Distinct Functional Interactions of Human Skn-1 Isoforms with Ese-1 during Keratinocyte Terminal Differentiation*

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Among the three major POU proteins expressed in human skin, Oct-1, Tst-1/Oct-6, and Skn-1/Oct-11, only the latter induced SPRR2A, a marker of keratinocyte terminal differentiation. In this study, we have identified three Skn-1 isoforms, which encode proteins with various N termini, generated by alternative promoter usage. These isotypes showed distinct expression patterns in various skin samples, internal squamous epithelia, and cultured human keratinocytes. Skn-1a and Skn-1d1 bound the SPRR2A octamer site with comparable affinity and functioned as transcriptional activators. Skn-1d2 did not affect SPRR2A expression. Skn-1a, the largest protein, functionally cooperated with Ese-1/Elf-3, an epithelial-specific transcription factor, previously implicated in SPRR2A induction. This cooperativity, which depended on an N-terminal pointed-like domain in Skn-1a, was not found for Skn-1d1. Actually, Skn-1d1 counteracted the cooperativity between Skn-1a and Ese-1. Apparently, the human Skn-1 locus encodes multifunctional protein isoforms, subjected to biochemical cross-talk, which are likely to play a major role in the fine-tuning of keratinocyte terminal differentiation.

The epidermis constitutes the interface between the organism and the environment and provides protection against physical, chemical, and microbial damage. The major epidermal cell type, the keratinocyte, engages in a tightly regulated process of terminal differentiation, which is essential for the protective barrier of the skin and is reflected in the process of terminal differentiation, which is essential for the formation of cytoskeleton, desmosomes, and cornified cell envelopes. As a matter of fact many genetic and acquired human dermatoses have been linked to mutations or aberrant expression of these proteins (2, 3).

Whereas the importance of structural proteins in safeguarding the integrity of epidermis and internal squamous epithelia is becoming well understood, little is yet known about the regulatory processes that are involved. Although ubiquitous transcription factors contribute to keratinocyte-specific gene expression (4–7), the complex balance between proliferation, stratification, and cornification is likely to be coordinated by cell type-specific proteins. Good candidates for such a function are the POU domain transcription factors, a family of more than 40 homeodomain-containing proteins involved in cell differentiation and tissue specification (8, 9). The characteristic POU domain consists of two conserved regions, a POU-specific domain and a POU homeodomain, connected by a hypervariable linker region. The entire POU domain is required for DNA binding. The octamer 5′-ATGCAAAT-3′ is the most frequent target for POU domain proteins (10).

Major POU domain factors in skin are Oct-1, Tst-1/Oct-6, and Skn-1/Oct-11 (11). The ubiquitous Oct-1 is expressed in both proliferating and differentiating epidermal keratinocytes, whereas Oct-6 and Skn-1 are primarily expressed in suprabasal layers. Skn-1 is selectively expressed in the epidermis (12–15). In vivo ablation of murine Skn-1 did not reveal a specific function for this gene, mainly due to redundancy with Oct-6 (12). Recently however, the use of in vitro raft cultures disclosed a regulatory role of Skn-1 in keratinocyte proliferation and differentiation (16).

A further degree of regulatory complexity is due to the fact that at least several POU genes give rise to various isoforms, with specific functional properties and expression patterns (e.g. Oct-1, Oct-2, Brn-3, and Pit-1) (8). Also, the rat Skn-1 gene was shown to generate two functionally distinct transcripts, Skn-1a and Skn-1i (13). Here we show that the human homologue expresses three isoforms that differentially affect the expression of the SPRR2A cornified envelope precursor gene, a marker of keratinocyte terminal differentiation, whose regulation has been extensively studied (5, 17). This isotype-specific selectivity in SPRR2A regulation, which varied from activation to repression, depended on the differential interaction of the Skn-1 isoforms with the epithelium-specific Ets factor Ese-1 (18).

EXPERIMENTAL PROCEDURES

Screening of a Keratinocyte cDNA Library—A human keratinocyte cDNA library constructed in Lambda ZAP II (Stratagene) (19) was screened with probes from the POU domains of Oct-1 and Oct-2 (20, 21) at a stringent hybridization temperature. This yielded among others two independent Skn-1 clones. Plasmid DNA was isolated by in vivo

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exon with Exonux M13 helper phage (Stratagene). 5′-Rapid Amplification of cDNA Ends (RACE)—5′-RACE was performed on poly(A) RNA isolated from cultured normal human keratinocytes essentially according to a previously described method (22).

Briefly, cDNA synthesized from 1 μg of poly(A) RNA, primed with a 5′-biotinylated antisense oligonucleotide specific for Skn-1 (5′-biotin-GAAACCTCTTCTCAGAGTCGGCCG), was purified on Dynabeads coated with streptavidin (Dynal) and ligated to a 5′-phosphorylated and 3′-3-aminopropyl-ether-blocked RACE-anchor (5′-phosphate-GGCGGGCCTGTGACTGGAGAAGCCCHOHCHNH₂). PCR primer with various reverse Skn-1 primers (1, ACACAAATCTCTACGAGGTTGGTAGG, 2, AACCACGCGAACCCCATCCTCTGT, 3, GAGGAGCCGCTTGTGTTCTGTTGGA; positions in GenBank accession number AF133895 are 814, 651, and 394, respectively) and a RACE primer complementary to the anchor (GGGTTTTCCCAGTCACCAGCAAGCGGCCGCGCC) was performed with a 2.5:1 mixture of Pwo polymerase (Roche Applied Science) and Taq polymerase (HT Biotechnology Ltd.) for 40 cycles (20 min at 94 °C, 30 min at 60 °C, 2 min at 72 °C). Fragments were cloned in pBluescript II SK (+) (Stratagene).

Inverse PCR—Genomic DNA was isolated from either skin, COS-1 cells or mouse 3T3 fibroblasts by proteinase K digestion and protein extraction. One μg of DNA was digested with either BamHI or Sau3AI and ligated with T4 DNA ligase (Amersham Biosciences). Ligated DNA was used in a PCR reaction with primers designed to contain restriction sites at the 5′ end to facilitate subsequent cloning (sianish sense primer, CGATGTTCCGAGAAGA; antisense primer, AAGATCCACAGACGACCCTG; mouse sense primer, AAGATCCACAGACGACCCTG; mouse antisense primer, AAGAGCTTTTGTCGTCAGGAAG). 

Semiquantitative RT-PCR and RNA Blotting—Trizol reagent (Invitrogen) was used to isolate total epidermal RNA from tissue obtained either after breast reduction or circumcision. Total RNA (200 ng) was reverse-transcribed with Super-RT (SphaeroQ) and random hexamers primed with various reverse Skn-1 primers (1, ACCAAATCTCTACGAGGTTGGTAGG, 2, AACCACGCGAACCCCATCCTCTGT, 3, GAGGAGCCGCTTGTGTTCTGTTGGA; positions in GenBank accession number AF133895 are 814, 651, and 394, respectively) and a RACE primer complementary to the anchor (GGGTTTTCCCAGTCACCAGCAAGCGGCCGCGCC) was performed with a 2.5:1 mixture of Pwo polymerase (Roche Applied Science) and Taq polymerase (HT Biotechnology Ltd.) for 40 cycles (20 min at 94 °C, 30 min at 60 °C, 2 min at 72 °C). Fragments were cloned in pBluescript II SK (+) (Stratagene).

Cell Culture, Transient Transfections, CAT and Luciferase Assays—HaCaT cells were grown in DMEM with 10% bovine calf serum (HyClone). Confluent cultures were transfected by incubating 5 cm culture dishes for 2 h with 5 μg of reporter plasmid (CAT or luciferase), 2.0 μg of Rous sarcoma virus expression plasmids (including compensating amounts of empty Rous sarcoma virus vector), and 40 μg of N-[1-(2,3-dioleoyloxy)propyl]-N,N-trimethylammonium salts transfection reagent. Monolayers were washed with phosphate-buffered saline and incubated for 24 h in culture medium. CAT assays were performed as described (5). Luciferase activity was measured with the luciferase assay system (Promega) essentially as previously described (28). All transfections were performed at least in triplicate.

The SPPR2A minimal promoter-driven CAT plasmid pSG55 has been previously described (5). Luciferase plasmids were constructed in pGL3 (Promega) and contained the following SPPR2A promoter inserts, all derived from previously described plasmids (5): pSG550-wt (minimal promoter), pSG55; pSG5300-Elts mutant, pSG212; pSG57- octamer mutant, pSG185.

RESULTS

Structural Characterization and Organization of Skn-1 Isoforms—Searching the human genome data base at NCBI with the previously described human Skn-1a cDNA (GenBank accession number AF133895) disclosed one sequence (GenBank accession number AF001150), encompassing the complete human gene. The 2868-bp cDNA sequence comprises 13 exons and extends over a 70-kilobase genomic region (Fig. 1A). The characteristic POU domain is encoded by exons 7–10. The screening of a human keratinocyte cDNA library with a POU domain probe and 5′ RACE (see “Experimental Procedures”) identified three Skn-1 isoforms, namely the previously described Skn-1a (29) and two novel variants, Skn-1d1 and Skn-1d2. Comparison with the genomic sequence revealed that the 5′ end of Skn-1d1 and Skn-1d2 corresponded to sequences in introns 5 and 7, respectively (Fig. 1A). The absence of other introns in these transcripts confirmed the mRNA origin of both clones.

The human Skn-1d1 transcript is homologous to the Skn-1i variant of rat (13) and is compared in Fig. 1B with the corresponding simian and rodent sequences. The translation initiation codon previously identified in the rat (codon 1) is not present in the human sequence. Both primate genes contain an AUG codon (codon A), which is in-frame with the Skn-1a coding sequence. However, this start codon is not likely to be functional since it is followed by a termination codon UAG (codon B). The screening of a human keratinocyte cDNA library with a POU domain probe and 5′ RACE (see “Experimental Procedures”) identified three Skn-1 isoforms, namely the previously described Skn-1a (29) and two novel variants, Skn-1d1 and Skn-1d2. Comparison with the genomic sequence revealed that the 5′ end of Skn-1d1 and Skn-1d2 corresponded to sequences in introns 5 and 7, respectively (Fig. 1A). The absence of other introns in these transcripts confirmed the mRNA origin of both clones.

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The sequence of the Skn-1d2 transcript, which initiates in intron 7, revealed 3 intron-encoded AUG codons in-frame with the POU domain sequence but followed by a termination codon at position 154 (Fig. 1C). Potential start codons for open reading frames are found in exon 8 (codon D and/or exon E).

DNA Binding Activity of the hSkn-1 Variants—The identity of the Skn-1 expression plasmids and their coding potential were verified by producing proteins in vitro with the TNT reticulocyte lysate system (Promega) (Fig. 2B). Skn-1a (pPOU-
117) migrated at the predicted molecular mass (47.5 kDa) and bound to the \textit{SPRR2A} octamer site (Fig. 2C). The Skn-1d2 isoform (pPOU-121) generated two different products (Fig. 2B). The slow migrating product has an apparent molecular mass of 25 kDa, which is in accordance with a protein initiating at codon D in the POU-specific domain (Fig. 1C). The 21.5-kDa product corresponds to a protein starting at initiation codon E. None of these Skn-1d2 isoforms is capable of binding to the \textit{SPRR2A} octamer site (Fig. 2C), which is likely due to the partial deletion of the POU-specific domain (Fig. 1A).

The wild type \textit{Skn-1d1} transcript (plasmid pPOU-123) codes for a major polypeptide of 35 kDa and two minor products of 33 and 31 kDa (Fig. 2B). To investigate translational initiation of Skn-1d1 proteins more precisely, constructs with mutations in codons A, B, or C (Fig. 1B and 2A) were generated. Fig. 2B shows that translation of the major Skn-1d1 polypeptide most likely initiates at codon C (a weak CUG initiation codon). Indeed, pPOU-137, in which this codon was changed into an efficient AUG initiator, yielded high levels of a product of 35 kDa, identical to the largest polypeptide from the wild type transcript. This cDNA was used in transfection experiments.

The minor 33- and 31-kDa proteins were not investigated further.

Electrophoretic mobility shift assays were performed to compare the binding affinity of Skn-1a (pPOU-117) and Skn-1d1 (pPOU-137) to the \textit{SPRR2A} octamer site. Labeled double-stranded \textit{SPRR2A} octamer oligonucleotide was incubated with reticulocyte lysate programmed with either Skn-1a or Skn-1d1. As shown in Fig. 2D, competition with increasing amounts of unlabeled binding site revealed that both Skn-1 variants bound the \textit{SPRR2A} octamer site with similar affinities.

Transactivation Potential of Skn-1 Isoforms—In Fig. 3A, the \textit{SPRR2A} proximal promoter, fused to the CAT reporter and encompassing all cis-elements necessary for expression during keratinocyte terminal differentiation (5), was transiently transfected into HaCaT cells, which contain low levels of endogenous POU proteins (16). We first evaluated the transactivation potential of Oct-1, Oct-6/Tst-1, and Skn-1a, the major POU domain proteins expressed in skin (11), and of Oct-2, a lymphoid-specific transcription factor (21), also expressed in cultured human keratinocytes (12). Oct-1 and Oct-2 did not affect base-line expression of \textit{SPRR2A}, Oct-6 repressed promoter activity by $\sim$70%, and Skn-1a was the only POU domain

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protein tested that mediated gene activation (3–4-fold induction, Fig. 3A).

To investigate the relative contribution of the individual Skn-1 isoforms in the regulation of the SPRR2A gene, the transactivation potential of the three variants was determined by transfecting increasing amounts of isotype-specific expression plasmids into HaCaT cells, programmed with an SPRR2A-luciferase construct. Both Skn-1a and Skn-1d1 up-regulated the SPRR2A promoter by 3–4-fold. The saturation kinetics were, however, different because lower amounts of Skn-1a were needed to reach a plateau, indicating that Skn-1a can transactivate the SPRR2A promoter more efficiently than Skn-1d1 (Fig. 3B). Skn-1d2 had no effect on SPRR2A promoter activity, even at higher doses. Collectively, these results indicate that among several POU domain proteins only Skn-1a and Skn-1d1 are able to activate the SPRR2A promoter in an in vitro transient transfection experiment. Hence, it was important to investigate whether a similar direct relation existed also in vivo between Skn-1a/d1 and SPRR2A expression.

**Correlation between SPRR2A and Skn-1a Expression in Vivo**—Initially, a panel of 50 different RNA samples from various human tissues was analyzed with a Skn-1-specific probe (3′-UTR), which detects all isoforms. Skn-1 expression was restricted to a few stratified squamous epithelia including epidermis, cervix, and foreskin (results not shown). To compare the expression pattern of the three human Skn-1 variants with SPRR2A expression, semiquantitative RT-PCR with isotype-specific primer sets was performed on RNA isolated from either total skin, epidermis, foreskin, cervix, and cultured primary keratinocytes. RNA from uterus, which does not contain SPRR2A expression, semiquantitative RT-PCR with isotype-specific primer sets was performed on RNA isolated from either total skin, epidermis, foreskin, cervix, and cultured primary keratinocytes. RNA from uterus, which does not contain SPRR2A expression, was used as a negative control. The results in Fig. 4A show that Skn-1a was expressed in all squamous epithelia (lanes 1–6 and 8) and in cultured keratinocytes (lane 7), although at different levels. Expression of Skn-1d1 was more heterogeneous, as it was not detected in the skin and cervix samples from lanes 1 and 8, respectively. Relatively high expression levels of Skn-1d1 (already visible after 25 cycles) were found in the epidermal RNAs from lanes 2, 3, and 5. Skn-1d2 expression was, in general, similar to the one of Skn-1a. The absence of Skn-1 transcripts in uterus correlated well with the absence of SPRR2A expression. However, no clear correlation was found in the other samples between the expression level of one of the Skn-1 isoforms and SPRR2A expression, indicating the involvement of other transcription factors. Indeed, previous work from our laboratory has shown that expression of SPRR2A relied on interdependent regulatory promoter ele-
Fig. 4. A, semiquantitative RT-PCR analysis of Skn-1, Ese-1, SPRR2A, and GAPDH expression in various human tissues and cells. RNA isolated from total skin (lane 1), isolated epidermis (lanes 2–5), foreskin (lane 6), cultured primary keratinocytes (lane 7), cervix (lane 8), and uterus (lane 9) were analyzed with SPRR2A, Ese-1, GAPDH, and isormspecific Skn-1 primers. PCR was performed with 25, 30, and 35 cycles, and products were either detected with gene-specific probes (Skn-1 isoforms and SPRR2A) or by direct ethidium bromide staining (Ese-1 and GAPDH). For GAPDH only the 30-cycle product is shown. B, in situ hybridization performed with digoxigenin-labeled Skn-1, SPRR2A, and Ese-1-specific RNA probes. A foreskin section was analyzed with sense (a, c, e) and antisense (b, d, f) probes. Bar, 100 μm.

To investigate whether Skn-1 and Ese-1 co-localize in vivo, we monitored the stratum-specific expression of Skn-1, Ese-1, and SPRR2A in sections of foreskin (Fig. 4B). Whereas Skn-1 (the 3′-UTR probe used detects all isoforms) was present in most suprabasal layers, Ese-1 expression was confined to the more differentiated layers. Most importantly, the distribution of both factors overlapped with the expression of SPRR2A. Because both octamer and Ets binding sites in the promoter are essential for SPRR2A expression (5), we questioned whether Skn-1 isoforms and Ese-1 had the potential to cooperate in SPRR2A transactivation.

Ese-1 Selectively Cooperates with Skn-1a in the Transactivation of the SPRR2A Promoter—HaCat cells were transfected with the SPRR2A-luciferase reporter construct (PSG350) together with increasing amounts of the different transcription factors either alone or in various combinations (Fig. 5). The total amount of transfected expression plasmid was kept constant by compensating with the empty vector. Skn-1a, Skn-1d1, and Ese-1 induced the same amount of transactivation (Fig. 5A) only the 30-cycle product is shown. B, in situ hybridization performed with digoxigenin-labeled Skn-1, SPRR2A, and Ese-1-specific RNA probes. A foreskin section was analyzed with sense (a, c, e) and antisense (b, d, f) probes. Bar, 100 μm.

Fig. 5D shows the effect of Skn-1d1, Skn-1d2, or Ets-2 on the cooperative activation of the SPRR2A promoter by Skn-1a and Ese-1. Although neither Skn-1d2 nor Ets-2 was able to affect the cooperativity between Skn-1a and Ese-1, a clear drop in promoter activity was observed when Skn-1d1 was included. Apparently Skn-1d1 is able to compete with Skn-1a for promoter binding even in the presence of Ese-1, resulting in a complete abrogation of the synergistic effect. In the case of Ets-2 the situation is different; Ets-2 can at least partially down-modulate the activity of Skn-1a in the absence of Ese-1 (Fig. 5C) but not in its presence (Fig. 5D). Furthermore, it is shown that mutations in either Ets (PSG390) or the octamer binding site (PSG527) resulted in a complete inhibition of SPRR2A promoter activity, in agreement with our previously published results (5).

Taken together, our results show that although both Skn-1a and Skn-1d1 could transactivate the SPRR2A promoter, only Skn-1a was able to functionally cooperate with Ese-1, resulting in enhanced transactivation. Skn-1d1 was able to counteract this functional cooperativity.

DISCUSSION

In this study, we have identified and characterized three isoforms of the human Skn-1/Oct-11 gene and assessed their ability to regulate the human SPRR2A gene. The promoter of this gene has been well characterized in the past, and its activation during keratinocyte differentiation has been well documented. It encompasses an octamer binding site, that is recognized by Skn-1 and is essential for promoter activity together with three other transcriptional control elements bound by, respectively, the Ets, Irf, and Klf transcription factor families (5). Among these, only the Ets binding activity has previously been identified as the epithelial-specific Ets factor, Ese-1 (18). Our previous finding, that destruction of a single binding site results in a complete loss of promoter activity, has stressed the importance of signal integration and transcription factor cooperativity in the regulation of this gene (5). Here, we have
used this well documented facet of SPRR2A regulation to analyze the regulatory abilities, including possible synergistic/antagonistic activities, of the three different Skn-1 isoforms that we have identified.

The human Skn-1 gene produces three mRNA species, encoding proteins with various N termini (Fig. 6A). The two shorter mRNA variants are the result of internal promoter usage and initiate within introns 5 and 7. The observation that the three transcripts are differentially expressed in various skin samples and in cultured human keratinocytes (Fig. 4A) indicates that the corresponding promoters function independently and are subjected to selective regulation. Although the Skn-1d1 and Skn-1d2 variants originate within introns, termination codons prevent the addition of specific N-terminal (“intron”-encoded) sequences to the proteins. Consequently, Skn-1d1 and Skn-1d2 can be viewed as N-terminal deletions of Skn-1a.

Skn-1a, which encodes the full-length protein, is identical to the mRNA previously described by others (29) and is highly homologous to its rat and mouse orthologues (13, 14). Skn-1a transcripts are expressed in all human skin samples analyzed.
(Fig. 4A). The higher level of expression in epidermal samples (lanes 2–5) as compared with a total skin preparation (lane 1) correlates with the epidermal expression of this gene (Fig. 4B). Our analysis clearly shows that SPRRA2 expression is not strictly linked with either Skn-1A or Ese-1 expression, but it clearly correlates with the presence of both factors. For instance, low levels of Ese-1 in uterus do not induce SPRRA2 expression due to the absence of Skn-1a (Fig. 4A, lane 9). However, weak expression of Skn-1a can be compensated by the presence of Ese-1 and results in efficient SPRRA2 expression in cultured keratinocytes and cervix (lanes 7–8). These in vivo expression data are in line with our transient transfection experiments, which have established cooperativity of Skn-1a and Ese-1 in SPRRA2 promoter transactivation (Fig. 5) and corroborate the previously identified necessity for signal integration in the regulation of SPRRA2 (5).

Skn-1d1 did not have the ability to synergize with Ese-1. Because Skn-1a and Skn-1d1 exhibited comparable DNA binding activities with the SPRRA2 octamer site, the functional cooperativity between Skn-1a and Ese-1 is likely to be mediated by a specific domain in Skn-1a, which is not present in Skn-1d1. Hence, the 122-amino acid N-terminal part of Skn-1a, which is absent in Skn-1d1 (Fig. 6A), was screened for possible structural domains by using 3D-PSSM (31), a fold recognition program at Imperial Cancer Research Fund (London) (www.sbg.bio.ic.ac.uk/3dpssm). This search has revealed between amino acids 35 and 121 a region with significant similarity to the canonical sterile α motif/pointed domain (Fig. 6B). This fold, which is found in many different proteins, including for instance the Ets and p53 transcription factor families, functions essentially as a protein-protein interaction interface (32, 33). It might seem tempting to speculate that the pointed-like domain (PLD) in Skn-1a interacts directly with the Ese-1 pointed domain. However, such an interaction would not account for the highly specific cooperativity between Skn-1a and Ese-1 that we have observed. Ets-2 also contains a pointed domain; it has the ability to bind to the SPRRA2 Ets site, but it does not synergize with Skn-1a. Consequently, it seems more likely that the Skn-1a pointed-like domain contacts a protein domain in Ese-1, which is specific for this factor.

The human Skn-1d1 mRNA is conserved among rodents and primates; however, its coding potential has clearly changed during evolution. Although in primates the region encoded by intron 5 is not translated (due to in-frame termination codons), it encodes in rat and mouse an N-terminal 32-amino acid domain (Fig. 6A) that is responsible for the inhibitory activity of the Skn-1i isoform (13). Skn-1d1 also inhibits SPRRA2 promoter activity in the presence of Skn-1a and Ese-1 (Fig. 5D), although it had the ability to activate the same promoter when present on its own (Fig. 3). Furthermore, in our in vivo analysis, high Skn-1d1 expression was inversely related to high SPRRA2 expression. For instance, in the epidermal samples of lanes 2, 3, and 5, where high expression of Ese-1, Skn-1a, and Skn-1d1 is monitored, expression of SPRRA2 is clearly lower than in foreskin (lane 6), where high expression of Skn-1a and Ese-1 contrasts with low expression of Skn-1d1. Similarly, comparison of lanes 7 and 8 (cultured keratinocytes and cervix) reveals that similar levels of Skn-1a and Ese-1 lead to lower expression of SPRRA2 in the cultured cells (lane 7) due to the presence of Skn-1d1. The mechanisms by which hSkn-1d1 and mSkn-1i inhibit gene expression differ, however, fundamentally. Whereas the mSkn-1i inhibitory domain acts in cis and inhibits DNA binding (13), the human counterpart has the same DNA binding affinity as Skn-1a. Due to this property it can compete with Skn-1a for octamer binding and interfere in this way with the cooperative activation of SPRRA2 by Skn-1a and Ese-1. The differential effect of Skn-1d1 on SPRRA2 expression in the presence or absence of Skn-1a/Ese-1 is also interesting from a different point of view. It might actually shed light on several unexplained findings concerning the relative transactivation potential of Skn-1a and various truncated constructs, including an N-terminal deletion (29, 34). In these experiments the outcome depended greatly on the specific promoter that was tested. Although the N-terminal deletion of Skn-1a induced K10 and HPV1a promoter activity, no discernible effect was observed on HPV-18, and K14 was inhibited. Our results suggest that such variable outcomes can be expected and that they are likely to depend mainly on the specific transcription factor occupancy of the promoter that is analyzed. This is especially true for promoters subjected to strict combinatorial gene regulation, such as SPRRA2 (5) and most likely also for the various promoters mentioned above. Consequently, to be able to fully appreciate the outcome of transcription factor truncation experiments, a reasonable knowledge of the regulatory configuration of the promoter that is analyzed is a prerequisite. A similar complexity has also recently been observed for the profilaggrin promoter and is discussed by the authors (7).

The human Skn-1d2 isoform codes for two proteins lacking the first 27 or 53 amino acids of the POU-specific domain, whereas the POU homeodomain is left intact (Fig. 6A). It has previously been shown that both subdomains are required for DNA binding (35). This probably explains why Skn-1d2 neither bound to the SPRRA2 octamer site nor affected promoter activity. However, Skn-1d2 was widely expressed, with transcripts detected in all skin samples analyzed. This feature suggests that Skn-1d2 might have a physiological role, which does not depend on DNA binding. Indeed, POU domain proteins have been shown to regulate transcription also through protein-protein contacts, in a DNA binding-independent manner. These interactions are often mediated by the POU-specific, the POU-homeodomain, or both domains and target other transcription factors, co-regulators, basal factors, and chromatin components (for a recent review, see Ref. 36). For instance, down-regulation of keratin 14 is mediated by the POU domain of either Skn-1a or Oct-6 and does not involve DNA binding to the K14 promoter (34). In the case of Oct-1, several protein-protein interactions are mediated solely via the POU homeodomain (36). Consequently, it is possible that the Skn-1d2-encoded proteins, which still have intact POU homeodomains, have the ability to affect gene expression by similar protein-protein interactions. More experiments will be needed to unravel a possible regulatory function of the Skn-1d2 isoforms.

Our work describes for the first time a functional interaction between the Skn-1a and Ese-1 transcription factors. The differential cross-talk of Skn-1a and Skn-1d1 with Ese-1 highlights the complexity of combinatorial gene regulation during keratinocyte terminal differentiation. The strict dosage of Skn-1 isoforms is likely to guarantee both the fine-tuning of the process of epidermal maturation and its adaptation to external and environmental hazards.

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