Transcription Factor AP-2 Interacts with the SUMO-conjugating Enzyme UBC9 and Is Sumolated in Vivo*

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Received for publication, March 22, 2002, and in revised form, May 14, 2002
Published, JBC Papers in Press, June 18, 2002, DOI 10.1074/jbc.M202780200

The members of the AP-2 family of transcription factors are developmentally regulated and have distinct yet overlapping functions in the regulation of many genes governing growth and differentiation. All AP-2 factors appear to be capable of binding very similar DNA recognition sites, and the determinants of functional specificity remain to be elucidated. AP-2 transcription factors have been shown to act both as transcriptional activators and repressors in a promoter-specific manner. Although several mediators of their activation function have been suggested, few mechanisms for the repression or down-regulation of transcription have been described. In a two-hybrid screen for proteins interacting with AP-2 factors, we have identified the UBC9 gene that encodes the E2 (ubiquitin carrier protein)-conjugating enzyme for the small ubiquitin-like modifier, SUMO. The interaction domain resides in the C-terminal half of AP-2, which contains the conserved DNA binding and dimerization domains. We have detected sumolated forms of endogenous AP-2 in mammalian cells and have further mapped the in vivo sumolation site to conserved lysine 10. Transient transfection studies indicate that sumolation of AP-2 decreases its transcription activation potential, and we discuss the possible mechanisms for the observed suppression of AP-2 transactivation.

The AP-2 transcription factor family consists of four members, AP-2α, AP-2β, AP-2γ, and the recently described AP-2δ, each encoded by a separate gene (1–4). AP-2 proteins homodimerize through a unique C-terminal helix-span-helix motif and bind palindromic DNA recognition sequences (consensus 5′-GCCN3GGC-3′) through the basic domain that lies immediately N-terminal of the dimerization motif (Refs. 5 and 6; see Fig. 2A). The dimerization/DNA binding region is highly conserved between the AP-2 isoforms. AP-2 factors appear to execute crucial, overlapping (7, 8), yet distinct functions during embryonic development (9–11) and malignant transformation (12–14). Accordingly, these factors have been shown to regulate many genes related to growth and differentiation including the proto-oncogene ERBB2 (15), estrogen receptor (16), E-cadherin (17), and the cell cycle inhibitor p21WAF1 (18), and it has been suggested that they may fulfill a dual role, not only specifying gene expression patterns but also determining the balance between cellular growth and death (19).

Many proteins have previously been suggested to interact with and coactivate the AP-2 transcription factors, for example the general coactivator PC4 (20) and poly ADP-ribose polymerase (21). In addition, we have recently reported that two members of the CITED (CREB-binding protein (CBP)/p300-interacting transactivator with ED-rich tail) family of proteins, CITED2 (22) and CITED4 (23), specifically interact with and coactivate the AP-2α, -δ, and -γ isoforms of the family. It is also apparent, however, that AP-2 factors are able to repress expression of certain genes, particularly during cellular differentiation. For example, AP-2α contributes to adipogenesis by silencing expression of the pleiotropic activator C/EBPα during the early stages of adipocyte differentiation (24). In this context AP-2 factors might be expected to interact with a different set of cofactors, yet no corepressor proteins or mechanisms of down-regulation of AP-2 activity through a protein-protein interaction or protein modification have been described to date.

We aimed to investigate further how the transcriptional activity of AP-2 factors is regulated and how their transcriptional effects are mediated by cofactors and other proteins. To this end we set up a two-hybrid screen with AP-2γ as bait to identify novel AP-2-interacting proteins. Here we describe the isolation of UBC9 as an AP-2 interactor and as a covalent SUMO-conjugating enzyme. SUMO peptides are small ubiquitin-like modifiers that become covalently conjugated through an isopeptide linkage to lysine residues in a subset of cellular proteins via a series of enzymatic steps that closely parallels yet is distinct from the process of protein ubiquitination (for review, see Ref. 25). The enzymes involved in SUMO conjugation have been identified in yeast and more recently in mammalian cells (26–29). Sumolation occurs in the nucleus and requires a distinct E1-activating enzyme from that mediating ubiquitination, a SUMO-specific E2-conjugating enzyme, UBC9, that is also involved in substrate recognition, plus, at least in some instances, a substrate-specific E3 ligase. SUMO modification has been shown to have varying consequences on the target proteins, and here we present evidence that sumolation of AP-2 transcription factors down-regulates their transcriptional activation potency.

EXPERIMENTAL PROCEDURES

Plasmid Constructs—All plasmid constructs were verified by sequencing. Full-length human AP-2α, AP-2β, and AP-2γ cDNA sequences plus the AP-2γ deletion variants (see Fig. 2A) were subcloned into the GAL4 DNA binding domain fusion vector pGBT9 (CLONTECH). The mammalian expression constructs pcDNA3.1-AP2α and pcDNA3-CITED2 plus the Bluc and 3xAP2-Bluc reporter constructs have been described previously (22). The K10R mutation in AP-2γ was created using the GeneEditor kit (Promega) and, for mam-

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† The abbreviations used are: E2, ubiquitin carrier protein; E1, ubiquitin-activating enzyme; E3, ubiquitin-protein isopeptide ligase; IAA, iodoacetamide; NEM, N-ethylmaleimide.
malian transfections, was introduced into pcDNA3.1-AP2γ such that the construct was otherwise identical to the wild-type AP-2γ construct. Full-length coding sequences for human SUMO-1 and UBC9 were obtained by PCR using human Universal QUICK-Clone cDNA (CLONTECH) as the DNA template and subcloned into the yeast two-hybrid GAL4 activation domain fusion vector pGAD424 (CLONTECH) or the mammalian expression vector pSG5 (Stratagene). The C-terminal non-conjugatable SUMO-1 mutant, SUMO-1AC, was also created by PCR and identically cloned into pSG5. The UBC33/C93S mutant was obtained from R. T. Hay and introduced into the pSG5 and pGAD424 vectors to create expression constructs otherwise identical to the wild-type UBC9 constructs.

Yeast Two-hybrid Screen and Assays—The bait plasmid pGBT9-AP-2γ and a human placental oligo-tGt-primed GAL4 activation domain fusion library (library vector pACT2, CLONTECH) were transformed into the yeast strain HF7c (CLONTECH) by simultaneous cotransformation using the Alkaline-Cation yeast transformation kit (Bio101). Positive clones were isolated and further analyzed as instructed in the CLONTECH Matchmaker manual. To perform quantitative two-hybrid tests between known proteins, two yeast expression plasmids were simultaneously cotransformed into the yeast strain SFY526 (CLONTECH) as above. Liquid β-galactosidase assays were performed according to the CLONTECH Matchmaker manual and repeated at least three times, and the average, with the S.D. presented as error bars, is shown.

Cell Culture, Transient Transfections, and Dual Luciferase Assays—MDA MB 436, MCF7, T47D, and HepG2 cells were maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum, 60 μg/ml penicillin, and 100 μg/ml streptomycin. For MDA MB 436 and MCF7 cells the medium was further supplemented with 10 μg/ml insulin. All cells were cultured in 10% CO2 at 37 °C. Transient transfection assays were performed using TransFast transfection reagent (Promega) according to the manufacturer’s instructions. The cells were transfected on 12-well plates when 60–70% confluent. The amounts of reporter constructs added to cells in each individual transfection were 0.5 μg of 3xAP2-Bluc or Bluc and 0.5 μg tk-renilla (Promega). The total amount of DNA transfected was normalized using the appropriate empty vectors. Cells were harvested after 48 h, and lysates were assayed using the dual luciferase reporter assay system (Promega) and a Bio-Orib 1251 luminometer (Labtech). Firefly luciferase readings were normalized to tk-renilla luciferase values. All the transfection experiments were repeated at least three times; the average, with the S.D. presented as error bars, is shown.

Antibodies and Western Blotting—The 6E4/4 mouse monoclonal antibody was raised against bacterially produced AP-2 protein and specifically recognizes the C terminus of AP-2γ (to be described elsewhere). Whole cell extracts were prepared by lysis in radioimmune precipitation assay buffer (50 mM Tris-HCl, 150 mM NaCl, 0.5% sodium deoxycholate, 0.1% SDS, 1% Nonidet P-40). Where indicated, isopeptidase inhibitors were included at 200 μM iodoacetamide (IAA) and 100 μg/ml N-ethylmaleimide (NEM). Alternatively, cell pellets were directly resuspended in SDS loading buffer at 85 °C and boiled immediately. SDS-PAGE, gel transfers (Bio-Rad Mini Trans-Blot transfer cell) to Hybond-ECL nitrocellulose (Amersham Biosciences), and primary and secondary antibody incubations were performed using standard techniques. The chemiluminescent detection of horseradish peroxidase-conjugated secondary antibodies was performed using the West Femto Max sensitivity substrate kit (Pierce) according to the manufacturer’s instructions. To reprobe for normalization, membranes were stripped in 2% SDS, 62.5 mM Tris-HCl, pH 6.7, 100 μM β-mercaptoethanol for 30 min at 55 °C and then rebloked before incubation with the goat polyclonal Ku-70 antibody (C-19, Santa Cruz).

RESULTS

AP-2 Transcription Factors Interact with UBC9—To identify novel proteins that interact with the members of the AP-2 family of developmentally regulated transcription factors, we performed yeast two-hybrid screen. As a bait we used the full-length coding sequence for human AP-2γ into the GAL4 binding domain fusion vector pGAD9 and cotransformed it into the yeast strain HF7c together with a placental cDNA library fused N-terminal to the coding sequence of the GAL4 activation domain. The HF7c yeast strain contains two integrated GAL4-dependent reporter genes, HIS3 for metabolic selection and β-galactosidase for color detection. From the 1 × 106 cDNA clones screened, we isolated several positive clones.

Upon sequencing the strongest positive clone, isolated twice, we found that it encoded a full-length cDNA for UBC9, the conjugating enzyme for human small ubiquitin-like modifier 1 SUMO-1 (26, 30).

Having identified UBC9 as a novel AP-2γ-interacting protein in our two-hybrid screen, we then tested whether this was specific for AP-2γ or whether the AP-2α and AP-2β isoforms were also capable of UBC9 interaction. Yeast two-hybrid liquid β-galactosidase assays showed that UBC9 was indeed capable of interacting with all three of these AP-2 isoforms (Fig. 1). The specificity of these interactions was further confirmed when essentially the same result was obtained on performing the two-hybrid assays in the reciprocal configuration such that AP-2 proteins were expressed as a GAL4 activation domain fusion protein and UBC9 as a GAL4 DNA binding domain fusion protein (data not shown).

UBC9 Interacts with the C-terminal Half of AP-2γ—To map the region in AP-2 responsible for the UBC9 interaction, we created various deletions of the AP-2γ-coding sequence (Fig. 2A) and subcloned them into the pGBT9 vector so that the deletion variants were expressed as GAL4 DNA binding domain fusion proteins within yeast cells. Using these deletions in yeast two-hybrid assays, we mapped the UBC9-interacting region in AP-2γ to the C-terminal half encompassing the DNA binding and dimerization domains (ΔN220, Fig. 2B, column B). The C-terminal deletion variant ΔC181 of AP-2γ that contains the proposed transcriptional activation domain alone did not interact with UBC9 (Fig. 2B, column I). The removal of 37 C-terminal amino acids (ΔC413 and ΔN220C413, Fig. 2B, columns H and D, respectively) also disrupted this interaction, whereas the form of AP-2γ lacking only the C-terminal 20 amino acids (ΔN220C430, Fig. 2B, column C) was still capable of interacting with UBC9. In electrophoretic mobility shift assays and protein-protein interaction experiments we have shown that the ΔC413 variant is incapable of dimerization, whereas the ΔC430 deletion can dimerize to a similar degree as the wild-type AP-2γ (data not shown). The ΔN220 deletion is still capable of both DNA binding and dimerization and interacts with UBC9 in two-hybrid assays, but further N-terminal deletion to ΔN292 abolishes the UBC9 interaction (Fig. 2B, column E). ΔN292 is analogous to the ΔN278 mutation of AP-2α that can still dimerize but lacks the basic domain and is, hence, defective in DNA binding and acts in a dominant-negative fashion in transfection assays (5). Taken together, these data imply that the UBC9 interaction region in AP-2 overlaps...
Fig. 3. AP-2γ is sumolated in vivo. Whole cell extracts from AP-2γ-overexpressing MCF7 cells were prepared either in the presence (+) or absence (−) of the isopeptidase inhibitors IAA and NEM or by direct lysis in hot SDS sample buffer (S) and analyzed by Western blot probed with an AP-2γ-specific antibody (upper panel) or with a Ku-70-specific antibody (lower panel, loading control). Lane 1, in vitro translated (ivt) AP-2γ; lane 2, cell lysate prepared without IAA and NEM; lane 3, cell lysate prepared in the presence of IAA and NEM; lane 4, cells lysed directly in SDS sample buffer.

It is evident that only a small proportion of the total AP-2 population in cells is sumolated, which is again consistent with other proteins, including the transcription factors p53 (34, 35) and c-Jun (36), which are known to be targets for sumolation. We have similarly detected sumolated forms of both AP-2γ and AP-2α in other cell lines, including T47D, which endogenously express AP-2 factors at high levels (data not shown).

Lysine 10 Is the Site of AP-2γ Sumolation in Vivo—The consensus site for SUMO conjugation in target proteins has recently been defined as a tetrapeptide ψKxE (where ψ is a hydrophobic residue) that surrounds the acceptor lysine in substrate proteins (37). Within the primary amino acid sequence of AP-2 isoforms we have identified a stretch of amino acids (33), presumably because SUMO conjugation creates a branched structure in the polypeptide chain that slows down the migration of the sumolated protein on SDS-PAGE. Consistent with this, the size of unmodified AP-2γ is ~49 kDa, whereas the slower migrating sumolated form runs at ~65 kDa.

We have similarly detected sumolated forms of both AP-2γ and AP-2α in other cell lines, including T47D, which endogenously express AP-2 factors at high levels (data not shown).

Lysine 10 was identified as the site of SUMO conjugation to arginine in AP-2γ to create the mutant K10R. Expression plasmids for either wild-type AP-2γ or the K10R mutant were transfected into the low AP-2-expressing cell line, MDA MB 436, together with SUMO-1 expression plasmids. Whole cell extracts were prepared in the presence of isopeptidase inhibitors (IAA and NEM) from cells transfected with either AP-2γ variant and from control cells transfected with an empty vector and analyzed by Western blotting. The sumolated form of AP-2γ could only be detected when the wild-type but not the K10R mutant was exogenously expressed (Fig. 4, compare lanes 3 and 4).
further supporting the idea that all three AP-2 isoforms tested here are capable of both UBC9 interaction and subsequent SUMO conjugation. By mutational analysis, it has been demonstrated that isoleucine and valine are favored as the hydrophobic amino acids in the ψ position of the tetrapeptide recognition motif (37), thus indicating that the three AP-2 isoforms may be optimal substrates for SUMO conjugation.

Overexpression of SUMO-1 Suppresses the Transcriptional Activity of AP-2—Essentially all SUMO-1 in mammalian cells is conjugated to target proteins, particularly to the RanGAP1 nuclear pore protein (32), and the proportion of free SUMO-1 is consequently very small. We, therefore, attempted to exogenously increase the amount of SUMO-1 within cells to test whether this would have an effect on AP-2 activity, as monitored by an AP-2-dependent reporter construct in transient transfection assays. Indeed, when we cotransfected increasing amounts of a SUMO-1 expression plasmid into the low AP-2-expressing MDA MB 436 cell line (Fig. 6A, columns 1–3) or in the high AP-2-expressing T47D cell line (Fig. 6B, columns 1–3). We speculate that the endogenous activity of the UBC9 enzyme may be saturating, thus not readily modulated by transient transfection. We then utilized a sumolation-defective mutant form of UBC9, UBC9/C93S (27).

In cotransfection experiments this variant led to increased levels of AP-2-dependent transcriptional activation in both MDA MB 436 and T47D cells (Fig. 6, A (columns 1, 5, and 6) and B (columns 1, 4, and 5). Our yeast two-hybrid assays confirmed that, whereas UBC9/C93S is indeed severely compromised in its interaction with SUMO-1 (Fig. 6C, columns 1–3), it does still interact with AP-2 as efficiently as wild-type UBC9 (Fig. 6C, columns 4–6). We suggest, therefore, that the overexpressed mutant form of UBC9 can act in a dominant-negative fashion by interacting with AP-2 within cells, thus preventing the access of endogenous UBC9 capable of SUMO-1 conjugation. This would result in a decrease in the proportion of sumolated forms of AP-2 and, hence, increase transcriptional activation by unmodified AP-2.

**DISCUSSION**

The AP-2 transcription factors are required for normal growth and differentiation during mammalian development and have been linked to the etiology of human breast cancer through their regulation of ERBB2 and estrogen receptor gene expression (3, 14–16). It is therefore important to establish how AP-2 factors mediate their transcriptional effects and how their activity is modulated. We sought to identify proteins that are involved in this process through their interaction with AP-2 factors. Here we have identified a novel interaction partner for AP-2, namely the SUMO-conjugating enzyme UBC9, and examined the functional consequences of such an interaction.

By deletion mutation analysis we have mapped the interaction interface with UBC9 to the C-terminal regions of the AP-2γ protein, which is highly conserved between the members of the AP-2 family. It is consistent, therefore, that two other AP-2 isoforms, AP-2α and AP-2β, were also capable of UBC9 interaction. The deletion of the 37 C-terminal amino acids of AP-2γ abolishes both dimerization and UBC9 interaction. On the other hand, when the AP-2γ C terminus is kept intact and, thus, dimerization-competent but the N-terminal deletion is
Fig. 5. SUMO-1 overexpression suppresses AP-2 transcriptional activation potency. A, SUMO-1 overexpression suppresses exogenous AP-2 activity. MDA MB 436 cells were transfected with the 3xAP2-Bluc reporter plasmid either alone (column 1) or with 1.0 μg of pcDNA3-AP2γ (columns 2–4) plus pSG5-SUMO-1 at 0.5 μg (column 3) or 2.0 μg (column 4). B, conjugation-defective variant of SUMO-1 cannot suppress AP-2γ transcriptional activity. MDA MB 436 cells were transfected with the 3xAP2-Bluc reporter plasmid either alone (column 1) or with 1.0 μg of pcDNA3-AP2γ (columns 2–4) plus expression plasmids (2.0 μg) for either wt SUMO-1 (column 3) or the non-conjugatable mutant, SUMO-1A1C (column 4). C, SUMO-1 overexpression suppresses endogenous AP-2 activity. AP-2-expressing T47D cells were transfected with the reporter plasmids 3xAP2-Bluc (columns 1 and 2) or Bluc, which lacks the AP-2-responsive elements (columns 3 and 4), either alone or with 2.0 μg of the pSG5-SUMO-1 expression plasmid, as indicated. D, The K10R mutant form of AP-2γ cannot be suppressed by SUMO-1 overexpression. MDA MB 436 cells were transfected with the 3xAP2-Bluc reporter plasmid either alone (column 1) or with 1.0 μg of either pcDNA3-AP2γ (columns 2 and 3) or pcDNA3-AP2γ/K10R (columns 4 and 5) plus 2.0 μg of the pSG5-SUMO-1 expression plasmid, as indicated. E, CITED2-mediated co-activation of AP-2γ can also be suppressed by SUMO-1 overexpression. HepG2 cells (which express little or no AP-2 or CITED proteins) were transfected with the 3xAP2-Bluc reporter plasmid either alone (column 1) or with 0.1 μg of either pcDNA3-AP2γ (columns 2–4) or pcDNA3-AP2γ/K10R (columns 5–7) and 0.5 μg of pcDNA3-CITED2 (columns 3, 4, 6, and 7) plus 1.0 μg of the pSG5-SUMO-1 expression plasmid, as indicated.

extended to remove the basic region that mediates contacts with DNA, the UBC9 interaction is also lost. We propose a model where UBC9 interacts with the DNA binding, basic domains of AP-2 factors but only does so when the factors are in a dimerized form.

The function, activity, stability, or subcellular localization of many proteins can be altered after their synthesis by post-translational covalent modifications such as phosphorylation, acetylation, and ubiquitination. The only suggested post-translational modification of the AP-2 transcription factors before this study has been protein kinase A-mediated phosphorylation (38, 39). Having established that the SUMO-conjugating enzyme UBC9 interacts with AP-2 factors, we examined whether they could consequently be modified by sumolation. We succeeded in demonstrating that a proportion of endogenous AP-2 does indeed exist in a sumolated form in mammalian cells. We could detect only one major SUMO-conjugated form of AP-2 and have defined lysine 10 near the N terminus of AP-2γ, conserved between AP-2α, -β, and -γ, as the site of sumolation in vivo. We note that the most recent member of the AP-2 family, AP-2δ, does not contain the conserved, sumotatable lysine at position 10 or any other sequences conforming to the sumolation consensus motif, whereas the region of proposed UBC9 interaction remains highly conserved in AP-2δ (4). Future studies will investigate whether the AP-2δ isoform is refractory to transcriptional suppression by sumolation and whether its activity is in any way modulated by an interaction with UBC9.

Interestingly, the site of sumolation in AP-2γ does not overlap or lie adjacent to the UBC9 interaction site, as appears to be the case with many other sumolated proteins (40, 41). We envisage that the N terminus of AP-2 must fold back toward the more C-terminal UBC9 interaction region to allow UBC9-mediated SUMO-conjugation, but structural studies will be required to clarify the precise spatial dynamics of AP-2 sumolation. We have also attempted to set up a reconstituted assay to sumolate AP-2 in vitro using human recombinant AP-2, SUMO-1, UBC9, and the SAE1 and SAE2 subunits of the SUMO-activating enzyme, but we have only achieved very inefficient sumolation of AP-2 in vitro. It has recently been demonstrated that the enzymatic machinery for sumolation, similar to the ubiquitination system, utilizes the activity of an E3 ligase component of the enzyme cascade for optimal SUMO

2 J. J. Eloranta and H. C. Hurst, unpublished results.
Sumolation of Transcription Factor AP-2

Covalent modification of cellular proteins by SUMO regulates various cellular processes such as nuclear transport and cell cycle progression (for review, see Ref. 25). Unlike ubiquitination, SUMO conjugation of proteins does not target them for proteasome-mediated degradation. Sumolation of transcription factors has been shown to have a range of differing effects on their activity. For example, sumolation of the tumor suppressor p53 (34, 35) and the heat shock factor HSF2 (47) have been shown to modulate their activity. For example, sumolation of the tumor suppressor p53 (34, 35) and the heat shock factor HSF2 (47) have been shown to modulate their activity.

Sumolation, this may imply that AP-2 is primarily conjugated with SUMO-1 in vivo.

Modulating the levels of wild-type UBC9 within cells showed no effect on AP-2-dependent transcription, and we speculate that the endogenous levels of UBC9 are not limiting in the cell lines used in this study. Interestingly, when the UBC9 mutant C93S is exogenously overexpressed in mammalian cells, the AP-2-dependent levels of reporter gene activity were elevated. This mutant lacks conjugation activity because cysteine 93, which is required to form a thioester linkage with SUMO peptides before their transfer to target lysines, has been replaced with serine. Because the C93S mutant is still able to associate efficiently with AP-2, the observed transcriptional enhancement is likely to be a consequence of decreased AP-2 sumolation by endogenous wild-type UBC9.

Covalent modification of cellular proteins by SUMO regulates various cellular processes such as nuclear transport and cell cycle progression (for review, see Ref. 25). Unlike ubiquitination, SUMO conjugation of proteins does not target them for proteasome-mediated degradation. Sumolation of transcription factors has been shown to have a range of differing effects on their activity. For example, sumolation of the tumor suppressor p53 (34, 35) and the heat shock factor HSF2 (47) have been reported to increase their transactivation capacity, whereas conjugation of SUMO to c-Jun (36) or androgen receptor (48) leads to decreased transcriptional activation. We have shown that AP-2 belongs to the latter group of SUMO-modified transcription factors, where sumolation has a negative effect on transcriptional potency. The mechanism of this effect is at present uncertain; however, our transfection assay results collectively point to SUMO modification down-regulating AP-2 transcription activation activity rather than it leading to the factors becoming transcriptional repressors. Thus, it is more likely that sumolation leads to a weakening or an abolition of the interactions between AP-2 and transcriptional coactivators than to an enhanced interaction with transcriptional corepressors. Because sumolation is known to alter the subcellular localization of many target proteins, such as RanGAP1 (31, 33), promyelocytic leukemia protein PML (49), and the p53-related

modification of at least some substrates (42–44). It thus seems conceivable that specific substrates, such as AP-2, require a SUMO ligase activity for efficient modification, although at present the identity of the putative AP-2 E3 ligase remains unknown. We have tested whether PIAS1, the recently identified SUMO E3 ligase for the transcription factor p53 (44), has any influence on AP-2-dependent transcription in transfection assays but have failed to see any significant effect.2

By exogenously increasing cellular levels of free SUMO-1 we have shown that AP-2 transcriptional activation can be suppressed. SUMO-mediated transcriptional suppression is also effective on CITED2-coactivated AP-2-mediated transcription. Invertebrates only have a single SUMO gene, whereas three members of the SUMO family have been described in vertebrates, SUMO-1 and the close homologues SUMO-2 and SUMO-3 (45). All three SUMO species share the same conjugation machinery, and the UBC9-conjugating enzyme appears to show no preference between them (46). It remains unclear whether the functional consequences of SUMO-2/3 conjugation differ from those of SUMO-1 conjugation. We have investigated whether, similar to SUMO-1, exogenous overexpression of SUMO-2 and SUMO-3 can also have a suppressive effect on AP-2-activated transcription. Indeed, both SUMO-2 and SUMO-3 appeared capable of suppressing AP-2 activity to a similar degree as SUMO-1 in transient transfection assays2; thus, for AP-2, the functional consequences of SUMO-1 conjugation seem similar to those of SUMO-2 and -3. However, SUMO-2 and SUMO-3 have been shown to be able to form polymeric chains (48), and because we have not detected a range of slower migrating forms of AP-2 indicating its multisumolation, this may imply that AP-2 is primarily conjugated with SUMO-1 in vivo.
protein p73 (50), another possibility is that sumolated forms of AP-2 become localized into an inactive subnuclear compartment. Future studies will investigate these possibilities. In summary, this study represents the first description of a mechanism for down-regulation of the activity of the AP-2 family of transcription factors through a protein-protein interaction and subsequent post-translational modification.

Acknowledgments—We thank Claire Ibbit for technical assistance, Joanna Desterro and Ron Hay for the UBC9/C93S clone and useful discussions, Georges Vassaux, Rob Orford, and Despina Xanthaki for critical reading of the manuscript, and Zoe Leech for help with manuscript submission.

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J. Biol. Chem. 2002, 277:30798-30804.
doi: 10.1074/jbc.M202780200 originally published online June 18, 2002

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