Plk3 Functionally Links DNA Damage to Cell Cycle Arrest and Apoptosis at Least in Part via the p53 Pathway*

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Polo-like kinase 3 (Plk3, previously termed Pkr) contributes to regulation of M phase of the cell cycle (Ouyang, B., Pan, H., Lu, L., Li, J., Stambrook, P., Li, B., and Dai, W. (1997) *J. Biol. Chem.* 272, 28646–28651). Plk3 physically interacts with Cdc25C and phosphorylates this protein phosphatase predominantly on serine 216 (Ouyang, B., Li, W., Pan, H., Meadows, J., Hoffmann, I., and Dai, W. (1999) *Oncogene* 18, 6029–6036), suggesting that the role of Plk3 in mitosis is mediated, at least in part, through direct regulation of Cdc25C. Here we show that ectopic expression of a kinase-active Plk3 (Plk3-A) induced apoptosis. In response to DNA damage, the kinase activity of Plk3 was rapidly increased in an ATM-dependent manner, whereas that of Plk1 was markedly inhibited. Recombinant Plk3 phosphorylated in vitro a glutathione S-transferase fusion protein containing p53, but not glutathione S-transferase alone. Recombinant Plk1 also phosphorylated p53 but on residues that differed from those targeted by Plk3. Co-immunoprecipitation and pull-down assays demonstrated that Plk3 physically interacted with p53 and that this interaction was enhanced upon DNA damage. In vitro kinase assays followed by immunoblotting showed that serine 20 of p53 was a target of Plk3. Furthermore, expression of a kinase-defective Plk3 mutant (Plk3K52R) resulted in significant reduction of p53 phosphorylation on serine 20, which was correlated with a decrease in the expression of p21 and with a concomitant increase in cell proliferation. These results strongly suggest that Plk3 functionally links DNA damage to cell cycle arrest and apoptosis via the p53 pathway.

In mammals, DNA damage induced by ionizing radiation or UV light is detected by two serine/threonine kinases known as "mutated in ataxia telangiectasia" (ATM) and "ATM-related" (ATR) (3–5). Depending on the extent of DNA damage, cells either undergo cell cycle arrest or initiate apoptosis, responses that are at least partly mediated by p53 (4–6). Activated ATM and ATR phosphorylate p53 on serine 15 (7–9), thereby contributing to the activation of the tumor suppressor protein. The kinases Chk1 and Chk2, which act downstream of ATR and ATM, respectively (10–12), are reported to phosphorylate p53 in vitro on serine 20 (13–14); this residue is located within the domain of the protein that interacts with HDM2, resulting in stabilization of the normally short-lived p53 protein in response to DNA damage (15).

Polo family kinases also play a role in the DNA damage response (11, 16, 18). Cdc5, a polo homolog in budding yeast, is modified in its mobility on denaturing gels in response to DNA damage, and this modification is dependent on MEC1, Rad53 (a Chk homolog), and Rad9 (16). In addition, a functionally defective Cdc5 mutant suppresses a Rad53 checkpoint defect, whereas overexpression of Cdc5 overrides checkpoint-induced cell cycle arrest (11). Moreover, DNA damage appears to interfere with the activation of Plk1 in mammals, resulting in down-regulation of the kinase activity of this protein (18). On the other hand, expression of Plk1 mutants that are nonresponsive to DNA damage overrides G2 arrest. Mammalian Plk3 is a structural homolog of Plk1 (19), and its expression is down-regulated in several types of cancer (19, 20). Both Plk1 and Plk3 can rescue the temperature-sensitive phenotype of yeast Cdc5 mutants (1, 21). However, evidence indicates that Plk3 functions differently from Plk1 in regulation of cell proliferation and oncogenesis in mammalian cells (1, 2, 20).

We have previously shown that Plk3 phosphorylates Cdc25C on serine 216 (2), a site that is also targeted by Chk1 and Chk2 (10, 22). Phosphorylation of serine 216 of Cdc25C is inhibitory, which is due to sequestration of the protein phosphatase in the cytoplasm by 14-3-3 protein (24). In this report, we have provided evidence indicating that Plk3 is involved in DNA damage checkpoint response and that it may target p53 in vivo through regulation of phosphorylation on serine 20. A model is proposed that explains the mechanism of action of Plk3 during genotoxic stress-induced activation of the DNA damage checkpoint, which results in cell cycle arrest and/or apoptosis.

**EXPERIMENTAL PROCEDURES**

*Immunoblotting and Pull-down Assays—Various cell lines were obtained from ATCC, except for the GM00637 cell line, which was originally from the Coriell Institute for Medical Research. Cells treated with adriamycin (100 μM) for 30 min were collected and lysed (1). Equal amounts (50 μg) of protein lysates from various cell lines were analyzed by SDS-polyacrylamide gel electrophoresis followed by immunoblotting with antibodies to human Plk3 (Pharmingen) (2, 25), α-tubulin (Sigma), and p53 (Santa Cruz Biotechnology, Inc. (Santa Cruz, CA) and Phar-}

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The abbreviations used are: ATM, mutated in ataxia telangiectasia; ATR, ATM-related; GST, glutathione S-transferase; NTA, nitrilotriace-
rylated p53 (New England Biolabs). The p53 antigens were detected with horseradish peroxidase-conjugated goat secondary antibodies (Sigma) and enhanced chemiluminescence reagents (Amersham Pharmacia Biotech). Each experiment was repeated at least three times.

**Co-immunoprecipitation Analysis**—GM00637 cells lysates (1 mg of protein) were incubated for 30 min at 4 °C in a humid chamber for 90 min. After reaction, cells were stained with 4,6-diamidino-2-phenylindole (1 mg/ml) for 5 min. Fluorescence microscopy was performed on an Olympus ATTO microscope (Spot Diagnostic Instrument), and images were captured using a digital camera using Image System Spot RT software. GM00637 cells transfected with Plk3-A, Plk3K52R, or the vector alone for 1 day were also collected for genomic DNA isolation. The isolated DNA (10 μg/lane) was analyzed on agarose gels.

**RESULTS**

Plk3 is a structurally conserved protein serine/threonine kinase playing an important role in the regulation of M phase function (1). A recent study indicates that overexpression of Plk3 induces chromatin condensation and programmed cell death (25). To confirm the role of Plk3 in induction of apoptosis, we transfected GM00637 fibroblast cells with a plasmid construct expressing kinase-active Plk3 (Plk3-A) (1, 2). DNA fragmentation analyses (Fig. 1) revealed that transfection of Plk3-A, but not the vector alone, induced significant DNA fragmentation, indicative of apoptosis. Transfection of Plk3K52R in which lysine 52 was replaced with arginine also induced DNA fragmentation, albeit it was less significant than that of Plk3-A (Fig. 1B).

Recent studies have shown that phosphorylation of Cdc25C is inhibitory (22, 24). The observations that Plk3 phosphorylates serine 216 of Cdc25C (2) and that polo family kinases contribute to regulation of the DNA damage checkpoint (11, 16, 18) prompted us to investigate whether the kinase activity of Plk3 is affected in cells subjected to DNA damage. Immunocomplex kinase assays with α-casein as substrate revealed that the kinase activity of Plk3 was increased more than 10-fold by exposure of A549 cells to the DNA-damaging agent adriamycin (Fig. 2, A and B). A549 cells were used because they expressed good levels of Plk3. The activation of Plk3 in response to other genotoxic stresses such as UV or H2O2 was also detected (data not shown). Immunoblot analysis indicated (Fig. 2A) that p53 kinase antigens were not increased upon adriamycin treatment, suggesting that the increase of Plk3 kinase activity was due to a post-translational mechanism(s). Given that the kinase activity of Plk1 has been shown to be down-regulated during activation of the DNA damage checkpoint (18) and that the antibody to Plk3 used for our immunocomplex kinase assay did not cross-react with human Plk1 (Fig. 2C), we measured the kinase activities of both Plk1 and Plk3 in the same A549 cells treated with adriamycin. Whereas little endogenous Plk3 kinase activity was detected under control conditions (Fig. 2D, lane 1), activation of Plk3 was apparent 10 min after exposure of the cells to adriamycin; in contrast, Plk1 was constitutively active under basal conditions (Fig. 2D, lane 4), and its activity was markedly inhibited in response to adriamycin treatment (lanes 5 and 6). Thus, Plk1 and Plk3 appear to be differentially regulated in response to DNA damage.

To examine whether the DNA damage-induced activation of Plk3 is dependent on ATM, we exposed A549 cells that had been pretreated with caffeine, which inhibits the kinase activities of ATM and ATR, not adriamycin. Caffeine not only blocked the activation of Plk3 by adriamycin, but also inhibited the basal kinase activity of this protein (Fig. 3, A and B). To further confirm the dependence of Plk3 activation on ATM, we analyzed the Plk3 kinase activity in an ATM-deficient cell line (ATCC number CRL-7201) that had been treated with adriamycin for various times. No increase in Plk3 activity upon DNA damage was detected in the ATM-deficient cells (Fig. 3, C and D, lanes 2–4).

**DNA Fragment End Labeling Assay**—End labeling of DNA fragments was performed using a kit purchased from Oncogene Research Products (Boston, MA) according to the protocol provided by the manufacturer. Briefly, GM00637 cells cultured on cover slips were transfected with Plk3-A expression construct or with the vector alone for 18 h. The transfected cells as well as the untransfected parental cells were washed once with phosphate-buffered saline and then sequentially fixed in 4% paraformaldehyde and 80% ethanol for 10 min each. The fixed cells were rehydrated in Tris-buffered saline for 10 min and then treated with proteinase K (2 mg/ml) for 4 min. The treated cells were incubated in the labeling reaction mixture containing terminal deoxynucleotidyltransferase at 37 °C in a humid chamber for 90 min. After reaction, cells were stained with 4,6-diamidino-2-phenylindole (1 mg/ml) for 5 min. Fluorescence microscopy was performed on an Olympus ATTO microscope (Spot Diagnostic Instrument), and images were captured using a digital camera using Image System Spot RT software. GM00637 cells transfected with Plk3-A, Plk3K52R, or the vector alone for 1 day were also collected for genomic DNA isolation. The isolated DNA (10 μg/lane) was analyzed on agarose gels.

**Immunocomplex Kinase Assays**—Immunocomplex kinase assays were performed essentially as described (1). In brief, A549 or ATM-deficient (ATCC number CRL-7201) (23) cells were exposed to adriamycin for various times, lysed, and subjected to immunoprecipitation with antibodies to Plk3 or to Plk1 (Zymed Laboratories Inc.). The resulting precipitates were resuspended in kinase buffer (10 mM Hepes, pH 7.4, 10 μM MnCl2, 5 mM MgCl2), and the kinase reaction was initiated by the addition of [γ-32P]ATP (2 μCi) (Amersham Pharmacia Biotech). After incubation for an additional 2 h at room temperature or overnight at 4 °C. Proteins were then added to each immunoprecipitation mixture, and the incubation was continued for 1 h at room temperature. Immunoprecipitates were collected by centrifugation, washed three times with the cell lysis buffer, and subjected to immunoblot analysis with a monoclonal antibody to Plk3.

**Tryptic Peptide Mapping**—Tryptic peptide mapping was performed essentially as described (2, 26). In brief, 32P-labeled p53 was excised from dried SDS-polyacrylamide gels and eluted into extraction buffer (50 mM NH4HCO3, 1% SDS, 50 mM 2-mercaptoethanol). After removal of debris by centrifugation, the eluted protein was supplemented with 100 μg of acetylated bovine serum albumin (Sigma) and then precipitated by the addition of trichloroacetic acid. The protein precipitate was recovered by centrifugation, washed once with ethanol, dissolved in performic acid (95% formic acid, 30% H2O2) 9:1 (v/v), and lyophilized. The dried protein was resuspended in 50 mM NH4HCO3, pH 8.0, and subjected to digestion with 1-1-tosylamide-2-phenylethyl chloromethyl ketone-treated trypsin (Sigma). The resulting peptides were applied to thin layer chromatography plates and fractionated by electrophoresis followed by chromatography as described (26) with a Multiphor II (Amersham Pharmacia Biotech) apparatus. The plates were then air-dried and subjected to autoradiography. The mapping experiment was repeated at least three times.

**Transient Transfection**—HeLa cells were transfected, using the LipofectAMINE method (Life Technologies, Inc.), with pCR259Plk3 K52R (1, 20), pCDNA3-p53, pCDNA3-p53K52R, or the empty vectors (Invitrogen). The serine 20 mutant (serine 20 was replaced with alanine) of p53 was obtained via site-directed mutagenesis using a kit purchased from Stratagene according to the protocol provided by the supplier. One day after transfection, cells were treated with or without 100 μM adriamycin for 1 h. Cell lysates were prepared and blotted for Plk3, p53, or p21 expression. Two different antibodies to p53 were used. One was from Santa Cruz Biotechnology (DO-1, the recognition epitope of which is between residues 11 and 25), and the second one was from Pharmingen (G59-12). To determine the effect of p53 or Plk3K52R on overall cell growth, HeLa cells transfected with p53, p53K52R, and/or Plk3K52R were cultured in medium containing G418 (600 μg/ml) and maintained for 2 weeks. Colonies formed were visualized after staining with 0.125% crystal violet solution. As an alternative method to determine cell proliferation, triplicate transfected cells that were maintained in G418-containing medium (600 μg/ml) for 1 week were subject to 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide reduction assay as described (27). ODs at 570 nm were determined for each sample. Each treatment was repeated at least three times.
In addition to their reported phosphorylation of p53 on serine 20 (14, 29, 30), Chk1 and Chk2 phosphorylate serine 216 of Cdc25C (11, 22), phosphorylation of which is thought to be partly responsible for G2 arrest during activation of the DNA damage checkpoint. Given that Plk3 phosphorylates the same residue of Cdc25C (serine 216) as that targeted by Chk1 and Chk2, we investigated whether Plk3 also phosphorylates p53 in vitro. Recombinant histidine-tagged Plk3-A (His6-Plk3-A) phosphorylated GST-p53 (Fig. 4A, lane 2) but not GST alone (lane 5), indicating that Plk3 targets the p53 moiety of GST-p53. Plk3K52R phosphorylated GST-p53 to a greatly reduced extent (Fig. 4A, lane 3) compared with that observed with Plk3-A (lane 2). Recombinant (His6)-Plk1 also phosphorylated GST-p53 (Fig. 4A, lane 9) but not GST alone (data not shown). Unlike His6-Plk3, His6-Plk1 exhibited a high level of autophosphorylation activity (Fig. 4A, lane 9).

The biological functions of mammalian Plk3 and Plk1 appear to differ (2, 19, 20, 32, 33), although both mammalian proteins complement Cdc5 temperature-sensitive mutants of budding yeast (1, 21). Overexpression of murine Plk1 results in oncogenic transformation (33), whereas ectopic expression of human Plk3 inhibits cell growth by inducing apoptosis (25). Thus, our observation that both Plk3 and Plk1 phosphorylate p53 suggested that these two kinases may target different residues of the tumor suppressor protein. Whereas p53 phosphorylated by Plk3 yielded two major phosphopeptides in phosphopeptide mapping analysis, p53 phosphorylated by Plk1 yielded four major phosphopeptides (Fig. 4B). Further analysis of the phosphopeptide maps indicated that peptides a and e were specific to Plk3-phosphorylated p53 and that peptides b, c, and d were unique to Plk1-phosphorylated p53.

Our observation that Plk3 phosphorylates p53 in vitro suggested that the two proteins might physically interact in vivo. Co-immunoprecipitation experiments (Fig. 5A) revealed that both antibodies (from two different sources) to p53, but not control antibodies to CD45, precipitated Plk3 from GM00637 cell lysates. Furthermore, Ni2+-NTA resin conjugated with His6-Plk3, but not Ni2+-NTA resin alone, precipitated p53 from the cell lysates (Fig. 5B). The amount of p53 precipitated by the His6-Plk3-conjugated resin was markedly increased by prior exposure of the cells to adriamycin (Fig. 5B, lanes 3 and 4), although treatment with this drug for 30 min did not affect the total amount of p53 present in the cells (lanes 5 and 6). Proteins eluted from both His6-Plk3-conjugated resin and control resin were also blotted for the presence of phospho-p53. Fig. 5B shows that His6-Plk3 resin precipitated serine 20-phosphorylated p53 and that the amount of the phosphorylated p53 pulled down by His6-Plk3 was significantly increased following adriamycin treatment. These results thus suggest that activation of p53 promotes its interaction with Plk3.

Chk1 and Chk2, as well as Plk3, phosphorylate Cdc25C in serine 216 (2, 10, 22). The direct interaction of Plk3 with p53 and the observation that Chk1 and Chk2 also phosphorylate p53 on serine 20 prompted us to examine whether the serine 20 residue of p53 was a phosphorylation target of Plk3. In vitro kinase assays followed by immunoblotting showed (Fig. 5C, lane 2) that purified GST-p53 was not recognized by an antibody to serine 20-phosphorylated p53. However, GST-p53 phos-
 phosphorylated in vitro by Plk3 exhibited a strong phosphoserine 20 epitope (lane 3). Furthermore, when an equal amount of GST-p53S20A, a mutant in which serine 20 was replaced with alanine, was used as an in vitro substrate, no phosphoserine 20 epitope was detected (Fig. 5C, lane 4). Given that p53 serine 20 phosphorylation and Plk3 activation are induced by a variety of genome toxic stresses, these observations suggest that the serine 20 residue may be a direct target of Plk3.

To establish Plk3 serine 20 phosphorylation of p53 in vivo, we transfected both p53 and p53S20A expression plasmids into HeLa cells, which constitutively express Plk3 but not p53. Western blot analysis showed (Fig. 6A) that transfected p53, but not p53S20A mutant protein, was constitutively phosphorylated on serine 20. DNA damage induced by adriamycin significantly enhanced serine 20 phosphorylation of the transfected p53, indicating that the pathway leading to p53 serine 20 phosphorylation was intact, albeit endogenous p53 was inactivated in this tumor cell line.

To determine the effect of change in Plk3 activity on p53 phosphorylation, HeLa cells were transfected with plasmid constructs expressing p53 and Plk3K52R. Western blot analysis showed (Fig. 6B) that p53 was expressed and phosphorylated on serine 20 and that expression of Plk3K52R significantly reduced the level of p53 phosphorylation on serine 20 (lane 6). To determine the consequence of serine 20 phosphorylation of p53 on its target gene expression, we measured p21 level in cells expressing p53 and/or the Plk3 dominant mutant. We have consistently observed (Fig. 6B) that while little p21 was detected in parental HeLa cells or cells transfected with vectors alone (lanes 1 and 2), ectopic expression of p53 greatly induced p21 expression (lane 3). Expression of p53S20A also induced p21 expression that was about 25% of that induced by wild-type p53 (lane 4). Further, expression of the Plk3K52R greatly reduced the ability of p53 to induce p21 expression (compare lanes 3 and 6), which was consistent with the reduced serine 20 phosphorylation of p53 as well as p53 protein levels. These observations thus clearly demonstrate that phosphorylation of p53 on serine 20 plays a significant role in activating p21 expression.

We then determined the colony-forming efficiency of HeLa cells that were cotransfected with Plk3K52R and p53. Fig. 7A shows that p53 suppressed colony formation of HeLa cells. Plk3K52R alone also suppressed the colony formation. However, when cotransfected with p53, Plk3K52R significantly blocked p53-mediated suppression of colony formation of HeLa cells. Independent assays using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide method confirmed that Plk3K52R effectively blocked the ability of p53 to inhibit HeLa cell proliferation (Fig. 7B). It is interesting that Plk3K52R also had an effect on p53S20A in terms of cell growth. This effect was likely to be mediated by inhibition of phosphorylation of other serine/threonine sites by Plk3K52R because Plk3 phosphorylates p53 on multiple sites in vitro (Fig. 4B).

**DISCUSSION**

Our results demonstrate that Plk3 contributes to DNA damage checkpoint activation, which is at least partly mediated by regulating phosphorylation of p53. The adriamycin-induced activation of Plk3 was caffeine-sensitive and not observed in...
ATM-deficient cells, indicating the participation of ATM. Phosphorylation is thought to play an important role in regulation of the stability and activity of p53. This study suggests that serine 20 of p53 may be an in vivo target of Plk3 during DNA damage checkpoint activation because recombinant Plk3 phosphorylates p53 in vitro, resulting in a strong phosphoserine 20 epitope (Fig. 5C) and because it also interacts with serine 20-phosphorylated p53. Furthermore, expression of Plk3K52R in HeLa cells results in decreased phosphorylation of cotransfected p53, which was correlated with a significant reduction in p21 expression (Fig. 6B) with a concomitant increase in cell proliferation (Fig. 7).

Together with previous observations (2), our data suggest a simple and somewhat redundant set of mechanisms for DNA damage-induced signal transduction between ATM or ATR and effector molecules, resulting in cell cycle arrest and apoptosis (Fig. 8). According to this model, Plk3 may act in parallel with Chk1 and Chk2, downstream of ATM or ATR. It is possible that Plk3 preferentially transduces signals generated by a specific genotoxic stress, just as Chk1 and Chk2 are differentially activated by UV radiation and ionizing radiation, respectively (6). Alternatively, Plk3 may be activated by Chk2 (and/or Chk1), given that Cdc5 acts downstream of Rad53 in yeast (16). Plk3 may thus integrate the signals from ATM-Chk2 and ATR-Chk1 and induce cell cycle arrest or apoptosis by phosphorylating both p53 and Cdc25C. Consistent with this second scenario, Plk3 is activated by the ionizing radiation-mimetic drug adriamycin, UV radiation, and oxidative stress.2

A third possibility also exists to explain our observations; namely Chk1 and Chk2 preferentially target serine 20 of p53. In this case, p53, phosphorylated on serine 20 by Chk1 and Chk2, interacts with high affinity to Plk3, resulting in phosphorylation of p53 on additional sites. This would also place Plk3 downstream of Chk1/Chk2 in the DNA damage checkpoint pathway. It is possible that phosphorylation of p53 on other sites enforces the DNA damage checkpoint, but is not absolutely required. Although we observed that Plk3K52R expression reduced levels of serine 20 phosphorylation of p53, overexpression of this mutant protein may result in more efficient interaction with p53, potentially blocking access of p53 to

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Chk1 or Chk2. Therefore, the in vivo role of Plk3 in regulating serine 20 phosphorylation remains to be established, which would require obtaining Plk3 null cells.

Several other residues of p53 are potential sites of phosphorylation by Plk3. Phosphorylation of threonine 18 is induced by DNA damage, and a casein kinase 1-like enzyme is thought to phosphorylate this residue in A549 cells (31). However, the identity of this casein kinase 1-like enzyme is unknown. Given that Plk3, a casein-phosphorylating kinase, is activated by DNA damage, Plk3 may also target threonine 18 of p53. Moreover, given the apoptosis-inducing function of Plk3 (Fig. 1) and the observations both that residues 43–63 of p53 are necessary for induction of apoptosis by p53 (23) and that serine 46 phosphorylation is important for the transactivation of apoptosis-inducing genes by p53 (17), serine 46 may be another potential site of phosphorylation of p53 by Plk3.

Overexpression of Plk3-A induced a significant level of DNA fragmentation in GM00637 cells (Fig. 1), which is consistent with an early finding that Plk3 induces chromatin condensation and apoptosis (25). Given that p53 is an in vitro substrate of Plk3-A, it is reasonable to predict that Plk3-induced apoptosis may be mediated through phosphorylation and activation of p53. However, we were unable to detect enhanced serine 20 phosphorylation of p53 in HeLa cells cotransfected with Plk3-A and p53 expression constructs (data not shown). One explanation for this discrepancy is that the Plk3-A clone is missing a short nucleotide sequence encoding about 30 amino acid residues at the amino terminus when compared with that of murine counterpart (19). Although it is active in vitro, Plk3-A may be much less so than the cellular Plk3 toward in vivo targets. Thus, it is possible that Plk3-A may not behave as a "wild-type" protein in vivo, resulting in only a partial response to the upstream activators. Alternatively, HeLa cells may lack a cofactor(s) that is required for activation of Plk3, or the pathway leading activation of Plk3 is compromised.

It is interesting to note that Plk3K52R also suppressed colony
Fig. 7. Plk3K52R blocks p53-mediated suppression of cell proliferation. A, HeLa cells transfected with p53, p53S20A, and/or Plk3K52R expression constructs were cultured in medium containing G418. Colonies were visualized after staining with crystal violet after 2 weeks’ incubation. B, HeLa cells transfected with p53, p53S20A, and/or Plk3K52R expression constructs were cultured in the medium containing G418. After 1 week’s incubation, cell proliferation rate was measured by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. Relative growth rates (A570) were summarized from three independent experiments.

Fig. 8. A model for the regulation of cell cycle progression by Plk3 in response to DNA damage. Dashed arrows indicate the direct relationship between these two proteins is not confirmed experimentally.

formation (Fig. 7), which may be partly due to the fact that this mutant protein retains some residual kinase activities toward p53 (Fig. 4A) and other in vitro substrates such as Cdc25C (2) and α-casein.2 Given that Plk3-A differs from Plk3K52R by only one amino acid (lysine 52) and that it is more efficient than Plk3K52R in inducing cell cycle arrest and apoptosis (Fig. 1B),2 it is reasonable to conclude that the kinase activity of Plk3 is involved in the regulation of cell proliferation. On the other hand, as shown by the study of Conn et al. (25), the COOH-terminal half of Plk3 also plays an important role in induction of apoptosis. In addition, our recent studies indicate that Plk3 is concentrated at the centrosomal region during interphase and that Plk3 appears to be involved in the regulation of microtubule dynamics as well as centrosome function.3 Therefore, Plk3 may regulate cell proliferation through multiple mechanisms.

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REFERENCES
1. Ouyang, B., Pan, H., Lu, L., Li, J., Stambrook, P., Li, B., and Dai, W. (1997) J. Biol. Chem. 272, 28646–28651
2. Ouyang, B., Li, W., Pan, H., Meadows, J., Hoffmann, I., and Dai, W. (1999) Nat. Genet. 20, 398–400
3. Westphal, C. H. (1997) Curr. Biol. 7, R789–R792
4. Nakamura, Y. (1998) Nat. Med. 4, 1231–1232
5. de Klein, A., Muijtjens, M., van Os, R., Verhoeven, Y., Smit, B., Carr, A. M., Lehmann, A. R., and Hoeijmakers, J. H. (2000) Curr. Biol. 20, 479–482
6. Caspari, T. (2000) Curr. Biol. 20, R315–R317
7. Tibbetts, R. S., Brumbaugh, K. M., Williams, J. M., Sarkaria, J. N., Chiby, W. A., Shieh, S. Y., Taya, Y., Prives, C., and Abraham, R. T. (1999) Genes Dev. 13, 152–157
8. Nakagawa, K., Taya, Y., Tamai, K., and Yamaizumi, M. (1999) Mol. Cell. Biol. 19, 2829–2834
9. Khanna, K. K., Keating, K. E., Kozlov, S., Scott, S., Gatei, M., Hoshin, K., Taya, Y., Gabrielli, B., Chao, D., Lees-Miller, S. P., and Lavin, M. F. (1999) Nat. Genet. 20, 498–503
10. Matsuoka, S., Huang, M., and Elledge, S. J. (1998) Science 282, 1893–1897
11. Sanchez, Y., Bachant, J., Wang, H., Hu, F., Liu, D., Tetzlaff, M., and Elledge, S. J. (1999) Science 286, 1166–1171
12. Tominaga, K., Morisaki, H., Kaneo, Y., Fujimoto, A., Tanaka, T., Ohtsubo, M., Hirai, M., Okayama, H., Ikeda, K., and Nakanishi, M. (1999) J. Biol. Chem. 274, 31463–31467
13. Hirao, A., Kong, Y. Y., Matsuoka, S., Wakeham, A., Ruland, J., Yoshida, H., Liu, D., Elledge, S. J., and Mak, T. W. (2000) Science 287, 1824–1827
14. Shieh, S. Y., Ahn, J., Tamai, K., Taya, Y., and Prives, C. (2000) Genes Dev. 14, 289–300
15. Chehab, N. H., Malikzay, A., Appel, M., and Halazonetis, T. D. (2000) Genes Dev. 14, 278–288
16. Cheng, L., Hunke, L., and Hardy, C. F. J. (1998) Mol. Cell. Biol. 18, 7360–7370
17. Oda, K., Arakawa, H., Tanaka, T., Matsuda, K., Tanikawa, C., Mori, T., Nishimori, H., Tamai, K., Tokino, T., Nakamura, Y., and Taya, Y. (2000) Cell 102, 849–862
18. Smits, V. A., Klempnauer, R., Arnaud, L., Rijken, G., Nigg, E. A., and Medema, R. H. (2000) Nat. Cell Biol. 2, 672–676
19. Li, B., Ouyang, B., Pan, H., Reissmann, P. T., Slamon, D. J., Arceci, R., Lu, L., and Dai, W. (1996) J. Biol. Chem. 271, 19402–19408

3 Q. Wang, S.-Q. Xie, J. Chen, K. Fukasawa, F. Traganos, Z. Darzynekiewicz, M. Jhanwar-Uniyal, and W. Dai, submitted for publication.
20. Dai, W., Li, Y., Ouyang, B., Pan, H., Reissmann, P., Li, J., Wiest, J., Stambrook, P., Gluckman, J. L., Noffsinger, A., and Bejarano, P. (2000) *Genes Chromosomes Cancer* 27, 332–336.
21. Lee, K. S., and Erikson, R. L. (1997) *Mol. Cell. Biol.* 17, 3408–3417.
22. Sanchez, Y., Wong, C., Thoma, R. S., Richman, R., Wu, Z., Piwnica-Worms, H., and Elledge, S. J. (1997) *Science* 277, 1497–1501.
23. Zhu, J., Zhang, S., Jiang, J., and Chen, X. (2000) *J. Biol. Chem.* 275, 39927–39934.
24. Peng, C. Y., Graves, P. R., Thoma, R. S., Wu, Z., Shaw, A. S., and Piwnica-Worms, H. (1997) *Science* 277, 1501–1505.
25. Conn, C. W., Hennigan R. F., Dai W, Sanchez, Y., and Stambrook, P. (2000) *Cancer Res.* 60, 6826–6831.
26. Boyle, W. J., van der Geer, P., and Hunter, T. (1991) *Methods Enzymol.* 201, 119–149.
27. Hussain, R. F., Nouri, A. M., and Oliver, R. T. (1993) *J. Immunol. Methods* 160, 89–96.
28. Sarkaria, J. N., Busby, E. C., Tibbetts, R. S., Roos, P., Taya, Y., Karnitz, L. M., and Abraham, R. T. (1999) *Cancer Res.* 59, 4375–4382.
29. Haggman, M. J., Wojno, K. J., Pearsall, C. P., and Macoska, J. A. (1997) *Urology* 50, 643–647.
30. Kagan, J., Stein, J., Babaian, R. J., Joe, Y. S., Pisters, L. L., Glassman, A. B., von Eschenbach, A. C., and Troncoso, P. (1999) *Oncogene* 11, 2121–2126.
31. Sakaguchi, K., Saito, S., Higashimoto, Y., Roy, S., Anderson, C. W., and Appella, E. (2000) *J. Biol. Chem.* 275, 9278–9283.
32. Knecht, R., Elez, R., Oechler, M., Solbach, C., von Ilberg, C., and Strehlhardt, K. (1999) *Cancer Res.* 59, 2794–2797.
33. Smith, M. R., Wilson, M. L., Hamanaka, R., Chase, D., Kung, H., Longo, D. L., and Ferris, D. R. (1997) *Biochem. Biophys. Res. Commun.* 19, 397–405.