Conditional Knockdown of Proteasomes Results in Cell-cycle Arrest and Enhanced Expression of Molecular Chaperones Hsp70 and Hsp40 in Chicken DT40 Cells

Tomoko Tanahashi-Hori, Nobuyuki Tanahashi, Keiji Tanaka, and Tomoki Chiba

The 26 S proteasome is an evolutionarily conserved ATP-dependent protease complex that degrades poly-ubiquitinated proteins and plays essential roles in a critical part of cellular regulation. In vertebrates, the roles of the proteasome have been widely studied by use of specific inhibitors, but not genetically. Here, we generated a cell line Z<sup>-/-</sup>/Z-HA, in which the expression of the catalytic subunit of the proteasome, Z (p2) could be manipulated. This cell line expresses exogenous Z protein under the control of a tetracycline-repressible promoter in a Z-nullizygous genetic background. Treatment of these cells with doxycycline inhibited Z expression and, hence, the function of the proteasome. The latter resulted in accumulation of poly-ubiquitinated proteins and concomitant induction of molecular chaperones Hsp70 and Hsp40. These results suggest a synergistic role for the proteasome with these molecular chaperones to eliminate misfolded or damaged proteins in vivo. Furthermore, knockdown of the proteasome induced apoptotic cell death following cell-cycle arrest at G<sub>M</sub> phase. Our Z<sup>-/-</sup>/Z-HA cell line would be useful for evaluating proteolytic processes catalyzed by the proteasome in many biological events in vertebrate cells.

The 26 S proteasome with a molecular mass of ~2500 kDa consists of the central 20 S protease (catalytic core) and two outer 19 S regulatory particles (alias PA700), functioning as a protein-destroying machine responsible for energy-dependent proteolysis (1, 2). The 20 S proteasome is composed of two copies of 14 different subunits: 7 distinct <i>α</i> and 7 distinct <i>β</i> type subunits. It is a barrel-like particle formed by the axial stacking of four rings made up of two outer <i>α</i>-rings and inner <i>β</i>-rings, being associated in the order of <i>α</i><sub>3</sub><i>β</i><sub>7</sub>. Three out of seven <i>β</i>-type subunits of each inner ring have catalytically active threonine residues at their N termini, and these active sites reside in a chamber formed by the centers of the abutting <i>β</i>-rings. The eukaryotic 20 S proteasome has at least three different catalytic activities against synthetic peptide substrates; i.e. a trypsin-like, chymotrypsin-like, and caspase-like (or peptidylglutamyl-hydrolyzing) activities, that contribute to the hydrolysis of multiple peptide bonds in a single polypeptide by a coordinated mechanism (1, 3, 4).

To date, yeast proteasomal mutants and membrane-permeable inhibitors have been used to determine in vivo functions of proteasomes, which have created diverse arrays of evidence on the biological importance of proteasomes such as the cell cycle, immune response, signaling cascades, and protein quality control in various eukaryotes (5, 6). Indeed, budding yeast mutants that lack some peptidase activities have contributed greatly to our understanding of the involvement of proteasomes in the degradation of many unstable key proteins (7), but their application to higher organisms has not been tested. Various substrate-related pepidyl compounds such as MG-132 and Z-L<sub>V</sub>SV have been devised as potent inhibitors of proteasomes (8, 9), but caution must be exercised in their use for interpreting proteasome functions, because they inhibit not only proteasomes but also other proteases. In contrast to these compounds, new microbial metabolites, such as lactacyclin and eponemycin, were found to induce selective inhibition of proteasomes that do not affect other proteases examined so far (10, 11). However, although these metabolites bind to active threonine residues of proteasomes, the possibility that they inhibit other as-yet-unidentified threonine protease(s) cannot be ruled out completely. Therefore, genetic approaches capable of manipulating proteasomal activities are still required to determine the in vivo functions of proteasomes in higher organisms such as vertebrate cells.

For this purpose, we disrupted proteasome subunit Z (formally designated p2) gene (<i>cpsmb7</i>) in chicken B cell line DT40 then established Z<sup>-/-</sup>/Z-HA cells that express a tetracycline-repressible HA<sup>1</sup>-tagged Z protein (Z-HA). This construct could manipulate proteasome levels in vertebrate cells by repressing the Z-HA by doxycycline (Dox) treatment. Using these cells, we found that reduction of proteasomes caused not only G<sub>M</sub> arrest during cell-cycle progression but also induction of apoptosis. Moreover, our results surprisingly showed that reduced proteasomes functions induced the expression of major molecular chaperones Hsp70 and Hsp40, suggesting a potential link...
between proteasome-mediated proteolysis and stress response for protein homeostasis in the cell.

**EXPERIMENTAL PROCEDURES**

**Plasmid Constructs**—Partial chicken Z cDNA was obtained from DT40 cells-derived mRNAs by reverse transcription-PCR method. The primers (5’-GACACGAGGGCGACCGAAGGGATG-3’ and 5’-GGCGCTGCTCAAGGTAAGTCCAGT-3’) were synthesized based on expressed sequence tag sequence (AJ397675). The full-length cDNA and genomic DNA were obtained by screening chicken muscle cDNA and genomic DNA libraries (Stratagene). The Z targeting vectors were designed by replacing the DNA segment that encompasses exon 1 to exon 3, with drug-resistant cassettes for blasticidin (Bsd), puromycin (Puro), and phleomycin (Bleo). A hemagglutinin (HA) tag was fused to the 3’-end of chicken Z cDNA coding regions by PCR amplifications. HA-tagged chicken Z cDNA (Z-HA) was inserted into the pUHD10–3 vector at the EcoRI site and the 5’-end sequence was replaced with the HindIII site by a HindIII linker. To construct a tetracycline (tet)-regulatable Z expression vector, IIA-dependent promoter flanked with HA-tagged chicken Z cDNA were recovered from pUHD10–3-Z-HA by digestion with HindIII and inserted into the HindIII site of pTet2-Neo vector (Clontech) that encode tet-repressible tTA (tetR-VP16). The resulting plasmid (pTet2-Neo-tetZ-HA) expresses Z-HA protein under the control of the tetII-VPI (12).

**Cell Culture and Transfection**—DT40 cells were cultured in RPMI1640 medium containing 10% (v/v) fetal bovine serum, 5% (v/v) chicken serum, 10 μM 2-mercaptoethanol, and antibiotics (penicillin and streptomycin) at 39.5 °C under 5% CO2. Cells were electroporated at 25 microfarads and 550 V (Bio-Rad) as described previously (13). Stable transformants were selected with each drug at the following concentrations: 2.0 mg/ml Geneticin (Sigma), 0.5 mg/ml puromycin (Sigma), and 20 mg/ml phleomycin (Bleo). A hemagglutinin (HA) tag was fused to the 3’-end of chicken Z cDNA (Z-HA) was inserted into the pUHD10–3 vector at the EcoRI site and the 5’-end sequence was replaced with the HindIII site by a HindIII linker. To construct a tetracycline (tet)-regulatable Z expression vector, IIA-dependent promoter flanked with HA-tagged chicken Z cDNA were recovered from pUHD10–3-Z-HA by digestion with HindIII and inserted into the HindIII site of pTet2-Neo vector (Clontech) that encode tet-repressible tTA (tetR-VP16). The resulting plasmid (pTet2-Neo-tetZ-HA) expresses Z-HA protein under the control of the tetII-VPI (12).

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**Antibodies**—Anti-chicken Z polyclonal antibody was raised in rabbits using a purified recombinant His-tagged Z protein expressed in Escherichia coli BL21. Anti-Hsp70 (MBL), anti-Hsp40 (Stress Gen), anti-actin (Chemicon), anti-Weel 1 (Santa Cruz Biotechnology), anti-polypeptide (MBL), horseradish peroxidase-conjugated anti-rabbit and anti-mouse IgG antibodies (Amersham Biosciences) were purchased.

**Western Blot Analysis**—Cells were lysed in 50 mM Tris-HCl (pH 8.0) containing 0.1% Triton X-100 and protease inhibitor mixture (Roche Molecular Biochemicals). Following a brief sonication, the extracts were cleared by centrifugation and subjected to 10–20% SDS-PAGE (14). After transfer onto polyvinylidene difluoride membranes (Millipore), proteins were detected by specific antibodies with the ECL method (Amerham Biosciences). Protein concentration was measured by the method of Bradford with bovine serum albumin as a standard (15).

**Southern Blot Analysis**—Genomic DNAs were isolated by using a DNeasy tissue kit (Qiagen). Genomic DNA (15 μg) was digested with EcoRI, separated in a 0.7% (w/v) agarose gel, and transferred onto a Hybond N nylon membrane (Amersham Biosciences). The membrane was hybridized with 32P-labeled probe (Clal-EcoRI fragment indicated in Fig. 1A), washed at high stringency, and then autoradiographed.

**Northern Blot Analysis**—Total RNAs were isolated by using an RNeasy Mini kit (Qiagen). Approximately 15 μg of total RNAs was separated and transferred onto a Hybond N+ nylon membrane (Amersham Biosciences). The membrane was hybridized with 32P-labeled probe (ClaI-EcoRI fragment indicated in Fig. 1A), washed at high stringency, and then autoradiographed.

**Glycerol Gradient Fractionation**—Cells were lysed in 25 mM Tris-HCl (pH 7.5) containing 1 mM dithiothreitol with 2 mM ATP by sonication, and the lysates were centrifuged at 15,000 × g for 50 min. The supernatants were subjected to glycerol gradient centrifugation with 10–40% glycerol in the above buffer. After centrifugation at 83,000 × g for 2 h using a Beckman SW28 rotor, the gradient was separated into 30 fractions of 1 ml each (16).
**Assay of Peptidase Activity**—Hydrolysis of the synthetic peptides, succinyl-Leu-Leu-Val-Tyr-7-amino-4-methylcoumarine (Suc-LLVY-AMC), t-butyloxycarbonyl-Leu-Arg-Ang-AMC (Boc-LRR-AMC), and carboxbenzoxyl-Leu-Leu-Glu-AMC (Z-LLL-AMC) was measured under the presence or absence of 0.05% SDS as described previously (17). One unit of peptidase activity was defined as the amount that degraded 1 nmol of a given fluorogenic peptide per minute.

**Assay of \(^{35}\)S/ODC Degradation Activity**—\(^{35}\)S-Labeled ornithine decarboxylase (ODC) was produced in vitro by translating rat ODC mRNA in rabbit reticulocyte lysates with \(^{35}\)S-labeled Met and Cys (PerkinElmer Life Sciences) and then immunopurified. The degradation of ODC was assayed as described previously (17). In brief, \(^{35}\)S-labeled ODC (3000 cpm) was incubated with antizyme, ATP, and enzyme solution in buffer containing the ATP regeneration system at 37 °C for 1 h. The reaction mixtures were then precipitated with trichloroacetic acid, the radioactivity of the trichloroacetic acid-soluble fraction was measured, and the activity was expressed as a percentage of total ODC radioactivity added.

**Flow Cytometric Analysis**—Cells were fixed in 70% ethanol in phosphate-buffered saline at 4 °C. Fixed cells were washed in phosphate-buffered saline, incubated with 0.25 mg/ml RNase A at 37 °C, and stained with 10 μg/ml propidium iodide at 4 °C. DNA contents were measured by a flow cytometry and cell cycle profiles were analyzed by the Expo ADC analysis program (Beckman Coulter).

**Immunofluorescence and TUNEL Assay**—Cells were fixed in 1% paraformaldehyde. For immunofluorescence analysis, anti-HA monoclonal antibody (BAbCO) and Alexa Fluor 594 goat anti-mouse IgG antibody (Molecular Probes) were used. Nuclei were counterstained with TOTO3 (Molecular Probes). Apoptotic cells were detected by TdT-mediated dUTP-biotin nick end-labeling (TUNEL) assay using an Apoptag kit (Intergen). The assay was performed according to instructions provided by the manufacturer. Fluorescence images were obtained using a confocal laser microscope (Zeiss and Bio-Rad).

## RESULTS

### Genetic Manipulation of Proteasome Function
To examine the cellular roles of proteasomes in vertebrates, we generated a cell line that could genetically manipulate the level of the proteasome subunit that confers a peptidase activity. Chicken B cell line DT40 is advantageous for this purpose, because of its efficiency rate of homologous recombination (13). Full-length chicken Z cDNA was obtained by screening a chicken muscle cDNA library using the partial cDNA fragment obtained by reverse transcription-PCR. The full-length chicken Z cDNA deduced a protein of 277 amino acids (accession number AB098728), displaying 57.4% and 83.8% identities with S. cerevisiae and human, respectively, at the amino acid level. To disrupt the proteasome Z gene (cpsmb7), chicken Z genomic DNA was isolated from chicken genomic DNA library, and the targeting vectors were constructed as shown in Fig. 1A. The vectors were designed to create a null allele by replacing the DNA segment that encompasses exon 1 to exon 3, which encodes the first 85 amino acids, including the essential catalytic site (threonine 44 in the exon 2), with drug-resistant cassettes. DT40 cells that contain three functional Z genes (cpsmb7) were successively transfected with each targeting vector, and the homologously recombined clones were identified by genomic Southern blot (Fig. 1B). Because the null mutant was expected to be lethal, we transfected the tet-regulatable Z-HA expression vector (pTAT2-Neo-tetZ-HA vector), in which the expression of Z protein could be shut off by Dox treatment, and isolated their stable transformants after the first allele was disrupted by Bsd construct. The second and third loci were disrupted by Puro and Bleo constructs, respectively. Finally, we obtained the Z/−/−/Z-HA clone that expressed Z-HA in a Z-nullizygous genetic background. The genomic Southern blots of representative clones are shown in Fig. 4B. Homologous recombination was identified as appearance of new 5.2-, 12-, and 11.5-kb bands corresponding to the targeted alleles generated by the Bsd, Puro, and Bleo constructs, respectively.

We then tested the effect of Dox treatment on Z-HA expression in Z/−/−/Z-HA cells. Dox treatment of Z/−/−/Z-HA cells reduced the mRNA and protein expression of Z-HA to undetectable levels at 24 h after Dox treatment (Fig. 1, C and D). Western blot with anti-Z antibody detected both precursor and mature forms of Z-HA. The latter form migrated below the size of the former (Fig. 1D). It is known that the catalytic subunits are synthesized as precursor forms and processed into the mature form during the assembly of the 20 S proteasome complex (18). The mature form co-sedimented with the 20 S proteasome, whereas the precursor form was detected in the lighter fraction when the cell extracts were fractionated by glycerol density gradient centrifugation (see Fig. 3, lower panels), suggesting that Z-HA is processed and incorporated into the 20 S proteasome.

**Depletion of Z Subunit Resulted in Loss of Proteasome Activity**—Based on yeast studies, Pup1p, which corresponds to Zp2, is known to confer trypsin-like activity (19, 20). In the next step, we tested whether loss of Z results in specific loss of trypsin-like activity. After Dox treatment, cells were serially collected at the indicated times and cell fractions were prepared. The cell fractions were first tested for their ability to degrade ODC, a proteasome-specific substrate independent of ubiquitination. As shown in Fig. 2A, ODC-degrading activities gradually decreased upon Dox treatment. These effects were not seen in Dox-un-treated cells or Dox-treated wild-type DT40 cells. Testing for the specificity of peptidase activities showed that not only trypsin-like activity, but also other peptidase activities...
chymotrypsin-like and caspase-like activities were reduced (Fig. 2, B–D).

To further confirm that these peptidase activities represent proteasome-specific activities, Z1/1/Z-HA cells were treated with or without Dox for 30 h and the cell extracts were further fractionated by glycerol density gradient centrifugation (10–40% glycerol from fractions 1 to 30) as described under “Experimental Procedures.” After fractionation, aliquots (20 μl) of individual fractions were used for an assay of three peptide hydrolysis with (solid circles) or without (open circles) 0.05% SDS. The degradation of 35S-ODC (crosses) was also assayed. Elution positions of purified 20 and 26 S proteasomes are shown. Lower panel, Western blot analysis. Proteins in 200 μl of each fraction were precipitated with acetone, subjected to SDS-PAGE, and stained by Western blot analysis using an anti-chicken Z antibody. Numbers correspond to fraction numbers in the upper panels. The solid and open arrowheads point to the mature and precursor forms of Z, respectively, similar to Fig. 1D.

Western blot analysis revealed that the mature form of Z protein migrated in the fractions 11–19 with 20–26 S proteasomes (Fig. 3, lower panels). The precursor form of Z in wild-type cell extracts migrated at lighter fractions 7–11. These fractions corresponded to 16 S pre-proteasomal particles as reported previously (18). On the other hand, the precursor Z-HA in Dox-untreated Z1/1/Z-HA cell extracts was recovered in fractions 1–5. The precursor Z-HA observed in fractions 1–5 might be the free form based on its exogenous overexpression. Nonetheless, most mature Z-HA was recovered in the fractions containing sediments of 20–26 S proteasomes, indicating that Z-HA is assembled into these proteasomes. Of note, in the Z1/1/Z-HA cells with Dox, the mature Z-HA was reduced, and

![Fig. 3. Sedimentation velocity analysis of wild-type and Z1/1/Z-HA cell extracts.](http://www.jbc.org/Downloadedfrom)
revealed cell death at 30 h after Dox treatment (Fig. 5B). To examine whether this was due to apoptosis, we performed TUNEL analysis (Fig. 5D). The apoptotic cells had large nuclei and less Z-HA expression. Taken together, these results indicated that loss of Z-HA and proteasomal activities result in cell-cycle arrest at G2/M phase followed by cell death. It is worth noting that residual Z-HA was present mainly in the cytoplasm as a punctate-like structure and to a lesser extent in the nuclei of Dox-treated cells, whereas it was uniformly present in the cytoplasm and rather abundantly in the nuclei of untreated cells (Fig. 5D).

Enhanced Expression of Hsp70 and Hsp40 in Proteasome-defective Cells—It is known that damaged proteins and/or misfolded proteins are rapidly eliminated from the cells by the ubiquitin-proteasome system. Indeed, proteasome inhibitors induce accumulation of such abnormal proteins in the cells and trigger signals that up-regulate the expression of certain molecular chaperones in the cells (22). Given that the expression of proteasomes can be reduced in Z−/−/Z-HA cells, we next examined whether Z depletion could up-regulate the expressions of major molecular chaperones, Hsp40 and Hsp70 in Z−/−/Z-HA cells. As shown in Fig. 6 (lower panels), Hsp40 and Hsp70 were consistently up-regulated by Z depletion in Z−/−/Z-HA cells. In contrast, these effects were not observed in Dox-treated wild-type DT40 cells (Fig. 6, upper panels). These results suggested that these molecular chaperones are up-regulated upon loss of proteasome function.

**DISCUSSION**

The proteasome is a multifunctional protease complex and essential for cell viability. We generated Z−/−/Z-HA cell line in which the expression of proteasome subunit Z could be manipulated by Dox. This cell line expressed Dox-repressible Z protein, tagged with C-terminal HA peptide. The C-terminal HA-tag did not interfere with the wild-type function of Z, because (i) Z-HA complemented the lethality of Z null-phenotype (Fig. 5A) and (ii) Z-HA was processed in mature form and incorporated into the proteasome complex (Figs. 1D and 3D). Depletion of Z-HA resulted in inhibition of three types of proteasomal peptidase activities and ODC-degrading activity (Figs. 2 and 3). Furthermore, considerable accumulation of poly-ubiquitinated cellular proteins was observed in vivo (Fig. 4). This is consistent with the fact that cellular poly-ubiquitinated proteins accumulate in the yeast proteasomal temperature-sensitive mutants under restrictive temperature (23). Taken together, these results suggest that the Z subunit is essential for the integrity of proteasome.

The proteasome is the major protease for poly-ubiquitinated proteins and known to degrade many cell-cycle regulators during the cell cycle progression. Most of the yeast proteasome subunit mutants, although not all, exhibit cell-cycle arrest at the G2/M phase rather than the G1/S phase (24). Our data also indicate that the major function of the proteasome in cell-cycle regulation is required at the G2/M phase rather than the G1/S phase. The essential substrates to be degraded by the proteasome at G2/M phase were not characterized in this study; however, we observed accumulation of Wee1 kinase in Z-HA knockdown cells (Fig. 5C). The Wee1 kinase is known to phosphorylate cyclin-dependent kinase 1 and thus plays a critical role in the checkpoint mechanism by inhibiting cyclin-dependent kinase 1 activity (21). Whether Wee1 kinase is one of the essential substrates or simply accumulates due to cell-cycle arrest at G2/M phase remains to be elucidated.

The ubiquitin-proteasome system has been implicated in quality control of proteins in the cytosol and endoplasmic reticulum (ER). Accumulation of unfolded proteins in the ER induces the unfolded protein response (UPR), which (i) halts
the translation of newly synthesized proteins, (ii) enhances the expression of molecular chaperones, and (iii) back translocates unfolded protein in the ER to the cytosol for proteasome degradation (25). The last process is called ER-associated protein degradation among the UPR reactions (26). In the present study, our results showed that inhibition of the proteasome enhances the expression of Hsp70 and Hsp40. The latter is known to collaborate with the former for folding newly synthesized and damaged proteins (27). How the cells sense the level of the proteasome and induce these molecular chaperones is unknown at present. However, this process is most likely due to enhanced UPR, because failure of protein degradation results in accumulation of abnormal proteins. Furthermore, the expression of molecular chaperone might be further enhanced following failure of the ER-associated protein degradation pathway.

Ubiquitin and proteasome-dependent protein degradation play essential roles in various biological events as mentioned in the introduction. In this regard, recent studies in the ubiquitin field reveal novel functions for ubiquitin in various biological events such as endocytosis, DNA repair, transcriptional regulation, and kinase activation (28). Whether these events do or do not involve proteasome-dependent degradation remain to be elucidated by genetic means, because proteasome inhibitors are known to inhibit some of the above biological events, such as endocytosis (29). Furthermore, many de-ubiquitinating enzymes that counteract with ubiquitination are vital in the cells (28). Thus, Z-/-/Z-HA cells could be used as a tool for examining the function of proteasomes in various cellular events that, at least, involve ubiquitination.

Acknowledgments—We thank Y. Yamaguchi-Iwai for providing the parental DT40 cell line, the drug-resistance cassettes, and the pUHD10–3 vector and Y. Murakami for providing ODC and antizyme.
We also thank K. Iwatsuki, T. Yasuda, and all members of the Tanaka laboratory for the advice and technical assistance.

REFERENCES

1. Coux, O., Tanaka, K., and Goldberg, A. L. (1996) Annu. Rev. Biochem. 65, 801–847
2. Baumeister, W., Watz, J., Zuhl, F., and Seemuller, E. (1998) Cell 92, 367–380
3. Grill, M., Dittrich, L., Lowe, J., Stock, D., Bochtler, M., Bartunik, H. D., and Huber, R. (1997) Nature 386, 463–471
4. Unno, M., Mizushima, T., Morimoto, Y., Tomisugi, Y., Tanaka, K., Yasuoka, N., and Tsukihara, T. (2002) Structure 10, 609–618
5. Hershko, A., and Ciechanover, A. (1998) Annu. Rev. Biochem. 67, 425–479
6. Pickart, C. M. (2001) Annu. Rev. Biochem. 70, 503–533
7. Hochstrasser, M. (1997) Annu. Rev. Genet. 30, 405–439
8. Rock, K. L., Gramm, C., Rothstein, L., Dick, L., Hwang, D., and Goldberg, A. L. (1994) Cell 78, 761–771
9. Bogyo, M., McMaster, J. S., Gaczynska, M., Tortorella, D., Goldberg, A. L., and Ploegh, H. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 6629–6634
10. Fenteany, G., Standaert, R. F., Lane, W. S., Choi, S., Corey, E. J., and Schreiber, S. L. (1995) Science 268, 729–731
11. Arendt, C. S., and Hochstrasser, M. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 7156–7161
12. Sherman, M. Y., and Goldberg, A. L. (2001) Neuron 29, 15–32
13. Russell, P., and Nurse, P. (1994) Cell 79, 559–567
14. Kominami, K., DeMartino, G. N., Moonaw, C. R., Slaughter, C. A., Shimbara, N., Fujimura, M., Hisamatsu, H., Tanahashi, N., Shimizu, Y., Tanaka, K., and Toh-e, A. (1995) EMBO J. 14, 3105–3115
15. Ghislain, M., Udvardy, A., and Mann, C. (1993) Nature 366, 358–362
16. Ma, Y., and Hendershot, L. M. (2001) Cell 107, 827–830
17. Ploegh, H. (1997) Annu. Rev. Biochem. 66, 358–362
18. Weissman, A. M. (2001) Nat. Rev. Mol. Cell Biol. 2, 169–178
19. Madshus, I. H. (2002) J. Cell Biol. 5, 843–854
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J. Biol. Chem. 2003, 278:16237-16243.
doi: 10.1074/jbc.M301331200 originally published online February 19, 2003

Access the most updated version of this article at doi: 10.1074/jbc.M301331200

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