SUMO-1 Conjugation to Human DNA Topoisomerase II Isozymes*

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Topoisomerase I-mediated DNA damage induced by camptothecin has been shown to induce rapid small ubiquitin-related modifier (SUMO-1) conjugation to topoisomerase I. In the current study, we show that topoisomerase II-mediated DNA damage induced by teniposide (VM-26) results in the formation of high molecular weight conjugates of both topoisomerase IIα and IIβ isoforms in HeLa cells. Immunological characterization of these conjugates suggests that both topoisomerase IIα and IIβ isoforms are conjugated to SUMO-1. The involvement of SUMO-1/UBC9 in the modification of topoisomerase II and SUMO-1/UBC9. Surprisingly, ICRF-193, which does not induce topoisomerase II-mediated DNA damage but traps topoisomerase II into a circular clamp conformation, is also shown to induce similar SUMO-1 conjugation to topoisomerase II isoforms. In addition, we show that both oxidative and heat shock stresses, which can cause protein damage, rapidly increase nuclear SUMO-1 conjugates. These studies raise the question on whether SUMO-1 conjugation to topoisomerases is an indirect result of a DNA damage response or a direct result because of protein conformational changes.

In this communication, we show that TOP2-mediated DNA damage induced by teniposide (VM-26), a TOP2-specific poison (27), also results in rapid accumulation of SUMO-1-TOP2 conjugates. In addition, ICRF-193, which is known not to induce TOP2-mediated DNA damage but to trap topoisomerase II into circular clamp conformation, is also shown to induce rapid accumulation of SUMO-1-TOP2 conjugates. Furthermore, heat shock stress, which is known to cause protein unfolding, also significantly increases SUMO-1 conjugation to nuclear proteins. These results suggest that the signal that triggers SUMO-1 conjugation to topoisomerase II may result from the conformational change (the circular clamp conformation) of topoisomerase II.

EXPERIMENTAL PROCEDURES

Materials—ICRF-193 was purchased from ICN Biomedicals. Protein A-Sepharose bead 4B and GST-Sepharose beads were purchased from Amersham Pharmacia Biotech. The Matchmaker II yeast two-hybrid system and human placenta cDNA library were purchased from CLONTECH. Mouse monoclonal anti-SUMO-1 antibody (anti-GMP1) was purchased from Zymed Laboratories Inc. Laboratories. Rabbit antiserum against the hTOP2β isozyme were obtained from Dr. Jaulang Hwang (Academia Sinica, Taiwan). Staphylococcal 87 nuclease was purchase from Roche Molecular Biochemicals.

Cloning of hTOP2β cDNA—The full-length of hTOP2β cDNA was cloned from human U937 cells by reverse transcriptase-polymerase

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The abbreviations used are: TOP, topoisomerase; SUMO, small ubiquitin-related modifier; E1, ubiquitin-activating enzyme; E2, ubiquitin-conjugating enzyme; GST, glutathione S-transferase; h, human; NEM, N-ethylmaleimide; AMP-PNP, adenosine 5'-β,γ-imino-triphosphate; RanGAP, RanGTPase-activating protein; VM-26, teniposide.

Human SUMO-1 (small ubiquitin-related modifier) (also named UBL1 (5), PIC1 (6), GMP1 (7), SMT3C (8), and sentrin (9) in the literature) is a ubiquitin-like protein, which shares about 18% identity to ubiquitin (10). The yeast homologue of SUMO-1, Smt3p, was first identified as a suppressor of mif2 mutation (11). SUMO-1 and Smt3p share about 52% identity (12). They share a similar activation and conjugation pathway with ubiquitin but employ distinct sets of E1 and E2 enzymes (13–19). The E1 enzymes for activating human SUMO-1 and yeast Smt3p are the heterodimeric proteins, SAE1/SAE2 and Aos1p/Uba2p, respectively (13, 15). UBC9 is the only E2 identified for SUMO-1/Smt3p, whereas a dozen E2 enzymes have been identified for ubiquitin in yeast (20, 21). yUBC9 and hUBC9 share extensive sequence similarity. Moreover, hUBC9 can be a functional substitute for yUBC9 in yeast (22). Recently, some proteases, which specifically activate SUMO-1/Smt3p precursors and cleave SUMO-1/Smt3p from their protein conjugates and are distinct from isopeptidases for ubiquitin conjugates, have also been identified in yeast and mammalian cells (23–25). Whereas the primary role of ubiquitin-protein conjugation is to facilitate protein degradation by the 26 S proteasome (26), the role of SUMO-1-protein conjugation is not clear. Many proteins such as RanGAP1, PML, IκBα, RAD51, RAD52, centromere proteins, and p53, which have diverse functions have been shown to interact with UBC9/SUMO-1 or be modified by SUMO-1 (see review in Ref. 12).

In this communication, we show that TOP2-mediated DNA damage induced by teniposide (VM-26), a TOP2-specific poison (27), also results in rapid accumulation of SUMO-1-TOP2 conjugates. In addition, ICRF-193, which is known not to induce TOP2-mediated DNA damage but to trap topoisomerase II into circular clamp conformation, is also shown to induce rapid accumulation of SUMO-1-TOP2 conjugates. Furthermore, heat shock stress, which is known to cause protein unfolding, also significantly increases SUMO-1 conjugation to nuclear proteins. These results suggest that the signal that triggers SUMO-1 conjugation to topoisomerase II may result from the conformational change (the circular clamp conformation) of topoisomerase II.
SUMO-1 Conjugation to Topoisomerase II

RESULTS

hTOP2α and hTOP2β Interact with hUBC9—Using hTOP2β as the bait in a yeast two-hybrid screen (see "Experimental Procedures"), we have identified 12 positive clones. Sequencing analysis of these clones have revealed that 9 of 12 of these clones encodes full-length hUBC9. The interaction between hUBC9 and hTOP2β was also confirmed by quantitative liquid β-galactosidase assay. The interaction between hTOP2β (both wild type and active site mutant Y821F) and hUBC9 gave rise to β-galactosidase activity, which was about 100-fold higher compared with hUBC9 alone without the bait (Table I). However, no positive interaction between the C-terminal domain of hTOP2β (amino acids 991–1621) and hUBC9 was demonstrated in the filter assay. UBC9 is the E2 enzyme for SUMO-1 conjugation (16). It has been reported that some proteins, which are covalently modified by SUMO-1 also interact with SUMO-1 noncovalently (9, 29). Consequently, we have also tested the interaction between hTOP2β and SUMO-1 using the yeast two-hybrid assay. As shown in Table I, hTOP2β also interacts strongly with SUMO-1 in the β-galactosidase filter assay.

To confirm the interaction between hUBC9 and hTOP2β, we constructed and purified a GST-hUBC9 fusion protein and performed a GST pull-down assay and co-immunoprecipitation assay. Using the GST pull-down assay, we showed that both hTOP2β and hTOP2α interacted with hUBC9 (Fig. 1A). GST-hUBC9 pulled down both hTOP2α and hTOP2β from nuclear extracts and purified protein preparations (Fig. 1, lanes 3 and 4). As a control using GST alone, neither hTOP2 isozyme was pulled down (Fig. 1A, lanes 1 and 2). This result suggested that the interaction between hTOP2 and GST-hUBC9 was specific. This specific interaction between hTOP2 and hUBC9 was further confirmed by the co-immunoprecipitation assay. In this assay, GST-hUBC9 was mixed with the nuclear extract prepared from human 2RA cells (WI38 cells transformed with SV40 T antigen). As shown in Fig. 1B (lane 3), antibodies that recognize both hTOP2α and hTOP2β isoforms co-immunoprecipitated GST-hUBC9 from nuclear extract (Fig. 1B, lane 3). In contrast, HA antibodies (used as a control) did not immunoprecipitate GST-hUBC9, which remained in the supernatant fraction (Fig. 1B, compare lanes 2 and 4).

hTOP2 Isozymes Are Covalently Modified by SUMO-1 in HeLa Cells Treated with VM-26—To demonstrate a functional interaction between hTOP2 and SUMO-1/UBC9, we have tested the possibility that hTOP2 can be covalently modified by SUMO-1. As shown in Fig. 2A (lane 1), no SUMO-1-hTOP2 conjugates in HeLa cells were detectable by immunoblotting (Fig. 1A, lanes 1 and 2). However, upon VM-26 treatment, some higher molecular weight species were detectable in HeLa cells treated with anti-hTOP2 antibodies (anti-hTOP2α, anti-hTOP2β, and anti-SUMO-1 antibodies) as well as with anti-HA antibody (Fig. 2A, lane 3). This result suggested that these high molecular weight species could be SUMO-1-hTOP2 conjugates.

To test whether both hTOP2 isoforms were covalently modified by SUMO-1, antibodies specific for each hTOP2 isoform were used to perform immunoblotting analysis. As shown in Fig. 2B, high molecular species were detectable in HeLa cells treated with VM-26 using isozyme-specific antibodies against either isoform.

Table I

| Prey          | Bait                  | β-galactosidase units | Liquid Assay | Filter Assay |
|---------------|-----------------------|-----------------------|--------------|--------------|
| hUBC9         | hTOP2β (wt)           | 17.7                  | ++           | ++           |
| hUBC9         | hTOP2β (C-terminal)   | nd                    | –            | –            |
| SUMO-1        | hTOP2β (wt)           | 0.7                   | nd           | ++           |
| hUBC9         | hTOP2β (Y821F)        | 19.4                  | ++           | ++           |

* +++, visible blue color developed within 30 min.
+ n/d, not determined
–, no visible blue color after 18 h.
SUMO-1 Conjugation to Topoisomerase II

hTOP2 Isozymes Are Covalently Modified by SUMO-1 in HeLa Cells Treated with ICRF-193—ICRF-193 belongs to a different class of hTOP2 inhibitors (30). Unlike VM-26, which induces topoisomerase II-mediated DNA cleavage, ICRF-193 inhibits the catalytic activity of hTOP2 by trapping TPO2 into circular protein clamps in the presence of ATP (31). Interestingly, covalent modification of hTOP2 by SUMO-1 was also observed in HeLa cells treated with ICRF-193 (Fig. 3A). As shown in Fig. 3A (compare lanes 1 and 2), a similar group of high molecular weight species (see the bracket labeled hTOP2-SUMO-1 conjugates) were detected by anti-hTOP2αβ antibodies using lysates prepared from HeLa cells treated with ICRF-193. The same membrane filter was stripped and rebotted with anti-SUMO-1 antibodies (Fig. 3A, lanes 3 and 4). Again, similar high molecular weight species were observed (see bands in the bracketed region in Fig. 3A), suggesting that these high molecular weight species could be covalent SUMO-1-hTOP2 conjugates.

To verify that those conjugates induced in HeLa cells treated with ICRF-193 were covalent SUMO-1-hTOP2 complexes, we immunoprecipitated hTOP2 isoforms from HeLa cell lysates using isozyme-specific antibodies (Fig. 3B). The immunoprecipitates were then immunoblotted with either anti-hTOP2αβ antibodies (Fig. 3B, lanes 3–6) or anti-SUMO-1 antibodies (Fig. 3B, lanes 9–12). The same high molecular weight species (see bracketed regions) in immunoprecipitates were detectable using either hTOP2αβ antibodies or anti-SUMO-1 antibodies, suggesting that these high molecular weight species contained both SUMO-1 and hTOP2 in the aggregate, these results suggest that the high molecular weight species induced by either ICRF-193 or VM-26 are SUMO-1-hTOP2 conjugates.

SUMO-1-conjugated hTOP2 in Cells Treated with ICRF-193 Is Not Covalently Linked to DNA—In our current study, we have shown that both ICRF-193 and VM-26, which represent two distinct types of TOP2 inhibitors, induce SUMO-1 conjugation to hTOP2. Previous studies have demonstrated that the majority of SUMO-1-TOP2 conjugates in cells treated with camptothecin are covalently linked to DNA (3, 4). To test whether SUMO-1-hTOP2 conjugates in cells treated with either ICRF-193 or VM-26 were covalently linked to DNA, the effect of nuclease (Staphylococcal S7 nuclease) treatment of cell lysates was tested (Fig. 4). As shown in Fig. 4A (lanes 1 and 2), in the absence of nuclease treatment, no SUMO-1 conjugates were detectable in HeLa cells treated with VM-26. The decrease in the band intensities of hTOP2α and hTOP2β (Fig. 4A, lane 2) was because of the formation of significant fractions of hTOP2 into covalent complexes with DNA, which were too large to enter gel (32). Upon nuclease treatment, these covalent complexes (either retained in the well or migrated as smears) were released from DNA, and consequently more hTOP2 species including those high molecular weight SUMO-1-hTOP2 conjugates were detected as distinct bands (Fig. 4A, lane 4).
However, when a similar experiment was performed using ICRF-193 instead of VM-26, those high molecular weight species were detectable with or without nuclease treatment (Fig. 4B, compare lanes 2 and 4). In addition, the amounts of unconjugated hTOP2 species (hTOP2α and hTOP2β) were about the same with or without nuclease treatment (Fig. 4B, compare lanes 2 and 4), indicating that ICRF-193, in contrast to VM-26, does not induce the formation of covalent hTOP2-DNA complexes. These experiments suggest that SUMO-1-hTOP2 conjugates formed in cells treated with VM-26 but not with ICRF-193 are covalently linked to chromosomal DNA. Consistent with this interpretation, we have also shown that the formation
fractionation, a duplicate gel was stained with Coomassie Blue and shown in Fig. 7C. We have also monitored the levels of ubiquitin conjugates in HeLa cells with or without heat shock treatment. The same gel as shown in Fig. 7A was stripped and reblotted with anti-ubiquitin antibodies. As shown in Fig. 7B, heat shock greatly increased the levels of ubiquitin conjugates in both the cytoplasmic (C) and nuclear (N) fractions.

The effect of heat shock on the increase in SUMO-1 conjugates may be because of two possibilities; one is that heat shock could partially unfold some nuclear proteins making them better substrates for SUMO-1/UBC9. The other is that heat shock could either stimulate the activity of UBC9 and/or inhibit the activity of the de-SUMOylation enzyme, resulting in the increase in the steady state level of SUMO-1 conjugates. To test the latter possibility, we have performed the following experiment (Fig. 8). Previous studies have demonstrated that TOP1-mediated DNA damage induced by camptothecin specifically induces accumulation of SUMO-1-hTOP1 conjugates (4). As shown in Fig. 8, HeLa cells treated with camptothecin resulted in the formation of a ladder of bands, which have previously been identified to be SUMO-1-hTOP1 conjugates (Fig. 7, lane 2). These SUMO-1-hTOP1 conjugates did not increase in abundance upon heat shock as would be expected if the latter possibility were true. They in fact dramatically decreased in their abundance (Fig. 8, compare lanes 2 and 4). The decrease in abundance of SUMO-1-hTOP1 conjugates is probably because of heat (45 °C)-induced reversal of hTOP1 cleavable complexes followed by de-SUMOylation (data not shown). This result thus argues against the latter possibility and favors the former possibility that unfolding of nuclear protein induced by heat shock directly signals SUMO-1 modification.

**DISCUSSION**

SUMO-1, a ubiquitin-like protein, has been identified to be conjugated to many important cellular proteins (for review, see Ref. 34). Consequently, SUMOylation has been suggested to be involved in diverse biological functions such as regulation of ubiquitin conjugation in the case of IκBα (35), regulation of protein transport in the case of RanGAP1 (36–38), the formation of PML oncogenic domains (PODs) (nuclear bodies) in the case of PML (39, 40), regulation of transcriptional activation in the case of p53 (41, 42), and regulation of apoptosis in the case of the FAS (9). Our recent studies have suggested that SUMO-1-hTOP1 conjugates may be involved in the repair of topoisomerase I-mediated DNA damage (4).

In this study, we show that VM-26 induces SUMO-1 modification of both hTOP2 isozymes, hTOP2α and hTOP2β, in HeLa cells. The role of UBC9 and SUMO-1 in this system has been further corroborated by the demonstration of physical interactions between hTOP2 and UBC9 and between hTOP2 and SUMO-1. VM-26 is a TOP2 poison, which traps DNA-hTOP2 in a ternary complex in which TOP2 is covalently linked to the 5′-phosphoryl ends of the double strand break (27). We have also demonstrated that VM-26-induced SUMOylation of hTOP2 is greatly reduced in CEM/VM-1 cells, which are defective in VM-26-induced formation of the ternary complex (Fig. 5). Consequently, the effect of VM-26 on SUMOylation of hTOP2 is because of the formation of these ternary complexes rather than an unidentified side effect(s) of VM-26. However, it is unclear whether SUMO-1 conjugation to hTOP2 is signaled by hTOP2 because of its conformational changes and/or an indirect response because of a DNA damage response.

To differentiate between these two possibilities, another TOP2 inhibitor, ICRF-193, was used. ICRF-193 belongs to a different class of TOP2 inhibitors that inhibit the catalytic activity of TOP2 without trapping the covalent ternary complex (i.e. no topoisomerase II-mediated DNA damage). In the pres-
ence of ATP, ICRF-193 is known to lock TOP2 into a circular clamp conformation (31). In this study, we have shown that ICRF-193 also induces SUMO-1 conjugation to hTOP2. As expected, the SUMO-1-hTOP2 conjugates induced by ICRF-193, unlike those induced by VM-26, were not covalently linked to DNA. This result suggests that the signal for SUMO-1 conjugation to hTOP2, at least in the case of ICRF-193, is not from DNA damage.

Previous studies have also demonstrated that the formation of the circular protein clamp could either enhance hTOP2 interaction with SUMO-1 and/or UBC9 or bring SUMO-1 and UBC9 into proper geometry on the enzyme surface for effective SUMO-1 conjugation to hTOP2. Alternatively, the circular clamp conformation could increase the $k_{cat}$ of the SUMO-1 conjugation reaction. We are in the process of testing these possibilities in an in vitro SUMOylation system. Clearly, further experiments are necessary to establish whether SUMO-1 conjugation to hTOP2 is signaled by TOP2 conformational change(s) or an unknown mechanism(s) (e.g., a DNA damage response).

We have also examined the effect of some stress conditions on SUMO-1 conjugation in HeLa cells. These stress conditions are known to cause protein unfolding (e.g., heat shock) and/or protein damage (e.g., H$_2$O$_2$ and NEM). Among the various stress conditions, heat shock and H$_2$O$_2$ treatment are most

**Fig. 6. Stress-induced SUMO-1 conjugates in HeLa cells.** A, heat shock stimulates SUMO-1 conjugation in HeLa cells. HeLa cells cultured at 37 °C (lane 1) were shifted to 45 °C for 10 min (lane 2) and then lysed by the alkali lysis procedure as described under “Experimental Procedures.” The samples were analyzed by immunoblotting with anti-SUMO-1 antibodies. The 90-kDa protein band marked by * is SUMO-1-modified RanGAP1. The unconjugated 18-kDa SUMO-1 was revealed by 10% SDS gel (lower panel). B, H$_2$O$_2$ elevates the levels of SUMO-1 conjugates. HeLa cells (lane 1) were treated with 10 (lane 2) and 50 mM H$_2$O$_2$ (lane 3) for 5 min and then processed for immunoblotting with anti-SUMO-1 antibodies as described in A. C, NEM induces SUMO-1 conjugation in HeLa cells. HeLa cells (lane 1) were treated with either 0.001 (lane 2) or 0.01 mM (lane 3) NEM for 30 min. Samples were analyzed by immunoblotting with anti-SUMO-1 antibodies as described in A. D, HeLa cells were irradiated with various UV doses at 0 (lane 1), 1 (lane 2), 5 (lane 3), 25 (lane 4), 200 (lane 5), and 2000 (lane 6) J/m$^2$. After irradiation, cells were processed for immunoblotting with anti-SUMO-1 antibodies. E, HeLa cells (lane 1) were treated with either 10 μM (lane 2) or 50 μM (lane 3) cisplatin for 30 min. Cells were then processed for immunoblotting with anti-SUMO-1 antibodies.
effective in increasing the levels of SUMO-1 conjugates in the nucleus. NEM exhibited less effect compared with heat shock and H2O2. Within 10 min of heat shock at 45 °C, the levels of SUMO-1 conjugates in HeLa cells were greatly elevated. Concomitantly, the unconjugated SUMO-1 pool was rapidly exhausted. Except for the 90-kDa SUMO-1-conjugated RanGAP1, all SUMO-1 conjugates were shown to be in the nuclear fraction. The simplest explanation is that heat shock induces partial unfolding of some nuclear proteins. These unfolded proteins trigger their SUMO-1 conjugation. However, it is also possible that SUMO-1 conjugates may exist in a dynamic state being constantly formed and de-SUMOylated. In this case, heat shock might increase the steady state level of SUMO-1 conjugates by affecting the enzymes (e.g. UBC9 and the SUMO-1-specific protease) involved in SUMOylation and de-SUMOylation. If this were true, the levels of all SUMO-1 conjugates should be elevated upon heat shock. However, careful inspection of Fig. 6A has revealed that heat shock appears to induce additional SUMO-1 conjugates without affecting some of the existing SUMO-1 conjugates. In addition, the level of camptothecin-induced SUMO-1-hTOP1 conjugates was decreased rather than increased after heat shock treatment. These results argue against an effect of heat shock on SUMOylation and/or de-SUMOylation enzymes. Thus, it seems plausible that heat shock may cause unfolding of certain nuclear proteins that directly signal their SUMO-1 conjugation. The effect of H2O2 can be similarly explained. H2O2 is known to damage both DNA and protein via reactive oxygen species. These damaged proteins may somehow signal SUMO-1 conjugations. This result may be particularly significant, because cells are constantly under oxidative stress. SUMOylation may thus be evolved as a defense mechanism for repair/removal of damaged nuclear proteins. The effect of UV irradiation on SUMOylation is interesting. At low doses of UV, little effect on SUMOylation was observed. However, at high doses (over 200 J/m²), SUMOylation of nuclear proteins was significantly increased. It has been reported that UV irradiation increases SUMOylation of p53 (40, 41). Our preliminary study has also demonstrated that UV induces SUMOylation of hTOP1.2 UV irradiation is known to damage both nucleic acids and proteins (45). Whether the effect of UV on SUMOylation of nuclear proteins is because of DNA damage or protein damage requires further clarification. It should be pointed out that whereas our studies have demonstrated the involvement of SUMO-1 in modifying topoisomerases and nuclear proteins, we cannot rule out the possibility that SUMO-2 and SUMO-3 may also be involved (8, 46). In fact, we have shown that camptothecin can induce SUMO-2/3-hTOP1 conjugates in addition to SUMO-1-hTOP1 conjugates in mammalian cells.2

The fate of SUMO-1-modified nuclear proteins remains unclear. SUMO-1 conjugates could be destined for degradation by interacting with the ubiquitin/26 S proteasome pathway or for repair/refolding by some unknown mechanisms. Our current studies have demonstrated that topoisomerases and nuclear proteins, we cannot rule out the possibility that SUMO-2 and SUMO-3 may also be involved (8, 46). In fact, we have shown that camptothecin can induce SUMO-2/3-hTOP1 conjugates in addition to SUMO-1-hTOP1 conjugates in mammalian cells.2

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2 Y. Mao and L. F. Liu, unpublished results.
repair of topoisomerase-mediated DNA damage and more generally in regulating nuclear protein functions.

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REFERENCES
1. Wang, J. C. (1996) Annu. Rev. Biochem. 65, 635–692
2. Liu, L. F., Dunn, P., Lin, C. T., D’Arpa, P., and Wu, J. (1996) Ann. N. Y. Acad. Sci. 803, 44–49
3. Desai, S. D., Liu, L. F., Vazquez-Abad, D., and D’Arpa, P. (1997) J. Biol. Chem. 272, 24159–24164
4. Mao, Y., Sun, M., Desai, S. D., and Liu, L. F. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 4076–4081
5. Shen, Z., Pardington-Prymyn, P. E., Ceneaux, J. C., Mozis, R. K., and Chen, D. J. (1996) Genomics 36, 271–279
6. Boddy, M. N., Howe, K., Eltis, L. D., Solomon, E., and Freemont, P. S. (1996) Oncogene 13, 971–982
7. Matunis, M. J., Coutavas, E., and Blobel, G. (1996) J. Cell Biol. 135, 1457–1470
8. Lapenta, V., Chiurazzi, P., van der Spek, P., Pizzuti, A., Hanaoka, F., and Brahe, C. (1997) Genomics 40, 362–366
9. Okura, T., Gong, L., Kamitani, T., Wada, T., Okura, I., Wei, C. F., Chang, H. M., and Yeh, E. T. (1996) J. Immunol. 157, 4277–4281
10. Mahajan, R., Delphin, C., Guan, T., Gerace, L., and Melchior, F. (1997) Cell 88, 97–107
11. Melsh, P. B., and Koshland, D. (1995) Mol. Biol. Cell 6, 793–807
12. Saitoh, H., Pu, R. T., and Dasso, M. (1997) Trends Biochem. Sci. 22, 374–376
13. Johnson, E. S., Schwienhorst, I., Dohmen, R. J., and Blobel, G. (1997) EMBO J. 16, 5509–5519
14. Okuma, T., Honda, R., Ichikawa, G., Tsunagari, N., and Yasuda, H. (1999) Biochem. Biophys. Res. Commun. 260, 362–366
15. Desterro, J. M., Rodriguez, M. S., Kemp, G. D., and Hay, R. T. (1999) J. Biol. Chem. 274, 10618–10624
16. Desterro, J. M., Thomson, J., and Hay, R. T. (1997) FEBS Lett. 417, 297–300
17. Schwartz, S. E., Matuschewski, K., Liakopoulos, D., Scheffner, M., and Jentsch, S. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 560–564
18. Lee, G. W., Melchior, F., Matunis, M. J., Mahajan, R., Tian, Q., and Anderson, P. (1998) J. Biol. Chem. 273, 6503–6507
19. Zhao, W., and Blobel, G. (1997) J. Biol. Chem. 272, 26799–26802
20. Chen, P., Johnson, P., Sommer, T., Jentsch, S., and Hochstrasser, M. (1993) Cell 74, 357–369
21. Gillon, T., Chomskey, O., and Kunka, R. G. (1998) EMBO J. 17, 2759–2766
22. Wang, Z. Y., Qiu, Q. Q., Seufert, W., Taguchi, T., Testa, J. R., Whitmore, S. A., Callen, D. F., Walsh, D., Shenk, T., and Deuel, T. F. (1996) J. Biol. Chem. 271, 24811–24816
23. Li, S. J., and Hochstrasser, M. (1999) Nature 398, 246–251
24. Suzuki, T., Ichiyama, A., Saitoh, H., Kawakami, T., Omata, M., Chung, C. H., Kimura, M., Shimbara, N., and Tanaka, K. (1999) J. Biol. Chem. 274, 31131–31134
25. Gong, L., Millas, S., Maul, G. G., and Yeh, E. T. (2000) J. Cell Biol. 149, 3355–3359
26. Hochstrasser, M. (1996) Annu. Rev. Genet. 30, 405–439
27. Liu, L. F. (1989) Annu. Rev. Biochem. 58, 351–375
28. Wasserman, R. A., Austin, C. A., Fisher, L. M., and Wang, J. C. (1993) Cancer Res. 53, 3591–3596
29. Gong, L., Kamitani, T., Fujise, K., Caskey, L. S., and Yeh, E. T. (1997) J. Biol. Chem. 272, 28198–28204
30. Andoh, T., and Ishida, R. (1998) Biochim. Biophys. Acta 1400, 155–171
31. Roca, J., Ishida, R., Berger, J. M., Andoh, T., and Wang, J. C. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 1781–1785
32. Hsiang, Y. H., and Liu, L. F. (1989) J. Biol. Chem. 264, 9713–9715
33. Fernandes, D. J., Danks, M. K., and Beck, W. T. (1990) Biochemistry 29, 4235–4241
34. Kretz-Rein, C., and Tanguay, R. M. (1999) Biochim. Biophys. Acta 1445, 299–309
35. Desterro, J. M., Rodriguez, M. S., and Hay, R. T. (1998) Mol. Cell. 2, 233–239
36. Mahajan, R., Gerace, L., and Melchior, F. (1998) J. Cell Biol. 140, 259–270
37. Matunis, M. J., Wu, J., and Blobel, G. (1998) J. Cell Biol. 140, 499–509
38. Saitoh, H., Sparrow, D. B., Shiomi, T., Pu, R. T., Nishimoto, T., Mohan, T. J., and Dasso, M. (1998) Curr. Biol. 8, 121–124
39. Kamitani, T., Kito, K., Nguyen, H. P., Wada, H., Fukuda-Kamitani, T., and Yeh, E. T. (1998) J. Biol. Chem. 273, 26675–26682
40. Muller, S., and Dejean, A. (1999) J. Virol. 73, 5137–5143
41. Gostissa, M., Hengstermann, V. F., Sandy, P., Schwartz, S. E., Scheffner, M., and Del Sal, G. (1999) EMBO J. 18, 6462–6471
42. Rodriguez, M. S., Desterro, J. M. P., Lain, S., Midgley, C. A., Lane, D. P., and Hay, R. T. (1999) EMBO J. 18, 6455–6461
43. Danks, M. K., Schmidt, C. A., Deneka, D. A., and Beck, W. T. (1989) Cancer Commun. 1, 101–109
44. Mao, Y., Yu, C. H., Hsieh, T. S., Nitiss, J. L., Liu, A. A., and Liu, L. F. (1999) Biochemistry 38, 10793–10800
45. Tyrrell, R. M. (1996) EXS 77, 255–271
46. Chen, A., Mannen, H., and Li, S. S. (1998) Biochem. Mol. Biol. Int. 46, 1161–1174
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