The Ubiquitin-conjugating Enzymes UbcH7 and UbcH8 Interact with RING Finger/IBR Motif-containing Domains of HHARI and H7-AP1*

(Received for publication, April 9, 1999, and in revised form, July 2, 1999)

The primary role of the protein ubiquitinylation pathway is the targeting of intracellular substrate proteins for degradation (1). In this process, ubiquitin is first activated in an ATP-dependent step forming a thioester bond with an ubiquitin-activating enzyme (E1). Ubiquitin is then transferred to an ubiquitin-conjugating enzyme (E2), retaining the high energy thioester bond. Thereafter, the E2, alone or in conjunction with an ubiquitin-protein ligase (E3), catalyzes the final attachment of ubiquitin to the target protein (2). Ubiquitin itself can then serve as a ubiquitinylation substrate, resulting in the generation of polyubiquitylated proteins possibly with the aid of an E4 (3). Finally, ubiquitylated proteins are recognized and degraded by the 26 S proteasome.

It has been proposed that the selection of an individual protein for proteasomal degradation via the ubiquitin pathway requires a unique combination of an E2 and E3. In S. cerevisiae, some 13 E2s or E2-related proteins have been identified, and many more are present in higher eukaryotes (2). E2s are characterized by a conserved catalytic domain of approximately 150 amino acid residues. Despite their functional redundancy, individual E2s appear to be involved in different cellular processes and, therefore, in the ubiquitinylation of different substrate proteins. The distinct substrate specificity of E2s is at least in part explained by the observation that different E2s interact with different E3s.

Four classes of E3 have been identified to date; in contrast to E2s, they exhibit no overt sequence homology. These are yeast UBR1 and its mammalian homologues (4), mammalian E6-AP and members of an E6-AP-related family of E3s termed hect domain proteins (homologous to E6-AP C terminus) (2, 5, 6), mammalian E6-AP and members of an E6-AP-related family of E3s termed hect domain proteins (homologous to E6-AP C terminus) (2, 5, 6), a hetero-oligomeric protein complex termed the cyclosome or anaphase promoting complex (7, 8), and the Skp1/Cdc53/F-box (SCF) complexes (9–11). Although SCF complexes, for instance, do not appear to have a catalytic role in protein ubiquitinylation (9–11), hect domain family members have been suggested to catalyze directly the final attachment of ubiquitin to substrates proteins (2, 12). Despite their different mode of action, it appears that a common feature of all E3s is to specifically interact with distinct E2s. For instance, hect domain family members interact specifically in vitro with UbcH5 and/or UbcH7 and related E2s (5, 6, 12–15) through the ~350 amino acid C-terminal hect domain. Their functional biological importance is indicated by the findings that loss of function of the E6-AP gene, UBE3A, results in Angelman syndrome (16, 17), and disruption of the mouse hect E3 gene encoding Itchy results in abnormalities in the immune system (18).

Over the past decade, it has become clear that many cellular proteins are targeted for degradation by the ubiquitinylation pathway. However, the respective E2s and E3s mediating their ubiquitinylation have been determined for only a few proteins. Furthermore, although several distinct classes of E3 have been identified to date, it seems likely that novel classes of E3s, or other proteins involved in mediating the substrate specificity of E2s, remain to be discovered. Homology cloning has clearly proven successful in the identification of additional members of proteins.
specific E3 families such as the hect family. However, because of the lack of homology between the different classes of E3, other interaction-based approaches must be employed to identify novel E3s and/or ubiquitinylating “ancillary proteins.” We therefore employed the yeast two-hybrid system to isolate proteins that interact with UbcH7, a human E2 that in vitro appears to be involved in the ubiquitylation of various proteins including the tumor suppressor p53 (13, 19–22).

**EXPERIMENTAL PROCEDURES**

**Yeast Two-hybrid Screen for UbcH7-interacting Proteins**—The GAL4-based yeast two-hybrid screening technique (23) was employed to identify UbcH7-interacting proteins. The “bait” plasmid was constructed by cloning the open reading frame of UbcH7 (13, 20, 21) into the vector pGADT7 (GAL4-TAD-DNA-BD, TRP1, ampR) (CLONTECH) as a GAL4 DNA-binding domain fusion protein (pGBlUbcH7). A human testis cDNA library was used to screen this vector (GAL4-TAD-DNA-BD, LEU2, ampR, HA epitope tag) (CLONTECH) to identify UbcH7-interacting proteins. The bait plasmid was transformed into S. cerevisiae strain H7Tc (MATa, ura3-52, his3-200, ade2-101, lys2-801, trp1-901, leu2-3, gal4–542, gal80–538, lys2::GAL1::GAL1, ade2–101, lys2–801, trp1–901, leu2–3, 112, gal4–542, gal80–538, LYS2::GAL1, ade2–101, lys2–801, trp1–901, leu2–3, 112, canr, gal4–542, gal80–538, LYS2::GAL1, ade2–101, lys2–801, trp1–901, leu2–3, 112, canr, gal4–542, gal80–538, URA3::GAL1, HIS3, LYS2::GAL1 (63–64, 75–76) (CLONTECH). Colonies were selected on minimal synthetic medium without leu-1 medium in the absence of tryptophan. Yeast clones harboring the bait plasmid were then sequentially transformed with the “prey” library. Positive clones, potentially harboring UbcH7-interacting species, were identified by their capacity to grow on media without tryptophan, leucine, and histidine and the detection of β-galactosidase activity.

Plasmids were isolated from positive yeast clones, selecting for pACT2 DNA library plasmids by transformation of the leucine auxotroph Escherichia coli strain HB101. Plasmids were then isolated by PCR using flanking vector oligonucleotide primers, and clones were then grouped by size and analyzed by cross-hybridization.

To confirm the specificity of the interactions, pACT2 library plasmids were transformed into the yeast host strain harboring no plasmid, yeast containing the prey library only, yeast containing the prey library but not the plasmid, and pGBlUbcH7. Only those library plasmids demonstrating a requirement for the pGBlUbcH7 plasmid for induced expression of the lacZ and HIS3 reporter genes were considered for further analysis. To assess the specificity of the interaction, UbcH7 was cloned into the GAL4 activation domain plasmid pGAD424 (GAL4-TDAD-ACTAD, AD, LEU2, ampR), and inserts derived from the pACT2 cDNA library were cloned into the pGBT9 vector for β-galactosidase activity. Positive clones were isolated and sequenced.

**Northern Blot Analysis**—Multiple human tissue Northern blots (GenBankm accession number AJ243190). Positive clones were isolated and sequenced. Northern blots were hybridized with radiolabeled HHARI or H7-AP1 DNA probes. DNA was generated by PCR from the inserts of the appropriate pACT2 clones and radiolabeled with [α-32P]dCTP using the Megaprobe DNA labeling system (Amersham Pharmacia Biotech).

**Isolation of Full-length cDNA Clones**—To isolate full-length cDNA clones of human homologue of Drosophila ariadne (HHARI), a human fetal brain cDNA library (Oregene) was screened by PCR using oligonucleotide primers corresponding to nucleotides 591–611 (CATGAGCGAGGAGGTATTAC) and 889–867 (dTCTAGAGCTAGCAGCGGAGCTAGCAGGCG) of the full-length sequence (GenBankm accession number AF072832). Positive clones were isolated and sequenced.

**RESULTS**

**Isolation of the Human Homologue of Drosophila melanogaster Ariadne Protein**—0.5 × 10⁹ independent cDNA clones of a human testis cDNA library were screened for interaction with pGBlUbcH7. Two groups of cross-hybridizing clones were identified and initially termed H7-AP (UbcH7-associated protein) type I clones (seven cross-hybridizing clones) and H7-AP2 clones (six clones). The specificity of the interaction was confirmed by performing a “vector-swap” experiment in which UbcH7 was expressed from the pGBl vector and the H7-AP1 or H7-AP2 cDNA inserts from the pACT2 construct.

The longest H7-AP2 clone of 1605 nucleotides encompassed amino acids 93–557 (Fig. IA). A full-length clone, encoding the entire open reading frame of 557 amino acids (Fig. IA) and constituting a protein of approximately 61 kDa, was obtained by screening a human fetal brain cDNA library. A database search with the full-length H7-AP2 protein revealed that the encoded protein showed striking similarity (72% identity) to the D. melanogaster ariadne protein (Fig. IA). Hence, H7-AP2 was renamed HHARI. A second Drosophila homologue, of lower identity, which we term ariadne2, and a partial murine orthologue were also identified (Fig. IA). The deduced HHARI protein sequence displays several notable primary structural characteristics: a highly acidic domain and a poly(Gly) tract in the
N-terminal region of the protein and three Cys/His-rich motifs located centrally. Two of these motifs, CXHXCX_xCXHXCX_xCXHX_xCX and CXHXCX_xCXHXCX_xCX, encompassing amino acids 186–236 and 344–375, respectively, correspond to the consensus sequence of the RING finger motif, CX_HX_ (9–39)_C (28). The central Cys/His-rich region has recently been termed the “in between RING fingers” (IBR) motif (residues 257–317) (29).

The largest of the H7-AP1 clones of 2366 nucleotides encompassed the C-terminal 734 amino acids of an open reading frame (Fig. 1B). A database search using this cDNA revealed an exact match to the 3’ region of a partial cDNA clone of 5404 base pairs encoding an open reading frame of 1753 amino acids (GenBank™ accession number AB014608). Amino acids 453–482 of the predicted coding region again represent a RING finger motif, CX_HX_ (9–39)_C (28). Furthermore, an extended Cys-rich, RING finger-like motif was also identified between amino acids 273 and 422, CXHXCX_xCXHXCX_xCX, encompassing amino acids 236–344 (Fig. 1B). This extended motif includes an IBR domain between residues 358 and 422.

Expression and Chromosomal Mapping of HHARI and H7-AP1—To assess the expression pattern of H7-AP1 and HHARI, Northern blot analysis was performed using the respective cDNAs as a probe. Three HHARI transcripts of approximately 2.2, 2.7, and 6.5 kb were observed in all tissues, although the 2.2-kb species was weakly expressed except in testis, where it is the predominant transcript (Fig. 2A). A predominant 6.5-kb transcript was also observed in peripheral blood lymphocytes. Particularly high levels of the 6.5-kb transcript were observed in skeletal muscle, spleen, testis, and ovary, and high levels of the 2.7-kb transcript were observed in skeletal muscle and testis. A predominant H7-AP1 transcript of approximately 9 kb was observed in all tissues (Fig. 2B), although relatively higher levels were observed in brain, kidney, and testis.

The chromosomal localization of the HHARI gene was determined by radiation hybrid mapping using oligonucleotide primer pairs derived from both the coding region and the 3’ untranslated region. The mapping data for HHARI were in complete agreement, localizing the gene to the long arm of chromosome 15, 2.84 cR from the marker WI-3873 and 2.9 cR from the marker 79 cR from the top of chromosome 15. The mapping data for H7-AP1 were mapped to chromosome 6p12–21.1 by fluorescence in situ hybridization.

Interaction of HHARI and H7-AP1 with Different Human E2s in Vitro—To test whether the observed interaction of UbcH7 with HHARI and H7-AP1 in the yeast two-hybrid system can also be observed in vitro, HHARI and H7-AP1 were expressed as GST fusion proteins in E. coli. The respective GST fusion proteins were then incubated with different radiolabeled...
E2s generated in the rabbit reticulocyte lysate system and the amount of bound E2s determined in a co-precipitation analysis. The GST-HHARI fusion protein associated with UbcH7 and the closely related UbcH8 (approximately 25% of input radiolabeled E2 was bound to HHARI) but not with the unrelated E2s UbcH1 or UbcH5 (Fig. 3). A similar pattern of interactions was observed with H7-AP1 (data not shown).

Next, the ability of GST-HHARI (118–557) to interact with endogenous cellular UbcH7 was tested. Protein extracts were prepared from HeLa cells and incubated with GST-HHARI bound to glutathione-Sepharose beads. Bound proteins were then separated by SDS-PAGE (Fig. 4, α-UbcH7, lane A), and the presence of UbcH7 was detected by Western blotting using a polyclonal rabbit antisera raised against UbcH7. This demonstrated that cellular UbcH7 specifically bound to GST-HHARI. The specificity of this interaction is demonstrated by the observation that UbcH7 did not bind to GST alone or to an unrelated GST fusion protein encompassing the C-terminal 200 amino acids of the adenomatous polyposis coli (APC) protein (Fig. 4, α-UbcH7, lanes G and C, respectively). The binding specificity is further confirmed by the observation that, as expected, the cellular EB1 protein formed a stable complex with the GST-APC fusion protein but not with GST-HHARI (Fig. 4, α-EB1, lanes L, G, A, and C).

A RING Finger and IBR Motif of the HHARI Protein and an IBR Motif of H7-AP1 Are Necessary for UbcH7 Binding—To delineate the region of HHARI that mediates the interaction with UbcH7, a series of N- and C-terminal mutants of HHARI were prepared as GST fusion proteins (see Fig. 5A, fusion proteins A–J). These constructs were then assayed for their ability to associate with radiolabeled UbcH7. The results indicated that the UbcH7 binding region maps to amino acids 118–293 of HHARI, which encompasses the N-terminal RING finger motif (amino acids 186–236) and part of the IBR motif (amino acids 269–327) (see Figs. 1 and 5B).

A second series of GST-HHARI deletion constructs was then used to fine map the E2 binding domain (Fig. 5, fusion proteins K–U). Results from these binding assays (Fig. 5B) indicated that the minimal UbcH7 binding domain lies between residues 167–293. Although the N-terminal RING finger motif was necessary for UbcH7 binding, a fusion protein containing this motif alone (GST-HHARI (118–242) fusion protein P, Fig. 5) failed to bind the E2. This indicated that both the N-terminal RING finger motif and part of the IBR domain are required for stable interaction with UbcH7.

Similar studies on H7-AP1 revealed that a larger polypeptide sequence (residues 1–441) was required for interaction with UbcH7. This sequence does not incorporate the classic RING finger motif that lies between residues 453–482 (CX_4CX_3CXHXCX_2CX_2CX_2CX_2C) but contains an extended cysteine-rich sequence interspersed with histidine residues (CXHXCX_2CX_2CX_2CX_2CXHXCX_2CX_2CX_2CX) which includes the IBR motif between residues 358–422. No other recognized motif is present between residues 1–441.
UbcH7 and UbcH8 Interact with RING Finger/IBR Proteins

FIG. 6. Full-length UbcH7 is required for stable complex formation with HHARI. A series of TXD fusion proteins were constructed containing regions of UbcH7 and assayed for their capacity to form a stable association with GST-HHARI fusion protein (full-length UbcH7 contains 154 amino acid residues). Bound TXD-UbcH7 fusion proteins were analyzed by SDS-PAGE and Western blotting using chemiluminescent detection as described under “Experimental Procedures.” Lanes + and – indicate thioredoxin-UbcH7 fusion proteins incubated with GST-HHARI or GST alone as a negative control, respectively. Lanes 1 contain 5% of the input UbcH7 fusion protein used in each binding assay. Molecular mass markers are indicated in kDa.

DISCUSSION

We have identified two novel human proteins, HHARI and H7-AP1, that interact with UbcH7 and the closely related E2 UbcH8 but not with the unrelated E2s UbcH1 and UbcH5. No overt structural similarity was identified between them other than the presence of RING finger and IBR motifs. RING finger- and IBR motif-containing domains are proposed to mediate protein-protein interactions (28, 29). Indeed, an N-terminal RING finger domain and part of the IBR motif of HHARI and the IBR motif of H7-AP1 are required for interaction with UbcH7 and UbcH8, although other, as yet undescribed, structural motifs may contribute to these interactions. While this article was under review, it was reported that another RING finger protein, termed Rbx1, recruits the E2 UBC3/CDC34 to SCF complexes (30–32). Thus, it appears that distinct subclasses of RING finger- or IBR motif-containing proteins represent a new family of proteins that specifically interact with distinct E2 enzymes.

In support of the hypothesis that RING finger- or IBR motif-containing proteins commonly play a role in protein ubiquitylation, the presence of these motifs has previously been observed in proteins associated with the ubiquitin/proteasome system. Arabidopsis PRT1 (which, like HHARI, contains two RING finger motifs) appears to be involved in the degradation of proteins via the N-end rule pathway (33). A subunit of APC, APC11, is very similar to Rbx1 (32, 34). Drosophila Sina and its human orthologue Siah contain a RING finger and are involved in the degradation of Tramtrack (35, 36) and DCC (deleted in colon cancer) (37, 38), respectively. Of note, the RING finger of Siah is required to induce DCC proteolysis (37). Finally, the Mdm2 proto-oncoprotein, which mediates the ubiquitylation and subsequent degradation of p53 (39–41), contains a RING finger motif that is required for p53 degradation (39, 41, 42). Although it has not yet been demonstrated, it is tempting to speculate that the RING finger motifs of the above mentioned proteins are directly involved in the interaction with their cognate E2s.

The ability of HHARI and H7-AP1 to interact with UbcH7 may indicate that these proteins function as E3s or as part of E3 complexes in UbcH7-dependent ubiquitylation. UbcH7 has previously been demonstrated to interact functionally with members of the hect E3 family, in vitro (6, 13). These E3s are characterized by their ability to accept activated ubiquitin from UbcH7 or UbcH5 in the form of a thioester complex. However, such an activity was not observed for HHARI (data not shown). Thus, if HHARI and H7-AP1 have E3 activity, they are likely to function similar to SCF complexes (9–11), namely as “docking proteins,” by bringing the target protein into juxtaposition for direct ubiquitylation by UbcH7. Interestingly, RING finger domains are often found in the component proteins of multiprotein complexes (28). HHARI contains two RING fingers and one IBR motif, suggesting that it has the potential to interact specifically with several different proteins. Indeed, using metabolically labeled cell extracts, we observed that a number of cellular proteins could specifically bind to HHARI (not shown). Whether these proteins represent potential targets for HHARI/ UbcH7-facilitated ubiquitylation is presently unclear.

Alternatively, HHARI and H7-AP1 may serve as substrates for UbcH7-mediated ubiquitylation. Preliminary results suggest that, at least in vitro, HHARI and H7-AP1 are not ubiquitylated by UbcH7 (not shown). However, this does not exclude HHARI and H7-AP1 as substrates for UbcH7 because it is possible that in the in vitro system a factor(s) is missing that is required for ubiquitylation of these proteins. Interestingly, Siah and Mdm2 have been reported not only to promote the degradation of DCC and p53, respectively, but also to mediate their own degradation, at least under certain conditions (37, 38, 42). Thus, even if ubiquitylation of HHARI and H7-AP1 is observed in vitro or in vivo, this will not exclude the possibility that HHARI and H7-AP1 are actively involved in the ubiquitylation of so far unknown cellular proteins.

HHARI probably represents the direct human orthologue of Drosophila ariadne as it displays a remarkably high sequence similarity (72% amino acid identity). This high sequence conservation implies that these proteins play an essential role in fundamental cellular processes. Indeed, mutant alleles that result in truncation or point mutation of the RING finger domain of Drosophila ariadne are recessive lethal at the pupal stage, demonstrating a severe disorganization of the central nervous system (43). These data, together with the results presented in this study, suggest that the phenotype may be attributable to aberrant ubiquitylation. Finally, it is interesting to note that the HHARI gene maps just telomeric of the UBE3A gene on human chromosome 15q24, a region recently associated with a syndrome characterized by severe mental retardation, spasticity, and tapetoretinal degeneration (44).
Acknowledgments—We thank Drs. J. Askham and E. Morrison for providing the GST-APC fusion protein and anti-EB-1 antibodies.

REFERENCES

1. Hershko, A., and Ciechanover, A. (1998) Ann. Rev. Biochem. 67, 425–479
2. Scheffner, M., Smith, S., and Jentsch, S. (1998) in Ubiquitin and the Biology of the Cell (Peters, J.-M., Harris, J. R., and Finley, D., eds) pp. 65–91, Plenum Press, London
3. Koeogl, M., Hoppe, T., Schlenker, S., Ulrich, H. D., Mayer, T. U., and Jentsch, S. (1999) Cell 96, 635–644
4. Varsharsky, A., Byrd, C., Davydov, I. V., Dehmen, R. J., Du, F., Ghislain, M., Gonzalez, M., Grigoryev, S., Johnson, E. S., Johnsson, N., Johnston J. A., Kwon, Y. T., Levy, F., Lomovskaya, O., Madura, K., Ota, I., Rue menapf, T., Shadrer, T. E., Suzuki, T., Turner, G., Waller, P. R. H., Webster, A., and Xie, Y. (1998) in Ubiquitin and the Biology of the Cell (Peters, J.-M., Harris, J. R., and Finley, D., eds) pp. 223–278, Plenum Press, London
5. Huisbrugse, J. M., Scheffner, M., Beaudenon, S., and Howley, P. M. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 2563–2567
6. Schwarz, S. E., Rosa, J. L., and Scheffner, M. (1998) J. Biol. Chem. 273, 12148–12154
7. King, R. W., Peters, J. M., Tugendreich, S., Rolfe, M., Ito, H., Tyers, M., Elledge, S. J., and Harper, J. W. (1997) Mol. Cell. Biol. 17, 8683–8688
8. Sudakin, V., Ganoth, D., Dahan, M., Hershko, J., Luca, F., Mayer, A., Smith, C. E., Siegel, N. R., Schwarz, S. E., Rosa, J. L., and Scheffner, M. (1998) J. Biol. Chem. 273, 2725–2730
9. Kumar, S., Kao, W. H., and Howley, P. M. (1997) J. Biol. Chem. 272, 15458–15464
10. Naka taya, S., Jensen, J. P., and Weissman, A. M. (1997) J. Biol. Chem. 272, 15085–15092
11. B. 252 urger, J. Butting, K., Dittrich, B., Groos, S., Lich, C., Sperling, K., Stork, B., and Reis, W. (1997) Am. J. Hum. Genet. 61, 88–93
12. Matsuura, T., Sutcliffe, J. S., Fang, P., Galjaard, R.-J., Jiang, Y.-H., Benton, C. S., Rommens, J. M., and Beaudet, A. L. (1997) Nat. Genet. 15, 74–77
13. Perry, W. L., Hustad, C. M., Swing, D. A., O’Sullivan, T. N., Jenkins, N. A., and Copeland, N. G. (1998) Nat. Genet. 18, 143–146
14. Blumenfeld, N., Gony, H., Mayer, A., Smith, C. E., Siegel, N. R., Schwarz, A. L., and Ciechanover, A. (1994) J. Biol. Chem. 269, 8674–8681
15. Robinson, P. A., Leek, J. P., Thompson, J., Carr, I. M., Bailey, A., Maynihan, T. P., Colletta, P. L., Lench, N. J., and Markham, A. F. (1995) Mammalian Genome 6, 725–731
16. Moynihan, T. P., Cole, C. G., Dunham, I., O’Neil, L., Markham, A. F., and Robinson, P. A. (1998) Genomics 51, 124–127
17. Gross-Mesi laty, S., Reinstein, E., Bercovich, B., Tobias, K. E., Schwartz, A. L., Kahana, C., and Ciechanover, A. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 8058–8063
18. Chien, C.-T., Bartel, P. L., Steen glanz, R., and Fields, S. (1991) Proc. Natl. Acad. Sci. U. S. A. 74, 5463–5467
19. Schneider, R., Eckerskorn, C., Lottspeich, F., and Schweiger, M. (1996) EMBO J. 15, 1431–1435
20. Scheffner, M., Huisbrugse, J. M., and Howley, P. M. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 8797–8801
21. Su, L. K., Burrell, M., Gyruris, J., Brent, R., Wiltshire, R., Trent, J., Vogelstein, B., and Kinzler, K. W. (1996) Cancer Res. 56, 2972–2977
22. Towbin, H., Staehelin, T., and Gordon, J. (1979) Proc. Natl. Acad. Sci. U. S. A. 76, 4350–4354
23. Freemont, P. S. (1993) Annu. N. Y. Acad. Sci. 684, 174–192
24. Morett, E., and Bork, P. (1999) Trends Biochem. Sci. 24, 229–231
25. Skowyra, D., Roe p, D. M., Kamura, T., Kon, M. N., Skowyra, D., Moreland, R. L., Ilipoulos, O., Lane, W. S., Kaelin Jr., W. G., Elledge, S. J., Conaway, R. C., Harper, J. W., and Conaway, J. W. (1999) Science 284, 657–661
26. Skowyra, D., Koepp, D. M., Kamura, T., Conrad, M. N., Skowyra, D., Moreland, R. L., Ilipoulos, O., Lane, W. S., Kaelin Jr., W. G., Elledge, S. J., Conaway, R. C., Harper, J. W., and Conaway, J. W. (1999) Science 284, 662–665
27. Tyers, M., and Willems, A. R. (1999) Science 284, 601–604
28. Potuschak, T., Starly, S., Schlegelhoffer, F., Becke, F., Nejinskaia, V., and Bachmair, A. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 7904–7908
29. Zachariae, W., Shevchenko, A., Andrews, P. D., Ciosk, R., Galovo, M., Stark, M. J. R., Mann, M., and Nesmy, F. K. (1998) Science 270, 1216–1219
30. Wang, A. H., Neufeld, T. P., Kwan, E., and Rubin, G. M. (1997) Cell 90, 459–467
31. Li, S., Li, Y., Carthew, R. W., and Lai, Z.-C. (1997) Cell 90, 469–478
32. Hu, G., and Fearon, E. R. (1999) Mol. Cell. Biol. 19, 724–732
33. Hu, G., Zhang, S., Vidal, M., La Baer, J., Xu, T., and Fearon, E. R. (1997) Genes Dev. 11, 2701–2714
34. Haupt, Y., Maya, R., Kazaz, A., and Oren, M. (1997) Nature 387, 296–299
35. Kubbutat, M. H. G., Jones, S. N., and Vousden, K. H. (1997) Nature 387, 299–303
36. Honda, R., Tanaka, H., and Yasuda, H. (1997) FEBS Lett. 420, 25–27
37. Kubbata, M. H., Ludwig, R. L., Levine, A. J., and Vousden, K. H. (1999) Cell Growth Differ. 10, 87–92
38. Aguilar, M., Oliveros, M., Martinez-Padron, M., Barbas, J. A., and Ferrus, A. (1998) Adv. Con. Res. 39, 522A
39. Mitchell, S. J., McMule, D. P., Campbell, D. A., Lench, N. J., Mueller, R. F., Bondy, S. E., and Markham, A. F. (1998) Am. J. Hum. Genet. 62, 1070–1076