Herc5, an Interferon-induced HECT E3 Enzyme, Is Required for Conjugation of ISG15 in Human Cells*

Anahita Dastur, Sylvie Beaudenon1, Melissa Kelley2, Robert M. Krug, and Jon M. Huibregtse3

From the Institute for Cellular and Molecular Biology, Section of Molecular Genetics and Microbiology, The University of Texas at Austin, Austin, Texas 78712

Type 1 interferons play an essential role in innate immunity (1). One of the many genes strongly activated by IFN-α/β codes ISG15, a 15-kDa ubiquitin-like protein (Ubi) (2, 3). Like ubiquitin (Ub), Ubs are linked to target proteins via isopeptide bonds between their terminal carboxyl group and lysine side chains of target proteins (4). The fact that ISG15 is expressed and conjugated in IFN-α/β-stimulated cells and lipopolysaccharide-stimulated cells implies that ISG15 conjugation is likely to mediate an important component of the innate immune response. This is supported by the finding that the influenza B virus NS1B protein specifically blocks ISG15 conjugation (5).

The biochemical effect of ISG15 on target proteins is unknown; however, the recent identification of a large number of target proteins (6) provides opportunities for determining both the function of ISG15 and its role in the innate immune response. Also essential for functional studies is the identification of the complete set of enzymes required for ISG15 conjugation. As with Ub conjugation, it is presumed that a cooperating set of E1, E2, and E3 enzymes, in addition to possible accessory factors, will be required for ISG5 conjugation. The ISG15 E1 and E2 enzymes have been identified. Ube1L is a single-subunit enzyme 62% similar to the Ub E1 enzyme (5), and UbcH8 is the major, if not exclusive, E2 enzyme for ISG15 (7, 8). The genes encoding both Ube1L and UbcH8 are, like ISG15, transcriptionally activated by IFN-α/β (5, 7–9), suggesting that the entire conjugation system might be coordinately regulated. A candidate E3 enzyme for ISG15 conjugation emerged from mass spectrometry-based identification of ISG15 target proteins (6).

Proteomics analyses of SUMO- and Ub-conjugated proteins have shown that enzymatic components of Ub/Ubl conjugation pathways are often auto-conjugated (10, 11), and consistent with this, Ube1L and UbcH8 were identified as ISG15-modified proteins. In addition, a single HECT E3, Herc5, was identified as an ISG15-modified protein, suggesting that this enzyme might also be a component of the ISG15 conjugation pathway. We show here that Herc5 is required for the conjugation of ISG15 to a broad spectrum of target proteins in human cells.

EXPERIMENTAL PROCEDURES

Cell Culture, Plasmids, Antibodies, and siRNAs—HeLa and 293 cells were grown in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum. Constructs for expression of Ube1L, UbcH8, and ISG15 were described previously (6). A plasmid containing the complete Herc5/CER1 open reading frame (12) was provided by M. Ohtsubo (Hiroshima University). The Herc5 open reading frame and mutants were subcloned into a pcDNA3 vector containing an amino-terminal HA tag. Anti-p56 antibody was provided by Ganes Sen (Cleveland Clinic), anti-MxA antibody by Otto Haller (University of Freiburg), and ISG15 antibody by Ernest Borden (Cleveland Clinic). siRNAs were supplied by Dharmacon, Inc., and sequences are shown for those targeting human Herc5 (5A, 5B, 5C), Herc6 (6A, 6B), and both Herc5 and Herc6 (5/6): 5A, GAAUGAAGCAUAAGCUAAU; 5B, GACACAACUAUAAUUCUAUU; 5C, UAAGGACACUGACAGUUUUU; 6A, UACCCCAAGAAUUAUAAUU; 6B, GAAGUGCCCGGUAUAAAGA; 5/6, GAGGCUAAUGUUCUAAGUUAU. E6AP (14) and UbcH8 (7, 8) siRNAs were validated previously.

Reverse Transcriptase (RT)-PCR and Microarray Analyses—Total RNA for RT-PCR was isolated using the PARIS kit (Ambion, Inc.). SuperscriptII reverse transcriptase (Invitrogen) was used for cDNA synthesis, which was used as a template in subsequent PCR reactions. Initially, a range of PCR cycles was performed using cDNA from IFN-β-treated or untreated cells to determine the linear range of amplification for each gene, and these parameters were used for PCR shown in Figs. 1A and 2A. Microarray analyses were performed as described previously (14). Elements chosen for analysis were screened for several data quality standards, including minimum intensity and pixel consistency.

Transfection Experiments—siRNAs transfections were carried out using Oligofectamine (Invitrogen), at a final concentration of 100 nM siRNA. Cells were treated with IFN-β (1,000 units/ml; Berlex) for the indicated times. Protein extracts were made in buffer containing 0.1 M Tris (pH 7.0), 0.1 M NaCl, 1% Nonidet P-40, and 1 mM dithiothreitol. To
detect ISG15 conjugates, 30 μg of total cell proteins were separated by SDS-PAGE, transferred to nitrocellulose membranes, and probed with anti-ISG15 antibody. Plasmid DNA transfections were performed with HeLa cells at 80% confluence using Genejuice transfection reagent (Novagen). Plasmids expressing Ube1L (0.25 μg), UbcH8 (0.25 μg), and ISG15 (0.5 μg) were transfected with or without Herc5-expressing plasmids (0.5 μg). Cells were harvested and lysed 48 h post-transfection and ISG15 conjugates were analyzed as described above.

RESULTS

Herc5 is one of six human Herc proteins (HECT and RCC1), defined by a carboxyl-terminal HECT domain and by one or more RLDs (RCC1-like domains) (15). Herc5 was a candidate for being a component of the ISG15 conjugation pathway based on the fact that it was identified as an ISG15-modified protein (6), and it belongs to the HECT family of E3 enzymes (16), some of which can interact with UbcH8 (8, 17). To determine whether Herc5 plays a significant role in overall ISG15 conjugation, two synthetic double-stranded siRNAs (designated 5A and 5B) were designed to target Herc5 mRNA. The Herc6 protein is 49% identical to Herc5, and two siRNAs (6A and 6B) were therefore designed to target Herc6, as well as one siRNA that would simultaneously target both Herc5 and Herc6 (5/6). RT-PCR confirmed that transfection of the Herc5- and Herc6-specific siRNAs reduced Herc5 and Herc6 mRNAs levels, respectively, in IFN-β-treated HeLa cells (Figs. 1A). siRNA 5/6 reduced both Herc5 and Herc6 mRNAs, and as shown below (Fig. 3C), all three siRNAs against Herc5 efficiently knocked down expression of Herc5 at the protein level. HeLa cells were transfected with the Herc5 or Herc6 siRNAs or, as a negative control, an siRNA against the E6AP HECT E3 (14), and 6 h after transfection, IFN-β was added for an additional 48 h. Total cell extracts were prepared and ISG15 conjugates were analyzed by immunoblotting with an antibody against ISG15. Induction of ISG15 and high molecular weight ISG15 conjugates was observed in IFN-β-treated cells (Fig. 1B, compare lanes 1 and 2). Both of the Herc5-specific siRNAs and the siRNA that targeted Herc5 and Herc6 (5/6) resulted in a dramatic decrease in overall ISG15 conjugates (lanes 3–5), while neither of the Herc6-specific siRNAs or the E6AP siRNA led to a discernible decrease in ISG15 conjugates (lanes 6–8). Similar results were observed in 293 cells (data not shown).

Combinations of Herc5 and Herc6 siRNAs were also transfected together, on the premise that potential Herc6-dependent effects might be more evident following reduction of Herc5 activity (Fig. 1C). For these experiments, a third Herc5 siRNA (5C) was used that elicited only a partial reduction in Herc5 activity (compare lanes 1–3). Co-transfection of Herc6 siRNA 6A with either Herc5 siRNA 5A or 5C did not elicit any further decrease in ISG5 conjugates relative to the Herc5 siRNAs alone. The effect of Herc5 and Herc6 siRNAs on conjugation of ISG15 to two specific target proteins (6), p56 and MxA, was also examined (Fig. 1D). The identification and validation of the IFN-β-induced ISG15-conjugated forms of both of these proteins was described previously (6). Consistent with the effect of Herc5 siRNAs on total ISG15 conjugates, siRNAs that targeted Herc5 blocked conjugation to both p56 and MxA, while Herc6-specific siRNAs did not. We conclude that Herc5 plays a major role in mediating overall ISG15 conjugation to a broad spectrum of target proteins. We cannot rule out that Herc6 might also function in the ISG15 conjugation pathway, but if so, it clearly plays a minor role relative to Herc5.

We determined whether expression of Herc5 and/or Herc6 was regulated by IFN-β by microarray gene expression analyses, comparing IFN-β-treated cells to untreated cells at various time points following addition of IFN-β (3, 6, and 21 h). Fig. 2A shows representations of the microarray elements corresponding to ISG15, Ube1L, UbcH8, Herc5, and Herc6 cDNAs, along with elements corresponding to three genes not expected to be affected by IFN-β treatment (E115, UbcH7, and E6AP). The induction of ISG15, Ube1L, and UbcH8 was evident, along
Herc5 Is Required for ISG15 Conjugation

Figure 2. Herc5 and Herc6 expression is induced by IFN-β. A, microarray gene expression analysis was performed, comparing HeLa cells treated with IFN-β for the indicated time periods (0, 3, 6, and 21 h) to untreated HeLa cells. cDNA from IFN-β-treated and untreated cells were labeled with Cy5 (red) and Cy3 (green), respectively. Microarray elements corresponding to the indicated genes are shown. B, bar graph representation of the microarray data shown in A, illustrating the time course and magnitude of induction for each of the indicated genes.

with both Herc5 and Herc6, with maximal induction over this time course at 21 h post-IFN-β treatment. This corresponds to the beginning of maximal accumulation of ISG15 conjugates (6, 18). Expression of E1Ub, UbcH7, and E6AP was not affected by IFN-β treatment. Fig. 2B shows that the time course and magnitude of Herc5 and Herc6 induction was similar to that of Ube1L and UbcH8. The regulation of Herc5 and Herc6 expression by IFN-β is consistent with the demonstrated importance of Herc5 in ISG15 conjugation, as well as a potential minor role for Herc6.

Co-transfection of plasmids expressing ISG15, Ube1L, and UbcH8 leads to ISG15 conjugation in non-IFN-treated HeLa cells, although at a lower level than observed in IFN-β-treated cells (6). To determine whether this level of conjugation was due to a significant basal level of Herc5 expression, we transfected Herc5 siRNAs prior to co-transfection of plasmids expressing ISG15, Ube1L, and UbcH8. As shown in Fig. 3A, transfection of Herc5 siRNAs (5A, 5B, or 5/6) did indeed block ISG15 conjugation, while Herc6 siRNAs did not, indicating that a basal level of Herc5 expression is responsible for ISG15 conjugation in this context.

We also determined whether co-transfection of a Herc5-expressing plasmid would further boost the level of ISG15 conjugates seen in non-IFN cells. In HeLa cells, transfection of a Herc5-expressing plasmid boosted ISG15 conjugation ~3-fold over that seen with transfection of ISG15, Ube1L, and UbcH8 plasmids (Fig. 3B). Importantly, the Herc5 active-site mutant (C994A) did not stimulate ISG15 conjugation, indi-
Herc5 Is Required for ISG15 Conjugation

Herc5 is a HECT E3 ligase that is required for ISG15 conjugation. It was previously shown to play a role in promoting ubiquitin conjugation by IFN-α/β. Herc5 is required for conjugation of ISG15 to a broad range, and potentially the dominant function in the ISG15 system. While several mammalian species appear to have direct Herc5 homologs (primates, cow, dog), rodents (mice, rats) do not. It was speculated that Herc5 is evolutionarily the most recent Herc family member and resulted from duplication of the physically adjacent Herc6 gene (15). It is conceivable that Herc6 plays a major role in ISG15 conjugation in rodents. In addition to Herc5 and Herc6, our microarray analysis identified several TRIM (tripartite motif) proteins that were induced by IFN-β. While we did not detect an effect of Herc6 siRNAs on overall ISG15 conjugation, it is possible that Herc6 plays a minor role in ISG15 conjugation, perhaps targeting a limited set of proteins compared with Herc5. Further biochemical comparisons of Herc5 and Herc6 will be important for identifying the determinants of Herc5 that confer its dominant function in the ISG15 system.

While several mammalian species appear to have direct Herc5 homologs (primates, cow, dog), rodents (mice, rats) do not. It was speculated that Herc5 is evolutionarily the most recent Herc family member and resulted from duplication of the physically adjacent Herc6 gene (15). It is conceivable that Herc6 plays a major role in ISG15 conjugation in rodents. In addition to Herc5 and Herc6, our microarray analysis identified several TRIM (tripartite motif) proteins that were induced by IFN-β. While we did not detect an effect of Herc6 siRNAs on overall ISG15 conjugation, it is possible that Herc6 plays a minor role in ISG15 conjugation, perhaps targeting a limited set of proteins compared with Herc5. Further biochemical comparisons of Herc5 and Herc6 will be important for identifying the determinants of Herc5 that confer its dominant function in the ISG15 system.

While several mammalian species appear to have direct Herc5 homologs (primates, cow, dog), rodents (mice, rats) do not. It was speculated that Herc5 is evolutionarily the most recent Herc family member and resulted from duplication of the physically adjacent Herc6 gene (15). It is conceivable that Herc6 plays a major role in ISG15 conjugation in rodents. In addition to Herc5 and Herc6, our microarray analysis identified several TRIM (tripartite motif) proteins that were induced by IFN-β. While we did not detect an effect of Herc6 siRNAs on overall ISG15 conjugation, it is possible that Herc6 plays a minor role in ISG15 conjugation, perhaps targeting a limited set of proteins compared with Herc5. Further biochemical comparisons of Herc5 and Herc6 will be important for identifying the determinants of Herc5 that confer its dominant function in the ISG15 system.

While several mammalian species appear to have direct Herc5 homologs (primates, cow, dog), rodents (mice, rats) do not. It was speculated that Herc5 is evolutionarily the most recent Herc family member and resulted from duplication of the physically adjacent Herc6 gene (15). It is conceivable that Herc6 plays a major role in ISG15 conjugation in rodents. In addition to Herc5 and Herc6, our microarray analysis identified several TRIM (tripartite motif) proteins that were induced by IFN-β. While we did not detect an effect of Herc6 siRNAs on overall ISG15 conjugation, it is possible that Herc6 plays a minor role in ISG15 conjugation, perhaps targeting a limited set of proteins compared with Herc5. Further biochemical comparisons of Herc5 and Herc6 will be important for identifying the determinants of Herc5 that confer its dominant function in the ISG15 system.

While several mammalian species appear to have direct Herc5 homologs (primates, cow, dog), rodents (mice, rats) do not. It was speculated that Herc5 is evolutionarily the most recent Herc family member and resulted from duplication of the physically adjacent Herc6 gene (15). It is conceivable that Herc6 plays a major role in ISG15 conjugation in rodents. In addition to Herc5 and Herc6, our microarray analysis identified several TRIM (tripartite motif) proteins that were induced by IFN-β. While we did not detect an effect of Herc6 siRNAs on overall ISG15 conjugation, it is possible that Herc6 plays a minor role in ISG15 conjugation, perhaps targeting a limited set of proteins compared with Herc5. Further biochemical comparisons of Herc5 and Herc6 will be important for identifying the determinants of Herc5 that confer its dominant function in the ISG15 system.

While several mammalian species appear to have direct Herc5 homologs (primates, cow, dog), rodents (mice, rats) do not. It was speculated that Herc5 is evolutionarily the most recent Herc family member and resulted from duplication of the physically adjacent Herc6 gene (15). It is conceivable that Herc6 plays a major role in ISG15 conjugation in rodents. In addition to Herc5 and Herc6, our microarray analysis identified several TRIM (tripartite motif) proteins that were induced by IFN-β. While we did not detect an effect of Herc6 siRNAs on overall ISG15 conjugation, it is possible that Herc6 plays a minor role in ISG15 conjugation, perhaps targeting a limited set of proteins compared with Herc5. Further biochemical comparisons of Herc5 and Herc6 will be important for identifying the determinants of Herc5 that confer its dominant function in the ISG15 system.

While several mammalian species appear to have direct Herc5 homologs (primates, cow, dog), rodents (mice, rats) do not. It was speculated that Herc5 is evolutionarily the most recent Herc family member and resulted from duplication of the physically adjacent Herc6 gene (15). It is conceivable that Herc6 plays a major role in ISG15 conjugation in rodents. In addition to Herc5 and Herc6, our microarray analysis identified several TRIM (tripartite motif) proteins that were induced by IFN-β. While we did not detect an effect of Herc6 siRNAs on overall ISG15 conjugation, it is possible that Herc6 plays a minor role in ISG15 conjugation, perhaps targeting a limited set of proteins compared with Herc5. Further biochemical comparisons of Herc5 and Herc6 will be important for identifying the determinants of Herc5 that confer its dominant function in the ISG15 system.

While several mammalian species appear to have direct Herc5 homologs (primates, cow, dog), rodents (mice, rats) do not. It was speculated that Herc5 is evolutionarily the most recent Herc family member and resulted from duplication of the physically adjacent Herc6 gene (15). It is conceivable that Herc6 plays a major role in ISG15 conjugation in rodents. In addition to Herc5 and Herc6, our microarray analysis identified several TRIM (tripartite motif) proteins that were induced by IFN-β. While we did not detect an effect of Herc6 siRNAs on overall ISG15 conjugation, it is possible that Herc6 plays a minor role in ISG15 conjugation, perhaps targeting a limited set of proteins compared with Herc5. Further biochemical comparisons of Herc5 and Herc6 will be important for identifying the determinants of Herc5 that confer its dominant function in the ISG15 system.

While several mammalian species appear to have direct Herc5 homologs (primates, cow, dog), rodents (mice, rats) do not. It was speculated that Herc5 is evolutionarily the most recent Herc family member and resulted from duplication of the physically adjacent Herc6 gene (15). It is conceivable that Herc6 plays a major role in ISG15 conjugation in rodents. In addition to Herc5 and Herc6, our microarray analysis identified several TRIM (tripartite motif) proteins that were induced by IFN-β. While we did not detect an effect of Herc6 siRNAs on overall ISG15 conjugation, it is possible that Herc6 plays a minor role in ISG15 conjugation, perhaps targeting a limited set of proteins compared with Herc5. Further biochemical comparisons of Herc5 and Herc6 will be important for identifying the determinants of Herc5 that confer its dominant function in the ISG15 system.

While several mammalian species appear to have direct Herc5 homologs (primates, cow, dog), rodents (mice, rats) do not. It was speculated that Herc5 is evolutionarily the most recent Herc family member and resulted from duplication of the physically adjacent Herc6 gene (15). It is conceivable that Herc6 plays a major role in ISG15 conjugation in rodents. In addition to Herc5 and Herc6, our microarray analysis identified several TRIM (tripartite motif) proteins that were induced by IFN-β. While we did not detect an effect of Herc6 siRNAs on overall ISG15 conjugation, it is possible that Herc6 plays a minor role in ISG15 conjugation, perhaps targeting a limited set of proteins compared with Herc5. Further biochemical comparisons of Herc5 and Herc6 will be important for identifying the determinants of Herc5 that confer its dominant function in the ISG15 system.

While several mammalian species appear to have direct Herc5 homologs (primates, cow, dog), rodents (mice, rats) do not. It was speculated that Herc5 is evolutionarily the most recent Herc family member and resulted from duplication of the physically adjacent Herc6 gene (15). It is conceivable that Herc6 plays a major role in ISG15 conjugation in rodents. In addition to Herc5 and Herc6, our microarray analysis identified several TRIM (tripartite motif) proteins that were induced by IFN-β. While we did not detect an effect of Herc6 siRNAs on overall ISG15 conjugation, it is possible that Herc6 plays a minor role in ISG15 conjugation, perhaps targeting a limited set of proteins compared with Herc5. Further biochemical comparisons of Herc5 and Herc6 will be important for identifying the determinants of Herc5 that confer its dominant function in the ISG15 system.

While several mammalian species appear to have direct Herc5 homologs (primates, cow, dog), rodents (mice, rats) do not. It was speculated that Herc5 is evolutionarily the most recent Herc family member and resulted from duplication of the physically adjacent Herc6 gene (15). It is conceivable that Herc6 plays a major role in ISG15 conjugation in rodents. In addition to Herc5 and Herc6, our microarray analysis identified several TRIM (tripartite motif) proteins that were induced by IFN-β. While we did not detect an effect of Herc6 siRNAs on overall ISG15 conjugation, it is possible that Herc6 plays a minor role in ISG15 conjugation, perhaps targeting a limited set of proteins compared with Herc5. Further biochemical comparisons of Herc5 and Herc6 will be important for identifying the determinants of Herc5 that confer its dominant function in the ISG15 system.

While several mammalian species appear to have direct Herc5 homologs (primates, cow, dog), rodents (mice, rats) do not. It was speculated that Herc5 is evolutionarily the most recent Herc family member and resulted from duplication of the physically adjacent Herc6 gene (15). It is conceivable that Herc6 plays a major role in ISG15 conjugation in rodents. In addition to Herc5 and Herc6, our microarray analysis identified several TRIM (tripartite motif) proteins that were induced by IFN-β. While we did not detect an effect of Herc6 siRNAs on overall ISG15 conjugation, it is possible that Herc6 plays a minor role in ISG15 conjugation, perhaps targeting a limited set of proteins compared with Herc5. Further biochemical comparisons of Herc5 and Herc6 will be important for identifying the determinants of Herc5 that confer its dominant function in the ISG15 system.

While several mammalian species appear to have direct Herc5 homologs (primates, cow, dog), rodents (mice, rats) do not. It was speculated that Herc5 is evolutionarily the most recent Herc family member and resulted from duplication of the physically adjacent Herc6 gene (15). It is conceivable that Herc6 plays a major role in ISG15 conjugation in rodents. In addition to Herc5 and Herc6, our microarray analysis identified several TRIM (tripartite motif) proteins that were induced by IFN-β. While we did not detect an effect of Herc6 siRNAs on overall ISG15 conjugation, it is possible that Herc6 plays a minor role in ISG15 conjugation, perhaps targeting a limited set of proteins compared with Herc5. Further biochemical comparisons of Herc5 and Herc6 will be important for identifying the determinants of Herc5 that confer its dominant function in the ISG15 system.
broad spectrum of target proteins in human cells will facilitate analysis of effects of ISG15 conjugation on target proteins and elucidation of the role of ISG15 conjugation in anti-viral and anti-microbial responses.

Acknowledgment—We thank Chen Zhao for critical reading of the manuscript and helpful discussions.

REFERENCES
1. Biron, C. A., and Sen, G. C. (2001) in Field Virology (Knipe, D. M., and Howley, P. M., eds) pp. 321–351, Lippincott Williams & Wilkins, Philadelphia
2. Farrell, P. J., Broeze, R. J., and Lengyel, P. (1979) Nature 279, 523–525
3. Haas, A. L., Ahrens, P., Bright, P. M., and Ankel, H. (1987) J. Biol. Chem. 262, 11315–11323
4. Jentsch, S., and Pyrowolakis, G. (2000) Trends Cell Biol. 10, 335–342
5. Yuan, W., and Krug, R. M. (2001) EMBO J. 20, 362–371
6. Zhao, C., Denison, C., Huibregtse, J. M., Gygi, S., and Krug, R. M. (2005) Proc. Natl. Acad. Sci. U. S. A. 102, 10200–10205
7. Kim, K. I., Giannakopoulos, N. V., Virgin, H. W., and Zhang, D. E. (2004) Mol. Cell. Biol. 24, 9592–9600
8. Zhao, C., Beaudenon, S. L., Kelley, M. L., Waddell, M. B., Yuan, W., Schulman, B. A., Huibregtse, J. M., and Krug, R. M. (2004) Proc. Natl. Acad. Sci. U. S. A. 101, 7578–7582
9. Nyman, T. A., Matikainen, S., Sareneva, T., Julkunen, I., and Kakkinen, N. (2000) Eur. J. Biochem. 267, 4011–4019
10. Denison, C., Rudner, A. D., Gerber, S. A., Bakalarski, C. E., Moazed, D., and Gygi, S. P. (2005) Mol. Cell. Proteomics 4, 246–254
11. Peng, J., Schwartz, D., Elias, J. E., Thoreen, C. C., Cheng, D., Marisichly, G., Roelofs, J., Finley, D., and Gygi, S. P. (2003) Nat. Biotechnol. 21, 921–926
12. Mitsui, K., Nakashima, M., Okatsu, S., Norwood, T. H., Okabayashi, K., Miyamoto, C., Tanaka, K., Yoshimura, A., and Ohitsuho, M. (1999) Biochem. Biophys. Res. Commun. 266, 115–122
13. Puig, O., Caspary, F., Rigaut, G., Rutz, B., Bouveret, E., Bragado-Nilsson, E., Wilm, M., and Seraphin, B. (2001) Methods (Orlando) 24, 218–229
14. Kelley, M. L., Keiger, K. E., Lee, C. J., and Huibregtse, J. M. (2005) J. Virol. 79, 3737–3747
15. Hochrainer, K., Mayer, H., Baranyi, U., Binder, B., Lipp, J., and Kroismayr, R. (2005) Genomics 85, 153–164
16. Huibregtse, J. M., Scheffner, M., Beaudenon, S., and Howley, P. M. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 2563–2567
17. Kumar, S., Kao, W. H., and Howley, P. M. (1997) J. Biol. Chem. 272, 13548–13554
18. Liu, M., Li, X. L., and Hassel, B. A. (2003) J. Biol. Chem. 278, 1594–1602
19. Salvat, C., Wang, G., Dastur, A., Lyon, N., and Huibregtse, J. M. (2004) J. Biol. Chem. 279, 18935–18943
20. Huang, L., Kinnucan, E., Wang, G., Beaudenon, S., Huibregtse, J. M., and Pavletich, N. P. (1999) Science 286, 1321–1326
21. Nuber, U., and Scheffner, M. (1999) J. Biol. Chem. 274, 7576–7582
22. Johnson, E. S. (2004) Annu. Rev. Biochem. 73, 355–382
23. Urano, T., Saito, T., Tsukui, T., Fujita, M., Hosoi, T., Muramatsu, M., Ouchi, Y., and Inoue, S. (2002) Nature 417, 871–875
24. Xu, L., Yang, L., Moitra, P. K., Hashimoto, K., Rallabhandi, P., Kaul, S., Meroni, G., Jensen, J. P., Weissman, A. M., and D’Arpa, P. (2003) Exp. Cell Res. 288, 84–93