Proteins of the poly(ADP-ribose) polymerase (PARP) family play a wide array of functions, covering virtually every aspect of DNA metabolism and function, most notably with the response to DNA damage, transcription, and the maintenance of genomic stability. Here we report the identification and characterization of a novel PARP family member, PARP10 (FLJ14464 or hypothetical protein LOC84875). Overexpression of PARP10 results in loss of cell viability, although down-expression by short hairpin RNA leads to unknown. PARP10 exists as the major consumer of NAD metabolism and function, most notably with the response to DNA damage, transcription, and facilitating DNA repair, and regulating gene transcription, and facilitating DNA repair, and remains as the major consumer of NAD** accounting for more than 90% poly(ADP-ribose) production (1–3). Eighteen members of the PARP family have been defined (4), including PARP1, PARP2 (5), PARP3 (6), PARP4 (VPARP, AAD47250) (7), PARP5a (tankyrase-1, GenBank™ accession number NP_003738) (8), PARP5b (tankyrase-2, GenBank™ accession number XP_018395), PARP8 (FLJ21308; XP_018395), PARP9 (RAL; GenBank™ accession number NP_116178; BAB55067), PARP11 (GenBank™ accession number AAAP1391), PARP12 (zin signaling), PARP13 (CAE11868), PARP14 (KIAA1286; GenBank™ accession number XP_291055), PARP15 (GenBank™ accession number XP_093336), and PARP16 (GenBank™ accession number XP_113792; FLJ20509). With the exception of PARP members 1–5, the biological functions of most of these members are completely unknown. PARP2 is also part of the DNA repair pathway (5). Moreover, when both parp-1 and parp-2 genes are disrupted, the mouse embryo dies early in development at the onset of gastrulation. A specific female embryonic lethality is observed when parp-1 null and parp-2 heterozygous mice were created, associated with specific X-chromosome instability (12). PARP3 is present in the centrosome throughout the cell cycle and is always localized to the daughter centriole. Overexpression of PARP3 prevents centriole duplication and causes a G1/S cell cycle block (13). VPARP (PARP4) is a component of cytoplasmic vault particle. During mitosis it co-localizes with the mitotic spindle; it is also found in the nucleus and in the telomeres together with TEP1 (14). Tankyrase 1 associates with TRF1 and regulates telomere length (15). It is also found in the centrosomes and at nuclear pores. 2,3,7,8-tetrachloro-dibenzo-p-dioxin-induced PARP (TPARP; PARP7), BAL protein (PARP9), and hyprophilic proteins of mouse (BAC25913, GenBank™ accession numbers XP_291055 and NP_766481), rat (GenBank™ accession numbers XP_216963 and XP_224241), and human PARP10, PARP11, PARP12, PARP13, PARP14, and PARP15 belong to a closely related subfamily of PARP (16). Another TPARP-related protein, human ZAP (PARP13) (GenBank™ accession number NP_064004), may be involved in the resistance to viral infection. Studies of the rat homolog indicate that the protein may primarily function to inhibit viral gene expression and induces an innate immunity to viral infection (17).

CDK10 was discovered serendipitously during our investigation of functions of CDK-phosphorylated Eg5 protein. The phosphorylation of threonine residue 927 (Thr-927) of Eg5 by CDK1 has been shown important for targeting to the microtubule spindle and for the functions of Eg5 in mitosis (18, 19). To further study the functions of Eg5 by Thr-927 phosphorylation, we have developed phospho-specific antibodies to a peptide with sequence surrounding phospho-Thr-927. In addition to the phospho-Eg5 detected during mitosis, we observed a cross-reactive species with a slightly higher molecular weight that appears during the G1/S and M phase. We identify and functionally characterize the polypeptide as a newly defined member of the PARP superfamily, PARP10 (also known as FLJ14464). It localizes both in the cytoplasm and the nucleus, with a subpopulation that concentrates in the nucleolus during late G1/S and acquires CDK2-dependent phosphorylation by CDK2-cyclin E in vitro. CDK-phosphorylated PARP10 is absent in growth-arrested cells. These results suggest that PARP10 functions in cell proliferation and may serve as a marker for proliferating cells.

Poly(ADP-ribose) polymerase-1 (PARP1) catalyzes the covalent attachment of ADP-ribose units from NAD* to itself and to a number of proteins involved in chromatin architecture (e.g. histones H1, H2B, high mobility group proteins, lamin B) and DNA metabolism (DNA replication factors), resulting in the loss of their affinity for DNA (1). The catalytic domain of PARP1 is located in the 40-kDa fragment of the C-terminal region, which shares homology and defines the PARP superfamily (2). PARP1 has been shown to participate in fundamental biological activities, including safeguarding genomic integrity, regulating gene transcription, and facilitating DNA repair, and remains as the major consumer of NAD metabolism accounting for more than 90% poly(ADP-ribose) production (1–3). Eighteen members of the PARP family have been defined (4), including PARP1, PARP2 (5), PARP3 (6), PARP4 (VPARP, AAD47250) (7), PARP5a (tankyrase-1, GenBank™ accession number NP_003738) (8), PARP5b (tankyrase-2, AAK82330) (9), PARP5c (tankyrase 3, BAB14665), PARP6 (BAB14092), PARP7 (TPARP; CAB45747) (10), PARP8 (FLJ21308; XP_018395), PARP9 (RAL; GenBank™ accession number NP_116178; BAB55067), PARP11 (GenBank™ accession number AAAP1391), PARP12 (zin signaling), PARP13 (CAE11868), PARP14 (KIAA1286; GenBank™ accession number XP_291055), PARP15 (GenBank™ accession number XP_093336), and PARP16 (GenBank™ accession number XP_113792; FLJ20509). With the exception of PARP members 1–5, the biological functions of most of these members are completely unknown. PARP2 is also part of the DNA repair pathway (5). Moreover, when both parp-1 and parp-2 genes are disrupted, the mouse embryo dies early in development at the onset of gastrulation. A specific female embryonic lethality is observed when parp-1 null and parp-2 heterozygous mice were created, associated with specific X-chromosome instability (12). PARP3 is present in the centrosome throughout the cell cycle and is always localized to the daughter centriole. Overexpression of PARP3 prevents centriole duplication and causes a G1/S cell cycle block (13). VPARP (PARP4) is a component of cytoplasmic vault particle. During mitosis it co-localizes with the mitotic spindle; it is also found in the nucleus and in the telomeres together with TEP1 (14). Tankyrase 1 associates with TRF1 and regulates telomere length (15). It is also found in the centrosomes and at nuclear pores. 2,3,7,8-tetrachloro-dibenzo-p-dioxin-induced PARP (TPARP; PARP7), BAL protein (PARP9), and hyprophilic proteins of mouse (BAC25913, GenBank™ accession numbers XP_291055 and NP_766481), rat (GenBank™ accession numbers XP_216963 and XP_224241), and human PARP10, PARP11, PARP12, PARP13, PARP14, and PARP15 belong to a closely related subfamily of PARP (16). Another TPARP-related protein, human ZAP (PARP13) (GenBank™ accession number NP_064004), may be involved in the resistance to viral infection. Studies of the rat homolog indicate that the protein may primarily function to inhibit viral gene expression and induces an innate immunity to viral infection (17).
CDK-dependent Activation of PARP10

ribose) polymerase activity, although the holomolecule depends on its phosphorylation by CDK2-cyclin E for enzyme activity. Together, our results suggest that cell cycle-dependent phosphorylation of PARP10 by CDK2 plays crucial functions in cell proliferation.

EXPERIMENTAL PROCEDURES

Cell Culture—HeLa, HaCaT, and SK-N-MC cells were cultured in Dulbecco’s modified Eagle’s medium, supplemented with 1 mM l-glutamine, 100 units/ml penicillin, 100 µg/ml streptomycin, and 10% (v/v) fetal bovine serum (Hyclone) in an humidified atmosphere of 5% CO₂ at 37 °C. All the above-mentioned reagents, with the exception of fetal calf serum, were purchased from Invitrogen.

Antibodies and Immunoadsorption—Monoclonal antibodies to PARP10 (clone 19F7), Eg5 (clone 9D1), and tubulin α (10D8) were generated by FO myeloma cell fusion with BALB/c splenocytes immunized with recombinant proteins corresponding to the N-terminal 144-amino acid region of PARP10, the 320–530-amino acid region of Eg5, and full-length tubulin α, respectively. Monoclonal antibodies against hNopp140 and nucleolin were gifts from Dr. N. S. Yeh, and the human autoimmune serum against RNApol was a gift from Dr. G. R. Harvey. The mouse anti-PCNA1 (clone PC10) was obtained from BioLegend, and anti-tubulin β was from NeoMarkers. Direct cell lysates were harvested with 4× SDS sample buffer containing 6 M urea at 95 °C, then subjected to SDS-PAGE, and blotted onto polyvinylidene difluoride membranes (Millipore). Membranes were blocked with 1% BSA in TBS, 0.1% Tween 20 for 1 h at room temperature and hybridized with the primary antibody overnight at 4 °C. After washing three times with TBS, 0.1% Tween 20, the membrane was incubated with the corresponding horseradish peroxidase-conjugated secondary antibody diluted in TBS, 0.1% Tween 20 for 1 h at room temperature. The blots were visualized using the enhanced chemiluminescence system (PerkinElmer Life Sciences). For immunoprecipitation assays, cells were treated with 5 mM okadaic acid and 100 mM calcium A for 30 min and then harvested in 4× RIPA buffer supplemented with protein phosphatase inhibitor set II (all from Calbiochem), 40 µM sodium orthovanadate (Sigma), and Complete Protease Inhibitors Mixture (Roche Applied Science). Approximately 5 µg of antibody cross-linked to protein G beads were incubated for 6 h with the lysates diluted to 1× RIPA with all the supplements included.

Immunofluorescence Staining—Cells were seeded at 50% confluency on acid-treated glass coverslips, fixed with 4% paraformaldehyde in PBS for 10 min, permeabilized for 5 min with 0.5% Triton X-100, then blocked in PBS containing 0.1% BSA and 0.1% Triton X-100 for 30 min, and incubated with the indicated antibodies for 1 h. Cells were washed three times with PBS and then incubated with goat anti-mouse IgG or goat anti-rabbit IgG secondary antibody coupled to Alexa® 488 or Alexa® 594 dyes (Molecular Probes) or Texas Red-conjugated goat anti-human IgG (The Jackson Laboratory) for 40 min. Cells were counterstained with DAPI to highlight the nuclei and examined under a Leica DM RD immunofluorescence microscope. Images were captured with a cool CCD, processed by CoolSNAP software (Roper Scientific Inc.).

In Vitro CDK Phosphorylation Assay—In vitro-activated CDKs were purified from insect SF21 cells by co-infection of recombinant baculoviruses encoding human CDKs and His-tagged cyclins using standard nickel column techniques, and calibrated to equal kinase activity toward myelin basic protein substrate protein by liquid scintillation assay. In vitro phosphorylations of fusion proteins were carried out by incubating 100 ng of the purified substrate proteins in a total volume of 20 µl with recombinant kinase for 30 min at 30 °C in kinase buffer (50 mM Tris (pH 7.4), 10 mM MgCl₂, 6 mM EGTA, and 10 µM ATP).

Chromosome Spreads—HeLa cells were treated with 0.5 µg/ml colcemid for 60 min, harvested by trypsinization, hypotonically swollen in 10 mM Tris-HCl (pH 7.4), 10 mM NaCl, and 5 mM MgCl₂, and sedimented onto coverslips for 15 s at 900 × g. Chromosomes were swollen for an additional 15 min in 25% PBS, fixed in 3.7% formaldehyde in 25% PBS for 10 min, and permeabilized with 0.5% Nonidet P-40 in 25% PBS for 10 min. Samples were blocked with 1% bovine serum albumin (BSA) in PBS and then processed as described under “Immunofluorescence Staining.”

PARP Activity Assay—The cDNA sequence encoding the catalytic domain (amino acids 823–1025) of PARP10 was amplified by PCR and inserted into the pPRET B expression vector. The His-tagged protein was purified on a nickel column. Reactions contained 0.5 µg of purified recombinant protein or endogenous PARP10 immunoprecipitated with 19F7. Poly(ADP-ribosyl)ation activity assays were carried out as described previously (20), with incubation carried out at 25 °C for 30 min in 50 µl of assay buffer (50 mM Tris-HCl (pH 8.0), 4 mM MgCl₂, 0.2 mM dithiothreitol, and 1 mM NAD⁺). Some assays contained the PARP inhibitor 3-aminobenzamide (3AB) at 1 mM final concentration or 0.1 µCi of [³²P]NAD⁺ (Amersham Biosciences).

shRNA Construction—The DNA sequence corresponding to PARP10 nucleotides 215–236 was subcloned into the RNA interference-ready pSIREN-RetroQ vector (BD Biosciences) and mixed at 10:1 molar ratio with pEGFP-C1 (Clontech) for transfection into HeLa cells using Lipofectamine 2000 (Invitrogen) according to the manufacturer’s instructions. Suppression of PARP10 after specific time points was analyzed by Western blotting, as described previously, and by RT-PCR using the AccessQuick RT-PCR system (Promega). For PARP10, the specific primers used were 5’-ATGGTTGCAATGGGGAGCCA-GA-3’ and 5’-TCCAGGTCAACTCCAGCAACA-3’; for human glyceraldehyde-3-phosphate dehydrogenase, the primers were 5’-CATCAGTCCCACCAGAGCTTGGA-3’ and 5’-TACTCCTTGGAGGCCATGTAGCAGTCAT-3’.

Flow Cytometry—Transfected HeLa cells were harvested at the times indicated, stained with propidium iodide (Roche Applied Science), and analyzed by Epics Elite Flow Cytometer (Coulter). The multicyle (M cycle) program for cell cycle distribution (Phoenix Flow Systems, Inc.) was used to analyze the data.

RESULTS

The T927-101 Antibody Recognizes Both Phosphorylated Eg5 and PARP10—In an attempt to explore the functional implications of CDK phosphorylation on the Thr-927 residue of human Eg5, we raised a rabbit polyclonal antibody using synthetic phosphopeptide as antigen (Fig. 1A). The affinity-purified T927 antibody recognized two distinct bands with apparent molecular masses of 110 and 130 kDa in Western blot assays using direct cell lysates from HeLa cells (Fig. 1B, left panel). Probing with the monoclonal anti-Eg5 antibody 9D1 indicated that the 110-kDa band corresponds to the Eg5 protein phosphorylated at Thr-927 during mitosis (Fig. 1B, middle panel). Because the anti-Eg5 antibody cannot recognize the 130-kDa band, we reasoned that it might represent a cross-reactive species possessing sequence context similarity to the original antigen peptide, and thus, we conducted a search in public data bases for proteins with similar molecular weight and sequence surrounding the immunizing peptide. A single candidate emerged as the recently characterized protein PARP10, formerly known as hypothetical protein FLJ14464. To verify if PARP10 is indeed the cross-reactive species, we generated a monoclonal antibody (clone 19F7) against the N-terminal 144-amino acid region of PARP10, and we observed the detection of a 130-kDa signal by Western blot assay (Fig. 1C, bottom panel).
CDK-dependent Phosphorylation of PARP10—In light of the consensus CDK site present on PARP10, in addition to the cell cycle-dependent pattern of phosphorylation recognized by the T927-101 antibody, we tested whether CDK activity would also be responsible for the PARP10 phosphorylation at Thr-101. To avoid the secondary effects that may emerge from prolonged incubation with the CDK inhibitors roscovitin and olomoucine, immunofluorescence staining was performed with synchronized interphase cells to restrict the study on Thr-101 phosphorylation acquired during late G1 phase. The phosphorylation of nucleolar PARP10 is undetectable when the cells were treated with CDK inhibitors roscovitin (Rosc.) or Me2SO (DMSO) for 6 h prior to fixation and immunofluorescence staining. Anti-Nopp140 antibody staining was used as a positive control for nucleolus. Nuclei were counterstained with DAPI. B, phosphorolysis of recombinant PARP10 by CDK2-cyclin E. The N-terminal 144 amino acids of PARP10 were expressed as GST fusion recombinant in E. coli, purified by glutathione beads, and incubated with baculovirus-derived CDK2-cyclin E or CDK1/cyclin B as described under “Experimental Procedures.” The CDK2-cyclin E-phosphorylated PARP10 can be recognized by T927-101 antibody. αGST, anti-GST antibody.

Phosphorylated PARP10 Is a Stable Component of the RNAPolI Module—Because hNopp140 is a structural component of the RNAPolI transcription module throughout the interphase, its co-localization with T927-101 staining suggests that phosphorylated PARP10 may be associated with the RNAPolI machinery (Fig. 3A). Chromatin spread assay also revealed the co-localization of phosphorylated PARP10 with RNAPolI in nucleolar organizing regions during mitosis (Fig. 3B). To assess whether phosphorylated PARP10 is a stable component of the
CDK-dependent Activation of PARP10

We next proceeded to evaluate the possible relationship between CDK-dependent phosphorylation and the enzymatic activity of PARP10. We found that endogenous PARP10 immunoprecipitated from HeLa cell lysates showed very low PARP activity in vitro (Fig. 4C, P10 only). However, a brief incubation with recombinant CDK2-cyclin E prior to PARP assay enhances poly(ADP-ribose)ylation activity (Fig. 4C, P10 + CDK) to about 8-fold, which is a level comparable to the reaction, indicating the formation of ADP-ribose polymers. In parallel, Western blot using anti-His antibody shows that similar mounted to the reaction, indicating the formation of ADP-ribose polymers.

FIGURE 3. Association of the phosphorylated PARP10 with RNAPolI modules. A, Thr-101-phosphorylated PARP10 co-localized with hNopp140 in a subpopulation of interphase HeLa cells. Asynchronously growing HeLa cells were doubly stained with T927-101 and hNopp140 antibodies. hNopp140 is a structural protein that interacts with the pol I transcription machinery. In mitotic cells, only phosphorylated PARP10 retained the dotted pattern in close association with the condensed chromosomes, although in some interphase cells the situation is reversed and the nucleoli stained solely with hNopp140. B, Thr-101-phosphorylated PARP10 co-localizes with the pol I-containing nucleolar organizing regions during mitosis. Chromatin spread preparations of metaphase HeLa cells were stained with anti-pol I and T927-101 antibodies. Confocal microscopy images were pseudocolored using Adobe Photoshop. C, Thr-101-phosphorylated hNuPARP co-fractionates with purified nucleoli. Nucl. Pol, nucleolus; Nuc, Nop140; Nuc, nucleolar fraction; NP34, high density nuclear fraction; NP88, whole nuclear fraction. Dap, detergent-extractable cytoplasmic fraction; Cyt8, soluble cytoplasmic extraction. Ku80 was used as a nuclear marker. Tubulin was used as a cytoplasmic marker.

nucleolus, we purified nucleoli from exponentially growing HeLa cells by sucrose gradient fractionation. Indeed, Western blot analysis revealed that phosphorylated PARP10 is highly enriched in the nucleolar fraction (Fig. 3C, Nuc). Together, our results indicate that Thr-101-phosphorylated PARP10 co-purifies with the nucleoli and associates with RNAPolI modules.

CDK2 Phosphorylation Enhances PARP10 Activity—Sequence comparison reveals a PARP catalytic domain structure located in the C-terminal portion of PARP10, conserving the most important secondary structures as well as some of the critical residues for NAD+ binding (Fig. 4A). To determine whether PARP10 possesses PARP activity, a recombinant protein comprising its entire PARP-catalytic domain was expressed in E. coli as a His-tagged fusion protein. Because most of the PARP superfamily members have been shown to catalyze auto-poly(ADP ribosyl)ation, an in vitro assay for PARP activity was carried out using the N-terminal 250 amino acids of PARP10 as acceptor protein of the ADP-ribose moieties. In Fig. 4B, the anti-poly(ADP-ribose) antibody (aPAR) detects a ladder of lower mobility bands when 1 μl of the substrate NAD+ was supplemented to the reaction, indicating the formation of ADP-ribose polymers. In parallel, Western blot using anti-His antibody shows that similar amounts of recombinant proteins were loaded in each assay mixture (Fig. 4B, aHis). Furthermore, when the PARP-specific inhibitor 3AB was included, the ADP-ribosylation reaction was blocked, suggesting that the activity of PARP10 is analogous to PARP1-catalyzed poly(ADP-ribose)ylation. These results indicate that PARP10 can catalyze auto(poly ADP-ribo)sylation to itself and is thus a bona fide member of the PARP superfamily.
In this study, we detailed the characterization of a novel member of the PARP superfamily, PARP10, whose suppression induces delayed G₁ progression and loss of cell viability. The gene encoding PARP10 is located on the long arm of chromosome 8q24.3. A PARP domain is found in the C terminus of PARP10 as revealed by sequence comparison, and we show that it catalyzes auto-poly(ADP-ribosyl)ation in vitro by using the N-terminal 250-amino acid portion as an acceptor protein. In contrast, histone H3 was not modified by PARP10 (data not shown). The endogenous substrates of PARP10 remain to be identified.

A major finding in our studies is the localization of CDK-activated PARP10 to the nucleolus. The nucleolus is a dynamic nuclear domain that provides compartmentalization for diverse nuclear functions, including most importantly, the biogenesis of ribosomes. In higher eukaryotic cells, the nucleolus is assembled at the beginning of interphase, maintained during interphase, and dispersed into smaller organizing components during mitosis (21). Even if its structural organization appears to be nondissociable from its function in ribosome biogenesis, the mechanisms that govern the formation and maintenance of the nucleolus have not been elucidated. Several lines of evidence have indicated that CDK activity is indispensable for the building of functional nucleoli after mitotic disassembly. Furthermore, inhibition of CDKs in interphase cells causes hampered prerRNA processing and induces a dramatic disorganization of the nucleolus. Thus, mechanisms governing both formation and maintenance of functional nucleoli involve CDK activities, coupling ribosome biogenesis to the controls of cell cycle progression (22). The discoveries of PARP10 and its phosphorylation by CDK2 in the nucleolus may account for part of the roles of CDKs in nucleolar function and/or formation. Our present results suggest that CDK2 may be responsible for the alteration of specific nucleolar functions of PARP10. Together with the observation that the phosphorylation at Thr-101 is absent in quiescent or differentiated cells, a link between the function of phosphorylated PARP10 with ribosomal
CDK-dependent Activation of PARP10

DNA replication/integrity and cell proliferation could be envisioned. In addition, we also showed that phosphorylated PARP10 associates with the RNApolI machinery in nucleolar organizing regions. During mitosis, most nucleolar proteins disperse throughout the cell, although all known basal factors required for transcription initiation remain associated with the rDNA loci in condensed chromosomes (23). The selective retention of the RNApolI transcription apparatus could be a regulatory mechanism aimed at the rapid assembly into the pre-initiation complexes when cells re-enter the G1 phase of the cell cycle. Nevertheless, our preliminary data showed that PARP10 is not required for the initiation of RNApolI transcription, and nuclease treatment assays indicate a DNA-dependent association of PARP10 with the chromosomes (data not shown). Thus, in contrast to the knock-out phenotypes of parp-1−/− (1), parp-2−/− (12), vparp−− (14), or knockdown of tankyrase (24), it is likely that PARP10 is an essential gene during early embryonic development. Furthermore, the level of cytoplasmic PARP10 does not seem to change when cells get differentiated. This is strikingly different from the appearance of the CDK2-dependent phosphorylation of PARP10 in the nucleolus of proliferating cells. Apart from the localization of the phosphorylated form in the nucleolus of proliferating cells, the level of PARP10 may be indispensable in the homeostasis of terminally differentiated cells. Functions of PARP10 in the cytoplasm, nucleoplasm, and nucleolus may be segregated but may all contribute to the smooth cell cycle progression.

The phosphorylated PARP10 is very susceptible to phosphatase activity, as demonstrated by the fact that phosphorylated PARP10 can only be detected by Western blot when the cells were lysed directly with hot SDS sample buffer. This suggests that the phosphorylation and dephosphorylation of PARP10 may be tightly regulated in a temporal and spatial manner. In an attempt to resolve the importance of Thr-101 phosphorylation, we performed site-directed mutagenesis of PARP10. In transient transfection experiments, we observed that modification on the Thr-101 residue is not required for nucleolar localization of PARP10 (data not shown). Notably, overexpression of wild type, T101A, and the phosphomimetic T101E mutants resulted in cell death (data not shown). This also points to the fact that although the activity of PARP10 is tightly regulated by CDK phosphorylation, the homeostasis of its level is equally highly maintained. Down-regulation of PARP10 by shRNA causes delayed G1 progression and cell death (Fig. 5), a result that remains reproducible using different shRNA targets as well as double knockdowns (data not shown). Thus, in contrast to the knock-out phenotypes of parp-1−/− (1), parp-2−/− (12), vparp−− (14), or knockdown of tankyrase (24), it is likely that PARP10 is an essential gene during early embryonic development. Furthermore, the level of cytoplasmic PARP10 does not seem to change when cells get differentiated. This is strikingly different from the appearance of the CDK2-dependent phosphorylation of PARP10 in the nucleolus of proliferating cells.

FIGURE 5. Knockdown of PARP10 by shRNA causes delayed G1 progression and loss of cell viability.

A, HeLa cells were analyzed for the expression of PARP10 mRNA and protein level at specific time points after transfection with either shRNA expression vector or control plasmid. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA and tubulin α protein levels were used as controls for RT-PCR and Western blot assays for quantitation of PARP10 knockdown cells at specific time points after transfection as sorted by co-expressing GFP intensity. Luc control, nonspecific shRNA control construct encoding for a target sequence to the firefly luciferase gene. Data represent the means of two independent experiments.

B, quantitation of PARP10 knockdown cells and tubulin α protein levels were used as controls for RT-PCR and Western blot assays for monitoring the suppression of PARP10 levels.

C, DNA content of GFP-expressing cells was measured by propidium iodide staining and flow cytometry at the time points indicated. Percentage of cells in each cell cycle stage is shown; note the gradual accumulation of G1 cells with PARP10 shRNA expression.

FIGURE 6. PARP10 phosphorylation is suppressed in growth-arrested/differentiated cells.

HaCaT cells were triggered to differentiate by confluency and prolonged culture (14 days) in high Ca2+ medium. Normal nucleolar biogenesis can be visualized by the compact nucleolin staining in all cells (upper two panels); most cells triggered to growth arrest/differentiation lost the punctate nuclear staining for the proliferation marker PCNA1, as well as the T927-101 signal (lower two panels).
Members of the PARP family show a similarity of 29–60% between their PARP domains. Generally, they do not resemble each other outside the catalytic domain, suggesting diverse cellular functions other than the well known role of caretakers of the genome. This is found exactly in members of the PARP7 subfamily to which PARP10 belongs (Fig. 4A). Members of this emerging subfamily display strikingly divergent cellular functions, underscoring their unique primary structure of the PARP domain exemplified by PARP7, BAL protein (PARP9), and PARP10. Although each subfamily member possesses poly(ADP-ribose) polymerase activity, their endogenous substrates under various physiological conditions remain to be identified. Apart from the C-terminal PARP domain, PARP10 contains a putative RNA recognition motif and a ubiquitin interaction motif (4). The domain organization of PARP10 suggests that it may interact with RNA/DNA, and its stability may be regulated by ubiquitin-mediated degradation.

A 960-amino acid mouse protein (BAC25913) and a 985-amino acid rat protein (GenBank™ accession number XP_216963) of unknown functions are closely related to PARP10. These two proteins share significant overall sequence homology (50% identity) with PARP10. Other members of the PARP7 subfamily include PARP9, PARP12, PARP13, and a handful of other hypothetical proteins. PARP7 may have functions in the induction of long term potentiation (25) and in T cells from progressing tumors3 and has been implicated in malignant B lymphoma migration (11). In addition to the PARP domain, it also contains his-macro and SEC14 domains that may function in the chromatin structure dynamics and transcription. PARP12 protein possesses a CCCH zinc finger and two WWE domains, suggesting a role in RNA processing (16). PARP13 has been shown to have antiviral activity (17). Together, members of the PARP7 subfamily have functions as diverse as those of the PARP1 family.

Our findings that the activity of RNAPolI-associated PARP10 can be enhanced through CDK2 phosphorylation suggests that it may play crucial role in the regulation of rDNA dynamics. On the basis of these results, future studies on the functions of PARP10 promise valuable advances to the present understanding on the relationship between nucleolar dynamics and cell cycle regulation.

3 R. A. Kurt, R. M. Bernstein, S. F. Schluter, J. Park, J. Marchalonis, and E. Akporiaye, GenBank™ accession number U55178.

Acknowledgments—We thank Ko-chun Ko, Li-ping Tu, and Rui Chao for technical assistance. We thank Ming-Yue Lee for reading the manuscript. We also thank the Helix Research Institute of Japan for the cDNA clone FLJ14464.

Addendum—During preparation of this manuscript, Yu et al. (26) reported the identification of PARP10 as a Myc-interacting protein.

REFERENCES

1. Shall, S., and de Murcia, G. (2000) Mutat. Res. 460, 1–15
2. Smith, S. (2001) Trends Biochem. Sci. 26, 179–183
3. Shall, S. (2002) BioEssays 24, 197–201
4. Amo, J. C., Splenhauer, C., and de Murcia, G. (2004) BioEssays 26, 882–893
5. Berghammer, H., Ebner, M., Marksteiner, R., and Auer, B. (1999) FEBS Lett. 449, 259–263
6. Johansson, M. (1999) Genomics 57, 442–445
7. Kickhoefer, V. A., Stephen, A. G., Harrington, L., Robinson, M. O., and Rome, L. M. (1999) J. Cell Biol. 146, 917–928
8. Smith, S., Giriat, I., Schmitt, A., and de Lange, T. (1998) Science 282, 1484–1487
9. Lyons, R. J., Deane, R., Lynch, D. K., Ye, Z. S., Sanderson, G. M., Eyre, H. J., Sutherland, G. R., and Daly, R. J. (2001) J. Biol. Chem. 276, 17172–17180
10. Ma, Q., Baldwin, K. T., Renzelli, A. J., McDaniel, A., and Dong, L. (2001) Biochem. Biophys. Res. Commun. 289, 499–506
11. Aguiar, R. C., Yakuhashi, Y., Kharbanda, S., Salgia, R., Fletcher, J. A., and Shipp, M. A. (2000) Blood 96, 4328–4334
12. Menissier-de Murcia, J., and de Murcia, G. (1999) J. Biol. Chem. 274, 17860–17868
13. Augustin, A., Spenhauer, C., Durand, H., Menissier-de Murcia, J., Piel, M., Schmitt, A. C., Apiou, F., Vonesch, J. L., Kock, M., Bornens, M., and De Murcia, G. (2003) J. Cell Sci. 116, 1551–1562
14. Liu, Y., Snow, B. E., Kickhoefer, V. A., Erdmann, N., Zhou, W., Wakeham, A., Gomez, M., Rome, L. H., and Harrington, L. (2004) Mol. Cell. Biol. 24, 5314–5323
15. Smith, S., and de Lange, T. (2000) Curr. Biol. 10, 1299–1302
16. Kato, M., and Katoj, M. (2003) Int. J. Oncol. 23, 541–547
17. Gao, G., Guo, X., and Goff, S. P. (2002) Science 297, 1703–1706
18. Blangy, A., Lane, H. A., d’Herin, P., Harper, M., Kress, M., and Nigg, E. A. (1999) Cell 83, 1159–1169
19. Sawin, K. E., and Mitchison, T. J. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 4239–4293
20. Smith, S., and de Lange, T. (1999) J. Cell Sci. 112, 3649–3656
21. Hernandez-Verdun, D., Roussel, P., and Gebran Younes, J. (2002) J. Cell Sci. 115, 2265–2270
22. Siriri, V., Hernandez-Verdun, D., and Roussel, P. (2002) J. Cell Biol. 156, 969–981
23. Moss, T., and Stefanovsky, V. Y. (2002) Cell 109, 545–548
24. Dynak, J. N., and Smith, S. (2004) Science 304, 97–101
25. Matsuo, R., Murayama, A., Saitoh, Y., Sakaki, H., and Inokuchi, K. (2000) J. Neurochem. 74, 2239–2249
26. Yu, M., Schreek, S., Cerni, C., Schamberser, C., Lesnieciewicz, K., Porchez, E., Verhoeven, J., Walensmann, G., Grotzinger, J., Kremmer, E., Metha-Rao, Y., Mertsching, J., Kraft, R., Austen, M., Luscher-Firzlaff, J., and Luscher, B. (2005) Oncogene 24, 1982–1993