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A Conserved Transcription Motif Suggesting Functional Parallels between *Caenorhabditis elegans* SKN-1 and Cap'n'Collar-related Basic Leucine Zipper Proteins*

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In *Caenorhabditis elegans*, the predicted transcription factor SKN-1 is required for embryonic endodermal and mesodermal specification and for maintaining differentiated intestinal cells post-embryonically. The SKN-1 DNA-binding region is related to the Cap'n'Collar (CNC) family of basic leucine zipper proteins, but uniquely, SKN-1 binds DNA as a monomer. CNC proteins are absent in *C. elegans*, however; and their involvement in the endoderm and mesoderm suggests some functional parallels to SKN-1. Using a cell culture assay, we show that SKN-1 induces transcription and contains three potent activation domains. The functional core of one domain is a short motif, the DIDLID element, which is highly conserved in a subgroup of vertebrate CNC proteins. The DIDLID element is important for SKN-1-driven transcription, suggesting a likely significance in other CNC proteins. SKN-1 binds to and activates transcription through the p300/cAMP-responsive element-binding protein-binding protein (CBP) coactivator, supporting the genetic prediction that SKN-1 recruits the *C. elegans* p300/CBP ortholog, CBP-1. The DIDLID element appears to act independently of p300/CBP, however, suggesting a distinct conserved target. The evolutionarily preservation of the DIDLID transcriptional element supports the model that SKN-1 and some CNC proteins interact with analogous cofactors and may have preserved some similar functions despite having divergent DNA-binding domains.

During development, establishment of cell fates frequently involves conserved regulatory pathways. In the early *Caenorhabditis elegans* embryo, endodermal (intestinal) fates are specified by GATA family transcription factors (END-1, END-3, ELT-2) that are related to those that mediate endoderm development in *Drosophila* (Serpent) and vertebrates (GATA-4/5/6) (1–3). This program is triggered by maternally expressed SKN-1, a predicted transcription factor (4) that also specifies mesodermal lineages (pharynx and some body wall muscle) (5). The presence of consensus SKN-1-binding sites adjacent to the *end-1* gene along with the timing of its expression suggests that SKN-1 may activate *end-1* directly (2). SKN-1 is also required in the endoderm post-embryonically, to prevent differentiated intestinal cells from undergoing severe atrophy (5).

Apparent SKN-1 orthologs have not been identified outside of nematodes, but in its DNA-binding region, SKN-1 is related to a subgroup of basic leucine zipper transcription factors (4, 5), the CNC[1] proteins. A basic DNA-binding region at the SKN-1 COOH terminus is particularly similar to those of CNC proteins, but SKN-1 lacks a zipper dimerization domain (Fig. 1, *A* and *C*) and, uniquely, binds DNA as a monomer (4). Its DNA binding requires the adjacent α-helical "CNC region" (Fig. 1, *A* and *C*) (4, 6–8), which is otherwise found only in CNC proteins. An adjacent SKN-1 element that is lacking in CNC proteins, the NH_2-terminal arm (Fig. 1, *A* and *C*), contributes additional binding affinity and specificity (6, 9). These similarities and differences pose the question of whether SKN-1- and CNC-related proteins simply bind DNA through related but divergent mechanisms or might share a closer functional relationship.

Like SKN-1, many CNC proteins are involved in the development or function of endodermal or mesodermal cells. *Drosophila* CNC is required for specification of pharyngeal segments (10), and vertebrate $p45^{NF\text{-}E2}$ is involved in hematopoiesis (11). In mice, different knockouts of the *Nrf1* (NF-E2 related factor-1; also LCRF-1/TC11) gene either cause a fetal liver microenvironment defect (12) or appear to block cell-to-cell induction of the mesoderm, a function usually ascribed to endodermal cells (13). Both NRF1 and NRF2 directly induce expression of detoxification enzymes (14–17), a pathway that is markedly stimulated in the liver and intestine (14). Supporting the idea that some CNC protein functions might parallel those of SKN-1, neither they nor their dimerization partners, the Maf basic leucine zipper proteins (18, 19), appear to be encoded in the complete *C. elegans* genome (data not shown).

Genetic evidence suggests that SKN-1 may interact functionally with CBP-1, the *C. elegans* ortholog of the p300/CBP transcription coactivators. (20). p300/CBP proteins are metazoan histone acetyltransferases that are involved in developmental and inducible gene expression and that are recruited by numerous activators (21), including the CNC protein $p45^{NF\text{-}E2}$ (22). *C. elegans* CBP-1 is required for specification of all non-neuronal embryonic developmental lineages (20). Endodermal differentiation can be restored in embryos that lack either CBP-1 or SKN-1, however, by inhibition of histone deacetylases

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[1] The abbreviations used are: CNC, Cap'n'Collar; CBP, cAMP-responsive element-binding protein-binding protein; CMV, cytomegalovirus; TK, thymidine kinase; CAT, chloramphenicol acetyltransferase; GST, glutathione *S*-transferase; PCAF, p300/CBP-associated factor.

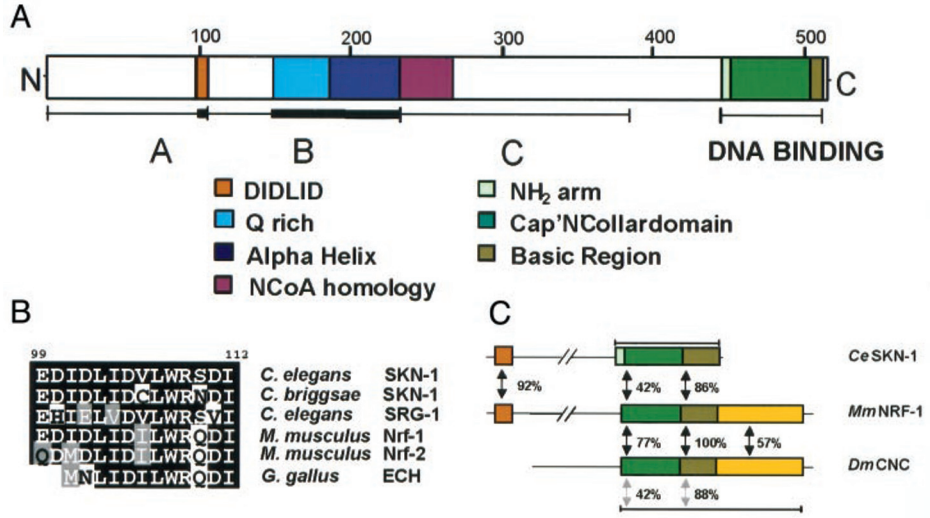

FIG. 1. **SKN-1 sequence domains.** *A*, full-length SKN-1 sequence domains are indicated by *shaded boxes*. Separable transactivation domains (A, B, and C) are shown below the diagram, with their major functional units demarcated by a *heavy line*. *B*, shown is a sequence comparison of DIDLID motifs, with SKN-1 residue numbers indicated. Conserved amino acids are indicated by *gray boxes*, and nonconserved changes by *white boxes*. The *Gallus gallus* (chicken) ECH DIDLID element constitutes the NH_2 terminus of the protein. *C*, shown are similarities between SKN-1 and CNC proteins. *Black arrows* indicate percent similarity (BLOSUM62 matrix) between adjacent proteins within sequence domains that are colored as described for *A* and within the leucine zipper (*yellow*). *Gray arrows* beneath *Drosophila melanogaster CNC*, which lacks a DIDLID element, show percent similarity to SKN-1. SKN-1 is comparably similar to other CNC proteins within the corresponding regions (not shown). *Bars* span the DNA-binding domains of SKN-1 (*top*) and CNC proteins (*bottom*). *Ce*, *C. elegans*; *Mm*, *Mus musculus*.

(20). This suggests that in the endoderm, SKN-1 might recruit the CBP-1 histone acetyltransferase activity directly. It remains possible, nevertheless, that lack of histone deacetylases simply restores expression of downstream genes independently of possible histone acetyltransferase recruitment by SKN-1.

We have addressed these issues by investigating how SKN-1 regulates transcription. *C. elegans* cell lines have not been developed, but given the conservation of the eukaryotic mRNA transcription machinery (23), SKN-1 would be predicted to be active in mammalian cells. Using transfection assays, we show here that SKN-1 is a powerful activator of transcription that contains three transactivation domains. The functional core of one domain consists of a short sequence (the DIDLID element) (Fig. 1*B*) that is specific to SKN-1-related proteins and to the NRF group of CNC proteins. The p300/CBP proteins appear to be direct cofactors of SKN-1, but not to be critical for the activity of the DIDLID element. The conservation and transcriptional function of the DIDLID element suggest that SKN-1 and the NRF CNC proteins share a common transcriptional protein target in addition to p300/CBP and, during evolution, may have maintained some parallel functions in endodermal cells.

EXPERIMENTAL PROCEDURES

Plasmid Constructions—For analysis of full-length SKN-1, a FLAG epitope was added at its NH_2 terminus. A *Spe*I site was first created by polymerase chain reaction, which added a linker of LV prior to the initial methionine, and then the FLAG epitope (DYKNDDDKDP) was added as an R1/*Spe*I fragment prior to cloning into the CMV-based vector CS2 (24). To delete the DIDLID motif (ΔDID), an SKN-1-(113–391) fragment was generated by polymerase chain reaction and then substituted for residues 100–391. Activation domain B was disrupted (ΔAD(B)) by deleting a restriction fragment encoding residues 154–205. The double deletion (ΔDID,AD(B)) was made by swapping appropriate restriction fragments. Each deleted region was sequenced. Point mutations were constructed with the QuickChange kit (Stratagene) and confirmed by sequencing. Gal4-SKN-1 fusions were constructed by creating *Bam*HI/*Xba*I sites at the ends of SKN-1 sequences using the polymerase chain reaction. These were linked to the COOH terminus of the Gal4 DNA-binding domain within pSG424 (25), creating an intervening linker of ISRA. Junctions were sequenced for accuracy. The polymerase chain reaction was performed using *Pfu* polymerase (Stratagene). The SKN-1/TK-CAT reporter was constructed by blunting and multimerizing the SK1 oligonucleotide (4), which corresponds to the preferred SKN-1-binding site. A fragment with four SK1 sites in the same orientation was then ligated into a blunted *Sal*I site in the TK-CAT reporter (26).

Transfections and Analysis—HeLa cells were used in all transfections and maintained in Dulbecco's modified Eagle's medium plus 10% fetal calf serum. Cells were transfected transiently by calcium phosphate for 18 h at 3% CO_2 and then placed in 5% CO_2 and harvested 24–26 h later. Transfections for CAT assays were performed in six-well plates and brought to 10 μg of total DNA with Bluescript. Transfections for Western blotting and electrophoretic mobility shift assays were performed in 100-mm plates using 20 μg of total DNA. CAT assays were performed as described (25). An internal control was omitted because some SKN-1 constructs influenced overall gene expression, but not transfection efficiency (data not shown), presumably through squelching. Samples were normalized for total protein concentration. Six independent values were generated for each data point, of which a representative is shown in each figure. Each fell in the linear range for CAT and represented an average of three experiments unless otherwise indicated, with error bars showing S.D. values. Cotransfection of a β-galactosidase vector revealed that in these assays, E1A expression did not cause detectable apoptosis (data not shown).

Protein Analysis—Lysates for Western and electrophoretic mobility shift assay analyses were prepared as described (27), and SKN-1 was expressed by *in vitro* translation using Promega kits. For Western blotting, proteins were separated on a 7.5% gel and transferred to nitrocellulose membranes (Schleicher & Schüll), which were probed with an anti-SKN-1 monoclonal antibody (a gift of J. Priess) and visualized by enhanced chemiluminescence (Amersham Pharmacia Biotech). Electrophoretic mobility shift assays were carried out as described (9) with an extract equivalent of 10% of a 100-cm plate/lane. Binding of SKN-1 to a ^{32}P-labeled SK1 oligonucleotide (4) was assayed and was competed either by excess unlabeled SK1 DNA or by a mutant site (MSK1) to which SKN-1 does not bind specifically (4). Glutathione *S*-transferase (GST)-p300 proteins were expressed as described (28) and then coupled to glutathione-agarose beads (Sigma) and incubated for 2 h with ^{35}S-labeled SKN-1 in 25 mM Hepes, 250 mM NaCl, 25 mM EDTA, 0.1% Nonidet P-40, and protease inhibitor mixture as described (28). Beads were washed in phosphate-buffered saline plus 0.1% Nonidet P-40 five times, and then proteins were eluted in sample buffer and electrophoresed on an SDS-polyacrylamide gel.

RESULTS

A Short Transactivation Motif Specific to SKN-1 and Some CNC-related Basic Leucine Zipper Proteins—When expressed

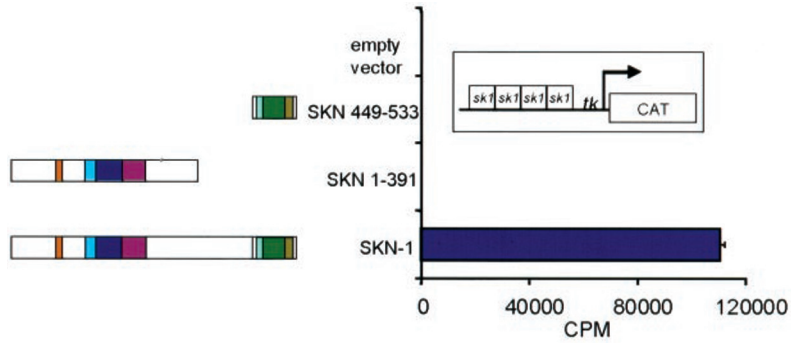

FIG. 2. **Activation of transcription by SKN-1.** Full-length SKN-1 (0.5 μg) and the indicated mutants were assayed by transient transfection for activation of the SKN-1/TK-CAT reporter (2.0 μg). SKN-1 sequence domains are indicated as described in the legend to Fig. 1.

by transfection in human (HeLa) cells, SKN-1 strongly activated a reporter containing four SKN-1-binding sites (SKN-1/TK-CAT) (Fig. 2), but not the corresponding control reporter lacking those sites (data not shown). SKN-1/TK-CAT was not activated by the SKN-1 DNA-binding domain alone (SKN-1-(449–533)) or by SKN-1 residues 1–391, which encompass its similarity to a *C. elegans* SKN-1-related gene (SRG-1) (5) outside of the DNA-binding domain (data not shown) (Fig. 2). When fused to the yeast Gal4 DNA-binding domain, however, residues 1–391 (Gal4-SKN-(1–391)) strongly activated a Gal4-based reporter (E1B-CAT) (Fig. 3*A*) (28). They did not activate a control reporter lacking Gal4 sites (data not shown), suggesting that this SKN-1 region can bind and recruit transcription complexes. Residues 1–448 similarly constituted a powerful activator that was comparable to the viral protein VP16 (Fig. 3*A*).

Outside of their DNA-binding domains, SKN-1, NRF1, and NRF2 are highly related within a short NH_2-terminal motif, which we refer to as "DIDLID" after part of its sequence (Fig. 1, *A* and *B*). This motif is also present in SRG-1 and an SKN-1 homolog in the nematode *Caenorhabditis briggsae* (Fig. 1*B*), but among CNC proteins, it appears to be restricted to NRF1 and NRF2 orthologs (data not shown). The DIDLID element appears to be present only in SKN-1- and CNC-related proteins, and SKN-1 and NRF1 are more closely related within it (92%) (Fig. 1, *B* and *C*) than in their DNA-binding basic regions (86%), suggesting that it has a specific and conserved function. The NRF2 DIDLID element is located adjacent to a domain that is lacking in SKN-1 and that can retain NRF2 in the cytoplasm, but the DIDLID element is not involved in this interaction (29). The DIDLID element includes alternating charged and hydrophobic residues (Fig. 1*B*), but is not predicted to form an amphipathic α-helix. It is reminiscent, however, of short helical protein-protein interaction modules that are involved in transcription, such as the L*XX*LL motif in nuclear receptor co-activators (30, 31) and the LDFS motif in E2A proteins (32), suggesting that the DIDLID element might also have a transcriptional function.

Supporting this idea, activation by the SKN-1 NH_2 terminus was not substantially diminished by COOH-terminal deletions to position 112 (Gal4-SKN-(1–112)), but was abrogated by further removal of the DIDLID element (Gal4-SKN-(1–98)) (Fig. 3*A*). Within residues 1–112, only the 14-residue DIDLID element activated significantly on its own, to ~10% of the level induced by SKN-1-(1–391) or VP16 (Gal4-SKN-(99–112)) (Fig. 3*A*), suggesting that it forms the functional core of the SKN-1 NH_2-terminal activation domain (domain A) (Fig. 1*A*). Deletion of the DIDLID motif from full-length SKN-1 (ΔDID) decreased transcription of SKN-1/TK-CAT by 60% (Fig. 4*A*), but did not impair SKN-1 expression or DNA binding (Fig. 4, *B* and *C*). Transactivation by SKN-1 was comparably decreased by mutation of its third glutamic acid to alanine (D105A), but not arginine (D105R) (Fig. 4*A*), suggesting that the hydrophilicity of this residue might be more important than its charge. In

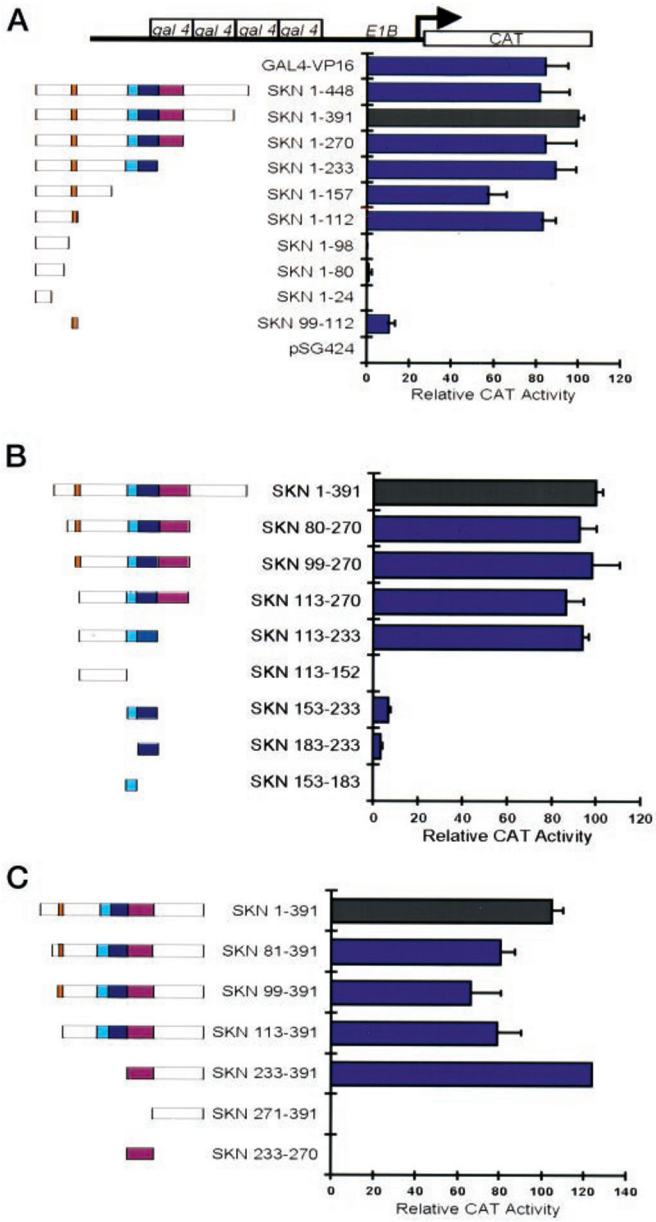

FIG. 3. **SKN-1 activation domains.** *A*, delineation of domain A. The SKN-1 residues shown were fused to the Gal4 DNA-binding domain within the pSG424 vector, These Gal4-SKN-1 fusion expression constructs (100 ng) were assayed by transfection for activation of the Gal4/E1B-CAT reporter (2 μg), which was inactive on its own (not shown). Each experiment was standardized to the activity of Gal4-SKN-(1–391) (100%; shown as *black bars*), for which the counts/min ranged between 80,000 and 200,000. SKN-1 sequence domains are indicated as described in the legend to Fig. 1. *B*, domain B comparison of Gal4-SKN-(1–391) with the indicated Gal4-SKN-1 fusions, assayed as described for *A*. *C*, domain C analysis of COOH-terminal Gal4-SKN-1 deletions, performed as described for *A*.

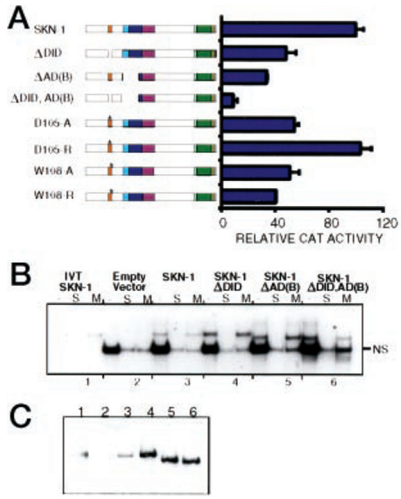

FIG. 4. **Analysis of activation domains in full-length SKN-1.** *A*, activity of SKN-1 mutants. Vectors expressing SKN-1 or the indicated mutants (2.0 μg) were assayed for activation of the SKN-1/TK-CAT reporter (1.0 μg) (see Fig. 2). Sequence domains are indicated as described in the legend to Fig. 1. *B*, DNA binding by SKN-1 mutants. Extracts from cells transfected with the indicated SKN-1 derivatives were tested by electrophoretic mobility shift assay along with *in vitro* translated (*IVT*) SKN-1 for binding to a labeled SKN-1-binding site (4). Only bound complexes are shown, with a nonspecific species indicated by *NS*. In the indicated samples, a 500-fold excess of unlabeled SKN-1-binding site (*S*) or a nonspecific sequence (*M*) was added to the assay mixture. *C*, expression of SKN-1 derivatives, assayed by Western blotting of the six protein samples analyzed in *B*. By this assay, the DIDLID point mutants analyzed in *A* were also expressed comparably to SKN-1 (not shown).

contrast, alteration of the conserved tryptophan to either Ala (W108A) or Arg (W108R) decreased transcription markedly (Fig. 4*A*), indicating that interactions involving the DIDLID element are specific.

Activation of Transcription by Other SKN-1 Regions—Considerable transcriptional activity remained after deletion of the DIDLID element (ΔDID) (Fig. 4*A*), and analysis of internal deletions (Fig. 3*B*) revealed a second SKN-1 activation domain (domain B) (Fig. 1*A*), which is centered around a Glu-rich region (residues 153–183) and a predicted amphipathic α-helix (residues 184–233). The α-helical region alone had independent activity (SKN-1-(183–233)), which appeared to be enhanced by the presence of the Glu-rich region (SKN-1-(153–233) and the residues immediately NH_2-terminal to it (SKN-1-(113–233)) (Fig. 3*B*). Transactivation by full-length SKN-1 was diminished by ~65% by disruption of the Glu-rich and α-helical regions (ΔAD(B)) and by ~90% by concurrent deletion of the DIDLID element (ΔDID,AD(B)) (Fig. 4*A*). Neither deletion impaired SKN-1 expression or DNA binding (Fig. 4, *B* and *C*), indicating that domain B is important for activation of transcription by SKN-1. We investigated the remaining activity of the ΔDID,AD(B) mutant (Fig. 4*A*) by analyzing NH_2-terminal deletions of Gal4-SKN-(1–391) (Fig. 3*C*). Residues 271–391 are weakly similar (20%) to sequences near the NH_2 terminus of NCoA-1 (31), a nuclear hormone receptor coactivator that binds p300/CBP and the p300/CBP-associated factor (PCAF), which is also a histone acetyltransferase (21). Together, the SKN-1/NCoA-1 homology region and downstream sequences constituted a third activation domain: SKN-1-(233–391) (Fig. 3*C*) and domain C (Fig. 1*A*).

Contributions of p300/CBP Proteins to SKN-1 Function—The model that *C. elegans* CBP-1 cooperates directly with SKN-1 predicts that recruitment of p300/CBP would be important for its activation of transcription in human cells. This is particularly likely because CBP-1 is related to p300 throughout its length, especially within its predicted functional domains (20). Supporting this idea, *in vitro* translated SKN-1 bound specifically to GST fusion proteins that contain either the NH_2- or COOH-terminal region of human p300 (Fig. 5*A*), each of which interacts with numerous transcription activators (21). In contrast, SKN-1 did not bind to the p300 center (Fig. 5*A*), which contains the histone acetyltransferase domain and generally does not bind directly to activators (21). SKN-1-dependent transcription was increased by expression of p300 in increasing amounts (up to 7-fold) (Fig. 5*B*) and was decreased by ~80% by expression of the adenovirus E1A 12 S protein (Fig. 5*C*), which binds and inhibits both p300/CBP and PCAF (21, 33–35). SKN-1-dependent transcription was also inhibited by an E1A mutant that does not bind the retinoblastoma (Rb) protein (pM47AI24), but not by one that does not bind p300/CBP (RG2) (Fig. 5*C*).

These findings raise the question of whether p300/CBP proteins are required by the DIDLID element or other SKN-1 activation domains. Coexpression of p300 significantly enhanced the activity of either domain B or domain C, but not that of domain A (Fig. 5*D*), suggesting that p300/CBP protein levels are not limiting for function of the core DIDLID element (Fig. 1*A*). Each SKN-1 activation domain, but not VP16, was inhibited by both E1A and the E1A Rbmut protein (Fig. 5*E*), which does not bind the Rb protein. None were repressed by the E1A ΔCR1 mutant (Fig. 5*E*), which lacks conserved region 1 and does not bind either p300/CBP or PCAF. In contrast, the SKN-1 activation domains differed in the extent to which they were inhibited by E1A mutants that are impaired for binding to either p300/CBP proteins (p300mut) or PCAF (E55) individually. Neither of these last two E1A mutants repressed domain B, but each retained a partial effect on domains A and C (Fig. 5*E*). They also differed from each other in their effects on domain A, which was repressed by ~50% by E1A p300mut (Fig. 5*E*), supporting the idea that the DIDLID element targets a factor that is distinct from p300/CBP.

DISCUSSION

The presence of a DNA-binding domain in SKN-1 (4) and its localization to nuclei (36) suggested previously that SKN-1 is likely to regulate transcription. We have shown here that SKN-1 is a potent activator of transcription when it binds its cognate site (Fig. 2). SKN-1 interacts with two regions of human p300 *in vitro* (Fig. 5*A*), and p300/CBP proteins contribute to its activity (Fig. 5, *B* and *C*). Given the extensive similarity between p300/CBP proteins and their *C. elegans* ortholog CBP-1, these data support the model that SKN-1 may recruit CBP-1 directly to promoters as a cofactor *in vivo* (20). Overexpression of p300 potentiated SKN-1 activation of domains B and C (Fig. 5*D*), suggesting that it can be recruited directly or indirectly by them. Domain B was not inhibited by E1A mutants in which binding to either p300/CBP (p300mut) or PCAF (E55) in particular was impaired (Fig. 5*E*), implicating each of these histone acetyltransferases in its activity. Domain C was partially inhibited by these E1A mutants (Fig. 5*E*), however, suggesting either that it may require both histone acetyltransferases simultaneously or might act on an independent E1A target. The activity of domain A, which contains the DIDLID element (Fig. 1*A*), was not enhanced significantly by p300 expression (Fig. 5*D*), and was inhibited by E1A p300mut (Fig. 5E), suggests that it has a target that is distinct from p300/CBP.

The conservation of the DIDLID element across evolution (Fig. 1, *B* and *C*) supports the model that SKN-1 and the CNC proteins evolved from a common precursor (4). This conservation is particularly striking because the DIDLID motif is separate from the DNA-binding domain and was maintained despite divergences in how these proteins bind DNA (Fig. 1*C*).

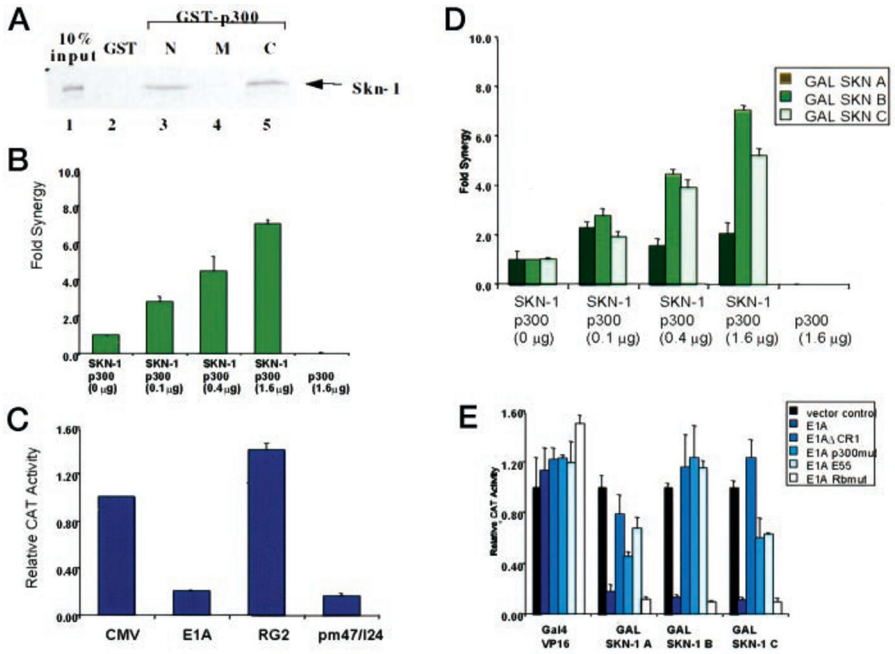

FIG. 5. **Involvement of p300/CBP proteins in SKN-1-driven transcription.** *A*, binding of p300 regions to SKN-1. *In vitro* translated SKN-1 was assayed for binding to GST or GST fusions with human p300 residues 1–596 (*N*), 744–1571 (*M*), or 1572–2370 (*C*) and compared with input protein as described (28). *B*, effect of p300 on full-length SKN-1 activity. Full-length SKN-1 (0.2 μg) was cotransfected with the SKN-1/TK-CAT reporter (2 μg), the indicated amounts of CMV p300 (28), and sufficient empty CMV expression vector to bring the total vector amount to 1.6 μg of CMV. p300 alone did not activate the reporter (not shown). *Synergy* refers to the relative increase in SKN-1 activity (set at 1). *Bars* represent the average of two data points. *C*, inhibition of full-length SKN-1 by E1A. SKN-1 (250 ng) was cotransfected along with 200 ng of CMV E1A, empty CMV vector, or the indicated E1A mutants (28) and assayed for activation of the SKN-1/TK-CAT reporter as described for *B*. *D*, differential dependence of SKN-1 activation domains on p300/CBP. Gal4-SKN-1 domain A (residues 1–112), Gal4-SKN-1 domain B (residues 113–233), and Gal4-SKN-1 domain C (residues 234–391) were transfected at limiting amounts (0.005 μg) in the presence of increasing amounts of CMV p300 and 5 μg of Gal4/E1B-CAT reporter. The total amount of CMV expression vector was brought to 1 6 μg. In this experiment, the activity of each SKN-1 construct was at least 5-fold lower than when 0.1 μg was transfected (see Fig. 3) and was set at 1.0. *Bars* represent an average of four data points. *E*, repression of SKN-1 activation domains by E1A. 2 μg of Gal4/TK-CAT reporter were transfected along with 0.1 μg of SKN-1 effector plasmid and 0.2 μg of Rous sarcoma virus vector or the indicated E1A derivative. The values represent an average of two data points. SKN-1 activation was set at 1, and the reporter plasmid background was essentially 0. Rous sarcoma virus E1A ΔCR1 contains a deletion of E1A residues 40–80 (37); E1A p300mut (TK460) lacks residues 64–68 (38); E1A E55 has Ala substitutions across residues 56–60 (33); and E1A Rbmut (TK496) contains Ala at positions 38–44 (37).

Point mutants in the DIDLID element dramatically decreased SKN-1-driven transcription in mammalian cells (Fig. 4*A*), supporting the idea that it mediates a highly specific interaction that is common to these proteins. In apparent contrast to our findings, an NRF2 fragment that contained the DIDLID element appeared to lack transcriptional activity in Gal4 fusion assays (29). This particular NRF2 fragment also included the inhibitory domain that can retain NRF2 in the cytoplasm (29), however, suggesting that in this context, the DIDLID element might have been masked or not present in the nucleus.

Our findings suggest that SKN-1 and CNC proteins may have preserved some parallel functions in the endoderm and mesoderm. Also supporting this idea, in all of these proteins, hydrophobic residues on the CNC region surface are conserved that are not predicted to influence folding or DNA binding, but instead form a pocket, suggesting a common protein-protein interaction (8). The CNC protein most analogous to SKN-1 may be NRF1, which contains DIDLID (Fig. 1, *B* and *C*) and appears to be involved in endodermal and mesodermal differentiation and regulation of detoxification genes (13, 15, 16). These similarities suggest a particularly intriguing possibility, that the little understood requirement for SKN-1 to maintain the viability of differentiated intestinal cells (5) might involve functions that parallel the role of the NRF1 and NRF2 proteins in antioxidant responses.

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