The Novel Murine Ca\(^{2+}\)-binding Protein, Scarf, Is Differentially Expressed during Epidermal Differentiation*

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Calcium (Ca\(^{2+}\)) signaling-dependent systems, such as the epidermal differentiation process, must effectively respond to variations in Ca\(^{2+}\) concentration. Members of the Ca\(^{2+}\)-binding proteins play a central function in the transduction of Ca\(^{2+}\) signals, exerting their roles through a Ca\(^{2+}\)-dependent interaction with their target proteins, spatially and temporally. By performing a suppression subtractive hybridization screen we identified a novel mouse gene, Scarf (skin calmodulin-related factor), which has homology to calmodulin (CaM)-like Ca\(^{2+}\)-binding protein genes and is exclusively expressed in differentiating keratinocytes in the epidermis. The Scarf open reading frame encodes a 148-amino acid protein that contains four conserved EF-hand motifs (predicted to be Ca\(^{2+}\)-binding domains) and has homology to mouse CaM, human CaM-like protein, hClp, and human CaM-like skin protein, hClsp. The functionality of Scarf EF-hand domains was assayed with a radioactive Ca\(^{2+}\)-binding method. By Southern blot and computational genome sequence analysis, a highly related gene, Scarf2, was found 15 kb downstream of Scarf on mouse chromosome 13. The functional Scarf Ca\(^{2+}\)-binding domains suggest a role in the regulation of epidermal differentiation through the control of Ca\(^{2+}\)-mediated signaling.

Keratinocytes follow a complex program of differentiation from the basal layer to the spinous and granular layers of the epidermis, which ultimately leads to the formation of a water-impermeable barrier. The final phase in epidermal differentiation is characterized by granular cell death, destruction of organelles, covalent cross-linking of cornified envelope precursors by Ca\(^{2+}\)-dependent transglutaminases (TGases), and attachment of lipid molecules to the cross-linked envelopes (1). This program is accomplished through specific transcriptional induction and repression of structural and enzymatic differentiation-specific markers (2). The differentiation process can be recapitulated partially in mouse keratinocytes cultured in vitro by increasing the Ca\(^{2+}\) concentration in the culture medium (3) and is associated with the activation of protein kinase C (PKC) (4). This produces a situation that mimics the endogenous Ca\(^{2+}\) gradient present in the skin, with low extracellular levels surrounding the basal cells and increasing levels toward the upper granular layers (5). The ability to induce differentiation in primary cultured keratinocytes, along with the presence of a Ca\(^{2+}\) gradient, underscores the importance of this ion and the Ca\(^{2+}\)-dependent signaling pathways in the epidermis.

A crucial role for the transduction of the Ca\(^{2+}\) signal is accomplished by members of the Ca\(^{2+}\)-binding proteins, which are characterized by the presence of a common helix-loop-helix structural motif in their Ca\(^{2+}\)-binding domain, termed EF-hand (6). These proteins function by undergoing conformational changes upon binding of Ca\(^{2+}\), allowing the association and regulation of activity of a range of specific target proteins. EF-hand-containing proteins have been described in epidermis; these include the large proteins profilaggrin and reteplin (7, 8), and the small proteins Scarf, profilaggrin, and reteplin (7, 8), which present EF-hand motifs in the NH2-terminal region followed by multiple tandem repeats and members of the dimeric EF-hand S100 multigenic family (9–11). The best studied of the Ca\(^{2+}\)-signaling proteins is CaM, a small, highly conserved, and ubiquitous protein that is crucial to many Ca\(^{2+}\)-dependent processes in eukaryotes (6, 12). The functions of many proteins involved in cell signaling by phosphorylation/dephosphorylation or in the modulation of intracellular levels of second messengers are reportedly CaM-dependent (12). Recently, several Ca\(^{2+}\)-binding proteins with structural homology to CaM have been reported: calcium-binding proteins (CaBPs) (13, 14), hClp (15), and the human skin-specific hClsp (16, 17).

We present the cloning, genomic structure, expression, and functional assay for a novel mouse Ca\(^{2+}\)-binding protein that we have termed Scarf. The Scarf open reading frame (ORF) codes for a small protein with four EF-hand Ca\(^{2+}\)-binding domains. The Scarf gene and protein are differentially expressed in vivo in the spinous and granular layers of the epidermis. We also present the identification of a second highly homologous gene, Scarf2, which localizes 15 kb downstream from Scarf in the mouse chromosome 13. We propose that Scarf and Scarf2 are new members of the CaM-like proteins with potential roles in the Ca\(^{2+}\)-dependent epidermal differentiation process.

EXPERIMENTAL PROCEDURES

Suppression Subtractive Hybridization (SSH)—An SSH screen was performed following the instructions of the manufacturer (Clontech) using basal cell RNA as a “driver” and suprabasal cell RNA as a “tester” (18, 19). The primary mouse basal and suprabasal keratinocytes were obtained from neonatal skins that were trypsinized overnight at 4 °C and then separated by a discontinuous Percoll gradient (20). From this screen, we identified a partial cDNA sequence (231 bp), which corresponded to a partial coding region of a novel mouse gene we called Scarf.
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**Cloning and Genomic Sequences Analysis of Scarf and Scarf2**—A genomic DNA region of ~120 kb was cloned through screening of a mouse UCSC/UCB Library (Genome Systems Inc.) using an 0.9-kb Scarf cDNA as a probe. Comparison of the genomic and cDNA sequences determined the 5’- and 3’-end boundaries, the absence of intronic regions in the Scarf gene, and the identification of a Scarf-related gene that we termed Scarf2 (GenBank™ accession no. AY293058). For sequence homology searches we used a BLAST analysis (basic local alignment search tool), made available through the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov/BLAST).

**Cell Culture**—Primary mouse keratinocytes were isolated from trypsinized newborn BALB/c mouse skins and cultured as reported previously (19). Keratinocytes were cultured in medium with 0.05 mM Ca2+ to maintain a basal-like population of undifferentiated cells (3). Cells were treated with different inhibitors at 10 μM concentration: a PKC inhibitor, GF109203X; a PKA inhibitor, H89; and a CaM kinase II inhibitor, KN62. Differentiation was achieved by increasing the Ca2+ concentration in the medium to 0.12 mM.

**Northern Blots and Gene Expression**—Total RNA was isolated from basal and suprabasal keratinocytes and mouse primary keratinocytes differentiated in vitro using TRIzol (Invitrogen). The RNA samples (1–3 μg) were electrophoresed in 1% agarose-methymercuryhydroxide gels, electrobotted to nylon membranes, and hybridized according to Church and Gilbert (21). An mRNA dot blot and Northern blot for mouse tissues were purchased from Clontech and used according to the instructions of the manufacturer. The mouse-specific probes used were: the 5’- and 3’-untranslated regions for loricrin (LOR), keratin 5 (K5), and keratin 1 (K1) (kindly provided by Dr. Yuspa and Dr. Compton). For the differentiation marker involucrin (INV), the expressed sequence tag clone AA798100 was utilized. The coding region of CaM kinase was used as a probe was obtained by PCR with the following gene-specific oligonucleotides: Forward, 5’-ctccttcctctctgctcaagc-3’; and Reverse, 5’-ctccttcctctctgctcaagc-3’. The Scarf coding region was obtained by PCR using: Forward, 5’-gctccttcctctctgctcaagc-3’; and Reverse, 5’-gctccttcctctctgctcaagc-3’. A glyceraldehyde-3-phosphate dehydrogenase cDNA probe was used to control for RNA integrity (22).

For the RT-PCR reactions, cDNA was synthesized by Superscript II kit (Invitrogen) using 0.2 μg of mRNA isolated from the total RNA of suprabasal keratinocytes with an Oligotex kit (Qiagen). The oligonucleotides used were: Forward Scarf, 5’-tacagagaggaataagttgacg-3’; and Reverse Scarf, 5’-gctcagagaggaataagttgacg-3’. A negative control, an RT-PCR reaction was performed with no cDNA input. PCR reactions were 40 cycles of denaturation at 94°C for 30 s, annealing at 60°C for 30 s, and extension at 72°C for 1 min.

**Radioactive In Situ Hybridization**—RNA probes corresponding to the sense and antisense strands of mouse Scarf partial cDNA (202–447 bp) were prepared using T7 and Sp6 RNA polymerase. In situ hybridization was performed at high stringency on consecutive sagittal sections of 8-μm-thick paraffin-embedded mouse tissues. The probes were hybridized to appropriately dehydrated and rehydrated mouse tissues using the Biogenex Biostain kit (Biogenex) according to the manufacturer’s protocol and visualized with an autoradiography film technique.

**Protein Expression and Purification**—Scarf coding sequence was obtained by PCR and subcloned into the BamHI site of the pET-28c(+) vector (Novagen). The recombinant protein was expressed in BL21(DE3) and contained a His tag and a T7 tag at the NH2 terminus. The soluble recombinant protein was purified by nickel-nitrilotriacetic acid spin columns (Qiagen). The soluble recombinant protein was renatured in 50 mM Tris-HCl (pH 8.0), 10% glycerol, 100 mM NaCl, and 1 mM EDTA. The recombinant protein was then purified using a Ni-NTA agarose column (Qiagen). We analyzed the predicted protein sequence using the Swiss Institute of Experimental Cancer Research tools (www.isrec.isb-sib.ch), and MacVector version 6.5.3. Mutations in the EF-hand motifs to generate the Scarf mutants were performed using the QuikChange site-directed mutagenesis kit (Stratagene). The conserved first Asp residue of each EF-hand motif (sequence GAC) was replaced with a Gly residue (GCC sequence). Mutant recombinant proteins were purified on nickel-nitrilotriacetic acid spin columns (Qiagen). mEF1–4 represent the letters. The open triangle indicates the site of splicing (exon/intron boundary) in the Scarf2 gene. Asterisks denote the termination codons (TGA) and the putative amino acid sequences of Scarf and Scarf2. A, B, and C, comparison of the amino acid sequence (B) and percentage of homology (C) at the nucleotide and amino acid level among several CaM-like calcium-binding proteins.

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**Fig. 1. Characterization of Scarf and Scarf2.** A. Nucleotide sequences of Scarf cDNA (899 bp) and Scarf2 ORF (423 bp) were aligned with the predicted amino acid sequences. The double underlined nucleotides indicate the canonical poly(A) addition signal in Scarf. Numbers on the right indicate base pairs. Putative EF-hand motifs are in bold (113–344 bp). The upstream sequence was obtained by performing rapid amplification of cDNA ends (5’-RACE, Clontech) utilizing a gene-specific oligonucleotide (5’-tgaaacagcggccggtcgc-3’). The complete mRNA sequence was deposited in GenBank™ (accession no. AY293058).
mutated forms of Scarf in each EF-hand 1–4 motif, respectively. Anti-Scarf antibody against the full-length protein was generated in hens (Aves Laboratories).

Recombinant hC1p was obtained by subcloning a PCR fragment containing the ORF into the BamHI site of pET-28a(+) vector (Novagen). The oligonucleotides used were: Forward, 5’-gaggatcacaggeggag- gagctgact-3’, and Reverse, 5’-ggggatccatggccggt-gccggatccatggccggt-3’. PCR reactions were performed for 30 cycles of denaturation at 94 °C for 30 s, annealing at 58 °C for 30 s, and extension at 72 °C for 1 min. Purification of the recombinant hC1p was performed as mentioned above. hC1p recombinant protein was kindly provided by Dr. Strehler.

**Immunohistochemistry and Western Blot Analysis—**Immunohistochemical analysis was performed on paraffin-embedded 8-μm sections of murine neonatal skin. The deparaffinized sections were treated with a 1:2000 dilution of the anti-Scarf primary antibody overnight at 4 °C and a 1:200 dilution of anti-IgY fluorescein isothiocyanate-conjugated secondary antibody (Aves Laboratories) for 1 h at room temperature. Sections were visualized by fluorescence microscopy.

For Western blot analysis, basal and suprabasal keratinocytes were incubated separately in lysis buffer (10 mM Hepes (pH 7.5), 150 mM NaCl, 1% Triton X-100, 10 mM phenylmethylsulfonyl fluoride) and centrifuged at 14,000 rpm for 20 min. Soluble fractions were collected, and insoluble pellets were resuspended in 6M urea buffer (6 M urea, 50 mM Tris (pH 8.3), 150 mM NaCl, 20 mM EDTA, 0.05% SDS). 25 μg of total protein extract or 1 μg of recombinant CaM-like Ca2+-binding proteins were resolved by SDS-PAGE, transferred onto a nitrocellulose membrane, and probed using anti-Scarf primary antibody (1:5000) and horseradish peroxidase-conjugated goat anti-chicken IgG secondary antibody (1:2000; Aves Laboratories).

**Calcium Binding Assay—**Calcium binding was assayed by the micro method described by Kawasaki et al. (24). Briefly, 3 μg of the recombinant proteins were blotted onto a polyvinylidene difluoride membrane and washed with two changes of methanol for 5 min, after which the membrane was soaked in 10 μM CaCl2, 50 mM Tris-HCl (pH 7.5), 100 mM NaCl, and incubated for 1 h with 10 μCi of 45Ca2+ in the same buffer. The filter was washed twice for 30 s at room temperature in a solution containing 10 μM CaCl2, 50 mM NaCl, 20 mM Tris-HCl (pH 7.5), and 10% methanol, dried, and exposed to an x-ray film. The autoradiograms were quantitated on a densitometer. The ability to bind Ca2+ by the recombinant proteins was assayed in three independent experiments.

**RESULTS**

Scarf Cloning and Genomic and Protein Characterization—A cDNA differentially expressed in suprabasal keratinocytes was obtained from an SSH screen. Scarf 899-bp cDNA (Fig. 1A) contains an ORF that encodes a protein of 149 amino acids with four EF-hand domains (Fig. 1A, bold letters) and a predicted molecular mass of 16.7 kDa. As is characteristic of other members of the CaM-like Ca2+-binding proteins, Scarf is an acidic protein (pI = 4.59) with no cysteine or tryptophan residues. A particular difference with calcium-binding proteins (13, 14) is that Scarf does not present putative sites for myristoylation. Comparison of the four EF-hand motifs of Scarf and other CaM-like proteins showed high homology in the EF-2, -3, and -4 motifs, with EF-1 being the most divergent (Fig. 1A). Protein sequence comparisons determined that Scarf presented 56.7% similarity to mouse CaM (mCaM), 64.2% to hC1p, and 64.9% to hC1p (Fig. 1C). A phylogenetic analysis of several Ca2+-binding proteins shows that Scarf and hC1p belong to the same subgroup and are more distantly related to CaM and hC1p (data not shown).

A genomic DNA region of ~120 kb was cloned by screening of a mouse VJ/129 BAC library using an 0.9-kb Scarf cDNA probe. Comparison of the genomic and cDNA sequences determined the 5′- and 3′-end boundaries and the absence of intronic regions in the Scarf gene. Southern blot analysis of the BAC clone helped us identify the existence of a second gene with homology to Scarf. We termed this second gene Scarf2.

Computational analysis of the Scarf2 genomic sequence proposed that a complete ORF would be derived after the splicing of a putative small intronic region. To determine whether the gene was expressed in keratinocytes and whether the transcript was the product of a splicing event, Scarf2-specific oligonucleotides encoding sequences surrounding the putative intronic region (Fig. 2A) were utilized to perform RT-PCR with mouse keratinocytes mRNA. As shown in Fig. 2A (lower panel),
a fragment was obtained, and after subcloning and subsequent sequencing we determined that the predicted intronic sequence had been spliced to generate the Scarf2 mRNA transcript. As a control reaction, RT-PCR was performed with the same set of primers and no input cDNA. Therefore, Scarf2 is expressed in keratinocytes; the mRNA sequence and putative ORF is presented in Fig. 1A.

The Scarf2 gene localized 15.3 kb downstream of Scarf (Fig. 2B), and the high degree of homology between Scarf and Scarf2 extended to regions upstream and downstream of the ORF for both genes. These regions contain potential proximal promoter and regulatory sequences (Fig. 2B). The upstream regions (Fig. 2B, striped boxes) are 1.6 kb in length and 96% homologous. The downstream regions (Fig. 2B, gray boxes) are 1.2 kb in length and 89.5% homologous.

A mouse gene with homology to hClp (mClp) localized ~33 kb upstream from the Scarf gene. Comparison of the genomic organization of the CaM-like genes in human chromosome 10 and the syntenic region on mouse chromosome 13 is shown in Fig. 2B. Human and mouse show similar genomic arrangement, with Scarf and hClp being potential homologs and the differences being that hClp is in reverse transcriptional orientation and the apparent absence of a Scarf2 homolog of human chromosome 10.

**Scarf Expression during Development and in Cultured Primary Keratinocytes**—Using Northern blot analysis and radioactive in situ hybridization, we examined the pattern of Scarf expression during development and in mouse primary keratinocytes. In situ hybridization of sagittal sections of 15-day (Fig. 3, A and B) and 16-day (Fig. 3, C and D) mouse embryos with antisense Scarf probes (Fig. 3, A and C), showed epidermis-specific expression at both developmental stages. The expression was not detected in the basal layer, but was clearly seen within the spinous and granular layers in the stratified epidermis of 16-day embryos (Fig. 3, E and F). Expression was also detected in the dorsum of the tongue (Fig. 3, H and I) and in the vibrissae of a 16-day mouse embryo (Fig. 3, J and K). Scarf expression was restricted to the differentiated layers of the stratified epidermis in neonatal skin (Fig. 3, L and M). No expression was detected with the use of Scarf sense probe (Fig. 3, B, D, G, and N).

Using a commercial Northern blot of total embryo mRNA at different developmental stages, Scarf was first detected at 15 days of embryogenesis and dramatically increased by day 17 (Fig. 4A). Sites of expression detected on a dot blot and Northern blot of different adult tissues (Clontech) were thyroid (data not shown) and skeletal muscle (Fig. 4B). Using the commercial blot, no expression was detected in heart, brain, spleen, lung, liver, kidney, or testis (Fig. 4B). With the hybridization of the commercial blot to a glyceraldehyde-3-phosphate dehydrogenase probe to determine loading amounts and integrity of the mRNA, it became apparent that there was less input from the spleen, lung, and testis samples. For this reason, it is not possible to clearly assess the expression level of Scarf in these tissues.

In the epidermis, Scarf expression was restricted to the suprabasal differentiated cells obtained by Percoll gradient of preparations from neonatal skins (Fig. 4C). Several epidermis-specific markers were utilized to validate the Percoll separation method for basal (B) and suprabasal (SB) cells (Fig. 4C).
The K5 marker is restricted to the basal fraction, whereas the markers INV, LOR, and K1 are primarily in the suprabasal fraction. The ubiquitously expressed CaM mRNA was detected in both proliferating and differentiated keratinocytes (Fig. 4C).

The expression of Scarf was also studied in cultured primary keratinocytes induced to differentiate in vitro. Scarf expression was detectable in low Ca\(^{2+}\) (0.05 mM) and showed a 4-fold increase 24 h after induction of differentiation by raising the Ca\(^{2+}\) concentration to 0.12 mM and a further increase by 48 h (Fig. 4D). Northern blots were also hybridized with basal cell-specific marker (K5) and suprabasal differentiation-specific markers (K1, LOR, and INV). The Scarf expression pattern in cultured keratinocytes mimics that of early differentiation markers such as INV, whereas the late epidermal differentiation marker, LOR, was detected 48 h after Ca\(^{2+}\) switch.

Treatment of cultured cells with different kinase (PKC, PKA, CaM kinase II) inhibitors showed a specific down-regulation of Scarf expression by treatment with a PKC inhibitor, GF109203X (Fig. 4E). We also determined the down-regulation for other epidermal factors (INV, K1, and LOR), where expression is dependent on the PKC signaling pathway. No effect on Scarf expression was seen with treatment with PKA inhibitor H89 and CaM kinase II inhibitor KN62.

Scarf Ca\(^{2+}\)-binding Protein in Differentiated Keratinocytes—To assess the expression of Scarf protein in epidermis, we performed immunohistochemistry on paraffin sections of mouse neonatal skin with a polyclonal antibody generated in hens against the full-length recombinant protein (rScarf). Scarf protein was clearly detected from the spinous layers to anucleated cells of the stratum corneum in neonatal skin sections using a fluorescein isothiocyanate-conjugated secondary antibody against hen (shown in green, Fig. 5A). Propidium iodide counter-stain determined the localization of the nuclei (Fig. 5A, shown in red).

In Western blot assays, anti-Scarf polyclonal antibody detected rScarf and the endogenous Scarf protein in the soluble fraction of suprabasal cells (Fig. 5B). No Scarf was detected in the insoluble fractions of either basal or suprabasal keratinocytes. Using several recombinant CaM-like proteins in a Western blot analysis, Scarf antibody weakly detected recombinant hClsp but had no cross-reactivity to either hClp or CaM (Fig. 5C).

To investigate the capability of Scarf to bind Ca\(^{2+}\) and the functionality of each of the EF-hand motifs, we performed Ca\(^{2+}\)-binding assays. Scarf mutants were obtained by substituting the conserved first Asp residue of each EF-hand motif (sequence GAC) with a Gly residue (GCC sequence) to generate mtEF1, mtEF2, mtEF3, and mtEF4. Parallel binding assays were performed with CaM as control, several CaM-like Ca\(^{2+}\)-binding proteins (CaM, hClsp, and hClp), rScarf, and each recombinant mutant. Quantitation of the autoradiograms with a densitometer show that Scarf is able to bind Ca\(^{2+}\) (Fig. 6A) and that mutations in each of the EF-hand motifs lead to a diminished ability to bind Ca\(^{2+}\), with mutation of the EF-2 domain showing the highest effect (Fig. 6B). The data presented are the mean from three independent experiments ± S.D. (Fig. 6B).
Identification of differentially expressed proteins during epidermal stratification will help us elucidate the necessary precursors and the series of complex steps that are required for the formation of a functional water barrier in the skin. By performing an SSH screen between the undifferentiated and differentiated cells of the neonatal mouse epidermis, we identified a novel murine Ca\(^{2+}\)-binding protein gene we termed Scarf. Sequencing of the full-length cDNA determined that Scarf contained regions that would translate into putative EF-hand Ca\(^{2+}\)-binding motifs. The presence of four EF-hand motifs and the degree of homology to CaM indicate that Scarf belongs to the family of CaM-like Ca\(^{2+}\)-binding proteins.

In the in situ hybridization data, we showed that Scarf mRNA was expressed throughout the differentiated layers of the embryonic developing epidermis. In neonatal epidermis, the Scarf gene was also differentially expressed in the spinous and granular layers. An 0.9-kb mRNA Scarf transcript was detected in suprabasal cells obtained from neonatal epidermis by discontinuous Percoll gradients and in primary keratinocytes differentiated in vitro. Scarf expression was similar to that of other early differentiation markers such as INV and was dependent on PKC signaling.

The Scarf transcript encodes a small acidic protein of 148 amino acids detected with anti-Scarf-specific antibodies in the suprabasal layers of the epidermis. Scarf EF-hand motifs maintain the highly conserved residues present in the canonical 12 residue EF-hand motif (DXD(D/N/G/X)II/IV)XXE) (12). The Ca\(^{2+}\)-binding ability and functionality of each Scarf EF-motif were tested. Our results indicate that Scarf is able to bind Ca\(^{2+}\) and mutations in each EF-hand lead to a decreased ability to bind Ca\(^{2+}\), with mutations in the EF-2 hand motif being the most effective. For the CaM-like protein hClsp, it has recently been reported that its four EF-hand motifs bind Ca\(^{2+}\), but the motifs have been defined as two Ca\(^{2+}\)-Mg\(^{2+}\) high affinity binding motifs (EF-1 and EF-2) and two low Ca\(^{2+}\)-binding motifs (EF-3 and EF-4) (25). In CaM, mutations of the EF-2 motif decreased its affinity for several of its known target proteins: smooth and skeletal muscle myosin light chain kinases, adenyl cyclase, and plasma membrane Ca\(^{2+}\)-ATPase (26). It remains to be determined whether Scarf EF-hands have differential affinity for Ca\(^{2+}\), whether the motifs are independent or mutually interactive, and what physiological relevance this has for its capacity to interact with its specific target proteins in the mouse epidermis. Targets for other members of the CaM-like binding-protein family have been reported, with hClp binding a human unconventional myosin X (27), CaBPs binding the inositol trisphosphate receptor (Ins-P\(_3\)-R) (28), and hClsp binding TGase3 (17). TGases are Ca\(^{2+}\)-dependent enzymes that catalyze the N-(γ-glutamyl)lysine isopeptide bonds that occur during the cross-linking of precursors to form the specialized cornified cell envelope in the epidermis (1). The Ca\(^{2+}\)-dependent binding of hClsp to TGase3 is a potential way of regulating epidermal differentiation through the modulation of TGase3 enzyme activity (16). Alternatively, members of the S100 EF-hand-containing protein family are TGase enzymatic targets (11, 29), leading to a mechanism for regulating the S100 protein functions in the epidermis.

Based on sequence homology and genomic localization, we propose that Scarf is a potential mouse homolog of hClsp. However, certain relevant distinctions between Scarf and hClsp have been determined: (a) hClsp was reported as a late granular differentiation marker expressed only in the very late stages of human keratinocyte differentiation and detected in...
lungs (16, 17), whereas Scarf is expressed early in the differentiation process throughout the spinous and granular layers of the stratified epidermis and is also detected in thyroid and skeletal muscle, and (b) Scarf has a slightly extended central helix when compared with hClsp (16), making it more similar to CaM. For CaM, the central helix serves a fundamental role, allowing the amino- and carboxyl-terminal domains to swing around and permitting interactions with specific target proteins (6, 12). The focus of our future studies will be to establish whether the slightly extended central helix in Scarf allows for the binding to targets other than the factors that interact with hClsp and to determine the role of these interactions in the control of the Ca\textsuperscript{2+}-dependent epidermal differentiation.

In this study we have also identified a gene with high homology to Scarf, which we termed Scarf2, that localizes −15 kb downstream on mouse chromosome 13. Sequence analysis of the genomic regions 5′ and 3′ to each gene showed that the homology extended to the putative proximal promoter and 3′-regions. We hypothesize that the bigenic cluster arose by recent duplication in the mouse, because we were unable to localize by genomic sequence scanning a counterpart to Scarf2 in the human genome. We detected a transcript for Scarf2 in mouse keratinocytes by RT-PCR but not in Northern blots with samples of total RNA from basal and suprabasal cells isolated from neonatal skins or in commercial blots (data not shown), suggesting that Scarf2 is expressed at lower levels than Scarf. Analysis of the Scarf2 putative ORF indicated that it encodes a Ca\textsuperscript{2+}-binding protein, and based on a comparison with the canonical consensus sequence for the EF-hand motif and the critical role of the residues at positions 1 and 12, we hypothesize that the Scarf2 EF-1 and -4 motifs will be inactive or present altered Ca\textsuperscript{2+} binding. How these differences characterize Scarf2 function remains to be determined, as does the role of the highly homologous Scarf and Scarf2 and their interaction with target proteins in the transduction of the Ca\textsuperscript{2+}-dependent regulatory signals during the epidermal differentiation process.

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