SNIP1 Inhibits NF-κB Signaling by Competing for Its Binding to the C/H1 Domain of CBP/p300 Transcriptional Co-activators*

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SNIP1 is a 396-amino acid nuclear protein shown to be an inhibitor of the TGF-β signal transduction pathway and to be important in suppressing transcriptional activation dependent on the co-activators CBP and p300. In this report we show that SNIP1 potently inhibits the activity of NF-κB, which binds the C/H1 domain of CBP/p300, but does not interfere with the activity of transcription factors such as p53, which bind to other domains of p300, or factors such as VP16, which are independent of these co-activators. Inhibition of NF-κB activity is a function of the N-terminal domain of SNIP1 and involves competition of SNIP1 and the NF-κB subunit, RelA/p65, for binding to p300, similar to the mechanism of inhibition of Smad signaling by SNIP1. Immunohistochemical staining shows that expression of SNIP1 is strictly regulated in development and that it colocalizes, in certain tissues, with nuclear staining for RelA/p65 and for p300, suggesting that they may regulate NF-κB activity in vivo in a spatially and temporally controlled manner. These data led us to suggest that SNIP1 may be an inhibitor of multiple transcriptional pathways that require the C/H1 domain of CBP/p300.

We recently described a novel nuclear inhibitor of transcription called SNIP1 (Smad nuclear interacting protein 1), which was cloned and characterized on the basis of its ability to inhibit the TGF-β signal transduction pathway (1). TGF-β is the prototypic member of a large family of structurally related cytokines, including the TGF-βs, activins, and bone morphogenetic proteins, which regulate various aspects of cellular activity, including cell growth and differentiation (2, 3). Moreover, these cytokines are involved in the control of important cell functions, including tissue repair, extracellular matrix deposition, and immune response through the transcriptional regulation of a diverse set of gene targets (2, 4). To date, Smad proteins are the only identified direct transducers of signals from TGF-β family members, being phosphorylated by the type I receptor kinases and then translocated to the nucleus in a complex with the common mediator, Smad4, to regulate transcription of target genes (5, 6). A yeast two-hybrid screen designed to identify proteins interacting with Smad1, a receptor-activated Smad downstream of the bone morphogenetic protein receptors, identified the C-terminal domain of SNIP1 (1). This C-terminal portion of SNIP1 (SNIP1-C), which contains a forkhead-associated domain (7), has been shown to have no discernible biological activity, to date. However, the N-terminal domain, SNIP1-N, which contains a bi-partite nuclear localization signal, was shown to strongly inhibit Smad-dependent transcriptional activity by competing with Smad4 for binding to the co-activator CBP/p300 (1). This competitive mode of transcriptional regulation suggests that expression of SNIP1 might set a threshold for the TGF-β response in a particular cell type based on the ratio of SNIP1 to nuclear Smad4 and on levels of the transcriptional co-activators CBP/p300.

The nuclear proteins CBP and p300 have been shown to be essential for transcriptional activating activity of a large number of structurally and functionally diverse transcription factors that include the Smad proteins (8–11). Although CBP was first isolated as a cofactor for the transcription factor CREB (12), and p300, by its association with the adenoviral-transforming protein E1A (13), the two proteins are now considered to be orthologous (14). They act as crucial scaffolds to bring together transcription factors from the upstream region of the promoter with basal transcriptional machinery in the transcriptional initiation complex. As such, they function as essential co-activators for a wide variety of transcription factors, physically linking them to the basal transcriptional machinery. In addition to possessing intrinsic histone acetyltransferase activity, these proteins are also able to interact with other histone acetyltransferases such as pCAF and steroid receptor co-activators (15). CBP and p300 also contain three highly conserved cysteine-histidine-rich zinc finger motifs (C/H1, C/H2, and C/H3), which constitute the binding sites for a wide variety of transcription factors, including the Smad proteins. Thus, the C/H1 domain was shown to interact with a unique domain of Smad4 essential for its transactivating activity and called the Smad activation domain (SAD) of Smad4 (11), whereas the C/H3 domain is the locus of interaction with the MH2 domain of receptor-activated Smads (8, 10, 16).

The binding of SNIP1 to the C/H1 domain of these co-activators suggested to us that transcriptional inhibition by SNIP1 might not be limited to Smad complexes but might also extend to other transcription factors that bind to the same domain,
such as nuclear factor-κB (NF-κB)/Rel (14). NF-κB proteins are dimeric, sequence-specific transcription factors that regulate cellular responses to pro-inflammatory stimuli as well as to external stresses such as ionizing radiation (17). Five members of the NF-κB/Rel family have been identified in mammalian cells, including NF-κB1 (p50 and its precursor p105), NF-κB2 (p52 and its precursor p100), c-Rel, RelA/p65, and RelB (18, 19, 20), with RelA/p65/p50 constituting the most abundant heterodimers in cells. Of these family members, only RelA/p65, RelB, and c-Rel have transcriptional activation domains, and only RelA/p65 and c-Rel have been implicated in embryogenesis (21, 22). NF-κB is retained in the cytoplasm in an inactive form by its interaction with the inhibitory protein IκB. Upon activation of the pathway, IκB is phosphorylated by members of the recently identified IκB kinase family, which signal the ubiquitination of IκB and trigger its subsequent degradation by the 26S proteasomal pathway. The degradation of IκB unmasks the nuclear localization signal of NF-κB, which then translocates to the nucleus to activate transcription (18–20).

In this report we show that SNIP1 is likely an inhibitor of multiple transcriptional pathways that require the C/H1 domain of CBP/p300. We demonstrate that, although SNIP1 does not inhibit the activity of transcription factors that transactivate through other domains of p300 or that possibly utilize p300 indirectly, such as p53, Sp1, or the herpes simplex virion protein 16 (VP16 (23–26)), it potently inhibits the activity of NF-κB, which requires the C/H1 domain of CBP/p300. Moreover, inhibition of NF-κB activity is a function of the N-terminal domain of SNIP1 and involves competition of SNIP1 and the NF-κB subunit, RelA/p65, for binding to p300, as well as direct binding and sequestration of RelA/p65 by SNIP1, similar to the mechanism previously described for inhibition of Smad signaling by SNIP1. We also show that, during mouse embryogenesis, the expression of SNIP1 protein is strictly regulated, overlapping with nuclear staining for RelA/p65 and p300 in certain tissues, consistent with our hypothesis that it may regulate access of a specific subset of transcription factors to the co-activators p300 and CBP in selected cells, thus imparting another level of regulation of patterns of gene expression in embryogenesis.

EXPERIMENTAL PROCEDURES

Cell Lines, Antibodies, and Expression Constructs—Mouse mammary gland NMuMg cells and human embryonic kidney (HEK) 293 cells were maintained in Dulbecco’s modified Eagle’s medium (Life Technologies) with 10% fetal bovine serum (Life Technologies) and antibiotics (100 units of penicillin and 100 μg of streptomycin per ml). Antibodies used in these experiments were as follows: mouse monoclonal α-α-HA (clone 12CA5), rabbit polyclonal α-p300 (clone N15), α-p300 (clone C20), Santa Cruz Biotechnology, Santa Cruz, CA), α-p65 (1226) (27), and rabbit polyclonal α-NF-κB p65 (Santa Cruz catalog number sc-109). Antibody against SNIP1 (rabbit polyclonal 1413-B) was a kind gift of Dr. Steve Tronick (Santa Cruz Biotechnology). Gal4-p300 constructs used for luciferase assays were previously described (28). Gal4-p65 (29); Gal4-p53, Gal4-VP16, and Gal4-Sp1 (28); pGL-2kB (30); HIV NF-κB luciferase (31); and 4X AP-1-Luc (32) have been previously described.

Immunoprecipitation and Western Blot—To detect interactions of transfected proteins in vivo, 293 cells were transfected with the indicated constructs, using the calcium phosphate method (33). After 24 h, cells were switched to 0.2% serum overnight and lysed in 0.5 ml of Triton X-100 lysis buffer B (25 mM Hepes, pH 7.5; 150 mM NaCl; 10% glycerol; 5 mM EDTA; 1% Triton X-100) in the presence of phosphatase and protease inhibitors. Lysates were either directly separated by SDS-polyacrylamide gel electrophoresis and transferred onto Immobilon-P membrane (Millipore), and/or first immunoprecipitated with the indicated antibody. Proteins were detected by Western blotting using the appropriate horseradish peroxidase-conjugated secondary antibody, and visualized by chemiluminescence (Pierce).

In Vitro Binding Assay—GST fusion protein constructs expressing the various SNIP1 deletion proteins were expressed in bacteria, quantitated by SDS-PAGE, and stained using CLECODE Blue Stain (Pierce) following affinity purification on glutathione beads (Amersham Pharmacia Biotech). Equal amounts of fusion protein were incubated in Triton X-100 lysis buffer B with in vitro transcribed and translated full-length RelA/p65 made with TnT Master mix (Promega) according to the manufacturer’s protocol. Products were incubated with the SNIP1 GST fusion proteins for 2 h, washed with the same lysis buffer, separated on SDS/PAGE gels, and subjected to autoradiography using X-Omat MR films (Kodak).

Recombinant protein expression and GST pull down experiments using GST RelA/p65 (1–306) (GST p65(RHD)) and GST RelA/p65 (428–551) (GST p65(TAD)) were performed essentially as described previously (1). GST RelA/p65 (428–551) (GST p65(RHD)) has been described previously (29, 34). GST RelA/p65 (428–551) (GST p65(TAD)) was created by subcloning the corresponding fragment of RelA generated by PCR into pGEX CD1, a form of pGEX KT (29, 35) with a modified polylinker.

Transcriptional Assays—The reporter construct pGSE1B, which contains the Gal4 recognition sites driving expression of the luciferase gene, was co-transfected into NMuMg cells together with various constructs expressing truncated SNIP1 proteins and/or expression vectors for fusion proteins of the Gal4 DNA-binding region linked to p300, RelA/p65, p50, Sp1, and VP16. Two different NF-κB-responsive reporter vectors (pGL-2x-b and HIV NF-κB Luc) were used to test the ability of SNIP1 to inhibit the activation of these promoters by either p53, expression vectors for NF-κB, or with 10 ng/ml TNF-α, respectively, in NMuMg cells. Transfection was done using LipofectAMINE (Life Technologies, Inc.) in NMuMg cells and the calcium phosphate method in 293 cells (36). Treatment with TNF-α was carried out in 0.2% serum for 16–24 h prior to harvest. Luciferase assays were performed as previously described, co-transfecting pSV-b-galactosidase to allow for normalization of transfection efficiency (37). The total amount of transfected DNA was standardized by addition of pcDNA3 control vector as required. All assays were performed in triplicate and represented as the mean (±S.E.) of three independent transfections.

Immunohistochemistry—SNIP1 protein was localized on 5-μm sections of formalin-fixed, paraffin-embedded tissues from either mouse 14- or 18-day mouse embryos or 5-day neonates. The staining was performed using the Optimax Plus 2.0 automated cell staining system with research software from BioGenex (San Ramon, CA). Following deparaffinization and blocking of endogenous peroxidase, nonspecific protein binding was blocked with a solution containing 1% bovine serum albumin and 5% goat serum for 1 h. Sections were incubated for 2 h with affinity-purified anti-SNIP1, anti-NF-κB p65 (Santa Cruz), or anti-p300 (clone N15) at 5 μg of IgG/ml in TBS/1% bovine serum albumin. Rabbit IgG (5 μg/ml) was used as a negative control. Antibody-antigen complexes were detected using the Vectastain Elite ABC peroxidase kit (Vector Laboratories) according to the manufacturer’s instructions. After a 30-min incubation with biotinylated secondary antibody, and a 30-min incubation with ABC reagent, a 5-min reaction with H2O2 (BioGenex) was used to detect the bound peroxidase. Carazzi 3,3’-diaminobenzidine hematoxylin was used as the counterstain.

RESULTS

SNIP1 Inhibits the Activity of Transcription Factors, Which Require the C/H1 Domain of CBP/p300—We previously demonstrated that SNIP1 is able to suppress the transcriptional activity of Smad4. Moreover, this transcriptional inhibition was dependent on the ability of SNIP1 to compete with Smad4 for binding to the C/H1 domain of CBP/p300 (1). To refine the region on which SNIP1 exerts its action on p300, we assessed the ability of various domains of SNIP1 to inhibit the transcriptional activity of various domains of p300 fused to the DNA-binding domain of the yeast transcriptional activator Gal4. As shown in Fig. 1A, SNIP1-FL and SNIP1-N inhibit the activity of Gal4-p300 (full-length) and Gal4-p300 (aa 192–700), both of which contain the C/H1 domain. However, Gal4-p300 (aa 192–700) does not inhibit the activity of Gal4-p300 (aa 1700–2414), containing only the C/H2 and C/H3 domains. These data confirm that SNIP1 is a specific inhibitor of the C/H1 domain of p300.

Because SNIP1 had previously been shown to inhibit the activity of Gal4-p65 (1), a heterologous fusion protein containing only the transcriptional activation domain (TAD) of RelA/p65 (304–551), which interacts with the N-terminal (C/H1) domain of p300 and CBP (38), we next investigated which
domain of SNIP1 would harbor this inhibitory activity. Both SNIP1-FL and SNIP1-N, but not SNIP1-C, inhibited the activity of Gal4-p65 (Fig. 1B), analogous to the pattern previously observed for inhibition of Smad activity by SNIP1 (1). Specificity of this inhibition was shown by lack of effect of either SNIP1-FL or SNIP1-N on the transcriptional activity of Gal4-p53, Gal4-Sp1, or Gal4-VP16, each of which are independent of the N-terminal regions of CBP/p300 but depend instead on C-terminal domains, including the bromodomain and the C/H3 domain (23–25) (Fig. 1B). Inhibitory effects of SNIP1 on the activity of Gal4-p65 were shown to be specific and dose-dependent, with concentrations of SNIP1-FL that inhibit Gal4-p65 by 75% having no effect on the transcriptional activity of Gal4-p53 (Fig. 1C). Together, these results demonstrate that SNIP1 inhibits the C/H1 domain of p300 and possibly a subset of transcription factors that require this domain of p300.

To assess whether SNIP1 would also inhibit full-length RelA/p65 and not simply its TAD, we examined the effects of SNIP1 on the reporter pGL-2×B, which contains two copies of the NF-κB binding site linked to a basal promoter. Luciferase activity of pGL-2×B activated by co-transfection of p50 and RelA/p65 expression vectors was inhibited by SNIP1-FL and SNIP1-N, whereas SNIP1-C again had no effect (Fig. 2). Activation of this reporter construct was inhibited by co-transfection of E1A, but not the mutant E1A, which is unable to bind to p300, suggesting that it is p300-dependent (Fig. 2). Activation of a naturally occurring NF-κB-responsive promoter construct, HIV-NF-κB-luciferase, with TNF-α, which activates endogenous NF-κB (39), was also strongly inhibited by both SNIP1-FL and SNIP1-N, but not SNIP1-C (Fig. 3A). Consistent with the proposed mechanism of competition between SNIP1 and RelA/p65 for binding to the C/H1 domain of p300, overexpression of p300 was able to reverse the inhibition of this promoter by SNIP1 (Fig. 3B). Further supporting the proposed mechanism, the C-terminal domain of p300, spanning amino acids 1700–2414 and lacking the C/H1 domain, which binds SNIP1, was unable to reverse the inhibition (Fig. 3B). These data show that SNIP1 is able to inhibit the NF-κB signal transduction path-
way and that this inhibition can be rescued by the addition of the C/H1 domain of p300, thus demonstrating that the transcriptional inhibition by SNIP1 occurs not only in the TGF-β signal transduction pathway but also in other pathways, such as the NF-κB, that similarly require the C/H1 domain of p300.

**SNIP1 Interferes with the Interaction of RelA/p65 and CBP/p300**—Because SNIP1 was shown to inhibit Smad-dependent signaling by competing with Smad4 for binding to the C/H1 domain of p300, and because the RelA/p65 subunit of NF-κB also recruits p300 to its transcriptional activation complex through the C/H1 domain (38), we investigated whether the mechanism whereby SNIP1 suppresses the transcriptional activation of the TNF-α/NF-κB signal transduction pathway also involved direct competition for binding to p300. HEK293 cells were transfected with RelA/p65 and increasing amounts of SNIP1, and the interaction between RelA/p65 and endogenous p300 was examined. Immunoprecipitation of endogenous p300 followed by Western blotting with an anti-RelA/p65 antibody to detect transfected and endogenous RelA/p65 showed that overexpression of SNIP1 diminished the interaction between p300

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**FIG. 2.** SNIP1 inhibits transcriptional activation of an NF-κB reporter. HEK293 cells were co-transfected with 50 ng of pGL-2κB/well of a 6-well plate and 1 μg/well of HA-SNIP1 constructs, E1A or an E1A mutant. Expression vectors for p50 and RelA/p65 subunits of NF-κB complex were either not added (white bars) or added (black bars) at a final concentration of 4 μg each/well and analyzed for luciferase activity. Results are expressed as means (±S.D.) of triplicate assays, normalized for transfection efficiency using β-galactosidase activity.

**FIG. 3.** SNIP1 inhibits transcriptional activation of the HIV-NF-κB reporter by TNF-α and this inhibition is reversed by p300. A, HEK293 cells were co-transfected with 50 ng of HIV NF-κB-luciferase with indicated amounts of HA-SNIP1 and its deletions. TNF-α was either not added (white bars) or added (black bars) at a final concentration of 10 ng/ml, 24 h before lysis and analyzed for luciferase activity. B, HEK293 cells were co-transfected with 50 ng of HIV NF-κB-luciferase with indicated amounts of HA-SNIP1 alone or with indicated amount of p300 expression vectors. TNF-α was either not added (white bars) or added (black bars) at a final concentration of 10 ng/ml, 24 h before lysis and analyzed for luciferase activity. All results are expressed as means (±S.D.) of triplicate assays, normalized for transfection efficiency using β-galactosidase activity.
and RelA/p65 in a dose-dependent manner (Fig. 4A). SNIP1-N was as effective as full-length SNIP1 in preventing the RelA/p65–p300 interaction, whereas SNIP1-C had little effect (Fig. 4B). These data suggest that transcriptional inhibition by SNIP1 is dependent on its ability to compete with particular transcription factors for binding to the C/H1 domain of p300.

The N Terminus of SNIP1 Interacts Directly with the N-terminal RHD Domain of RelA/p65—To investigate whether SNIP1 and RelA/p65 might interact with each other directly, in addition to their ability to compete for binding to a common site on CBP/p300, we utilized in vitro binding assays with in vitro transcribed/translated SNIP1 and RelA/p65 (Fig. 5A). As shown in Fig. 5B, SNIP1 interacts with full-length RelA/p65 through its N-terminal, nuclear localization signal-containing domain (GST-

The complementary experiment was done to determine the region of RelA/p65, which binds SNIP1. The N-terminal Rel homology domain (RHD; GST-p65(1–306)), but not the C-terminal TAD (GST-p65(428–551)) interacted with full-length in vitro transcribed/translated SNIP1, whereas luciferase, used as a negative control, showed no interaction (Fig. 5C). These data show that SNIP1 can directly bind the N-terminal RHD of RelA/p65 in addition to its ability to compete for the binding of the C-terminal RelA/p65 activation to the C/H1 region of CBP/p300.

Expression of SNIP1 Is Highly Regulated during Development—Because these data suggest that SNIP1 can attenuate signaling through several different pathways that converge on transcription factors, which bind to the C/H1 domain of CBP/p300, we investigated whether expression of SNIP1 might be regulated during development, where it has been shown that signaling is often orchestrated in a tempo-spatial-specific pattern. Immunohistochemical staining of selected tissues of mouse embryos and neonates with antibodies shown to be specific for SNIP1 (1) showed that expression of endogenous SNIP1 is tightly regulated in vivo. In general, the staining seen in tissues correlates with the pattern of SNIP1 mRNA expression seen in Northern blots (1), with the highest expression in muscle and lowest in lung (not shown). A role for SNIP1 as a regulator of differentiation is suggested by the striking increase in levels of expression in tissues such as intestine (Fig. 6, D–F) and liver (not shown) of the neonate as compared with its expression in 14- or 18-day embryos. For example, very few epithelial cells in the gut of the 14- or 18-day mouse embryo express SNIP1 (not shown), whereas ∼30% of the epithelial cells in the intestinal villi of the neonate show nuclear staining for SNIP1 (Fig. 6D). In contrast to the liver, kidney, and intestine, where SNIP1 expression increases as the cells differentiate, in the skin of 18-day embryos, SNIP1 staining is seen predominantly in the less differentiated, proliferating basal cells of the epidermis (Fig. 6A). Similarly, in the growth plate of the femur of 5-day-old mice, staining for SNIP1 is seen primarily in resting chondrocytes and decreases as cells differentiate (data not shown).

To examine whether the expression patterns of CBP/p300 and RelA/p65 might be similar to that of SNIP1, skin of 18-day embryos and intestine of 5-day neonates tissues with prominent staining for SNIP1 were stained with antibodies against p300 and RelA/p65. In the intestinal epithelium, nuclear staining for p300 and RelA/p65 showed a pattern similar to that observed for SNIP1 (Fig. 6, D–F). In the skin, a highly selective and strongly overlapping pattern of nuclear staining of SNIP1 and p300 is seen in the basal cells of the epidermis (Fig. 6, A and B). Whereas staining for cytoplasmic RelA/p65 is strong in the more differentiated stratum spinosum, nuclear staining, likely to correlate with transcriptional activity, is seen in the basal cells of the epidermis as well as in the dermis. Together, these data suggest that the competition of binding of RelA/p65 and SNIP1 for p300/CBP characterized in vitro might have significance in development and, by inference, also in disease.

DISCUSSION

The transcriptional co-activators CBP and p300 interact with a wide variety of transcriptional activators and repressors through distinct domains to provide a physical link between these factors and the basal transcriptional machinery, including TFIIA, TFIIB, and TFIID (14). CBP/p300 also recruit other histone acetyltransferases such as p/CAF and steroid receptor co-activators to the transcriptional complex and coordinate transcriptional pathways with chromatin remodeling via their intrinsic histone acetyltransferase activity (15). Of three cysteine-histidine-rich zinc finger motifs, the C/H1 and C/H3 do-
mains and the CREB-binding domain are the sites of interaction of most transcription factors with these co-activators. The C/H1 domain has been identified as the site of interaction of many transcriptional activators, including Smad4, RelA/p65, MRG1, Ets-1, and STAT-2 (11, 14, 40, 41), and we have now shown that this domain binds the transcriptional inhibitor SNIP1 (1). Although p53 has also been shown to bind to this domain, recent data suggest that this binding, which occurs through its core domain, is important for the stability of p53, contributing to regulation of its nuclear export and degradation via a ubiquitin-mediated pathway; the transactivating activity of p53 is dependent on binding of its transactivation domain to the C/H3 domain of p300, consistent with the lack of SNIP1 inhibition of Gal4-p53 (23).

To our knowledge, SNIP1 is the only transcriptional repressor binding to the C/H1 domain of p300. As we demonstrated previously, the binding of SNIP1 to the C/H1 domain of p300/ CBP can compete with the binding of the transcription activation SAD domain of Smad4 (11), resulting in inhibition of TGF-β-dependent signaling (1). We now show that the inhibitory activity of SNIP1 is not limited to the TGF-β pathway, in that NF-κB-dependent transcription, which is dependent on binding of its RelA/p65 subunit to the C/H1 domain of CBP/p300, is also inhibited by SNIP1. This inhibition is based on the ability of SNIP1 to compete for binding of the RelA/p65 subunit to CBP/p300, as well as, on the ability of SNIP1 to bind RelA/p65 directly through its N-terminal RHD (Fig. 7). The role of the direct binding of SNIP1 to the RHD is unclear at present, because our data show that SNIP1 and SNIP1-N also inhibit the activity of Gal4-p56Δ, which lacks this domain but which retains the CBP/p300-interacting domain (Fig. 1, B and C). The ability of overexpressed p300 to overcome the inhibition by SNIP1 of an NF-κB-responsive reporter gene further supports the importance of this competition in the mechanism of inhibition (Fig. 3B). Also consistent with the hypothesis that SNIP1 inhibits by competing for binding of transcription factors requiring the C/H1 domain of CBP/p300 is the ability of SNIP1 to inhibit activation of an AP-1 reporter construct (4X-AP-1-Luc).
c-Jun, a component of the AP-1 complex, interacts with the C/H1 domain of p300 and requires p300 for its transcriptional activity (42). Based on these data, we propose that SNIP1 may serve as a potent inhibitor of certain other transcription factors similarly dependent on binding to the C/H1 domain of CBP/p300.

The mechanism of transcriptional inhibition by SNIP1 is distinct from that of other transcriptional inhibitors such as TGIF, a homeodomain protein that acts in part via its ability to recruit histone deacetylases to the transcriptional complex (43, 44), or the oncogenes Ski and SnoN (45–48), which also bring histone deacetylases to the complex via their ability to recruit the nuclear corepressor, NcoR. Rather, our data suggest that the binding of the N-terminal domain of SNIP1 to CBP/p300 can compete with the binding of transcriptional activators such as Smad4 or RelA/p65 to this same domain. Therefore, expression of SNIP1 confers a level of regulation that is highly contextual, being dependent not only on the relative levels of SNIP1 and the transcriptional activators, such as Smad4 and RelA/p65, but also on the cellular levels of CBP/p300.

Recent data suggest that the demand for CBP/p300 exceeds its supply and that competition for binding to CBP/p300 is rate limiting in certain instances, playing an important role in growth regulation (49). Indeed, CBP/p300 has been shown to mediate antagonistic cross-talk between various signal transduction pathways such as AP-1 and nuclear hormone receptors (50), AP-1 and JAK/STAT pathways (51), or NF-κB and p53 (33). Overexpression of CBP/p300 relieves this negative cross-talk, suggesting that competition for binding to CBP/p300 plays a role in determining which pathways predominate in terms of gene activation. Our data now show that binding of SNIP1 to the C/H1 domain of CBP/p300 can regulate the availability of these co-activators in a sequence-specific manner,
rendering them unavailable to a subset of transcriptional activators that bind to that same region. Overexpression of CBP/p300 can alleviate this competition and restore transcriptional activation, similar to the antagonistic cross-talk between signaling pathways, which is also dependent on limiting amounts of the co-activators (50). Conversely, overexpression of SNIP1 in the presence of a constant level of CBP/p300 and transcription factor (NF-κB or Smad4) suppresses binding of RelA/p65 (Fig. 4A) or Smad4 (1) to the co-activators in a dose-dependent manner and correlates with suppressive effects on transcription. Factors such as SNIP1 might thus serve as an additional mechanism to fine-tune the transcriptional activating activity of these proteins, resulting in suppression of certain pathways while being permissive for others.

Developmental processes are highly regulated, and a wealth of data now demonstrates that mechanisms exist for the selective activation or inhibition of particular pathways in a temporal and spatially specific manner. In Drosophila, Rel/NF-κB homologues, Dorsal, Dif, and Relish, have been shown to regulate embryonic pattern formation, muscle development, immunity, and hematopoiesis (21, 32), and in avian embryos, c-Rel plays a critical role in patterning of the developing limb bud (22, 32). Mice null for RelA/p65 die by about embryonic bud (22, 32). Mice null for RelA/p65 die by about embryonic

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