Proc. Natl. Acad. Sci. USA
Vol. 96, pp. 2147–2152, March 1999
Cell Biology

p53 regulates a G_2 checkpoint through cyclin B1

(cell cycle/cdc2/mitosis/cancer)

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Edited by Arthur B. Pardee, Dana–Farber Cancer Institute, Boston, MA, and approved December 21, 1998 (received for review October 13, 1998)

ABSTRACT **The p53 tumor suppressor controls multiple cell cycle checkpoints regulating the mammalian response to DNA damage. To identify the mechanism by which p53 regulates G_2, we have derived a human ovarian cell that undergoes p53-dependent G_2 arrest at 32°C. We have found that p53 prevents G_2/M transition by decreasing intracellular levels of cyclin B1 protein and attenuating the activity of the cyclin B1 promoter. Cyclin B1 is the regulatory subunit of the cdc2 kinase and is a protein required for mitotic initiation. The ability of p53 to control mitotic initiation by regulating intracellular cyclin B1 levels suggests that the cyclin B-dependent G_2 checkpoint has a role in preventing neoplastic transformation.**

The p53 gene encodes an important tumor suppressor whose inactivation is considered to be a critical step in tumorigenesis (1). Consistent with this idea, loss of p53 function is associated with an increased susceptibility to spontaneous and carcinogen-induced tumorigenesis in mice (2, 3). The p53 protein is a phosphoprotein transcription factor that activates the transcription of genes containing the consensus DNA sequence 5′-Pu-Pu-Pu-C-A/T-T/A-G-Py-Py-Py-3′ (Pu, purine; Py, pyrimidine) (4). p53 also can repress the activity of promoters lacking the consensus-binding site (5). Wild-type p53 is a suppressor of cell growth, and targets for p53 transactivation include genes involved in growth arrest or apoptosis (6, 7), whereas targets for transcriptional repression generally are involved in growth promotion (8).

p53 suppresses tumor growth by two distinct mechanisms. First, p53 induces apoptosis, a tightly regulated pathway of programmed cell death involving changes in mitochondrial physiology, cysteine protease activation, and DNA degradation (9–11). Overexpression of p53 induces apoptosis (12), and p53 inactivation leads to a decrease in apoptosis caused by γ-radiation (13, 14). p53 also prevents growth by halting the cell cycle. p53 inhibits G_1/S transition in cells exposed to DNA-damaging agents by causing accumulation of $p21^{CIP1/WAF1}$ (6, 15), a protein that binds to and inactivates the cyclin-dependent kinases necessary for initiating DNA synthesis (16). p53 also regulates a mitotic spindle checkpoint that prevents DNA synthesis before chromosome segregation (17). In addition, p53 controls a G_2 checkpoint and expression of wild-type p53 can prevent G_2/M transition in mouse and human cell lines (18–20). Loss of these checkpoints in p53 deficient cells can lead to polyploidy (17, 21), chromosomal breaks (3), and centrosome amplification (22), indicating that the checkpoints contribute to the maintenance of DNA integrity.

To study G_2 regulation by p53, we have established a human cell line, Ts-SKOV3, that stably expresses a temperature-sensitive p53 allele and undergoes G_2 arrest at 32°C. Using this cell line we have found that p53 arrests cell cycle in G_2 by lowering intracellular levels of cyclin B1, a protein absolutely required for mitotic initiation.

MATERIALS AND METHODS

Cell Lines. Ts-SKOV3 is a derivative of the parental SKOV3 ovarian cancer line (American Type Culture Collection, ATCC; HTB 77) that has been stably transfected with pSVneo and pUL-53–3, a plasmid containing a genomic copy of the mouse $p53^{val135}$ gene under the control of the p53 promoter. Ts-SKOV3 was generated while two of us (J.M.L. and J.L.A.A.) were postdoctoral fellows in the laboratory of Alan Bernstein (Mt. Sinai, Toronto). Saos-2 was obtained from ATCC, and A431 was a gift of Maria Rozakis-Adcock (Hamilton Regional Cancer Center).

Cell Cycle Analysis. Cells were removed from the plate by using 3 mM EDTA/PBS, which was washed twice in PBS and incubated in 1 ml of PBS containing 50 μg/ml of propidium iodide (Sigma), 10 μl Triton-X 100, and 66 units/ml of RNase (GIBCO/BRL) on ice for 30 min. Flow cytometry was performed by using an Epics-Profile II (Coulter). A total of 1.0×10^4 nuclei were analyzed at a flow rate of approximately 100 nuclei per sec. Quantitation of cell cycle distribution was performed by the M-CYCLE Supply Software program.

Western and Northern Blotting. For Western blotting, 1×10^6 cells were lysed directly in 100 μl of 1× reducing SDS sample buffer (NEB) and incubated at 100°C for 5 min. Twenty microliters of the boiled extract was run on a 12% polyacrylamide gel, transferred to nitrocellulose (Schleicher & Schuell) using a Bio-Rad semidry transfer apparatus. The blot then was incubated with an antibody against p53 (Ab-3, Oncogene Science), cyclin B1 (Oncogene Science), cdc2 (Santa Cruz Biotechnology), or pTyr-cdc2 (NEB, Beverly, MA) as per the manufacturer's directions. For Northern blotting, 1×10^7 cells were lysed and RNA was isolated by using the RNeasy kit as per the manufacturer's instructions (Qiagen). Ten micrograms of total RNA was run on 1.2% denaturing agarose gels containing 37% formaldehyde and transferred to S&S Nytran (Mandel Scientific Comp., Guelph, Canada). The blot was incubated for 15–30 min at 42°C in prehybridization solution (40% formamide/0.96 M Na_2HPO4, pH 7.2/5 M NaCl/15% SDS/0.25 M EDTA in diethyl-pyrocarbonate H_2O). The boiled probe and fresh hybridization solution then was added and the mixture was incubated at 42°C overnight. Blots were washed (*i*) 2× SSC/0.1% SDS at room temperature (RT), (*ii*) 0.5× SSC/0.1% SDS at RT, and (*iii*) 0.1% SSC/0.1% SDS at 43°C.

MPM-2 Detection. Antibody capture ELISA detection of MPM-2 epitope is based on a previously reported protocol

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Abbreviations: RT, room temperature; CAT, chloramphenicol acetyltransferase.

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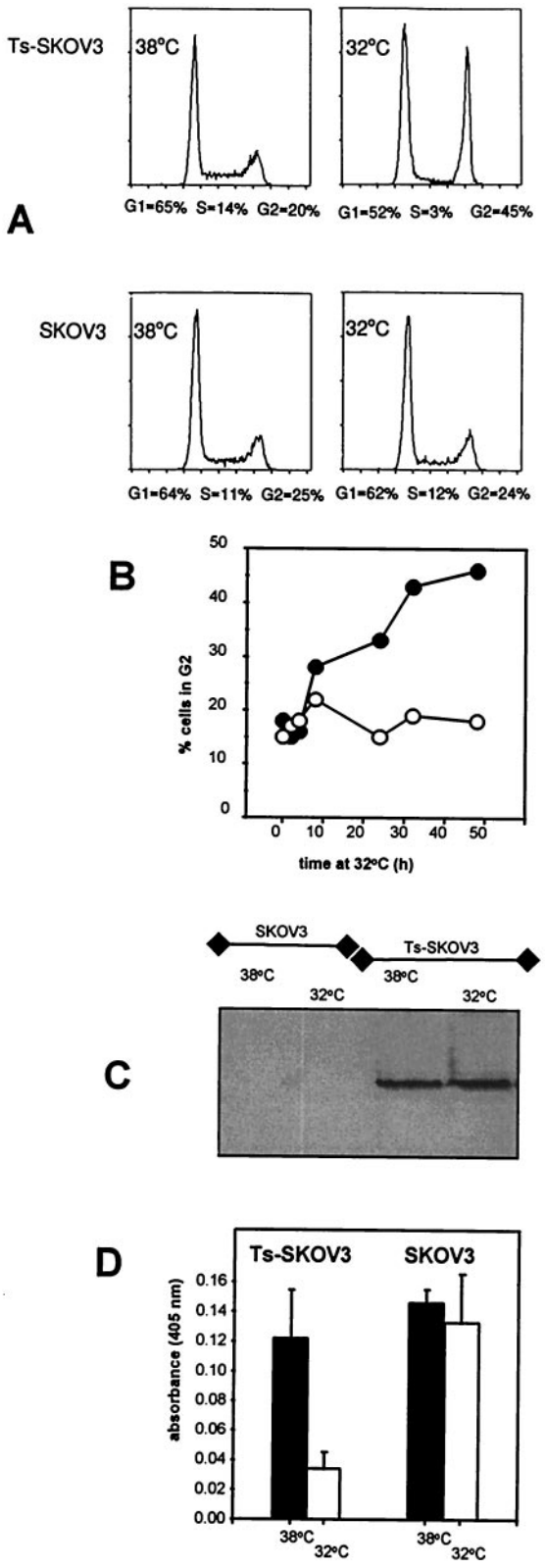

FIG. 1. Ts-SKOV3 cells undergo G_2 arrest at 32°C. (*A*) A propidium iodide (PI) DNA-staining profile of Ts-SKOV3 and parental SKOV3 cells at 38°C and after 48 hr at 32°C. At 32°C the majority of Ts-SKOV3 cells are either in G_1 or G_2. A 32°C incubation has no effect on the cell cycle profile of the parental SKOV3 cells. The percentage of cells in each phase of the cell cycle, as determined by the M-CYCLE program, is indicated. (*B*) The fraction of Ts-SKOV3 cells in G_2 increases as a function of time at 32°C (●). There is no change in the G_2 fraction in parental SKOV3 cells as a function of time (○). (*C*) Ts-SKOV3 expresses substantial amounts of the $p53^{val135}$ protein at 38°C and 32°C. The parental SKOV3 line expresses no detectable mutant or wild-type p53 protein. (*D*) MPM-2 reactivity, as measured by ELISA, is reduced in Ts-SKOV3 cells at 32°C relative to 38°C. MPM-2 reactivity in SKOV3 cells is similar between 32°C and 38°C.

(23). Cells were lysed in 80 mM sodium-β-glycerol phosphate/20 mM EGTA/15 mM $MgCl_2$, pH 7.3/lysis buffer (LB) at 2×10^7/ml. Extract was diluted 1:25 in LB, and 200 μl of extract was added to a single well of a 96-well plate for 2 hr at RT, and the plate was washed 3× with PBS. Wells were blocked with 5% BSA/0.02% sodium azide/PBS assay buffer (AB) for 2 hr at RT. Two hundred microliters of a 1:1,000 dilution of MPM-2 antibody (Upstate Biotechnology, Lake Placid, NY) in AB was added to each well and incubated for 2 hr at RT and washed three times with PBS. Two hundred microliters of a 1:1,000 dilution of alkaline phosphatase-conjugated goat anti-mouse IgG (Gibco) was added for 2 hr at RT and washed three times with PBS p-nitrophenol phosphate. Bound antibody was detected by using 100 μl of 1 mg/ml PNPP in 10 mM DTT/0.5 mg/ml $MgCl_2$ (pH 9.5) for 30 min at RT, and OD_{405} was measured. Background (no secondary antibody added) was subtracted from all measurements.

Histone H1 Kinase Assays. Cells (5×10^5) were removed from a 100-mm plate using cold 3 mM EDTA in PBS. Cells were washed two times in PBS and lysed in 500 μl of 1% Nonidet P-40/50 mM Tris·HCl, pH 7.4/5 mM EDTA/150 mM NaCl/2 μM leupeptin/400 μm phenylmethylsulfonyl fluoride/5 μg/ml aprotinin. Ten microliters of α-cdc2 antibody (Santa Cruz Biotechnology) was added, and the mixture was incubated overnight at 4°C. Fifty microliters of 10% protein-A Sepharose beads (Pharmacia) then was added for 1 hr to overnight. Immunoprecipitated cdc2 was washed three times in lysis buffer, and the beads were incubated with 10 μl of 2 mg/ml Histone H1 (20 mM MOPS, pH 7.2/25 mM glycerol phosphate/5 mM EGTA/1 mM sodium orthovanadate/1 mM DTT), 10 μl of a non-cdc2 kinase inhibitor mixture (20 μM protein kinase C inhibitor peptide, 2 μM protein kinase A inhibitor peptide, and 20 μM compound R24571). The reaction was started by adding 9 μl 75 mM magnesium chloride/500 μM ATP containing 1 μl of 100 μCi [^{32}P]ATP and incubated at 30°C for 10 min. Twenty-five microliters of the reaction mixture was spotted onto P81 phosphocellulose paper and washed 10 times with 0.75% phosphoric acid.

Cyclin B1 Reporter Assays. For cyclin B1/chloramphenicol acetyltransferase (CAT) activity assays, 2×10^5 cells were plated into a 60-mm Nunc dish and transfected the following day with 10 μl of Superfect and 5 μg of the cyclin B1 CAT promoter construct with a 5-hr Superfect incubation. Twenty-four hours after the beginning of the transfection protocol, cells were lysed in 200 μl of lysis buffer, and 150 μl of the extract (normalized for protein concentration) was added to 10 μl of [^{3}H]chloramphenicol and 5 μl of *n*-butyrl CoA. Samples were incubated overnight and xylene was extracted twice. For cyclin B1 promoter activity in Ts-SKOV3 at 37°C and 32°C, 2×10^5 cells were plated at 37°C and transfected the next day. Five hours after transfection at 37°C, cells were moved to 32°C and CAT activity was measured at various times thereafter.

Cyclin B1 Overexpression. Ts-SKOV3 cells (5×10^5) were plated into a 100-mm Nunc dish and transfected the following day with 30 μl of Superfect, 3.6 μg of an I-Ak^k marker, and 11.4 μg of either cyclin B1, pDNA3, or cdc2 plasmids. Six hours later cells were either left at 38°C or moved to 32°C. Thirty-four hours after the temperature shift, cells were removed from the plate using 3 mM EDTA/PBS and incubated with a specific I-Ak^k antibody conjugated to Ferrous oxide microbeads as per the manufacturer's instructions (Miltenyi Biotec, Auburn, CA). Transfected cells were magnetically separated by using a Miltenyi MAC column as per the manufacturer's instructions. Flow cytometry was performed on a Coulter Epics XL.

RESULTS

A Human Cell Line That Undergoes p53-Dependent G_2 Arrest. Ts-SKOV3, which stably expresses the mouse $p53^{val135}$ allele under the control of the p53 promoter, is derived from the p53-deficient human ovarian SKOV3 cell line. At 37–38°C the $p53^{val135}$ protein has oncogenic properties and cooperates with ras in primary cell transformation (24). At 32°C, on the other hand, the $p53^{val135}$ protein undergoes a conformational change and acquires properties of a wild-type p53 protein (25). As shown in Fig. 1*A*, Ts-SKOV3 cells undergo a G_2 arrest, as determined by an increase in cells with a 4 N DNA content, when incubated at 32°C. There is also an arrest in G_1 (2 N DNA), which is commonly observed in other cell types expressing the $p53^{val135}$ allele at 32°C (26). At 32°C, the fraction of Ts-SKOV3 cells in G_2 increases as a function of time; approximately 45% of Ts-SKOV3 cells are in G_2 after 30 hr at 32°C (Fig. 1*B*). The parental SKOV3 continues to grow normally at 32°C (Fig. 1*A*), and there is no change in the number of SKOV3 cells in G_2 (Fig. 1*B*). As shown in Fig. 1*C*, Ts-SKOV3 cells express equivalent levels of the p53 protein at both 38°C and 32°C and there is no detectable p53 protein in parental SKOV3 cells at either temperature.

To further examine the G_2 arrest, we measured the appearance of the MPM2 epitope in Ts-SKOV3 cells at 38°C and at 32°C. MPM2 is a phosphoprotein epitope that appears on multiple proteins during mitotic metaphase but is not present in the proteins of G_1, S, or G_2 cells (27). As shown in Fig. 1*D*, total protein extracts from Ts-SKOV3 cells show diminished MPM2 reactivity at 32°C relative to 38°C, as determined by antibody-capture ELISA. There is no change in MPM2 reactivity between SKOV3 cells at 32°C and 38°C. Thus, substantially fewer Ts-SKOV3 cells are in metaphase at 32°C relative to 38°C. Taken together, Fig. 1 *A–D* indicates that Ts-SKOV3 cells undergo p53-dependent cell cycle arrest in G_2 phase.

p53-Dependent G_2 Arrest Is Associated with a Decrease in cdc2 Activity. To determine a mechanism for p53-dependent G_2 arrest, we measured cdc2 kinase activity in Ts-SKOV3 cells at 32°C. cdc2 induces mitosis by phosphorylating and activating the enzymes regulating chromatin condensation, nuclear membrane breakdown, and mitosis-specific microtubule reorganization (28). Because entry of cells into mitosis requires a threshold level of cdc2 kinase activity (29), we reasoned that p53 might inhibit mitotic initiation by attenuating cdc2 kinase activity. As shown in Fig. 2*A*, cdc2 kinase activity decreases markedly in Ts-SKOV3 cells grown at 32°C, coincident with the G_2 arrest. There is no substantial change in cdc2 activity in the parental SKOV3 cell line grown at 32°C (Fig. 2*A*). The decrease in cdc2 activity in Ts-SKOV3 at 32°C is not the result of substantial changes in cdc2 protein levels or inhibitory tyrosine phosphorylation (Fig. 2*B*). Tyrosine phosphorylation of cdc2 at Y15 inhibits kinase activity (30) and is one of the mechanisms by which human cells inhibit mitosis after exposure to DNA-damaging agents (31). Thus, p53 inhibits mitosis and cdc2 kinase activity independently of cdc2 protein levels or inhibitory tyrosine phosphorylation.

p53-Dependent G_2 Arrest Is Associated with a Decrease in Cyclin B1 mRNA and Protein. Because cdc2 kinase activity requires that cdc2 binds to cyclins A or B (30), we investigated whether the p53-mediated decrease in cdc2 activity and mitotic arrest in Ts-SKOV3 cells might result from changes in cyclin protein levels. Cyclin A regulates the initiation and maintenance of DNA synthesis whereas B cyclins control mitosis (32, 33). Cyclin B mRNA and protein levels are low in G_1 and S phase, and a threshold level of cyclin B in G_2 is a prerequisite for mitotic initiation (34). There are two human B-type cyclins, B1 and B2. Cyclin B2 is not essential for mouse development, and mice homozygous for a targeted deletion of the cyclin B2 gene are viable, fertile, and develop normally (35). On the

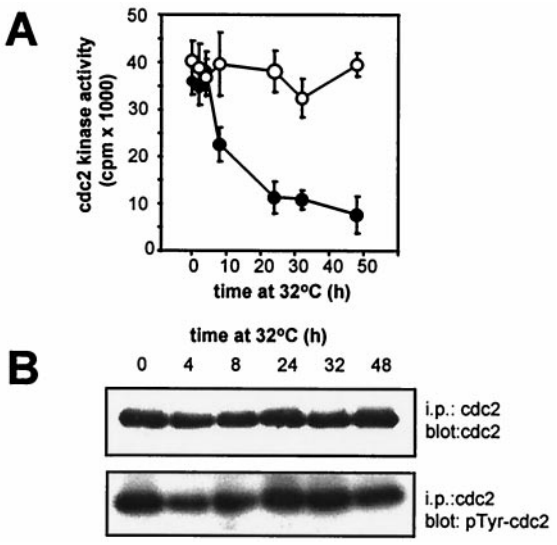

FIG. 2. G_2 arrest in Ts-SKOV3 cells is associated with a decrease in cyclin B1/cdc2 kinase activity. (*A*) Cdc2 kinase activity, as measured using Histone H1 as a substrate, as a function of time at 32°C. Cdc2 activity decreases in Ts-SKOV3 (●) but not SKOV3 (○) at 32°C. (*B*) The decrease in cdc2 kinase activity is not a result of a change in cdc2 protein levels or cdc2 inhibitory tyrosine phosphorylation as determined by Western blotting.

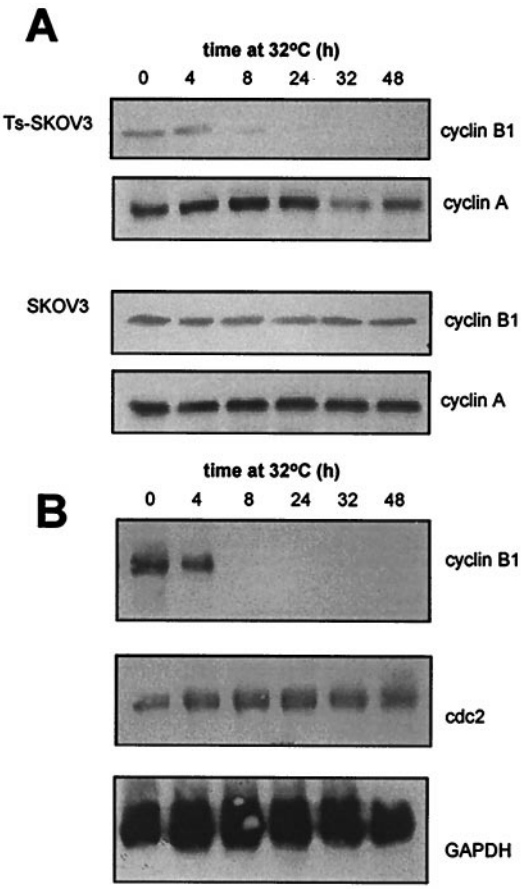

FIG. 3. G_2 arrest in Ts-SKOV3 is associated with a decrease in cyclin B1 protein and mRNA. (*A*) Total cyclin B1 protein levels, as determined by Western blotting, decrease in Ts-SKOV3 cells at 32°C. There is no change in cyclin B1 protein in SKOV3 cells at 32°C. There is a small change in cyclin A protein levels in Ts-SKOV3 at 32°C. (*B*) Total cyclin B1 mRNA decrease as a function of time in Ts-SKOV3 cells grown at 32°C. Cdc2 mRNA levels are unchanged at 32°C. Glyceraldehyde-3-phosphate dehydrogenase is used as a loading control.

other hand, homozygous deletion of cyclin B1 leads to death *in utero* (35), consistent with the idea that cyclin B1 likely is the primary regulator of mammalian mitosis. As shown in Fig. 3*A*, there is a substantial decrease in total cyclin B1 protein in Ts-SKOV3 grown at 32°C. Cyclin B1 protein levels do not change in SKOV3 at 32°C. There is also a small decrease in cyclin A protein levels at 32°C. As shown in Fig. 3*B*, the decrease in cyclin B1 protein is the result of a decrease in cyclin B1 mRNA, which is undetectable in Ts-SKOV3 cells 8 hr after the temperature shift to 32°C. The kinetics of disappearance of both the message and protein are similar. There is no change in cdc2 mRNA levels in Ts-SKOV3 cells grown at 32°C (Fig. 3).

Cyclin B1 Expression Rescues p53-Mediated G_2 Arrest. To determine whether the decrease in cyclin B1 mRNA was the primary mechanism by which p53 causes G_2/M arrest and decreases cdc2 kinase activity, we transiently introduced human cyclin B1 to SKOV3 cells grown at 32°C. We reasoned that if p53-mediated G_2/M arrest is dependent on a decrease in cyclin B1 levels, increasing cyclin B1 levels would overcome the G_2 arrest of Ts-SKOV3 cells at 32°C. Moreover, if a decrease in cyclin B1 causes the p53-mediated decrease in cdc2 kinase activity, cyclin B1 expression will also increase cdc2 activity. As detailed in *Materials and Methods*, Ts-SKOV3 cells were transiently transfected with cyclin B1 and I-Ak^k as a transfection marker. Expression of cyclin B1 and I-Ak^k is under the control of promoter/enhancer elements of the cytomegalovirus promoter, whose activity is not regulated by p53. Cells were shifted to 32°C and transfected cells were selected with an I-Ak^k antibody and subsequently analyzed for DNA content. As shown in Fig. 4, introduction of cyclin B1 abolishes p53-mediated G_2/M arrest evident in Ts-SKOV3 cells at 32°C. Ts-SKOV3 cells transfected with cyclin B1 no longer arrest in G_2, but now halt cell cycle in G_1. Protein levels of cyclin B1 and cdc2 for each selected population are shown in Fig. 4*B*. Levels of cyclin B1 protein in cyclin B1-transfected cells is 5- to 8-fold higher than in untreated cells. Cdc2 protein levels in cdc2-transfected cells are 2- to 4-fold higher than in untreated cells. Cyclin B1/cdc2 kinase activity is shown in Fig. 4*C*. Cyclin B1 rescues the p53-dependent drop in cdc2 kinase activity in Ts-SKOV3 cells at 32°C. A cdc2 expression plasmid had little effect on the G_2 arrest, consistent with the idea that the abundance of cdc2 is not the limiting factor during the G_2/M arrest. Introduction of cyclin B1 had no effect on cell cycle distribution in Ts-SKOV3 cells grown at 38°C. Thus, the p53-mediated decrease in cyclin B1 protein levels leads to G_2 arrest and the decrease in cdc2 kinase activity.

p53 Decreases the Activity of the Human Cyclin B1 Promoter. To identify a mechanism by which p53 could cause a decrease in cyclin B1 protein and mRNA, we determined whether p53 could regulate the activity of the cyclin B1 promoter. To this end, we transiently transfected Ts-SKOV3 and SKOV3 cells with a CAT reporter construct containing 1,050 bp of the human cyclin B1 promoter and measured CAT activity at 37°C and 32°C. This promoter contains the cis-DNA sequences necessary for cell cycle-specific transcription of the cyclin B1 gene (36). As shown in Fig. 5*A*, the activity of the cyclin B1 promoter is reduced in Ts-SKOV3 cells at 32°C relative to Ts-SKOV3 cells at 37°C. Thus, the decrease in cyclin B1 mRNA in Ts-SKOV3 cells at 32°C likely is a result of a p53-mediated decrease in cyclin B1 promoter activity. Cyclin B1 promoter activity is unchanged in SKOV3 cells between 37°C and 32°C. Moreover, wild-type p53 decreases the activity of the B1 promoter in transient transfection assays in SKOV3 cells (Fig. 5*B*). p53 also decreases cyclin B1 promoter activity in the mouse Friend erythroleukemia cell line DP16, the human ovarian carcinoma cell line Saos-2, and the human epithelial carcinoma line A431 (Fig. 5*B*).

DISCUSSION

The p53 protein controls cell cycle checkpoints in G_1, M, and G_2. In this report, we have demonstrated that p53 controls a G_2 checkpoint by decreasing intracellular levels of cyclin B1 and decreasing cyclin B1 promoter activity. The physiological importance of the p53-regulated G_2 checkpoint is a matter of some controversy. Some cell lines do not undergo G_2 arrest after ectopic p53 expression (26), and γ-radiation-induced G_2/M arrest occurs in p53-deficient cell lines (37). However, many mouse, human, and rat cell lines will halt cell cycle in G_2 after p53 expression (18–20), demonstrating that p53 is indeed a G_2 regulator. The ability of p53 to prevent mitosis may, however, vary between cell lines.

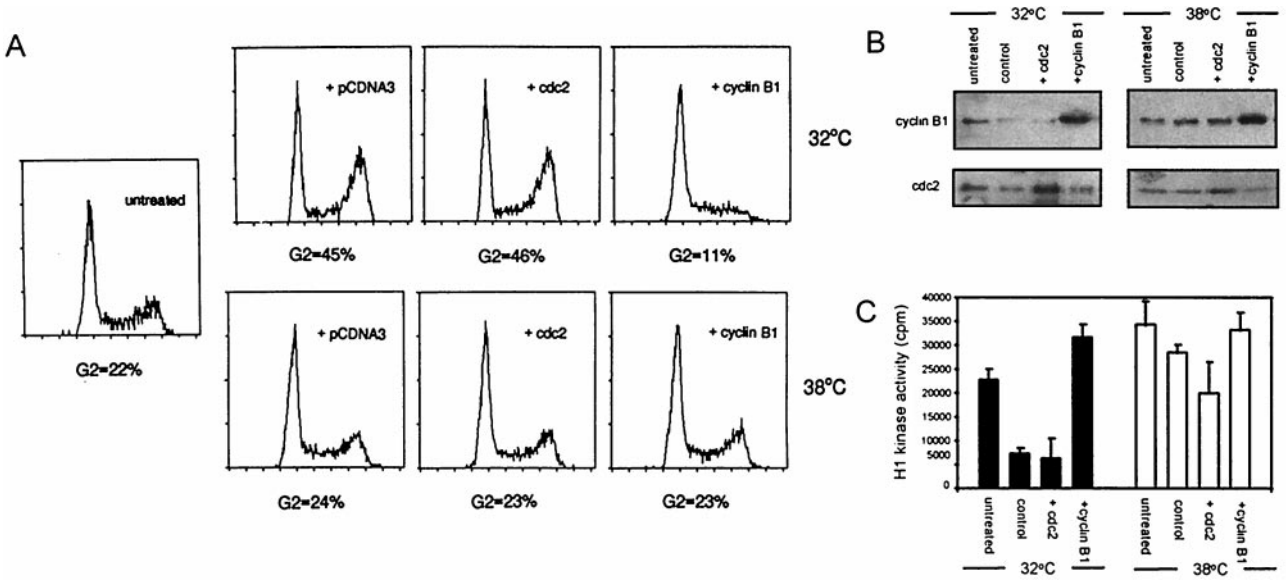

FIG. 4. Rescue of p53-mediated G_2 cell cycle arrest by cyclin B1 overexpression. (*A*) Transient transfection of cyclin B1 (cytomegalovirus promoter) into Ts-SKOV3 cells abolishes the G_2 arrest that occurs at 32°C in control (pCDNA3) transfected cells. Transfection of cdc2 has no effect on the G_2 arrest. Transient transfection of cyclin B1 has no effect on the cell cycle profile of Ts-SKOV3 cells at 38°C. (*B*) Total protein levels of cyclin B1 and cdc2, as determined by Western blot, for each transfection condition in *A*. (*C*) Cyclin B1/cdc2 kinase activity, as determined by Histone H1 phosphorylation, for each condition in *A*.

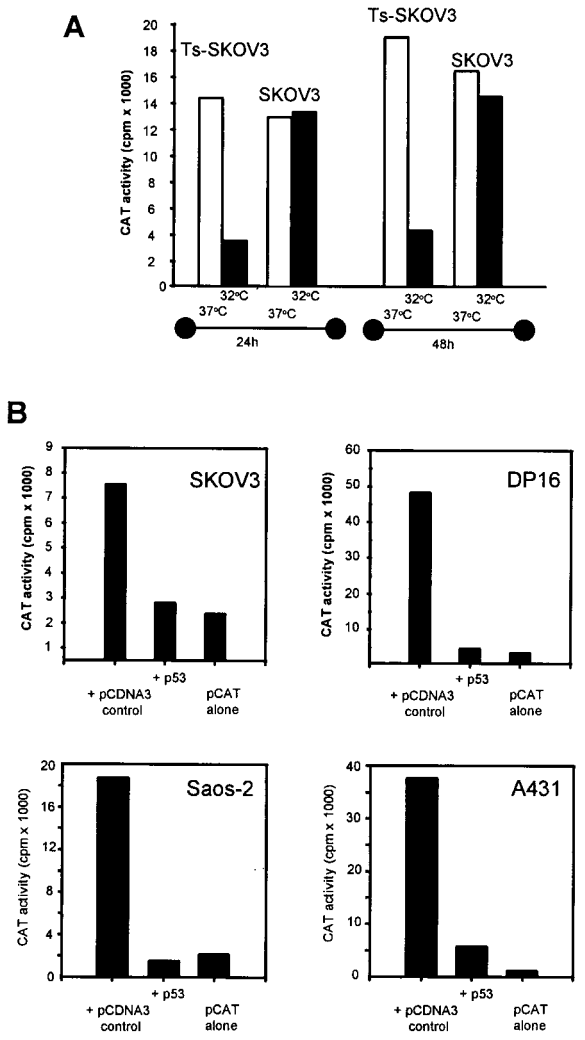

FIG. 5. p53 decreases the activity of the cyclin B1 promoter. (*A*) The activity of the cyclin B1 promoter (36) (1,050 bp 5′ of transcription initiation) transiently transfected into Ts-SKOV3 cells is markedly lower at 32°C (solid bars) than at 37°C (open bars) 24 and 48 hr after transfection. Promoter activity is not altered substantially in SKOV3 cells between 37°C (open bars) and 32°C (solid bars). (*B*) Wild-type p53 can suppress the activity of the cyclin B1 promoter in transient transfection assays in SKOV3, DP16, Saos-2, and A431 cells. Assays were performed at 37°C, and all data points are the mean of duplicate CAT measurements.

The cyclin B1-dependent checkpoint that we have described is not the only mechanism by which p53 controls mitotic initiation. In the human colorectal cancer cell line HCT116, p53 inhibits G_2/M progression, at least in part, through the 14-3-3σ protein (38); 14-3-3σ is a member of the 14-3-3 protein family, whose yeast homologues *rad24* and *rad25* are well characterized mitotic regulators in *Saccharomyces pombe* (39). After DNA damage, HCT116 cells accumulate 14-3-3σ mRNA in a p53-dependent manner, and overexpression of 14-3-3 leads to G_2 arrest, consistent with the idea that p53-dependent G_2 arrest in HCT116 cells is the result of 14-3-3 σ expression. It is likely that the 14-3-3σ protein prevents mitotic initiation by inactivating cdc25C, a phosphatase that activates cdc2 by dephosphorylating it at tyrosine 15. However, we do not observe any alterations in cdc2 tyrosine phosphorylation during G_2 arrest in Ts-SKOV3 cells, making it unlikely that p53 regulates mitosis in Ts-SKOV3 cells through cdc2 tyrosine phosphorylation and the cdc25 phosphatases. In addition, overexpression of cyclin B1 but not cdc2 rescues Ts-SKOV3 cells from p53-mediated G_2 arrest, consistent with the idea that cyclin B1 is the limiting factor in G_2 arrest. Thus, at least two p53-dependent pathways can contribute to G_2 arrest: an increase in 14-3-3σ levels and a decrease in the intracellular levels of cyclin B1.

Because we have found that p53 can inhibit cyclin B1 promoter activity, it is likely that the p53-mediated decrease in cyclin B1 mRNA is the result of a reduction in the rate of cyclin B1 transcription. The mechanism by which p53 regulates cyclin B1 promoter activity has yet to be determined. p53 could prevent cyclin B1 transcription by binding to and preventing the function of cyclin B1-specific transcription factors or could interact directly with cyclin B1 promoter DNA. There is no obvious p53 consensus-binding site in the cyclin B1 promoter, suggesting that p53 regulates B1 transcription without direct interaction with promoter DNA. The cyclin B1 promoter lacks a consensus TATA box, and p53 is known to repress the activity of other TATA-less and TATA-containing promoters by interacting the TATA box-binding protein (TBP) and preventing transcriptional initiation (5, 40). However, there may be TBP-independent mechanisms of p53-mediated transcriptional repression (41), and p53 binds to and interferes with the functions of the SP1- (42) and CCAAT-binding proteins (43). Transcription of the human cyclin B1 gene is activated by USF (36) and NF-Y (44) proteins and can be repressed by MyoD (45). p53 could regulate cyclin B1 promoter activity directly or indirectly through any or all of these transcription factors.

The inhibition of cyclin B1 transcription and concomitant mitosis by p53 could have an important role in controlling tumor development. For example, the majority of human breast cancer cell lines overexpress cyclin B1, raising the possibility that a failure to control cyclin B1 promoter activity and cyclin B1 accumulation might be a prerequisite for tumor growth (46). In addition, a decrease in cyclin B1 levels also is associated with p53-dependent induction of senescence (20), raising the possibility that p53 could regulate senescence through cyclin B1. Therefore, it will be of interest to determine whether $p53^{-/-}$ tumors generally overexpress cyclin B1 and whether cyclin B1 overexpression can overcome p53-mediated senescence. A further investigation into the coordinate role that p53 and cyclin B1 have in tumorigenesis and quiescence will shed further light on these issues.

We thank Lisa Porter, Vivian Leong, and Drs. Alan Bernstein, Curt Harris, Linda Ko, Carol Prives, Maria Rozakis Adcock, Gurmit Singh, and Xin Wang for helpful discussion and critical reading of this manuscript. We thank Karen Dougherty for secretarial assistance. This work is supported by funds to J.M.L. from the Medical Research Council of Canada, the Leukemia Research Fund, Cancer Care Ontario, and the Hamilton Regional Cancer Centre.

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