Modification of CCAAT/Enhancer-binding Protein-β by the Small Ubiquitin-like Modifier (SUMO) Family Members, SUMO-2 and SUMO-3*

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The small ubiquitin-like modifier, or SUMO,1 proteins are a group of polypeptides that are conjugated to lysine residues in target proteins in much the same manner as ubiquitin (for a review, see Ref. 1). To date, three family members have been identified in higher eukaryotes: SUMO-1, SUMO-2, and SUMO-3. In humans and mice, the three isoforms appear to be ubiquitously expressed. SUMO-1 has been the most extensively characterized; homologues have been found in organisms ranging from yeast to humans. Glycine 97 in this 101-amino-acid protein is conjugated to a lysine in target proteins (2, 3) utilizing activating and conjugating enzymes similar to those in the ubiquitin conjugation pathway (4). The consensus sequence, (LV)XKXE, for sumoylation has been defined (see Ref. 5 for a review). Targets of SUMO-1 include the GTPase-activating protein RanGAP1 (10), the promyelocytic leukemia gene product (PML) (6), IκBα (7), the transcription factors AP-2 (8), and several members of the C/EBP family (9). Sumoylation of these proteins leads to localization of RanGAP1 at the nuclear pore (5, 10) and localization of promyelocytic leukemia gene product into discrete bodies in the nucleus (5, 6). Modification of IκBα by SUMO antagonizes ubiquitination and subsequent degradation of this inhibitor of the transcription factor NFκB (7). Recently, the repression domain I of C/EBPα has been demonstrated to be modified by SUMO-1 (9); this modification is proposed to be important for the inhibitory function of this domain. These authors also show that conserved SUMO target sequences are present in C/EBPα, C/EBPβ, and C/EBPδ and that these isoforms can be conjugated to SUMO-1 (9).

SUMO-2 and SUMO-3 are 66% homologous to SUMO-1; SUMO-2 and SUMO-3 are 97% identical to each other (11). Despite the fact that the SUMO family members are very closely related, SUMO-2 and SUMO-3 have the ability to form poly(SUMO) chains owing to the fact that their amino acid sequence contains a SUMO target consensus (4). Because SUMO-1 does not contain a target lysine for sumoylation, it appears to be conjugated to proteins as a monomer (12). Antibodies currently available cannot distinguish between SUMO-2 and SUMO-3 (4), but it is assumed that since SUMO-2 and -3 have such a high degree of sequence similarity, the two proteins have overlapping functions. Elucidation of their differences awaits further study. Little is known about the function of SUMO-2/-3 within cells. Saitoh and Hinchey (13) described that oxidative stress, heat shock, or UV irradiation caused a dramatic increase in the amount of SUMO-2/-3 incorporated in high molecular weight complexes within Cos-7 cells. At the current time, the identities of the proteins modified by SUMO-2/-3 in response to stress are unknown.

C/EBPβ is a member of the basic leucine zipper family of transcription factors. The protein is transcribed from an intronless gene but gives rise to three protein isoforms due to alternative translation initiation at three in-frame ATG initiator codons (14). In the human, the full-length C/EBPβ begins at the first in-frame methionine and consists of the entire 346 amino acids (this is 297 in the rat and mouse). The second isoform begins at the second in-frame methionine, which is 23 amino acids (or in rat/mouse, 21 amino acids) downstream from the first. The third isoform begins at the final in-frame methionine that is at position 198 in humans. The structure of C/EBPβ is such that the transactivation domain resides in the N-terminal region. The DNA binding and protein dimerization domains, the basic region and leucine zipper, reside in the C-terminal end. The first two isoforms consist of both the activation and DNA binding/dimerization domains and differ only by a 23-amino-acid N-terminal truncation. The third isoform, however, lacks the N-terminal activation domain while retaining the DNA binding/dimerization domain. This protein acts as a transcriptional repressor due to the fact that it can homodimerize or heterodimerize with the larger C/EBPβ isoforms and/or occupy the same C/EBPβ DNA elements within promoters of target genes.

We have previously observed that C/EBPβ-1 and C/EBPβ-2 are differentially expressed between non-dividing, normal breast cells and dividing breast cells, either normal or neoplas-
tic (15). Furthermore, introduction of C/EBPβ-2 into the normal, immortal mammary epithelial cell line, MCF10A, causes anchorage-independent growth, acquisition of invasive potential, and transition to an epithelial to mesenchymal state (16). This phenotype is not observed with the introduction of C/EBPβ-1. Konew-Leutz and Leutz (17) have demonstrated that C/EBPβ-1 (in their work, the rat protein termed LAP") could recruit the chromatin remodeling complex Swi-Snf to the promoters of genes important in the differentiation of myeloid cells. The second isoform, C/EBPβ-2 or LAP, was unable to perform this function (17). Our results, along with results from other laboratories, support the theory that these two activator isoforms, despite differing by only 21–23 amino acids, behave very differently within cells.

Here, we demonstrate that C/EBPβ is modified by the SUMO family members SUMO-2 and SUMO-3. Furthermore, this modification is specific to the first isoform of C/EBPβ family members SUMO-2 and SUMO-3. Mutation of this lysine does not affect the punctate nuclear localization of C/EBPβ-1; however, this lysine is critical for the failure of C/EBPβ-1 to activate the cyclin D1 promoter. In addition, we demonstrate that the modification of C/EBPβ-1 by SUMO is dependent on the integrity of the N-terminal amino acids present in C/EBPβ-1, absent in C/EBPβ-2.

**EXPERIMENTAL PROCEDURES**

**Expression Vectors**—Hemagglutinin (HA)-tagged SUMO-2 and HA-tagged SUMO-3 expression vectors were a kind gift of Dr. Ron Hay (University of St Andrews, St Andrews, UK). The expression vectors for T7-tagged C/EBPβ-1 and T7-tagged C/EBPβ-2 have been described previously as has the pG5-cyclin D1 reporter construct and the empty CMV vector (15, 16). The C/EBPβ-1 lysine 173 to alanine mutant was constructed as follows: an expression vector encoding a T7-tagged, 541-base pair C terminus mutant of C/EBPβ-1 was obtained as follows. The pRSETC-N-terminal mutant-NF-IL6 was digested with BamHI and EcoRI to release a 75-base pair fragment encompassing lysine 173. The vector was purified from the nucleotide fragment using gel electrophoresis and ligated into the linearized, absent in pRSETB LAP541. The fragment was isolated from the vector BstI described in Ref. 18) with mutant was constructed by digesting wild-type pRSETB LAP (designated T7 antibody-conjugated agarose beads, followed by immunoblotting (IB) with polyclonal HA antibody. In B, the blot from A was stripped and reprobed with antibody to C/EBPβ, demonstrating that equivalent amounts of transfected proteins are expressed and precipitated.

**Immunofluorescence**—Cells were fixed in 3.7% formaldehyde in phosphate-buffered saline (PBS), permeabilized with 0.1% Triton X-100 in PBS, and blocked with 5% bovine serum albumin in PBS. Coverslips were incubated with T7 tag antibody (Novagen, Madison, WI) at a 1:10,000 dilution in 2% bovine serum albumin, 0.1% Triton X-100 in PBS for 1 h at room temperature. Coverslips were

**Cells and Culturing Conditions**—Cos-7 cells were a gift from Dr Steve Hann, Vanderbilt University. The cells were maintained in Dulbecco’s modified Eagle’s medium plus 10% calf serum (Colorado Serum Co., Denver, CO). Human mammary epithelial cells (HMECs) were purchased from Clonetics (Walkersville, MD) and maintained in mammary epithelial cell growth medium from the same company. NIH3T3 cells were purchased from the ATCC and maintained in Dulbecco’s modified Eagle’s medium plus 10% Colorado calf serum. All cells were kept in a humidified chamber at 37°C with 5% CO2.

**Immunofluorescence**—HMECs were transfected with 5 μg of expression vectors of C/EBPβ-1 or C/EBPβ-2 Lys to Ala mutant using Geneporter (GeneTHERAPY Systems, Inc.) as per the manufacturer’s instructions. After 24 h, cells were plated onto polylysine-coated coverslips. Cells were allowed to sit down onto the coverslips and then fixed using 3% formaldehyde in phosphate-buffered saline (PBS), permeabilized with 0.1% Triton X-100 in PBS, and blocked with 5% bovine serum albumin in PBS. Coverslips were incubated with T7 tag antibody (Novagen, Madison, WI) at a 1:10,000 dilution in 2% bovine serum albumin, 0.1% Triton X-100 in PBS for 1 h at room temperature. Coverslips were
washed extensively with 0.1% Triton X-100 in PBS and then incubated with goat anti-mouse secondary antibody conjugated to Alexa 594 (Molecular Probes, Eugene OR) at a 2 µg/ml dilution in 2% bovine serum albumin, 0.1% Triton X-100 for 1 hour at room temperature in the dark. Coverslips were again washed extensively with 0.1% Triton X-100 in PBS and then rinsed with deionized water before staining with Hoechst dye at a 1 µg/ml in PBS concentration for 20 min at room temperature. Coverslips were washed with PBS and then mounted onto slides using Poly-Aquamount (Polysciences, Inc., Warrington, PA). Fluorescence was visualized using a Zeiss axiophot upright fluorescence microscope equipped with a low light CCD camera.

Transfections and immunoprecipitations—NIH3T3 cells were transfected with either 2 µg of expression vector only (CMV4) or 2 µg of C/EBPβ-1, C/EBPβ-2, or C/EBPβ-1 Lys to Ala mutant expression vectors plus 2 µg of pGL3-cyclin D1 reporter vector using Novafector liposomes (VennNova, Pompano Beach, FL). Luciferase assays were carried out as described previously (15). A portion of the lysates was combined with 2× SDS sample buffer, boiled, and subjected to 10% SDS-PAGE. Western analysis was performed as described previously (15) using the T7 tag antibody at a 1:20,000 dilution.

Cos-7 cells were transfected with 5 µg of indicated expression vectors using NovaFector liposomes as per the manufacturer’s instructions. After 30–36 h, cells were harvested at 4 °C in PBS plus 0.1 mM sodium vanadate, spun at 840 × g, and resuspended, on ice in radioimmune precipitation buffer (20 mM Tris, pH 7.5, 50 mM NaCl, 0.5% Triton X-100, 0.5% sodium deoxycholate, 0.1% SDS, 1 mM EDTA) plus protease and phosphatase inhibitors (1 µg/ml aprotenin, 1 µg/ml leupeptin, 1 mM phenylmethylsulfonyl fluoride, 10 mM sodium molybdate, 0.1 mM sodium vanadate, 10 mM β-glycerol-phosphate). Cell extracts were sonicated and added to T7 monoclonal antibody-conjugated agarose beads (Covance, Berkeley, CA) that were washed previously with radioimmune precipitation buffer plus protease/phosphatase inhibitors. Following an overnight incubation at 4 °C, the beads were collected, extensively washed, and subjected to immunoblotting as described previously (15).

**FIG. 2.** The integrity of the N terminus of C/EBPβ-1 is necessary for efficient incorporation of SUMO-2/3, and lysine 173 in the human protein is the major site of SUMO-2/3 modification. Transient transfections were performed in Cos-7 cells using expression vectors for HA-tagged SUMO-2 and SUMO-3 and T7-tagged C/EBPβ isoforms. Immunoprecipitations (IP) were then performed using T7 antibody-conjugated agarose beads followed by western analyses utilizing HA antibody (A and C) or T7 antibody (B and D). B and D depict blots A and C, respectively, stripped and reprobed with T7 antibody to demonstrate that equivalent amounts of the proteins are expressed and immunoprecipitated. IB, immunoblots. WT, wild type. MW, apparent molecular weight.

**FIG. 3.** C/EBPβ antibody immunoprecipitates a 97-kDa protein from HMECs. HMECs were radiolabeled using [35S]methionine and subjected to immunoprecipitation using guinea pig serum directed to the full-length C/EBPβ protein. Immune serum precipitated a 97-kDa protein (lane 2), whereas preimmune serum failed to precipitate this form (lane 1).
SUMOylation of C/EBPβ-1 by SUMO-2/-3

RESULTS

SUMO-2 and SUMO-3 Are Conjugated to C/EBPβ-1, but Not C/EBPβ-2—Given the fact that we observed a consensus SUMO-targeting sequence in C/EBPβ (this observation was confirmed while this work was in progress (9)), we decided to investigate whether the two activator isoforms could be modified by SUMO-2/3. We utilized Cos-7 cells to perform co-transfection experiments with expression vectors for HA-tagged SUMO-2 or HA-SUMO-3 together with T7-tagged C/EBPβ-1 or C/EBPβ-2. Following the transfection, proteins were immunoprecipitated using the T7 monoclonal antibody conjugated to agarose beads. The immunoprecipitates were then subjected to immunoblotting with an HA tag antibody. As seen in Fig. 2, muta-
tations in three amino acids, conserved between species, of the N-terminal extension of C/EBPβ forms human C/EBPβ differ only by a 23-amino-acid truncation of the extreme N terminus of C/EBPβ-1. We next wanted to ascertain whether these amino acids were important for the striking difference we observed between the sumoylation of C/EBPβ-1 and C/EBPβ-2. To address this question, we made mutations in three amino acids, conserved between species, of the N-terminal extension of C/EBPβ-1 (Table I). We next performed immunoprecipitation experiments utilizing this mutant together with the wild-type construct. As seen in Fig. 2, mutation of these amino acids greatly reduced conjugation of SUMO-2/-3 to C/EBPβ-1. In Fig. 2, A and C, lanes 3 and 4 depict tagged C/EBPβ-1 and the tagged C/EBPβ-1 N-terminal mutant trans-
fected into Cos-7 cells together with SUMO-2 (A) and SUMO-3 (C), immunoprecipitated with T7 antibody, and immunoblotted with an HA tag antibody. In this particular experiment, poly-
sumoylated wild-type C/EBPβ-1 containing even more than three chains (Fig. 2, C, lane 3, higher molecular mass species of ~116 kDa) is observed. However, mutation of the N-terminal amino acids markedly reduces the ability of both SUMO-2 and

Fig. 4. Mutation of lysine 173 to alanine does not affect sub-nuclear localization of C/EBPβ-1. HMECs were transfected with 5 ng of expression vector encoding either wild-type C/EBPβ-1 (C and D) or the Lys to Ala C/EBPβ-1 mutant (A and B). Transfected protein was detected by immunofluorescence using the T7 tag monoclonal antibody followed by Alexa 594-conjugated secondary antibody (A and C). Nuclear localization was confirmed by co-staining with Hoechst (B and D).

Anti-HA polyclonal antibody from Clontech was used to probe the membranes at a 1:500 dilution. Anti-C/EBPβ antibody (Santa Cruz Biotechnology) was used at a 1:2000 dilution. Anti-T7 tag antibody (Novagen) was used at a 1:1000 dilution. Membranes were stripped using Re-Blot Plus from Chemicon International (Temecula, CA). HMECs were radiolabeled with [35S]methionine, and immunoprecipitations were performed as described previously (15) with the exception being that guinea pig immune serum, raised to the full-length C/EBPβ-1, was stripped and reprobed with anti-C/EBPβ antibody (Fig. 1A) but not into C/EBPβ-2 and SUMO-2 or -3 are expressed but not when C/EBPβ-2 and SUMO-2 or -3 are expressed (Fig. 1B), consistent with sumoylation of C/EBPβ-1.

Interestingly, the apparent molecular mass of the sumoy-
lated C/EBPβ-1 is ~82 kDa (Fig. 1, A and B, lanes 1 and 2). This indicates that there are likely at least three of the ~10–11-kDa SUMO proteins incorporated in C/EBPβ-1 (the apparent molecular mass of C/EBPβ-1 in our SDS-PAGE system is 55 kDa).

Disruption of the N-Terminal Extension of C/EBPβ-1 Reduces SUMO Incorporation—As stated earlier, the two activator iso-

Fig. 5. Lysine 173 is critical for the failure of C/EBPβ-1 to activate the cyclin D1 promoter in a transient transfection. In A, NIH3T3 cells were transfected with expression vectors encoding C/EBPβ-1, C/EBPβ-2, or the Lys to Ala mutant C/EBPβ-1 and a cyclin D1 promoter/luciferase reporter vector and luciferase activity determined as described in Ref. 15. Data are the average of three experi-
ments, each performed in duplicate. In B, transfected cell extracts were subject to immunoblotting with T7 tag antibody, and levels of trans-
fected proteins from a representative experiment are depicted. MW, apparent molecular weight.
SUMO-3 to be incorporated into C/EBPβ-1 despite the fact that equivalent amounts of the C/EBPβ proteins are expressed and immunoprecipitated (as seen in the same blots (Fig. 2, B and D), stripped, and reprobed with T7 tag antibody). These data indicate that the integrity of the N terminus of C/EBPβ-1 is crucial for sumoylation.

**Lysine 173 Is a Target of SUMO-2/3**—With the evidence that C/EBPβ-1 is being modified by SUMO-2/3, we next wanted to ascertain whether the consensus lysine, at position 173 in the human protein, was the primary target. We developed a mutant form of C/EBPβ-1, in which the lysine 173 was changed into an alanine. Utilizing this construct in co-transfection experiments, we observed an ablation of incorporation of SUMO-2/3 into the C/EBPβ-1 protein. As seen in Fig. 2, A and C, lanes 5, there is no incorporation of SUMO-3 and very little incorporation of SUMO-2 into the lysine 173 to alanine mutant despite the fact that the proteins are synthesized and immunoprecipitated (Fig. 2, B and D). This slight amount of SUMO-2 incorporated into the C/EBPβ-1 Lys to Ala mutant may represent sumoylation at a cryptic site within the protein. These results serve to indicate that lysine 173 is the critical residue being modified by SUMO-2/3.

**Antibody Directed to C/EBPβ Immunoprecipitates a 97-kDa Protein from HMECs**—Work from our laboratory has demonstrated previously that normal HMECs placed in culture from reduction mammaplasty express both activator isoforms of C/EBPβ (15). Antibody directed to the entire C/EBPβ protein immunoprecipitates proteins consistent with the molecular weight of C/EBPβ-1 (p55) and C/EBPβ-2 (p42) from [35S]methionine radiolabeled HMECs (Fig. 3). This antibody also immunoprecipitates a 97-kDa form of C/EBPβ from these cells. Because this isosform is larger than the sumoylated C/EBPβ-1 observed in Fig. 1 (in which both C/EBPβ-1 and the SUMO proteins carry epitope tags), it is likely that the C/EBPβ we observe in these cells is conjugated to more SUMO chains than the exogenously expressed C/EBPβ in Fig. 1 but could correspond to the higher molecular weight SUMO-3-conjugated species seen in Fig. 2C, lane 3 (which likely carries 1 T7 and 4 HA epitope tags).

**Lysine 173 Does Not Affect Nuclear Localization of C/EBPβ-1**—SUMO proteins are known to affect cellular localization of target proteins. We have observed that C/EBPβ-1 has a specific, punctate nuclear staining pattern. We hypothesized that the sumoylation of C/EBPβ-1 may be responsible for targeting the transcription factor to these areas. To address this question, we transfected HMECs with expression vectors for either wild-type C/EBPβ-1 or the C/EBPβ-1 lysine 173 to alanine mutant. Very small quantities (5 ng) of DNA were used to ensure that exogenous protein did not overwhelm the sumoylation conjugation capacity of the cells. Transfected protein expression levels reflected those seen with endogenous C/EBPβ-1 protein (data not shown). As seen in Fig. 4, the staining pattern of the wild-type protein (C) mirrored that of the lysine 173 to alanine mutant (A). Hoechst staining demonstrates that the expression of C/EBPβ-1 is nuclear (Fig. 4, B and D). Thus, it appears that mutation of the sumoylation target does not affect subnuclear localization of C/EBPβ-1.

**Lysine 173 Is Critical for the Failure of C/EBPβ-1 to Activate the Cyclin D1 Promoter**—Previously, we demonstrated that C/EBPβ-1, but not C/EBPβ-2, can activate the cyclin D1 promoter (15). Recently, Kim *et al.* (9) demonstrated that the lysine targeted by SUMO-1 (lysine 134 in the rat protein) was necessary for proper function of the repression domain I in a C/EBPβ-1-Gal4 fusion protein reporter assay. We wanted to investigate whether the conserved human lysine 173 was necessary for the failure of C/EBPβ-1 to activate this promoter. Using transient transfections in NIH-3T3 murine fibroblasts, we observed that mutation of this lysine caused C/EBPβ-1 to behave similarly to C/EBPβ-2 using the cyclin D1 promoter/luciferase reporter construct. Whereas C/EBPβ-2 activates this construct above basal levels (CMV4), C/EBPβ-1 does not (Fig. 5). Mutation of lysine 173 in C/EBPβ-1 results in activation of the cyclin D1 construct. All transactivator proteins were expressed at equivalent levels as shown in Fig. 5B. These results indicate that lysine 173 is critical for the function of the repression domain of C/EBPβ-1 using this promoter construct.

**DISCUSSION**

C/EBPβ-1 and C/EBPβ-2 differ by only 21–23 amino acids, yet evidence continues to accumulate that they are functionally distinct. In 1999, Kowenz-Leutz and Leutz (17) demonstrated that the largest form of C/EBPβ, in their work termed LAP*, was capable of recruiting the Swi-Snf chromatin remodeling complex to the promoters of differentiation-specific genes in myeloid cells. The second isoform, LAP or C/EBPβ-2, was unable to recruit this complex to activate genes within the chromatin context. Furthermore, these authors demonstrated that the extreme N terminus of full-length C/EBPβ was necessary, but not sufficient, to recruit the Swi-Snf complex (17). Our laboratory has found differences in the expression and function of C/EBPβ-1 and C/EBPβ-2 in mammary epithelial cells. We have demonstrated that C/EBPβ-2 is associated with dividing cells, either normal or cancerous, and that overexpression of this isoform causes transformation of normal mammary epithelial cells in culture (15). This transformation is not observed with introduction of C/EBPβ-1; moreover, we have demonstrated that this isoform is lost from breast cancer cell lines.

The work presented here expands on the observation that C/EBPβ-1 and C/EBPβ-2 are functionally distinct by demonstrating that C/EBPβ-1, but not C/EBPβ-2, can be conjugated to the SUMO family members, SUMO-2 and SUMO-3. The intact N terminus of C/EBPβ-1 is needed, at least in part, for this conjugation, as evidenced by the reduction of SUMO-2/3 incorporation when conserved amino acids within the N-terminal tail are mutated. We are, at the present time, unsure as to the mechanism by which these amino acids are important for conjugation of SUMO-2/3. Given the observation that this tail is necessary, but not sufficient, for the interaction of C/EBPβ-1 with the large, multisubunit Swi-Snf complex (17), it is possible that the amino acids are involved in creating a structural change of the entire transcription factor, which distinguishes C/EBPβ-1 from C/EBPβ-2. Alternatively, the N terminus could be bound by a “bridging” factor necessary for interaction with components of the Swi-Snf complex and for interaction with the SUMO conjugation machinery. Unfortunately, there are currently no structural studies of the entirety of C/EBPβ; studies examining the differences in the structure of these two activator proteins will prove interesting.

Mutation of lysine 173 mostly ablates the incorporation of SUMO-2/3 into C/EBPβ-1. This sequence matches the SUMO target consensus and is likely the major site of conjugation of SUMO-2/3 as well as SUMO-1 (9). C/EBPβ-2, but not C/EBPβ-1, is able to activate the cyclin D1 promoter despite the fact that both proteins are able to bind a consensus C/EBP site within the promoter (15). We extend this observation by demonstrating that lysine 173 is critical for the inability of C/EBPβ-1 to activate this promoter. Furthermore, we extend the observation made by Kim *et al.* (9), who used a Gal4/C/EBPβ activation domain fusion protein to show that the conserved lysine 134 in the rat was necessary for function of the
repression domain. When the authors mutated the lysine to an alanine, they observed an increase in transactivation potential at a Gal4 binding site reporter construct (9). This is an expansion of earlier works, describing an increase in activation potential of a C/EBPβ-Gal4 fusion protein when certain domains (termed repression domains or conserved regions) were deleted (19, 20). Here, we show that mutation of this conserved lysine in the context of the entire protein disrupts the repression functions of C/EBPβ-1 at the cyclin D1 promoter. The apparent sumoylation of this lysine does not appear to influence subnuclear localization of C/EBPβ-1. Likely, sumoylation is affecting protein-protein interactions, such as with co-activators, co-repressors, or chromatin remodeling machinery, or blocking the transactivation potential via an intramolecular interaction. Recently, SUMO-dependent repression of the coactivator p300 function was shown to be mediated by recruitment of HDAC6 (21). Whether histone deacetylases are involved in C/EBPβ-1 repression and whether their recruitment is SUMO-dependent will be interesting to determine in future studies. Although non-sumoylated C/EBPβ-1 (the lysine 173 mutant) behaves similarly to C/EBPβ-2 at the cyclin D1 promoter in a transient transfection assay, further studies will be necessary to determine whether non-sumoylated C/EBPβ-1 is functionally equivalent to C/EBPβ-2 at the entire set of genes that may be subject to C/EBPβ regulation.

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