Polypyrrimidine tract-binding protein 1 (PTBP1) and its brain-specific homologue, PTBP2, are associated with pre-mRNAs and influence pre-mRNA processing, as well as mRNA metabolism and transport. They play important roles in neural differentiation and glioma development. In our study, we detected the expression of the two proteins in glioma cells and predicted that they may be sumoylated using SUMOplot analyses. We confirmed that PTBP1 and PTBP2 can be modified by SUMO1 with co-immunoprecipitation experiments using 293ET cells transiently co-expressing SUMO1 and either PTBP1 or PTBP2. We also found that SUMO1 modification of PTBP2 was enhanced by Ubc9 (E2). The mutation of the sumoylation site (Lys137) of PTBP2 markedly inhibited its modification by SUMO1. Interestingly, in T98G glioma cells, the level of sumoylated PTBP2 was reduced compared to that of normal brain cells. Overall, this study shows that PTBP2 is posttranslationally modified by SUMO1. [BMB Reports 2014; 47(4): 233-238]

INTRODUCTION

The polypyrrimidine tract-binding (PTB) proteins are an important family of RNA-binding proteins that associate with pyrimidine-rich sequences. They are thought to be involved in cell-specific pre-mRNA alternative splicing, as well as mRNA stability, transport and translation (1). PTBP1 is present in most mammalian tissues but is replaced by paralogous protein PTBP2 in the nervous system and testes. The expression patterns and RNA-binding sites of [originally termed brPTB (2) or nPTB (3)] in the nervous system are different from those in mammalian tissues but is replaced by paralogous protein PTBP2 in its role as a regulator of pre-mRNA splicing. PTBP1 and PTBP2 are up-regulated in human glioma cells. Although the PTBP1 protein has been reported to be elevated in several glioma cell lines and WHO grade IV tumors (7, 12), there are few reports on the expression of PTBP2 in gliomas, especially exclusive: PTBP1 in glial cells and PTBP2 mostly in neurons (4, 5). The two PTB proteins are generally cooperatively involved in the regulation of neural development (6) and neural tumorigenesis (7). PTBP2 represses adult-specific splicing to regulate the generation of neuronal precursors in the embryonic brain (8). The loss of expression of first PTBP1 and then PTBP2 during embryonic development allows splicing of exon 18 and expression of postsynaptic density protein 95 late in neuronal maturation (9). However, in nonneuronal cells, the splicing of 3' terminal introns within genes encoding critical presynaptic proteins is repressed by PTBP1 (10). Although PTBP1 expression is absent in most neuronally derived tumor cells, strong up-regulation of PTBP1 expression in tumor cells of glial or primitive neuroectodermal origin suggests involvement of this protein in cellular transformation (11, 12).

In glioma cells, an increasing number of genes have been found to be involved in alternative splicing by PTB proteins, such as RTN4/Nogo (7) and pyruvate kinase M (13). However, the posttranslational modification mechanisms of PTBP1 and PTBP2 themselves, such as sumoylation, are still unknown. Small ubiquitin-like modifiers (SUMO1, SUMO2 and SUMO3) belong to a family of ubiquitin-like proteins that covalently attach to substrate proteins to regulate their functions. SUMO modification has been implicated in diverse processes, including protein stability, subcellular localization, and protein-protein interaction (14).

In this study, we have shown that both PTBP1 and PTBP2 can be sumoylated by SUMO1 in vitro and that PTBP2 is sumoylated in vivo in glioma cells. Our data demonstrate that while the level of the PTBP2 protein is up-regulated, the level of PTBP2 sumoylation is reduced in glioma cells. We hypothesized that the reduced sumoylation of PTBP2 may be associated with its nuclear-cytoplasmic shuttling and functional activity in glioma development. A refined understanding of the posttranslational control of PTB proteins may provide novel insights into how these modifications affect RNA processing.

RESULTS

PTBP1 and PTBP2 are up-regulated in human glioma cells

Although the PTBP1 protein has been reported to be elevated in several glioma cell lines and WHO grade IV tumors (7, 12), there are few reports on the expression of PTBP2 in gliomas, especially

Keywords: Glioma, PTBP1, PTBP2, SUMO1, UBC9
in glioma cell lines. As expected, western blotting showed that the PTBP1 protein was up-regulated in 4 glioma cell lines (T98G, A172, U251 and U87MG) compared with 2 normal human brain tissues. PTBP2 expression also showed a modest increase in glioma cells (Fig. 1A). Elevated levels of PTBP1 and PTBP2 were observed in grade III glioma tissues compared with normal brain tissues (Fig. 1B). We performed immunofluorescence on T98G and U87MG cells using antibodies that recognize only PTB or nPTB.

As seen by co-staining with the nuclear marker DAPI, PTBP1 signals predominately localized to the nuclei of glioma cells (Fig. 1C). On the other hand, PTBP2 was expressed both in the nucleus and cytoplasm (Fig. 1D).

**PTBP1 and PTBP2 can be modified by SUMO1 in 293ET cells**

Most SUMO-modified proteins contain the tetrapeptide motif ψ-K-x-D/E, where ψ is a hydrophobic residue, K is the lysine conjugated to SUMO, x is any amino acid (aa), and D/E is an acidic residue. To determine whether PTB proteins have potential SUMO modification sites, we performed a bioinformatic screen for high-probability sumoylation sites using the SUMOplot™ Analysis Program. SUMOplot™ is an excellent computational program that makes predictions of sumoylation sites based on similarity with the hydrophobic consensus motif and the degree of matching with known sumoylation sites from Ubc9-binding substrates. As shown in Fig. 2A and B, the program predicted three high-probability sumoylation sites at Lysines 48, 137, and 439 in PTBP1 and four high-probability sumoylation sites at Lysines 13, 48, 137, and 440 in PTBP2. We next wished to determine whether PTBP1 and PTBP2 do indeed undergo SUMO modification. We first examined the positive control Bmal1, a known SUMO1 target (15), in 293ET cells transiently expressing both Myc-Bmal1 and Flag-SUMO1. The cell lysates were immunoprecipitated using either an anti-Myc or an anti-SUMO1 antibody, followed by an immunoblot analysis. Two major bands (approximately 78 and 98 kDa) were detected in the immunoprecipitates compared with IgG (Fig. 2C). Mature human SUMO1 is an 11 kDa protein, but one SUMO1 conjugate appears to be approximately 20 kDa larger than the molecular weight of most substrates on the SDS-PAGE gel (16). These findings suggest that the 98 kDa immunoreactive band corresponds to Myc-Bmal1 (78 kDa) conjugated to one SUMO1 molecule. Next, we also constructed plasmids for the expression of Myc-tagged PTBP1 and PTBP2 (Fig. S1). By performing similar, co-immunoprecipitation experiments on 293ET cells, we verified that both Myc-PTBP1 and Myc-PTBP2 can be modified by SUMO1. Western blot analysis revealed a sumoylated protein band, approximately 20 kDa larger than unmodified Myc-PTBP1 or Myc-PTBP2 (Fig. 2D and E). Notably, Myc-PTBP2 showed a more specific SUMO1-modified band by co-immunoprecipitation analysis. Because the SUMO conjugating pathway depends on the activity of the E2 enzyme Ubc9 (17), we investigated whether the SUMO1 conjugation of PTBP2 also depends on Ubc9 (Fig. 2F, Fig. S2). Ubc9 expression significantly enhanced the levels of the PTBP2-SUMO1 conjugate in 293ET cells co-transfected with a combination of Myc-PTBP2, Flag-SUMO1 and V5-Ubc9. These results indicate that Ubc9 is required for the SUMO1 conjugation of PTBP2.

To further determine whether the 83-kDa band in Figures 2D-E was representative of Myc-PTBP2 (63 kDa) covalently conjugated to SUMO1, we constructed a SUMO1 ΔGG mutant that lacks the C-terminal di-glycine motif essential for covalent modification (Fig. 3A). The 83 kDa band completely disappeared in...
Fig. 2. PTBP1 and PTBP2 are sumoylated by SUMO1 in vitro. (A-B) The SUMOplot™ Analysis Program prediction and scoring of sumoylation sites in PTBP1 and PTBP2 proteins. (C-E) For the co-immunoprecipitation assay, Myc-tagged Bmal1 and Flag-tagged SUMO1 (positive control) (C), Myc-tagged PTBP1 and Flag-tagged SUMO1 (D), or Myc-tagged PTBP2 and Flag-tagged SUMO1 (E) were cotransfected into 293ET cells. Cell lysates were immunoprecipitated with either an anti-Myc or anti-SUMO1 antibody, and precipitates were again immunoblotted with the anti-Myc or anti-SUMO1 antibody. IgG served as a negative control. (F) Cells were cotransfected with Myc-tagged PTBP2, Flag-tagged SUMO1, and 2 μg (or 4 μg) of V5-tagged Ubc9, as indicated. Forty-eight hours after transfection, cell lysates were immunoprecipitated with anti-Myc antibody, and precipitates were analyzed by immunoblotting with anti-Myc and anti-SUMO1 antibodies.

the 293ET cells transfected with Myc-PTBP2 and the Flag-SUMO1ΔGG mutant (Fig. 3C). This result indicates that the 83 kDa immunoreactive band corresponds to a Myc-PTBP2-SUMO1 conjugate linked by an isopeptide bond via a C-terminal di-glycine motif in SUMO1.

### Lysine 137 in PTBP2 is the sumoylation site

Having confirmed that PTBP2 is a substrate for conventional SUMO1 modification, we next examined the potential sumoylation sites in PTBP2. Based on the SUMOplot analysis (Fig. 2B), we focused on the two highest probability potential sumoylation sites (K137 and K48) and generated two Myc-PTBP2 mutants in which these lysine residues were individually changed to arginine residues (K137R and K48R) (Fig. 3B). When each mutant was co-transfected with Flag-SUMO1, the Myc-PTBP2-SUMO1 conjugate (83 kDa) was only detected for the Myc-PTBP2 (K48R) mutant but not the Myc-PTBP2 (K137R) mutant (Fig. 3C). These results indicate that Lysine 137, a candidate sumoylation site found by the SUMOplot analysis, is the actual target site in PTBP2.
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Fig. 3. Lysine 137 of PTBP2 is sumoylated and PTBP2 sumoylation is reduced in glioma cells. (A) 293ET cells were transiently transfected with Myc-tagged vector, Flag-tagged SUMO1, SUMO1ΔGG (lacking the C-terminal di-glycine motif), or Myc-tagged PTBP2, followed by immunoblotting with anti-SUMO1, anti-Myc and anti-β-actin. (B) Myc-tagged PTBP2 or either of the lysine-to-arginine mutants (K48R or K137R), were cotransfected into 293ET cells with SUMO1 and Ubc9. Cell lysates were immunoblotted with an anti-Myc antibody. β-actin was used as a loading control. (C) Either Myc-tagged PTBP2, Myc-tagged PTBP2 (K48R or K137R) were cotransfected with SUMO1 into 293ET cells. Cell lysates were first immunoprecipitated and then immunoblotted with an anti-Myc antibody. Myc-tagged PTBP2 and SUMO1ΔGG were cotransfected into 293ET cells as a negative control. (D) The normal brain and T98G glioma cell line lysates were immunoprecipitated with an anti-PTBP2 antibody. Cells cotransfected with Myc-PTBP2 and SUMO1 were immunoprecipitated with an anti-Myc antibody as a positive control. The precipitates were immunoblotted with an anti-SUMO1 antibody. The arrow on the left indicates sumoylated Myc-PTBP2 in vitro, and the arrow on the right indicates sumoylated PTBP2 in glioma cells in vivo.

Sumoylated PTBP2 is reduced in T98G glioma cells
Finally, the in vivo level of sumoylated PTBP2 protein was assessed in glioma cells. We performed immunoprecipitation experiments on normal brain tissues and T98G glioma cells using an antibody that recognizes only PTBP2 (nPTB-IS2) (Fig. S1B), followed by an immunoblot analysis with an anti-SUMO1 antibody. The cell lysates of Myc-PTBP2 and SUMO1 cotransfectants were immunoprecipitated with an anti-Myc antibody as a positive control. The band representing in vivo sumoylated PTBP2 was detected at a size approximately 2-3 kDa smaller than the corresponding in vitro Myc-PTBP2-SUMO1 conjugate (Fig. 3D). In contrast to its expression in normal brain tissue, the level of sumoylated PTBP2 in T98G glioma cells was significantly reduced, despite the fact that global protein levels were higher in glioma cells.

DISCUSSION
In recent years, a great deal of progress has been made on the role of sumoylated proteins in the development of the nervous system, neuronal differentiation, synaptic formation, normal neuronal physiology and neurodegenerative diseases. SUMO modification of proteins is involved in the regulation of a large number of necessary cellular processes. In cases when sumoylation does not occur normally, the resulting impairment of these cellular processes likely has a large impact on the function of the nervous system. Several studies have shown that the brain (18), and brain tumors (19, 20) contain many SUMO1-modified proteins, most of which
have not yet been identified.

In this study, we have demonstrated that the neuronal-specific PTBP2 protein is posttranslationally sumoylated by SUMO1 in cells. We also found that the sumoylation level of the PTBP2 protein is reduced in glioma cells. PTBP2 is predominantly expressed in the brain but is also found in heart and skeletal muscle. Although it localizes to the nucleus and contains four RRM (RNA recognition motif) domains, PTBP2 functions as an RNA-binding protein associated in a complex that is involved in the regulation of exon splicing and the stabilization of mRNAs in the cytoplasm. According to a previous study, most SUMO-modified proteins are found in the nucleus. These results support our data that in T98G cells, PTBP2 is mostly localized to the cytoplasm and is sumoylated at lower levels. It is likely that sumoylation of PTBP2 is associated with its nuclear localization.

Sumoylation appears to be a highly selective process both with respect to the choice of substrates as well as to the timing of their modification. The deconjugating enzymes (SUMO-specific proteases; SENPs) play important roles in the SUMO cycle because they have a dual role as processing proteases for SUMO precursors and as isopeptidases for substrate de-sumoylation (21). To stably enrich for sumoylated proteins in our in vitro and in vivo experiments, cells and brain tissues were lysed in the presence of an isopeptidase inhibitor N-ethylmaleimide (NEM). We found that the immunoreactive band corresponding to sumoylated PTBP2 disappeared when wild-type SUMO1 was replaced with a ΔGG mutant (Fig. 3C). This effect was enhanced when Ubc9 was overexpressed (Fig. 2F). Next, a search was conducted for putative sumoylation sites in the PTBP2 protein using the SUMOplot Analysis Program. It is conceivable that sumoylation of PTBP2 at these sites induces a conformational change that modifies the protein’s function. A PTBP2 mutant with a lysine (K)-to-arginine (R) substitution at residue 48 (K48R) was still sumoylated, while a similar mutant with an arginine substitution at residue 137 (K137R) was not (Fig. 3C). This suggests that the lysine 137 residue in PTBP2 is the target for SUMO1 modification. Taken together, these findings clearly indicate that PTBP2 is modified by SUMO1. The sumoylation of PTBP2 at Lys137 may stimulate neuronal differentiation by PTBP2-mediated neuron-specific alternative splicing in the nucleus of differentiating neurons. However, in glioma cells, the decreased sumoylation at Lys137 causes export of PTBP2 to the cytoplasm. The change in PTBP2 subcellular localization may repress neuronal differentiation and lead to initiation and maintenance of tumor progression.

In conclusion, to our knowledge, this is the first report on sumoylation of polyypurimidine tract-binding proteins. PTBP2 plays a key role in neural development and glioma progression. Sumoylation modification might be important for regulating PTBP2 function in the brain.

MATERIALS AND METHODS

Human tissue samples

All human tissue samples from normal brains and gliomas were obtained from the Department of Neurosurgery, Beijing Tiantan Hospital. The tumor samples were classified according to the third edition of the histological grading of tumors of the nervous system published by the WHO in 2000. This study was approved by the Medical Ethics Committee of Tiantan Hospital.

Cell lines and cell culture

Human glioma cell lines T98G, U87MG and A172 were purchased from the American Type Culture Collection (ATCC) and cultured according to the guidelines recommended by the ATCC. The U251 cell line (from the Cell Center of Peking Union Medical College) was cultured in minimum essential medium supplemented with 10% fetal bovine serum. The human 293ET cell line was a gift from Dr. Chengyu Jiang (Peking Union Medical College, China). All cells were maintained at 37°C with 5% CO2.

Plasmid and transfection

The coding region of SUMO1 was amplified by PCR and inserted into the SfiI site in the pcDEF-Flag-tagged vector. The Flag-SUMO1 mutant (ΔGG, lacking a C-terminal di-glycine motif) was generated from the Flag-SUMO1 coding sequence. Myc-tagged human Bmal1 and PTBP1 were subcloned into the BamHI and EcoRI sites and the EcoRI and XhoI sites of the expression vector pcDNA4.0, respectively. Myc-PTBP2 was subcloned into the SfiI site of the expression vector pcDEF. The single lysine-to-arginine substitution mutants were generated from Myc-PTBP2 by using the QuickChange Site-Directed Mutagenesis Kit (Stratagene, USA). A plasmid expressing V5-Ubc9 was generated by inserting the coding region into the BamHI and XhoI sites of the pcDNA6.0 vector. 293ET cells were transfected with 4 μg plasmid DNA per 60 mm-diameter dish using the VigoFect reagent.

Immunoprecipitation and western blotting analysis

Whole cells or tissues were collected and homogenized with TNTE lysis buffer containing protease inhibitors and 20 mM N-ethylmaleimide. After incubation for 30 min on ice, the homogenate was centrifuged at 18,000 × g for 20 min. The supernatants were stored at −80°C until the immunoprecipitation assay was performed.

For the immunoprecipitation assay, equal amounts (1,200 ~ 1,500 μg) of the cell lysates were incubated with 1/100 (w/v) of the anti-Myc monoclonal antibody (Shanghai Genomics, China), anti-SUMO1 antibody (generated by our laboratory), or anti-PTBP2 polyclonal antibody (α-nPTB IS2) overnight at 4°C. Subsequently, the lysates were incubated with Protein A Sepharose (GE Healthcare, UK) while rotating for 3 h at 4°C. Immunoprecipitates were washed four times with lysis buffer without the protease inhibitor, eluted in 20 μl of 6× loading buffer at 98°C and analyzed by an immunoblot analysis.

The protein samples were separated by 10% SDS-PAGE gel, transferred to a nitrocellulose membrane (Amersham, USA) and processed for western blotting analyses with the anti-Myc (Flag or V5) tag (Shanghai Genomics, China), anti-SUMO1 (generated...
by our laboratory, anti-PTBP1 (PTBP2) [PTB-NT (α-PTBP IS2),
kind gifts from Douglas L. Black’s laboratory] and anti-β-actin
(Sigma, USA) antibodies.

**Immunohistochemistry and immunofluorescence**

Immunohistochemical analyses of PTBP1 and PTBP2 were con-
ducted using frozen section specimens of gliomas (Grade III)
and normal brain tissues. The sections were incubated with
PTB-NT or α-PTBP IS2 antibodies at 4°C overnight. Staining was
directed using a diaminobenzidine staining kit (Zhongshan,
China).

For immunofluorescence analysis, cells were fixed with 4% paraformaldehyde in phosphate-buffered saline and incubated with PTB-NT or α-PTBP IS2 antibodies. Cells were visualized with secondary antibodies conjugated with fluorescein (Zhongshan, China).

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**REFERENCES**

1. Sawicka, K., Bushell, M., Spriggs, K. A. and Willis, A. E. (2008) Polypyrimidine-tract-binding protein: a multifunctional RNA-binding protein. *Biochem. Soc. Trans.* 36, 641-647.

2. Polydorides, A. D., Okano, H. J., Yang, Y. L., Stefani, G. and Darnell, R. B. (2000) A brain-enriched polypyrimidine tract-binding protein antagonizes the ability of Nova to regulate neuron-specific alternative splicing. *Proc. Natl. Acad. Sci. U.S.A.* 97, 6350-6355.

3. Markovtsov, V., Nikolic, J. M., Goldman, J. A., Turck, C. W., Chou, M. Y. and Black, D. L. (2000) Cooperative assembly of an hnRNP complex induced by a tissue-specific homolog of polypyrimidine tract binding protein. *Mol. Cell Biol.* 20, 7463-7479.

4. Lillevall, K., Kulla, A. and Ord, T. (2001) Comparative expression analysis of the genes encoding polypyrimidine tract binding protein (PTB) and its neural homologue (brPTB) in prenatal and postnatal mouse brain. *Mech. Dev.* 101, 217-220.

5. Boutilier, P. L., Stool, P., Li, Q., Lin, C. H., Chawla, G., Ostrow, K., Shiue, L., Ares, M., Jr. and Black, D. L. (2007) A post-transcriptional regulatory switch in polypyrimidine tract-binding proteins reprograms alternative splicing in developing neurons. *Genes Dev.* 21, 1636-1652.

6. Keppetipola, N., Sharma, S., Li, Q. and Black, D. L. (2012) Neuronal regulation of pre-mRNA splicing by polypyrimidine tract binding proteins, PTBP1 and PTBP2. *Crit. Rev. Biochem. Mol. Biol.* 47, 360-378.

7. Cheung, H. C., Cha, T., Zhu, W., Baggerly, K. A., Tsavachidis, S., Krahe, R. and Cote, G. J. (2009) Splicing factors PTBP1 and PTBP2 promote proliferation and migration of glioma cell lines. *Brain* 132, 2277-2288.

8. Licatalosi, D. D., Yano, M., Fak, J. J., Mele, A., Grabinski, S. E., Zhang, C. and Darnell, R. B. (2012) Ptbp2 represses adult-specific splicing to regulate the generation of neuronal precursors in the embryonic brain. *Genes Dev.* 26, 1626-1642.

9. Zheng, S., Gray, E. E., Chawla, G., Porue, B. T., O’Dell, T. J. and Black, D. L. (2012) PSD-95 is post-transcriptionally repressed during early neural development by PTBP1 and PTBP2. Nat. Neurosci. 15, 381-388.

10. Yap, K., Lin, Z. Q., Khandelia, P., Friedman, B. and Makeyev, E. V. (2012) Coordinated regulation of neuronal mRNA steady-state levels through developmentally controlled intron retention. *Genes Dev.* 26, 1209-1223.

11. McCutcheon, I. E., Hentschel, S. J., Fuller, G. N., Jin, W. and Cote, G. J. (2004) Expression of the splicing regulator polypyrimidine tract-binding protein in normal and neoplastic brain. *Neuro. Oncol.* 6, 9-14.

12. Cheung, H. C., Corley, L. J., Fuller, G. N., McCutcheon, I. E. and Cote, G. J. (2006) Polypyrimidine tract binding protein and Notch1 are independently re-expressed in glioma. *Mod. Pathol.* 19, 1034-1041.

13. Clowers, C. V., Chatterjee, D., Wang, Z., Cantley, L. C., Vander Heiden, M. G. and Krainer, A. R. (2010) The alternative splicing repressors hnRNP A1/A2 and PTB influence pyruvate kinase isoenzyme expression and cell metabolism. *Proc. Natl. Acad. Sci. U.S.A.* 107, 1894-1899.

14. Hickey, C. M., Wilson, N. R. and Hochstrasser, M. (2012) Function and regulation of SUMO proteases. *Nat. Rev. Mol. Cell Biol.* 13, 755-766.

15. Lee, J., Lee, Y., Lee, M. I., Park, E., Kang, S. H., Chung, C. H., Lee, K. H. and Kim, K. (2008) Dual modification of BMAL1 by SUMO2/3 and ubiquitin promotes circadian activation of the CLOCK/BMAL1 complex. *Mol. Cell. Biol.* 28, 6056-6065.

16. Johnson, E. S. (2004) Protein modification by SUMO. *Annu. Rev. Biochem.* 73, 355-382.

17. Wilkinson, K. A. and Henley, J. M. (2010) Mechanisms, regulation and consequences of protein SUMOylation. *Biochem. J.* 428, 133-145.

18. Cimarro, H., Lindberg, C., Bomholt, S. F., Ronn, L. C. and Henley, J. M. (2008) Increased protein SUMOylation following focal cerebral ischemia. *Neuropharmacology* 54, 280-289.

19. Grant, M. M. (2010) Identification of SUMOylated proteins in neuroblastoma cells after treatment with hydrogen peroxide or ascorbate. *BMB Rep.* 43, 720-725.

20. Yang, W., Wang, L., Roehn, G., Pearlstein, R. D., Ali-Osman, F., Pan, H., Goldbrunner, R., Kranz, M., Harms, C. and Paschen, W. (2013) Small ubiquitin-like modifier 1-3 conjugation [corrected] is activated in human astrocytic brain tumors and is required for glioblastoma cell survival. *Cancer Sci.* 104, 70-77.

21. Mukhopadhyay, D. and Dasso, M. (2007) Modification in reverse: the SUMO proteases. *Trends. Biochem. Sci.* 32, 286-293.

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