S100 Proteins in the Epidermis

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The S100 proteins comprise a family of 21 low molecular weight (9–13 kDa) proteins that are characterized by the presence of two calcium-binding EF-hand motifs. Fourteen S100 protein genes are located within the epidermal differentiation complex on human chromosome 1q21 and 13 S100 proteins (S100A2, S100A3, S100A4, S100A6, S100A7, S100A8, S100A9, S100A10, S100A11, S100A12, S100A15, S100B, and S100P) are expressed in normal and/or diseased epidermis. S100 proteins exist in cells as anti-parallel hetero- and homodimers and upon calcium binding interact with target proteins to regulate cell function. S100 proteins are of interest as mediators of calcium-associated signal transduction and undergo changes in subcellular distribution in response to extracellular stimuli. They also function as chemotactic agents and may play a role in the pathogenesis of epidermal disease, as selected S100 proteins are markedly overexpressed in psoriasis, wound healing, skin cancer, inflammation, cellular stress, and other epidermal states.

Key words: calcium/epidermis/keratinocyte differentiation/psoriasin/S100/S100A2/S100A7/S100A8/S100A11/S100A15/wound healing
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S100 Proteins

The S100 proteins comprise a multigene family of low molecular weight proteins that engage in multiple functions in a wide variety of cell types and tissues (Heizmann *et al*, 2002; Donato, 2003). Many members of this family of gene are encoded in the epidermal differentiation complex (EDC) located on chromosome 1q21 (Volz *et al*, 1993; Hardas *et al*, 1996; Wicki *et al*, 1996). This region is of particular interest, since it encodes many genes (involucrin, filaggrin, trichoyalin, repetin, etc.) that are expressed in epidermal keratinocytes (Mischke *et al*, 1996; South *et al*, 1999). Thus, the finding that many S100 genes are clustered within the EDC has heightened interest in their role in the epidermis. Among the 21 S100 proteins that have been cloned to date, 11, including S100A2, S100A3, S100A4, S100A6, S100A7, S100A8, S100A9, S100A10, S100A11, S100A12, and S100A15, are expressed in the human epidermis or in cultured keratinocytes (Boni *et al*, 1997; Xia *et al*, 1997; Broome *et al*, 2003; Wolf *et al*, 2003). In addition, S100B is expressed in Langerhans cells and melanocytes (Boni *et al*, 1997; Shrestha *et al*, 1998; Park and Min, 2003) and S100P is expressed in Meissner's corpuscles (Del Valle *et al*, 1994) (Table I).

It is thought that S100 proteins serve as calcium sensor proteins that, upon activation, regulate the function and/or subcellular distribution of specific target proteins (Donato, 1999). S100 proteins are characterized by common structural motifs including two EF hands (helix-loop-helix calcium-binding domains) that are separated by a hinge region and flanked by amino- and carboxy-terminal domains (Fig 1*A*). The canonical C-terminal EF-hand binds calcium with a 100-fold higher affinity than the N-terminal, non-conventional, EF-hand (Gribenko and Makhatadze, 1998; Zimmer *et al*, 2003). The carboxy-terminal domain is variable among S100 proteins and is thought to provide the site responsible for the selective interaction of each individual S100 protein with specific target proteins (Kube *et al*, 1992; Seemann *et al*, 1996). Figure 1*B* shows the amino acid sequence of the S100 proteins known to be expressed in the epidermis. This alignment reveals the EF-hands, the hinge region, the α-helical domains, and the N- and C-terminal extensions.

S100 proteins exist in cells as anti-parallel homo- and heterodimers in which the monomers are held together by non-covalent interactions and are oriented by a 2-fold axis of rotation (Brodersen *et al*, 1998; Sastry *et al*, 1998; Ishikawa *et al*, 2000; Moroz *et al*, 2000) (Fig 1*C*). Helices I and IV, which flank the calcium-binding EF-hands, come together to form the dimer interface (Drohat *et al*, 1997, 1998). S100B, for example, exists as a non-covalent dimer in the presence or absence of calcium, suggesting that calcium is not required for dimer formation (Drohat *et al*, 1997). Thus, S100 proteins are likely to exist in cells as preassembled dimers (Zimmer *et al*, 2003). Upon calcium binding, the helices rearrange and a cleft forms in each monomer. The residues present in this cleft create a target protein recognition site (Brodersen *et al*, 1998; Sastry *et al*, 1998; Rety *et al*, 1999; Moroz *et al*, 2000; Rety *et al*, 2000; Donato, 2001; McClintock and Shaw, 2003). Because of the anti-parallel structure of the S100 dimer, target protein

Abbreviations: E-FABP, epidermal fatty acid binding protein; RAGE, receptor for advanced glycation endproducts

Table I. S100 proteins in epidermis

S100 name	Common names	Chromosome	Normal epidermal expression	Expression in skin disease	References[a]
S100A2	S100L, CaN19	1q21	Basal keratinocytes, eccrine duct, epithelial cells of sebaceous gland, hair follicle, cultured keratinocytes	Pigmented actinic keratosis, malignant melanoma, squamous cell carcinoma	Boni *et al* (1997), Xia *et al* (1997), Deshpande *et al* (2000), Zhang *et al* (2002), Ribe and McNutt (2003a)
S100A3	S100E	1q21	Endocuticle of hair	Cancer, hair damage and regeneration	Boni *et al* (1997), Kizawa *et al* (1998), Takizawa *et al* (1999), Kizawa *et al* (2002)
S100A4	CAPL, CaN19	1q21	Stem cell region of pelage follicle	Hair damage and regeneration	Shrestha *et al* (1998), Ito and Kizawa (2001), Ito *et al* (2002)
S100A6	Calcyclin, CACY, 2A9, PRA, CaBP, 5B10	1q21	Hair follicle-anagen sac, keratinocytes, Langherhan's cells, melanocytes, sweat glands	Melanoma, Spitz nevi	Boni *et al* (1997), Ito and Kizawa (2001), Ribe and McNutt (2003b)
S100A7	Psoriasin, PSOR1, BDA11, CAAF2	1q21	Minimal expression	Basal and suprabasal keratinocytes in psoriasis, wound healing, cancer	Hagens *et al* (1999b), Semprini *et al* (2002), Broome *et al* (2003)
S100A8	Calgranulin A, MRP8, CAGA, CGAg, p8, MAC387, 60B8Ag, L1Ag, CP-10, MIF, NIF	1q21	Minimal expression in epidermis, UVA induced in epidermis	Basal and suprabasal keratinocytes in psoriasis, wound healing, stress, hair follicle, Spitz nevi	Thorey *et al* (2001), Gebhardt and Breitenbach (2002), Semprini *et al* (2002), Broome *et al* (2003), Grimbaldeston *et al* (2003), Ribe and McNutt (2003b)
S100A9	Calgranulin B, MRP14, CAGB, CFAg, p14, MAC387, 60B8Ag, L1Ag, MIF, NIF	1q21	Minimal expression in epidermis,	Wounding, hair follicle, psoriasis, cancer	Gribenko and Makhatadze (1998), Schmidt *et al* (2001), Thorey *et al* (2001), Gebhardt and Breitenbach (2002), Semprini *et al* (2002), Broome *et al* (2003)
S100A10	Calpactin light chain, p11, CAL12, CLP11, p10, 42C	1q21	Basal and suprabasal keratinocytes, corneocyte precursor		Robinson *et al* (1997), Ruse *et al* (2001), Bierie *et al* (2003), Broome *et al* (2003), Ruse *et al* (2003)
S100A11	Calgizzarin	1q21	Basal and suprabasal keratinocytes, Langerhan's cells, corneocyte precursor		Robinson *et al* (1997), Ruse *et al* (2001), Broome *et al* (2003), Broome and Eckert (2004)
S100A12	Calgranulin C, p6, CAAF1, CGRP, corned-associated antigen	1q21	Basal keratinocytes, suprabasal keratinocytes, Langerhan's cells	Lesional psoriasis, melanoma, Spitz nevi	Mirmohammadsadegh *et al* (2000), Ribe and McNutt (2003b), Semprini *et al* (2002)
S100A15	–	1q21	Basal keratinocytes	Psoriasis	Wolf *et al* (2003)
S100B	S100β, NEF	21q22	Langerhan's cells, melanocytes	Chondroid syringoma, melanoma	Park and Min (2003), Ribe and McNutt (2003b)
S100P	–	4p16	Meissner's corpuscles		Del Valle *et al* (1994)
Profilaggrin	–	1q21	Suprabasal keratinocytes	Erythrokeratoderma	Dale *et al* (1985), Ishida-Yamamoto *et al* (1999)
Trichohyalin	–	1q21	Suprabasal keratinocytes, hair follicle	Alopecia areata	Presland and Dale (2000), Tobin *et al* (2003)
Repetin	–	1q21	Suprabasal keratinocytes		Krieg *et al* (1997)

[a]References refer to epidermal expression and skin disease columns.

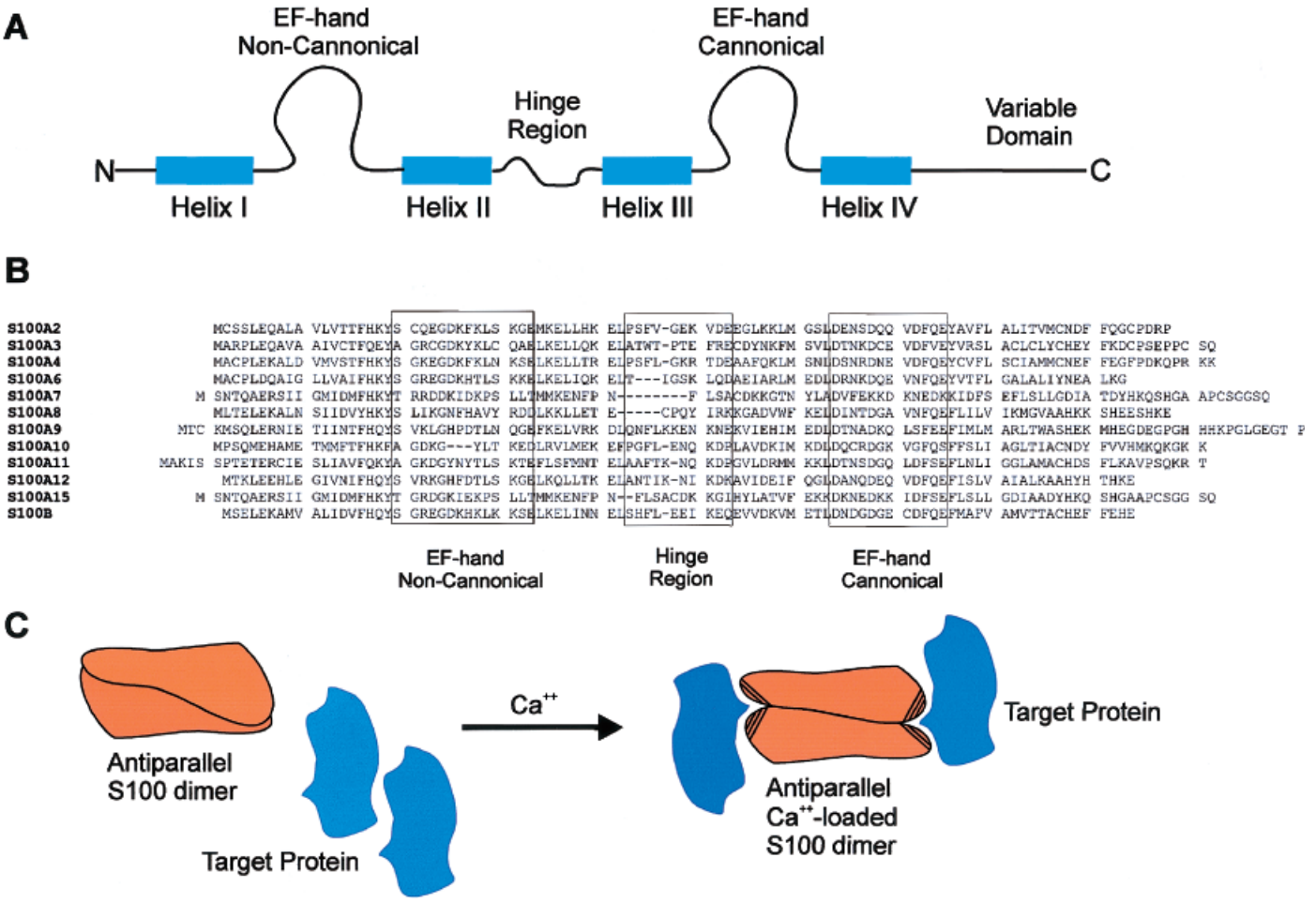

Figure 1
S100 protein structure. (*A*) The overall structure of each S100 protein family member includes four α-helical segments, two calcium-binding EF-hands (one non-canonical site binds calcium with low affinity, and one canonical), a central hinge region of variable length, and the C- and N-terminal variable domains. (*B*) The amino acid sequences of the human S100 proteins expressed in epidermis are aligned with the EF-hands and central hinge region indicated. All sequences are human and the accession numbers are S100A2, NM_005978; S100A3, BC012893; S100A4, NM_019554; S100A6, BC001431; S100A7, BC034687; S100A8, BC005928; S100A9, BC047681; S100A10, XM_001468; S100A11, X80201; S100A12, NM_005621; S100A15, AY189119; S100B NM_006272. The ClustalW sequence comparison program, http://decypher.stanford.edu/index_by_algo.shtml, was used to align the protein sequences. The alignment was then further adjusted as described by Zimmer *et al* (2003). (*C*) Model of S100 protein/target protein interaction. S100 proteins exist as anti-parallel dimers. An increase in calcium concentration results in a conformation change in the dimer that results in exposure of a cleft, which forms the target protein binding site (cross-hatched). Once in the calcium-loaded state, each S100 protein dimer can interact with a target protein via its C-terminal domain. Thus, a single S100 protein dimer can ligate two target proteins.

monomers can bind on opposite ends of the S100 protein dimer. Thus, the S100 protein dimer is, in effect, a cross-bridge between the two target proteins (Fig 1*C*), and the ultimate complex is a heterotetramer composed of two S100 protein subunits and two target protein subunits. A calcium-dependent S100 protein conformation change is required for interaction with target proteins, a topic that is covered in depth in two recent reviews (Nelson and Chazin, 1998; Zimmer *et al*, 2003). There is presently, however, no direct evidence that calcium causes a change in S100 protein conformation in the intracellular environment. In fact, it can be argued that the affinity of the EF-hands for calcium (1–100 μM) (Baudier *et al*, 1986; Kligman and Hilt, 1988; Gribenko and Makhatadze, 1998; Rustandi *et al*, 1998) is below the level required for calcium sensitivity in the intracellular keratinocyte environment where calcium levels are approximately 100 nM (Hennings *et al*, 1989). Calcium-binding affinity for S100 proteins, however, is influenced by a variety of ions and other proteins (Rustandi *et al*, 1998; Zimmer *et al*, 2003). For example, S100B binding to a peptide fragment derived from p53 increases the affinity of calcium binding to S100B by 3-fold (Rustandi *et al*, 1998). Thus, predicting the intracellular affinity for calcium is difficult and it is widely believed, based on functional evidence, that these proteins are calcium responsive in cells. In a few cases, S100 proteins are calcium insensitive. For example, S100A10, due to mutations in the EF-hands, is perpetually in an "open conformational state" and, therefore, does not require calcium for activation (Gerke and Weber, 1985; Rety *et al*, 1999).

The main focus of many studies in keratinocytes has been localizing these proteins in normal and diseased epidermis. This information is summarized in Table I. Compared with other cell types (Heizmann and Cox, 1998; Donato, 1999), information regarding S100 protein function in keratinocytes is limited. Recent studies, however,

suggest potential roles for S100 proteins in epidermal wound repair, cancer, differentiation, and response to stress. The following paragraphs focus on the function of individual S100 proteins in the epidermis.

S100A2 Is an Oxidative Stress-Regulated Protein

S100A2 (S100L) is localized to the basal layer of normal human epidermis and hair follicles (Boni *et al*, 1997; Shrestha *et al*, 1998). The most common form of S100A2 is overexpressed in psoriasis, although some polymorphic S100A2 forms are not (Stoll *et al*, 2001). S100A2 has a strong tendency to form homodimers as measured by interaction in a yeast two-hybrid screen and by immunoprecipitation (Deshpande *et al*, 2000). Moreover, when exposed to oxidizing conditions, the homodimers become linked via disulfide bonds (Deshpande *et al*, 2000). In cultured normal human keratinocytes, S100A2 is found mainly in the nucleus (Zhang *et al*, 2002). H_2O_2 treatment of normal keratinocytes causes a relocation of S100A2 from the nucleus to the cytoplasm. This translocation is also observed when cells are exposed to an ionophore-dependent increase in intracellular calcium, and both the H_2O_2- and ionophore-dependent translocation is inhibited by treatment with reducing agent. S100A2 translocation occurs within 1 h after treatment with H_2O_2 and cell death follows within 24 h. Thus, S100A2 translocation is an early marker of oxidative stress-related keratinocyte cell death. The inhibition of translocation from nucleus to cytoplasm by reducing agent treatment suggests that disulfide-linked dimer formation is required for movement (Deshpande *et al*, 2000). S100A2 expression is markedly increased in ErbB-driven epidermal hyperplasia, and decreased in the absence of functional p53 in carcinoma cell lines and tumors (Xia *et al*, 1997); however, the biological importance of this S100 protein concentration change is not known.

S100A7 Is Overexpressed in Disease

S100A7, also called psoriasin, is distributed in the cytoplasm of keratinocytes in normal human epidermis and is present at the cell periphery in terminally differentiated keratinocytes (Broome *et al*, 2003). The peripheral distribution observed in differentiated cells may be important, since, under some conditions, S100A7 may be released from keratinocytes. Several S100 proteins are thought to be secreted (Hitomi *et al*, 1996; Katz and Taichman, 1999; Karimi-Busheri *et al*, 2002). Indeed, S100A7 has been shown to function as a chemotactic agent and as a cytokine (Hoffmann *et al*, 1994; Jinquan *et al*, 1996), and to attract CD4+ lymphocytes and neutrophils (Jinquan *et al*, 1996). Originally characterized as a marker of psoriasis, S100A7 overexpression is seen in many epidermal inflammatory diseases, including atopic dermatitis, mycosis fungoides, Darier's disease, and inflammatory lichen sclerosus and atrophicus (Madsen *et al*, 1991; Algermissen *et al*, 1996; Broome *et al*, 2003). The high level of expression in active psoriatic lesions has prompted investigators to suggest that S100A7 may have a chemotactic role in psoriasis (Jinquan *et al*, 1996). S100A7 expression is also increased in invasive skin cancers such as squamous cell carcinoma, and squamous carcinoma *in situ*, but not in basal cell carcinoma (Alowami *et al*, 2003).

S100A7 Expression Is Regulated by Various Agents

Topical treatment with all-trans retinoic acid results in a rapid increase in S100A7 level in the human epidermis, as does retinoid treatment of cultured keratinocytes (Tavakkol *et al*, 1994; Zouboulis *et al*, 1996). Calcium also increases keratinocyte S100A7 level (Hoffmann *et al*, 1994). In addition, recent studies indicate that UV light increases S100A7 expression in the human epidermis coincident with increases in adhesion protein (LFA1/ICAM-1) expression (Di Nuzzo *et al*, 2000). This increase in LFA-1/ICAM-1 and S100A7 level is associated with increased epidermal accumulation of CD4+ T cells in response to UV treatment (Di Nuzzo *et al*, 2000). Taken together, these studies suggest that S100A7 levels increase in response to inflammatory stress and that the S100A7 protein may function as a keratinocyte-derived chemotactic agent for immune cells (Fig 2*A*). Indeed, this may also be the role of S1007 in psoriasis.

S100A7 Interacts with Epidermal-Fatty Acid Binding Protein

As previously mentioned, S100 proteins are thought to regulate cell function by interacting with target proteins in a calcium-dependent manner. Thus, substantial effort has been directed to identify these target proteins in numerous systems. But less information is available in keratinocytes. Early studies identified epidermal fatty acid binding protein (E-FABP) as a candidate target of S100A7. Celis and colleagues first identified and cloned E-FABP as an overexpressed protein in the epidermis (Madsen *et al*, 1992). E-FABP is one member of a multigene family of proteins that are required for fatty acid solubility, influx across the plasma membrane, intracellular transport and storage, and metabolism (Hertzel and Bernlohr, 2000). Siegenthaler and colleagues confirmed that E-FABP level is elevated in psoriasis and showed that immunoprecipitation of psoriatic scale extracts with anti-E-FABP results in co-immunoprecipitation of S100A7 (Hagens *et al*, 1999b). Additional studies showed that nitrocellulose-immobilized E-FABP can bind S100A7 (Hagens *et al*, 1999a).

A recent study that examines S100A7 and E-FABP distribution and interaction in cultured normal human keratinocytes (Ruse *et al*, 2003) confirms and extends the observations of Siegenthaler *et al* (Hagens *et al*, 1999a, b). In this study, S100A7 and E-FABP were overexpressed in keratinocytes to produce a disease-like intracellular level of S100A7. Three lines of evidence suggest a possible functional interaction between S100A7 and E-FABP. First, overexpression of S100A7 results in increased levels of E-FABP and vice versa, suggesting post-translational stabili-

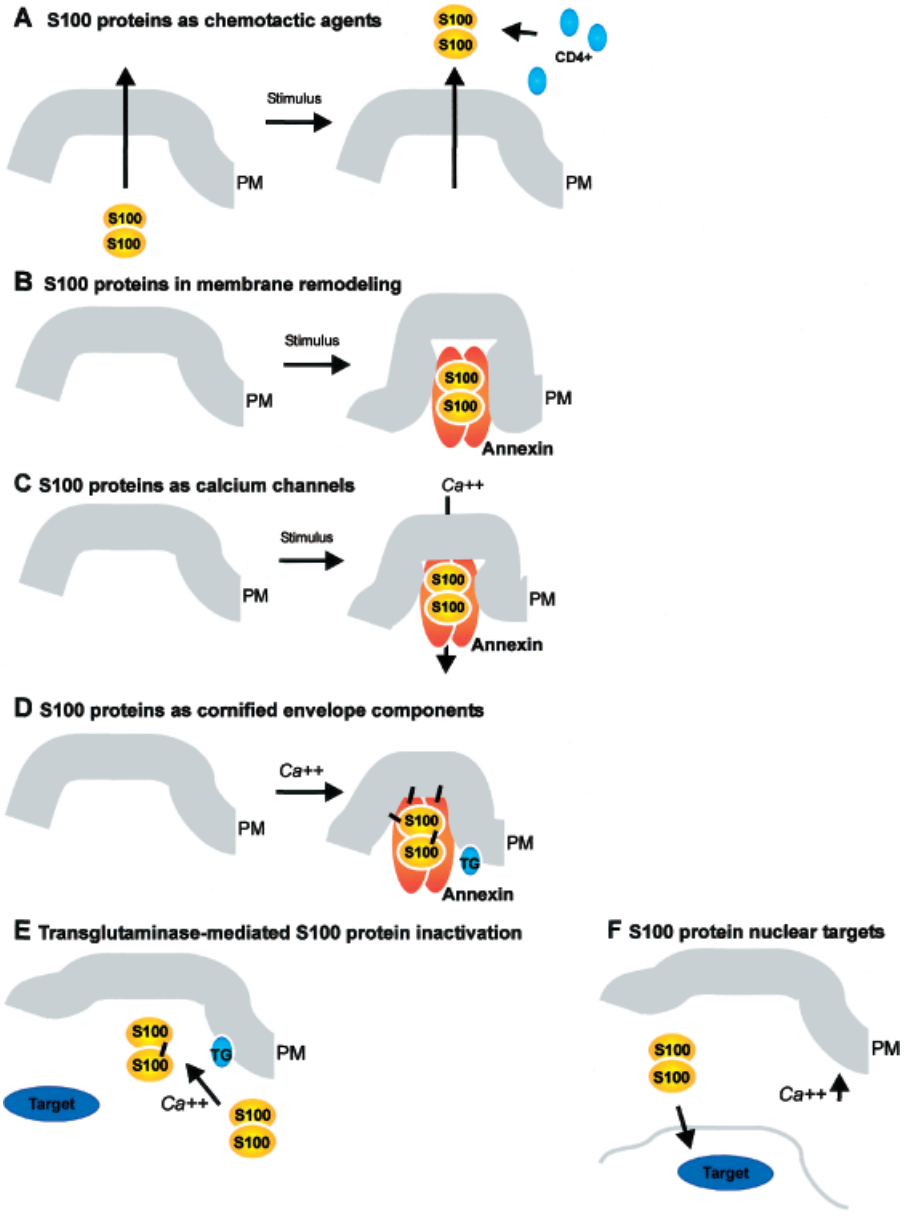

Figure 2
Possible S100 protein functions in keratinocytes. The plasma membrane (PM), S100 protein (S100), type I transglutaminase (TG), CD4 + lymphocytes, annexin, and S100 target protein (target) are indicated. The black lines in panel (*D*) and (*E*) represent covalent transglutaminase-mediated covalent inter-protein cross-links. All S100 dimers are anti-parallel and S100 and annexin are regarded as fully calcium-loaded following application of stimulus (see text for details).

zation (Ruse *et al*, 2003). Second, S100A7 and E-FABP co-immunoprecipitate—this suggests that they are part of a complex. In addition, individual E-FABP and S100A7 monomers are released after immunoprecipitation, indicating that the individual subunits of the complex are not modified as part of the interaction (Ruse *et al*, 2003). Third, S100A7 and E-FABP co-localize in resting cells in a diffuse cytoplasmic pattern. There are not overt changes in cell morphology in the presence of overexpressed S100A7 and/or E-FABP.

A potentially important finding of this study is that calcium/ionophore treatment causes movement of S100A7 and E-FABP to cell-associated peripheral structures that protrude from the cell surface to contact the substrate. Formation of these structures does not require S100A7 or E-FABP; however, both proteins localize in these structures following calcium/ionophore treatment. These structures also contain the focal adhesion proteins, paxillin and α-actinin, suggesting that S100A7 and E-FABP participate in focal adhesion-related functions. These studies may be physiologically meaningful, as immunohistological studies suggest that S100A7 redistributes from the cytoplasm to a cell peripheral location during keratinocyte differentiation in the epidermis (Broome *et al*, 2003; Ruse *et al*, 2003). It should be noted that although a change in S100A7/E-FABP distribution is observed when cells are treated with calcium, not all agents that regulate keratinocyte function promote S100A7 redistribution. Like calcium, okadaic acid treatment also causes S100A7 to move to peripheral focal adhesion-like structures. In contrast, no change in distribution is observed following treatment with phorbol ester, an agent known to promote keratinocyte differentiation (Ruse *et al*, 2003). It is interesting to speculate regarding the function of the S100A7/E-FABP complex. Although no specific function has been assigned to E-FABP, both free E-FABP and the S100A7/E-FABP complex bind oleic acid, suggesting a role

in oleic acid transport and metabolism (Hagens *et al*, 1999a). Oleic acid may have a role in inflammation, as topical application modulates epidermal Langerhans cells density (Touitou *et al*, 2002). The complex could also function in lipid metabolism and transport during epidermal barrier assembly and may also modulate the inflammatory response in psoriasis and other epidermal diseases.

S100A8 and S100A9 Are Stress-Induced Proteins

S100A8 and S100A9 form homo- and heterodimers and are frequently co-expressed (Teigelkamp *et al*, 1991). In normal epidermis, S100A8 and S100A9 are expressed at very low levels, although occasional expression is observed in the granular layer. Both proteins are highly overexpressed in psoriasis and are present in the basal, granular, and spinous layers (Broome *et al*, 2003). S100A8 and S100A9 may have a role in promoting and/or responding to the hyperproliferative state in the epidermis, as S100A8 and S100A9 expression is strongly induced within the first week after epidermal injury (Thorey *et al*, 2001). The increased expression of S100A8 and S100A9 in wound-associated keratinocytes may be related to the activated state of the keratinocytes and not caused by the accompanying inflammation. This idea is based on their observation that S100A8 and S100A9 levels are increased in the epidermis of activin-overexpressing mice that display epidermal hyperproliferation without inflammation. In addition, S100A8 and S100A9 are efficiently secreted from cultured immortalized (HaCaT) keratinocytes (Thorey *et al*, 2001). S100A8 and S100A9 are also released from neutrophils via a microtubule-dependent mechanism and may enhance inflammation by influencing leukocyte trafficking (Kerkhoff *et al*, 1999; Kerkhoff *et al*, 2001). Thus, S100A8 and S100A9 release from keratinocytes may initiate immune cell invasion that is further propagated by the release of S100A8 and S100A9 from incoming neutrophils (Fig 2*A*).

A recent study showed that ultraviolet A irradiation of mouse epidermis causes a concentration-dependent increase in S100A8 level (Grimbaldeston *et al*, 2003). Ultraviolet A irradiation produces an oxidizing environment that appears to influence S100 production. For example, an ultraviolet A-dependent increase in S100A8 expression is observed in mouse keratinocyte PAM212 cells and this increase is inhibited by treatment with Tempol, a superoxide enzyme-mimicking agent, suggesting a role for oxidative stress in the S100A8 induction (Grimbaldeston *et al*, 2003). Surprisingly, the level of S100A9, the heterodimeric partner of S100A8, did not increase along with S100A8. This suggests that S100A8 may function as a homodimer in this model. It is known that S100A8 homodimers can produce different responses compared with S100A8/S100A9 heterodimers (Newton and Hogg, 1998; Kerkhoff *et al*, 1999; Eue *et al*, 2002). The observation that S100A8 levels increase without a corresponding increase in S100A9 is interesting and differs from recent transgenic S100A9 knockout mouse studies showing that elimination of S100A9 results in a coordinate loss of S100A8 (Manitz *et al*, 2003). A recent study shows that S100A8 and S100A9 levels increase in response to a wide variety of skin stresses, including tape stripping, exposure to detergent, Vaseline application and UV exposure (Marionnet *et al*, 2003). In addition, tumor promoter treatment of mouse skin increases S100A8 and S100A9 level and glucocorticoid treatment inhibits this increase (Gebhardt *et al*, 2002). These results suggest that S100A8 and S100A9 may play a role in carcinogenesis. Taken together, these studies suggest a role for S100A8 and S100A9 in regulating the epidermal response to tissue injury, inflammation and disease.

S100A10, Annexin II and the Cell Periphery

S100A10 is present in both the cytoplasm and periphery in cells of the basal and spinous layers of the human epidermis (Broome *et al*, 2003). S100A10 homodimers bind two copies of annexin II to form the calpactin I heterotetramer (Nakata *et al*, 1990; Pigault *et al*, 1990). Unlike other S100 proteins, due to a unique EF hand structure, S100A10 is constitutively activated and binds to annexin II in a calcium-independent manner (Gerke and Weber, 1985; Rety *et al*, 1999). Calcium-activated annexin II binds to membranes (Harder and Gerke, 1993; Ma and Ozers, 1996), and has also been identified within the extracellular space in keratinocytes (Ma *et al*, 1994; Karimi-Busheri *et al*, 2002). The calpactin I complex appears to function to bring structures together (Gerke and Moss, 2002). Because of the propensity of annexin II to interact with and structure membrane lipids, it is not surprising that the calpactin I complex associates with the plasma membrane in keratinocytes or that S100A10 is incorporated as a cross-linked constituent of the keratinocyte cornified envelope (Robinson *et al*, 1997). The role of the membrane-associated calpactin I complex in keratinocytes is not well understood; however, it may function in remodeling the cell membrane during keratinocyte differentiation. The S100A10 dimer binds two annexin II target proteins, as in Fig 1*C*, to form the calpactin I heterotetramer. In the presence of calcium, the annexin II subunits of the calpactin I complex interact with the plasma membrane (Ma and Ozers, 1996). This interaction may function to remodel the membrane topography (Fig 2*B*) in a wide range of biological contexts including receptor internalization and endosome formation. In addition, the ratio of S100A10 to annexin II varies during differentiation and wound healing. S100A10 is present in all epidermal layers, whereas annexin II is preferentially expressed in the basal layers (Munz *et al*, 1997). The functional importance of this changing ratio requires additional study.

Like other annexin proteins, annexin II can form a calcium channel in reconstituted systems (Gerke and Moss, 2002). Thus, in addition to its membrane structuring function, the calpactin I complex may also form a plasma membrane-localized calcium channel that enhances calcium influx leading to calcium-dependent terminal differentiation (Fig 2*C*). S100A10 is a transglutaminase substrate (Ruse *et al*, 2001) and is found covalently incorporated into the cornified envelope (Robinson *et al*, 1997). Thus, it may have a role in cornified envelope assembly during terminal keratinocyte differentiation (Fig 2*D*).

S100A11 and Directed Movement to the Cell Periphery

S100A11 (S100C, calgizarrin) is located in the cytoplasm of basal epidermal keratinocytes and at the cell periphery in spinous layer cells (Broome *et al*, 2003). It is also expressed in cultured normal human keratinocytes (Broome and Eckert, 2004). The S100A11/annexin I complex is a heterotetramer consisting of two S100A11 and two annexin I proteins. Rety *et al* solved the structure of this complex, confirming a novel mode of interaction of S100A11 with annexin I, and suggesting a model whereby the calcium-regulated (annexin I/S100A11)$_2$ heterotetramer could function to organize membrane fusion events (Rety *et al*, 2000).

Results from our laboratory indicate that S100A11 is localized in the cytoplasm in resting cells and moves to the cell periphery in cultured keratinocytes following calcium challenge. This movement requires the presence of intact microtubules (Broome and Eckert, 2004). These studies are consistent with findings regarding S100 protein movement and export in other cell types. For example, Rammes *et al* noted that S100A8 and S100A9 are secreted independently of the classical Golgi/ER pathway (Rammes *et al*, 1997). Likewise, Roth *et al* demonstrated a microtubule requirement for S100A8/S100A9 redistribution in myelomonocytic cells (Roth *et al*, 1993). S100A11 membrane association and filament interaction has been reported by other investigators (Arcuri *et al*, 2002; Bianchi *et al*, 2003). These findings suggest that the relocation of S100A11 to the cell periphery that is observed during keratinocyte differentiation *in vivo* (Broome *et al*, 2003) may be physiologically relevant, as the (S100A11/annexin I)$_2$ heterotetramer may function to regulate and/or facilitate plasma membrane remodeling during terminal differentiation (Fig 2*B*). The presence of S100A10 at the cell periphery is consistent with previous observations that S100A11 is a cross-linked component of the cornified envelope, a structure that is assembled from membrane-associated constituents (Robinson *et al*, 1997) (Fig 2*D*). Moreover, annexin I is also a covalently cross-linked cornified envelope component (Robinson *et al*, 1997; Robinson and Eckert, 1998; Rety *et al*, 2000). S100A11 has also been reported to be present in the nucleus in some cultured cells and have a nuclear function (Sakaguchi *et al*, 2000). Indeed, some cells in human epidermal sections show *nuclear* anti-S100A11 staining (Broome and Eckert, 2004). Co-localization with Langerhans cell markers, however, suggest that this nuclear staining in the epidermis is contributed by epidermal Langerhans cells (Broome and Eckert, 2004).

Another feature of S100A11/annexin I function may also be important in keratinocytes. As mentioned above, almost all annexins display calcium channel activity in *in vitro* systems (Benz *et al*, 1996; Gerke and Moss, 2002) including annexin I and annexin II (Chen *et al*, 1993; Cohen *et al*, 1995; Benz *et al*, 1996; Burger *et al*, 1996). This activity has not been demonstrated in cells under normal intracellular conditions, but such activity may be possible under oxidizing and reduced pH conditions (Gerke and Moss, 2002). Thus, annexin-dependent channel activity may be possible in keratinocytes in the oxidizing, acidic environment observed during terminal keratinocyte differentiation.

The movement of S100A11 to the cell periphery during keratinocyte differentiation *in vivo* (Broome *et al*, 2003) and *in vitro* (Broome and Eckert, 2004), along with the finding that S100A11 is a cross-linked component of the cornified envelope (Robinson *et al*, 1997), suggests a differentiation-dependent membrane-associated function. Therefore, the (S100A11/annexin I)$_2$ heterotetramer may have three possible roles during terminal keratinocyte differentiation. The first is that of remodeling plasma membrane structures during terminal differentiation. Thus interaction of annexin with the plasma membrane lipids can join disparate segments of membrane or bend the membrane surface (Fig 2*B*). This may be important, for example, when lamellar bodies fuse with the plasma membrane in terminally differentiating cells. A second role is as a differentiation-activated calcium channel that permits calcium influx in terminally differentiating keratinocytes (Fig 2*C*). A gradient of increasing free calcium is known to exist as cells move from the basal to suprabasal layers during epidermal differentiation (Menon *et al*, 1985). A mechanism may exist whereby an increase in intracellular calcium activates S100A11/annexin I interaction and movement to the keratinocyte plasma membrane. Ultimately, insertion of this complex into the plasma membrane may create a porous environment that allows calcium to freely equilibrate between the cell interior and exterior compartments.

A third role of some S100 proteins (e.g., S100A10, S100A11) pertains to assembly of the cornified envelope (Fig 2*D*). The net result of the increased intracellular calcium is the calcium-dependent activation of type I transglutaminase—the enzyme that is responsible for cornified envelope assembly (Kim *et al*, 1992; Phillips *et al*, 1993). There is evidence indicating that S100A11 and annexin I are cross-linked constituents of the cornified envelope (Robinson *et al*, 1997). Thus, upon completion of their physiological function, these proteins may serve as structural components of the skin surface. In this context, it is interesting to note that some studies suggest that S100 proteins can be anti-microbial (Nisapakultorn *et al*, 2001). Thus, the covalently anchored S100 protein within the cornified envelope may also serve a protective function.

Specific information is available regarding this cross-linking. Type I transglutaminase is a calcium-activated, plasma membrane-anchored protein that assembles the covalent isopeptide cross-links that comprise the structure of the cornified envelope (Eckert *et al*, 1997; Nemes and Steinert, 1999). S100 proteins are efficient transglutaminase substrates that are cross-linked at lysine and glutamine residues that are located in the solvent-exposed N- and C-terminal flanking regions of these proteins (Robinson and Eckert, 1998; Ruse *et al*, 2001). The selection of these specific residues for cross-linking is consistent with the structure of the S100 protein dimer (i.e., a closed globular core with exposed N- and C-termini), as cross-linking generally is observed only in transglutaminase-accessible regions of proteins.

Transglutaminase may also have an additional role in regulating S100 protein/target protein interaction (Fig 2*E*). Our studies indicate that the S100A11 forms antiparallel, covalently cross-linked heterodimers in which the C-terminus of one monomer is linked via an isopeptide bond

to the N-terminus of the second monomer (Robinson and Eckert, 1998). Since the C- and N-termini are the sites of interaction with the annexin I target protein, it is very likely that cross-links at this location would inactivate S100A11 interaction with annexin I—thereby terminating function. Thus, transglutaminase-dependent cross-linking may act to terminate S100A11 function.

S100A11 has also been shown to have an interesting nuclear function. Sakaguchi *et al* showed the calcium-dependent movement of S100A11 to the nucleus of normal human keratinocytes and HaCaT cells (Sakaguchi *et al*, 2003). This movement involves S100A11 phosphorylation and association with nucleolin, a nuclear protein that shuttles between the cytoplasm and nucleus. S100A11 interaction with nucleolin in the nucleus results in the displacement of Sp1 and Sp3 from a nucleolin-Sp1/3 complex. The increased level of non-complexed nuclear Sp1/3 transcription factors is associated with increased expression of the p21 gene. The increased expression of p21, a cyclin-dependent kinase inhibitor, results in reduced cell proliferation. Thus, as shown in Fig 2*F*, S100A11 also has nuclear functions.

S100A12 and S100A15

S100A12 (calgranulin C, EN-RAGE) is a pro-inflammatory protein that interacts with the receptor for advanced glycation endproducts (RAGE). S100A12 is markedly over-expressed in various inflammatory diseases (Yang *et al*, 2001; Rouleau *et al*, 2003; Foell *et al*, 2003a, b) including psoriasis (Mirmohammadsadegh *et al*, 2000; Semprini *et al*, 2002). In involved psoriatic epidermis, S100A12 is expressed in the suprabasal epidermal layers (Mirmohammadsadegh *et al*, 2000; Semprini *et al*, 2002). S100A12 is called EN-RAGE, as it is a RAGE receptor ligand (Hofmann *et al*, 1999). RAGE is a member of the immunoglobulin superfamily of cell surface proteins that interact with a range of ligands, including advanced glycation endproducts (AGE), amyloid fibrils, S100A12, and amphoterin. The pathobiology observed in response to RAGE activation is enhanced by accumulation of RAGE ligands at pathologic sites, leading to further upregulation of the receptor and sustained RAGE-dependent cell activation. This eventually leads to cell dysfunction (Stern *et al*, 2002). It is not yet known whether RAGE is activated in psoriasis; however, this seems likely. Since RAGE functions as a progression factor, driving cellular dysfunction and enhancing the host response to tissue destruction, activation of RAGE by S100 proteins could contribute to an exacerbation of the psoriatic phenotype.

S100A15 is a recently cloned S100 protein that is 93% identical to S100A7 with lower levels of identify to S100A11 (34%) and S100A8 (29%) (Wolf *et al*, 2003). Compared with normal epidermis, S100A15 mRNA levels are slightly increased in non-lesional and markedly increased in lesional psoriasis. Little is presently known regarding the function of S100A15, but the close identity to S100A7 may suggest a similar role.

Overall role for S100 proteins S100 proteins comprise a family of homologous proteins that regulate a wide range of cellular processes. Although precise functional roles have not been assigned, our survey suggests some conclusions regarding the role of S100 proteins in keratinocytes. A common theme among S100 family members is an involvement in inflammatory processes. It is perhaps not surprising that the level of several S100 proteins, including S100A2, S100A7, S100A8, and S100A9, and S100A15, are markedly elevated in psoriasis. In this context, although it has not been convincingly demonstrated, these proteins may be exported from the cell and function as chemokines. Although only a few studies have directly tested the hypothesis that S100 proteins are key players in epidermal inflammatory disease (Jinquan *et al*, 1996), a significant body of circumstantial evidence suggests such a role. A second common theme is that S100 proteins function in membrane remodeling. They may play a role in the remodeling that occurs during keratinocyte differentiation—especially in reorganizing the keratinocyte surface. A third possible function is suggested by the fact that some S100 protein target proteins (e.g., annexins) function in the formation of calcium channels. S100 proteins, in conjunction with annexins, may function to facilitate the transmembrane influx of calcium that occurs during terminal differentiation. A fourth important observation that may have functional consequences is that S100 proteins are efficient transglutaminase substrates. This transglutaminase-dependent covalent protein modification results in S100 protein incorporation into the cornified envelope. Transglutaminase activity may also play a role in terminating S100 protein interaction with target proteins. A fifth theme is that intracellular movement is required for function. These studies are mapping the intracellular distribution of individual S100 proteins in keratinocytes and their movement in response to exogenous agents. These studies are also particularly important, since intracellular location can profoundly influence function. In this context, S100 proteins may move to locate their target proteins or they may function to carry target proteins to new intra- and extracellular locations. Finally, S100 proteins, covalently cross-linked in the cornified envelope, may protect against bacterial infection.

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