Regulation of P311 Expression by Met-Hepatocyte Growth Factor/Scatter Factor and the Ubiquitin/Proteasome System*

(Received for publication, August 3, 1999, and in revised form, November 11, 1999)

Gregory A. Taylor‡§, Eric Hudson‡§, James H. Resau‡§, and George F. Vande Woude**

From the §Advanced Bioscience Laboratories Basic Research Program and the **National Cancer Institute, NCI-Frederick Cancer Research and Development Center, Frederick, Maryland 21702

P311 is a mouse cDNA originally identified for its high expression in late-stage embryonic brain and adult cerebellum, hippocampus, and olfactory bulb. The protein product of P311, however, had not been identified previously, and its function remains unknown. We report here that P311 expression is regulated at multiple levels by pathways that control cellular transformation. P311 mRNA expression was decreased sharply in both neural and smooth muscle cells when the cells were transformed by coexpression of the oncogenic tyrosine kinase receptor Met and its ligand hepatocyte growth factor/scatter factor. The P311 mRNA was found to encode an 8-kDa polypeptide that was subject to rapid degradation by the lactacystin-sensitive ubiquitin/proteasome system and an unidentified metalloprotease, resulting in a protein half-life of about 5 min. These data suggest that P311 expression is dramatically decreased by several pathways that regulate cellular growth.

Met is a 190-kDa tyrosine kinase receptor that has been implicated in the etiology of a number of human cancers (for review see Ref. 1). Met initiates cancerous growth when it is co-expressed constitutively with its ligand, hepatocyte growth factor/scatter factor (HGF/SF)1 (2–5), or when it is activated through germ-line or somatic mutations in its tyrosine kinase domain (6). The former mechanism plays a role in the generation of many sarcomas (3), carcinomas (1), melanomas (7), and gliomas (8, 9), whereas the latter plays a role in the generation of familial and sporadic papillary renal cell carcinoma (6).

Our recent efforts have been aimed at understanding the ability of Met to initiate primary tumor growth and metastasis at the molecular level, and toward that end, we have identified several genes whose expression is regulated by Met action. Many of these genes encode extracellular matrix proteins or enzymes that degrade these proteins, and this underscores the profound effect that Met action has on the extracellular environment of the cell. For instance, Met not only decreases production of the extracellular matrix protein fibronectin, but it dramatically decreases cell surface integrin expression, thereby limiting interaction with matrix proteins2 (10). In addition, Met signaling increases production of proteases, such as urokinase, that degrade the extracellular matrix, (2) whereas Met signaling reduces the expression of tissue inhibitors of matrix metalloproteases, which are inhibitors of the matrix metalloproteases that degrade the extracellular matrix (11). These alterations are thought to increase invasive growth and deregulate cell cycle pathways.

In the studies described here we used differential display screening to identify other genes that may be important in Met-mediated tumorigenesis. We found a new Met-HGF/SF-regulated mRNA, P311 (12), that was down-regulated by Met signaling in several types of transformed cells. We found that this mRNA encoded a relatively small intracellular protein that was targeted for degradation by multiple proteolytic pathways including the ubiquitin/proteasome, resulting in an extremely short protein half-life. Therefore, expression of the P311 gene was under tight regulation by several mechanisms that regulate cellular growth.

EXPERIMENTAL PROCEDURES

Cells and Culture—SK-LMS, HCN-1A, HCN-2, U118, U373, DBTRG, SK-N-SH, SW-1783, and COS-7 cells were obtained from American Type Culture Collection (Manassas, VA) and maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum (v/v) (Life Technologies, Inc.). SK-LMS cells that stably express HGF/SF have been described previously (2). Normal human astrocytes (NHA) (Clonetics, San Diego, CA) were maintained in media provided by the manufacturer. HCN-1A and HCN-2 were induced to differentiate by exposure to 25 ng/ml neural growth factor (NGF) (Roche Molecular Biochemicals), 0.5 mM dibutyryl cAMP (Sigma), and 0.5 mM 3-isobutyl-1-methylxanthine (Sigma) for 3 days.

Northern Blotting—Total RNA was prepared from cultured cells using a standard acidic phenol extraction procedure (13). 15-µg RNA samples were separated on 1.2% agarose/formaldehyde gels and used for Northern blot analysis as described previously (14). The blots were probed with a 9 -untranslated region of the mouse P311 cDNA that was isolated by differential display screening (see below) or with a 1.2-kilobase pair PstI fragment of pHeGAP, a human glyceralddehyde-phosphate dehydrogenase plasmid (15). Probes were also generated from the human P311 cDNA protein coding region using polymerase chain reactions and appropriate primers, and their hybridization patterns were essentially identical to those of the differential display P311 fragment probe.

Differential Display Screening—Total RNA was isolated from SK-LMS or SK-LMS/HGF cells and used for differential display screening with the RNAimage kit 1 (GenHunter Corp., Nashville, TN) according to protocols supplied by the manufacturer. One cDNA fragment isolated using primers H-T11A and H-AP2 corresponded to a 280-nucleotide fragment of the 3’-end of the human P311 cDNA (GenBankTM U30521).

Expression Vectors—Using appropriate primers, SK-LMS cDNA, and polymerase chain reactions, the human P311 cDNA coding region was

* This research was sponsored in part by the NCI, Department of Health and Human Services under Advanced Bioscience Laboratories Contract NO1-CO-46000. 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.

¶ Current Address: Dept. of Medicine, Duke University, and Veterans Affairs Medical Center, Durham, NC 27705.

§ Current Address: Van Andel Research Inst., Grand Rapids, MI 49503.

** To whom correspondence should be addressed: Van Andel Research Inst., 201 Monroe Ave., N.W., Suite 400, Grand Rapids, MI 49503; Tel.: 616-235-8242; Fax: 616-235-8245; E-mail: george.vandewoude@vai.org.

1 The abbreviations used are: HGF, hepatocyte growth factor; SF, scatter factor; HCN, human cortical neuronal cells; NHA, normal human astrocytes; NGF, neural growth factor; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

2 G. A. Taylor and G. F. Vande Woude, unpublished data.
cloned into the pRC/CMV (Invitrogen, Carlsbad, CA) expression vector. The FLAG epitope tag, DYKDDDK, was cloned onto the 5′ or 3′ edge of this cDNA fragment by inserting sequences coding those amino acids into the polymerase chain reaction primers. The plasmids were transfected into cells using the SuperFect kit (Qiagen, Valencia, CA).

**Immunoprecipitation**—The immunoprecipitation protocol has previously been described in detail (16). Cells were plated onto 60-mm plates, grown to confluency, and labeled in Cys/Met-free Dulbecco’s modified Eagle’s medium (Life Technologies, Inc.) supplemented with 10% fetal bovine serum (v/v) and 0.5 μCi [35S]-EXPRE35S35S protein labeling mix (NEN Life Science Products). Cell lysates were prepared and used for immunoprecipitation with the monoclonal M2 anti-FLAG antibody (Eastman Kodak Co.), or rabbit polyclonal anti-P311 antisera raised against the peptide CGSSELRSPRISYLHFF, corresponding to the human P311 C-terminal sequence. Precipitated proteins were resolved by 10% tricine gel electrophoresis, and the resulting gels were used for autoradiography. In some experiments the cells were exposed to 10 μM lactacystin (Boston Biochem, Boston, MA), 2 μg/ml aprotinin, 0.68 μg/ml pepstatin A, 24 μg/ml leupeptin, 0.2 mg/ml (1:26 nm) o-phenanthroline, or 1.26 nm 4,7-phenanthroline prior to and during the labeling period.

**Immunofluorescence Assays**—NHA were grown on poly-l-lysine-coated, four-well glass chamber slides (12–50–51 SuperCell culture slides; Fisher) until they reached about 50% confluency. The cells were fixed in 1% paraformaldehyde (w/v) for 15 min, permeabilized with 0.2% Triton X-100 in phosphate-buffered saline for 10 min, and blocked with 10% goat serum (v/v), 0.1% Tween 20 (v/v) in phosphate-buffered saline for 1 h. The cells were then incubated for 60 min with 1:50 dilutions of a polyclonal anti-C-terminal P311 antibody (see “Immunoprecipitation”) or a polyclonal P311 antibody that recognized the internal sequence KRKLVPVPKEVRRK. In some experiments the cells were cotained with 1:400 dilutions of a mouse monoclonal anti-vinculin antibody (Sigma). Finally, the cells were incubated for 30 min with 1:100 dilutions of a fluorescein isothiocyanate-conjugated anti-rabbit antibody (Roche Molecular Biochemicals) and/or a rhodamine-conjugated anti-mouse antibody (Roche Molecular Biochemicals). The cells were observed and images were analyzed using a Zeiss LSM310 confocal laser-scanning microscope; it was configured with a 25-milliwatt argon internal HeNe laser. Nomarski images were made using the 543 laser and appropriate polarizer lenses.

**Nude Mouse Tumor Studies**—Nude mouse protocols have previously been described in detail (2). 10⁶ cells were injected subcutaneously into athymic nude mice that were monitored daily for tumor growth.

**Results**

**Identification of P311 as a Met-HGF/SF-regulated Gene**—To identify genes regulated by Met-HGF/SF signaling we used differential display screening to compare cDNAs from a cell line with low Met-HGF/SF signaling to the same cells engineered to have high signaling. The cells we chose were SK-LMS cells, which were originally derived from a human leiomyosarcoma; we have shown previously that they express high levels of Met but virtually no HGF/SF and in nude mouse tumor assays, that they are non-metastatic and only weakly tumorigenic, decreasing the tumor latency period from 10 to 5 weeks (2). In the present study we identified a differentially displayed cDNA fragment that was present at moderately high levels in parental SK-LMS cells and at substantially lower levels in a pool of SK-LMS/HGF cells (data not shown); we subsequently verified this result using the fragment as a probe for a Northern blot (Fig. 1). Cloning and sequencing revealed that the cDNA fragment corresponded to 280 nucleotides from the 3′-untranslated region of P311, a cDNA whose cloned homolog is highly expressed in late-stage embryonic brain and adult cerebellum, hippocampus, and olfactory bulb but for which no function has been described (12). The effect of HGF/SF in decreasing P311 expression in SK-LMS cells required constitutive exposure to the cytokine, in that exposure of parental SK-LMS cells to a single dose of HGF/SF did not appreciably lower P311 mRNA levels (data not shown). Because SK-LMS cells are transformed smooth muscle cells we also examined P311 expression in normal human intestinal smooth muscle cells, in which we found extremely high P311 mRNA levels (Fig. 1), suggesting a progressive decrease in P311 expression in increasingly more transformed smooth muscle cells.

We also measured P311 levels in tumors derived from SK-LMS and SK-LMS/HGF cells (Fig. 2). The moderately high P311 expression in the parental cells was decreased to very low levels in two tumor explants of these cells (Fig. 2), an effect that may be partially explained by increased Met-HGF/SF signaling in the tumor cells that we have demonstrated previously (2). In tumor explants from SK-LMS/HGF cells, P311 levels remained very low (Fig. 2). Therefore, low P311 expression correlated with increased Met-HGF/SF signaling and tumorigenic growth of SK-LMS cells.

**P311 Expression in Neural Cells**—Next we examined other cells for the effect of Met-HGF/SF on P311 expression. Because P311 is expressed at its highest levels in brain (12) we measured mRNA levels in NHA and in several glioma cell lines that express high levels of both Met and HGF/SF; autocrine Met-HGF/SF signaling in these gliomas is thought to contribute to their transformation (8). We found extremely high P311 expression in NHA cells (Fig. 3) at levels comparable with those in normal smooth muscle cells (Fig. 1 and data not shown), whereas each of the glioma cell lines had much lower expression (Fig. 3). In the case of U118 glioma cells the low P311 expression was due, at least in part, to Met-HGF/SF action, because addition of a neutralizing HGF/SF antibody to the cell’s culture medium increased P311 mRNA levels (data not shown).

We also examined whether terminal differentiation of normal neural cells resulted in loss of P311 expression just as transformation of neural cells did. For these experiments we used HCN-1A and HCN-2 cells; these are human cerebral cortical pre-neuronal cell lines that can be induced to differentiate into neuron-like cells by exposure to NGF (17). Both HCN-1A and HCN-2 cells expressed very high levels of P311 mRNA similar to those in NHA, but these levels were decreased when the cells were induced to differentiate by NGF treatment (Fig. 4). Therefore, differentiation, as well as transformation, of neural cells resulted in loss of P311 expression.

**Instability of the P311 Protein**—Prior to the studies described here the P311 protein product had not been identified, and in fact it was unclear whether the mRNA was translated in vivo (12). The human P311 cDNA contains three open reading frames, but only the first reading frame, which encodes a
68-amino acid polypeptide, is conserved among the human, mouse, and chicken P311 cDNAs (12). Therefore, to begin our studies of the protein we generated P311 expression vectors in which this putative protein coding sequence was linked to a FLAG epitope tag at either its N or C terminus. After transient expression of these vectors in COS-7 cells we were able to detect FLAG-P311 and P311-FLAG protein by immunoprecipitation with an anti-FLAG antibody (Fig. 5). However, detection of the protein was only possible if the cells were radiolabeled for very short periods of time (5–10 min); longer labeling times resulting in backgrounds too high to distinguish the 8-kDa band. This suggested that the protein was unstable, and we confirmed this in a pulse-chase experiment in which we found FLAG-P311 to decay with a half-life of about 5 min (Fig. 6).

Extremely rapid decay of P311 protein suggested that it may be targeted by a specific intracellular proteolysis system. One such system is the ubiquitin/proteasome system, which degrades proteins with PEST domains by covalently linking ubiquitin to lysine residues of the protein, thereby allowing recognition and degradation of the protein by the proteasome (for review see Ref. 18). Because P311 contains PEST domains as well as lysine residues we examined it for proteasome-mediated degradation by conducting a pulse-chase experiment in the presence of the proteasome inhibitor lactacystin (19) (Fig. 7). In the presence of this inhibitor essentially no degradation of FLAG-P311 took place during the 30-min period of the experiment (Fig. 7) demonstrating that FLAG-P311 was targeted by the proteasome system.

Next we examined expression of wild-type P311 that lacked a FLAG epitope tag. Two polyclonal antisera were raised against P311, one that recognized an internal sequence and another a C-terminal sequence. Both of them were able to...
immunoprecipitate the 8-kDa P311 band from NHA lysates, and this band was seen only in cell lysates and not in conditioned media (data not shown). We assessed the stability of wild-type P311 in a pulse-chase experiment following transient expression of wild-type P311 in COS-7 cells, and, as expected, it decayed very rapidly with a half-life of about 5 min (Fig. 8 and data not shown). Surprisingly, however, lactacystin did not block decay of the protein (Fig. 8), demonstrating that some other protease that had not been active against FLAG-P311 degraded wild-type P311. To determine the nature of this protease, we performed pulse-chase experiments in which cells were treated with lactacystin in combination with a series of protease inhibitors including o-phenanthroline, pepstatin, leupeptin, and aprotinin. Only the combination of lactacystin and the metalloprotease inhibitor, o-phenanthroline, was able to block degradation of P311 (Fig. 8 and data not shown). In contrast, a combination of lactacystin and 4,7-phenanthroline, a non-chelating analog of o-phenanthroline, did not block degradation, implying that the ability of o-phenanthroline to inhibit P311 degradation required its chelating capacity. Taken together, these data suggested that P311 was subject to rapid degradation by both the ubiquitin/proteasome system and an unknown metalloprotease. The reason that the addition of the FLAG epitope to P311 blocked degradation required its chelating capacity. Taken together, these data suggested that P311 was subject to rapid degradation by both the ubiquitin/proteasome system and an unknown metalloprotease. The reason that the addition of the FLAG epitope to P311 blocked degradation required its chelating capacity. Taken together, these data suggested that P311 was subject to rapid degradation by both the ubiquitin/proteasome system and an unknown metalloprotease. The reason that the addition of the FLAG epitope to P311 blocked degradation required its chelating capacity. Taken together, these data suggested that P311 was subject to rapid degradation by both the ubiquitin/proteasome system and an unknown metalloprotease. The reason that the addition of the FLAG epitope to P311 blocked degradation required its chelating capacity.

**P311 Detection in the Nucleus and Cytosol**—We examined the subcellular localization of endogenous P311 by immunocytochemistry of normal human astrocytes. Two different antisera that recognized distinct P311 regions showed similar patterns, with staining of nucleoli in all cells and staining of focal adhesions in some cells (Fig. 9C and data not shown). Focal adhesion staining occurred only along the leading edge of cells with an obvious motile morphology. Simultaneous staining with monoclonal antibodies recognizing the focal adhesion protein vinculin (20), showed colocalization of P311 and vinculin in the focal adhesions (Fig. 9C). In related biochemical experiments, we fractionated pulse-labeled cells into nuclear and cytosolic fractions and used each fraction for P311 immunoprecipitation. Again, P311 was present in both fractions with slightly higher levels in the cytosol (data not shown).

**The Effect of P311 Overexpression on Tumorigenicity**—To determine whether overexpression of P311 had a direct effect on the tumorigenicity of U118 cells we stably transfected them with the P311-FLAG expression vector. A pool of cells that expressed high levels of P311-FLAG primary antibodies (A) or both rabbit anti-P311 (C-terminal) and mouse anti-vinculin primary antibodies (B), followed by both fluorescein-conjugated anti-rabbit and rhodamine-conjugated anti-mouse secondary antibodies. The staining was analyzed and enhanced with a Zeiss LSM310 confocal laser scanning microscope in the confocal mode. P311 staining is shown in green, vinculin staining in red, and P311/vinculin co-staining in yellow or orange. Nomarski images of the cells (B and D) correspond to the antibody-stained cells (A and C).
Therefore, P311 overexpression did not interfere with the tumorigenic growth of U118 cells.

DISCUSSION

We have shown that P311 expression is tightly regulated at several levels by mechanisms that control cellular growth and transformation. At the mRNA level we found that P311 expression was reduced substantially in both smooth muscle and neural cells transformed by coexpression of Met and its ligand, HGF/SF. Signaling through Met initiates transformation of a wide variety of mesenchymal and epithelial cells (1), but the down-stream molecular pathways that are important in their transformation have not been completely delineated. Regulation of P311 expression is thus a novel pathway that could be of importance for Met-induced tumor growth. Overexpression of P311 in U118 glioma cells, in which Met-HGF/SF signaling is active, did not interfere with tumorigenic growth, implying that P311 does not have a dominant tumor suppressor function in that context. However, the strong correlation between loss of P311 expression and tumorigenesis suggests that low P311 could be a contributing factor to tumorigenesis. In addition, P311 may be a useful marker for distinguishing between normal and transformed smooth muscle or neural tissue.

We also found that P311 expression was controlled at the level of protein stability. In both smooth muscle and neural cells the protein had a half-life of 5 min or less, with the rapid degradation being directed by two pathways: the ubiquitin/proteasome system and a metalloprotease whose identity we have not determined. Ubiquitin is a 76-residue protein that is covalently linked to lysines of proteins with PEST sequences, thereby tagging the proteins for degradation by the proteasome (18). This pathway is thought to regulate major biological processes including the cell cycle, cellular differentiation, and neuronal function (18). For instance, the p53 tumor suppressor protein is subject to ubiquitin-proteasome degradation as a result of its association with mdm2, and this profoundly influences p53 function and consequently, cellular growth (21, 22). A second protein subject to rapid turnover by the proteasome is p35, a neuronal-specific activator of cyclin-dependent kinase 5, that drives neuronal migration and development of the mammalian cortex (23). Thus, P311 represents another protein that has links to neuronal function and cellular transformation/differentiation and that is turned over rapidly by the proteasome. Although our studies propose the involvement of P311 in cellular growth and differentiation, the function of the protein remains unknown. The primary amino acid sequence of P311 is apparently novel among proteins in the current databases and thus suggests no function. In the immunostaining experiments the protein was present in both the nucleus and the cytosol. The nuclear localization was found in all cells, but the cytosolic localization occurred only in those cells with a motile morphology, with the protein concentrated along the periphery of the cells in focal adhesions. This suggests that the protein could be involved in the processes of gene expression or cellular motility or could possibly form a link between the two. The apparent increase in P311 concentrations in motile cells may represent another level at which its expression is regulated. It may also explain why the overall concentration of P311 in a population of cells seems to be quite low and precluded attempts to detect the protein by Western blotting. Based on our studies, future work should address the potential roles of P311 in cellular differentiation, transformation, and motility.

Acknowledgments—We thank Michelle Reed and Ave Cline for assistance in preparing the manuscript and Richard Frederickson for assistance in preparing the figures.

REFERENCES

1. Jeffers, M., Rong, S., and Vande Woude, G. F. (1998) J. Mol. Med. 76, 505–513
2. Jeffers, M., Rong, S., and Vande Woude, G. F. (1996) Mol. Cell. Biol. 16, 1115–1125
3. Rong, S., Jeffers, M., Resau, J. H., Tsarfaty, I., Oskarsson, M., and Vande Woude, G. F. (1996a) Cancer Res. 56, 5355–5360
4. Rong, S. Oskarsson, M., Faletto, D., Tsarfaty, I., Resau, J. H., Nakamura, T., Rosen, E., Hopkins, R. F. R., and Vande Woude, G. F. (1995b) Cell Growth Different. 4, 563–569
5. Rong, S., Segal, S., Anver, M., Resau, J. H., and Vande Woude, G. F. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 4731–4735
6. Schmidt, L., Duh, R.-M., Chen, F., Kishida, T., Glenn, G., Choyke, P., Scherer, S. W., Zhuang, Z., Lubensky, J., Dean, M., Allimont, R., Chidambaram, A., Bergerheim, U. R., Felits, J. T., Casadevall, C., Zamaaron, A., Bernues, M., Richard, S., Lips, C. J. M., Walter, M. M., Tsu, L.-C., Gei, L., Orcutt, M. L., Stackhouse, T., Lipan, J., Sife, L., Brauch, H., Decker, J., Niehans, G., Hughson, M. D., Much, H., Sterkel, S., Lerman, M. I., Linehan, W. M., and Zbar, B. (1997) Nat. Genet. 16, 68–73
7. Saito, K., Takahashi, H., Sawada, N., and Parsons, P. G. (1994) J. Pathol. 174, 191–199
8. Koekchepour, S., Jeffers, M., Rulong, S., Klineberg, E., Taylor, G., Hudson, E. A., Resau, J. H., and Vande Woude, G. H. (1997) Cancer Res. 57, 5391–5396
9. Laterriere, J., Nam, M., Rosen, E., Rao, J. S., Lamxus, K., Goldberg, I. D., and Johnston, P. (1997) Lab. Invest. 76, 565–577
10. Taylor, G. A., Jeffers, M., Webb, C. P., Koo, H.-M., Anver, M., Sekiguchi, K., and Vande Woude, G. F. (1998) Oncogene 17, 1179–1183
11. Koekchepour, S., Jeffers, M., Wang, P. H., Gong, C., Taylor, G. A., Roesler, L. M., Stearman, R., Vasselli, J. R., Stettler-Stevenson, W. G., Kaelin, W. G., Linehan, W. M., Kraus, R. D., Gnarra, J. R., and Vande Woude, G. F. (1999) Mol. Cell. Biol. 19, 5902–5912
12. Studler, J. M., Glowunski, J., and Levi-Strauss, M. (1993) Eur. J. Neurosci. 5, 614–623
13. Chomczynski, P., and Sacchi, N. (1987) Anal. Biochem. 162, 156–159
14. Stumpo, D. J., Graff, J. M., Albert, K. A., Greengard, P., and Blackshear, P. J. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 4012–4016
15. Tso, J. Y., Sun, X. H., Kao, T.-h., Reece, K. S., and Wu, R. (1985) Nucleic Acids Res. 13, 2485–2502
16. Taylor, G. A., Jeffers, M., Largeszepada, D. A., Jenkins, N. A., Copeland, N. G., and Vande Woude, G. F. (1996) J. Biol. Chem. 271, 20399–20405
17. Ronnett, G. V., Hester, L. D., Nye, J. S., and Snyder, S. H. (1994) Neuroscience 43, 1081–1099
18. Varshavsky, A. (1997) Trends Biochem. Sci. 22, 383–387
19. Dick, L. R., Cruickshank, A. A., Destree, T. A., Grenier, L., McCormack, T. A., Melandri, F. D., Nunes, S. L., Palombella, V. J., Parent, L. A., Plamondon, L., and Stein, R. L. (1997) J. Biol. Chem. 272, 182–188
20. Geiger, B., Tokuyasu, K. T., Dutton, A. H., and Singer, S. J. (1980) J. Cell Biol. 86, 4731–4735
21. Kubbutat, M. H., Jones, S. N., and Vousden, K. H. (1997) Nature 387, 299–303
22. Crook, T., Ludwig, R. L., Martzen, N. J., Willkomm, D., and Vousden, K. H. (1996) Virology 217, 285–292
23. Patrick, G. N., Zhou, P., Kwon, Y. T., Howley, P. M., and Tsai, L. H. (1998) J. Biol. Chem. 273, 24057–24064