the S100 protein family that dimerize are characterized by a symmetric homodimeric fold not found in other Ca^{2+}-binding proteins [63–67] (Fig. 1). These studies also indicate that dimerization of these proteins is mediated by hydrophobic contacts through individual helices IV (in an S100 monomer, helix IV is located just next the conventional Ca^{2+}-binding loop found in the C-terminal half), and that residues at the C-termini of

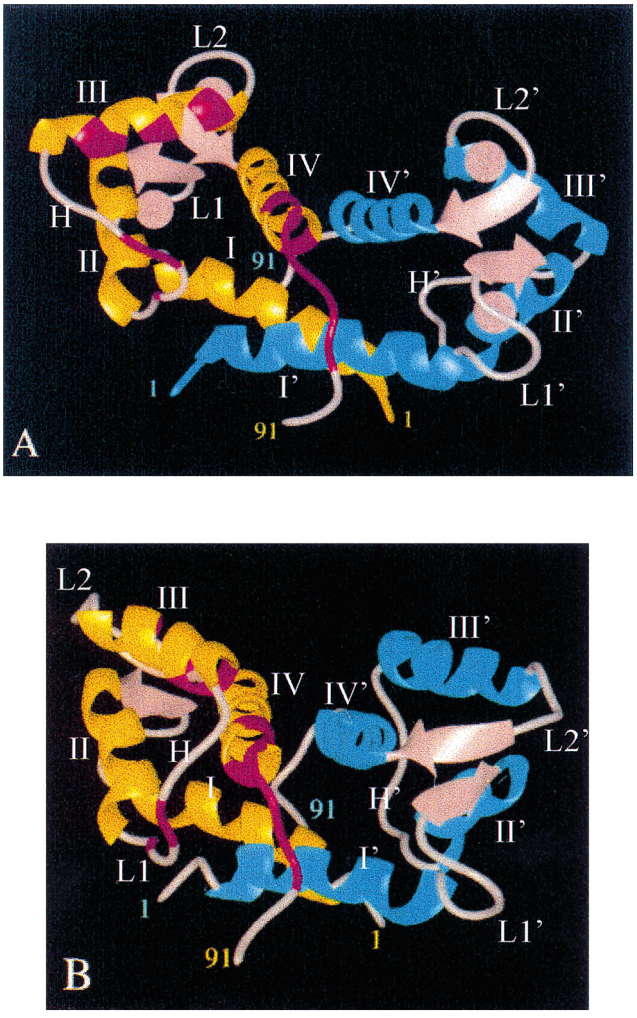


Fig. 1. Structure of the Ca^{2+}-loaded (A) and Ca^{2+}-free (B) $S100B_2$ dimer. One S100B monomer is in yellow and the other one is in blue. Helices are indicated by Roman numerals (I–IV in one monomer, and I′–IV′ in the other monomer). Binding of Ca^{2+} to each S100B monomer causes a reorientation of helix III relative to all other helices with consequent reorientiation of the hinge region (H). These changes result in the exposure to the solvent of a surface defined by residues (in magenta) in helices III and IV, the hinge region and the C-terminal extension [75]. Residues in helix I of the other monomer (I′) could also take part in the generation of the binding surface, as suggested by others [66,73]. Calcium ions are represented by light-pink dots within Ca^{2+}-binding loops (L1, L2 in one monomer, and L1′, L2′ in the other monomer). Note that the Ca^{2+}-loaded $S100B_2$ dimer is more extended than the Ca^{2+}-free dimer. Reproduced, with some additions, with permission from Fig. 7 in [75].

helix I (which precedes the non-conventional Ca^{2+}-binding loop in the N-terminal half) and residues in the C-terminal extension of individual subunits contribute to the dimer interface. The S100B dimer (and, by analogy, all other S100 homodimers) appears highly stable ($^{dimer}K_d < 500$ pM) because of the large number of hydrophobic residues in the dimer interface [68], suggesting that within cells most of S100 proteins do not exist as monomers (also see below).

The two monomers in the apo S100A6, the apo S100B and the apo S100A7 dimers are related by a two-fold axis of rotation. It is suggested that the mode of dimer formation in the cases of S100A6 and S100B and S100A7 applies to all the S100 proteins that dimerize [63,64]. The above studies [63,64] have identified two regions in individual S100 subunits that might be implicated in the formation of a binding region for S100 target proteins, the C-terminal extension and the hinge region. It is known that the C-terminal extension of S100A10 is implicated in the binding of its natural ligand, annexin II [69]. In one study [70], the C-terminal extension of S100A1 has been shown to be important for the binding of a synthetic peptide, TRTK-12, derived from a sequence found in the α subunit of the actin capping protein, CapZ, an S100A1- and S100B-binding protein [71,72] (Table 1), suggesting that the C-terminal extension of S100A1 (and, likely, S100B) is implicated in the recognition of CapZ. However, in another study residues in the C-terminal helix (helix IV) and residues V-8 to D-12 of the N-terminal helix (helix I) of S100B have been shown to be affected by TRTK-12, by NMR spectroscopy [73]. Since in the Ca^{2+}-free S100B dimer the N-terminal helix of one monomer passes close to the C-terminal helix of the opposite monomer [64,65], the C-terminal helix and the N-terminal helix of opposite monomers were suggested to form a discontinuous epitope acting as the CapZ-binding site on S100B (and, likely, S100A1) [73].

The major change induced by Ca^{2+} in S100B is a remarkable change of the interhelical angle between helices III and IV which flank the canonical Ca^{2+}-binding loop in the C-terminal half of S100B [66,74–77]. This change exposes a cleft, defined by residues in the hinge region, helices III and IV, and the C-terminal extension, which is buried in apo S100B and might thus be important for binding target proteins in the presence of Ca^{2+}. Also, the Ca^{2+}-induced change in the position of helix III of S100B causes significant changes in the structure and orientation of

Table 1
Summary of members of the S100 protein family and their target proteins, and suggested functions

Protein	Target proteins, references	Suggested functions, references
S100A1	CapZα [72], desmin [83,85], GFAP [84,85], p53 [88], annexins V and VI [96], tubulin [101,121], τ proteins [108,109], MyoD [111,132], E12 [132], fructose-1,6-bisphosphate aldolase [133], glycogen phosphorylase [134], phosphoglucomutase [135], twitchin kinase [136,137], Ndr [142], adenylate cyclase [144], membrane-bound guanylate cyclase [146–148,151], ryanodine receptor [158], F-actin [226]	Inhibits type III intermediate filament [83–85,179] and microtubule assemblies [120,121,123,160–162,176]; inhibits protein phosphorylation [88–92,106–111]; inhibits glycogen phosphorylase [134] and phosphoglucomutase [135] activities and stimulates aldolase [133], adenylate cyclase [144], guanylate cyclase [146–148,151], twitchin kinase [136,137] and Ndr kinase [142] activities; stimulates Ca^{2+}-induced Ca^{2+} release [156,158]; regulates transcription factors [111,131,132]
S100A2	Tropomyosin [103], F-actin [226]	Regulates F-actin-tropomyosin interactions [103]; suppresses tumor progression [353–355]; once secreted, chemoattracts eosinophils [59]
S100A3		
S100A4	Myosin [130,220–225,348,349], F-actin [222,225,226], tropomyosin [222]	Inhibits myosin phosphorylation [130]; confers Ca^{2+}-sensitivity to tropomyosin regulation of actomyosin ATPase activity [222]; plays a role in metastatic invasiveness [130,341–349]
S100A5		
S100A6	Tropomyosin [56], annexins II [82] and XI [71,94], glyceraldehyde-3-phosphate dehydrogenase [82], caldesmon [227]	Blocks the caldesmon-dependent inhibition of actomyosin ATPase activity [227]; regulates cell-cycle progression [333]
S100A7		Once secreted, chemoattracts $CD4^+$ lymphocytes [60]
S100A8	Microtubule protein [61]; vimentin [207,209], keratins [211,212]	Inhibits casein kinase II activity [152]; regulates myeloid cell maturation and function [152–154]; contributes to macrophage activation [212–214]; in the form of an S100A8/S100A9 complex binds unsaturated fatty acids likely to modulate their pro-inflammatory activity [302,303]; once secreted and in the form of an S100A8/S100A9 complex displays cytostatic [293,294] and antimicrobial [295] activities, inhibits macrophage activation [297] and immunoglobulin synthesis [298] by lymphocytes
S100A8-like, CP-10 protein		Once secreted, chemoattracts leukocytes [286–288,290,291]
S100A9	Microtubule protein [61]; vimentin [207,209], keratins [211,212]	Inhibits casein kinase II activity [152]; regulates myeloid cell maturation and function [152–154]; contributes to macrophage activation [212–214]; in the form of an S100A8/S100A9 complex binds unsaturated fatty acids likely to modulate their pro-inflammatory activity [302,303]; once secreted and in the form of an S100A8/S100A9 complex displays cytostatic [293,294] and antimicrobial [295] activities, inhibits macrophage activation [297] and immunoglobulin synthesis [298] by lymphocytes
S100A10	Annexin II [31–33,36,185–187,200], 85-kDa phospholipase A_2 [155], unidentified proteins of the cornified envelope [199], plasminogen [310]	Targets annexin II to membranes and F-actin [31–36,117,200]; in the form of the annexin II_2-$S100A10_2$ complex crossbridges F-actin to membranes [31–36,190]; inhibits annexin II phosphorylation [114–116]; stimulates F-actin bundling [117,190,191]; enhances the stimulatory effect of annexin II on GFAP assembly [126,127]; inhibits 85-kDa phospholipase A_2 activity [155]; regulates exocytosis and endocytosis (in the form of the annexin II_2-$S100A10_2$ heterotetramer) [188,189,193]; contributes to the formation of the cornified envelope in epidermal keratinocytes [199]; stimulates t-PA plasminogen activation [310]

Table 1 (*continued*)
Summary of members of the S100 protein family and their target proteins, and suggested functions

Protein	Target proteins, references	Suggested functions, references
S100A11	Annexin I [86,95,203], proteins of the cornified envelope likely including annexin I [199]	Inhibits annexin I phosphorylation [86,95],; contributes to the formation of the cornified envelope in epidermal keratinocytes likely in the form of an annexin I_2-S100A11$_2$ heterotetramer [199,203]; in the form of an annexin I_2-S100A11$_2$ heterotetramer has a role in endocytosis [204]
S100A12		
S100A13	Fibroblast growth factor-1/synaptotagmin aggregate [309]	Participates in fibroblast growth factor-1 release [309]
S100B	Tubulin [38,101,122,166], GFAP [38,84,85,112,179,196], vimentin [38,166], CapZα [71], retinal phosphoprotein p80 [78], desmin [83,196], p53 [88], neuromodulin (GAP-43) [90–92], neurogranin [92], MARCKS [92,106], annexins V [96], VI [96] and II [102,107], τ proteins [108,109], caldesmon [110,124,125,219], MyoD [111,132], E12 [132], fructose-1,6-bisphosphate aldolase [133], phosphoglucomutase [135], twitchin kinase [136], Ndr [142], adenylate cyclase [143], membrane-bound guanylate cyclase [146–148,151], calponin [218]	Inhibits protein phosphorylation [78,87–92,107–112]; inhibits type III intermediate filament [83–85,179] and microtubule [120,121,123,160–162,176] assemblies; regulates cell-cycle progression likely via interaction with transcription factors [88,111,132,271,311–313]; blocks the caldesmon [124,125]- and calponin [218]-dependent inhibition of actomyosin ATPase activity; inhibits fetal protein (α-actin and β-myosin) expression in striated muscle cells [131]; stimulates aldolase [133], phosphoglucomutase [135], Ndr kinase [142], adenylate cyclase [143], and guanylate cyclase [146–148,151] activities; once secreted, stimulates neurite outgrowth [50,51,236–239,241–244,272] and astrocyte proliferation [245] by enhancing Ca^{2+} fluxes [249] (at nM concentrations) or is toxic to neurons [267–270] and astrocytes [270] (at μM concentrations), participates in the pathophysiology of neurodegenerative disorders [250–262,284], participates in long term potentiation and memory processes [273–284]; inhibits cytotoxic lymphocyte proliferation and IFN-γ secretion [296]
S100P		
Calbindin 3 (D_{9k})		Buffers cytosolic Ca^{2+} [104]
Profilaggrin	Keratins [24,26]	Regulates lateral association of keratin intermediate filaments [24,26]
Trychohyalin	Keratins [25]	Regulates lateral association of keratin intermediate filaments [25]
Repetin	Keratins [27]	Regulates lateral association of keratin intermediate filaments [27]

the hinge region and the C-terminal extension [66,74,75]. These data appear to confirm the previous suggestion that the hinge region and the C-terminal extension might take part in the formation of a binding surface for the recognition of S100B target proteins [48,64]. However, whereas there is a substantial agreement between NMR spectroscopy and crystallography data about the structure of apo and Ca^{2+}-bound S100B [66,74,75,77], it was suggested that an S100B dimer would bind a target protein through a site made of helix IV of one monomer and helix IV of the other monomer [66]. This mode of interaction would predict a binding stoichiometry of 1 mol of ligand/mol of $S100B_2$ dimer [66], which is not the case. Actually, in those instances where binding of a target protein to an S100 protein had been accurately determined, a binding stoichiometry of 2 mol/mol of $S100_2$ has been reported (see above and below). However, other modes of S100 protein interaction with target proteins cannot be excluded. For instance, the S100A7 dimer has been suggested to wrap around its target proteins through individual C-terminal halves [67]. Differences in the S100A7 sequence (which is only ∼27% homologous to that of S100B) as well as in the reorientation of S100A7 helices might be responsible for differences in the mode of target protein recognition in S100A7 [67]. However, no data are available about S100A7 target proteins. Recent observations have implicated both the hinge region and the C-terminal extension of S100B in the interaction with and the activation of a membrane-bound guanylate cyclase activity as well

in the interaction of S100B with the retinal phosphoprotein, p80 [78]. Moreover, a recombinant S100A1 lacking the last nine residues was reported to be unable to interact with phenyl-Sepharose and TRTK-12 [39]. Also recently, it was found that a recombinant S100A1 lacking the C-terminal extension has no or a remarkably reduced ability to interact with a number of S100A1 (and S100B) target proteins, e.g. glial fibrillary acidic protein (GFAP) and tubulin as well as with the TRTK-12 peptide and a peptide derived from the tumor suppressor protein p53 [79], which again points to an important role of the C-terminal extension in Ca^{2+}-dependent, target protein recognition by S100A1 and, by analogy, S100B.

Discrepancies concerning the S100B regions implicated in target recognition, as outlined above, might be settled in light of recent X-ray crystallography data on the structure of S100A10, which, as mentioned above, is considered to be in a permanent 'Ca^{2+} on' state [31–33,36,69]: the hinge region and the C-terminal helix/extension of one monomer and the N-terminal helix of the other monomer would constitute a binding surface for the recognition of annexin II [80]. In this respect, the similarity between the structure of the $S100A10_2$ dimer (see Fig. 1A in [80]) and that of Ca^{2+}-loaded $S100B_2$ and $S100A6_2$ dimers (see Fig. 1C in [77] and Fig. 5B in [75], and Fig. 1A in [76], for S100B and S100A6, respectively) is remarkable. All the above observations would also explain the reason why S100 members that act to transduce the Ca^{2+} signal form homodimers and, eventually, heterodimers: regions of both monomers would contribute to generate their functional binding sites. In the case of S100A6 no major Ca^{2+}-induced structural changes were concluded to occur, raising questions about the proposed roles of S100 proteins as Ca^{2+} sensor proteins implicated in signal transduction [76]. Yet, the orientations of the hinge region, helix III and the C-terminal extension in S100A6 appear to be affected by Ca^{2+} binding [76], suggesting that in S100A6 also a binding surface, which is buried in the apo state, is exposed to the solvent in the Ca^{2+}-bound state. Differences in the primary sequences of S100B and S100A6, as well as differences in the length of the hinge region and the C-terminal extension in these proteins [48], might explain the specificity of target protein binding. Actually, while there is no information as to whether or not annexin XI and glyceraldehyde-3-phosphate dehydrogenase, two known S100A6 target proteins [81,82] (Table 1), also interact with S100B, it has been reported that S100A6 does not compete with S100A1 and S100B for binding to desmin [83].

The IF subunits, GFAP and desmin, two other S100A1- and S100B-binding proteins [83,84] (Table 1), likely interact with the TRTK-12-binding site on S100B and S100A1 since TRTK-12 blocks both these interactions and the inhibitory effects of S100A1 and S100B on GFAP assembly [85] and desmin assembly (unpublished data) into IFs. Also, the C-terminal extension of S100A11 is implicated in the binding of annexin I [86] (Table 1). Thus, it is possible that the C-terminal extension of any S100 member that acts as a Ca^{2+} sensor protein is implicated in the interaction with one or more targets. In addition, the hinge region, which connects the N-terminal half to the C-terminal half in any S100 protein, could also be implicated in the recognition of definite target proteins [63,64,75–77,87]. A synthetic peptide corresponding to the S-367 to E-388 C-terminal sequence of p53, which was proposed to contain the S100B-binding site on p53 and to correspond to the p53 phosphorylation site by protein kinase C (PKC) [88] (Table 1), binds to S100B in a Ca^{2+}-dependent manner [87]. Residues S-41, L-44, E-45, E-46 and I-47 in the hinge region, V-52, V-53, V-56 and T-59 in helix III, and A-83, C-84, H-85, E-86 and F-87 in the helix IV/C-terminal extension appear to be implicated in the Ca^{2+}-dependent p53 peptide binding to and/or reflect structural changes in S100B, as investigated by NMR spectroscopy [87]. Binding of S100B to the p53 peptide also inhibits its phosphorylation [89]. Interestingly, S100B also binds to and inhibits the phosphorylation of a peptide derived from neuromodulin [89], an S100B target protein [90–92] (Table 1), and the regulatory domain of PKC [89]. These three peptides display a certain amount of sequence identity [89] with the CapZα peptide, TRTK-12, suggesting that S100B can bind to proteins that possess the consensus sequence found in CapZα (i.e. (K/R)(L/I)*X*W*XX*IL), and that target proteins displaying this feature may be S100B-binding proteins.

Recent observations indicate that the p53 peptide also interacts with S100A1; that the S100A1 C-terminal extension plays a major role in this interaction; and that the p53 peptide blocks the S100A1

binding to GFAP and tubulin, and the inhibitory effect of S100A1 on GFAP polymerization [79]. All these findings suggest that the functional binding site on S100A1 and S100B monomers may be similar and that a number of S100A1 and S100B target proteins might share a common binding site on S100A1 and S100B. In further support of the possibility that the hinge region has a role in the formation of a binding surface on S100 proteins is the observation that a synthetic peptide derived from the hinge region of the S100A8-like CP-10 protein, a chemotactic protein, exhibits chemotactic properties similar to those of full-length protein [93] (Table 1). On the other hand, the N-terminal region (helix I) of S100A6 has been suggested to be essential for the binding of glyceraldehyde-3-phosphate dehydrogenase [81] and an annexin XI isoform [81,82,94] (Table 1). Helix I of one S100A6 monomer could contribute to the generation of a binding surface together with residues in helices III and IV and the hinge region of the other monomer (see Fig. 1 in [76]).

The lack of extensive sequence homology between annexins I, II and XI (the target proteins in the cases of S100A11, S100A10 and S100A6, respectively) (Table 1), on the one hand, and the α subunit of CapZ, GFAP, desmin, neuromodulin, tubulin, neurogranin, and p53 (which are S100A1 and S100B target proteins Table 1), on the other hand, and the target specificity reported for many S100 proteins (Table 1) suggest that individual components of the surface exposed to the solvent upon Ca^{2+} binding to S100B and S100A6 (and, likely, other S100 proteins) might play differential roles in the recognition of target proteins. For instance, this would explain the observations that the C-terminal extension of S100A11 in one report [86] and the N-terminal helix of S100A11 in another report [95] were implicated in annexin I binding; that deletion of the N-terminal helix of S100A6 abolishes the glyceraldehyde-3-phosphate dehydrogenase [82] and annexin XI [81] binding; and that the chemotactic potency of the synthetic peptide derived from the hinge region of the S100A8-like protein, CP-10, is much smaller than the full-length protein [93]. Probably, this might also explain recent observations on annexin VI binding to S100A1 and S100B and annexin VI blockade of the inhibitory effects of S100A1 and S100B on desmin and GFAP assemblies into IFs (Table 1): whereas there seems to be no major competition between annexin VI and desmin/GFAP for binding to either S100 protein, occupation of the annexin VI site alters the properties of the desmin or GFAP site and vice versa [96]. Also, whereas deletion of S100A1 C-terminal extension strongly reduces or abolishes the interaction of S100A1 with the GFAP, desmin, and tubulin [79], deleted S100A1 still binds annexin VI in a Ca^{2+}-dependent manner (unpublished observations). On the other hand, S100B has been recently proposed to interact with its target proteins through more than one site [97] and S100A1 has been suggested to utilize different mechanisms (i.e. different regions) for interacting Ca^{2+}-dependently with some target proteins and Ca^{2+}-independently with some other target proteins (e.g. muscle-specific aldolase A) [39]. However, no information is available at present about the region(s) of S100A1 (and, likely, S100B) that are implicated in the Ca^{2+}-independent interaction of these proteins with certain target proteins, aside from the observation that the C-terminal part of helix IV and the C-terminal extension are not required for Ca^{2+}-independent binding of S100A1 to aldolase A [39].

In conclusion, the available evidence indicates that the binding surface that is exposed upon Ca^{2+} binding to S100A6 and S100B (and eventually several other S100 members) might accommodate the binding a number of target proteins, with the different portions of the S100 molecule that have been implicated in the formation of the binding surface being more or less important depending on the target considered. Clearly, this mode of Ca^{2+}-dependent interaction of S100 proteins with their target proteins is quite different from that of calmodulin and troponin-C [98–100]: individual monomers in an S100 dimer open up to accommodate the target protein binding with resulting elongation of the whole dimer [75] (Fig. 1) (also see Fig. 3A in [76] and Fig. 5 in [66]). The location of target protein-binding sites on opposite sides of S100 homodimers (and, possibly, heterodimers) would permit an S100 dimer to crossbridge two homologous or heterologous S100 target protein. Examples of this are the annexin II_2-$S100A10_2$ and annexin I_2-$S100A11_2$ heterotetramers [31–33,36,86] (Table 1). Also, the stoichiometry of S100A1 and S100B binding to desmin, GFAP, tubulin, CapZ and p53 (1 mol of S100A1 or S100B/mol

of desmin, GFAP, tubulin, CapZ or p53) [71,72,83,84,101] suggests that each of the above S100A1 or S100B target proteins may bind to opposite sides on each S100A1 or S100B dimer. The same would apply to the S100A6/glyceraldehyde-3-phosphate dehydrogenase complex [82], the S100B/annexin II complex [102], the S100A6/annexin XI complex [81], the S100A2/tropomyosin complex [103], and the S100A1 or S100B/annexin VI and S100A1 or S100B/annexin V complexes [96].

Calbindin D_{9k} is the only S100 member identified so far that does not form dimers, acting as a Ca^{2+} modulator, rather than a Ca^{2+} sensor protein ([104] and references therein) (Table 1). This protein buffers cytosolic Ca^{2+} and no target proteins for it have been identified, implying that it is implicated in Ca^{2+} signal transduction to the extent to which it buffers cytosolic Ca^{2+}. The shorter helices I and IV and hinge region in calbindin D_{9k} and the smaller hydrophobic surface that becomes exposed upon Ca^{2+} binding, as compared to other S100 proteins (see Fig. 3 in [66]), might be responsible for the inability of calbindin D_{9k} both to form dimers and to interact with target proteins.

Recent crystallographic structure determinations suggest that, in addition to homodimers, S100A10 forms homotetramers in which the two homodimers are antiparallely connected via disulfide bridges between pairs of the C-61 residues of one each of the two S100A10 copies that form an $S100A10_2$ dimer, both in the absence and in the presence of the annexin II N-terminal tail [80]. However, information is lacking about functional implications of S100A10 tetramer formation.

A recent structural study of S100A8 and S100A9 suggests that $S100A8_2$ and $S100A9_2$ homodimers form independently of the absence or presence of Ca^{2+}, and that in solution containing both proteins S100A8 and S100A9 display a strong preference to associate as heterodimers [105]. It is suggested that the two monomers of the heterodimer are related by a two-fold axis of rotation, as is the case with $S100A6_2$, $S100B_2$, $S100A7_2$ and $S100A10_2$ dimers, that the functionally relevant form of S100A8 and S100A9 is a heterodimer, and that functional roles attributed to individual S100A8 and S100A9 proteins rely on formation of stable homodimers [105].

3. Putative functions of S100 proteins

Extensive work done in several laboratories has implicated S100 proteins in both intracellular and extracellular activities [1–3,8,47–49] (Table 1).

3.1. Intracellular functions of S100 proteins

3.1.1. Regulation of protein phosphorylation

Ca^{2+}-dependent and, in two cases, Ca^{2+}-independent inhibition of protein phosphorylation by S100 proteins in vitro has been documented since 1982 [1–3]. This inhibition results from a direct interaction of S100 proteins with kinase substrates rather than with kinases. Among the proteins whose phosphorylation is inhibited by S100 proteins are the myristoylated alanine-rich C kinase substrate (MARCKS) also known as 87-kDa protein [106], neuromodulin (GAP-43) [90–92], annexin II [107], and neurogranin [92], microtubule-associated τ proteins [108,109], caldesmon [110], MyoD [111], p53 [88,89], GFAP and vimentin [112], and the MARCKS-like retinal phosphoprotein p80 [78] (in all these instances S100A1 and/or S100B are the S100 members implicated), and annexins I and II, the phosphorylation of which is inhibited by their ligands, S100A11 and S100A10, respectively [86,95,113–116] (Table 1). However, the functional correlates of S100 protein-dependent inhibition of protein phosphorylation are not fully elucidated.

S100B-dependent inhibition of the tumor suppressor protein p53 phosphorylation might be part of S100B-dependent protection of p53 (see Section 3.2.2).

Changes in the phosphorylation state of annexins I and II might alter the association of these proteins with their targets (reviewed in [34,35,117]) (also see Section 3.1.4).

MARCKS is abundant in neurons [106] and was proposed to crosslink actin to membranes. In principle, in those neurons that have been reported to express S100A1 and/or S100B [10–15], these latter proteins might intervene in the regulation of the proposed role of MARCKS as an actin-crosslinking factor via inhibition of its phosphorylation (but no information is available at present in this respect).

Phosphorylation of τ proteins might represent a means to control MT formation (phosphorylation

of MT-associated proteins results in a decreased ability of these proteins to promote tubulin polymerization into MTs [118,119]). In this context, inhibition of τ protein phosphorylation by S100A1 and S100B might contribute to the regulation of the state of assembly of MTs (Table 1). These findings would complement in vitro studies showing that S100A1 and S100B inhibit tubulin polymerization [120,121] by binding to unassembled tubulin [101,122] and stimulate MT disassembly by increasing the Ca^{2+}-sensitivity of preformed MTs [123] (see Section 3.1.4) (Table 1).

S100A1 and/or S100B binding to caldesmon (a cytoskeletal protein abundant in smooth muscle cells and expressed in other cell types) and the ensuing inhibition of caldesmon phosphorylation results in reversal of caldesmon-dependent inhibition of actomyosin ATPase activity [110,124,125] (Table 1). Yet, no data are available on colocalization of S100A1 and/or S100B with caldesmon.

Inhibition of GFAP and vimentin phosphorylation by S100B might reflect interaction of S100B with a stretch of residues in the N-terminal domain of the above IF subunits [85] (also see Section 3.1.4).

S100A10 inhibits annexin II phosphorylation [114–116] (Table 1). As anticipated, S100A10 forms a heterotetrameric complex with annexin II in which an $S100A10_2$ dimer crossbridges two copies of annexin II [31–33,36]. This complex interacts with artificial membranes, promotes chromaffin granule aggregation and fusion, bundles F-actin, and rescues the ability of GFAP to assemble into IFs at pH values >6.7 in a Ca^{2+}-dependent manner [34,35,117, 126,127]. S100A10 colocalizes with annexin II [35]. Phosphorylated annexin II_2-$S100A10_2$ requires a higher Ca^{2+} concentration for inducing lipid vesicle aggregation and does not bind F-actin, but its binding to lipid vesicles and chromaffin granules remains unchanged [34,35,117]. In one case, annexin II_2-$S100A10_2$ phosphorylation by PKC was reported to result in dissociation of the annexin II_2-$S100A10_2$ complex and in a striking annexin II-dependent stimulation of chromaffin granule fusion [128]. These data indicate that S100A10 regulates the annexin II activities which, in turn, depend on the phosphorylation state of annexin II (also see Section 3.1.4). Although S100A1 and/or S100B also inhibit annexin II phosphorylation in a Ca^{2+}-independent manner like S100A10 [107], but bind annexin II in a Ca^{2+}-dependent manner [102], there is no evidence that S100A1 and/or S100B can act as substitutes for S100A10.

Analogously, since phosphorylation of annexin I results in an enhancement of its phospholipid-binding activity [129], S100A11, which binds to and inhibits annexin I phosphorylation [86,95] (Table 1), might likewise regulate annexin I association with membranes and other targets (also see Section 3.1.4).

S100A4 inhibits the PKC-dependent phosphorylation of myosin heavy chain [130] thereby putatively modulating the cytoskeleton dynamics in metastatic cells (Table 1) (see Section 5).

The ability of several S100 proteins to regulate the phosphorylation state of a number of kinase substrates suggests that these proteins have a role in signal transduction, particularly by linking elevation of the cytosolic Ca^{2+} concentration to the phosphorylation state of the above mentioned S100 target proteins. Further supporting this possibility is the recent finding that S100B is induced in rat myocardium post-infarction concomitantly with the down-regulation of a fetal protein, skeletal α-actin, and that transfection of an S100B expression vector into cultured neonatal rat cardiomyocytes inhibits the β-PKC-mediated induction of skeletal α-actin and embryonic β-myosin heavy chain, another fetal protein, by norepineprine and phenylephrine [131]. These data suggest that S100B might act as a negative regulatory element that limits the hypertrophic response following myocardial infarction via inhibition of a β-PKC-dependent phosphorylation of an unknown factor [131]. It is interesting to note that S100A1 and S100B bind to the basic helix-loop-helix (bHLH) sequence found in the transcription factors MyoD and E12 [111,132] and that at least S100A1 inhibits MyoD phosphorylation [111] (Table 1). Binding of S100A1 and S100B to the bHLH sequence in MyoD is regulated by N-terminal MyoD sequences, which apparently render the bHLH motif unaccessible to S100A1 and S100B (and calmodulin) [132]. On this basis, S100A1 and S100B might have a role in the regulation of the activity of certain transcription factors (Table 1). It is not known at present whether the factor suggested to be inhibited by S100B in myocardium post-infarction [131] is the tissue-specific MyoD or some other transcription fac-

tor. Also unknown is the role, if any, of S100A1 in the regulation of hypertrophic response to and the sequence of events leading to expression of S100B in myocardium post-infarction.

The ability of some S100 proteins to inhibit protein phosphorylation by interacting with kinase substrates rather than with kinases could represent a means to finely regulate specific steps of those signalling pathways in which phosphorylatable S100 target proteins have a role.

3.1.2. Regulation of enzyme activity

No enzymatic activity has been ascribed to any member of the S100 family thus far. By contrast, several S100 proteins have been shown to regulate some enzyme activities. S100A1 and S100B were shown to interact with a brain fructose-1,6-bisphosphate aldolase stimulating its V_{max} [133], whereas S100A1, but not S100B, was reported to bind to and to inhibit glycogen phosphorylase [134] (Table 1). Also, S100A1 inhibits and S100B stimulates phosphoglucomutase [135] (Table 1). These findings suggest that S100A1 and S100B might have a role in the regulation of energy metabolism. The finding that S100A1 is abundantly expressed in slow-twitch skeletal muscle cells and cardiomyocytes [1–3] has been put in relation to a possible role of this protein in preventing fatiguability of slow-twitch skeletal muscle cells by reducing glycogen breakdown [135].

Recently, S100A1 and, to a much smaller extent, S100B were shown to interact with and to stimulate the sarcomeric, myosin-associated giant kinase twitchin in a Ca^{2+}- and Zn^{2+}-dependent manner [136] (Table 1). S100A6 and calmodulin, though able to interact with twitchin, do not activate its kinase activity [137]. The ability of S100A1 to regulate the twitchin kinase activity appears relevant in consideration of the abundance of S100A1 in striated muscle cells and its localization near the Z discs ([138,139], and unpublished observations) to which twitchin is also linked. As the S100A1-binding site on twitchin has been identified [136], the regulatory activity of S100A1 on this kind of kinase might be of physiological relevance. Yet, the available genomic databases indicate that invertebrates do not express S100 proteins and, accordingly, attempts to identify S100A1 or its mRNA in the nematode *C. elegans* proved unsuccessful (M. Garbuglia and R. Donato, unpublished observations). Twitchin is a member of a family of giant protein kinases involved in the regulation of muscle contraction and the mechanoelastic properties of the sarcomere in invertebrates [140,141]. The corresponding vertebrate protein is titin [140,141], to which S100A1 also binds (M. Garbuglia and R. Donato, unpublished observations). Aside from regulatory effects of S100A1 on twitchin/titin kinase activity, which could be relevant to the general economy of muscle contraction, the interaction of S100A1 with members of this sarcomeric protein family might be important in the context of the role of twitchin/titin as the molecular entity responsible for the mechanoelastic properties of skeletal muscles [140,141]. Conclusions in this respect must await information about S100A1-dependent regulation of titin kinase activity and/or titin-based mechanoelastic properties of vertebrate skeletal muscles as well as clear evidence of localization and/or Ca^{2+}-dependent translocation of S100A1 to the center part of the sarcomere where the catalytic domain of twitchin/titin is located [140,141].

S100B and, to a smaller extent, S100A1 stimulate Ndr, a nuclear serine/threonine protein kinase important in the regulation of cell division and cell morphology, in a Ca^{2+}-dependent manner [142] (Table 1). S100B interacts with a basic/hydrophobic motif within the N-terminal regulatory domain of Ndr, and S100B binding can be inhibited by a synthetic peptide derived from this Ndr region [142]. Since S100B has been implicated in the regulation of cell cycle (see Section 4), the S100B/Ndr interactions could be of physiological relevance.

S100A1 and S100B were reported to regulate the adenylate cyclase activity [143,144], apparently via an interaction with a G-protein [145] (Table 1). Further analysis is required to draw definite conclusions about the physiological relevance of these observations.

Stimulation of a membrane-bound guanylate cyclase activity in photoreceptor outer segments by S100A1 and S100B in the presence of micromolar levels of free Ca^{2+} in vitro was also reported [146–148] and S100B was shown to be identical to and interconvertible with CD-GCAP (calcium-dependent guanylate cyclase-activating protein) [148] (Table 1). It is known that activation of photoreceptor outer segment membrane-bound guanylate cyclase has an

important role in phototransduction, serving to increase the range of intensities to which the rod can respond, and that several Ca^{2+}-binding proteins are implicated in phototransduction [149,150]. S100A1- and S100B-stimulated membrane-bound guanylate cyclase was localized to photoreceptor disc membranes, photoreceptor cell bodies, and retinal Müller cells, by ultracytochemistry [151]. Activation of this enzyme by either S100 protein in the outer segment of photoreceptors proved dependent on Ca^{2+} (with much smaller free Ca^{2+} concentrations required in the case of S100B than S100A1) and the dark [146,147,151]. In contrast, S100A1/B-dependent activation of membrane-bound guanylate cyclase activity in photoreceptor cell bodies and retinal Müller cells was dependent on Ca^{2+} and independent of light- or dark-adaptation [151]. The possibility that S100A1 and S100B might have a role in the regulation of membrane-bound guanylate cyclase activity is supported by the finding that both proteins are present in the outer segments of photoreceptors and are co-localized with that enzyme to disc membranes [151]. Interestingly, the membrane-bound guanylate cyclase activity stimulated by either S100 protein is insensitive to the natriuretic peptides, which activate another member of the membrane-bound guanylate cyclase family in other retinal cells [150]. These data suggest that S100B and, eventually, S100A1 might play a role in dark-adaptation of photoreceptors in vertebrates or, alternatively, S100A1 and S100B may transduce a different kind of signal in photoreceptors and Müller cells via stimulation of a membrane-bound guanylate cyclase. As mentioned above, the hinge region and the C-terminal extension have been implicated in the recognition of membrane-bound guanylate cyclase by S100B [78].

The S100A8/S100A9 heterodimer inhibits casein kinase I and II, two enzymes that phosphorylate topoisomerase I and RNA polymerases I and II [152] (Table 1). These findings suggest that S100A8 and S100A9 and/or the S100A8/S100A9 heterodimer might regulate myeloid cell maturation and function. It is important to note that S100A8 and S100A9 expression in monocytes occurs in early stages of differentiation [153] and in infiltrating monocytes, but not in resident tissue macrophages [154]. This suggests that the expression of S100A8 and S100A9, formation of the S100A8/S100A9 complex, and S100A8/S100A9-dependent regulation of casein kinase I and II activity are related to a definite functional stage of macrophages and participate in the inflammatory response (see Section 3.2.4).

S100A10 interacts with cytosolic (85-kDa) phospholipase A_2 and inhibits its activity [155] (Table 1). Interaction occurs at the C-terminal region of the enzyme and the inhibitory effect is independent of the presence or absence of the natural S100A10 ligand, annexin II, suggesting that the phospolipase A_2 site might not comprise the S100A10 C-terminal extension. Inhibition of cytosolic phospholipase A_2 activity by S100A10 appears of physiological importance since S100A10 associates with this enzyme in vivo, the formation of the S100A10/phospholipase A_2 complex results in a decreased release of archidonic acid, and antisense inhibition of S100A10 expression results in an increased phospholipase A_2 activity and a parallel increase in arachidonic acid release by cultured cells [155]. These data point to a role of S100A10 in the inflammatory response; specifically, they point to an anti-inflammatory role of this protein.

With the exception of membrane-bound guanylate cyclase [78], there is no information at present about the location of the site(s) on S100 proteins that recognize(s) the above enzyme or enzyme regulatory proteins.

3.1.3. Regulation of Ca^{2+} homeostasis

S100A1 is localized to triades in striated muscle cells [139] and stimulates Ca^{2+}-induced Ca^{2+} release in skeletal muscle cells [156] (Table 1). A similar stimulatory effect was reported for a mixture of S100A1 and S100B consisting of ~20% S100A1 and ~80% S100B [157]. These latter studies also showed that the S100A1/B mixture blocks the inhibitory effect of ruthenium red on Ca^{2+}-induced Ca^{2+} release and increases the binding affinity of ryanodine for its receptor, suggesting a direct binding of these proteins to the ryanodine receptor [157]. Consistently, direct S100A1 binding to the ryanodine receptor, the molecular entity responsible for Ca^{2+}-induced Ca^{2+} release, has been recently documented [158] (Table 1). S100A1, which binds to purified ryanodine receptor at both nanomolar and micromolar Ca^{2+} levels, increases the affinity of ryanodine binding to its receptor at nanomolar Ca^{2+} concentrations

and, under these conditions, increases the open probability of the ryanodine receptor channel [158]. Binding of S100A1 to the ryanodine receptor has been mapped to residues 1861–2155, 3774–3874, and 4425–4621, although with different Ca^{2+} requirements [158]. The ryanodine receptor polypeptide defined by residues 4425–4621, which binds S100A1 at nanomolar Ca^{2+} concentrations, but much less so at millimolar Ca^{2+} concentrations, and binds calmodulin with a low affinity, has been implicated in the Ca^{2+}-dependent regulation of the Ca^{2+} release channel (see [158] for literature). Together, these data strongly suggest the possibility that S100A1 has a role in intracellular Ca^{2+} homeostasis in skeletal muscle cells by directly activating the ryanodine receptor. In this context, it is interesting to note that S100A1 is down-regulated in failing human heart [159], a finding that has been put in relation to a reduced Ca^{2+}-induced Ca^{2+} release from sarcoplasmic reticulum in heart failure [159]. The S100A1 region implicated in the binding of the ryanodine receptor is not known at present.

3.1.4. Regulation of the dynamics of cytoskeleton components

All three major constituents of cytoplasmic cytoskeleton, i.e. MTs, IFs and microfilaments (MFs), and tropomyosin and myosin appear to be targets of S100 proteins. S100A1 and S100B have been reported to inhibit MT protein and purified tubulin assemblies in the presence of micromolar concentrations of free Ca^{2+} and to increase the Ca^{2+} sensitivity of preformed MTs, in a pH-dependent manner in vitro [120–123,160–162] (Table 1). In particular, S100A1 and S100B cause a strikingly fast disassembly of preformed MTs in the presence of Ca^{2+} and thus could be two of the factors responsible for the so-called Ca^{2+}-induced catastrophic MT disassembly ([163] and references therein). Immunocytochemical analyses have documented the association of S100B with axonemal MTs, centrioles, basal bodies, the mitotic spindle, the centrosomes, and the center part of the midbody in telophase cells (the centrosomes and the center part of the midbody are MT nucleation centers [164,165]) as well as with cytoplasmic MTs [1,2,38,166], observations that support the idea that S100B might have a role in avoiding excess tubulin polymerization, in the regulation of tubulin-dependent stimulation of the ATPase activity associated with MAPs (the MT-associated proteins) [167], in remodelling of MTs at sites where the free Ca^{2+} concentration is expected to be relatively high, e.g. the cytoplasm facing plasma membranes and membranes of Ca^{2+} stores [168,169], or in the modulation of tubulin-dependent regulation of signalling pathways [170]. Together with the observation that a fraction of S100B exists in a membrane-bound state [37], and interacts with artificial membranes [171], effects of S100B on the MT dynamics might be relevant in the regulation of MT-endosome interactions, which are considered important in the mechanism of fusion of endosomes [172], transport from early endosomes to late endosomes [173], and apical recycling [174]. In this regard, it is interesting to note that S100B was also localized to Golgi membranes and other perinuclear membranes by immunocytochemistry [38,166, 175]. The presence of S100B at MT nucleation centers (the centrosomes and the center part of the midbody in dividing cells) supports the possibility that S100B regulates the nucleation of MTs. Actually, S100A1 and S100B inhibit MT assembly by interfering with both the nucleation and the elongation of MTs in vitro [120,176]. In contrast, in spite of its in vitro effects, S100A1 seems not to be localized to MTs or MT nucleation centers ([166,177], and unpublished observations), suggesting that this protein might intervene in the regulation of MT dynamics by interacting with unassembled tubulin. Interestingly, recent evidence suggests that inhibition of S100A1 synthesis in PC12 cells results in an increase in tubulin levels and the number of neurites extended in response to NGF, and a decrease in cell proliferation [178] (Table 1). The increase in tubulin levels is due to an increase in unpolymerized tubulin at constant levels of polymerized tubulin [178], which appears in line with the finding that S100A1 inhibits tubulin assembly [120–122]. The observed decrease in proliferation of $S100A1^{-}$ PC12 cells is suggested to depend on lack of effects of S100A1 on growth regulatory factors [178], the identity of which remains to be established. As mentioned above, S100A1 interacts with certain transcription factors thereby blocking their interaction with DNA [111,132]: absence of S100A1 might result in deregulation of the gene program underlying PC12 cell proliferation and activation of gene program(s) implicated in cell differentiation.

Direct binding of S100A1 and S100B to desmin and GFAP, two proteins that self-assemble to form the type III IFs specific to muscle and glial cells, respectively, results in a dose-dependent inhibition of desmin and GFAP assemblies and stimulation of desmin and GFAP IF disassembly, in the presence of a few micromolar free Ca^{2+} levels [83,84,179] (Table 1). The S100 protein effect is stoichiometric (1 mol of desmin or GFAP/mol of S100A1 or B monomer with an affinity of 0.5–1 μM), resulting in inhibition of nucleation and elongation of desmin and GFAP IFs, as well as in stimulation of the polymerized-unpolymerized desmin and GFAP exchange [83–85,179] (Fig. 2). Other S100 proteins (S100A6 and S100A10) do not bind to desmin or affect desmin or GFAP assembly [83,126]. The inhibitory effects of S100A1 and S100B on desmin and GFAP assemblies can be blocked by annexin VI via annexin VI binding to either S100 protein [96]. Annexin V, which also interacts with S100A1 and S100B, has no effects on the above S100 effects on IF formation [96]. S100A1 and S100B bind to a stretch of residues found in the GFAP and desmin N-terminal domain which shares a high sequence identity with the S100A1/S100B-inhibitory peptide TRTK-12 [85] and contains a motif, the RP-box motif, shown to be important for the formation of GFAP, desmin and vimentin IFs [180] (Fig. 2). S100B has been localized to IFs in both untreated cells and in Triton-cytoskeletons of cultured Schwann cells, astrocytes, rat L6 myoblasts and myotubes, and renal cells ([38,166,181], and unpublished observations). By similar approaches, in mature skeletal muscle cells and in chick embryo myotubes S100A1 has been localized to structures associated with Z discs, the sarcolemma and the fascia adherens of the intercalated discs, as well as to the sarcoplasm facing the sarcolemma and the sarcoplasmic reticulum ([138,139], and unpublished observations). However, whereas S100A1 appears to be located close to sites where desmin IFs are also found (e.g. the Z discs in mature striated muscle cells), no association of this protein with IFs in rat L6 myoblasts and myotubes could be documented ([166,177], and unpublished observations). This suggests that S100A1 is closely located, but not linked to IFs, and/or that S100A1 might intervene in the regulation of IF dynamics by interacting with unassembled subunits, as it seems to be the case with

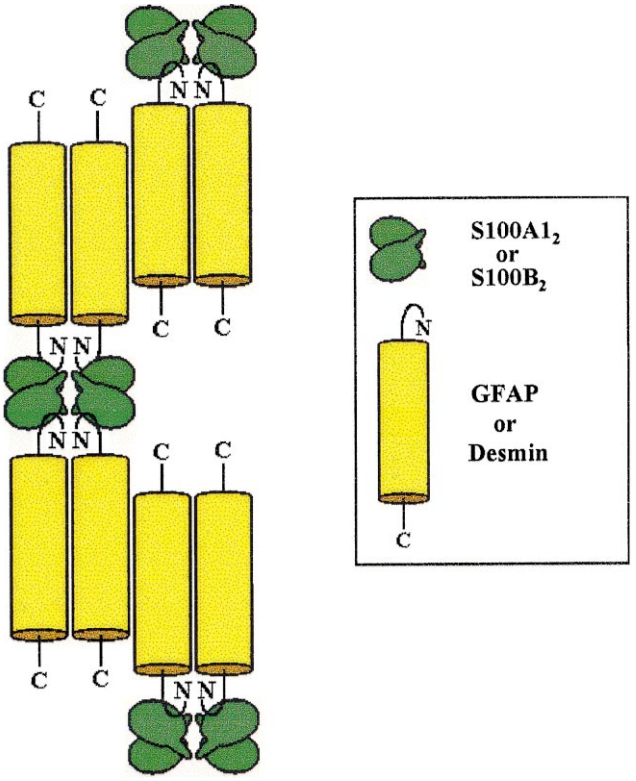


Fig. 2. Schematic representation of the Ca^{2+}-dependent interaction between $S100A1_2$ or $S100B_2$ dimers and desmin or GFAP tetramers. A pair of $S100_2$ dimers crossbridges two desmin or GFAP dimers by interacting with the desmin or GFAP N-terminal domain [85,196]. Binding of either S100 protein to the N-terminal domain results in inhibition of desmin or GFAP assembly into type III intermediate filaments. Formation of macrocomplexes composed of four desmin or GFAP subunits plus four copies of S100A1 or S100B monomers was observed by chemical crosslinking [85,195]. A similar mode of interaction, i.e. crossbridging of two homologous or heterologous target proteins by an S100 dimer, could apply to most, if not all, S100-target protein complexes that form in a Ca^{2+}-dependent manner (see text). An analogous mode of interaction could apply to those S100-target protein complexes that form in a Ca^{2+}-independent manner (see Fig. 7 in [39]).

tubulin. Altogether, these observations suggest that S100A1 and S100B could have a role in the regulation of the state of assembly of type III IFs, albeit with different mechanisms of action. On the one hand, S100A1 and S100B might be important in avoiding excess IF assembly and contributing to the appropriate orientation of IFs, and, on the other hand, they might participate in remodelling IFs in mitosis, cell locomotion and shape change (e.g. in development), and eventually, in skeletal muscle

cells, during the cycle of contraction-relaxation. Interestingly, astrocytes in the brain of mutant mice expressing a much reduced amount of S100B exhibit larger amounts of GFAP IFs [182], a finding that has been put in relation [182] to the ability of S100B to inhibit GFAP IF assembly.

As mentioned above, three large molecular weight proteins, profilaggrin and trichohyalin and repetin, that are expressed in keratinocytes and are involved in the lateral association of keratin IFs in differentiated epithelial cells, contain a long stretch of residues highly homologous to S100 proteins [24–27] (Table 1). Since S100A1 and S100B are also expressed in a restricted number of epithelial cells [183] and S100A7, S100A8, S100A9 and S100A10 are also expressed in some epithelial cells [3], it would be of interest to see whether these proteins are also involved in the regulation of keratin IFs (type I and II IFs).

S100A10 is the natural ligand of annexin II with which it forms an annexin II_2-$S100A10_2$ heterotetramer [31–33,36]. Binding of S100A10 to annexin II targets annexin II to the cortical cytoskeleton of fibroblasts and adrenal chromaffin cells [184–186], besides remarkably reducing the Ca^{2+} requirement for phospholipid binding and chromaffin granule aggregation and fusion by annexin II [187–189], and favors the annexin II-dependent bundling of F-actin [31,190,191] (Table 1). The F-actin-binding site on annexin II is located in the annexin II core domain [31,190]. While the F-actin-bundling activity of the annexin II_2-$S100A10_2$ heterotetramer might be related to the reorganization of F-actin in exocytosis [34,35,117,188,191–193], some uncertainty exists as to the physiological relevance of this annexin II_2-$S100A10_2$ heterotetramer activity because annexin II does not colocalize with stress fibres [194] and the annexin II_2-$S100A10_2$ heterotetramer and F-actin are coexpressed in several other cell types not characterized by exocytotic events [35]. Yet, the fact that S100A10 induces a redistribution of annexin II within cells [184–186] and inhibits annexin II phosphorylation [114–116] strongly suggests that this protein regulates annexin II activities, including annexin II association with cytoskeleton constituents. Moreover, the annexin II_2-$S100A10_2$ heterotetramer might be implicated in docking F-actin to plasma membranes (see below). The annexin II_2-$S100A10_2$ heterotetramer binds to both unassembled and assembled GFAP in the presence of Ca^{2+} [195] and rescues the ability of GFAP to assemble into typical IFs under conditions in which assembly is inhibited (e.g. at $pH > 6.7$) [126,127] (Table 1). While the annexin II moiety is the molecular entity responsible for these effects, the integrity of the annexin II_2-$S100A10_2$ heterotetramer is required for optimal effects [126], indicating that S100A10 binding to annexin II is a prerequisite for annexin II-GFAP interactions. The annexin II_2-$S100A10_2$ heterotetramer interacts with the GFAP rod domain [196], which is critical for the formation of dimers/tetramers of IF subunits [197,198], whereas the GFAP-binding site on annexin II is tentatively located at the transition between the annexin II N-terminal tail and domain I of the annexin II core [126]. The stimulatory effect of the annexin II_2-$S100A10_2$ heterotetramer on GFAP assembly is counteracted by S100A1/B, the inhibitory effect of which is in turn counteracted by the annexin II_2-$S100A10_2$ heterotetramer [127]. The opposite effects of S100A1/B and the annexin II_2-$S100A10_2$ heterotetramer appear to depend on the different location of the S100A1/S100B and the annexin II_2-$S100A10_2$ heterotetramer sites on GFAP [85,196]. Whether or not the annexin II_2-$S100A10_2$ heterotetramer associates with GFAP IFs in vivo is not known at present. S100A10 has been identified as one precursor of the cornified envelope of cultured epidermal keratinocytes [199]. Interestingly, annexin II was not found associated with S100A10 in that structure, and this represents one of the rare examples of presence of S100A10 on a cell structure without its accompanying protein, annexin II. It is suggested that annexin II, by virtue of its membrane phospholipid-binding property, might target S100A10 to the cornified envelope (a crosslinked sheath of protein that forms beneath the plasma membrane during the final stages of epidermal keratinocyte differentiation), and detaches from S100A10 during envelope assembly [199]. While the identity of S100A10 target protein(s) in the cornified envelope is not known, one or more of the proteins detected in the cornified envelope might be a target protein for S100A10. If so, given the mode of assembly of S100A10 and annexin II into the annexin II_2-$S100A10_2$ heterotetramer [31–33,36,200], one might speculate that the $S100A10_2$ dimer interacts with its target protein(s) in the enve-

lope, while still bound to annexin II, through a site that does not comprise the S100A10 C-terminal extension, and that annexin II detaches from S100A10 because of that interaction. This scenario differs substantially from that proposed for translocation of the annexin II_2-$S100A10_2$ heterotetramer to membranes [186] and stimulation of GFAP assembly [126]. Complex formation between S100A10 and annexin II is required for the Ca^{2+}-dependent association of annexin II with membranes [184–189] where the annexin II_2-$S100A10_2$ heterotetramer could mediate the association of F-actin with the membrane lipid bilayer and/or membrane-membrane associations that precede the actual membrane fusion [189,201,202]. Also, annexin II needs to be associated with S100A10 for maximal stimulatory effect of GFAP assembly [126].

S100A11 also has been identified as one component of the cornified envelope of cultured epidermal keratinocytes where it is likely bound to annexin I [199] (Table 1). S100A11 is suggested to be targeted by annexin I to the keratinocyte plasma membrane in a Ca^{2+}-dependent manner and thus made available for crosslinking [199]. S100A11 dimers, trimers, tetramers and larger multimers can be generated in vitro upon treatment with a transglutaminase and similar S100A11 oligomers can be extracted from the cornified envelope of cultured epidermal keratinocytes, pointing to in vivo occurrence of S100A11 oligomerization [203]. It is suggested that transglutaminase crosslinks S100A11 to the N-terminal domain of annexin I in the presence of Ca^{2+} [203]. Targeting of S100A11 to endosomes by annexin I has also been reported [204]. However, it not clear whether annexin I-dependent translocation of S100A11 to the keratinocyte cornified envelope and/or endosomes serves to anchor S100A11 to specific membrane sites where this protein regulates cytoskeleton constituents, or this event is unrelated to the organization of the membrane skeleton. Also, it is not clear how the proposed transglutaminase-dependent crosslinking of S100A11 to annexin I [203] results in the formation of S100A11 oligomers within the cornified envelope. Overall, there is no information about the functional role of S100A11 dimers/oligomers within the keratinocyte cornified envelope. The annexin I_2-$S100A11_2$ heterotetramer has been suggested to serve to interconnect membranes of different endosomal compartments, i.e. to hold endosomal membranes in place for their actual fusion and/or to organize the endosomal compartment [204] (Table 1). The available evidence suggests that S100A11 forms annexin I_2-$S100A11_2$ heterotetramers in a Ca^{2+}-dependent manner, in which the $S100A11_2$ dimer binds a copy of annexin I on opposite sides [86,95]. As the $S100A11_2$ dimer is a stable structure and S100A11 binding to annexin I depends on Ca^{2+}, S100A11 would be kept onto membranes as long as the Ca^{2+} concentration remains relatively high, with detachment of the whole $S100A11_2$ dimer from annexin I and, hence, membranes upon decrease in the Ca^{2+} concentration. Thus, S100A11 would serve to render the annexin I activity as a membrane crossbridging agent possible, in a Ca^{2+}-regulated manner.

S100A8, S100A9, and S100A12, which is an S100A9 isoform that lacks the first four amino acids and is the product of an alternative translation of a single mRNA species [41,205–210], translocate to plasma membranes and vimentin IFs in monocytes and granulocytes upon elevation of cytosolic Ca^{2+} concentration [207,209] (Table 1). Also, S100A8 and S100A9 translocate to keratin IFs upon elevation of the free Ca^{2+} concentration in epithelial cells [211]. Phosphorylation of S100A9 and S100A12 increases the extent of this translocation as well as the Ca^{2+}-binding affinity of these proteins, and translocation of S100A8 to membranes and cytoskeleton depends on prior complexation with S100A9 [209,212]. PKC-independent and PKC-dependent phosphorylation of S100A8 was reported [212]. Particularly, PKC-dependent phosphorylation of S100A8 is suggested to play a modulatory role in the translocation of this protein to membranes during neutrophil activation [213]. Phosphorylated S100A8 and S100A9 display increased affinity for Ca^{2+}. Translocation of S100A8/S100A9 to plasma membranes and cytoskeleton might be put in relation to the roles of plasma membranes and the cytoskeleton in migration, chemotaxis, degranulation, phagocytosis and respiratory burst, all of which are characteristic of activated granulocytes and monocytes. Accordingly, complex formation between S100A8 and S100A9, which appears essential for these proteins to take part in the general process of granulocyte and monocyte activation, occurs in a subpopulation of activated macrophages during e.g. allograft

rejection, despite individual proteins are being expressed in all monocytes under these conditions [214]. Thus, occurrence or non-occurrence of S100A8/S100A9 complex appears to reflect distinct functional stages of macrophages with special reference to the mode S100A8/S100A9 transduces the Ca^{2+} signal during the various steps of the inflammatory response (also see Section 3.2.4). S100A8 and S100A9 are also expressed in resident, activated microglial cells in brain inflammatory processes and Alzheimer's disease [215], as well as in ischemic brain lesions [216]. In the latter case, expression of these proteins in microglial cells is restricted to the first three days post infarction, pointing to a specific role of S100A8 and S100A9 during the early phase of tissue reaction. While it is suggested that the S100A8/S100A9 complex modulates Ca^{2+}-dependent interactions between IFs and membranes [207,209], there is no information as to whether or not S100A8 and/or S100A9 affect IF dynamics and IF interactions with membranes and/or other cytoskeleton components. Also, the functional implications of these interactions are still to be elucidated. Recently, activation of PKC in monocytes has been shown to result in translocation of the S100A8/S100A9 complex to MTs [61]. See below for implications of secreted S100A8/S100A9 as well as the S100A8-like protein, CP-10, in the inflammatory response.

S100A1/B binds to the actin-associated proteins, caldesmon and calponin [110,124,125,217–219] (Table 1). Binding of S100A1/B to caldesmon reverses the caldesmon inhibitory effect on actomyosin ATPase activity [110,124,217]. Interaction occurs with a stretch of residues (residues 669–737) located in the C-terminal domain of caldesmon [217], which is suggested to contain a sequence around W-692 similar to the consensus sequence found in CapZα from which the S100A1- and S100B-inhibitory peptide, TRTK-12 was derived [71,72]. This observation further supports the possibility that S100A1/B recognizes and regulates target proteins exhibiting the consensus sequence (K/R)(L/I)*X*W*XX*IL. The list of these target proteins includes CapZα, GFAP, desmin (and, likely, vimentin), p53, neuromodulin, neurogranin, and caldesmon, thus far (see Section 2.2 and Table 1). Similarly, S100B binding to the N-terminal 22-kDa fragment of calponin relieves the calponin-dependent inhibition of actomyosin ATPase activity [217].

A number of S100 proteins interact with tropomyosin and muscle and non-muscle myosin. S100A4 interacts with muscle and non-muscle myosin heavy chain [220–225] (Table 1). S100A4 binds to non-muscle tropomyosin in NIH 3T3 cells and is found associated with this cytoskeleton protein in MF bundles [222]. Binding of S100A4 to MFs and colocalization of S100A4 with MFs in other cell types were also reported [222,225,226]. Binding of S100A4 was reported to occur to the region in tropomyosin containing residues 39–107 [222] (Table 1). It is suggested that binding of S100A4 might confer Ca^{2+} sensitivity to tropomyosin regulation of actomyosin ATPase activity [222]. Recently, S100A4 has been reported to inhibit PKC-dependent phosphorylation of myosin heavy chain at S-1917, a residue that is found in a C-terminal stretch of myosin residues (residues 1909–1937) to which S100A4 binds [130]. It is suggested that S100A4 might play a role in the dynamics of myosin filaments (which in turn depends on the phosphorylation state of this cytoskeleton molecule) and, hence, in metastatic invasiveness [130] (see Section 5). S100A6 binds to muscle tropomyosin in vitro [56], but no functional correlates of this interactions have been reported. Instead, as with S100A1/B, binding of S100A6 to caldesmon reverses the inhibitory effect of caldesmon on actomyosin ATPase activity [227]. It is not known whether S100A6 associates with caldesmon in vivo or S100A6 and S100A1/B share the binding site on caldesmon. S100A2 binds to tropomyosin with a 1:1 stoichiometry in an epithelial cell line and undergoes redistribution upon differentiation of these cells [103] (Table 1). In particular, S100A2 appears to translocate from the cytoplasm to microvilli, where it colocalizes with MFs, in cells induced to differentiate from a fibroblast phenotype to an epithelial phenotype. However, S100A2 does not colocalize with tropomyosin in these cells. It is suggested that S100A2 might be important to prevent association of tropomyosin to MFs in microvilli (which in fact lack tropomyosin) and, thus, to play a role in the organization of the actin cytoskeleton via its interaction with tropomyosin. In support of this possibility is the finding that in vivo association of S100A2 with tro-

pomyosin is transient and depends on elevation of cytosolic Ca^{2+} [103].

Thus far, three observations suggest that S100B and/or S100A1 proteins could play a role in the regulation of the state of assembly of MFs. (i) C6 glioma cells in which the expression of S100B had been selectively inhibited by antisense oligonucleotide techniques, were observed to display a more organized MF network [228], suggesting an inhibitory effect of S100B on MF formation. However, there is no evidence presented of association of S100B with G-actin or F-actin or of effects of S100B on actin assembly. Since $S100B^-$ C6 cells divide at reduced rate as compared to the $S100B^+$ counterpart, it is possible that the more organized MF network found in $S100B^-$ cells results from a less undifferentiated state of the latter cells [228]. (ii) Recently, the α-subunit of the actin capping protein, CapZ (a 35-kDa protein that associates with CapZβ, a 32-kDa protein, to form a dimer that caps F-actin and acts as a nucleation center for MFs [229–232]), was identified as a target of S100B and S100A1 [71,72] (Table 1). The identification of CapZα as an S100A1/B-binding protein was obtained through the previous isolation by Ca^{2+}-dependent affinity chromatography on S100B-Sepharose of a subfamily of peptides displaying sequence identity to a consensus sequence contained in the C-terminal half of CapZα [71]. In addition to direct binding of S100A1/B to CapZα (K_d in the nanomolar range in the presence of submillimolar Ca^{2+} concentrations), a synthetic peptide, TRTK-12, obtained from the consensus sequence found in CapZα, was shown to bind to S100A1 and S100B with a stoichiometry of 1 mol of peptide/mol of S100A1 or B monomer and to inhibit the interaction of S100A1 and S100B with CapZα in a dose-dependent manner and stoichiometrically [71,72]. These observations suggest that CapZα might be regarded as a physiological target of S100A1 and S100B and, hence, that S100A1 and S100B might be involved in the regulation of the state of assembly of MFs by interacting with CapZα. At present, however, experimental data on functional implications of the S100A1/B-CapZα interactions are lacking and no information is available about colocalization of S100A1/B with CapZ. The above observations also suggest that the synthetic peptide, TRTK-12, might represent a powerful tool for analyzing several aspects of the functional implications of S100A1 and S100B. Also, TRTK-12 might be used in studies aimed at identifying the structure of the site(s) on S100A1/B that mediate the interaction of these proteins with their target proteins. As mentioned above, the C-terminal extension of S100A1 was implicated in the recognition of TRTK-12 [70,79] and use of TRTK-12, which inhibits the interaction of S100A1 and S100B protein with GFAP and desmin and blocks the ability of S100A1 and S100B to inhibit GFAP and desmin assemblies into IFs in vitro [85], permitted the identification of the putative S100A1- and S100B-binding site on GFAP and desmin [85]. (iii) S100A1 was reported to bind Ca^{2+}-dependently to F-actin in vitro and to translocate to stress fibres upon elevation of cytosolic Ca^{2+} concentration in cultured smooth muscle cells [226]. The functional correlates of this interaction are not known at present.

All the above observations strongly suggest that one general role of S100 proteins is to regulate cell morphology, the dynamics of certain cytoskeleton constituents, and the reciprocal relationships of cytoskeleton elements via their direct and/or indirect interactions with MTs, IFs, MFs, myosin, and/or tropomyosin. The dimeric structure of S100 proteins renders these proteins potential candidates to cross-bridge individual cytoskeleton components to each other and/or to membranes, and/or to anchor some enzymes (aldolase, glyceraldehyde-3-phosphate dehydrogenase, and phosphoglucomutase), shown to be S100 target proteins [82,133,135], to the cytoskeleton. See Section 5 for possible implications of S100A4 interaction with MFs, myosin and tropomyosin in cancer.

3.2. Extracellular activities of S100 proteins

3.2.1. Neurotrophic effects of the S100B disulfide crosslinked dimer

S100B is secreted by glial [57,58] and pituitary folliculostellate [233] cells, and adipocytes treated with stimulators of lipolysis [234,235]. Secretion of S100B by glial cells has attracted much attention in view of possible extracellular effects of this protein in the nervous system. Indeed, a protein factor with ability to promote neurite extension was identified as a disulfide crosslinked form of the S100B dimer [50], and

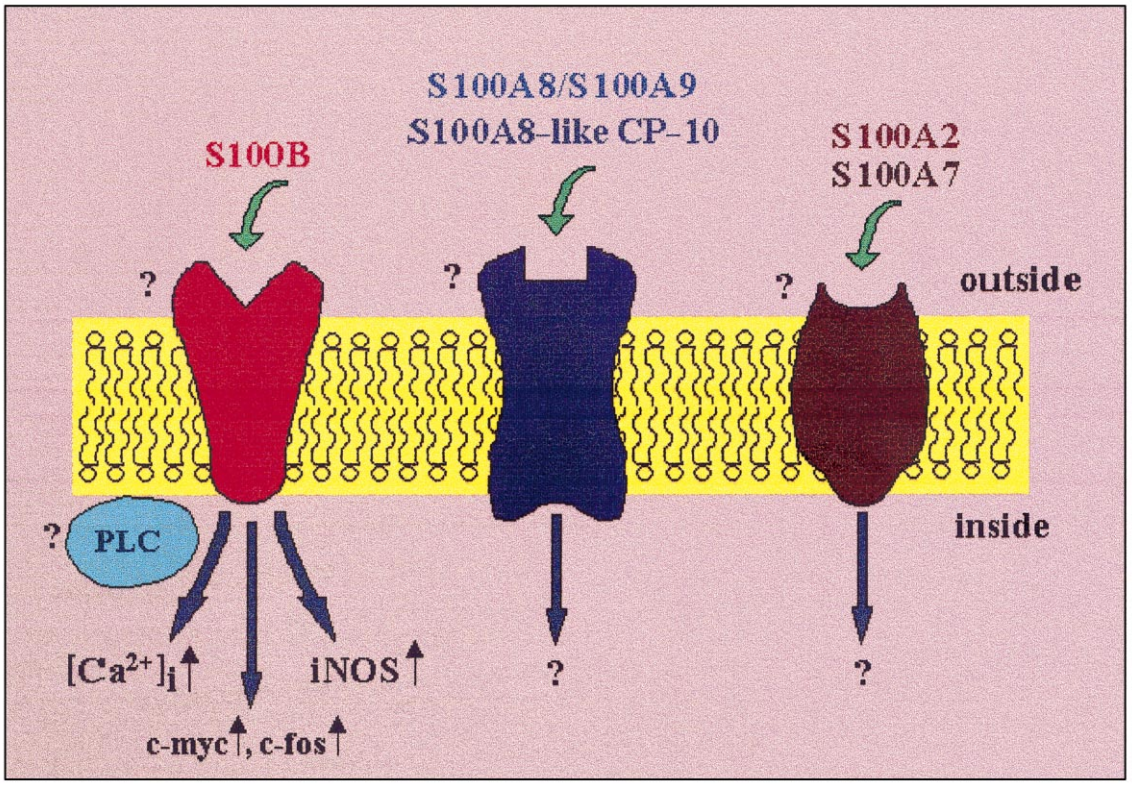


Fig. 3. Schematic representation of putative receptors for S100B, S100A8/S100A9, the S100A8-like CP-10 protein, S100A2, and S100A7. Interaction of S100B with a putative receptor on responsive cells would cause a release of Ca^{2+} from internal stores likely via a phospholipase C (PLC)-dependent mechanism as well as up-regulation of proto-oncogenes [249], and NO production via inducible nitric oxide synthase (iNOS) [270]. The signalling pathways activated by the S100A8/S100A9, the S100A8-like CP-10 protein, S100A2, and S100A7 interaction with responsive cells are not known. Question points (?) denote absence of information about the existence and/or identity of receptors for any released S100 protein shown to affect activities in target cells, and about the signalling pathway(s) activated within responsive cells following S100 protein interaction. Grouping several S100 proteins acting on target cells from outside is not to mean that released S100 proteins share a common plasma membrane receptor.

S100B was shown to exert this effect on specific neuronal populations [51,236–241] and to enhance survival of neurons during development [237–239] and after injury [241] (Table 1). Also, S100B antisense mRNA blocks the serotoninergic nerve sprouting in the central nervous system induced by S100B [242]. Moreover, S100B prevents motor neuron degeneration in newborn rats after sciatic nerve section [243] and local administration of S100B stimulates regeneration of injured rat sciatic nerve [244], in vivo. All these observations point to a physiological role of secreted S100B as a neurotrophic factor, also in consideration that this S100B activity occurs at nanomolar concentration of the protein. In addition to a paracrine effect of (glial) S100B on neurons, which, as anticipated, could be important during both development and nerve regeneration, S100B appears to exert an autocrine effect on astrocytes, since the protein stimulates glial proliferation in vitro with concomitant expression of growth-associated proto-oncogenes [245] (Fig. 3). However, little is known about the mechanisms through which S100B is secreted and exerts its effects on responsive cells. S100B lacks a signal peptide sequence and is suspected to be released from glial cells via a mechanism similar to that whereby other factors (e.g. fibroblast growth factors, ciliary neurotrophic factor, interleukins-1α and 1β, and human endothelial growth factor) are secreted or released [52]. The formation of the disulfide crosslinked S100B dimer is accelerated by the presence of high Ca^{2+} and lipids [52]. It is known that S100B interacts with lipids in the presence of Ca^{2+} and changes the structural properties of lipid bilayers [171]. Thus, disulfide crosslinked S100B

dimers could form as a consequence of S100B-Ca^{2+}-lipid interactions during the release process [52]. The S100B/A1 heterodimer also interacts with Ca^{2+} and lipids [30], but is devoid of neurotrophic activity as are other S100 family members. What seems to make the difference is the presence of two cysteine residues, C-68 and C-84, which are specific to S100B and are essential for both the formation of S100B disulfide crosslinked dimers and S100B neurotrophic effects [51]. An important issue that remains to be elucidated is represented by the molecular mechanism underlying the S100B neurotrophic effect and stimulatory effect of glial proliferation. Thus far, no evidence has been presented for the existence of an S100B receptor on S100B target cells. However, circumstantial evidence suggests that S100B interacts with specific binding sites of protein nature on synaptic plasma membranes [246–248]. Binding of S100B to receptive cells results in an increase in the intracellular free Ca^{2+} concentration from internal Ca^{2+} stores via activation of phospholipase C and the ensuing formation of IP_3 [249] (Fig. 3). While these observations suggest that the (yet undiscovered) S100B receptor is linked to a definite intracellular signal pathway, it is not known how elevation of cytosolic Ca^{2+} transduces the S100B binding into trophic effects on responsive neurons and stimulation of glia proliferation. Although, as anticipated, adipocytes also release S100B [234,235], there is no information about the functional meaning of this release. Also, it is not known whether other S100B-expressing cells release the protein.

3.2.2. S100B in neurodegenerative disorders

While nanomolar concentrations of brain extracellular S100B may be beneficial as outlined above, micromolar levels of the protein may have deleterious effects. For instance, high levels of S100B have been detected in brains from patients with Down's syndrome and with Alzheimer's disease [250], the levels of S100B are elevated in the activated astrocytes associated with amyloid-containing plaques in Alzheimer's disease [251–253], and a progressive association of both microglia overexpressing interleukin-1α and astrocytes overexpressing S100B with $\tau 2^{+}$ neurofibrillary tangle stages has been documented [254]. Also, a selective up-regulation of S100B expression has been detected in human temporal lobe epilepsy [255] and increased expression of S100B and its mRNA in rat and human brain occurs with age [256,257]. These observations, together with the findings that the human gene coding for S100B is located on chromosome 21q22.3 [258] with consequent overdosage of S100B in Down's syndrome, and that β-amyloid stimulates the synthesis of both S100B mRNA and S100B protein in C6 glioma cells and primary astrocyte cultures [259], suggest that S100B has a role in the pathogenesis of brain disorders associated with Alzheimer's disease and Down's syndrome. Probably, participation of S100B in the neuropathological changes typical of Down's syndrome is a late event, since no significant differences in the accumulation of S100B mRNA and protein can be detected during infancy in Down's syndrome and control subjects [260]. Also, whereas chronic elevation of S100B does not appear to be sufficient to induce formation of β-amyloid plaques in the mouse brain [261], β-amyloid precursor protein and its mRNA were reported to increase in neuronal cultures exposed to S100B [262]. On this basis, secreted S100B is currently viewed as a cytokine which, like interleukin-1 [263,264], promotes neuronal survival al low concentrations but is neurotoxic at high levels. As a cytokine, S100B would take part not only in the pathophysiology of neurodegenerative disorders typical of Alzheimer's disease and Down's syndrome, but also in brain inflammatory diseases eventually ending in Alzheimer-like disorders (see [265,266] for reviews) (Table 1).

S100B would produce its neurotoxic effects in vitro by inducing apoptosis of both neuronal [267–269] and glial cells [270,271] (Table 1). In one study, exposure of PC12 neuronal cells to a mixture of brain S100B/A1 caused a sustained elevation of cytosolic Ca^{2+} with concomitant appearance of apoptotic bodies and DNA fragmentation typical of apoptosis [267]. Elevation of cytosolic Ca^{2+} was shown to depend on a transmembrane capacitative flux followed by depletion of internal Ca^{2+} stores [267]. This Ca^{2+} increase could be blocked by nifedipine, a specific L-type Ca^{2+} channel blocker, and completely abolished by pretreatment of cells with thapsigargin, an inhibitor of endoplasmic reticulum Ca^{2+}-ATPase [267]. Also, nerve growth factor prevented the S100B/A1 toxic effects on PC12 cells [268]. Toxic effects of S100B/A1 on neuronal cells were ascribed to binding

of S100B/A1 to high affinity sites on neuronal plasma membranes [267]. In one other study, S100B was shown to induce astrocyte apoptotic death by induction of the mRNA of inducible nitric oxide synthase (iNOS) and elevation of this enzyme activity [270] (Table 1 and Fig. 3). While S100B does not induce the expression of iNOS in neurons, induction and activation of this enzyme in astrocytes by exogenous S100B would be responsible for the S100B toxic effects on neurons (and astrocytes) via diffusion of NO [269]. The ability of S100B to interact with the tumor suppressor protein p53 and to block its thermal-dependent oligomerization [88], and the recent observation that S100B cooperates with p53 to cause cell growth arrest and apoptosis [271] have been suggested to indicate a role of S100B in the regulation of cell growth. However, by the same mechanism overexpression of S100B and altered Ca^{2+} homeostasis might contribute to neurodegenerative disorders [271]. Recent observations suggest that micromolar concentrations of (extracellular) S100B activate cultured microglia cells (C. Adami, G. Sorci, E. Blasi, F. Bistoni, and R. Donato, manuscript in preparation). On the other hand and interestingly, S100B has been recently shown to protect cells from oxidative damage by sequestering copper ions [42]. Together, these data would suggest that as long as S100B is being kept within cells at physiological levels of expression, the protein would exert a protective effect, whereas once secreted its local concentration would dictate its beneficial or detrimental effects. Also recently, oxidized monomeric S100B (i.e. intrachain disulfide crosslinked S100B monomer), that forms on exposure of the protein to peroxynitrite anion ($OONO^-$), was shown to act as a potent astrocyte mitogenic factor at subnanomolar concentrations, its effect being much larger than that exerted by the disulfide crosslinked $S100B_2$ dimer [272]. Whether or not intrachain disulfide crosslinked S100B monomer can form in vivo is not known yet. S100B exists as a homodimer even at very low concentrations [68], and oxidation of monomeric and dimeric S100B was shown not to occur under reducing conditions [272], suggesting that within cells intrachain and interchain disulfide crosslinking of S100B requires a remarkable alteration of the redox status of C-68 and C-84 residues. While the non-reducing conditions found extracellularly might favor the formation of intrachain and interchain disulfide crosslinked S100B species, such an event has been questioned, based on structural studies [77]. Yet, since administration of S100B to cultured astrocytes, neurons, and microglia causes definite (trophic, mitogenic, toxic, and regulatory) effects [51,236–240,243–245,267–270,272], and unpublished observations), and since storage of S100B in the absence of reducing agents results in the formation of disulfide crosslinked S100B dimers [52], future analyses should elucidate the mechanism whereby intrachain and/or interchain disulfide crosslinked S100B is being generated in vivo. As mentioned above, interaction of S100B with phospholipid bilayers (e.g. during the process of S100B release to the extracellular space) might alter the S100B conformation thereby determining disulfide crosslinking in the protein [30,52]. Interestingly, oxidized S100B is an excellent substrate of casein kinase II in vitro, the protein being phosphorylated at S-62, i.e. within its C-terminal Ca^{2+}-binding loop, whereas S100A1 is not phosphorylated [272]. Phosphorylation of S100B requires prior oxidation, $OONO^-$ being the most potent oxidizing agent among those tested [272]. While phosphorylated S100B maintains its ability to stimulate glial cell proliferation in an in vitro assay, attempts to detected phosphorylated S100B in a glial cell line proved unsuccessful [272]. Thus, it is at present uncertain whether or not phosphorylation of S100B does occur in vivo or is functionally relevant.

3.2.3. S100B and cognitive behavior

S100B has been implicated in the modulation of learning and memory (Table 1). Injection of an anti-S100 protein antiserum (likely an anti-S100B antiserum) into the rat brain was shown to cause memory deficits for tasks involving transfer of handedness [273], maze learning [274], and alimentary conditioned reflex [275], and injection into the hippocampus inhibited long-term potentiation [276,277]. Also, injection of monoclonal antibodies to S100A1 and S100B into chick brain was shown to interfere a few minutes after injection with the sequence of events leading to the consolidation of passive avoidance memory in a temporally distinct manner [278]. According to these studies, extracellular S100B would play a role in memory processes, but the mechanism whereby S100B acts is far from being

elucidated. The fact that S100A1 also is implicated in learning is difficult to explain since there in no evidence presented that S100A1 is released by glial or neuronal cells. On the other hand, transgenic mice overexpressing S100B show behavioral abnormalities. S100B transgenic mice display decreased T-maze spontaneous alternation rate [279] and novelty-induced female-specific hyperactivity [280]. Also, overexpression of S100B impairs, but does not abolish, the ability to solve a spatial task [281], and interferes with the spatial and temporal exploratory pattern [282,283]. These observations were put in relation to the above described changes in S100B levels in Alzheimer's disease and Down's syndrome brains, but, again, the molecular mechanism(s) underlying these abnormalities is(are) far from being elucidated. Brains from S100B transgenic mice show a higher density of dendrites in the hippocampus at the earliest stages of postnatal development, compared to controls, and a significant loss of dendrites by one year of age suggestive of the presence of neurofibrillary tangles [284]. Again, S100B seems to be beneficial at physiological concentrations, and detrimental at higher concentrations, assuming that overexpression of S100B is accompanied by an increased release and accumulation of this protein in the brain extracellular space, for which there is no conclusive evidence presented thus far. Neuromodulin, an S100B target protein (Table 1), has an important role in neuronal development and plasticity (reviewed in [285]). Inhibition of neuromodulin phosphorylation by S100B [90–92] might result in regulation of the suggested activity of this protein in neuronal development and plasticity, as PKC-dependent phosphorylation of neuromodulin is linked with both nerve-terminal sprouting and long-term potentiation [285]. However, at present there is no information on cellular and subcellular colocalization of neuromodulin and S100B nor is there any evidence that overexpression of S100B is accompanied by a blockade of the suggested role of neuromodulin in nerve-terminal sprouting.

3.2.4. S100 proteins and inflammation

A murine 10-kDa chemotactic protein, CP-10, displaying 59% amino acid sequence identity to human S100A8, exhibits potent chemotactic activity for polymorphonuclear leukocytes and monocytes both in vitro and in vivo (reviewed in [286]; also see [287,288]). Although the cDNA sequence of murine CP-10 is identical to that of murine S100A8 [289] and both proteins have been associated with inflammation, several differences were reported concerning the biological activity of the two proteins [286]. Thus, CP-10 is being referred to as the S100A8-like CP-10 protein in the present review. This protein is constitutively expressed in neutrophils, is not expressed by resting or activated lymphocytes, by fibroblasts or by unstimulated endothelial cells, but is expressed in macrophages and endothelial cells activated by interleukin-1 and lipopolysaccharide (LPS) [287–291]. It is suggested that the S100A8-like CP-10 protein may contribute to amplification of leukocyte recruitment [287] (Table 1). This implies that the protein is being released by the cells that express it. In fact, LPS-primed and challenged macrophage RAW cells secrete the S100A8-like CP-10 protein [40], and a synthetic peptide derived from the hinge region of the protein acts as a chemotactic agent [93]. The S100A8-like CP-10 protein shows many of the characteristics of pure chemoattractants, including stimulation of actin polymerization in and shape change of target cells without degranulation or oxidative burst, similar to transforming growth factor-β1 [286,292]. As with all other S100 members shown to be released into the extracellular space, the mechanism whereby the S100A8-like CP-10 protein is secreted is largely unknown (also see below).

Other S100 members have been reported to display chemotactic activity, i.e. S100A2, which was shown to be active on eosinophils at subnanomolar concentrations and to interact with high affinity with specific binding sites on the surface of these cells [59], and S100A7, which is chemotactic for $CD4^+$ lymphocytes [60] (Table 1).

In addition to chemotactic activities, some S100 members display cytostatic or antimicrobial activities. Thus, extracellular S100A8/S100A9 have been reported to inhibit immature, mononmyelocytic HL-60 cell proliferation and mitogen-stimulated lymphocyte proliferation [293,294], and to have cytostatic activity towards bacteria/fungi [295]. Recent evidence suggests that administration of S100B to a cytotoxic lymphocyte cell line results in a dose-dependent decrease in proliferation rate and inhibition of interferon-γ secretion [296]. Also, S100A9 inhibits macro-

phage activation, likely in cooperation with other factors such as interleukin-10 and transforming growth factor-β [297], and inhibits immunoglobulin synthesis by lymphocytes in vitro [298]. Thus, S100A8/S100A9, that appear to play a role in monocyte/macrophage maturation [152–154], once secreted actually display anti-inflammatory activities, as opposite to the pro-inflammatory activity of the S100A8-like CP-10 protein. Release of monocyte S100A8/S100A9 is inhibited by interleukin-10 and interleukin 4, but not by interleukin-3 [299]. Yet, it has been recently reported that local skin inoculation with the S100A8/S100A9 complex in the presence of Ca^{2+} caused extensive eosinophil accumulation [300], and sustained increases in S100A8 and S100A9 mRNA expression were observed in infiltrating neutrophils in the early stage of the delayed type hypersensitivity skin response (1 day) followed by a decrease in neutrophil S100A8 and S100A9 content by 2–3 days [301]. These data were interpreted as indicative of a pro-inflammatory role of the two S100 proteins [300,301]. Thus, further information is required to precisely assess the biological role of secreted S100A8 and S100A9.

Together, these observations suggest that, following secretion or release into the extracellular space, some S100 members might have a regulatory (either activatory or inhibitory) role in the inflammatory response. It is not known at present how these S100 proteins interact with target cells. As to the signal transduction pathways that are being activated in response to S100 interaction with target cells, the few available data indicate that extracellular S100B causes a sustained increase in cytosolic Ca^{2+} via activation of phospholipase C [249], stimulate the expression of proto-oncogenes [245], and induce iNOS in astrocytes [270]. No information is available at present about the signalling pathways activated by the interaction of S100A8/S100A9, the S100A8-like CP-10 protein, and S100A7 with responsive cells (Fig. 3).

S100A8 and S100A9 have been recently reported to be secreted by monocytes after activation of PKC via a MT-dependent pathway, i.e. a mechanism different from the classical endoplasmic reticulum/Golgi route and the alternative secretory pathway of the interleukin-1β-type [61]. However, the relationship between MT network integrity and S100A8/S100A9 secretion remains to be elucidated.

Binding of the S100A8/S100A9 heterocomplex, but not individual proteins, to unsaturated fatty acids has been put in relation to the regulatory role of this protein complex in inflammation [302,303]. Since S100A8 and S100A9 are up-regulated in keratinocytes induced to differentiate by extracellular Ca^{2+} and in psoriatic keratinocytes [304–308], complexation of the S100A8/S100A9 heterodimer with unsaturated fatty acids could represent a way to modulate the pro-inflammatory action of arachidonic acid or to target the heterodimer to membranes during the S100A8/S100A9 secretion process [302,303].

Recently, S100A13 has been found associated with an aggregate made of fibroblast growth factor-1 and synaptotagmin-1 and suggested to play a role in the regulation of the release of this aggregate [309] (Table 1). Since fibroblast growth factor-1 has been implicated in inflammation, S100A13 was postulated to be a functional component of the fibroblast growth factor-1 release pathway [309]. However, it is not known whether S100A13 is also released into the extracellular space and/or S100A13 serves to target the fibroblast growth factor-1-synaptotagmin aggregate to plasma membranes during the release process.

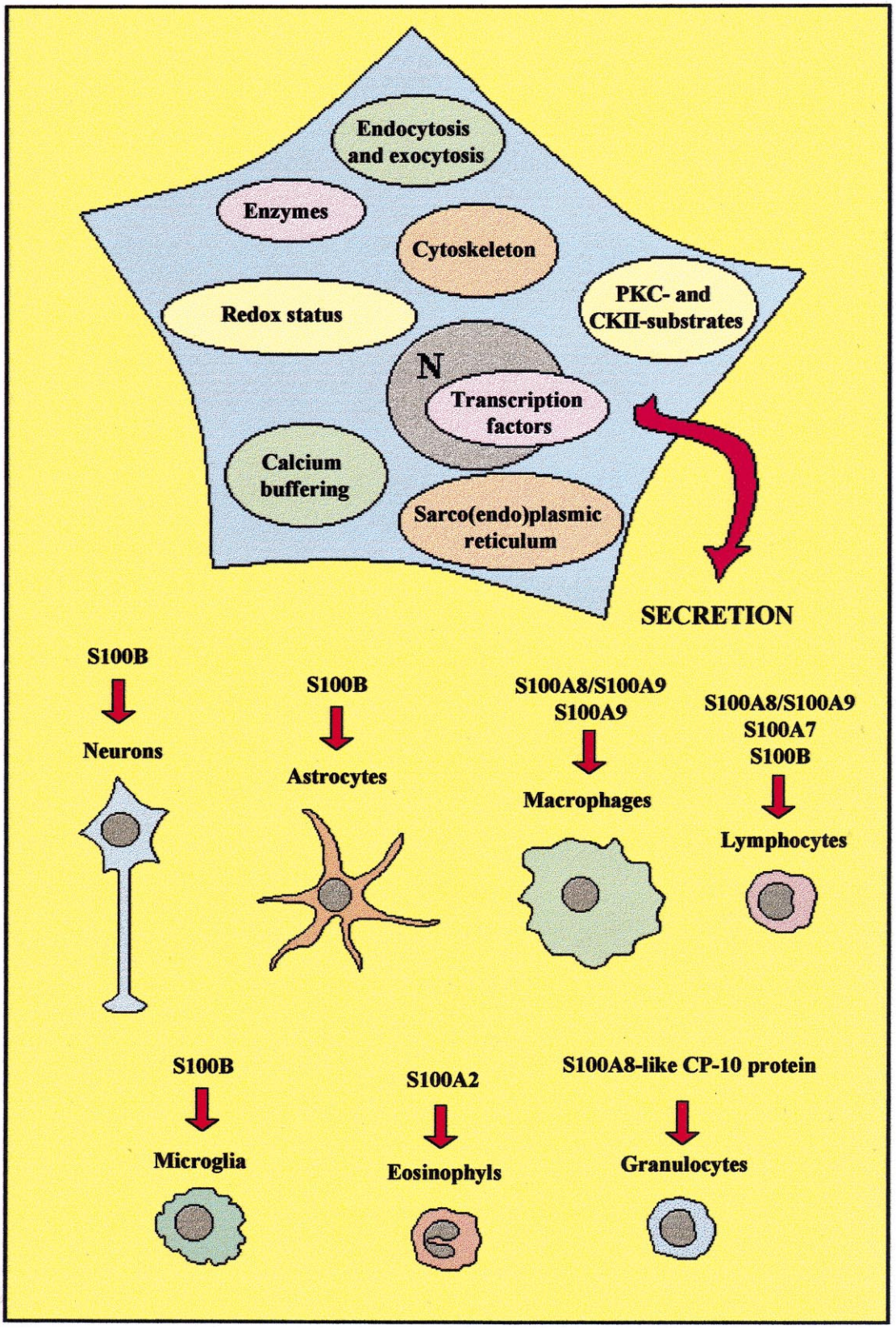
3.2.5. S100A10 and plasminogen activation

S100A10 has been reported to bind to plasminogen via lysine residues (K-95 and K-96) in its C-terminal extension and to stimulate tissue-type plasminogen activator (*t*-PA)-dependent plasminogen activation either alone or as a complex with annexin II [310] (Table 1). Consistently, a synthetic peptide derived from S100A10 C-terminal extension blocks the stimulation of *t*-PA-dependent plasminogen activation [310]. Also, S100A10 was shown to be essential for the protection of plasmin and t-PA from inactivation by α_2-antiplasmin and plasminogen activator inhibitor type 1, respectively [310]. Thus, S100A10 might play a role in the regulation of the extrinsic pathway of blood coagulation. It was suggested that S100A10 might be located on endothelial cell surface, but the mechanism whereby S100A10 (and annexin II) is being translocated outside the cell is still elusive [310]. Since the S100A10 C-termi-

nal extension is critical for complexation with annexin II [31–33,36,69] and its last two lysine residues are essential for S100A10 to interact with plasminogen [310], then S100A10 should be complexed with annexin II in such a way as K-95 and K-96 are available for interaction with plasminogen.

4. Regulation of expression of S100 proteins

S100B is synthesized during the G_1 phase of the cell cycle in glioma and melanoma cells [311,312] with accumulation of the protein in the G_0 phase and cell differentiation [313,314]. Synthesis of S100B might thus be important for progression of glioma and melanoma cells through the cell cycle. Accordingly, inhibition of S100B synthesis results in a decreased rate of glial cell proliferation [228]. The recent observation that S100B stimulates the activity of Ndr [142], a kinase shown to be important for cell division, also appears in line with the possibility that S100B may have a role in the progression of certain cell types through the cell cycle. Any putative role of S100B in cell-cycle progression likely is developmentally regulated since the protein accumulates during differentiation in cell cultures [313,314]. Also, an S100 family member, probably S100B, accumulates at a very fast rate in the mouse nervous system during the second and third postnatal week [315], and in rat, rabbit and guinea pig cerebrum during the third and fourth postnatal week [316–318], in coincidence with the maximal increment in the number of synapses [319]. Decreased expression of both S100B protein [320] and S100B mRNA [321] in crushed or transected rat sciatic nerve with restoration of their normal levels following nerve regeneration has been documented. De-differentiation and proliferation of Schwann cells in injured nerves were proposed as the cause of the above changes [320,321]. Thus, S100B might be important in both cell proliferation and cell maturation by acting on different target proteins. These observations also suggest that the S100B gene expression is under complex transcriptional regulation. Previous and recent work has shown that the rat, mouse and human S100B gene is regulated by both positive and negative sequences located upstream in the 5′ flanking DNA regions [322–324]. The human S100B promoter is contained within the −167/+697 region of the gene and its expression is variable among a number of cell lines, but is not restricted to a definite cell type or species [321]. Unlike the positive regulatory elements of the rat and mouse S100B gene that were reported to be specific to glial cells [322,323], the positive regulatory elements of the human gene appear cell line-specific [324]. Also, while a previous study concluded that the negative regulatory elements of the mouse S100B gene function only in non-glial cells [322], the human S100B gene expression may be constitutively repressed in all cell types by negative regulatory elements, implying that the expression of S100B in a given cell type requires induction of an appropriate factor that counters the action of that element [324]. Such a mechanism would allow a controlled expression of S100B in definite cell types or in the same cell type depending on the cell's functional states. The recent observations that S100B is being expressed in cardiomyocytes post-infarction [131] and in replicating myoblasts and immature myotubes [175], whereas it is substantially absent in mature striated muscle cells [138,139], appear to support the above view. Also, the presence of both positive and negative regulatory elements upstream of the human S100B promoter would provide a means to achieve an optimal S100B expression level in a given cell [324]. Several factors have been shown to regulate the S100B expression. (i) Increases in the intracellular levels of cAMP resulting in an increased synthesis of S100B in rat C6 glioma cells [325–328], and cAMP have been reported to control the expression of the S100B gene in parallel with the expression of the GFAP gene in the subpopulation of RT4 neuroglial cell line capable of acquiring a glial-like phenotype, but not in the subpopulation capable of acquiring a neuronal-like phenotype [328]. A putative cAMP response element has been found in the human S100B gene [329]. (ii) Dexamethasone causes a biphasic response in the levels of S100B mRNA in cultured rat hippocampal astrocytes, i.e. an increase during the first 24 h of treatment which is followed by a significant decrease thereafter, with no changes in the levels of intracellular S100B during the first 24 h and a significant decrease thereafter [330]. (iii) Basic fibroblast growth factor-2 also causes a decrease in the S100B mRNA levels in cultured rat cerebral cortex astrocytes after a short-term treatment which is fol-


Endocytosis and exocytosis
Enzymes
Cytoskeleton
Redox status
PKC- and CKII-substrates
N
Transcription factors
Calcium buffering
Sarco(endo)plasmic reticulum
SECRETION
S100B
Neurons
S100B
Astrocytes
S100A8/S100A9
S100A9
Macrophages
S100A8/S100A9
S100A7
S100B
Lymphocytes
S100B
Microglia
S100A2
Eosinophyls
S100A8-like CP-10 protein
Granulocytes

lowed by a remarkable increase after a long-term treatment likely due to changes in the rate of gene transcription [331]. These changes are accompanied by only a slight, if any decrease in the S100B protein content of astrocytes during the first 3 days of treatment, which is followed by a several-fold increase thereafter [331]. (iv) In contrast, in the same cells (cortical astrocytes) interleukin-1β causes suppression of S100B mRNA after both short- and long-term treatment with no significant decrease in the S100B protein levels [331], and treatment with both, basic fibroblast growth factor-2 and interleukin-1β, results in a synergistic effect [331]. Thus, whereas basic fibroblast growth factor-2 appears to regulate the expression of S100B, no definite role can be ascribed to interleukin-1β as far as S100B expression is concerned. (v) β-Amyloid stimulates the synthesis of both S100B mRNA and S100B protein in C6 glioma cells and primary astrocyte cultures [259]. See above for implications of increased S100B expression in neurodegenerative disorders.

S100A6 was originally identified in fibroblasts induced to progress from the G_0 to the G_1 phase of the cell cycle and thus suggested to be involved in cell-cycle progression [332]. However, as in the case of S100B, the increase in S100A6 mRNA during retinoic acid-induced cell differentiation (but not in cells induced to stop proliferating by serum starvation), suggests that the S100A6 gene also is under complex transcriptional regulation and that the S100A6 gene has a bifunctional role, being expressed in both proliferating and differentiating cells [333]. The increased expression of S100A6 in proliferating cells depends on the binding of the heat-shock element and SV40 enhancer to the promoter region of the S100A6 gene [334], whereas the increased expression of the protein in retinoic acid-treated, differentiating cells depends on increased binding of the transcription factor AP-1 to the −161/−135 promoter region of the S100A6 gene [335].

The murine S100A1 gene also is under complex transcriptional control [336]. A positive regulatory element was found in the S100A1 promoter region in a skeletal muscle cell line between −1600/−1081. In a glial cell line and in a neuronal cell line these same sequences were negative and neutral, respectively, suggesting that this region has a major role in targeting S100A1 expression to specific cell types. As with S100B [228], inhibition of S100A1 synthesis results in a decreased rate of proliferation of PC12 cells [178]. Interestingly, although less active that S100B, S100A1 also stimulate the Ndr kinase activity [142].

Up-regulation of the S100A4 and S100A10 mRNAs in proliferating Schwann cells in crushed or transected rat sciatic nerve with their subsequent down-regulation after nerve regeneration has been reported [321]. These observations suggest that S100A4 and S100A10 have a (yet unknown) role in de-differentiated, proliferating Schwann cells. The genes of these two proteins have been shown to be induced by nerve growth factor in cultured neurons [337].

5. S100 proteins in tumor cells

S100A4, S100A6, S100A7 and S100B are up-regulated in several human tumor cells ([22,23,335,338, 339], and references therein). S100A4 has attracted much attention in this regard. S100A4 was originally identified as a protein induced when cultured rat mammary epithelial cells change to an elongated myoepithelial-like cell [340]. However, elevation of S1004 or its mRNA can be observed in serum-induced increase in growth rate of cultured murine fibroblasts [341], oncogene- or carcinogen-induced transformation of murine fibroblasts [342], or rat kidney cells [343]. A positive correlation was found between the metastatic potential of murine mammary adenocarcinoma cell lines and the level of expression of S100A4 mRNA [344,345] and B16 melanoma cells [346]. Also, expression of S100A4 in MMTV-*neu* transgenic mice induces metastasis of mammary tu-

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Fig. 4. Summary of intracellular and extracellular functional roles of S100 proteins. Within cells S100 proteins may participate in the regulation of a large variety of cell activities, as indicated. Members of the S100 family that have been shown to be released into the extracellular space, albeit with mechanisms that remain to be elucidated, would exert effects on a number of cell types, thereby regulating their activities as detailed in the text (also see Fig. 3).

mors [347] and expression of antisense RNA to S100A4 gene in high-metastatic A11 cells suppresses cell motility and in vitro invasiveness [348]. These findings strongly suggest that S100A4 has an important role in the acquisition of metastatic phenotype, although the molecular mechanism whereby S100A4 operates is largely unknown [349]. Interaction of S100A4 with non-muscle tropomyosin and myosin [130,222,223] and F-actin [224–226] and structural changes in anchoring junctions [345] have been proposed as possible mechanism by which S100A4 increases the invasiveness of tumor cells. The recent observation that S100A4 inhibits myosin heavy chain phosphorylation by PKC [130] and previous studies showing that PKC-dependent phosphorylation of myosin heavy chain results in inhibition of myosin heavy chain assembly [350,351] may indicate that S100A4 is implicated in the regulation of cell motility and, hence, invasiveness of metastatic cells via interaction with myosin heavy chain. Demethylation of a CD3δ enhancer in the first intron of the murine S100A4 gene has been proposed as a possible mechanism of increased expression of this protein in metastatic cells [352].

S100A1 and S100B, which are known to be overexpressed in human cancers [22], have been suggested to have a role in the activation of the Ndr kinase activity, and a positive correlation has been found between hyperactivation of Ndr and overexpression of S100B in melanomas [142].

By contrast, S100A2 in markedly down-regulated in breast tumor biopsies and can be re-expressed in mammary carcinoma cells by azadeoxycytidine treatment [353]. These findings suggest that S100A2 might have a tumor suppressor function [354], a suggestion supported by the observation that the proximal promoter region of the S100A2 gene is unmethylated in normal but hypermethylated in tumorigenic cells [355]. Overall, these data indicate that, although the genes coding for S100A1 to S100A13 are tightly clustered on the human chromosomal region 1q21 [356,357], individual S100 genes in this chromosomal region are differentially regulated.

6. Conclusions

S100 proteins have been implicated in the regulation of several intracellular activities, e.g. protein phosphorylation, enzymes involved in energy metabolism, the cAMP and cGMP signalling pathways, the inflammatory response, the cycle of contraction-relaxation, Ca^{2+} homeostasis, the dynamics of cytoskeleton constituents, and cell proliferation (including neoplastic transformation) and differentiation (summarized in Fig. 4). No knock-out animal model has been obtained thus far for any S100 member. Thus, it is difficult at present to draw conclusions as to whether or not any putative function attributed to an S100 protein in vitro also occurs in vivo. However, in vitro effects of S100 proteins on protein phosphorylation, some enzyme activities, Ca^{2+} homeostasis, activities of certain transcriptional factors and cytoskeleton dynamics likely reflect in vivo regulatory functions of some S100 members. Although in a limited number of cases some S100 members share certain target proteins, in most cases target protein specificity has been documented, suggesting that individual S100 members display specific regulatory properties. Accordingly, a high degree of cellular specificity has been observed in terms of protein expression. With a few exceptions, S100 proteins likely exist as homodimers (and heterodimers, in some cases) within cells. Structural data indicate that in dimeric S100 proteins, the two monomers are related by a two-fold axis of rotation, thus exhibiting a three-dimensional structure not found in other Ca^{2+}-binding proteins. These data and other experimental evidence suggest that, upon Ca^{2+} binding, S100 dimers crossbridge two homologous or heterologous target proteins, as in an S100 dimer at least two binding sites are located on opposite sides. The available evidence suggests that several regions contribute to the formation of functional (either Ca^{2+}-dependent or Ca^{2+}-independent) binding sites on monomers of an S100 dimer, which could explain the ability of a given S100 protein to interact with distinct and structurally unrelated target proteins. Also, the relative concentrations of an S100 protein and its target protein(s) at a specific cell site, as well as the affinity of their binding, would be critical to the regulatory effects of S100 proteins. Some S100 members are released or secreted and act extracellularly. Released S100 proteins appear to exert trophic or toxic effects on, e.g. neurons and astrocytes, depending on their concentration, act as chemoattrac-

tants towards leukocyte recruitment, modulate the proliferation of certain target cells, or regulate the activation state of, e.g. macrophages. Thus, some S100 proteins might act from outside the cell to participate in tissue organization during development, the inflammatory response, and/or tissue remodelling in specific pathological states. However, the mechanism of S100 protein secretion/release, the factors that regulate S100 protein secretion/release, and the molecular mechanisms whereby released S100 proteins interact with and the signalling pathways they activate within their target cells are largely unknown at present. While further analyses should ascertain the physiological relevance of the in vitro functions of S100 proteins thus far discovered and the mechanisms whereby individual S100 members operate, S100 proteins appear to play important regulatory roles both within and outside cells.

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