Lithium affects several enzymatic activities, however, the molecular mechanisms of lithium actions are not fully understood. We previously showed that LiCl interacts synergistically with all-trans-retinoic acid to promote terminal differentiation of WEHI-3B D⁺ cells, a phenomenon accompanied by the recovery of the retinoid-induced loss of retinoic acid receptor α protein pools. Here, we demonstrate the effects of LiCl on proteasome-dependent degradation of retinoic acid receptor α proteins. LiCl alone, or in combination with all-trans-retinoic acid, increased cellular levels of ubiquitinated retinoic acid receptor α and markedly reduced chymotryptic-like activity of WEHI-3B D⁺ 20 S and 26 S proteasome enzymes. Neither KCl nor all-trans-retinoic acid affected enzyme activity, whereas NaCl produced a modest reduction at relatively high concentrations. In addition, LiCl inhibited 20 S proteasome chymotryptic-like activity from rabbits but had no effect on tryptic-like activity of the 26 S proteasome. This effect has significant consequences in stabilizing the retinoic acid receptor α protein levels that are necessary to promote continued differentiation of leukemia cells in response to all-trans-retinoic acid. In support of this concept, combination of proteasome inhibitors β-clastolactacystin or benzoylloxycarbonyl-Leu-Leu-Phe with all-trans-retinoic acid increased differentiation of WEHI-3B D⁺ cells in a manner that was analogous to the combination of LiCl and all-trans-retinoic acid.

Several mammalian enzymes are known targets of lithium including inositol monophosphatase, glycosen synthase kinase-3, and several phosphomonoesterases; however, the mechanism(s) by which lithium affects cellular events remains unclear (1). Pharmacological doses of lithium cause stabilization of bipolar disorder, developmental defects, subclinical hypothyroidism, and increased neutrophil production (2–4). Lithium has been proposed to impede G-protein-coupled signaling cascades because of a depletion of intracellular inositol by direct inhibition of inositol monophosphatase (5). Lithium also inhibits glycosen synthase kinase, which can lead to signaling of the wnt pathway through β-catenin (6). In addition, we have shown previously that lithium chloride, in combination with all-trans-retinoic acid (ATRA), causes synergistic induction of the differentiation of WEHI-3B D⁺ myelomonocytic leukemia cells and reverses the down-regulation of retinoic acid receptor α protein (RARα) produced by ATRA (7, 8). Recently, the down-regulation of RARα by ATRA has been reported to occur in acute promyelocytic leukemia and in breast cancer cells by specific targeting of the receptors to the proteasome-dependent degradation pathway (9, 10). These observations suggested that lithium may exert some of its effects by targeting a component of the proteasome-dependent degradation system.

The implication of proteasome-dependent degradation mediating important cellular events has been the subject of numerous studies to date (for example, see Refs. 11–13). Proteasomes are ubiquitous, multicatalytic complexes responsible for nonlysosomal, ubiquitin-dependent proteolytic activity (14). They are highly conserved polysubunit complexes organized into a 20 S catalytic core or a 26 S complex containing the catalytic core plus an associated regulatory complex (15). Both 20 S and 26 S proteasome particles exhibit at least five different proteolytic activities (16, 17). The proteasome plays important roles in cell cycle regulation and differentiation; thus, the degradation of cyclins and cyclin-dependent kinases (p21 and p27) as well as transcription factors (NF-κB and IκB) and tumor suppressors (p53) occur via the ubiquitin-proteasome pathway (18). During the differentiation of hematopoietic cells, changes in subcellular distribution, subunit composition, and enzymatic activity of proteasomes have been reported (19–22). In malignant hematopoietic cells and breast cancer cells, concentrations of proteasomes have been reported to be abnormally high and localized in the nucleus of leukemia cells (23). Thus, proteasome regulation is fundamental to the maintenance of normal cellular processes. Indeed, proteasome inhibitors activate apoptosis in human HL60 leukemia cells (24), and proteasome inhibitors have shown recently antagonism activity, with the potent boronic acid proteasome inhibitor PS-341 currently undergoing a clinical trial (25, 26). Recently, Gianni et al. (27) have shown that the c-Abl tyrosine kinase inhibitor STI571 enhances retinoid-induced differentiation of acute promyelocytic leukemia (APL) NB4 cells, and that regulation of the proteasome plays a role in this process.

In addition to the known enzymes affected by lithium, we demonstrate in this report that both the 20 S and 26 S forms of mammalian proteasomes are targets of lithium inhibition. Because the proteasome enzyme is intricately involved in the maintenance of many cellular processes, lithium chloride provides a unique method of regulation. An example of the signif-

* This research was supported in part by the United States Public Health Service Grant CA-05817 from the National Cancer Institute. 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.

† To whom correspondence should be addressed: Dept. of Pharmacology, Yale University School of Medicine, 333 Cedar St., New Haven, CT 06520. Tel.: 203-785-4533; Fax: 203-737-2045; E-mail: alan.sartorelli@yale.edu.

1 The abbreviations used are: ATRA, all-trans-retinoic acid; RARα, retinoic acid receptor α; APL, acute promyelocytic leukemia; TPA, 12-O-tetradecanoylphorbol-13-acetate; AMC, 7-amino-4-methylcoumarin.

The Journal of Biological Chemistry Vol. 276, No. 46, Issue of November 16, pp. 42722–42727, 2001
Printed in U.S.A.

Inhibition of 20 S and 26 S Proteasome Activity by Lithium Chloride

IMPACT ON THE DIFFERENTIATION OF LEUKEMIA CELLS BY ALL-TRANS-RETINOIC ACID

Received for publication, July 13, 2001, and in revised form, September 6, 2001
Published, JBC Papers in Press, September 12, 2001, DOI 10.1074/jbc.M106583200

Anna M. Rice and Alan C. Sartorelli‡
From the Department of Pharmacology and Developmental Therapeutics Program, Cancer Center, Yale University School of Medicine, New Haven, Connecticut 06520

This paper is available on line at http://www.jbc.org
Inhibition of Proteasome-dependent RARα Degradation by LiCl

icance of this action is seen in the differentiation of WEHI-3B D⁺ leukemia cells, where LiCl prevents the degradation of RARα protein pools produced by ATRA that are critical to promoting retinoid-induced leukemia cell differentiation (8). Because the structure and function of RARα are critical to the success of ATRA in the therapy of APL, which is often characterized by only short term remission rates and the development of retinoid resistance (28), the prevention of receptor loss by LiCl may allow more complete cell differentiation and longer remissions in APL patients.

EXPERIMENTAL PROCEDURES

Cell Culture and Differentiation—WEHI-3B D⁺ cells were cultured in suspension in McCoy's 5A-modified medium supplemented with 15% fetal calf serum. Differentiation was induced with ATRA and LiCl at a cell density of 5 × 10⁵ cells/ml. The degree of differentiation was assessed 72 h post-induction and was measured by the capacity of cells to reduce nitro blue tetrazolium following TPA stimulation as described previously (8). In some experiments, cells were incubated with β-clastolactacystin (BioMol, Plymouth Meeting, PA) or benzyloxycarbonyl-Leu-Leu-Phe to induce differentiation.

Immunoprecipitation and Western Blotting—RARα was immunoprecipitated from 100 μg of cell lysates prepared in radioimmunoprecipitation buffer with 2 μg of anti-RARα antibody (Santa Cruz Biotechnology, Inc., Santa Cruz, CA) for 12 h by separation by electrophoresis on a 10% SDS-polyacrylamide gel followed by transfer to nitrocellulose. Ubiquitin-containing proteins were identified by Western blot analysis using mouse anti-ubiquitin antibody (Santa Cruz Biotechnology) followed by ECL detection (Amersham Pharmacia Biotech UK, Ltd., Little Chalfont, Buckinghamshire, UK). RARα and ubiquitinated RARα species were quantified by densitometric scanning of blots and analysis using ImageQuant software.

Protein Purification and Activity Analysis—Proteasomes were partially purified from 2 × 10⁶ WEHI-3B D⁺ cells by homogenization in 50 mM Tris-HCl, pH 8.0, 1 mM MgCl₂, 1 mM dithiothreitol, 2 mM ATP, 10 mM creatine phosphokinase, 20% glycerol. The homogenate was centrifuged for 20 min at 10,000 × g, the resulting supernatant was centrifuged at 100,000 × g for 1 h, and the pellet corresponding to the 20 S proteasome was resuspended in 50 mM Tris-HCl, 5 mM MgCl₂, 2 mM ATP, 20% glycerol. The supernatant was further centrifuged for 5 h at 100,000 × g, and the pellet (20 S proteasome) was resuspended in 50 mM Tris-HCl, 5 mM MgCl₂, 2 mM ATP, 20% glycerol.

The proteolytic activity of WEHI-3B D⁺ 20 S and 26 S proteasomes was determined by the measurement of fluorescence generated from chymotryptic cleavage of the fluorogenic substrate Suc-Leu-Leu-Val-Tyr-AMC (CalBiochem, La Jolla, CA) activity was measured at 1.2 ng/µg of protein before the addition of substrate. Fluorescence release was measured for 60 min at 1-min intervals. Purified rabbit 20 S proteasome (Calbiochem, La Jolla, CA) was used as a positive control. The 50% inhibitory concentration (IC₅₀) was determined from plots of the ratio of velocities with and without inhibitor at various concentrations of inhibitor.

In Vitro Translation and Processing of RARα—[³⁵S]Methionine-labeled RARα protein was generated by in vitro coupled transcription/translation from pGem3zf(+)RARα plasmid containing the cDNA of the mouse RARα gene using a rabbit reticulocyte lysate system (Promega, Madison, WI) in the presence of 0.4 mM [³⁵S]methionine (20 μCi) (Amersham Pharmacia Biotech). After completion, the reaction was treated with 10 mM N-ethylmaleimide for 10 min at room temperature followed by the addition of dithiothreitol at a final concentration of 6 mM. The processing of labeled protein was modified from the degradation procedure described by Lodha et al. (30). RARα-labelling reaction (4.5 µl) was incubated at 37 °C in degradation buffer (50 mM Tris-HCl, pH 8.0, 0.5 mM MgCl₂, 1 mM dithiothreitol, 2 mM ATP, 10 mM creatine phosphokinase, 10 mM creatine phosphate, 5 mM ATP, 5 µM ubiquitin) for 30 min in the presence or absence of additional 26 S proteasomes (5 µg) partially purified from

FIG. 1. Accumulation of polyubiquitinated RARα species in WEHI-3B D⁺ cells produced by treatment with ATRA and LiCl. A, immunoprecipitation of RARα from WEHI-3B D⁺ cells treated for various times with ATRA, LiCl, or vehicle alone (control) followed by Western blot analysis for ubiquitin. Loading was standardized by using equal cell numbers. Data are representative of three independent experiments. B, densitometric analysis of bands indicated as polyubiquitinated RARα species in A. C, immunoprecipitation followed by Western blotting for ubiquitin. C, control; β-clastolactacystin (1 μM).

WEHI-3B D⁺ cells. Degradation reactions were subjected to 10% SDS-polyacrylamide gel electrophoresis followed by phosphorimaging analysis of radiolabeled RARα bands.

RESULTS

Accumulation of Ubiquitinated RARα following Exposure to ATRA and LiCl—In APL cell lines, both PML/RARα and RARα proteins were shown to be down-regulated by ATRA in a proteasome-dependent manner (9), and in MCF7 breast cancer cells, ATRA similarly induced proteasome-dependent degradation of the receptor (10). Therefore, we explored the mechanism by which LiCl maintained steady-state levels of RARα protein by measuring the patterns of ubiquitination of RARα after treatment with LiCl and ATRA, both alone and in combination in WEHI-3B D⁺ cells. Polyubiquitinated RARα species were identified as multiple bands migrating at high molecular masses (Fig. 1A) with the exception of ubiquitinated proteins (between 97 and 66 kDa), which migrated similarly to nonspecific proteins that were detected by RARα antibody. The treatment of cells with 3 µM ATRA for 1 h caused a transient 4-fold increase in polyubiquitinated RARα species. Polyubiquitination decreased by 3 h and returned to control levels at 24 h (Fig. 1A and B). In contrast, LiCl at 5 mM increased the level of polyubiquitinated RARα steadily over a 24-h period resulting in a 5.5-fold increase in polyubiquitinated RARα over untreated cell RARα polyubiquitination. This accumulation was even more pronounced when ATRA was combined with LiCl (Fig. 1C). Accumulated polyubiquitinated bands were similar to the amounts present in the cells treated with the potent proteasome inhibitor β-clastolactacystin (Fig. 1C).

The accumulation of polyubiquitinated RARα proteins upon ATRA treatment confirms that the retinoid induces receptor ubiquitination, targeting RARα proteins for proteasomal degradation. LiCl alone maintained increased levels of highly ubiquitinated
receptors and, in combination with ATRA, blocked the degradation of ubiquitinated receptors in a manner analogous to that produced by the proteasome inhibitor β-clastolactacystin.

Specific Inhibition of Proteasome Chymotryptic-like Activity of WEHI-3B D⁺ Cells by LiCl—The accumulation of polyubiquitinated RARα species produced by treatment with LiCl alone suggested that proteasome-dependent degradation of these receptors was relatively inactive in WEHI-3B D⁺ cells. To gain information on proteasomal activity in these cells, 20 S and 26 S proteasomes were partially purified from WEHI-3B D⁺ cells, and chymotryptic-like activity was assessed. Cleavage of the peptide substrate Suc-Leu-Leu-Val-Leu-AMC by the 20 S proteasome in the presence of 0.035% SDS resulted in fluorescence at 460 nm. 20 S proteasomes from WEHI-3B D⁺ cells displayed activity similar to that of commercially available rabbit 20 S proteasomes (95% purity) (Fig. 2A). The presence of increasing concentrations of LiCl decreased the rate of chymotryptic activity of WEHI-3B D⁺ cells and rabbit 20 S proteasomes (Fig. 2B and C). LiCl at 2.5 mM significantly reduced peptide cleavage after 1 h by WEHI-3B D⁺ 20 S proteasomes, whereas 10 mM LiCl reduced fluorescence in this system to background levels. A plot of $V/V_o$ versus the concentration of LiCl from 0–10 min demonstrated an IC₅₀ for WEHI-3B D⁺ 20 S proteasomes of 4 mM (Fig. 2D). Furthermore, proteasome complexes partially purified from cells pretreated with 5 mM LiCl exhibited decreased activity in the absence of additional LiCl in the in vitro assay (Fig. 2E). LiCl inhibited WEHI-3B D⁺ 26 S proteasomes to a similar extent (Table I), lowering the $V_{max/\sec}$ by more than 50% at 2.5 mM.

To determine the specificity of proteasome inhibition by LiCl, the activity was measured in the presence of 1–10 mM NaCl, KCl, or 3–10 μM ATRA. No effect was observed on 20 S proteasome activity by the presence of 1–5 mM NaCl (Fig. 3A); however, 10 mM NaCl decreased activity slightly. In contrast, neither KCl nor ATRA significantly affected the rate of chymotryptic-like activity of 20 S proteasomes (Fig. 3B and C). These observations suggest that LiCl has a unique effect on both WEHI-3B D⁺ and rabbit proteasome activity, and that this inhibitory effect probably is responsible for the observed accumulation of polyubiquitinilated RARα species following exposure of WEHI-3B D⁺ cells to LiCl.

LiCl at concentrations as high as 10 mM had no significant effect on the tryptic-like activity of partially purified 26 S proteasomes.
proteasomes from WEHI-3B D+ cells (Fig. 4). Thus, LiCl exhibited specificity for inhibition of chymotryptic-like protease activity.

LiCl Inhibits the Direct Proteasomal Degradation of RARα Protein Substrates in Vitro—The combination of ATRA and LiCl acts synergistically to promote the terminal differentiation of WEHI-3B D+ cells (8). Because LiCl inhibits proteasome activity, we postulate that at least a portion of the mechanism by which LiCl enhances the differentiation of WEHI-3B D+ cells produced by ATRA is attributed to the inhibition of proteasomal-targeted degradation of RARα. This action results in the restoration of RARα protein pools depleted by the retinoid.

To assess whether RARα is a direct substrate for degradation by the proteasome, we measured the proteolytic cleavage of in vitro translated [35S]methionine-labeled RARα protein by 26 S proteasomes partially purified from WEHI-3B D+ cells. The addition of 26 S proteasomes to [35S]methionine-labeled RARα proteins translated by rabbit reticulocyte lysate caused a marked decrease in radiolabel associated with RARα protein (Fig. 5). Only 7.5% of RARα protein remained after exposure to proteasomes. However, in the presence of 5 mM LiCl, degradation by 26 S proteasomes was dramatically reduced, maintaining 80% of control levels of radiolabeled receptor protein. This observation illustrates that RARα proteins are direct substrates of the proteasome, and that LiCl prevents proteolytic degradation in a manner independent of other cellular components.

Induction of WEHI-3B D+ Cell Differentiation by Proteasome Inhibitors—To determine whether inhibition of proteasome activity by LiCl in intact cells is involved in the mechanism by which LiCl promotes the differentiation of WEHI-3B D+ cells by ATRA, the dependence of retinoid-induced differentiation on proteasome function was assessed.

The extent of terminal differentiation was measured by determining the percentage of nitro blue tetrazolium-positive cells after 3 days of treatment of WEHI-3B D+ cells with either β-clasto-actacyclin, an irreversible proteasome inhibitor, or the peptide aldehyde inhibitor benzyloxycarbonyl-Leu-Leu-Phe. Proteasome inhibitors had no significant effect on differentiation when used alone. However, in combination with ATRA, β-clasto-actacyclin at 1 μM and 5 μM increased the degree of differentiation in a concentration-dependent manner from 27.7 ± 7.5% for the retinoid alone to 46.3 ± 4.9% and 65.0 ± 4.0%, respectively (Table II). A combination of LiCl with ATRA induced 74 ± 6% cell differentiation, a 2.7-fold increase over differentiation induced by ATRA alone, and benzyloxycarbonyl-Leu-Leu-Phe at 1 μM also exhibited synergism in combination with ATRA, inducing 62.0 ± 1.5% terminal differentiation.

DISCUSSION

In the present investigation, we report a new site of action of lithium, demonstrating that LiCl specifically inhibits the chymotryptic-like activity of both the 20 S and 26 S proteasome. An analysis of the rate of ubiquitination of RARα in the
WEHI-3B D⁺ leukemia cell system demonstrated transient increases in polyubiquitination of RARα induced by ATRA in WEHI-3B D⁺ cells, which was increased by the addition of LiCl. In addition to preventing the loss of ubiquitinated RARα species, LiCl alone was able to markedly reduce the enzymatic activity of the proteasome from WEHI-3B D⁺ cells. These results suggest that LiCl does not act to enhance ubiquitination but produces an accumulation of ubiquitinated RARα proteins by blocking ubiquitin-dependent degradation. We have also shown that other salts do not affect proteasome activity in a manner analogous to LiCl, suggesting a relatively unique effect on the proteasome by lithium (Fig. 3).

Although we have observed a pronounced effect of LiCl on the isolated proteasome enzyme, we have also shown a reduction in the activity of proteasomes from cells pretreated with LiCl before purification (Fig. 2). This finding suggests that LiCl may affect the proteasome structure irreversibly. It remains unclear how LiCl affects the proteasome; however, it is possible that LiCl causes spot-denaturation or small conformational changes at the chymotryptic-like active site. Alternatively, LiCl may induce the release of one or more of the subunits of this multisubunit complex.

We have demonstrated that the effect of LiCl on proteasome enzymatic activity is related to its ability to potentiate retinoid-induced cell differentiation. Since activity of proteasomes toward the RARα protein substrate in a system separate from cell-signaling mechanisms was inhibited by LiCl (Fig. 5), we conclude that the effects of LiCl on proteasome activity directly relates to the regulation of the retinoid receptor shown to be key to ligand-induced terminal differentiation.

Evidence to suggest that LiCl affects the proteasome in situ is seen when peptide inhibitors of the proteasome were used in combination with ATRA to differentiate WEHI-3B D⁺ cells. Because the increase in differentiation obtained was similar to that observed with LiCl + ATRA, we conclude that the modulation of ATRA-induced differentiation by LiCl occurs, at least in part, through the inhibition of the proteasome. Interestingly, the degree of differentiation produced by the peptide inhibitors of the proteasome in combination with ATRA was slightly less than that achieved by the LiCl/ATRA combination. The use of proteasome inhibitors as antitumor agents is currently being evaluated as an approach to the therapy of cancer (25, 26, 31), suggesting that lithium at therapeutic doses may have use in the treatment of malignant diseases other than APL.

The ubiquitin-dependent proteasome has been reported to localize more prominently in the nucleus of leukemia cells as well as expressed at much higher levels in these neoplastic cells than in normal cells (23). Therefore, proteasome location and function may be implicated in maintaining the proliferative state, and regulation of the proteasome may play a key role in cancer cell growth. Furthermore, one can speculate that in cancerous cells that are not ATRA-responsive but have supranormal levels of proteasome activity, the inhibition of the proteasome by LiCl may reduce cellular proteasome activity levels to those normally found in non-malignant cell types and thereby decrease proliferative activity. In WEHI-3B D⁺ leukemia cells, the disruption of the ATRA-induced targeting of RARα to the proteasome by LiCl appears to be important for promoting optimal terminal differentiation. Thus, upon treatment of WEHI-3B D⁺ cells with the combination of ATRA and LiCl, the accumulation of polyubiquitinated adducts appears to signal the cell to rebuild the pool of RARα protein and RARα mRNA accumulation occurs (8). The rebuilding of the RARα protein pool may be attributed to an increase in the back reaction of the ubiquitination enzyme system (i.e. ubiquitin hydrolysis), an increase in the rate of mRNA translation, or a combination of both events.

The effects of LiCl on proteasome activity are likely not to be specific to leukemia cells and, therefore, may be beneficial in the differentiation therapy in combination with ATRA of a broad spectrum of cancers. The identification of this new cellular target of lithium may also help answer questions on the mechanism of lithium action implicated in non-cancer-related cellular processes.

Acknowledgment—We thank Dr. Kathleen Holtz for invaluable scientific discussions.

REFERENCES
1. Piel, C. J., and Klein, P. S. (2001) Annu. Rev. Pharmacol. Toxicol. 41, 789–813
2. Price, L. H., and Heninger, G. R. (1994) N. Engl. J. Med. 331, 591–598
3. Kao, K. R., Masui, Y., and Elinson, B. P. (1986) Nature 321, 371–373
4. Boggs, D. R., and Joyce, R. A. (1983) Semin. Hematol. 20, 129–138
5. Hailer, L. M., and Sherman, W. R. (1986) J. Biol. Chem. 261, 10901–10901
6. Klein, P. S., and Melton, D. A. (1996) Dev. Biol. 176, 8455–8459
7. Sokoloski, J. A., Li, J., Nigam, A., and Sartorelli, A. C. (1993) Leuk. Res. 17, 403–410
8. Finch, R. A., Li, J., Chou, T. C., and Sartorelli, A. C. (2000) Blood 96, 2262–2268
9. Zhu, J., Gianni, M., Kopf, E., Honore, N., Chelbi-Alix, M., Koken, M., Quignon, F., Rochette-Egly, C., and de The, H. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 14807–14812
10. Tanaka, T., Rodriguez de la Concepcion, M. L., and De Luca, L. M. (2001) Biochem. Pharmacol. 61, 1347–1355
11. Pagano, M., Tan, S. Y., Theoret, A. M., Beer-Romero, P., DelSal, G., Chao, V., Yew, P. R., Draetta, G. F., and Rolfe, M. (1995) Science 269, 682–685
12. Palombella, V. J., Rando, O. J., Goldberg, A. L., and Maniatis, T. (1994) Cell 78, 773–785
13. Ferdeux, A., Gonzalez, F., Sun, L., Kodeske, T., and Johnston, A. (2001) Mol. Cell 7, 981–991
14. Matthews, W., Driscoll, J., Tanaka, K., Ichihara, A., and Goldberg, A. L. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 2597–2601
15. Bochtler, M., Ditzel, L., Groll, M., Hartmann, C., and Huber, R. (1999) Annu. Rev. Biophys. Biomol. Struct. 28, 295–317
16. Finch, R. A., Li, J., Chou, T. C., and Sartorelli, A. C. (2000) Blood 96, 2262–2268
17. Bochtler, M., Ditzel, L., Groll, M., Hartmann, C., and Huber, R. (1999) Annu. Rev. Biophys. Biomol. Struct. 28, 295–317
18. Orlofski, M. (1996) Biochemistry 35, 10288–10297
19. Orlofski, M., Cardozo, C., and Michael, C. (1995) Biochemistry 32, 1563–1572
20. Hershko, A., and Ciechanover, A. (1998) Annu. Rev. Biochem. 67, 425–479
21. Frisam, T., Levitovs, V., and Masucei, M. G. (2000) Int. J. Cancer 88, 881–888
22. Henry, L., Baz, A., Chateau, M. T., Scherrer, K., and Bureau, J. P. (1996) Cell Proliferation 29, 589–607
23. Henry, L., Baz, A., Chateau, M. T., Caravano, R., Scherrer, K., and Bureau, J. P. (1997) Anat. Cell Pathol. 15, 131–144
24. Shimbara, N., Ono, E., Yone, S., Ogura, T., Tashima, N., Shono, M., Tamura, T., Yasuda, H., Tanaka, K., and Ichihara, A. (1992) J. Biol. Chem. 267, 18160–18169
25. Kumasari, R., Tanaka, K., Inamura, N., Sone, S., Ogura, T., Matsumoto, T., Tachikawa, T., Shin, S., and Ichihara, A. (1999) Proc. Natl. Acad. Sci. U. S. A. 97, 7071–7075
26. Drexler, H. C. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 855–860
25. Teicher, B. A., Gulshan, A., Herbst, R., Palombella, V. J., and Adams, J. (1999) *Clin. Cancer Res.* 5, 2638–2645
26. Adams, J., Palombella, V. J., Sausville, E. A., Johnson, J., Destree, A., Lazarus, D. D., Mass, J., Pien, C., Prakash, S., and Elliott, P. J. (1999) *Cancer Res.* 59, 2615–2622
27. Gianni, M., Kalac, Y., Ponzanelli, I., Rambaldi, A., Terao, M., and Garattini, E. (2001) *Blood* 97, 3234–3243
28. Lin, R. J., Egan, D. A., and Evans, R. M. (1999) *Trends Genet.* 15, 179–184
29. Gazdzinski, M., Rock, K. L., Spies, T., and Goldberg, A. L. (1994) *Proc. Natl. Acad. Sci. U. S. A.* 91, 9213–9217
30. Loda, M., Cukor, B., Tam, S. Y., Lavin, P., Fiorentino, M., Draetta, G. F., Jessup, J. M., and Pagano, M. (1997) *Nat. Med.* 3, 231–234
31. Hideshima, T., Richardson, P., Chauhan, D., Palombella, V. J., Elliott, P. J., Adams, J., and Anderson, K. C. (2001) *Cancer Res.* 61, 3071–3076