Resistance to Diverse Drugs and Ultraviolet Light Conferred by Overexpression of a Novel Human 26 S Proteasome Subunit*

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We have investigated the usefulness of the fission yeast Schizosaccharomyces pombe as a model organism for the discovery of novel modes of drug resistance in human cells. In fission yeast, overexpression of the essential pad1+ gene confers pleiotropic drug resistance through a pathway involving an AP-1 transcription factor encoded by pap1+. We have identified POH1, a human pad1 homologue that can substitute fully for pad1+ and induce AP-1-dependent drug resistance in fission yeast. POH1 also confers P-glycoprotein-independent resistance to taxol (paclitaxel), doxorubicin, 7-hydroxy-staurosporine, and ultraviolet light when transiently overexpressed in mammalian cells. Poh1 is a previously unidentified component of the human 26 S proteasome, a multiprotein complex that degrades proteins targeted for destruction by the ubiquitin pathway. Hence, Poh1 is part of a conserved mechanism that determines cellular susceptibility to cytotoxic agents, perhaps by influencing the ubiquitin-dependent proteolysis of transcription factors.

Exposure of eukaryotic cells to any of a wide variety of cytotoxic agents can result in the appearance of multiply resistant subpopulations, a phenomenon that frequently limits the effectiveness of anticancer therapies. Much effort has been directed toward improved understanding of the molecular mechanisms that underlie such pleiotropic resistance. Many of these studies have used mammalian cell lines selected in vitro for their resistance to high concentrations of cytotoxic drugs. Under these circumstances the mechanism of resistance typically involves overexpression of P-glycoprotein (P-gp),1 a drug-excluding pump with broad specificity (1, 2). Unfortunately, P-gp overexpression fails to account for the majority of clinical drug resistances especially in solid tumors (3), and the key determinants of this phenomenon remain largely unknown.

We have taken an alternative route to the identification of human proteins potentially involved in pleiotropic drug resistance. The fission yeast Schizosaccharomyces pombe is established as a model organism in which powerful genetic approaches can be used to elucidate fundamental but complex eukaryotic processes such as mitosis (4). Overexpression of the pad1+ gene in S. pombe was recently found to confer moderate resistance to the protein kinase inhibitor staurosporine and a variety of other drugs including caffeine and the spindle poison thiabendazole (5–7). We have identified a human functional pad1 homologue (Poh1) that confers moderate resistance to chemotherapeutic drugs and also to ultraviolet light when transiently overproduced in mammalian cells. Poh1 is a previously unidentified component of the 26 S proteasome and genetic data suggest that Poh1-induced drug resistance is mediated through AP-1 transcription factors.

EXPERIMENTAL PROCEDURES

Molecular Cloning of POH1—Polymerase chain reaction amplification was performed using an HT29 human colon carcinoma cDNA library (kindly provided by H. Okayama, University of Tokyo) as template and the degenerate oligonucleotides 1) 5′-CTSGCHCTSC-T-5′ and 2) 5′-ACDSWTCTRDGGRT-5′, corresponding to the amino acid sequences LALLKM and DPIQSV found both in Pad1 and in the predicted product of the Caenorhabditis elegans cosmid clone F37A4.5 (5). The resulting products were used as template for a second round of amplification using the same primer 2 in combination with a 3rd primer, 5′-GARGTATGGGCTSAGTCT-3′, corresponding to the internal block of conserved amino acids EVMGLML. The resulting 300-base pair fragment was radioabeled and used to isolate full-length POH1 cDNAs from a Basinger human fibroblast cDNA library (also provided by H. Okayama) by standard procedures (8). Multiple clones with inserts of approximately 1.7 kilobase pairs were identified, which correspond to the POH1 open reading frame preceded by approximately 200 base pairs of 5′-untranslated sequence and followed by approximately 600 base pairs of 3′-untranslated sequence. One clone corresponded to a POH1 mRNA species with the same 5′ end but polyadenylated immediately 3′ to the translation stop codon. The complete nucleotide sequence of the open reading frame and 5′-untranslated region (GenBank™ accession number U86782) was determined on both strands using a Sequenase kit (United States Biochemical) according to the manufacturer’s instructions. A version of the POH1 open reading frame flanked by 5′ Start and 3′ Stop sites and tagged with the hemagglutinin epitope (YPHPDYVPA) was generated by polymerase chain reaction and cloned into the fission yeast vector pREP2X (kindly provided by S. Forsburg, The Salk Institute, La Jolla, CA), which is essentially identical to prep1 (9), containing 5′ Xhol and 3′ NotI sites in the polyclinker.

Antibodies and Immunochemistry—A polyclonal rabbit antisera against a GST-Poh1 fusion protein was raised using standard procedures (10) after cloning the POH1 open reading frame into pGEX4T-2 (Pharmacia Biotech Inc.) using the ECL method (Amersham Corp.) according to the manufacturer’s instructions.

Fission Yeast Methods—The strain HM123 (leu1–32, 7′) was used unless otherwise stated. Standard procedures for S. pombe genetics

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were followed (11), and all cultures were grown at 30 °C. The heterozygous diploid strain TP42 (\textit{D}pad1/\textit{D}pad1) (1) was used to construct haploid strains containing pREP-POH.

Transfections, Cytotoxicity Assays, and Analysis of Doxorubicin Uptake—A hemagglutinin epitope-tagged version of \textit{POH1} cDNA with the Kozak sequence 5'-CACC-3' immediately 5' to the initiator ATG was generated by polymerase chain reaction and inserted into the plasmid vector pcDNA3 containing an SV40 origin (Invitrogen) to give pcDNA3.POH1. COS-1 cells were transiently cotransfected by the calcium phosphate method (12) with either pcDNA3 (empty vector) or pcDNA3.POH1 in a ratio of 4:1 with the plasmid pOPRSV.CD2, which drives expression of a truncated version of rat CD2 (13). 24 h after transfection, cells were selected with anti-CD2 immunomagnetic beads (Dynal) according to manufacturer's guidelines. Cells transfected with pcDNA3.POH1 or pcDNA3 were used to prepare whole cell extracts for immunoblot analyses and to determine clonogenic survival curves. For clonogenic assays, 10^4 CD2 cells were seeded per plate; after 24 h, cells were exposed to the appropriate cytotoxic agent. Stock solutions of paclitaxel (Sigma), doxorubicin (Farmitalia), UCN-01 (kindly provided by E. Sausville, NCI, Bethesda), and hydrogen peroxide (Sigma) were made in complete medium and added to the cells at the final concentrations indicated. After drug exposure, the medium was removed and plates were washed with phosphate-buffered saline before the addition of fresh medium. For assessment of UV sensitivity, cells in phosphate-buffered saline were exposed to UVC (254 nm) by means of a Stratalinker™ 1800 (Stratagene) followed by the addition of fresh medium. After 10–14 days of growth, colonies were stained with crystal violet, and colonies with more than 30 cells were counted. For the assessment of cellular uptake of doxorubicin, 48 h after transfection, plates containing COS1/pcDNA3 or COS1/pcDNA3.POH1 cells were exposed to doxorubicin for 6 h, stained with fluorescein isothiocyanate-conjugated anti-CD2 antibodies and assessed by flow cytometry (FACScan, Becton Dickinson). The median fluorescence channel for the doxorubicin-derived signal (red) was measured in CD2^+ cells (green) containing pcDNA3 or pcDNA3.POH1.

Proteasome Purification—Lysates of logarithmically growing H1299 cells were prepared exactly as described (14) and were incubated with CNBr-activated Sepharose beads (Sigma) coated with the monoclonal antibody MCP-21 (14). The same procedure was carried out using mock-coated beads as a negative control. After extensive washing, the beads were boiled in SDS-polyacrylamide gel electrophoresis sample buffer and subjected to immunoblotting analysis using the anti-Poh1 rabbit antiserum and ECL detection. Biochemical purification of 26 S proteasomes from human erythrocytes was performed as described (15). Separation of 26 S and 20 S proteasomes by nondenaturing gel electrophoresis was essentially as described by Hendil et al. (14). HeLa cells were lysed by sonication for 5 s at 0 °C in 5 volumes of 50 mM Tris pH 7.4, 17% glycerol, and the lysate was centrifuged for 5 min at 14,000 × g at 4 °C. 20-μl samples of the supernatant were resolved by electrophoresis in gels containing 4.5% acrylamide and Tris borate buffer at 4 °C before transfer to nitrocellulose membranes and immunoblotting as described above.

RESULTS

A Human Functional Homologue of Fission Yeast Pad1—The sequence similarity between fission yeast Pad1 and a \textit{C. elegans} cosmid-encoded sequence (5) suggested that this determinant of drug resistance might be conserved in human cells. We exploited this sequence similarity to generate a hybridization probe by amplification using degenerate primers and hence clone a pad1 homologue (termed POH1 for pad one homologue) from a human cDNA library (Fig. 1A). The amino
FIG. 2. POH1 is a fully functional homologue of pad1+. Wild type (pad1+) and six independent Δpad1 strains of S. pombe containing pREP-POH, in which POH1 expression is controlled by the thiamine-repressible nmt1 promoter (9), were streaked in the positions indicated (C) onto selective minimal agar plates either lacking thiamine (nmt1 promoter derepressed (A) or containing 10 μM thiamine (nmt1 promoter repressed, B). D, expression of Pad1 and Poh1 proteins in fission yeast. Cell extracts were prepared from wild type (lanes 1, 2 and 3), Δpad1 (lane 4) or Δpap1 (lanes 5 and 6) strains containing plasmids pST23 (C-terminally truncated version of pad1−, lane 1), pST23–8 (an intact pad1+, lanes 2 and 5) or pREP-POH (lanes 3, 4 and 6). The extracts were analyzed by immunoblotting using a polyclonal antiserum raised against Pad1 (5) (indicated by bracket on left), which cross-reacts with Pad1, which although clearly related to Pad1 is unable to complement the Δpad1 strain (16).

POH1 Confers Pleiotropic Drug Resistance—High copy-number plasmids bearing pad1− confer pleiotropic drug resistance in fission yeast, in a manner that depends upon the presence of an otherwise inessential AP-1 transcription factor encoded by pap1− (5, 19). As shown in Fig. 3, wild-type cells containing pREP-POH became resistant to staurosporine, a protein kinase inhibitor, in the absence of thiamine (Fig. 3A, promoter derepressed) but not in the presence of thiamine (Fig. 3B, promoter repressed). Furthermore, as in the case of pad1−, the drug resistance phenotype induced by pREP-POH was no longer observed in the pap1 deletion (Δpap1) background (Fig. 3, A and B, lower halves), though the Δpap1 strain expressed high levels of Poh1 (Fig. 2D, lane 6) and was fully viable in the absence of the drug (Fig. 3C, lower half).

As overexpression of POH1 in fission yeast induced a drug resistance phenotype, we examined the possibility that the same might be true in mammalian cells. Our attempts to generate stable mammalian transfectants constitutively overexpressing Poh1 were not successful, so we chose instead to adopt a transient transfection approach. COS-1 cells were cotransfected with a plasmid encoding C-terminally truncated rat CD2 as a marker of transfection and either pcDNA3-POH1, an SV40 origin-containing plasmid in which POH1 expression is driven by the CMV promoter or the empty vector pcDNA3. Cells transiently overexpressing Poh1 or the vector control cells were isolated by means of immunomagnetic beads coated with an anti-CD2 monoclonal antibody and tested in clonogenic assays for their sensitivity to a variety of cytotoxic treatments. Western blots probed with a rabbit polyclonal antiserum raised against Poh1 demonstrated that the transiently transfected cells expressed approximately two-fold more Poh1 than the control cells (Fig. 5A). Comparisons of the sensitivities of the two populations to 7-hydroxystaurosporine (Fig. 4A), taxol (Fig. 4B), and doxorubicin (not shown) indicated that the Poh1-overexpressing cells were significantly resistant to each of these drugs when compared with the vector transfectants. The greatest effect was seen with 7-hydroxystaurosporine, where the Poh1-induced increase in IC50 was approximately 4-fold. In contrast, no significant differences in sensitivity to hydrogen peroxide (Fig. 4D) or ionizing radiation (not shown) were de-
influenced by intracellular accumulation of doxorubicin was not significantly after 6 h exposure to the drug (Fig. 6). The results indicate that transiently overexpressing resistance the accumulation of doxorubicin in COS-1 cells transiently transfected with either pcDNA3.POH1 (solid lines) or the empty vector pcDNA3 (dashed lines) and exposed to 7-hydroxystaurosporine (UCN-01; A), taxol (paclitaxel; B), ultraviolet light (C), or hydrogen peroxide (D). Survival at each dose was assessed in triplicate and the mean ± S.D. is shown. Where no error bar is present, the standard deviation is smaller than the plot symbol. Each curve is representative of at least two independent determinations.

Acquisition of multidrug resistance in mammalian cell lines is frequently accompanied by overexpression of the P-gp product of the MDR1 gene (1, 2, 20). To investigate the possibility that POH1 overexpression might lead to elevated MDR1 expression, P-gp levels were examined by Western blotting but were found to be similar in extracts of both populations of transiently transfected cells used for clonogenic assays (Fig. 5B). Thus POH1 induces multidrug resistance by a mechanism that does not involve P-gp overexpression. Moreover, POH1 overexpression also resulted in moderate but significant resistance to ultraviolet light (Fig. 4C), indicating that POH1-induced resistance is likely to be mediated at least in part through a mechanism unrelated to P-gp function.

To investigate further the nature of POH1-induced drug resistance the accumulation of doxorubicin in COS-1 cells transiently overexpressing POH1 was measured by flow cytometry after 6 h exposure to the drug (Fig. 6). The results indicate that intracellular accumulation of doxorubicin was not significantly influenced by POH1 overexpression, adding further weight to the argument that P-gp is not likely to be involved in the Poh1-responsive drug resistance pathway.

Poh1 is a Novel 26 S Proteasome Component—Given the similarity between Poh1 and S12/p40, we investigated the possibility that Poh1 might also be associated with the proteasome. The monoclonal antibody MCP-21, which recognizes a subunit of the core (20 S) human proteasome (14), was used to immunopurify proteasomes from lysates of human H1299 lung cancer cells. Immunoblotting analysis of these proteasome preparations showed that Poh1 was substantially enriched in the immunopurified material (Fig. 7A). The significance of this result was underlined by the finding that a biochemically purified 26 S proteasome preparation from human erythrocytes also contained Poh1 (Fig. 7B, lane 3). Approximately equivalent amounts of Poh1 were present in 50 μg of a crude fraction and 1 μg of the purified 26 S preparation (Fig. 7B, compare lanes 2 and 3), suggesting copurification of Poh1 alongside other 26 S components. A Coomassie-stained gel lane of the purified 26 S fraction indicated only a relatively low intensity of staining in the Poh1-containing region. This could indicate that Poh1 is not a major stoichiometric component of the proteasome, but it is also possible that Poh1 is less efficiently stained than other proteasome components under these conditions. To determine what fraction of total cellular Poh1 is associated with the proteasome, crude cellular lysates were fractionated by nondenaturing gel electrophoresis under conditions previously shown to separate intact 26 S and 20 S proteasome particles (14), the positions of which were revealed by immunoblotting with MCP-21 (Fig. 7C, lane 1). Blotting with affinity-purified anti-Poh1 antibodies indicated that all the detectable Poh1 is associated with the 26 S particles (Fig. 7C, lane 2). Thus very little Poh1 is present as free monomers or other relatively low molecular weight forms; this is also the case for the majority of the previously-characterized components of the 20 S proteasome (14). Taking these results together, we conclude that Poh1 is a previously uncharacterized component of the 19 S regulatory cap of the 26 S proteasome.

DISCUSSION

The validity of fission yeast as a model organism with which to investigate drug resistance in mammals is confirmed by our
Furthermore, tumor necrosis factor-induced killing of human POH1 permissive temperature. Interestingly, overexpression of the spindle poison methyl benzimidazole-2-yl carbamate at the permissive temperature confers resistance to cytotoxic insults is modified by overexpression of human cells. In COS cells the response to a specific subset of cytotoxic insults is modified by overexpression of Pad1, a key determinant of pleiotropic responses to a wide variety of stimuli. The demonstration that Pad1, a key determinant of pleiotropic resistance in *S. pombe* is functionally conserved as Poh1 in human cells. In COS cells the response to a specific subset of cytotoxic insults is modified by overexpression of POH1 in such a way that cell survival is favored. Alterations in P-gp expression or intracellular drug accumulation do not appear to be involved, but it is not clear at this stage if the POH1-induced survival advantage reflects a decreased propensity for cell death or an alteration in the processing of potentially lethal damage. POH1 can also induce drug resistance in fission yeast, which lacks the apoptotic cell death program, suggesting that modulation of apoptosis is unlikely to be solely responsible for POH1-induced resistance in mammalian cells.

Poh1 is a novel component of the 26 S proteasome, indicating that proteolysis is involved in determining the sensitivity of human cells to cytotoxic treatments. A number of recent reports lend weight to this idea. Mutations in the fission yeast proteasome components encoded by *mts2* and *mts3* (21, 22) are temperature-sensitive for growth and confer resistance to the spindle poison methyl benzimidazole-2-yl carbamate at the permissive temperature. Interestingly, overexpression of *POH1* in fission yeast also induces resistance to this drug. Furthermore, tumor necrosis factor-induced killing of human fibrosarcoma cells was modulated by proteasome inhibitors (23). We therefore consider it likely that Poh1 induces drug resistance by modulation of a mammalian proteasome activity.

**POH1 and AP-1 Transcription Factors**—The requirement for Pap1 in Pad1/Poh1-induced drug resistance in fission yeast and the similarity between Poh1 and Jab1 suggest that modulation of transcription could underlie the induction of drug resistance by the Poh1 proteasome component. Indeed, overexpression of Pap1 in fission yeast results in a pattern of drug resistance indistinguishable from that seen on overexpression of Pad1/Poh1 (5, 19). What then might be the significant targets of AP-1 that induce resistance to cytotoxic agents? Mammalian AP-1-responsive genes reported include those encoding glutathione S-transferase Yα and π, elevated levels of which could contribute to cellular resistance (24, 25). Similarly in budding yeast the promoter of the *GSH1* gene has been shown to be AP-1 responsive (26). This gene encodes γ-glutamylcysteine synthetase, which performs the rate-limiting step in glutathione biosynthesis and can influence cellular drug resistance. However, the observation in budding yeast of AP-1-induced resistance to nitrosoguanidine suggests that glutathione-independent mechanisms are also significant (27). In line with our observation of unaltered P-gp expression on *POH1* overexpression, AP-1-mediated drug resistance in budding and fission yeast was found to be independent of endogenous P-gp-related drug transporters (6, 28).

Both c-Jun and c-Fos are known to be degraded by the ubiquitin/proteasome pathway (29–32), and elevated expression of one or other of these AP-1 factors has been correlated with drug resistance in a number of instances (24, 33, 34). Ubiquitin-dependent degradation of c-Jun appears to be regulated in response to signals transduced by mitogen-activated protein kinase (35). In addition, murine cells lacking c-Fos were found to be sensitized to UV radiation (36), although DNA repair was not defective, suggesting a role for AP-1 factors in a pathway that modulates cell survival after exposure to a given level of cytotoxic insult. Our results suggest that Poh1, a component of the 26 S proteasome, also participates in such a pathway in mammalian cells. This pathway is potentially involved in clinical resistance to anticancer drugs, and our preliminary data indicate that Poh1 is widely but variably expressed in human cancer cell lines. The extent of the involvement of Poh1 in clinical drug resistance awaits further investigation.

A further direct connection between ubiquitin-dependent proteolysis and transcription factors arose with the recent description of budding yeast Sug1 and its mammalian counterpart (mSug1/Trip1/FZA-B) both as a transcriptional co-activator and as a component of the 26 S proteasome (37–39). Mammalian mSug1 associates with c-Fos via its leucine zipper (40) and also with a number of transcription factors of the nuclear receptor family (41). It is not clear at this stage whether Sug1 (and, by extension, Pad1/Poh1) acts as a proteasomal receptor for transcription factors that have been targeted for ubiquitin-dependent degradation, or if the 26 S proteasome component and transcription factors interact for other reasons. It is nonetheless intriguing that the c-Jun binding protein Jab1, which was identified as a transcriptional co-activator (16) is clearly a close relative of Pad1/Poh1, identified here as a component of the 26 S proteasome. It seems possible that coordination of proteolysis with transcriptional activation could be significant in the modulation of transcriptional responses to a wide variety of stimuli.

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REFERENCES
1. Riordan, J. R., Deuchars, K., Kartner, N., Alon, N., Trent, J., and Ling, V. (1985) Nature 316, 817–819
2. Kartner, N., Riordan, J. R., and Ling, V. (1983) Science 211, 1285–1288
3. Kaye, S. B. (1995) Am. J. Med. 99, 6A405–6A445
4. Nurse, P. (1990) Nature 344, 563–568
5. Shimamuki, M., Saka, Y., Yanagida, M., and Toda, T. (1995) J. Cell Biol. 131, 659–679
6. Usui, T., Yoshida, M., Honda, A., Beppu, T., and Horinouchi, S. (1995) Gene 153, 245–252
7. Kumada, K., Yanagida, M., and Toda, T. (1996) Mol. Gen. Genet. 250, 59–68
8. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York
9. Maundrell, K. (1993) Gene 123, 127–130
10. Harlow, E., and Lane, D. (1988) Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York
11. Moreno, S., Klar, A., and Nurse, P. (1991) Methods Enzymol.
12. Graham, F. L., and Van der Eb, A. J. (1973) Virology 52, 456–467
13. O’Connell, M. J., Norbury, C., and Nurse, P. (1994) EMBO J. 13, 4926–4937
14. Hendil, K. B., Kristensen, P., and Uerkvitz, W. (1995) Biochem. J. 305, 423–431
15. Claret, F. X., Hibi, M., Bhut, S., Toda, T., and Karin, M. (1996) Nature 383, 453–457
16. Dubiel, W., Ferrell, K., Dumdey, R., Standera, S., Prehn, S., and Rechsteiner, M. (1995) FEBS Lett. 363, 97–100
17. Tsurumi, C., DeMartino, G. N., Slaughter, C. A., Shimbara, N., and Tanaka, K. (1995) Biochem. Biophys. Res. Commun. 210, 600–606
18. Toda, T., Shimamuki, M., and Yanagida, M. (1991) Genes Dev. 5, 60–73
19. Juliano, R. L., and Ling, V. (1976) Biochem. Biophys. Acta 435, 152–162
20. Gordon, C., McGurk, G., Wallace, M., and Hastie, N. D. (1996) J. Biol. Chem. 271, 5704–5711
21. Gordon, C., McGurk, G., Dillon, P., Rosen, C., and Hastie, N. D. (1993) Nature 366, 355–357
22. Wang, C. Y., Mayo, M. W., and Baldwin, A. S. J. (1996) Science 274, 784–787
23. Moffat, G. J., McLaren, A. W., and Wolf, C. R. (1994) J. Biol. Chem. 269, 16397–16402
24. Pinkus, R., Bergelson, S., and Daniel, V. (1993) Biochem. J. 290, 637–640
25. Edeker, D., Moye Rowley, W. S., Wu, A. L., and Golin, J. (1994) Genetics 136, 505–515
26. Ciechanover, A., DiGiuseppe, J. A., Berovich, B., Orian, A., Richter, J. D., Schwartz, A. L., and Brodeur, G. M. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 139–143
27. Treier, M., Staszewski, L. M., and Bohmann, D. (1994) Cell 78, 787–798
28. Toda, T., Shimanuki, M., and Yanagida, M. (1991) Mol. Cell. Biol.
29. Guthrie, A., and Finley, D. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 8236–8240
30. Slaughter, C. A., Noda, C., and Tanaka, K. (1995) Mol. Cell. Biol. 15, 5682–5687
31. Tsurumi, C., Ishida, N., Tamura, T., Kakizuka, A., Nishida, E., Okumura, E., Kashimoto, T., Inagaki, M., Okazaki, K., Sagata, N., Ichihara, A., and Tanaka, K. (1995) Mol. Cell. Biol. 15, 5682–5687
32. Stancovski, I., Gonen, H., Orian, A., Schwartz, A. L., and Ciechanover, A. (1995) Mol. Cell. Biol. 15, 7106–7116
33. Tao, K. S., Godwin, A. K., Johnson, S. W., Oo, R. F., O’Dwyer, P. J., and Hamilton, T. C. (1995) Cancer Res. 55, 4367–4374
34. Rutke, M. K., Bergoltz, V. Z., Allan, W. P., and Yalowich, J. C. (1994) Biochem. Pharmacol. 48, 525–533
35. Schreiber, M., Baumann, B., Cotten, M., Ettinger, S. L., and Wagner, E. F. (1995) EMBO J. 14, 5338–5349
36. Swaffield, J. C., Bromberg, J. F., and Johnston, S. A. (1992) Nature 357, 596–597
37. Akiyama, K., Kagawa, S., Shimbara, N., DeMartino, G. N., Slaughter, C. A., Noda, C., and Tanaka, K. (1995) FEBS Lett. 363, 151–156
38. Rubin, D. M., Coux, O., Wefes, I., Hengartner, C., Young, R. A., Goldberg, A. L., and Finley, D. (1996) Nature 379, 655–657
39. Wang, W., Chevray, P. M., and Nathans, D. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 8236–8240
40. vom Baur, E., Zechel, C., Heine, M. J., Garnier, J. M., Vivat, V., Le Douarin, B., Gronemeyer, H., Champon, P., and Losson, R. (1996) FEBS J. 15, 110–112
41. Ciechanover, A., Hod, Y., and Herskho, A. (1978) Biochem. Biophys. Res. Commun. 81, 1100–1105