Coactivators and Corepressors of NF-κB in IκBa Gene Promoter*

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In this study, we investigated recruitment of coactivators (SRC-1, SRC-2, and SRC-3) and corepressors (HDAC1, HDAC2, HDAC3, SMRT, and NCoR) to the IκBa gene promoter after NF-κB activation by tumor necrosis factor-α. Our data from chromatin immunoprecipitation assay suggest that coactivators and corepressors are simultaneously recruited to the promoter, and their binding to the promoter DNA is oscillated in HEK293 cells. SRC-1, SRC-2, and SRC-3 all enhanced IκBα binding to the promoter DNA is oscillated in HEK293 assay. The data suggest that after NF-κB activation by TNF-α, SRC-1 and SRC-2 exhibit different patterns. After tumor necrosis factor-α treatment, SRC-1 signal was increased gradually, but SRC-2 signal was reduced immediately, suggesting replacement of SRC-2 by SRC-1. SRC-3 signal was increased at 30 min, reduced at 60 min, and then increased again at 120 min, suggesting an oscillation of SRC-3. The corepressors were recruited to the promoter together with the coactivators. The binding pattern suggests that the corepressor proteins formed two types of corepressor complexes, SMRT-HDAC1 and NCoR-HDAC3. The two complexes exhibited a switch at 30 and 60 min. The functions of cofactors were confirmed by gene overexpression and RNA interference-mediated gene knockdown. These data suggest that gene transactivation by the transcription factor NF-κB is subject to the regulation of a dynamic balance between the coactivators and corepressors. This model may represent a mechanism for integration of extracellular signals into a precise control of gene transactivation.

Transcriptional activity of NF-κB is regulated by transcription coactivators and corepressors, which are originally identified for nuclear receptors. The coactivators of NF-κB include p300/CBP,1 p/CAF, and p160 proteins (SRC-1, SRC-2, and SRC-3) (1–8). CBP/p300 and the p160 proteins have been shown to be involved in transactivation, and p/CAF is involved in transactivation by NF-κB50 (2). Interaction of p65 with p300/CAF requires p65 Ser276 phosphorylation (10), and Ser276 mutation inhibits p65 function (11). All of the three p160 proteins have been reported to participate in the transcriptional activation mediated by NF-κB; however, the relative importance of each isoform in NF-κB-mediated gene transcription remains to be investigated. Recent studies suggest that the function of a coactivator is not universal. It is determined by at least two factors: transcription factors and the promoter context. A coactivator may act as a corepressor in certain gene environments (12), and a corepressor (NCoR) has been reported to act as a coactivator in the regulation of gene transcription (13). To understand the relative importance of p160 proteins, we investigated the time course of p160 interaction with IκBα promoter by using the chromatin immunoprecipitation assay (ChIP) assay. The data suggest that after NF-κB activation by TNF-α, SRC-1 and SRC-2 exhibit opposite patterns of association with IκBα promoter. The SRC-3 is different from SRC-1 and -2 in that it exhibits an unique oscillation in association with the IκBα gene promoter.

The components of corepressor complex for NF-κB include SMRT, NCoR, HDAC1, HDAC2, and HDAC3 (14–19). In these corepressor proteins, SMRT and NCoR do not have an enzymatic activity, but they can trigger the catalytic activity of histone deacetylase for deacetylation of histone proteins (20). Although intracellular distribution of SMRT and NCoR is regulated by different signaling pathways (21), these two proteins are interchangeable in the inhibition of NF-κB activity. HDAC1–3 belong to the class I histone deacetylases that include HDAC1, -2, -3, -8, and -11 (22). The class II histone deacetylases include HDAC4, -5, -6, -7, -9, and -10. The catalytic activity of HDACs is required for deacetylation of histones and transcription factors in the regulation of transcription. HDAC1, HDAC2, and HDAC3 have all been reported to inhibit NF-κB; however, their roles in the regulation of NF-κB activity are highly controversial. HDAC1 and HDAC3 were shown to be

intrinsically associated with NF-κB and histone deacetylase activity, which is necessary to open the chromatin structure through an acetylation-induced conformation change in histone protein. However, the function of p160 protein is dependent upon CBP/p300 as p160 protein exhibits much less activity in the absence of CBP/p300 (9).

The most common active form of NF-κB is a heterodimer of two subunits, p65 (RelA) and p50 (NF-κB1). The subunit p65 contains an activation domain that binds to the coactivators for transcription initiation. The subunit p50 does not have an activation domain, but p50 can activate gene transcription through BCL3. The two subunits exhibit a different preference for p300/CAF and p/CAF. p300/CAF is required for p65-mediated transactivation, and p/CAF is involved in transactivation by NF-κB50 (2). Interaction of p65 with p300/CAF requires p65 Ser276 phosphorylation (10), and Ser276 mutation inhibits p65 function (11). All of the three p160 proteins have been reported to participate in the transcriptional activation mediated by NF-κB; however, the relative importance of each isoform in NF-κB-mediated gene transcription remains to be investigated. Recent studies suggest that the function of a coactivator is not universal. It is determined by at least two factors: transcription factors and the promoter context. A coactivator may act as a corepressor in certain gene environments (12), and a corepressor (NCoR) has been reported to act as a coactivator in the regulation of gene transcription (13). To understand the relative importance of p160 proteins, we investigated the time course of p160 interaction with IκBα promoter by using the chromatin immunoprecipitation assay (ChIP) assay. The data suggest that after NF-κB activation by TNF-α, SRC-1 and SRC-2 exhibit opposite patterns of association with IκBα promoter. The SRC-3 is different from SRC-1 and -2 in that it exhibits an unique oscillation in association with the IκBα gene promoter.

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1 The abbreviations used are: CBP, CREB-binding protein; CREB, cAMP-response element-binding protein; P/CAF, p300/CBP-associated factor; SRC, steroid receptor coactivator 1; ChIP, chromatin immunoprecipitation; TNF-α, tumor necrosis factor alpha; HDAC, histone deacetylase; NCoR, nuclear receptor corepressor; SMRT, silencing mediator for retinoid and thyroid hormone receptor; p/CAF, p300/CBP/co-integrator-associated protein; RNAi, RNA interference; Pol II, RNA polymerase II.

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involved in the inhibition of NF-κB activity (14, 18, 19, 23–26), but the relationship of the two deacetylases remains to be determined. We have investigated this issue using ChIP assays and evaluated their functions in the IkBa gene promoter. Our data suggest that HDAC1 and HDAC3 are both recruited to the IkBa promoter after NF-κB activation, but they can substitute for each other in a time-dependent manner.

**EXPERIMENTAL PROCEDURES**

**Cells and Reagents—**HEK293 cells were purchased from the American Type Culture Collection (ATCC). The cells were maintained in the Dulbecco's modified Eagle's medium culture medium supplemented with 5% fetal calf serum. Antibodies to IkBa (catalog number sc-371), p65 (sc-8008), p50 (sc-8414), SRC1 (sc-8995), SRC3 (sc-9119), SMRT (sc-1610), NCoR (sc-8994), and polymerase II (pol II) (sc-9001) were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). β-Actin (catalog number ab6265, HDAC2 (ab1770), and HDAC3 (ab2379) antibodies were obtained from Abcam (Cambridge, UK). HDAC1 antibody (catalog number H 6287) was from Sigma. SRC2 antibody (catalog number 06-986) was obtained from Upstate Biotechnology (Lake Placid, NY). The SRC-1 and SRC-3 expression vectors were kindly provided by Dr. Bert W. O'Malley at the Baylor College of Medicine. The SRC-2 (GRIP-1) vector was a gift from Dr. Michael R. Stallcup (University of Southern California). SMRT and NCoR, together with their RNAi expression vectors, were used as reported previously (27). HDAC1, HDAC2, and HDAC3 together with their RNAi expression vectors have been described elsewhere (28).

**Western Blotting—**Cells were treated with 20 ng/ml TNF-α after serum starvation in 0.5% bovine serum albumin-containing cell culture medium (29). Whole cell lysate protein was made in lysis buffer (1% Triton X-100, 50 mM KCl, 25 mM HEPES, pH 7.8, 10 µg/ml leupeptin, 20 µg/ml aprotinin, 125 µM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, 1 mM sodium orthovanadate) via sonication. The protein (100 µg) was boiled for 3 min, resolved in 6% mini-SDS-PAGE for 90 min at 100 volts, and blotted on the polyvinylidene difluoride membrane (catalog number 162-0184, Bio-Rad). After being preblotted in milk buffer for 20 min, the membrane was blotted with the first antibody for 1–24 h and the secondary antibody for 30 min. The horseradish peroxidase-conjugated secondary antibodies (catalog number NA934V or NA931, Amersham Biosciences) were used with chemiluminescence reagent (catalog number NEL-105, PerkinElmer Life Sciences) for generation of light signal. To detect multiple signals from one membrane, the signals were blotted with the same membrane using the mouse IkBa promoter (1–1 kb) in which four NF-κB binding sites have been identified. In all of the transient transfections, the internal control reporter is 0.1 µg/well of SV40-Renilla luciferase reporter plasmid, and the total DNA concentration was equalized in each well with a control plasmid. The luciferase assay was conducted using a 96-well luminometer with the dual luciferase substrate system (Promega). The luciferase activity was normalized with the internal control Renilla luciferase, and a mean value together with a standard error of the triplicate samples was used to determine the reporter activity. Each experiment was repeated at least three times.

**Statistical Analysis—**Each experiment was conducted at least three times with consistent results. The representative gel or blot from each experiment is presented in this study. In a reporter assay, a mean value and standard deviation of the triplicates were used to represent the reporter activity. The data were analyzed using student’s t test with significance $p < 0.05$.

**RESULTS AND DISCUSSION**

**SRC-1 Is a Coactivator for NF-κB in IkBa Promoter—**SRC-1 was the first 160-kDa nuclear receptor coactivator identified and is also known as nuclear coactivator 1 (NCoA-1) (32). Although SRC-1 has histone acetylase activity, its function is dependent on CBP (33, 34). SRC-1 facilitates transactivation by many nuclear receptors including the progesterone receptor, estrogen receptor, glucocorticoid receptor, thyroid hormone receptor, and retinoid X receptor (RXRα) (33). SRC-1 also participates in transactivation by the conventional transcription factors including NF-κB (1, 5), SP1, the chimeric Gal4-VP16 protein, and STAT5a (35, 36).
To evaluate the coactivator function of SRC-1 in NF-κB-mediated IκBα transcription, SRC-1 was investigated by using the ChIP assay. In this study, NF-κB is activated by TNF-α in HEK293 cells. Recruitment of SRC-1 was monitored during a time frame of 30–120 min after the addition of TNF-α (Fig. 1A). The association of SRC-1 with the IκBα gene promoter was increased gradually. The increase is associated with the DNA binding of NF-κB and the presence of RNA pol II, an indicator of transcriptional initiation. p65/DNA interaction is increased at 30 min, reduced at 60 min, and then increased again at 120 min during TNF treatment. This may reflect the asynchronous oscillations of p65 following TNF stimulation as reported recently (37). These data suggest that SRC-1 is recruited for gene transcription mediated by NF-κB.

SRC-1 function was examined by using the IκBα-luciferase report in transient transfection of HEK293 cells. Cotransfection of SRC-1 led to a significant increase in the IκBα reporter activity (Fig. 1B). Consistently, the protein abundance of endogenous IκBα was also doubled in this condition (Fig. 1C). The increase in IκBα protein and SRC-1 protein was determined in the transfected cells by Western blot. In a similar assay, NF-κB luciferase reporter was also enhanced by SRC-1 (Fig. 1D), suggesting that SRC-1 can serve as a coactivator for NF-κB regardless of promoter context.

**SRC-2 Is Replaced by SRC-1 after NF-κB Activation by TNF-α**—SRC-2 is also known as GRIP-1 (glucocorticoid receptor-interacting protein-1) (38) and TIF2 (transcriptional intermediary factor 2) (39). SRC-2 has been shown to be a coactivator for both class I and class II nuclear receptors (38). It shares high sequence homolog with SRC-1 (N-CoA1) and is also known as N-CoA2 (nuclear coactivator 2). SRC-2 is distributed in both cytoplasm and nucleus (40), and the distribution is regulated by cell differentiation. SRC-2 was reported as a coactivator of NF-κB (1).

Association of SRC-2 with the IκBα gene promoter was detectable in the absence of TNF-α treatment (Fig. 2A). It was reduced gradually in 293 cells after TNF treatment. This reduction is corresponding to an increase in SRC-1 signal, suggesting that SRC-2 is substituted by SRC-1 in the IκBα promoter after NF-κB activation. By examining intracellular distribution of SRC-2, we observed that SRC-2 protein was decreased in the nucleus but increased in the cytoplasm during TNF treatment (Fig. 2B). This suggests that replacement of SRC-2 by SRC-1 might be a result of the loss of SRC-2 in the nucleus. It is not clear why such a nuclear exclusion is induced by TNF-α. The exclusion might be related to phosphorylation of SRC-2, as was observed in SRC-1 (41). SRC-1 was shown to be phosphorylated by ERK. Since TNF-α induces activation of ERK, and SRC-2 shares a high level of homolog with SRC-1, it is likely that the nuclear exclusion of SRC-2 is related to ERK activation by TNF-α.

The function of SRC-2 was examined by using the IκBα reporter in a transient transfection. Overexpression of SRC-2 resulted in an increase in the reporter activity (Fig. 2C), and this was associated with an increase in IκBα protein, although knockdown of SRC-2 led to a decrease in IκBα protein (Fig. 2D). These results support that SRC-2 acts as a coactivator of NF-κB, and its major function might be related to the maintenance of basal level expression of IκBα. In the presence of TNF-α, SRC-1 replaces SRC-2 in the IκBα promoter.
for a robust transcriptional activation induced by NF-κB activation.

SRC-3 Exhibits Oscillation in Interaction with IκBα Promoter—SRC-3 was cloned independently by several laboratories in 1997 under different names including p/CIP (35), ACTR (42), AIB-1 (a gene amplified in breast cancer-1) (43), RAC-3 (receptor-associated coactivator 3) (44), and TRAM-1 (a thyroid hormone receptor activator molecule-1) (45). p/CIP is the mouse homolog of the human SRC-3 (46). SRC-3 shares 31 and 36% amino acid identity with SRC-1 and SRC-2, respectively (35). SRC-3 recruits CBP and p/CAF for generation of the transcription initiation complex (35, 42). Intracellular distribution of SRC-3 is regulated by extracellular signals including insulin (47) and TNF-α (7). In serum-free medium, SRC-3 is predominantly in the cytoplasm, whereas insulin or TNF-α results in SRC-3 nuclear translocation. SRC-3 was reported as a coactivator for NF-κB (7, 8). More interestingly, SRC-3 is associated with IκB kinase and is subject to phosphorylation by IκB kinase (7).

SRC-3 was examined in the same experimental conditions that were used for SRC-1. Interestingly, the pattern of SRC-3 signal is different from that of either SRC-1 or SRC-2 in the ChIP assay. SRC-3 recruitment was induced by TNF-α at 30 min (Fig. 3A). However, the association was reduced at 60 min and followed by another increase at 120 min. It is not clear why the SRC-3 signal follows this pattern of oscillation. Since this pattern of oscillation was observed in p65, the data suggest that SRC-3 may directly interact with p65. Therefore, its recruitment is strictly dependent on the presence of p65 in the promoter. It is also possible that SRC-3 may have a different function from SRC-1. It remains to be examined whether a nuclear exclusion contributes to the oscillation.

Functional analysis suggests that SRC-3 enhances NF-κB-mediated IκBα transcription. SRC-3 overexpression enhanced the IκBα reporter activity in a dose-dependent manner (Fig. 3B), whereas knockdown of SRC-3 decreased the IκBα reporter activity (Fig. 3C). The reporter activity is consistent with the IκBα protein levels under these conditions (Fig. 3D). These data suggest that although SRC-3 signal exhibits a different pattern from SRC-1 in the ChIP assay, SRC-3 still serves as a coactivator even when the transcription has been initiated.

The repressor activity of HDAC1 was confirmed with the IκBα reporter. Overexpression of HDAC1 led to reduction of
resulted in an enhancement in IκBα by TNF-α. Unlike HDAC1, the role of HDAC2 or HDAC3 is still controversy in the regulation of NF-κB. Our data support the hypothesis that HDAC3 can deacetylate p65 (18, 19, 49). Interestingly, the increase in HDAC3 is correlated with the decrease in p50, leading to NF-κB gene promoter.

HDAC2 Is Not a Corepressor for NF-κB in the IκBα Promoter—HDAC2 was reported to mediate glucocorticoid inhibition of p65-mediated transactivation (16, 17) or inhibit NF-κB p65-induced interleukin-8 transcription through association with HDAC1 (23). In this study, HDAC2/NF-κB interaction was investigated under the same condition as being used for HDAC1. The HDAC2 signal was induced by TNF-α in the ChIP assay (Fig. 5A). However, the induction was not as strong as for HDAC1. Modification of HDAC2 function by overexpression or knockdown failed to generate a significant impact on the IκBα reporter and protein activities (Fig. 5B). These data suggest that HDAC2 may not be involved in the regulation of NF-κB activity in the IκBα gene. This conclusion is supported by observations from other laboratories that although HDAC2 associates with p65 upon immunoprecipitation (19, 23, 48), HDAC2 does not exhibit catalytic activity (19).

HDAC3 Inhibits NF-κB-mediated Transcription—HDAC3 was reported to deacetylate NF-κB p65, leading to NF-κB nuclear export (18, 19). This activity of HDAC3 may limit transcriptional activity of NF-κB. However, unlike HDAC1, reports about HDAC3 are inconsistent with respect to its corepressor function for NF-κB (23, 25). In this study, association of HDAC3 was examined in a ChIP assay after NF-κB activation by TNF-α (Fig. 6A). Before TNF treatment, HDAC3 signal has already been detectable in the IκBα promoter. The signal was enhanced by TNF treatment at 30 min, peaked at 60 min, and then reduced by 120 min. If HDAC3 acts as an inhibitor of p65, recruitment of HDAC3 should lead to a reduction in gene transcription induced by NF-κB. Three published studies support that HDAC3 can deacetylate p65 (18, 19, 49). Interestingly, the increase in HDAC3 is correlated with the decrease in HDAC1 at 60 min. These data suggest that HDAC3 may be interchangeable with HDAC1 in the inhibition of NF-κB target gene transcription. This observation further supports our hypothesis that corepressor binds to NF-κB even when gene transcription is initiated.

Upon functional analysis, we observed that knockdown of HDAC3 led to a significant enhancement in the IκBα reporter activity (Fig. 6B), suggesting the negative role of HDAC3 in the regulation of NF-κB activity. The activity was confirmed by overexpression of HDAC3 that led to an inhibition of the reporter activity (Fig. 6C). These functional data suggest that HDAC3 is a corepressor for NF-κB in the IκBα gene promoter.

SMRT and NCoR Are Corepressors of NF-κB—SMRT and NCoR are two components of the nuclear corepressor complex in which they serve to activate the catalytic function of deacetylases. SMRT and NCoR contribute to the transcriptional repression in a transcription factor-specific manner (50). Data from an NCoR knock-out study suggests that NCoR may also act as a coactivator for expression of certain genes (13). An early study showed that SMRT inhibited the transcriptional activity of NF-κB (14). The inhibition was observed for both p65 and Gal4-p65, suggesting that the activation domain of p65 is targeted by SMRT. SMRT activity in the inhibition of gene transcription was abolished by the chemical inhibitor of histone deacetylase, trichostatin A (100 nM), suggesting that SMRT requires the catalytic function of HDACs for transcriptional inhibition. In the two major subunits of NF-κB, p50 exhibits a
stronger interaction with SMRT (14, 15). However, it was shown that SMRT failed to inhibit NF-κB activity in a reporter assay. Instead, NCoR was shown to inhibit the NF-κB reporter activity (23).

In ChIP assays, we observed that both SMRT and NCoR were recruited to the IκBα promoter after NF-κB activation (Fig. 7A). The signals were detectable before TNF-α treatment. TNF-α increased binding of both SMRT and NCoR to the promoter. SMRT and NCoR exhibited different binding patterns in the time course analysis. At 30 min, SMRT binding was stronger than that of NCoR. At 60 min, this relationship was reversed with NCoR overriding SMRT. At 120 min, SMRT and NCoR were both at a submaximum levels. This time-dependent change suggests that SMRT and NCoR may substitute among themselves for NF-κB activity. Since SMRT and HDAC3 exhibit similar patterns in the ChIP assay, SMRT may form a complex with HDAC3 in the inhibition of NF-κB activity. In contrast, NCoR and HDAC1 exhibit a similar signal pattern in the ChIP assay. NCoR is likely to form a corepressor complex with HDAC1. The ChIP data from SMRT and NCoR further support the model that corepressors bind to the gene promoter even when gene transcription is initiated.

Upon cotransfection, overexpression of SMRT or NCoR leads to inhibition of IκBα reporter (Fig. 7B). The inhibition was only observed in the presence of p50 in cotransfection, suggesting that an interaction of SMRT or NCoR with p50 is required for the inhibition in physiological conditions. These data also confirm that p50 is the major subunit in the NF-κB heterodimer to interact with SMRT or NCoR. Knockdown of either SMRT or NCoR by RNAi led to a dramatic enhancement of the IκBα reporter activity (Fig. 7C), confirming the corepressor functions of SMRT and NCoR.

Dynamic Interaction of Coactivators and Corepressors with the IκBα Promoter—It is believed that removal of corepressor components from nuclear receptors is associated with activation of ligand-bound receptor. Correspondingly, corepressor association leads to inactivation of nuclear receptor and inhibition of transcription. It remains to be determined whether this model also works for the DNA-specific transcription factor, like NF-κB. In this study, interaction of NF-κB with the coactivators and corepressors was analyzed systematically. The results suggest that activation of NF-κB by TNF-α not only results in coactivator binding but also triggers corepressor recruitment. More importantly, recruitment of the two classes of cofactors happens simultaneously (Fig. 8A). Binding of RNA pol II to NF-κB was induced by TNF-α, and this marks transcription initiation. Since the corepressors did not reduce pol II signal in the time frame of the current study, the result suggests that the coactivators are dominant in the control of transcription after NF-κB activation. These data demonstrate that association of corepressor is persistent and is not an “on or off” phenomenon. Thus, the transcription is determined by the balance between corepressors and coactivators. Coactivators will overcome the corepressor when an activation signal is integrated into the gene promoter. Otherwise, corepressor is dominant to minimize the gene transcription in prevention of gene leaking into the absence of stimulation. This may explain why gene transcription is tightly regulated in cells under physiological conditions.

Our data also suggest that there is a switch among the different members of coactivators in the course of gene transcription to drive the gene transcription. Such a switch is observed between SRC-1 and SRC-2 (Fig. 8A), suggesting that SRC-1 may be required for TNF-induced gene transcription and that SRC-2 is required for basal gene transcription. The data suggest that each isoform of p160 proteins has a stage-specific function in the regulation of gene transcription induced by NF-κB. Collectively, each of the three p160 proteins can substitute among themselves for NF-κB-mediated transcription. This explains why there is no function deficiency in NF-κB...
in the p160 isomorph knock-out mice, including SRC-1+/− (51, 52), SRC-2+/− (52, 53), and SRC-3+/− (46, 47).

This study suggests that corepressor complexes are also subject to exchange during the course of gene transcription induced by NF-κB (Fig. 8A). The binding patterns suggest that SMRT-HDAC1 and NCoR-HDAC3 form two different corepressor complexes. Although both complexes can inhibit NF-κB activity, it seems that they act at different time points along IκBα transcription induced by NF-κB. At 30 min, NCoR-HDAC3 complex is a major player, whereas at 60 min, SMRT-HDAC1 is the main corepressor complex. Similarly to the p160 coactivators, the corepressor switch ensures a precise control of gene transcription and efficient integration of signals from different sources into gene expression. Involvement of both forms of corepressor complexes provides a double check mechanism for the inhibition of NF-κB. Formation of the SMRT-HDAC1 complex may require the involvement of unknown proteins since association of these two proteins has not been observed in the process of protein purification. This study also provides evidence that HDAC1 and HDAC3 may use different mechanisms in the control of NF-κB activity as their binding patterns to the promoter are different. This study supports a more precise model for interactions of nuclear cofactors with NF-κB in the IκBα gene transcription. Although this study was done with NF-κB, the model of cofactor recruitment may also apply to other DNA-specific transcription factors. Finally, the promoter activity of the endogenous IκBα gene was determined through measuring mRNA expression using real-time reverse transcription-PCR. mRNA of IκBα was induced by TNF treatment (Fig. 8B). This induction was enhanced by coactivators (SRC-1, SRC-2, and SRC-3) and decreased by corepressors (HDAC1, HDAC3, SMRT, and NCoR) (Fig. 8C). This group of data suggests that cofactor binding (ChIP data) is consistent with its function in the regulation of gene transcription.

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**Cofactors of NF-κB in IkBα Gene**