The Human Cdc14 Phosphatases Interact with and Dephosphorylate the Tumor Suppressor Protein p53

(Liwu Li‡§, Mats Ljungman¶, and Jack E. Dixon‡

From the ‡Department of Medicine, Wake Forest University School of Medicine, Winston-Salem, North Carolina 27157 and the Departments of Biological Chemistry and §Radiation Oncology, University of Michigan Medical School, Ann Arbor, Michigan 48109-0606

The yeast Cdc14 phosphatase has been shown to play an important role in cell cycle regulation by dephosphorylating proteins phosphorylated by the cyclin-dependent kinase Cdc28/clb. We recently cloned two human orthologs of the yeast CDC14, termed hCDC14A and -B, the gene products of which share ~80% amino acid sequence identity within their N termini and phosphatase domains. Here we report that the hCdc14A and hCdc14B proteins interact with the tumor suppressor protein p53 both in vitro and in vivo. This interaction is dependent on the N termini of the hCdc14 proteins and the C terminus of p53. Furthermore, the hCdc14 phosphatases were found to dephosphorylate p53 specifically at the p34Cdc2/clb phosphorylation site (p53-phosphor-Ser315). Our findings that hCdc14 is a cyclin-dependent kinase substrate phosphatase suggest that it may play a role in cell cycle control in human cells. Furthermore, the identification of p53 as a substrate for hCdc14 indicates that hCdc14 may regulate the function of p53.

Cdc14 is a protein phosphatase conserved from yeast to man (1). Genetic analyses suggest that the yeast Cdc14 plays pleiotropic roles during the cell cycle, including the regulation of DNA replication and the exit from mitosis (2, 3). Recent studies indicate that Cdc14 can, in at least two ways, antagonize the cyclin-dependent kinase Cdc28/clb, which is the master regulator of the cell cycle in yeast (5). Firstly, Cdc14 dephosphorylates the substrates phosphorylated by Cdc28/clb (4). Secondly, Cdc14 dephosphorylates the transcription factor Swi5, resulting in the nuclear accumulation of Swi5 and the transactivation of the Cdc28/clb inhibitor Sic1 (6). The Cdc14 is itself regulated by compartmentalization in the nucleus. During the G1, S, G2, and early mitosis Cdc14 is anchored in the nucleolus and redistributed throughout the nucleus at the beginning of anaphase (7, 8).

Our laboratory has cloned two human orthologs of the yeast CDC14, termed hCDC14A and hCDC14B, the gene products of which share ~80% amino acid sequence identity within their N termini and phosphatase domains (1). Both hCDC14A and hCDC14B were found to be localized to the nucleus when over-expressed in mammalian cells. Using the yeast strain harboring a temperature-sensitive mutation in the CDC14 gene, we showed that introduction of either of the two human CDC14 genes could suppress the phenotype of the yeast cdc14 temperature-sensitive mutant. This finding suggests that the hCDC14s may perform functions in human cells similar to those performed by the Cdc14 in yeast.

Analogous to the inactivation of the yeast Cdc28/clb by Swi5-induced Sic1, the human p34Cdc2/clb kinase can in part be inactivated by the p53-regulated Cdk inhibitor p21WAF1 (9). Interestingly, p53 can be phosphorylated by human p34Cdc2/clb at serine 315 (10), parallel to the fact that Swi5 is phosphorylated by the yeast Cdc28/clb kinase (11). Because the yeast Cdc14 can dephosphorylate Swi5, we wanted to explore whether the hCdc14A and hCdc14B may dephosphorylate p53.

In this study, we report that the two hCdc14 phosphatases physically interact with the p53 protein both in vitro and in vivo. Such interaction involves the highly conserved N termini of the hCdc14 proteins and the C terminus of p53. Furthermore, both hCdc14 forms could specifically dephosphorylate the p34Cdc2/clb phosphorylation site of p53 (Ser315). We propose that hCdc14 phosphatases may, together with p34Cdc2 regulat p53 function by controlling the phosphorylation status of Ser315 of p53.

MATERIALS AND METHODS

Yeasts, Strains, Plasmids, and Recombinant Proteins—The Saccharomyces cerevisiae EGY48 strain was obtained from CLONTECH and cultured according to the manufacturer’s suggested condition (CLONTECH). To generate the pLexA-hCdc14B plasmid, the hCdc14B open reading frame (ORF) was cloned in-frame into the BamH1/XhoI restriction sites of the pLexA vector (CLONTECH). The pLexA-PTP3 plasmid was constructed by ligating the PCR product of the S. cerevisiae phosphatase PTP3 ORF in-frame into the EcoRI/XhoI restriction sites of the pLexA vector. The pB42AD-p53 plasmid was constructed by ligating the PCR product of the human p53 ORF in-frame into the EcoRI/XhoI restriction sites of the pB42AD vector (CLONTECH). The construction of the green fluorescent protein fusion plasmids pEGFP-hCdc14A, pEGFP-hCdc14As, and pEGFP-hCdc14B, as well as the plasmids pT7H-Cdc14A, pT7H-Cdc14B, pET-Cdc14Aa, and pET-Cdc14B were accomplished as described previously (1).

To make the pET-Cdc14A-150 and pET-Cdc14A-220–480 plasmids, the hCdc14A coding sequences from nucleotides 1 to 474 and 360 to 1740, respectively, were PCR-amplified and ligated in-frame into the NdeI/XhoI restriction sites of the pET23a vector (Novagen). The plasmids pET-Cdc14B-150 and pET-Cdc14B-220–450 were constructed by PCR amplifying the coding sequence of hCdc14B from nucleotide 1 to 690 and 450 to 1377, respectively, followed by ligation in-frame into the NdeI/XhoI restriction sites of the pET23a vector.

The GST-p53 fusion plasmid pGST-p53 was constructed by cloning human p53 ORF in-frame into the EcoRI/XhoI restriction sites of the pGEX-5X-1 vector (Amersham Pharmacia Biotech). To make pGST-p53-1–150, pGST-p53-150–290, and pGST-p53-290–393, the coding sequence of human p53 from nucleotides 1 to 630, 300 to 785, and 750 to 1179, respectively, were PCR-amplified and ligated in-frame into the EcoRI/
Cdc14 Proteins Dephosphorylate p53

RESULTS

Human Cdc14 Proteins Interact with p53 in Vivo—To study whether the hCdc14 proteins could interact with the p53 protein, we used the yeast two-hybrid assay. After co-transformation of pLexA-hCdc14B and pB42AD-p53 plasmids into the S. cerevisiae EG48 strain, the LEU reporter gene within the EG48 strain was activated and enabled the yeast cell to grow on selective media (see Materials and Methods for details). The colonies turned blue as a result of lacZ gene activation (Fig. 1A). Yeast cells transformed with either the EGFP-Cdc14A or EGFP-Cdc14B fusion construct (pEGFP-Cdc14A and pEGFP-Cdc14B). Immunoprecipitation of p53 was performed 48 h after transfection using anti-p53 antibodies. The immunoprecipitates were subjected to SDS-PAGE and Western blot analysis with anti-EGFP antibodies. As shown in lane 1 of Fig. 1B, a band corresponding in size to the EGFP-hCdc14A fusion protein (93 kDa) was detected on the blot, suggesting that EGFP-hCdc14A and p53 proteins co-existed in the immunocomplex. Lane 3 shows a band corresponding in size to the EGFP-hCdc14B fusion protein (82 kDa), suggesting that the hCdc14B protein also formed immunocomplexes with the p53 protein. Interestingly, p53 could also form immunocomplexes with EGFP-hCdc14As, which lacks the C-terminal domain (truncated from amino acid 365). This result suggests that hCdc14s interact with p53 via their highly conserved N-terminal domain.

To ensure that the interaction between the hCdc14 phosphatases and p53 was specific and unique, the 293 cells were transfected with either the EGFP vector alone or with another EGFP fusion construct coding for a putative dual specific protein phosphatase PTEN (pEGFP-PTEN). In these experiments, we could not detect any co-immunoprecipitation of either PTEN or EGFP with the anti-p53 antibody (expected size of 75 and 27 kDa, respectively) (lanes 4 and 5). Western blot with whole cell lysates indicated that all EGFP fusion proteins were expressed at similar levels (data not shown). Taken together, the results obtained with both the yeast two-hybrid system and

Figs. 1 Cdc14 proteins interact with p53. A, two-hybrid analysis of the interaction between hCdc14B and p53. Plasmids were transformed into S. cerevisiae EG48 strain as described under “Materials and Methods.” Transformants carrying different combinations of plasmids were plated onto SD –Ura, –His, –Trp, –Leu (SD-UHWL) plates. EGY48 cells carrying bait plasmids pLexA-hCdc14B, pEGFP-hCdc14As, or pEGFP-hCdc14B using LipofectAMINE according to the manufacturer’s instructions (Life Technologies, Inc.). 48 h after transfection, cells were lysed in a phosphatase-inhibited saline buffer containing 1% Triton X-100, 50 mM NaCl, 1 mM dithiothreitol, proteinase inhibitor mix (BMB). Endogenous p53 was immunoprecipitated using polyclonal anti-p53 antibody (BMB). The immunoprecipitates were washed with lysis buffer five times and subjected to SDS-PAGE analysis. The gel was blotted onto polyvinylidene difluoride membrane and probed with anti-EGFP monoclonal antibody (CLONTECH) to analyze whether any EGFP-hCdc14B fusion proteins may co-immunoprecipitate with p53.

In Vivo Expression of hCdc14 and Immunoprecipitation—The Epstein-Barr virus-transfected human cell line 293 was maintained in Dulbecco’s modified Eagle’s medium with 10% fetal bovine serum and 10 units/ml penicillin/streptomycin. Cells were transfected with the plasmids pEGFP-hCdc14A, pEGFP-hCdc14As, or pEGFP-hCdc14B using LipofectAMINE according to the manufacturer’s instructions (Life Technologies, Inc.). 48 h after transfection, cells were lysed in a phosphatase-inhibited saline buffer containing 1% Triton X-100, 50 mM NaCl, 1 mM dithiothreitol, proteinase inhibitor mix (BMB). Endogenous p53 was immunoprecipitated using polyclonal anti-p53 antibody (BMB). The immunoprecipitates were washed with lysis buffer five times and subjected to SDS-PAGE analysis. The gel was blotted onto polyvinylidene difluoride membrane and probed with anti-EGFP monoclonal antibody (CLONTECH) to analyze whether any EGFP-hCdc14B fusion proteins may co-immunoprecipitate with p53.

In Vitro Dephosphorylation of p53—Purified recombinant GST-p53 fusion proteins were phosphorylated in vitro by either p34

\[\text{cdc2} / \text{cyclin B}\] kinase or CKII according to the conditions provided by the manufacturer (New England Biolabs). The phosphorylated GST-p53 fusion proteins (300 ng) in 50 μl of glutathione-agarose beads were mixed with 1 μg of recombinant hCdc14A, hCdc14B, PTEN, YG4E, or bovine serum albumin in 20 mM Tris-HCl, pH 7.5, 100 mM NaCl, 1 mM EDTA, 10% glycerol, and 0.2 mM phenylmethylsulfonyl fluoride. After washing the agarose beads six times with the above buffer, SDS sample buffer was added, and the samples were boiled for 5 min and analyzed using SDS-PAGE. The gel was treated with Amplify (Amer-

In Vivo Expression of hCdc14 and Immunoprecipitation—The Epstein-Barr virus-transformed human cell line 293 was maintained in Dulbecco’s modified Eagle’s medium with 10% fetal bovine serum and 10 units/ml penicillin/streptomycin. Cells were transfected with the plasmids pEGFP-hCdc14A, pEGFP-hCdc14As, or pEGFP-hCdc14B using LipofectAMINE according to the manufacturer’s instructions (Life Technologies, Inc.). 48 h after transfection, cells were lysed in a phosphatase-inhibited saline buffer containing 1% Triton X-100, 50 mM NaCl, 1 mM dithiothreitol, proteinase inhibitor mix (BMB). Endogenous p53 was immunoprecipitated using polyclonal anti-p53 antibody (BMB). The immunoprecipitates were washed with lysis buffer five times and subjected to SDS-PAGE analysis. The gel was blotted onto polyvinylidene difluoride membrane and probed with anti-EGFP monoclonal antibody (CLONTECH) to analyze whether any EGFP-hCdc14B fusion proteins may co-immunoprecipitate with p53.

In Vitro Binding Assay—In vitro translated and 35S-labeled hCdc14 proteins were incubated with glutathione-agarose-immobilized GST-p53 fusion proteins at 4 °C for 2 h in PBST (4 mM NaH2PO4, 16 mM Na2HPO4, 100 mM NaCl, 0.5% Triton X-100, pH 7.4), 0.05% β-mercaptoethanol, 10% glycerol, and 0.2 mM phenylmethylsulfonyl fluoride. After washing the agarose beads six times with the above buffer, SDS sample buffer was added, and the samples were boiled for 5 min and analyzed using SDS-PAGE. The gel was treated with Amplify (Amer-}

Fig. 1. Cdc14 proteins interact with p53. A, two-hybrid analysis of the interaction between hCdc14B and p53. Plasmids were transformed into S. cerevisiae EG48 strain as described under “Materials and Methods.” Transformants carrying different combinations of plasmids were plated onto SD –Ura, –His, –Trp, –Leu (SD-UHWL) plates. EGY48 cells carrying bait plasmids pLexA-hCdc14B and pB42AD-p53 plasmids can grow and turn blue on such a plate. B, co-immunoprecipitation of p53 and human GFP-Cdc14 fusion proteins. 293 cells were transfected with pEGFP-hCdc14A, pEGFP-hCdc14As, pEGFP-hCdc14B, pEGFP-PTEN, or pEGFP-C2 (lanes 1–5) as described under “Materials and Methods.” Following immunoprecipitation with polyclonal anti-p53 antibody, the immunoprecipitated proteins were analyzed on SDS-PAGE and blotted with monoclonal anti-EGFP antibody. The expected positions of human GFP-hCdc14A, EGFP-hCdc14As, and EGFP-hCdc14B are marked.
of p53 are responsible for the interaction. A and B, 5 μl each of the in vitro translated and 35S-labeled hCdc14A, hCdc14A1–158, hCdc14A120–580 (A) or hCdc14B, hCdc14B1–230, hCdc14B150–459 (B) proteins were incubated with 50 μl (~300 ng) of agarose bead-immobilized GST-p53 fusion protein as described under “Materials and Methods.” After washing, proteins stably bound to the agarose beads were separated by SDS-PAGE, dried, and exposed to x-ray film. Input, 1 μl of the 50-μl in vitro translation reaction. C, SDS-PAGE of purified GST-p53 fusion proteins. D and E, 5 μl each of the in vitro translated and 35S-labeled hCdc14A (D) or hCdc14B (E) proteins were incubated with 50 μl (~300 ng) of agarose bead-immobilized GST or GST-p53 fusion proteins as described under “Materials and Methods.” After washing, proteins stably bound to the agarose beads were separated by SDS-PAGE, dried, and exposed to x-ray film. Input, 1 μl of the 50-μl in vitro translation reaction.

with the immunoprecipitation experiments demonstrated that the hCdc14A and hCdc14B phosphatases can physically interact with p53.

The N terminus of hCdc14 Proteins and the C Terminus of p53 Are Responsible for Their Interaction—To further confirm that the N terminus of hCdc14 interacts with p53, we performed binding assays using full-length recombinant GST-p53 proteins and various truncation mutants of hCdc14A and -B that had been synthesized by an in vitro translation procedure. Both hCdc14A1–158 and hCdc14B1–230 bound to GST-p53, whereas neither hCdc14A120–580 nor hCdc14B150–459 did (Fig. 2, A and B). As a control, we demonstrated that neither of these in vitro translated hCdc14 proteins interacted with the GST protein (data not shown).

We next performed in vitro binding assays to determine what domain of the p53 protein is involved in the interaction. 35S-Labeled hCdc14A and hCdc14B proteins, synthesized from pET-Cdc14A or pET-Cdc14B by in vitro translation, were incubated with recombinant proteins of GST fused to various substrates. As shown in Fig. 2, D and E, the full-length GST-p53 protein as well as the GST-p5350–393 bound well to hCdc14A and hCdc14B, retaining ~50% of the radioactivity of the input radiolabeled hCdc14s. In contrast, whereas neither hCdc14A120–580 nor hCdc14B150–459 had any phosphatase activity toward p53 phosphorylated at Ser315 or Ser392 (Fig. 3). As expected, the inactive mutant (hCdc14BCS) protein with its catalytic cysteine mutated to serine does not show any phosphatase activity against either site of p53. Two other unrelated phosphatases (PTEN and YG4E proteins) were used in the assay as controls. Like hCdc14, PTEN is a putative dual specific protein phosphatase with low activity against the artificial substrates p-nitrophenyl phosphate and 3-O-methylfluorescein phosphate (1). YG4E is an active protein phosphatase that exhibits strong activity against the substrates p-nitrophenyl phosphate and 3-O-methylfluorescein phosphate (data not shown). We found that neither PTEN nor YG4E could dephosphorylate GST-p53 phosphorylated at Ser315 or Ser392 (Fig. 3).

DISCUSSION

The yeast Cdc14 protein phosphatase plays important roles in the regulation of the cell cycle. As shown in the Fig. 4A, the yeast Cdc14 dephosphorylates several Cdc28/Cli1 substrates such as Hct1, Sic1, and Swi5. Cdc14-mediated dephosphorylation of Hct1 results in degradation of cyclin B (17), whereas dephosphorylation of the Cdc28/Cli1 kinase inhibitor Sic1 results in Cdc28/Cli1 accumulation (18). Cdc14-mediated dephosphorylation of the transcription factor Swi5 allows its nuclear translocation and subsequent transactivation of Sic1 (19). Taken together, the yeast Cdc14 appears to reverse the effects of Cdc28/Cli1 by directly dephosphorylating proteins phosphorylated by Cdc28/Cli1 (4, 17).

We recently cloned the human Cdc14 orthologs, hCDC14A
and hCdc14B (1). In this study, we found that hCdc14A and hCdc14B physically interact with the human tumor suppressor protein p53 both in vitro and in vivo. Such interaction specifically involves the highly conserved N termini of the hCdc14 proteins and the C terminus of the p53 protein. Furthermore, we showed that recombinant hCdc14A and hCdc14B specifically dephosphorylate p53 at Ser315, a site that has been shown to be phosphorylated by p34^Cdc2^/clb (10). No hCdc14 phosphatase activity was detected against p53 phosphorylated at the Ser392 site, which can be phosphorylated by casein kinase II (13). Other phosphatases tested, such as PTEN or YG4E, show no in vitro activity toward p53 phosphorylated at either Ser315 or Ser392. Our results that hCdc14s specifically dephosphorylate the p34^Cdc2^/clb phosphorylation site of p53 suggest that hCdc14 phosphatases may function analogously to the yeast Cdc14 by dephosphorylating substrate(s) of p34^Cdc2^/clb kinase.

p53 is a well studied tumor suppressor protein that plays important roles in the cellular response to DNA-damaging agents and other cellular stresses. p53 is normally present in low amounts in normal cells but accumulates in the cell nucleus in response to various cellular stresses (13). The nuclear accumulation of p53 leads to the transactivation of cyclin-dependent kinase inhibitor p21^WAF1^, causing cell cycle arrest at G1 phase as well as the G2/M phase (20). In addition to their roles in G1 cell cycle arrest, p53 and p21^WAF1^ have been suggested to be involved in the regulation of the mitotic exit checkpoint in mammalian cells (21). Loss or inactivation of p53 or p21^WAF1^ is associated with tetráploidy or aneuploidy due to the failure of cytokinesis (22–24). Furthermore, p53 appears to stimulate the exit of mitosis following a transient G2/M cell cycle arrest induced by various DNA-damaging agents in many cell types (25). Considering the fact that yeast Cdc14 facilitates mitotic exit by dephosphorylating Cdc28/clb substrates and in turn deactivating Cdc28/clb activity, we speculate that hCdc14-dependent dephosphorylation of p53 (and other p34^Cdc2^ substrates) may stimulate exit from mitosis in mammalian cells (Fig. 4B).

Human p53 has been shown to undergo numerous phosphorylations in vivo and in vitro. The phosphorylation status of p53 is determined by several kinases that target at least six different serines clustered in two domains on the protein (15). Two phosphorylated serines within the C terminus domain, Ser315 and Ser392, are phosphorylated by p34^Cdc2^/clb and casein kinase II, respectively (10, 15). p53 phosphorylation at Ser392 can enhance p53 sequence-specific DNA binding in vitro and is important for p53-mediated transcriptional activation in vivo (26). In addition, UV irradiation has been reported to increase the Ser392 phosphorylation (27, 28). Prolonged stability and/or activation of p53 by those phosphorylation events under stress conditions lead to G1/S or G2/M arrest. Several phosphatases including PP2A and PP5 have been inferred to inhibit p53 transcriptional activity by regulating the phosphorylation status of p53, although the targeting sites of these phosphatases are not known (29, 30). So far, the effect of Ser315 phosphorylation is not clear. Earlier studies showed that Ser315 phosphorylation increases the sequence-specific DNA binding capacity of p53, suggesting that Ser315 phosphorylation is an activating modification (10). However, mutation of the Ser315 residue has been reported to prolong the half-life of the p53 protein, suggesting that phosphorylation of Ser315 may target p53 for degradation (31). Contrary to those reports, recent studies in which Ser315 and other phosphorylation sites had been mutated suggest no role for the Ser315 in regulating the stability or activity of p53 (32, 33). Because the Ser315 site of p53 is located adjacent to its nuclear localization signal, it is conceivable that Ser315 phosphorylation may regulate p53 localization. Experiments studying the p53 localization showed that mutating Ser315 into alanine, which mimics constitutively dephosphorylated serine, does not affect the ability of p53 to translocate into the nucleus (34). However, it cannot be excluded from that study that phosphorylation of Ser315 may result in cytoplasmic retention of p53. In fact, the regulation of p53 nuclear export has been shown to be an important mechanism by which cells regulate p53 stability (35–37). Because the previous studies addressing p53 Ser315 phosphorylation were done using transient overexpression of p53 in transformed cell lines, they may fail to detect the effect of p53 Ser315 phosphorylation in normal cells, where only a minute amount of p53 is present. Further studies are needed to clarify the function of p53 Ser315 phosphorylation and its regulation by hCdc14 phosphatases.

In summary, our results demonstrate that hCdc14 proteins can interact with p53 and specifically dephosphorylate the p34^Cdc2^/clb-mediated Ser315 phosphorylation in vivo. Such findings suggest that Cdc14 may be an evolutionary conserved cyclin-dependent kinase phosphatase. Because the yeast Cdc14 phosphatase dephosphorylates multiple Cdc28/clb substrates (3, 4), it is possible that one or both of the human Cdc14 proteins can dephosphorylate multiple substrates phosphorylated by Cdks in mammalian cells. The fact that there are several closely related human p53 homologues recently being discovered (p73 and p63ket) raises an intriguing possibility that different hCdc14 proteins might regulate different p53 homologues. Further study regarding the regulation of hCdc14s will provide valuable insight toward the understanding of mammalian cell cycle control and the regulation of p53.

REFERENCES

1. Li, L., Ernsting, B. R., Wishart, M. J., Lohse, D. L., and Dixon, J. E. (1997) J. Biol. Chem. 272, 29403–29406
2. Hardy, C. F. (1989) Mol. Cell. Biol. 16, 1832–1841
3. Jaspersen, S. L., Charles, J. F., Tinker-Kulberg, R. L., and Morgan, D. O. (1998) Mol. Biol. Cell 9, 2803–2817
4. Visintin, R., Craig, K., Hwang, E. S., Prinz, S., Tyers, M., and Amon, A. (1998) Mol. Cell 2, 709–718
5. Mendenhall, M. D., and Hodge, A. E. (1998) Microbiol. Mol. Biol. Rev. 62, 1191–1243
6. Toya, J. H., Johnson, A. L., Donovan, J. D., Toone, W. M., and Johnston, L. H. (1997) Genetics 145, 85–96
7. Visintin, R., Hwang, E. S., and Amon, A. (1999) Nature 398, 818–823
8. Shou, W., Seol, J. H., Shevchenko, A., Baskerville, C., Moazed, D., Chen, Z. W., Yang, J., Shevchenko, A., Charbonneau, H., and Deshaies, R. J. (1999) Cell 97, 233–244
9. Nunez, A., Kaufman, W. K., Wilson, E., Thuley, E., Wang, M., and Amon, A. (1994) J. Cell Biol. 126, 743–758
10. Wang, Y., and Prives, C. (1995) Nature 376, 88–91
11. Moll, T., Tebb, G., Surana, U., Robitsch, H., and Nasmyth, K. (1991) Cell 66, 743–758
12. Gottlin, E. B., Xu, X., Epstein, D. M., Burke, S. P., Eckstein, J. W., Ballou, D. P., and Dixon, J. E. (1996) J. Biol. Chem. 271, 27445–27449
13. Ko, L. J., and Prives, C. (1996) Genes Dev. 10, 1054–1072
14. Bischoff, J. R., Friedman, P. N., Marshak, D. R., Proves, C., and Beach, D. (1990) Proc. Natl. Acad. Sci. U.S.A. 87, 4766–4770
15. Meek, D. W., Simon, S., Kikkawa, U., and Eckhart, W. (1990) EMBO J. 9, 3253–3260
16. Herrmann, C. P., Kraiss, S., and Montenarh, M. (1991) Oncogene 6, 877–884
17. Jaspersen, S. L., Charles, J. F., and Morgan, D. O. (1999) Curr. Biol. 9, 227–236
18. Verma, R., Feldman, R. M., and Deshaies, R. J. (1997) Mol. Cell 1, 47–57
19. Jans, P. (1996) J. Biol. Chem. 271, 709–718
20. Jans, D. A., Moll, T., Nasmyth, K., and Jans, P. (1995) J. Biol. Chem. 270, 17064–17067
Cdc14 Proteins Dephosphorylate p53

20. O'Connor, P. M. (1997) *Cancer Surv.* **29**, 151–182
21. Cross, S. M., Sanchez, C. A., Morgan, C. A., Schimke, M. K., Ramel, S., Idzerda, E. L., Raskind, W. H., and Reid, B. J. (1995) *Science* **267**, 1353–1356
22. Chang, T. H., Ray, F. A., Thompson, D. A., and Schlegel, R. (1997) *Oncogene* **14**, 2383–2393
23. Bunz, F., Dutriaux, A., Lengauer, C., Waldman, T., Zhou, S., Brown, J. P., Sedivy, J. M., Kinzler, K. W., and Vogelstein, B. (1998) *Science* **282**, 1497–1500
24. Barbeoule, N., Chadebech, P., Baldin, V., Vidal, S., and Valette, A. (1997) *Oncogene* **15**, 2867–2875
25. Guillouf, C., Roselli, F., Kirshnaraju, K., Moustacchi, E., Hoffman, B., and Lieberman, D. A. (1995) *Oncogene* **10**, 2263–2270
26. Hupp, T. R., Meek, D. W., Midgley, C. A., and Lane, D. P. (1993) *Nucleic Acids Res.* **21**, 3167–3174
27. Kapoor, M., and Loranzo, G. (1998) *Proc. Natl. Acad. Sci. U. S. A.* **95**, 2834–2837
28. Lu, H., Taya, Y., Ikeda, M., and Levine, A. J. (1998) *Proc. Natl. Acad. Sci. U. S. A.* **95**, 6399–6402
29. Yan, Y., Shay, J. W., and Wright, W. E., and Munby, M. C. (1997) *J. Biol. Chem.* **273**, 15220–15226
30. Zuo, Z., Dean, N. M., and Honkanen, R. E. (1998) *J. Biol. Chem.* **273**, 12250–12258
31. Lin, W. C., and Desiderio, S. (1998) *Science* **266**, 953–959
32. Ashcroft, M., Kibballat, M. H., and Vousden, K. H. (1999) *Mol. Cell. Biol.* **19**, 1751–1758
33. Hengstermann, A., Whitaker, N. J., Zimmer, D., Zentgraf, H., and Scheffner, M. (1998) *Oncogene* **17**, 2953–2961
34. Liang, S. H., Hong, D., and Clarke, M. F. (1998) *J. Biol. Chem.* **273**, 19817–19821
35. Stommel, J. M., Marchenko, N. D., Jimenez, G. S., Moll, U. M., Hope, T. J., and Wahl, G. F. (1999) *EMBO J.* **18**, 1660–1672
36. Freedman, D. A., and Levine, A. J. (1998) *Mol. Cell. Biol.* **18**, 7288–7293
37. Lain, S., Midgley, C., Sparks, A., Lane, E. B., and Land, D. P. (1999) *Exp. Cell. Res.* **248**, 457–472