SUMO-1 Modification of Human Transcription Factor (TF) IID Complex Subunits

INHIBITION OF TFIID PROMOTER-BINDING ACTIVITY THROUGH SUMO-1 MODIFICATION OF hsTAF5*

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The TFIID complex is composed of the TATA-binding protein (TBP) and TBP-associated factors (TAFs) and is the only component of the general RNA polymerase II (RNAP II) transcription machinery with intrinsic sequence-specific DNA-binding activity. Binding of transcription factor (TF) IID to the core promoter region of protein-coding genes is a key event in RNAP II transcription activation and is the first and rate-limiting step of transcription initiation complex assembly. Intense research efforts in the past have established that TFIID promoter-binding activity as well as the function of TFIID-promoter complexes is tightly regulated through dynamic TFIID interactions with positive- and negative-acting transcription regulatory proteins. However, very little is known about the role of post-translational modifications in the regulation of TFIID. Here we show that the human TFIID subunits hsTAF5 and hsTAF12 are modified by the small ubiquitin-related modifier SUMO-1 in vitro and in human cells. We identify Lys-14 in hsTAF5 and Lys-19 in hsTAF12 as the primary SUMO-1 acceptor sites and show that SUMO conjugation has no detectable effect on nuclear import or intranuclear distribution of hsTAF5 and hsTAF12. Finally, we demonstrate that purified human TFIID complex can be SUMO-1-modified in vitro at both hsTAF5 and hsTAF12. We find that SUMO-1 conjugation at hsTAF5 interferes with binding of TFIID to promoter DNA, whereas modification of hsTAF12 has no detectable effect on TFIID promoter-binding activity. Our observations suggest that reversible SUMO modification at hsTAF5 contributes to the dynamic regulation of TFIID promoter-binding activity in human cells.

To initiate mRNA synthesis, RNA polymerase II (RNAP II) must assemble in an ordered fashion with a set of general transcription factors, TFIIA, -IIB, -IID, -IIE, -IIF, and -IHF, to form a so-called preinitiation complex (PIC) at the core promoter region of protein-coding genes (1–3). The initial, and rate-limiting step for PIC assembly is binding of TFIID, a multiprotein complex composed of the TATA-binding protein TBP and at least 13 TBP-associated factors (TAFs), to core promoter sequence elements (1–5). In addition to sequence-specific DNA binding, the TFIID complex provides several enzymatic activities that all reside in its largest subunit TAF1 (6). These include histone acetyltransferase activity (7), protein kinase activity (8), and ubiquitin-activating/enzyme conjugating activity (9). How exactly TFIID enzymatic activities contribute to RNAP II transcription is still unknown.

In accordance with its crucial role in PIC assembly, TFIID is considered one of the key targets of transcription regulation pathways. Biochemical and genetic studies have shown that TFIID binding to promoters as well as the stability and functionality of TFIID-promoter complexes are subject to regulation by gene-specific activators and repressors (10, 11) and by an array of ubiquitous regulators of TFIID (TBP) activity, including NC2 (Dr1/DRAP1), Mot1/BTAF1, and the NOT complex (11–14). In addition, TFIID subunits are subject to post-transcriptional modifications. Earliest studies demonstrated that several subunits of TFIID are phosphorylated during mitosis and that TFIID isolated from mitotic cells fails to respond to transcription activators in vitro (15). Results of more recent studies demonstrated gene-specific effects upon hsTAF10 methylation by the SET9 protein methyltransferase (16) and that the C-terminal domain of TAF1 is a substrate for the protein kinase CKII (17). Exactly how and to what extent these TAF modifications modulate TFIID functions is not known.

In recent years, regulation of transcription factor activity through modification with SUMO proteins has attracted considerable attention (18–21). SUMO proteins are small ubiquitin-related modifiers that are conjugated to target proteins through an enzymatic pathway, which is similar to ubiquitylation, but which involves a distinct set of enzymes (22, 23). Little is known about the SUMO-proteasome role in TFIID function; however, one recent study demonstrated that SUMO modification modulates TFIID functions in the context of E1 ubiquitin-activating enzyme; E2, ubiquitin carrier protein; E3, ubiquitin-protein isopeptide ligase; HA, hemagglutinin; (HA)3, triple-HA epitope tag; aa, amino acids; NEM, N-ethylmaleimide; Ni-NTA, nickel-nitrioltriacetic acid; TBS, Tris-buffered saline; PBS, phosphate-buffered saline; eTFIID, FLAG:epitope-tagged TFIID complex; ATPγS, adenosine 5’-O-(thiotriphosphate).
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which a thioester bond is formed between its C-terminal glycine residue and a cysteine residue in the activating E1 enzyme (SAE1/2). Following transfer to a conjugating E2 enzyme (Ubc9), SUMO is covalently attached to a target protein lysine residue via isopeptide bond formation. Whereas SUMO E1 and E2 enzymes are sufficient to modify target proteins in vitro (23), several SUMO E3 ligases have been described that enhance transfer of SUMO from the E2 enzyme to specific substrates (22). Importantly, SUMO modification is a reversible and dynamic process, and SUMO conjugates can be removed from substrate proteins by SUMO-specific proteases (22).

SUMO modification occurs in many cases, but not exclusively, within a consensus motif ψKXE, where ψ is a large hydrophobic amino acid residue and X is any amino acid residue (22, 24). Here we investigated SUMO-1 modification of several TFIID subunits containing the ψKXE motif. We show that hsTAF5 and hsTAF12 are SUMO-1-modified in human cells. We further show that recombinant hsTAF1, hsTAF5, hsTAF12, and hsTBP can be SUMO-1-modified using a minimal SUMO conjugation system composed of recombinant E1 and E2 enzymes and SUMO-1. TAF5 and TAF12 can also be efficiently sumoylated in purified reconstituted TFIID complexes, whereas SUMO acceptor sites in TAF1 and TBP appear to become inaccessible upon assembly into TFIID. We identified the principal SUMO-1 acceptor sites in hsTAF5 and hsTAF12 and show that SUMO modification does not affect nuclear import or the nuclear distribution of hsTAF5 and hsTAF12 in human cells. Finally, we present results of in vitro DNA binding experiments showing that SUMO modification of purified human TFIID at hsTAF5 inhibits TFIID DNA-binding activity.

EXPERIMENTAL PROCEDURES

Cell Culture—HeLa cells were grown in Dulbecco’s modified Eagle’s medium, supplemented with 10% fetal bovine serum. HeLa-6His:Myc-SUMO-1 cells (25) were kindly provided by Peter O’Hare (Marie Curie Research Institute, Oxford, UK) and were grown in the presence of 2 μg/ml puromycin to select for 6His:Myc-SUMO-1 expression.

Antibodies—Rabbit polyclonal antibodies for hsTAF5, hsTAF6, hsTAF9, hsTAF12, and hsTBP were a kind gift of R. G. Roeder (The Rockefeller University, New York, NY). Mouse monoclonal antibodies for the HA epitope tag were purchased from Santa Cruz Biotechnology. Rabbit polyclonal antibody raised against the N-terminal part of VP22 (AGV31) has been described previously (26). Mouse monoclonal antibodies for the HA epitope tag for the 6His:epitope tag were purchased from Santa Cruz Biotechnology.

Plasmids—pTOG55Tdt (−1ATATA/+33) contains five binding sites for transcription factor II D (TFIID), GAL4 DNA in frame with the GAL4 activation domain. The GAL4 binding motif is accessible upon assembly into TFIID. We identified the principal SUMO-1 acceptor sites in hsTAF5 and hsTAF12 and show that SUMO modification does not affect nuclear import or the nuclear distribution of hsTAF5 and hsTAF12 in human cells. Finally, we present results of in vitro DNA binding experiments showing that SUMO modification of purified human TFIID at hsTAF5 inhibits TFIID DNA-binding activity.
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A, cell lysates of control HeLa cells (HeLa) or HeLa cells expressing 6His:Myc-SUMO-1 (HeLa-S/1) were passed over Ni-NTA affinity resin (Qiagen), and Ni-NTA eluates were analyzed with anti-hsTAF12 and anti-hsTAF5 antibodies. B, loss of hsTAF5 SUMO-1 modification in HeLa-6His:Myc-SUMO-1 cells upon HSV-1 infection and cold shock treatment. Whole cell lysates were analyzed by immunoblotting using antibodies for hsTAF5, hsSUMO-1, and the HSV-1 protein VP22. Asterisks denote the position of SUMO-1-modified proteins.

Fig. 1. TFIID subunits containing the consensus SUMO target sites ΨKXE. Schematic representation of the location of potential SUMO acceptor lysine residues relative to protein domains of interest: HAT, histone acetyltransferase activity; Ub E1/E2, ubiquitin-activating/conjugating enzyme activity; DBD, double bromodomain; CTK, C-terminal protein kinase activity; HFD, histone fold domain. (*) this study identifies Lys-14 in hsTAF5 and Lys-19 in hsTAF12 as bona fide SUMO-1 acceptor sites.

Fig. 2. SUMO-1 modification of hsTAF5 and hsTAF12 in human cells. A, cell lysates of control HeLa cells (HeLa) or HeLa cells expressing 6His:Myc-SUMO-1 (HeLa-S/1) were passed over Ni-NTA affinity resin (Qiagen), and Ni-NTA eluates were analyzed with anti-hsTAF12 and anti-hsTAF5 antibodies. B, loss of hsTAF5 SUMO-1 modification in HeLa-6His:Myc-SUMO-1 cells upon HSV-1 infection and cold shock treatment. Whole cell lysates were analyzed by immunoblotting using antibodies for hsTAF5, hsSUMO-1, and the HSV-1 protein VP22. Asterisks denote the position of SUMO-1-modified proteins.

RESULTS

hsTAF5 and hsTAF12 Are Modified by SUMO-1 in Human Cells—Protein sequence analysis revealed that several human TFIID subunits, including hsTBP, hsTAF1, hsTAF2, hsTAF4, hsTAF5, and hsTAF12, contain the sumoylation target consensus sequence ΨKXE (22, 24) (Fig. 1). We therefore wanted to investigate whether sumoylation of TFIID subunits occurs in human cells.

Detection of SUMO-1 conjugates is often difficult, because only a small percentage of cellular SUMO-1 target proteins is modified at any given time (22). For this reason, SUMO conjugation is generally investigated in cells that overexpress SUMO proteins, and, as a consequence, contain elevated levels of SUMO-1 conjugates. Detection of SUMO targets is further facilitated by expression of epitope-tagged versions of SUMO proteins, allowing for enrichment of SUMO conjugates from cell lysates by affinity chromatography methods.

To investigate the SUMO-1 modification of human TFIID subunits, we made use of a human cell line that constitutively expresses elevated levels of 6His:Myc-epitope-tagged human SUMO-1 protein (25). Cells were lysed in the presence of SDS and N-ethylmaleimide (NEM) to inhibit SUMO-specific proteases, and cell lysates were passed over nickel-charged agarose resin (Ni-NTA-agarose, Qiagen) to enrich proteins conjugated to 6His:Myc-SUMO-1. Analysis of Ni-NTA-bound fractions by immunoblotting using TAF-specific antibodies revealed immunoreactive bands with an apparent molecular weight expected for mono-sumoylated forms of hsTAF5 and hsTAF12 present in HeLa-6His:Myc-SUMO-1 cell lysates, but not in lysates of HeLa control cells (Fig. 2A, compare lanes 2 and 3). SUMO-1-modified hsTAF5, but not SUMO-1-modified hsTAF12, could also be detected in whole cell lysates of HeLa-6His:Myc-SUMO-1 cells (Fig. 2B and data not shown). Unfortunately, we were unable to obtain evidence for SUMO-1 modification of hsTAF12.
modification of other TFIID subunits with antibodies available to us.

Previous studies reported that stress conditions such as heat shock, serum starvation, and infection by viruses affect SUMO-1 conjugation in human cells (25, 35, 36). To investigate whether sumoylation of TFIID subunits in HeLa-6His:Myc:SUMO-1 is regulated, we examined the sumoylation status of endogenous hsTAF5 protein in response to different stress conditions, including heat shock, cold shock, serum starvation, and virus infection.

Heat treatment (1 h at 42 °C) and serum starvation (24 h at 37 °C) had no detectable effect on hsTAF5 sumoylation levels (data not shown). In contrast, cold shock (10 min at 4 °C) and infection with herpes simplex virus 1 (HSV-1) resulted in a dramatic loss of intracellular SUMO-1 conjugates and a corresponding loss of SUMO-1-modified hsTAF5 (Fig. 2B). Importantly, neither cold shock nor HSV-1 infection led to a detectable decrease in the absolute levels of cellular hsTAF5 protein. Thus, the observed loss of SUMO-1-modified hsTAF5 in response to stress can be attributed to either down-regulation of intracellular SUMO-1-activating/-conjugating activity and/or up-regulation of intracellular SUMO isopeptidase activity. In summary, these observations demonstrate that hsTAF5 and hsTAF12 are modified by SUMO-1 in human cells and that TAF SUMO-1 conjugation observed in HeLa-6His:Myc:SUMO-1 cells is a regulated event.

Recombinant hsTBP, hsTAF1, hsTAF5, and hsTAF12 Are Substrates for SUMO-1 Modification in Vitro—Next, we analyzed SUMO-1 modification of single recombinant TFIID subunits in vitro. Highly purified bacterially expressed recombinant hsTAF12 or hsTBP, or recombinant hsTAF1, hsTAF4, or hsTAF5 expressed by coupled in vitro transcription/translation, were incubated with a minimal SUMO-1 conjugation system composed of purified recombinant human E1 (SAE1/2) and E2 (UbI/UbC) enzymes, and purified mature SUMO-1 (aa 1–97). Immunoblot analysis of reaction products revealed SUMO-1 modification of hsTBP, hsTAF1, hsTAF5, and the 20-kDa variant of hsTAF12 (Fig. 3, A–D). SUMO-1 modification was not detected with hsTAF4 (Fig. 3D), which contains a SUMO consensus target site (Fig. 1), and with the 15-kDa variant of hsTAF12 (Fig. 3A), which lacks the N terminus of full-length TAF12 (28).

hsTAF1 contains two SUMO-1 consensus target sequences (Fig. 1), one located close to a region harboring histone acetyltransferase and ubiquitin-activating/-conjugating activity (E1/E2) activities (7, 9), and another located in close proximity to the hsTAF1 double-bromodomain region (37). To test whether SUMO modification of recombinant hsTAF1 occurs at a single site or at multiple sites, we divided hsTAF1 into three parts and expressed the corresponding protein fragments by coupled transcription/translation in vitro: an N-terminal fragment A spanning amino acid residues 1–500, which lacks consensus SUMO target sites, an internal fragment B (aa 501–1150), and C (aa 1151–1872) obtained by coupled in vitro transcription/translation was analyzed by SDS-PAGE and autoradiography. Asterisks indicate the position of SUMO-1-modified proteins.

**Fig. 3.** SUMO-1 modification of recombinant TFIID subunits in vitro. Purified bacterially expressed 20-kDa variants of 6His:hsTAF12 (A) and 6His:hsTBP (B), or in vitro expressed (HA)3-hsTAF5 (C), (HA)3-hsTAF4 and (HA)3-hsTAF1 (D) were subjected to SUMO-1 conjugation in vitro for 2 h at 37 °C. Reaction products were analyzed by immunoblotting using the antibodies indicated. E, SUMO-1 modification of S35-labeled hsTAF1 fragments A (aa 1–500), B (aa 501–1150), and C (aa 1151–1872) obtained by coupled in vitro transcription/translation was analyzed by SDS-PAGE and autoradiography. Asterisks indicate the position of SUMO-1-modified proteins.
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SUMO-1 modification of human TFIID subunits was investigated in a study involving recombinant expression of wild-type and mutant TAF5 and TAF12 proteins. The study utilized transient transfection in HeLa cells to analyze the intracellular localization and stability of these proteins in the context of the TFIID complex. The results demonstrated that SUMO-1 modification occurs at specific sites within these subunits, which are consistent with the idea that sumoylation contributes to the regulation of TFIID function.

**Identification of SUMO-1 acceptor sites in**: hsTAF12 and hsTAF5—Further investigations focused on hsTAF5 and hsTAF12, as these were the most relevant subunits for which we could demonstrate SUMO modification in human cells. In vitro experiments revealed that Lys-14 in hsTAF5 and Lys-19 in hsTAF12 are sites of SUMO-1 conjugation in human cells, transiently expressed wild-type and mutant TAF5 and TAF12 proteins in HeLa cells. SUMO modification was monitored using anti-HA antibodies, and transfected cells were analyzed by fluorescence microscopy.

**SUMO-1 Modification Does Not Affect hsTAF12 and hsTAF5 Intracellular Localization**—Previous studies had correlated SUMO modification with the recruitment of target proteins to specialized subnuclear domains, such as PML bodies. To address this issue, the study transiently expressed triple HA-epitope-tagged wild-type TAF5 and TAF12, as well as mutant TAF5K14R and TAF12K19R proteins. Immunostaining of transfected HeLa cells revealed that these proteins were localized predominantly in the nucleus, with a diffuse nuclear distribution excluding the nucleoli. Moreover, low levels of exogenous (HA)6:hsTAF5 were detected in the cytoplasm by immunoblotting.
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SUMO-1 Modification at TAF5 Inhibits DNA-binding Activity of Human TFIID—We used an immobilized promoter DNA template assay to investigate whether SUMO conjugation affects the DNA-binding activity of purified TFIID complex. We first asked if human TFIID could be SUMO-1-modified when bound to DNA. To this end, linear promoter DNA templates were immobilized on magnetic beads via a biotin-streptavidin link and incubated with highly purified f:TFIID complex. TFIID-promoter complexes were separated from unbound protein using a magnetic particle separator and incubated with our in vitro SUMO-1 conjugation system. After the in vitro sumoylation reaction, DNA-bound and unbound proteins were again separated and analyzed in parallel by immunoblotting. A small fraction (10–20%) of TFIID-promoter complexes dissociated during the incubation in sumoylation buffer (Fig. 7A). However, this was independent of ongoing sumoylation or the presence of individual components of the reconstituted human SUMO conjugation system (Fig. 7A, lanes 1–2 and 5–6 and data not shown). Furthermore, SUMO-1-modified forms of hsTAF5 and hsTAF12 could clearly be detected in the DNA-bound f:TFIID fraction (Fig. 7A, lanes 4 and 8). These results demonstrate that SUMO-1 can be conjugated to hsTAF5 and hsTAF12 in promoter-bound TFIID without affecting TFIID-promoter complex stability (Fig. 7A and data not shown).

Next, we tested whether sumoylation affects binding of purified TFIID complex to promoter DNA. f:TFIID was first subjected to in vitro SUMO-1 modification, followed by addition of immobilized promoter DNA template and further incubation for 60 min to assemble TFIID-promoter complexes. Free TFIID and TFIID-DNA complexes were separated and analyzed in parallel by immunoblotting. We found that in vitro SUMO conjugation was strongly inhibited in the presence of 15 mM ATPγS nucleotide analogue (Fig. 7B and data not shown). To prevent SUMO-1 conjugation during the DNA binding reaction, ATPγS was added along with the immobilized promoter DNA template (Fig. 7B, lanes 5–6 and 11–12).

When the DNA-binding reaction was carried out in the presence of sumoylated TFIID, DNA binding was partially rescued. In agreement with these data, we found that an antibody against SUMO peptides that bind to SUMO modified f:TFIID did not cross-react with f:TFIID when bound to DNA (Fig. 7A, lanes 6). These results suggest that TAF5, but not TAF12, is a target of SUMO-1 conjugation in vitro.
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In this study we investigated SUMO-1 modification of several human TFIID subunits containing the YKXE SUMO consensus target motif (22, 24). We demonstrate that the human TFIID subunits hsTAF5 and hsTAF12 are SUMO-modified in human cells and in vitro, both in isolation and in the context of purified human TFIID. We further observed in vitro sumoylation of recombinant hsTBP and hsTAF1. Interestingly, hsTAF1 contains at least two SUMO acceptor sites, one located close to the hsTAF1 HAT domain, and another in close proximity to the hsTAF1 double bromodomain (Fig. 1). However, so far we were unable to demonstrate in vitro SUMO conjugation to hsTAF1 or to hsTBP in the context of purified human TFIID complex.

Whether TFIID sumoylation at hsTAF1 and/or hsTBP can occur under more physiological conditions, for example in the presence of specific E3 ligase activity, remains a possibility that has to be investigated in the future.

Investigations into the functional consequences of SUMO modification within multiprotein complexes such as TFIID present a formidable technical challenge. In the simplest scenario, conjugation of SUMO to a specific target site may either directly or indirectly modulate subunit-specific TFIID functions, such as TAF interactions with regulatory proteins, TBP DNA-binding activity (see below), or one of the enzymatic activities residing in the TAF1 subunit. However, it is equally conceivable that a single SUMO modification can affect the entire TFIID complex, for example by altering its stability or by mediating its localization to specialized nuclear domains. On the other hand, SUMO modification at a single target site may have only very subtle effects, and multiple sumoylation events may be required to modulate TFIID functions. Finally, we note that global changes in cellular TFIID activity could be brought about by transient SUMO modification of isolated TFIID subunits, which may in turn affect TFIID complex assembly or the induction of dynamic changes in TFIID subunit composition seen during developmental processes (39, 40).

So far, we have no indication that SUMO modification affects the nuclear localization of TFIID. In human cultured cells, transiently expressed hsTAF5 and hsTAF12 point mutants lacking the SUMO acceptor lysine and SUMO-1-hsTAF5 and -hsTAF12 fusion proteins showed a nuclear localization pattern similar to their wild-type counterparts. However, the data obtained so far do not allow us to draw conclusions on the question whether changes in the nuclear distribution of TFIID can be brought about by simultaneous SUMO modification of
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TFIID is unique in that it is the only component of the general RNP II transcription machinery capable of sequence-specific DNA binding. TFIID interactions with core promoter DNA elements are crucial for RNP II transcription initiation complex assembly and are therefore tightly controlled by transcription regulatory pathways.

We investigated the effect of sumoylation on the DNA-binding activity of purified human TFIID complex using immobilized promoter DNA templates. Our data suggest that SUMO-1 conjugation to hsTAF5 interferes with TFIID binding to promoter DNA. In contrast, SUMO-1 conjugation to hsTAF12 subunit appears to have no detectable effect on TFIID DNA-binding activity. These observations suggest that reversible SUMO modification at the hsTAF5 subunit can contribute to the dynamic regulation of TFIID promoter-binding activity in human cells.

Surprisingly, prebound TFIID-promoter complexes can be SUMO-modified at both hsTAF5 and hsTAF12 without compromising their stability. This observation suggests that hsTAF5 sumoylation interferes with TFIID DNA interactions through a subunit other than hsTAF5 itself. Earlier studies suggested that the in vitro promoter-binding activity of purified human TFIID complex is largely dependent on sequence-specific TBP interactions with TATA box sequences (27, 41). Thus SUMO modification of TFIID at hsTAF5 may compromise TBP-DNA interactions.

Support for this model comes from structural analyses of purified human and yeast TFIID complexes by electron microscopy and digital image processing (43–46). These studies revealed a remarkable similarity in the overall architecture of human and yeast TFIID complexes, which resemble a molecular clamp formed of three major lobes connected by thin linking regions (45, 46). The location of TAFs within the yeast TFIID complex has been determined by immunolabeling experiments (43, 44). Importantly, yeast TFIID contains two copies of TAF5 (45), which form the bridging regions between the three lobes of the TFIID structure (Fig. 8) (44). The N termini of the two copies of TAF5 are located within lobe C in close proximity of each other, whereas the two TAF5 C termini, containing WD40 protein-protein interaction domains (48), extend into two different lobes A and B (Fig. 8) (43, 44). TAF12 forms a heterodimeric complex with TAF4 through specific histone fold domain interactions (4, 49). Yeast TFIID complexes can contain two TAF4/TAF12 submodules, which localize to lobes B and C of the TFIID structure (Fig. 8) (43). Fig. 8 shows a hypothetical model based on the yeast TFIID structure, which indicates the potential location of hsTAF5 and hsTAF12 SUMO target sites. The model predicts that SUMO conjugation to the N terminus of hsTAF5 occurs in lobe C in relation close proximity to TBP, consistent with the idea that hsTAF5 interferes with TBP DNA binding. Sumoylation of hsTAF12 may affect two distinct locations, one in lobe C close to the TAF5 N termini and one in lobe B, a good distance away from TBP. These observations suggest an additional level of complexity that must be considered when the functional relevance of SUMO modifications at individual TFIID subunits is investigated: SUMO modification of a particular TFIID subunit might potentially affect two distinct regions within the same TFIID structure.

Previous studies had shown that reversible SUMO conjugation modulates the activity of many promoter-specific transcription regulatory proteins in human cells (18, 19, 21). Our data extend these observations and provide the first evidence that SUMO modifications can directly affect the activity of the human TFIID complex, a key component of the general RNP II transcription machinery. Our observations are in broad agreement with recently published results of proteomic approaches in yeast, which identified several RNP II subunits as well as subunits of the yeast general transcription factors TFIIA, TFIID, TFIIE, and TFIIF as potential SUMO targets (50–52). Thus SUMO modification of general RNP II transcription factors appears to be a regulatory mechanism that is conserved throughout evolution.

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