Characterization of a Second Member of the Sentrin Family of Ubiquitin-like Proteins*

(Received for publication, October 1, 1997, and in revised form, January 22, 1998)

Tetsu Kamitani, Katsumi Kito, Hung Phi Nguyen, Taeko Fukuda-Kamitani, and Edward T. H. Yeh‡

From the Division of Molecular Medicine, Department of Internal Medicine, and the Research Center for Cardiovascular Diseases, Institute of Molecular Medicine for the Prevention of Human Diseases, University of Texas-Houston Health Science Center, Houston, Texas 77030.

* This work was supported in part by National Institutes of Health Grants HL-45851 and an American Heart Association Irene Grant HL-45851 and an American Heart Association established investigator award (to E. T. H. Y.) and by an Arthritis Foundation Irene Grant HL-45851 and an American Heart Association established investigator award (to T. K.). The costs of publication of this article were defrayed in part by the payment of page charges. This advertisement “in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† To whom correspondence should be addressed: Div. of Molecular Medicine, Dept. of Internal Medicine, University of Texas-Houston Health Science Center, 6431 Fannin, Suite 4.200, Houston, TX 77030. Tel.: 713-500-6660; Fax: 713-500-6647; E-mail: eyeh@heart.med.uth.tmc.edu.

© 1998 by The American Society for Biochemistry and Molecular Biology, Inc. Printed in U.S.A.

Sentrin is a novel ubiquitin-like protein that can be conjugated to other proteins in a manner analogous to ubiquitination. Two additional cDNA sequences that encode proteins highly homologous to sentrin have been reported to GenBank™. It is not known whether these sentrin-like proteins could also function as protein modifiers. In this report, a second member of the sentrin family was characterized in detail. Sentrin-2 is a 95-amino acid polypeptide that is 46% identical and 66% homologous to sentrin-1. Northern blot analysis showed that the sentrin-2 message was expressed in all tissues, but was barely detectable in the liver and placenta. The ability of sentrin-2 to conjugate to other proteins was tested by expressing hemagglutinin epitope-tagged sentrin-2 in COS cells. Western blot analysis showed that sentrin-2 could be transferred to other proteins in a pattern similar to that of sentrin-1 conjugation and had similar C-terminal processing. We further showed that both sentrin-1 and sentrin-2 could covalently modify RanGAP1, a Ran GTPase-activating protein critically involved in nuclear transport. Immunocytochemical analysis showed that sentrin-2 derivatives were highly enriched in the nucleus. Taken together, our results demonstrate that sentrin-2 is another protein modifier for the sentrinization pathway.

Sentrin was isolated in our laboratory because of its ability to interact with the death domains of both Fas and tumor necrosis factor receptor-1 (1). Overexpression of sentrin in mammalian cells protects them against anti-Fas or tumor necrosis factor-induced cell death (1). Analysis of the amino acid sequence of sentrin revealed a ubiquitin-like domain (residues 22–97), which is 18% identical and 48% homologous to human ubiquitin. In contrast to ubiquitin, sentrin contains 21 additional amino acids at the N terminus and 4 more amino acids at the C terminus (see Fig. 1). Using a COS cell expression system, we showed that the C terminus of sentrin is efficiently processed to allow sentrin to conjugate to other proteins via the conserved Gly-97 residue (2). However, the conjugation patterns between hemagglutinin (HA)3 epitope-tagged sentrin versus ubiquitin are entirely distinct (2). Furthermore, most of the sentrinized proteins appear to be localized in the nucleus, in contrast to the uniform expression of ubiquitin in both the nuclear and cytosolic compartments (2). Using sentrin as bait in the yeast two-hybrid system, we have identified Ubc9 as a preferred conjugating enzyme for the sentrinization pathway (3). In vitro translated sentrin could be precipitated by a glutathione S-transferase-Ubc9 fusion protein, but not by glutathione S-transferase. A β-mercaptoethanol-sensitive Ubc9-sentrin conjugate could also be identified in the in vitro binding assay. Substitution of the conserved Cys residue of Ubc9 by Ser abolished the formation of the Ubc9-sentrin conjugate. Thus, sentrin was able to form a thiol ester linkage with Ubc9 via the conserved Cys-93 residue. Recently, two other laboratories independently identified a novel ubiquitin-like protein called SUMO-1 or GMP-1 that could covalently modify RanGAP1, a Ran GTPase-activating protein critically involved in nuclear transport (4, 5). Remarkably, the amino acid sequences of SUMO-1 and GMP-1 are identical to that of sentrin (1). RanGAP1 exists as a 70-kDa monomer in the cytosol and is converted to a 90-kDa protein following sentrin modification. Thus, sentrinization of RanGAP1 is essential for the translocation of RanGAP1 from the cytosol to the nuclear pore complex to participate in nuclear protein import (4, 5). Taken together, sentrinization is a unique pathway for protein modification that is distinct from ubiquitination.

Data base research revealed the presence of sentrin homologues in Saccharomyces cerevisiae (GenBank™ accession number U27233; 50% identity and 74% similarity), Caenorhabditis elegans (X99600; 66% identity and 83% similarity), Arabidopsis thaliana (X99609; 49% identity and 64% similarity), and Oryza sativa (X99608; 48% identity and 66% similarity). The presence of sentrin homologues from A. thaliana to Homo sapiens suggests that sentrin is an evolutionary conserved protein that may have a specialized function in cellular metabolism. The yeast homologue of sentrin is termed Smt3, which is a high copy suppressor of the conditional lethal mif2 mutation, which, at nonpermissive temperature, shows increased mitotic chromosome instability, sensitivity to anti-microtubule drugs, and formation of aberrant spindles that break in half during anaphase (6, 7). We have preliminary results demonstrating that human sentrin could also suppress the yeast mif2 mutation,2 suggesting that human sentrin and yeast Smt3 are involved in a evolutionary conserved pathway of protein modification. Further analysis of the data base, and homologous to sentrin-1. Northern blot analysis showed that both sentrin-1 and sentrin-2 could covalently modify RanGAP1, a Ran GTPase-activating protein critically involved in nuclear transport. Immunocytochemical analysis showed that sentrin-2 derivatives were highly enriched in the nucleus. Taken together, our results demonstrate that sentrin-2 is another protein modifier for the sentrinization pathway.

* This work was supported in part by National Institutes of Health Grant HL-45851 and an American Heart Association established investigator award (to E. T. H. Y.) and by an Arthritis Foundation Irene Dugan arthritis investigator award (to T. K.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 The abbreviations used are: HA, hemagglutinin; PBS, phosphate-buffered saline; UCRP, ubiquitin cross-reactive protein; mAb, monoclonal antibody.

2 L. Gong, L. Caskey, and E. T. H. Yeh, unpublished results.
however, revealed two additional human cDNA sequences (X99584 and X99585) that are highly homologous to sentrin. This is of interest because there is only one sentrin homologue reported for all of the nonmammalian genera and species. It is not known whether any of these human cDNAs could encode functional proteins that also participate in the sentrinization pathway. In this report, we analyze a second member of the sentrin family, demonstrating the remarkably conserved modification patterns between these two sentrins.

EXPERIMENTAL PROCEDURES

Cell Lines and Culture Conditions—COS-M6 cells were maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum and antibiotics.

Antibodies—12CA5 (Boehringer Mannheim) and 16B12 (BAbCo, Richmond, CA) are mouse monoclonal antibodies to the peptide sequence YPYDVPDYA of influenza HA. Mouse anti-RH (specific for the amino acid sequence RGSHHHH) monoclonal antibody was purchased from QIAGEN Inc. (Santa Clara, CA). Rabbit anti-RanGAP1 antiserum was kindly provided by Frauke Melchior (The Scripps Research Institute).

Plasmid Construction and Transfection—To express HA-tagged proteins in COS-M6 cells, two vectors for amino-terminal tagging (pcDNA3/HA-N) and carboxyl-terminal tagging (pcDNA3/HA-C) were constructed as described previously (2). HA adaptor duplexes were inserted into pcDNA3 (Invitrogen, San Diego, CA), and then the cDNAs of ubiquitin, sentrin-1, Rad51, and sentrin-2 mutants were isolated by polymerase chain reaction using appropriate primers, followed by ligation into pcDNA3/HA-N or pcDNA3/HA-C. To express RGS(H)6-tagged RanGAP1 (RH-RanGAP1) in COS-M6 cells, the amino-terminal tagging vector (pcDNA3/RH-N) was constructed in the same way as pcDNA3/HA-N. The cDNA of RanGAP1 was isolated by polymerase chain reaction from a cDNA library of human testis and inserted into pcDNA3/HA-N to make pcDNA3/HA-RanGAP1. The sequence of each insert was confirmed by automated DNA sequencing. Plasmids were transfected into COS-M6 cells using LipofectAMINE (Life Technologies, Inc.). The transfected cells were harvested for Western blotting or immunostaining 16 h after transfection.

Northern Blot Analysis—A full-length cDNA fragment of human sentrin-1 or sentrin-2 from plasmid pcDNA3/sentrin-1-3A or pcDNA3/sentrin-2-3A, respectively, was labeled with [α-32P]dCTP with a Megaprime labeling kit (Amersham Pharmacia Biotech). The radioactive probe was hybridized with human multiple tissue Northern blots purchased from CLONTECH (Palo Alto, CA).

Western Blotting—Cells (1 × 106) were harvested, washed twice with cold PBS, and centrifuged. To prevent protein degradation, the cell pellet was immediately transferred into liquid nitrogen, and then the frozen pellet was treated at 45 °C for 1 h in 300 μl of 2% SDS treating solution containing 5% β-mercaptoethanol. DNA in the sample was sheared with a 25-gauge needle. After SDS-polyacrylamide gel electrophoresis, using 3 μl of the sample (equivalent to 1 × 106 cells) per lane, Western blotting was performed following the protocol of the ECL detection system (Amersham Pharmacia Biotech.). As a secondary antibody, horseradish peroxidase-conjugated anti-mouse IgG (Santa Cruz Biotechnology, Santa Cruz, CA) was used.

Nickel Precipitation of RH-tagged RanGAP1—To investigate the conjugation of sentrin-2 to RanGAP1, RH-RanGAP1 was coexpressed with HA-Rad51 (negative control), HA-ubiquitin, HA-sentrin-1 (positive control), or HA-sentrin-2 in COS-M6 cells by the cotransfection method. Since the sequence of the RH tag is RGSHHHHHHH, RH-RanGAP1 can be purified by nickel-charged beads (8). The total cell lysate of the transfectants expressing RH-RanGAP1 and HA-tagged protein was prepared in lysis buffer (6 m guanidine hydrochloride, 20 mM sodium phosphate, and 500 mM sodium chloride, pH 7.8). DNA in the sample was sheared with a 22-gauge needle, and then the lysate was centrifuged at 100,000 × g for 30 min at 15 °C. The supernatant was incubated with nickel-charged agarose resin beads (Invitrogen) for 1 h at room temperature. The beads were washed twice with washing buffer (8 M urea, 20 mM sodium phosphate, and 500 mM sodium chloride) (pH 7.8), followed by washing twice with washing buffer (pH 6.0). Finally, the beads were washed twice with PBS and treated in 2% SDS treating solution for SDS-polyacrylamide gel electrophoresis. The solubilized proteins were analyzed by Western blotting using anti-HA antibody (16B12) to detect RH-RanGAP1 conjugated to HA-tagged proteins and also using anti-RH antibody to detect every derivative of RH-RanGAP1.

Immunostaining—Immunohistochemical staining was performed by the avidin-biotin-horseradish peroxidase complex method (9) using the VECTASTAIN ABC kit system (Vector Laboratories, Inc., Burlingame, CA). Transfected COS-M6 cells on coverslips were fixed in 3.7% paraformaldehyde solution for 20 min and permeabilized in 0.1% Triton X-100 for 10 min at room temperature. After washing with PBS, the fixed cells were incubated with PBS containing 0.1% H2O2 for 10 min to quench endogenous peroxidase activity and then washed with PBS. The cells were incubated for 10 min with PBS containing 5% horse serum for blocking, followed by further incubation with anti-HA antibody (16B12) for 30 min at 37 °C. After rinsing with PBS, the cells were incubated with biotinylated anti-mouse IgG for 30 min at 37 °C, washed with PBS, and then treated with the avidin-biotin-horseradish peroxidase complex for 30 min at 37 °C. Finally, the enzymatic disclosing procedure was performed as reported previously (9).

RESULTS AND DISCUSSION

Sentrin-2 Is a Member of the Sentrin Family of Ubiquitin-like Proteins—Sentrin is a 101-amino acid polypeptide that is 18% identical and 48% homologous to ubiquitin (1). A database search revealed two human cDNA sequences (GenBank™ accession numbers X99584 and X99585) that are more closely related to sentrin than to ubiquitin. One of these sequences (X99585) encodes a 95-amino acid polypeptide that is 46% identical and 66% similar to sentrin. An identical polypeptide (X99585) encodes a 95-amino acid polypeptide that is 46% identical and 66% similar to sentrin. An identical polypeptide sequence is also encoded in a gene insertion in the p125 region of a cytopathic bovine viral diarrhea virus genome (U89439). Because this novel polypeptide shares several biochemical characteristics with sentrin (see below), we have renamed the original sentrin as sentrin-1 and the new protein as sentrin-2. Sentrin-1 was named UBL1 (10), PIC-1 (11), GMP-1 (5), SUMO-1 (4), and SMT3C (12) by other laboratories. Sentrin-2 was named SMT3B or GMP-related protein in these reports.

Fig. 1 shows the amino acid alignment of human sentrin-1, sentrin-2, and ubiquitin. As shown, sentrin-2 contains a ubiquitin domain, an N-terminal extension, and the conserved Gly-Gly residues for conjugation are boxed. The C-terminal double Gly-Gly residues for conjugation are boxed. The Lys residue at position 48 of ubiquitin, required for the formation of multimers, is indicated by an open triangle. The GenBank™ accession numbers for human sentrin-2 cDNA are T08096 and X99585.
A variety of tissues. Poly(A) hybridized with a 32P-labeled cDNA fragment of human sentrin-1 or sentrin-2. A β-actin probe was used as a control. kb, kilobases.

FIG. 2. Expression of human sentrin-1 and sentrin-2 mRNAs in a variety of tissues. Poly(A)* RNA (2 μg) from the indicated sources was run on a denaturing gel, transferred to a nylon membrane, and hybridized with a 32P-labeled cDNA fragment of human sentrin-1 or sentrin-2. A β-actin probe was used as a control. kb, kilobases.

FIG. 3. Comparison between sentrinization and ubiquitination in COS cell lysates. COS-M6 cells were transfected with empty vector, pcDNA3/HA-sentrin-1, pcDNA3/HA-sentrin-2, or pcDNA3/HA-ubiquitin. Total cell lysates were analyzed by Western blotting using anti-HA mAb (16B12 or 12CA5). The sentrin monomer and p90 are indicated by an asterisk. Molecular mass standards are expressed in kilodaltons.

FIG. 4. C-terminal processing and sentrinization of HA-tagged sentrin-2 mutants. HA-tagged wild-type sentrin-2 (Sen2) and the various mutants were transiently expressed in COS cells. The total cell lysates were analyzed by Western blotting with anti-HA mAb (16B12). Unconjugated sentrin-2 and p90 are indicated by a bracket and an arrowhead, respectively. Molecular mass standards are expressed in kilodaltons.

equivalent to Lys-48 in ubiquitin has been substituted with Gln in both sentrin-1 and sentrin-2. To determine the expression of sentrin-2 in human tissues, Northern blot analysis was performed using 32P-labeled human sentrin-1 or sentrin-2 cDNA as a probe. As shown in Fig. 2, the pattern of expression is quite similar for both sentrin-1 and sentrin-2, with a wide range of messenger levels across all the tissues examined and notably lower in the placenta, lung, liver, kidney, and pancreas.

Formation of Sentrin-2 Conjugates in COS Cells—Because sentrin-2 is highly homologous to sentrin-1, we sought to determine whether sentrin-2 could also be conjugated to other proteins in a manner analogous to sentrin-1. For this purpose, HA epitope-tagged sentrin-2 was expressed in COS cells as described previously (2). HA epitope-tagged sentrin-1 or ubiquitin was used as a control, and total cell lysates prepared from transfected COS cells were analyzed by Western blot analysis with anti-HA antibody. As shown in Fig. 3, both sentrin-1 and sentrin-2 monomers were clearly identified. In addition, sentrin-2 forms a number of conjugates similar to those of sentrin-1. Two anti-HA monoclonal antibodies were used in Western blot analyses. 12CA5 was more sensitive than 16B12 and could detect a 35-kDa band in both the sentrin-1- and sentrin-2-transfected lanes. The expression of the sentrin-2 monomer and conjugated proteins is always more prominent than the expression of sentrin-1. However, the conjugation patterns of sentrin-1 and sentrin-2 are similar, suggesting that they could modify common target proteins (see below).

C-terminal Processing Requirement of Sentrin-2—The C terminus of sentrin-2 contains 2 amino acids, Val-94 and Tyr-95, distal to the conserved Gly-92 and Gly-93 residues. For sentrin-2 conjugation to occur, this C-terminal tail must be cleaved. As shown in Fig. 4, when the HA tag was placed at the C terminus of sentrin-2, it was efficiently cleaved in transfected COS cells (lane 7). Substitution of Ala for Gly-93 rendered the C terminus of the sentrin-2 monomer more resistant to processing (lanes 5 and 8). Thus, only p90 was observed in lane 5. Similar observations have been made with the processing of the C terminus of sentrin-1 (2). Deletion of the C-terminal Gly-Val-Tyr residues (positions 93–95) of sentrin-2 abolished the conjugation activity of sentrin-2 (lane 3). Thus, the processing of the C terminus of sentrin-2 is identical to that of sentrin-1. However, it is not known whether the same enzyme is involved.

p90 Is Sentrin-2-conjugated RanGAP1—As shown in Figs. 3 and 4, the conjugation patterns of sentrin-1 and sentrin-2 are similar. Furthermore, a prominent 90-kDa band was observed in both sentrin-1- and sentrin-2-transfected samples. Since sentrin-1 has been shown to conjugate to RanGAP1 to form a 90-kDa protein (4, 5, 8), it was of interest to determine whether sentrin-2 could also be conjugated to RanGAP1. To test this hypothesis, the N terminus of RanGAP1 was tagged with the amino acid sequence RGSHHHHHH, termed RH tag, which allows for facile purification of the RanGAP1 fusion protein with nickel resin beads. Plasmids encoding HA epitope-tagged sentrin-1, sentrin-2, ubiquitin, and Rad51 were cotransfected with a plasmid encoding RH-RanGAP1 into COS cells as described above. Total cell lysates were precipitated first with nickel resin beads, followed by extensive washing. The precipitates were analyzed by Western blot analysis using anti-HA antibody. As shown in Fig. 5A (lanes 1–4), RanGAP1 could be modified by either sentrin-1 or sentrin-2, but not by ubiquitin or Rad51. The purified RH-
RanGAP1 derivatives were detected in a separate Western blot analysis utilizing anti-RH monoclonal antibody. As shown in lanes 5–8, both unmodified RanGAP1 and sentrinized RanGAP1 could be observed equally in all samples. It should be noted that p90 in lanes 5 and 6 was derived from RanGAP1 modified by native sentrin in COS cells. This was further supported by the observation of a doublet observed near p90 in the HA-sentrin-1- and HA-sentrin-2-transfected samples (lanes 7 and 8). In Fig. 5B, we show that sentrin-1 or sentrin-2 mutants (sentrin G) in which the second conserved Gly residue was removed could not form any conjugate, including p90. These mutants were used to further prove that the formation of p90 requires a covalent linkage between RanGAP1 and either sentrin-1 or sentrin-2. Plasmids encoding HA epitope-tagged deletion mutants were cotransfected with a plasmid encoding RH-RanGAP1 into COS cells, and the lysates were prepared as described above. As shown in Fig. 5C, the formation of sentrin and RanGAP1 conjugates requires the presence of the conserved Gly residue in the C terminus of the sentrin molecule.

To address the issue of relative incorporation of sentrin-2 into endogenous versus exogenous RanGAP1, HA-sentrin-2 and/or RH-RanGAP1 was expressed in COS cells. The normalized amounts of the entire extracts were probed with three different antibodies (anti-RanGAP1 to detect both endogenous and exogenous RanGAP1, anti-RH to detect exogenous RanGAP1, and anti-HA to detect exogenous sentrin-2). As shown in Fig. 6, all endogenous RanGAP1 proteins were already modified by endogenous sentrins (lanes 1 and 7). Expression of HA-tagged sentrin-2 did not increase the amount of modified RanGAP1 (lane 2). Coexpression of RH-tagged RanGAP1 with HA-tagged sentrin-2 increased the total amount of modified RanGAP1 (lane 3). However, >50% of the exogenous RanGAP1 proteins were not conjugated (lanes 3, 6, and 9). This was not due to the presence of a limiting amount of sentrin-2 because plenty of sentrin-2 monomers were present in the sample (lanes 3, 6, and 9). Thus, there appeared to be strict regulation of the total amount of sentrinized RanGAP1 in COS cells. It is possible that the amount of sentrinized RanGAP1 proteins is limited by their binding sites at the nuclear envelope. Interestingly, RH-tagged RanGAP1 appeared to conjugate to sentrin-2 more efficiently than other higher molecular mass proteins (lanes 8 and 9). Similar results were obtained when HA-tagged sentrin-1 was coexpressed with RH-tagged RanGAP1 (data not shown).

Predominant Nuclear Localization of Sentrin-2-modified Proteins—We have previously shown that sentrin-1-modified
proteins are localized predominantly in the nucleus (2). The subcellular localization of sentrin-2-modified proteins was determined as described in our earlier report (2). COS cells were also transfected with HA epitope-tagged sentrin-1, sentrin-2, or ubiquitin and stained with anti-HA antibody (16B12) as described previously (2). HA epitope-tagged sentrin-2 is expressed predominately in the nucleus, with minor cytosolic expression (data not shown). In contrast, HA epitope-tagged ubiquitin is distributed equally in both the nucleus and cytosol (data not shown). Thus, the subcellular localization of both sentrin-1 and sentrin-2 appears to be identical.

Protein modification by ubiquitin has been studied extensively in the last 2 decades (13, 14). Ubiquitination has been shown to play a critical role in antigen processing, cell cycle regulation, receptor endocytosis, and signal transduction (15–29). The complexity of the ubiquitination system is further compounded by the identification of other ubiquitin-like molecules, such as UCRP and sentrin. UCRP is a type 1 interferon-inducible protein that contains two ubiquitin domains (30). UCRP has been shown to be conjugated to a large number of intracellular proteins (31); however, the identities of UCRP-modified proteins have not been defined. The discovery of sentrin-1 and the demonstration of its ability to modify RanGAP1 add further excitement to this area of investigation (2, 4, 5). Our laboratory has recently characterized another ubiquitin-like protein, NEDD8, which could also form protein conjugates in a manner similar to protein ubiquitination (8). The results shown in this report establish sentrin-2 as the fourth member, after UCRP, sentrin-1, and NEDD8, of the ubiquitin-like protein modifiers.

Note Added in Proof—We have recently demonstrated that sentrin-3 can also be conjugated to other proteins (Kamitani, T., Nguyen, H.P., Kito, K., Fukuda-Kamitani, T., and Yeh, E.T.H. (1997) J. Biol. Chem. 272, 14001–14004).

REFERENCES
1. Okura, T., Gong, L., Kamitani, T., Wada, T., Okura, I., Wei, C. F., Chang, H. M., and Yeh, E. T. H. (1996) J. Immunol. 157, 4277–4281
2. Kamitani, T., Nguyen, H. P., and Yeh, E. T. H. (1997) J. Biol. Chem. 272, 14001–14004
3. Gong, L., Kamitani, T., Fujise, K., Caskey, L., and Yeh, E. T. H. (1997) J. Biol. Chem. 272, 28198–28201
4. Mahajan, R., Delphin, C., Guan, T., Gerace, L., and Melchior, F. (1997) Cell 88, 97–107
5. Matsunis, M. J., Coutavas, E., andBlobel, G. (1996) J. Cell Biol. 135, 1457–1470
6. Meluh, P. B., andKoshland, D. (1995) Mol. Biol. Cell 6, 793–807
7. Brown, M. T., Goetsch, L., and Hartwell, L. H. (1993) J. Cell Biol. 123, 387–401
8. Kamitani, T., Katsuki, K., Nguyen, R., and Yeh, E. T. H. (1997) J. Biol. Chem. 272, 28557–28562
9. Kamitani, T., Suzuki, H., and Yano, S. (1991) Clin. Immunol. Immunopathol. 58, 217–235
10. Shen, Z., Pardington-Purtsmun, P. E., Comeaux, J. C., Moyzis, R. K., and Chen, D. J. (1996) Genomics 36, 271–279
11. Boddy, M. N., Howe, K., Elkin, L. D., Solomon, E., and Freemont, P. S. (1996) Oncogene 14, 971–992
12. Lapenta, V., Chiurazzi, P., van der Spek, P., Pizzuti, A., Hanaoka, F., andBrahe, C. (1997) Genomics 40, 362–366
13. Hershko, A., and Ciechanover, A. (1992) Annu. Rev. Biochem 61, 761–807
14. Jentsch, S. (1992) Annu. Rev. Genet. 26, 179–207
15. Hochstrasser, M. (1996) Cell 84, 813–815
16. Rock, K. L., Gramm, C., Rothstein, L., Clark, K., Stein, R., Dick, L., Hwang, D., and Goldberg, A. L. (1994) Cell 76, 761–771
17. Murray, A. (1995) Cell 81, 149–152
18. Hopkin, K. (1997) J. Natl. Inst. Health Res. 9, 36–42
19. Cenciarelli, C., Wilhelm, K. G., Guo, A., andWeissman, A. M. (1996) J. Biol. Chem. 271, 8709–8713
20. Hiller, M. M., Finger, A., Schweiger, M., and Wolf, D. H. (1996) Science 273, 1725–1728
21. Johnson, E. S., Ma, P. C. M., Ota, I. M., and Varshavsky, A. (1995) J. Biol. Chem. 270, 17442–17456
22. Kim, T. K., and Maniatis, T. (1996) Science 273, 1717–1720
23. King, R. W., Peters, J. M., Taggndreisch, S., Rolfe, M., Hieter, P., andKirschner, M. W. (1995) Cell 81, 279–288
24. Lahav-Baratz, S., Sudakin, V., Ruderman, J. V., and Hershko, A. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 9303–9307
25. Mitch, W. E., and Goldberg, A. L. (1996) N. Engl. J. Med. 335, 1897–1905
26. Scheffner, M., Huibregtse, J. M., Vierstra, R. D., andHowley, P. M. (1993) Cell 75, 495–505
27. Strous, G. J., Van Kerkhof, P., Govers, R., Rotwein, P., andSchwartz, A. L. (1997) J. Biol. Chem. 272, 40–43
28. Treier, M., Stausszewski, L. M., andBohmann, D. (1994) Cell 78, 787–798
29. Lam, Y. A., Xu, W., DeMartino, G. N., and Cohen, R. E. (1996) Nature 385, 757–740
30. Haas, A. L., Ahrens, P., Bright, P. M., and Ankel, H. (1987) J. Biol. Chem. 262, 11315–11323
31. Loeb, K. R., and Haas, A. L. (1992) J. Biol. Chem. 267, 7806–7813