Small ubiquitin-related modifier-1 (SUMO-1) modification of the glucocorticoid receptor

Sha TIAN*, Hetti POUKKA*, Jorma J. PALVIMO*† and Olli A. JÄNNE*‡¹

*Biomedicum Helsinki, Institute of Biomedicine, University of Helsinki, P.O. Box 63 (Haartmaninkatu 8), FIN-00014 Helsinki, Finland, †Institute of Biotechnology, P.O. Box 56, University of Helsinki, FIN-00014 Helsinki, Finland, and ‡Department of Clinical Chemistry, P.O. Box 63, University of Helsinki and Helsinki University Central Hospital, FIN-00014 Helsinki, Finland

Small ubiquitin-related modifier-1 (SUMO-1) is covalently attached to many cellular targets to regulate protein–protein and protein–DNA interactions, as well as localization and stability of the target protein. The SUMO-1-conjugating E2 enzyme Ubc9 is known to interact with the glucocorticoid receptor (GR), a ligand-dependent transcription factor. In the present study, we show that GR is post-translationally modified by SUMO-1 (sumoylated) in a ligand-enhanced fashion. We identify experimentally three consensus SUMO attachment sites, two in the

N-terminal transactivation region and one in the ligand-binding domain of GR. The two N-terminal sites are the major acceptor sites for SUMO-1 attachment. Mutation of these sites enhances transcriptional activity of GR on minimal promoters, but has no clear effect on the more complex mouse mammary tumour virus promoter. Thus SUMO-1 modification of GR influences receptor function in a promoter context-dependent fashion.

Key words: nuclear receptor, sumoylation, transactivation.

INTRODUCTION

Glucocorticoids play important roles in the regulation of energy metabolism, development and homeostasis in human physiology. These hormones are also necessary for immune functions and stress responses via interactions with other transcription factors [1,2]. Glucocorticoids fulfil their tasks by binding the glucocorticoid receptor (GR), which is a ligand-dependent transcription factor belonging to the nuclear receptor superfamily. These receptors comprise three functional domains: a constitutively active N-terminal activation domain, a central DNA-binding domain and a C-terminal ligand-binding domain harbouring a second activation function. The receptors can be activated by steroid binding, after which they are translocated into the nucleus to bind to DNA-response elements as homodimers or heterodimers to activate transcription of target genes [3]. Mechanistic details of these processes are still being elucidated.

Transcriptional activity and stability of the receptors are affected by covalent post-translational modifications, such as phosphorylation, acetylation and ubiquitination. These modifications result in alterations of protein–protein interactions in multiprotein complexes, nuclear receptor DNA binding, subcellular structures and degradation [4–7]. Ubc9, the E2 conjugating enzyme for the small ubiquitin-related modifier-1 (SUMO-1, also known as sentrin, GMP1, phosphoinositidase C1 and Ubl1, or in yeast as Smt3), has been reported to interact with at least two steroid receptors, the androgen receptor (AR) and the GR, as assessed by yeast two-hybrid analysis. Ubc9 activates AR-dependent transcription; likewise, some of the functional consequences of Ubc9 and GR interaction have recently been addressed [8,9].

The sumoylation pathway resembles that of ubiquitin conjugation, but the enzymes catalysing the two processes are distinct [10]. Specifically, SUMO-1 is activated in an ATP-dependent manner by the E1 enzyme, consisting of an Aos1/Uba2 heterodimer [11]. Activated SUMO-1 is transferred to

Ubc9, the E2 enzyme, and subsequently attached to the ϵ -amino group of a specific lysine residue of the target protein [12]. *In vitro*, these two enzymic activities are sufficient to modify a number of SUMO-1 targets. In addition, SUMO-1 E3 ligases have been identified in the last year in yeast and mammalian cells [13–16]. There is also a family of SUMO-specific proteases that can cleave the isopeptide bond between SUMO-1 and the conjugated protein target; thus, SUMO-1 modification is reversible *in vivo*.

Sumoylation leads to diverse biological effects. SUMO-1 modification of RanGAP1 (GAP1, GTPase-activating protein 1) and Ran-binding protein 2 is involved in nucleocytoplasmic trafficking [17,18], and SUMO-1 conjugation is required for mitotic localization of RanGAP1 [19]. Attachment of SUMO-1 to promyelocytic leukaemia protein is found in subnuclear structures, and is also required for the promyelocytic leukaemia protein-mediated recruitment of other factors, such as SP100 and Daxx, into nuclear bodies [20–22]. SUMO-1 modification prevents inhibitory κ B- α from degradation through competition for the same lysine residue as that required for ubiquitination [23]. Many transcription factors, such as p53, c-Jun and c-Myb, have been identified as targets for SUMO-1 attachment [24,25]. We have shown previously [26] that the nuclear receptor AR is modified by SUMO-1 and that sumoylation influences the AR function. We have also found that another nuclear steroid hormone receptor, the GR, can be sumoylated *in vitro* [26]. In the present study, we have defined experimentally the sumoylation sites in the N-terminal transactivation domain and ligand-binding domain of GR and demonstrate that SUMO-1 modification affects the transcriptional activity of the receptor.

MATERIALS AND METHODS

Materials

Dexamethasone was purchased from Sigma. Mouse monoclonal anti-Flag antibody M2 and mouse monoclonal anti-SUMO-1

Abbreviations used: AR, androgen receptor; FBS, foetal bovine serum; GR, glucocorticoid receptor; K277R, Lys²⁷⁷ → Arg; LUC, luciferase; PR, progesterone receptor; SUMO-1, small ubiquitin-related modifier-1.

¹ To whom correspondence should be addressed at Biomedicum Helsinki (e-mail olli.janne@helsinki.fi).

(anti-GMP-1) were from Sigma and Zymed Laboratories (South San Francisco, CA, U.S.A.) respectively. Horseradish peroxidase-conjugated anti-rabbit IgG and anti-mouse IgG were from Zymed Laboratories.

Plasmid constructions

pCMV-Flag-hGR was generated by inserting PCR-amplified fragment encoding amino acids 2–148 of hGR into the *EcoRI*/*SaII* site of pFlag-CMV-2 (Sigma). The rest of hGR cDNA sequence was cut from pSG5-hGR [27] and inserted into the *SaII* site of pFlag-CMV-2. Point mutants Lys²⁷⁷ → Arg (K277R), Lys²⁹³ → Arg (K293R), Lys⁷⁰³ → Arg (K703R) and double (2K/R = K277R/K293R) and triple (3K/R = K277R/K293R/K703R) mutants of hGR were generated by the overlapping PCR mutagenesis strategy and inserted into the same vector. pSG5-His-SUMO-1 was a gift from Dr Anne Dejean (Pasteur Institute, Paris, France). The β -galactosidase expression plasmid pCMV β was from Clontech Laboratories (Palo Alto, CA, U.S.A.). pARE₂-TATA-LUC (LUC, luciferase) has been described previously [27]. pMMTV-LUC is a reporter construct that contains nucleotides –1146 to +88 of the mouse mammary tumour virus promoter in pGL3-Basic (Promega, Madison, WI, U.S.A.). pARE₄tk-LUC was made by inserting a duplicated C3(1) androgen response element into the *SmaI* site of pARE₂-tk-LUC vector [28].

Cell culture and transfections

COS-1 and HeLa cells were obtained from A.T.C.C. (Manassas, VA, U.S.A.) and cultured in Dulbecco's modified Eagle's medium supplemented with 10% foetal bovine serum (FBS), 25 units/ml penicillin and 25 units/ml streptomycin. For HeLa cells, non-essential amino acids were also added. For immunoprecipitation experiments, 3.5×10^5 COS-1 cells were seeded on to 6 cm dishes 24 h before transfection. The cells received fresh medium containing 10% charcoal-stripped FBS 2 h before transfection. Using the FuGene reagent (Roche Molecular Biochemicals, Mannheim, Germany), 1 μ g of Flag-hGR and mutant expression plasmids and 1.5 μ g His-SUMO-1 or pSG5 were transfected to recipient cells. The cells were supplied with 100 nM dexamethasone 18 h after transfection. For transactivation and transrepression assays, 4×10^4 COS-1 cells were seeded on to 12-well plates 24 h before transfection, and 4 h before transfection, the cells received fresh medium containing 10% charcoal-stripped FBS. Reporter plasmid (150 ng), pCMV β (50 ng) and indicated amounts of GR expression constructs were transfected. The cells received fresh medium containing 2% charcoal-stripped FBS and 100 nM dexamethasone or vehicle 18 h after transfection. After a 24 h culture, the cells were harvested and LUC and β -galactosidase activities were assayed as described in [28].

Immunoprecipitation and immunoblotting

COS-1 cells were collected in PBS containing 20 mM *N*-ethylmaleimide (Sigma), and cell extracts were prepared in modified RIPA buffer [50 mM Tris/HCl (pH 7.8)/150 mM NaCl/5 mM EDTA/15 mM MgCl₂/0.5% Nonidet P40/0.3% Triton X-100/0.5% sodium deoxycholate/1 mM dithiothreitol/1:200-diluted protease inhibitor cocktail/10 mM *N*-ethylmaleimide]. Immunoprecipitation with mouse monoclonal anti-Flag antibody M2 (1:2000 dilution) was performed as described in [27]. Bound proteins were released in 2 × SDS sample buffer, resolved on SDS/PAGE (7.5% acrylamide), transferred on to Hybond ECL[®] nitrocellulose membrane (Amersham Pharmacia Biotech, Arlington Heights, IL, U.S.A.), and the membrane incubated

with mouse monoclonal anti-SUMO-1 antibody (1:3000 dilution). The whole cell extracts from HeLa and COS-1 cells were resolved by electrophoresis on SDS/PAGE (7.5% acrylamide). Proteins were transferred on to Hybond ECL[®] nitrocellulose membrane that was incubated with mouse monoclonal anti-Flag antibody M2 (1:3000 dilution). The membrane was subsequently incubated with horseradish peroxidase-conjugated goat anti-mouse IgG antibody (Zymed Laboratories), and immunocomplexes were visualized by using the ECL[®] detection reagents (Amersham Pharmacia Biotech), according to the manufacturer's instructions.

RESULTS

GR is modified by SUMO-1

Expression vectors for Flag-tagged hGR and His₆-tagged SUMO-1 were transiently transfected into COS-1 cells, and cell extracts were prepared in the presence of 20 mM *N*-ethylmaleimide to inhibit *in vitro* SUMO-1 deconjugation [29,30]. Immunoblotting of the cell extracts with monoclonal anti-FLAG antibody showed four immunoreactive bands: a major band of approx. 94 kDa corresponding to the Flag-tagged unmodified GR and three less intense but more slowly migrating bands (≥ 150 kDa) (Figure 1A). Identical bands were detected with an antibody raised against GR, confirming that the bands indeed corresponded to different forms of GR (results not shown). To examine whether the slowly migrating bands represented GR modified by His-tagged SUMO-1, immunoprecipitations were performed with Flag antibody. Anti-SUMO-1 antibody detected the three bands of high molecular mass in the immunoprecipitates, whereas the 94 kDa unmodified GR band was not detected (Figure 1B). These results indicate that the slowly migrating bands represent SUMO-1-modified forms of GR and suggest that there are several SUMO-1 acceptor sites in the receptor.

Lys²⁷⁷ and Lys²⁹³ are the major GR sites modified by SUMO-1

SUMO-1 attachment to target proteins occurs at specific lysine residues, which are in most cases embedded in a consensus sequence Ψ KXE [31,32], where Ψ is a large hydrophobic amino acid and X any amino acid residue. In our previous work, we localized three sites in the hGR sequence that matched with the consensus SUMO-1 attachment motif. The sites Lys²⁷⁷ and Lys²⁹³ are in the N-terminal region, and Lys⁷⁰³ is in the ligand-binding domain [26]. To verify whether these residues are *in vivo* acceptors of SUMO-1, point mutations K277R, K293R and K703R were introduced into the Flag-tagged hGR. Immunoprecipitation of recipient COS-1 cell extracts revealed that K277R and K293R are poorly sumoylated in comparison with wild-type GR (Figures 1A and 1B, lanes 7–8 and 9–10). K703R mutation influenced GR sumoylation only marginally, indicating that Lys⁷⁰³ does not serve as an important *in vivo* acceptor of SUMO-1 (Figures 1A and 1B, lanes 11–12). When the point mutations were combined, SUMO-1 modification of GR was almost completely absent (3K/R, Figures 1A and 1B, lanes 13–14; 2K/R, results not shown). Even with the triple mutant, faint bands are still visible, suggesting that some sumoylation may occur at sites other than those conforming to the consensus sequence Ψ KXE.

Exposure of COS-1 cells to dexamethasone enhanced the extent of SUMO-1 modification of GR; this applied not only to wild-type GR (Figures 1A and 1B, lane 6 versus lane 5), but also to all receptor mutants (Figures 1A and 1B, lane 8 versus lane 7, lane 10 versus lane 9, lane 12 versus lane 11 and lane 14 versus lane 13). However, the pattern of SUMO-1 modification of the

Figure 1 Modification of GR by SUMO-1 at two major attachment sites

COS-1 cells and HeLa cells grown on 6 cm dishes were transfected with His-SUMO-1 or empty expression vector as indicated along with wild-type (wt) Flag-hGR, Flag-K277R, Flag-K293R, Flag-K703R, Flag-2K/R (K277R/K293R) or Flag-3K/R (K277R/K293R/K703R). The cells received 100 nM dexamethasone (Dex) or vehicle 16 h before harvesting. **(A)** COS-1 cell extracts (5%) were immunoblotted with anti-Flag antibody and **(B)** the remaining extracts were subjected to immunoprecipitation (IP) with anti-Flag antibody. The immunoprecipitates were Western-blotted (WB) with anti-SUMO-1 antibody. **(C)** HeLa cell extracts were immunoblotted with anti-Flag antibody.

wild-type and mutated receptor proteins was the same in the presence and absence of the hormone (Figure 1B). Increased sumoylation is not explained by differences in the amount of GR protein in the cells (Figure 1A). A likely explanation for these results is that the ligand promotes GR transfer into the nucleus, where Aosl/Uba2 and Ubc9 enzymes are predominantly located [33]. Sumoylation of GR was also examined in HeLa cells (Figure 1C). Similar to COS-1 cells, K277R and K293R are sumoylated less than wild-type GR in HeLa cells (Figure 1C, lanes 4–5 and 6–7). Likewise, Lys⁷⁰³ is not an important SUMO-1 acceptor in HeLa cells, since double mutation of the sumoylation sites (2K/R) is sufficient to eliminate GR sumoylation almost completely in these cells (Figure 1C).

Figure 2 Substitutions of the sumoylation sites modulate the activity of GR

(A) HeLa cells were transfected with 10 ng of expression vectors encoding wild-type (wt) Flag-GR and Flag-tagged mutants K277R, K293R, K703R, 2K/R (K277R/K293R) and 3K/R (K277R/K293R/K703R) together with the minimal pARE₂-TATA-LUC reporter (150 ng). The cells received 100 nM dexamethasone (+ Dex) or vehicle (– Dex) 18 h after transfection. Luciferase activities in cell extracts were adjusted to transfection efficiency by using β -galactosidase activity. The activity of wild-type (wt) GR (10 ng of plasmid DNA) in the presence of dexamethasone is set as 100, and the values represent Means $\pm$ S.D. for three independent experiments. **(B)** As in **(A)**, except that HeLa cells were transfected with 3–100 ng of GR expression plasmids as indicated. The amount of transfected DNA was balanced by the addition of an empty expression vector. Only values obtained in the presence of 100 nM dexamethasone are depicted. **(C)** Expression levels of wild-type GR, and 2K/R and 3K/R mutants in an experiment corresponding to results shown in **(B)**. GR was immunoblotted with anti-Flag antibody from the same lysates (pooled from triplicate wells) that were used for reporter gene assays.

Transactivation assays with the minimal ARE₂TATA-LUC reporter were employed in HeLa cells to examine whether substitutions in the SUMO-1 attachment sites of GR influence receptor function. Hormone response element in the ARE₂-TATA promoter also mediates GR-dependent signalling [34]. K277R is sumoylated as well as wild-type GR (Figure 1), and the activity of this mutant was somewhat lower than that of the wild-type receptor (Figure 2A), perhaps owing to the localization of the mutation in the ligand-binding domain of GR. K277R and K293R, which are poorly sumoylated, exhibited transcriptional activities 2.8- and 1.8-fold higher than wild-type GR respectively (Figure 2A). The 2K/R (K277R/K293R) and 3K/R (K277R/

Figure 3 Comparison of the effects of SUMO-1 attachment site mutations on GR activity with different promoters

(A) HeLa cells were transfected with pARE₄-tk-LUC reporter (150 ng) and increasing amounts (3, 10, 30 and 100 ng) of wild-type (wt) Flag-GR and 2K/R and 3K/R mutant expression vectors. The total amount of DNA was kept constant by adding empty pFlag-CMV-2 when necessary. (B) The same experiment was performed by using pMMTV-LUC reporter, which contains the region -1146/+88 of the mouse mammary tumour virus promoter in front of the LUC gene. The relative LUC values in the presence of 100 nM dexamethasone (Dex) and 10 ng of wild-type GR is set as 100. The values are means $\pm$ S.D. from three independent experiments.

K293R/K703R) mutants, which are poor targets for SUMO-1 attachment, displayed 9.3- and 8.4-fold higher activities than wild-type GR respectively. Importantly, the 2K/R and 3K/R mutants were constantly 4–9.3- and 3–8.4-fold, respectively, more active than wild-type GR over a wide range of expression plasmid (3–100 ng/well) (Figure 2B). The high transcriptional activity of 2K/R and 3K/R mutants cannot be explained by the amounts of expressed receptor protein, since if anything, the mutated receptors were expressed to levels lower than that of wild-type GR as assessed by immunoblotting (Figure 2C).

To examine whether SUMO-1 modification of GR function is dependent on the promoter sequence, the activity of wild-type GR and GR mutants (2K/R and 3K/R) was examined on two other promoters: the ARE₄-tk promoter and a more complex promoter, the MMTV promoter. When ARE₄-tk-LUC reporter was used, both GR mutants exhibited approx. 2-fold higher transcriptional activity than wild-type receptor (Figure 3A). By contrast, when the mouse mammary tumour virus promoter was used, these mutants were either slightly less active than or as

active as the wild-type GR (Figure 3B). Thus sumoylation influences the function of GR in a fashion that is dependent on the promoter context.

DISCUSSION

Similar to AR, GR was *in vitro* sumoylated in our previous experiments when Ubc9 is present in the reaction [26], but only one sumoylated GR form was identified in those experiments. This may be due to the previous reaction conditions that were geared to detect Ubc9-dependent SUMO-1 conjugation of AR. In the present study, we show that GR is covalently modified by SUMO-1 in intact cells and that dexamethasone facilitates sumoylation, probably by promoting the transfer of GR from cytoplasm to nucleus. The major sumoylation sites of human GR are the N-terminal Lys²⁷⁷ and Lys²⁹³ residues, whereas the third site, Lys⁷⁰³ residue in the ligand-binding domain, functions *in vivo* as a poor SUMO-1 acceptor.

The sumoylation sites (Lys²⁷⁷ and Lys²⁹³) in hGR are the same as the two synergy control motifs (Lys²⁹⁷/Lys³¹²) in the N-terminus of rat GR [35]. Iniguez-Lluhi and Pearce [35] showed that disruption of the two synergy control motifs selectively increases transcriptional activity of rat GR on a promoter with three copies of the tyrosine aminotransferase (TAT) hormone response element without influencing GR activity on a promoter with only one copy of the response element. Spacing of hormone response elements or their distance from the TATA box was not important for the function of the synergy control motifs [35]. In the present study we found, using promoter constructs different from those of Iniguez-Lluhi and Pearce, that transcriptional activity of the sumoylation-defective GR mutant is greater than that of wild-type GR from ARE₂-TATA and ARE₄-tk promoters. In principle, these results are in line with the findings of Iniguez-Lluhi and Pearce [35], indicating that the activity of sumoylation-defective GR is dependent on the promoter sequence, and they are also in concert with our previous results on AR, in that mutation of the sumoylation sites of AR increased transcriptional activity of this receptor on simple promoters severalfold [26]. In agreement with the results of Iniguez-Lluhi and Pearce [35], sumoylation-defective and wild-type GRs exhibited, in our study, indistinguishable activities on a more complex promoter, the MMTV promoter (Figure 3B), further substantiating the notion that sumoylation indeed influences GR function in a promoter context-dependent fashion. The underlying mechanisms remain to be elucidated.

N-terminal regions of most steroid receptors contain SUMO-1 attachment consensus motifs (Ψ KXE) [26], but experimental evidence that these sequences indeed function as *in vivo* sumoylation sites exists only for AR [25] and GR (present study). In this context, it is of interest to note that human progesterone receptor (PR) contains an inhibitory sequence in the N-terminal region, between residues 165 and 455 [36], and that the only SUMO-1 attachment motif of PR is located within this sequence, at residues 387–390 (Lys³⁸⁸) [26]. Currently, it is not known whether potential sumoylation of PR has any bearing on the function of this inhibitory sequence. SUMO-1-conjugated receptors are likely to be involved in protein–protein interactions and assembly or disassembly of protein complexes, such as those with co-activators [37] or other proteins involved in transcriptional regulation. Similar to AR and GR, sumoylation may also regulate negatively transcriptional activity of c-Jun and c-Myb [24,25]. SUMO-1 conjugation has been reported to both increase and decrease p53 transactivation function, and members of the PIAS protein family that act as SUMO E3 ligases repress transcriptional activity of p53 [38–40].

Taken together, sumoylation of steroid receptors is likely to play a role in the transcriptional activity of these proteins. Our results indicate that the previously identified synergy control motifs of GR [35] are the principal SUMO-1 acceptor sites of this receptor. Further studies are required to pinpoint the mechanisms by which sumoylation of steroid receptors is regulated and to identify protein complexes whose assembly and/or disassembly is dependent on the sumoylation status of the receptors.

We thank Pirjo Kilpiö, Leena Pietilä and Seija Mäki for technical assistance, and Anne Dejean and Andrii Domanskyi for the supply of plasmids. This work was supported by grants from the Academy of Finland, the Finnish Foundation for Cancer Research, the Sigrid Jusélius Foundation and Biocentrum Helsinki.

REFERENCES

- Karin, M. (1998) New twists in gene regulation by glucocorticoid receptor: is DNA binding dispensable? *Cell* (Cambridge, Mass.) **93**, 487–490
- Freedman, L. P. (1999) Increasing the complexity of coactivation in nuclear receptor signaling. *Cell* (Cambridge, Mass.) **97**, 5–8
- Glass, C. K. and Rosenfeld, M. G. (2000) The coregulator exchange in transcriptional functions of nuclear receptors. *Genes Dev.* **14**, 121–141
- Lange, C. A., Shen, T. and Horwitz, K. B. (2000) Phosphorylation of human progesterone receptors at serine-294 by mitogen-activated protein kinase signals their degradation by the 26 S proteasome. *Proc. Natl. Acad. Sci. U.S.A.* **97**, 1032–1037
- Li, X. Y., Boudjelal, M., Xiao, J. H., Peng, Z. H., Asuru, A., Kang, S., Fisher, G. J. and Voorhees, J. J. (1999) 1,25-Dihydroxyvitamin D3 increases nuclear vitamin D3 receptors by blocking ubiquitin/proteasome-mediated degradation in human skin. *Mol. Endocrinol.* **13**, 1686–1694
- Nawaz, Z., Lonard, D. M., Dennis, A. P., Smith, C. L. and O'Malley, B. W. (1999) Proteasome-dependent degradation of the human estrogen receptor. *Proc. Natl. Acad. Sci. U.S.A.* **96**, 1858–1862
- Wang, C., Fu, M., Angeletti, R. H., Siconolfi-Baez, L., Reutens, A. T., Albanese, C., Lisanti, M. P., Katzenellenbogen, B. S., Kato, S., Hopp, T. et al. (2001) Direct acetylation of estrogen receptor α hinge region by p300 regulates transactivation and hormone sensitivity. *J. Biol. Chem.* **276**, 18375–18383
- Kaul, S., Blackford, Jr, J. A., Cho, S. and Simons, Jr, S. S. (2002) Ubc9 is a novel modulator of the induction properties of glucocorticoid receptors. *J. Biol. Chem.* **277**, 12541–12549
- Poukka, H., Aarnisalo, P., Karvonen, U., Palvimo, J. J. and Jänne, O. A. (1999) Ubc9 interacts with the androgen receptor and activates receptor-dependent transcription. *J. Biol. Chem.* **274**, 19441–19446
- Melchior, F. (2000) SUMO—nonclassical ubiquitin. *Annu. Rev. Cell. Dev. Biol.* **16**, 591–626
- Johnson, E. S., Schwienhorst, I., Dohmen, R. J. and Blobel, G. (1997) The ubiquitin-like protein Smt3p is activated for conjugation to other proteins by an Aos1p/Uba2p heterodimer. *EMBO J.* **16**, 5509–5519
- Johnson, E. S. and Blobel, G. (1997) Ubc9p is the conjugating enzyme for the ubiquitin-like protein Smt3p. *J. Biol. Chem.* **272**, 26799–26802
- Kahyo, T., Nishida, T. and Yasuda, H. (2001) Involvement of PIAS1 in the sumoylation of tumor suppressor p53. *Mol. Cell* **8**, 713–718
- Johnson, E. S. and Gupta, A. A. (2001) An E3-like factor that promotes SUMO conjugation to the yeast septins. *Cell* (Cambridge, Mass.) **106**, 735–744
- Sachdev, S., Bruhn, L., Sieber, H., Pichler, A., Melchior, F. and Grosschedl, R. (2001) PIASy, a nuclear matrix-associated SUMO E3 ligase, represses LEF1 activity by sequestration into nuclear bodies. *Genes Dev.* **15**, 3088–3103
- Pichler, A., Gast, A., Seeler, J. S., Dejean, A. and Melchior, F. (2002) The nucleoporin RanBP2 has SUMO-1 E3 ligase activity. *Cell* (Cambridge, Mass.) **108**, 109–120
- Mahajan, R., Delphin, C., Guan, T., Gerace, L. and Melchior, F. (1997) A small ubiquitin-related polypeptide involved in targeting RanGAP1 to nuclear pore complex protein RanBP2. *Cell* (Cambridge, Mass.) **88**, 97–107
- Matunis, M. J., Wu, J. and Blobel, G. (1998) SUMO-1 modification and its role in targeting the Ran GTPase-activating protein, RanGAP1, to the nuclear pore complex. *J. Cell Biol.* **140**, 499–509
- Joseph, J., Tan, S. H., Karpova, T. S., McNally, J. G. and Dasso, M. (2002) SUMO-1 targets RanGAP1 to kinetochores and mitotic spindles. *J. Cell Biol.* **156**, 595–602
- Ishov, A. M., Sotnikov, A. G., Negorev, D., Vladimirova, O. V., Neff, N., Kamitani, T., Yeh, E. T., Strauss, III, J. F. and Maul, G. G. (1999) PML is critical for ND10 formation and recruits the PML-interacting protein daxx to this nuclear structure when modified by SUMO-1. *J. Cell Biol.* **147**, 221–234
- Muller, S., Matunis, M. J. and Dejean, A. (1998) Conjugation with the ubiquitin-related modifier SUMO-1 regulates the partitioning of PML within the nucleus. *EMBO J.* **17**, 61–70
- Seeler, J. S., Marchio, A., Losson, R., Desterro, J. M., Hay, R. T., Chambon, P. and Dejean, A. (2001) Common properties of nuclear body protein SP100 and TIF1 α chromatin factor: role of SUMO modification. *Mol. Cell. Biol.* **21**, 3314–3324
- Desterro, J. M., Rodriguez, M. S. and Hay, R. T. (1998) SUMO-1 modification of I κ B α inhibits NF- κ B activation. *Mol. Cell* **2**, 233–239
- Muller, S., Berger, M., Lehenbre, F., Seeler, J. S., Haupt, Y. and Dejean, A. (2000) c-Jun and p53 activity is modulated by SUMO-1 modification. *J. Biol. Chem.* **275**, 13321–13329
- Bies, J., Markus, J. and Wolff, L. (2002) Covalent attachment of the SUMO-1 protein to the negative regulatory domain of the c-Myc transcription factor modifies its stability and transactivation capacity. *J. Biol. Chem.* **277**, 8999–9009
- Poukka, H., Karvonen, U., Jänne, O. A. and Palvimo, J. J. (2000) Covalent modification of the androgen receptor by small ubiquitin-like modifier 1 (SUMO-1). *Proc. Natl. Acad. Sci. U.S.A.* **97**, 14145–14150
- Moilanen, A.-M., Poukka, H., Karvonen, U., Häkli, M., Jänne, O. A. and Palvimo, J. J. (1998) Identification of a novel RING finger protein as a coregulator in steroid receptor-mediated gene transcription. *Mol. Cell. Biol.* **18**, 5128–5139
- Ikonen, T., Palvimo, J. J. and Jänne, O. A. (1997) Interaction between the amino- and carboxyl-terminal regions of the rat androgen receptor modulates transcriptional activity and is influenced by nuclear receptor coactivators. *J. Biol. Chem.* **272**, 29821–29828
- Li, S. J. and Hochstrasser, M. (1999) A new protease required for cell-cycle progression in yeast. *Nature* (London) **398**, 246–251
- Suzuki, T., Ichiyama, A., Saitoh, H., Kawakami, T., Omata, M., Chung, C. H., Kimura, M., Shimbara, N. and Tanaka, K. (1999) A new 30-kDa ubiquitin-related SUMO-1 hydrolase from bovine brain. *J. Biol. Chem.* **274**, 31131–31134
- Sternsdorf, T., Jensen, K., Reich, B. and Will, H. (1999) The nuclear dot protein sp100, characterization of domains necessary for dimerization, subcellular localization, and modification by small ubiquitin-like modifiers. *J. Biol. Chem.* **274**, 12555–12566
- Johnson, E. S. and Blobel, G. (1999) Cell cycle-regulated attachment of the ubiquitin-related protein SUMO to the yeast septins. *J. Cell. Biol.* **147**, 981–994
- Rodriguez, M. S., Dargemont, C. and Hay, R. T. (2001) SUMO-1 conjugation *in vivo* requires both a consensus modification motif and nuclear targeting. *J. Biol. Chem.* **276**, 12654–12659
- Schoenmakers, E., Alen, P., Verrijdt, G., Peeters, B., Verhoeven, G., Rombauts, W. and Claessens, F. (1999) Differential DNA binding by the androgen and glucocorticoid receptors involves the second Zn-finger and a C-terminal extension of the DNA-binding domains. *Biochem. J.* **341**, 515–521
- Iniguez-Lluhi, J. A. and Pearce, D. (2000) A common motif within the negative regulatory regions of multiple factors inhibits their transcriptional synergy. *Mol. Cell. Biol.* **20**, 6040–6050
- Hovland, A. R., Powell, R. L., Takimoto, G. S., Tung, L. and Horwitz, K. B. (1998) An N-terminal inhibitory function, IF, suppresses transcription by the A-isoform but not the B-isoform of human progesterone receptors. *J. Biol. Chem.* **273**, 5455–5460
- Kotaja, N., Karvonen, U., Jänne, O. A. and Palvimo, J. J. (2002) PIAS proteins modulate transcription factors by functioning as SUMO-1 ligases. *Mol. Cell. Biol.* **22**, 5222–5234
- Gostissa, M., Hengstermann, A., Fogal, V., Sandy, P., Schwarz, S. E., Scheffner, M. and Del Sal, G. (1999) Activation of p53 by conjugation to the ubiquitin-like protein SUMO-1. *EMBO J.* **18**, 6462–6471
- Rodriguez, M. S., Desterro, J. M., Lain, S., Midgley, C. A., Lane, D. P. and Hay, R. T. (1999) SUMO-1 modification activates the transcriptional response of p53. *EMBO J.* **18**, 6455–6461
- Schmidt, D. and Muller, S. (2002) Members of the PIAS family act as SUMO ligases for c-Jun and p53 and repress p53 activity. *Proc. Natl. Acad. Sci. U.S.A.* **99**, 2872–2877

Received 10 July 2002; accepted 29 July 2002

Published as BJ Immediate Publication 29 July 2002, DOI 10.1042/BJ20021085