Characterization of the Regulatory Domains of the Human Skn-1a/Epoc-1/Oct-11 POU Transcription Factor*

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The Skn-1a POU transcription factor is primarily expressed in keratinocytes of murine embryonic and adult epidermis. Although some POU factors expressed in a tissue-specific manner are important for normal differentiation, the biological function of Skn-1a remains unknown. Previous in vitro studies indicate that Skn-1a has the ability to transactivate markers of keratinocyte differentiation. In this study, we have characterized Skn-1a's transactivation domain(s) and engineered a dominant negative protein that lacked this transactivation domain. Deletional analysis of the human homologue of Skn-1a with three target promoters revealed the presence of two functional domains: a primary C-terminal transactivation domain and a combined N-terminal inhibitory domain and transactivation domain. Skn-1a lacking the C-terminal region completely lost transactivation ability, irrespective of the promoter tested, and was able to block transactivation by normal Skn-1a in competition assays. Compared with full-length, Skn-1a lacking the N-terminal region demonstrated either increased transactivation (bovine cytokeratin 6 promoter), comparable transactivation (human papillomavirus type 1a long control region), or loss of transactivation (human papillomavirus type 18 long control region). The identification of a primary C-terminal transactivation domain enabled us to generate a dominant negative Skn-1a factor, which will be useful in the quest for a better understanding of this keratinocyte-specific gene regulator.

The epidermis is a regenerative tissue composed of several layers of keratinocytes with each layer representing an increased state of differentiation. The basal layer consists of proliferating undifferentiated keratinocytes that migrate outward into the nondividing suprabasal compartment and terminally differentiate. To ensure homeostasis in normal epidermis, a strict balance is maintained between the proliferation of keratinocytes and the rate of progression into the nondividing suprabasal compartment and terminal differentiation. In this tightly regulated differentiation process is the external tissue-specific manner are important for normal differentiation (1–3). Examples of these molecular markers include the keratin (K) intermediate filaments (4), structural proteins such as profilaggrin (5), and the substrates of keratinocyte transglutaminase (12), the cornified cell envelope proteins (small proline-rich proteins (SPRR) (6–8), involucrin (9), and loricrin (10, 11)). The proliferating basal keratinocytes express K5 and K14, and as keratinocytes move suprabasally and begin to differentiate, K5 and K14 expression is lost and K1 and K10 expression begins. The expression of keratinocyte transglutaminase as well as structural proteins such as involucrin, loricrin, profilaggrin, and SPRR also becomes evident at later stages of keratinocyte differentiation (1, 2). Detailed analysis of the regulatory regions of these genes have demonstrated the importance of a number of transcription factors (TFs), such as AP1 and AP2 (13–16). However, these TFs are also expressed in other organ systems and are not likely to ultimately determine which genes are uniquely expressed in keratinocytes. This has prompted a search for TFs that are only expressed in keratinocytes and are responsible for regulating expression of keratinocyte-specific genes during differentiation.

One TF that may play a role in regulating keratinocyte-specific gene expression is the POU TF Skn-1a/Epoc-1/Oct-11 (17–19). POU TFs are of particular interest because they are usually expressed in a tissue-specific manner and play an important role in the establishment of cell identity and cellular differentiation during development (20–24). POU TF family members, for instance, are essential for proper maturation of anterior pituitary cells (Pit-1) (25), B-cells (Oct-2) (26), and neuronal cells (Unc-86) (27). Since Skn-1a is primarily expressed in the epidermis, it may have an important regulatory role in both epidermal development and keratinocyte differentiation (17, 19). During mouse embryogenesis, a stratified epidermis appears by day 15 to 16. Skn-1a is expressed in the ectoderm underlying the periderm just prior to the sloughing off of the periderm and emergence of the stratified epidermis (17). In adult murine skin, Skn-1a expression is present primarily in differentiating suprabasal keratinocytes (17, 28). Correspondingly, in vitro studies have demonstrated that Skn-1a binds to and transactivates the promoter regions of keratinocyte genes involved in epidermal differentiation, such as human K10 (17) and SPRR-2A (29). Skn-1a also transactivates the long control region (LCR) of the human papillomaviruses (HPV) types 16, 18, 30, and 1a (31) and the expression

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s) AF162278.

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The abbreviations used are: K, cytokeratin; CAT, chloramphenicol acetyltransferase; HPV, human papillomavirus; LCR, long control region; ORF, open reading frame; SPRR, small proline-rich protein; TFs, transcription factors; UTR, untranslated region; RT-PCR, reverse transcription-polymerase chain reaction; bp, base pair(s); kb, kilobase(s) or kilobase pair(s); FISH, fluorescent in situ hybridization; EMSA, electromobility shift assay; DAPI, 4',6'-diamidino-2-phenylindole.
pattern of the early HPV gene products E6 and E7 parallels the bovine K6 gene, was a generous gift from H. Herrmann (40, 41). HPV-18-CAT reporter plasmid (p18F-4321) was a generous gift from C. Baker. A Smal/BamHI 1.0-kb fragment of the HPV-18 genome spanning the LCR and encompassing the nucleotides 6929–119 was subcloned into the HindIII site of pSB1 in the sense orientation. The HPV-1a LCR (ATCC), corresponding to nucleotides 3395–4370, was PCR amplified and subcloned into pCR2.1-TOPO (Invitrogen) and subsequently digested with Kpn1 and ligated into the Kpn1 site of pCATS basic Promega. The HPV-1a PCR primers utilized were as follows: 5′-gaggctcTCTTATATTTATATAATCATTT-3′ (sense) and 5′-tccceggTCTGACAGTCTACCTGTTATTT-3′ (antisense).

Northern Blot Analysis—Epidermal sheets were obtained by incubation with human foreskins with dispase (Gibco BRL/Grand Island, NY) for 90 min in a 0.5% (w/v) dispase solution.

FISH Analysis—The assay was performed on lymphocyte-derived chromosomes with a 1.7 kb hSkn-1a cDNA probe according to established procedures (Diagene). Thirty micrograms of total RNA was subject to standard 1% agarose gel electrophoresis with 660 m M formaldehyde as a denaturant. Transfer of the RNA was performed with Hybond N membranes with a 1.7 kb hSkn-1a cDNA probe according to the supplier’s guidelines (CLONTECH). The membranes were hybridized overnight at 42 °C with randomly primed probe and washed to a final stringency of 1× SSS, 0.1% SDS for 1 h at 50 °C. Phage DNA from double-positive plaques was obtained by conventional phenol-chloroform extraction and sequenced. A 1.0-kb partial hSkn-1a cDNA was synthesized by standard RT-PCR on total RNA derived from human foreskin epidermal sheets. Briefly, 1 μg of total RNA was used as template for reverse transcription with SuperScript II RT (Life Technologies, Inc.) as specified, followed by Pfu-based PCR (Stratagene). Thirty cycles of PCR were performed as follows: 45 s at 94 °C, 30 s at 50 °C, and 2 min at 72 °C. PCR cycles were followed by 10 min at 72 °C. Four independent RT-PCR products were cloned and sequenced. All sequencing reactions were performed by the Sanger method (38).

The primer pairs used to generate all products described are listed as follows: 310-bp probe, 5′-GTTAATCTCGGAGGCCATGCACG-3′ (sense) and 5′-GAAAATCGGGATGACACGAAG-3′ (antisense; RT); 451-bp probe, 5′-GGAGTTCGGAAGATTTTGCAAG-3′ (sense) and 5′-GATTGTCCTCCTTTGGTCG-3′ (antisense; RT); 3′-rapid amplification of cDNA ends, 5′-GCTGGAGAAGGGCTTGAAGT-3′ (sense) and 5′-GGCAGATCTTCCCTGCAG-3′ (nested sense); antisense primers (AP and UAP) were supplied by the manufacturer (Life Technologies, Inc.); hSkn-1a, 5′-GGAGGAAACGGACAGAACGT-3′ (RT primer), 5′-cagagctgggagggcgGGAAGGAGACCTGCTCCGC-3′ (antisense adaptor primer). The lowercase bases correspond to unique restriction sites added on the primers to enable proper subcloning of the PCR product.

Establishment of Reporter Plasmids—bK6-CAT, corresponding to 5.5 kb of the translation of the bovine K6 gene, was a generous gift from H. Herrmann (40, 41). HPV-18-CAT reporter plasmid (p18F-4321) was a generous gift from C. Baker. A Smal/BamHI 1.0-kb fragment of the HPV-18 genome spanning the LCR and encompassing the nucleotides 6929–119 was subcloned into the HindIII site of pSB1 in the sense orientation. The HPV-1a LCR (ATCC), corresponding to nucleotides 3395–4370, was PCR amplified and subcloned into pCR2.1-TOPO (Invitrogen) and subsequently digested with Kpn1 and ligated into the Kpn1 site of pCATS basic Promega. The HPV-1a PCR primers utilized were as follows: 5′-gaggctcTCTTATATTTATATAATCATTT-3′ (sense) and 5′-tccceggTCTGACAGTCTACCTGTTATTT-3′ (antisense).

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tured in Dulbecco’s modified Eagle’s medium with 4.5 g/liter glucose, 90%; fetal bovine serum, 10%; 1× antibiotic-antimycotic solution (Life Technologies, Inc.). CV-1 African green monkey kidney cell line was cultured in DMEM, 90%; fetal bovine serum 10%, 1× minimum essential media nonessential amino acids solution (0.1 mM solution); and 1× antibiotic-antimycotic solution.

**Transient Transfection Assays**—CaPO4 transfection assays were performed using the Modified Bovine Serum Transfection Kit (Stratagene). A total of 10 μg of plasmid DNA/100-mm tissue culture dish was utilized. Typically, 5 μg of any one of the Skn-1a expression vectors was co-transfected with 5 μg of empty pcDNA3 vector. Constant amounts of transfected DNA were also used for the competitive CAT assays. Cells were harvested 2–3 days post-transfection.

**RESULTS**

**Isolation of the hSkn-1a cDNA**—A combination of cDNA library screening along with 3′-rapid amplification of cDNA ends was used to clone the full-length human Skn-1a cDNA. A human primary keratinocyte agt11 cDNA library (CLONTECH) was screened with two probes derived from murine Skn-1a: one spanning a unique region within the 5′ portion of the ORF and the other spanning the POU domain (17). The resulting 2.4-kb cDNA demonstrated minimal divergence from the murine Skn-1a cDNA (Fig. 1A). A comparison of the ORF for both human and murine Skn-1a reveals that the human protein (hSkn-1a) is slightly larger than its murine counterpart by 6 amino acid residues (436 versus 430), but still shares approximately 90% overall identity. As anticipated, sequences within the POU domain only diverge within the hypervariable linker region (Fig. 1B). Sequence analysis of four independent clones of hSkn-1a revealed a polymorphism at residue 152 (Fig. 1, C and D). The nucleotide sequence for this particular codon was CAC or CGC with equal frequency. This A to G transition
lead to an amino acid substitution of histidine to arginine (Fig. 1C).

Northern blot analysis of total RNA derived from human foreskin epidermal sheets revealed that keratinocytes expressed two hSkn-1a transcripts estimated to be 2.4- and 3.5-kb in length (Fig. 1D). Our screening of a human cDNA library with murine Skn-1a yielded clones of rRNA cDNA in addition to Skn-1a. Thus, the 4.4-kb band on the Northern blot (which migrates at the same level as 28 S rRNA) most likely corresponds to nonspecific hybridization to 28 S rRNA.

Attempts to clone a human homologue of the previously described Skn-1i splicing variant were unsuccessful. All cDNAs obtained with a unique Skn-1i probe contained sequences homologous to Skn-1i, but did not contain a significant ORF due to frequent termination codons. Additionally, in vitro transcription and translation of the cDNAs in both orientations did not result in the synthesis of any detectable protein (data not shown). Northern blots also failed to reveal specific Skn-1i transcripts (data not shown). Therefore, a human homologue to murine Skn-1i may not exist.

Chromosomal Localization of hSkn-1a—To determine whether hSkn-1a was located at a chromosomal site where other keratinocyte-specific genes are located, FISH mapping and 4',6'-diamidino-2-phenylindole (DAPI) fluorescent dye multiband assay was performed on human chromosomes with a 1.7-kb hSkn-1a cDNA probe (42, 43). The DAPI banding pattern localized the hSkn-1a gene to the long arm of human chromosome 11 (Fig. 2A). Detailed mapping was determined by compiling the alignment of 10 independent photos of DAPI-stained chromosomes and FISH signals, which further localized the probe to human chromosome 11, region q23.3 (Fig. 2B).

Functional Domains of hSkn-1a—To localize the functional domains of hSkn-1a, we generated three constructs (Fig. 3). These constructs did not alter the conserved POU domain that is responsible for DNA binding, nuclear localization, and oligomerization (17, 23). The first construct contains an unaltered hSkn-1a cDNA (Fig. 3A). In the second construct, ΔNH3-Skn-1a, the N-terminal 120 amino acids were deleted and replaced with a FLAG tag (Kodak IBI) and an in-frame AUG at codon 121 (Fig. 3B). The third construct, ΔCOOH-Skn-1a, lacks the C-terminal 92 amino acids that have been replaced with a FL-Skn-1a tag and a stop codon immediately following the POU domain at codon 345 (Fig. 3C). To verify the integrity of the constructs, an in vitro transcription and translation assay with rabbit reticulocyte lysates was performed. All three constructs generated proteins that migrated at the predicted molecular masses of 48 kDa (FL-Skn-1a), 39 kDa (ΔCOOH-Skn-1a), and 36 kDa (ΔNH3-Skn-1a) in SDS-polyacrylamide gel electrophoresis (data not shown). To localize the transactivation domain of hSkn-1a, the ability of the deleted hSkn-1a constructs to transactivate the bovine K6 (bK6) promoter (equivalent to the human K10 promoter) (40, 41) was assessed in CAT cotransfection studies performed in both an epithelial cell line (HeLa S3) and nonepithelial cell line (NIH-3T3) (Fig. 4). Cotransfection of HeLa S3 cells (Fig. 4A) or NIH-3T3 cells (Fig. 4B) with the C-terminal deleted ΔCOOH-Skn-1a and bK6-CAT plasmids did not result in transactivation of bK6-CAT. By comparison, the full-length FL-Skn-1a transactivated bK6-CAT approximately 2-fold (HeLa) to 5-fold (NIH-3T3) above background. This indicated that the transactivation domain resides within the C-terminal 92 amino acids of the protein, downstream of the POU domain. In contrast, the N-terminal-deleted ΔNH3-Skn-1a protein demonstrated much greater transactivation of bK6-CAT than the full-length FL-hSkn-1a (Fig. 4). ΔNH3-Skn-1a was able to transactivate bK6-CAT approximately 6.5-fold (HeLa) to 12-fold (NIH-3T3) above background, suggesting the presence of a negative regulatory domain within the N-terminal region of the protein.

ΔCOOH-Skn-1a Retains DNA Binding Ability—Although the inability of ΔCOOH-Skn-1a to transactivate the bK6 promoter indicates the presence of a C-terminal transactivation domain, it is possible that the deleted construct was not able to bind DNA target sequences in the bK6 promoter because of conformational changes. To determine whether ΔCOOH-Skn-1a was able to bind DNA, EMSAs were performed with all three constructs and an octamer consensus sequence DNA probe (Fig. 5A (29)). Radiolabeled double-stranded DNA probe was incubated with either FL-Skn-1a, ΔNH3-Skn-1a, or ΔCOOH-Skn-1a derived from cell lines overexpressing these factors. Lanes 2, 5, and 8 correspond to band shifts of FL-Skn-1a, ΔNH3-Skn-1a, and ΔCOOH-Skn-1a, respectively. The full-length and deleted Skn-1a TEs both formed a complex with the DNA probe. The different band shift migration patterns resulted from the different molecular masses of the three constructs, with FL-Skn-1a followed in size by ΔCOOH-Skn-1a and ΔNH3-Skn-1a. A second, larger molecular mass shift
is seen with the double-stranded DNA probe and is due to binding with the ubiquitously expressed Oct-1 protein (Fig. 5A). The EMSA demonstrated that deletion of the C-terminal portion of the TF did not affect the ability of the protein to bind DNA. Additionally, the EMSA indirectly demonstrated that ΔCOOH-Skn-1a was present in cells at comparable levels to FL-Skn-1a and ΔNH3-Skn-1a. In order to directly assess the cellular accumulation of ΔCOOH-Skn-1a protein relative to ΔNH3-Skn-1a (which demonstrated increased transactivation of the bK6 promoter), a Western blot was performed on total cell extracts from NIH 3T3 cells overexpressing these two constructs (Fig. 5B). This assay detected bands of similar intensities at the predicted molecular masses for both ΔCOOH-Skn-1a (39 kDa) and ΔNH3-Skn-1a (36 kDa), which, in conjunction with the EMSA results, conclusively demonstrate that the loss of transactivation by ΔCOOH-Skn-1a is due to the absence of a transactivation domain.

hSkn-1a Target Promoter Comparison—POU TFs bind DNA targets with varying sequence composition, orientation, and spacing (23). This variation can determine what regulatory effect a POU TF has on a given promoter (23). Indeed, Skn-1a does recognize variable target sequences in different promoters (17, 29–31, 45). For example, murine Skn-1a has been shown to target and transactivate the HPV-1a LCR (31) and the HPV-18 LCR (30). In the HPV-1a LCR, murine Skn-1a recognizes octamer sequences that deviate slightly from the consensus sequence (5'-A(A/T)TATGC(A/T)AAT(T/A)T-3', core is indicated in bold type (31)), while in HPV-18 it preferentially binds to a nonconsensus sequence (5'-TGCATA(A/C)A-3') (30). Because of murine Skn-1a's multiple targets, we assessed the ability of the full-length Skn-1a and deleted Skn-1a TFs to transactivate these different HPV promoters.

hSkn-1a constructs were co-transfected into the CV-1 green monkey kidney-derived cells along with a CAT reporter plasmid driven either by the HPV-1a LCR (Fig. 6A) or the HPV-18 LCR (Fig. 6B). CV-1 cells were chosen because they do not express Skn-1a and are suitable for HPV LCR CAT assays (31). FL-Skn-1a transactivated both LCRs by 3.2-fold (HPV-1a) and

**FIG. 4.** **CAT assay with bK6-CAT in different cell lines.** HeLa S3 cells (A) or NIH-3T3 cells (B) were co-transfected with a bK6-CAT reporter plasmid and one of the three hSkn-1a expression vectors (Fig. 3). Activation of the bK6 promoter was tested by measuring the CAT activity of each co-transfection relative to that of transfection of the reporter plasmid alone (control groups). x axis, hSkn-1a constructs; y axis, fold transactivation above background control. Each bar graph represents one experiment with duplicate samples and is representative of additional experiments. Results demonstrating a statistically significant difference from control at p ≤ 0.01 (*) are indicated.
7.5-fold (HPV-18), comparable with what was initially observed with the bK6 promoter. Unlike the FL-Skn-1a construct, the ΔCOOH-Skn-1a construct did not transactivate the two viral promoters (Fig. 6, A and B), consistent with the bK6 promoter transactivation studies (Fig. 4). In contrast, the ability of ΔNH₂-Skn-1a to transactivate the HPV-1a and HPV-18 LCRs depended on which viral promoter is being tested. For HPV-1a LCR (Fig. 6A), ΔNH₂-Skn-1a and FL-Skn-1a had similar transactivating activity (3.5-fold versus 3.2-fold, respectively); however, ΔNH₂-Skn-1a is unable to transactivate the HPV-18 LCR (Fig. 6B), similar to the results obtained with ΔCOOH-Skn-1a. This is dramatically different from the increased transactivation of the bK6 promoter by ΔNH₂-Skn-1a (approximately 12-fold). In summary, FL-Skn-1a was consistently able to transactivate all three promoters and ΔCOOH-Skn-1a consistently lacked transactivation potential, while ΔNH₂-Skn-1a variably transactivated the three promoters (Figs. 4 and 6).

Functional Effect of ΔCOOH-Skn-1a by Competitive CAT Assay—Because ΔCOOH-Skn-1a did not transactivate the bK6 promoter, a competitive CAT assay was performed to determine whether ΔCOOH-Skn-1a could interfere with FL-Skn-1a transactivation of the bK6 promoter. NIH-3T3 cells were transiently co-transfected with FL-Skn-1a (○) or ΔNH₂-Skn-1a (●) and ΔCOOH-Skn-1a at various molar ratios along with the bK6-CAT reporter plasmid (Fig. 7). While ΔCOOH-Skn-1a is able to interfere with bK6 transactivation by FL-Skn-1a when both constructs are present in equimolar amounts (approximately 32% decrease in CAT activity relative to unchallenged FL-Skn-1a), the ability of FL-Skn-1a to transactivate the bK6 promoter was completely blocked by 3-fold molar excess of ΔCOOH-Skn-1a. This indi-
cates that ΔCOOH-Skn-1a is able to effectively compete with FL-Skn-1a for bK6 DNA target sites and prevent it from trans-activating the bK6 promoter. Thus, ΔCOOH-Skn-1a may be useful in exerting a dominant negative effect if overexpressed in in vivo model systems.

DISCUSSION

POU factors are implicated in cell lineage progression during early embryogenesis and tissue-specific cell maturation in later developmental stages (20). Skn-1a POU TF is primarily expressed in the epidermis (17, 19) and is a strong candidate gene for regulating keratinocyte-specific gene expression and keratinocyte differentiation. However, a biological function and mode of action for Skn-1a in epidermal differentiation has not yet been established. In vitro assays have demonstrated that Skn-1a can activate (K10) or inhibit (involutin) the expression of the keratinocyte-specific genes (17, 45). Yet, Skn-1a/i null mice develop normally and do not exhibit any overt phenotypic alterations (28), perhaps reflecting functional redundancy between Skn-1a/i and other ubiquitous POU TFs in skin. Consequently, the precise role of this keratinocyte-specific POU TF in epidermal differentiation still needs to be determined.

Elucidation of Skn-1a’s mechanism of action requires identification of functional domains by testing the effects of truncated Skn-1a constructs on different target promoters. To facilitate future in vitro studies with human keratinocyte organ cultures, we cloned the human Skn-1a homologue (hSkn-1a). hSkn-1a proved to be virtually identical to its murine counterpart. Interestingly, a human homologue of the previously described murine Skn-1i splice variant could not be detected. Deletion analysis of hSkn-1a revealed that it contained a primary transactivation domain downstream of the POU domain, within the C-terminal 92 amino acids. While FL-Skn-1a transactivated all promoters tested, regardless of which cell line was assayed, ΔCOOH-Skn-1a did not transactivate any of the promoters. Conversely, the N-terminal 120 amino acids of hSkn-1a contains either an inhibitory domain or a secondary transactivation domain, depending on the target DNA sequences in the different promoters. For the bK6 promoter, ΔNH3-Skn-1a demonstrated greater transactivation than FL-Skn-1a. However, deletion of the N-terminal 120 amino acids did not increase transactivation of the other promoters. ΔNH3-Skn-1a was incapable of transactivating the HPV-18 LCR, suggesting that either the N-terminal region contains a necessary transactivation domain for this promoter or that interaction of ΔNH3-Skn-1a with the nonconsensus DNA target site of HPV-18 LCR induces a conformational change in the POU domain preventing it from interacting with accessory factors necessary for proper promoter activation (46, 47). An earlier study, using a Skn-1a-LexA fusion protein approach, reported that the primary transactivation domain for the murine Skn-1a resides within the N-terminal 182 amino acids (31). Our data suggest

\[ \text{FIG. 6. CAT assay of hSkn-1a constructs on different promoters. The ability of the hSkn-1a constructs to transactivate two target promoters was evaluated by performing co-transfections of FL-Skn-1a, ΔNH3-Skn-1a, or ΔCOOH-Skn-1a with CAT reporter constructs being driven by HPV-1a LCR (A) or HPV-18 LCR (B). CAT activity was measured by determining the percent conversion of unacylated chloramphenicol to its acetylated forms for each individual sample relative to control transfections of the respective reporter plasmids alone. x axis, constructs used; y axis, fold transactivation above background control. Each bar graph represents one experiment with duplicate samples and is representative of additional experiments. Results demonstrating a statistically significant difference from control at } p \leq 0.01 (\ast) \text{ and at } p \leq 0.05 (\dagger) \text{ are indicated.} \]
that the critical domain for hSkn-1a transactivation lies in the C terminus, while a secondary transactivation domain in the N terminus is required for some promoters. This apparent discrepancy may reflect conformational changes resulting from fusion of Skn-1a fragments with the LexA DNA-binding domain or use of the LexA promoter as a target for the fusion protein.

POU TFs often have multiple transactivation domains (33, 35, 48–50). Oct-3/4, for instance, has two transactivation domains residing outside of the POU domain: a primary N-terminal transactivation domain and a secondary, cell-specific, C-terminal transactivation domain that is dependent on the POU domain itself (33–35). This dependence is lost if Oct-3/4’s C-terminal transactivation domain is fused to the heterologous POU domain itself (33–35). Oct-3/4, for instance, has two transactivation domains: 1) an invariant primary C-terminal domain and 2) a promoter-specific N-terminal domain. Additionally, we also demonstrate that the N-terminal 120 amino acids of hSkn-1a contain a promoter-specific inhibitory domain. The C-terminal truncated protein lacking the primary transactivation domain is capable of effectively competing with FL-Skn-1a in a dominant negative manner. Therefore, ACOOH-Skn-1a is a good candidate construct to effectively block and disrupt the function of endogenous Skn-1a in keratinocytes. The identification of important functional domains of hSkn-1a may help define its biological function in keratinocyte differentiation and epidermal homeostasis. Constructs with or without these defined domains can be inappropriately overexpressed or used to block endogenous Skn-1a in keratinocyte organ cultures and transgenic animal models to assess phenotypic and biochemical effects.

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