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Increased activity of p53 in senescing fibroblasts

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ABSTRACT **The p53 tumor-suppressor protein binds DNA and activates the expression of a 21-kDa protein that inhibits both the activity of cyclin-dependent kinases and the function of proliferating cell nuclear antigen. Since p21 expression has been reported to increase 10- to 20-fold as human diploid fibroblasts lose the ability to replicate, we examined the expression and activity of p53 during replicative aging. Similar levels of total p53 mRNA and protein were expressed in low-passage (young) and high-passage (old) cells but both DNA binding activity *in vitro* and transcriptional activity of p53 *in vivo* were increased severalfold in high-passage cells. While the basis of increased p53 activity is presently unclear, it is not correlated with differential phosphorylation or changes in p53–mouse double minute 2 gene product interactions. These results provide evidence for the activation of a protein involved in the control of cell cycle checkpoints during cellular aging, in the absence of increased expression.**

The p53 gene is commonly mutated in a wide spectrum of tumors (1, 2) and encodes a nuclear phosphoprotein that can function as a negative regulator of growth (3–6). One mechanism by which p53 is believed to act as a growth regulator is by binding to DNA in a sequence-specific manner (7–9) and activating the transcription (10) of growth-inhibitory genes such as the gene encoding p21, the 21-kDa inhibitor of both cyclin-dependent kinases (CDKs) and proliferating cell nuclear antigen (PCNA; refs. 11–14). The p53 protein also inhibits the expression of genes such as c-*fos* and c-*jun* that do not contain binding sites (15–17), most likely by interacting with the core transcriptional component TATA binding protein (18, 19). Additional target genes that contain binding sites and that are positively regulated by p53 include those encoding murine creatine phosphokinase, mouse double minute 2 (MDM-2), and GADD45 (20, 21).

In the cases of transcriptional induction of p21 by exogenous p53 (11) and radiation-induced growth arrest (22, 23), elevated levels of p53 protein are believed to be responsible for enhanced p53 function. In these situations, increased p53 has been proposed to constitute part of a cell cycle checkpoint in which p53-induced expression of p21 inhibits the activities of CDKs and of PCNA, blocking progression through the cell cycle until DNA repair has been completed (11, 12, 14).

Another experimental system in which elevated levels of p21 are seen is the aging human diploid fibroblast (HDF) (13). HDFs undergo a limited number of population doublings in culture that is proportional to the maximum lifespan of the species from which they were explanted and inversely proportional to the age of individual donors within a species (24, 25). Fibroblast replicative senescence appears to occur as a consequence of an intrinsic genetic program (25). This "senescence program" has been partially characterized by comparing gene expression in proliferation-competent fibroblasts to expression levels seen in aging fibroblasts. In general, gene expression remains relatively constant with increased *in vitro* age (27, 28), although genes encoding proteins that are believed to be important in regulating the passage of mammalian cells through checkpoints within the cell cycle have been found to have altered levels of expression in cells approaching senescence (29, 30). Of particular interest was the observation that p21, a protein capable of inhibiting normal cell growth, was expressed at very high levels in senescing HDFs (13). To better understand the basis for this increase, we examined the levels of p53 expressed in cells at different passage levels. In agreement with previous studies (27, 31), we find that the levels of p53 mRNA and protein do not change appreciably as cells age *in vitro*. In contrast, both p53 DNA binding activity and transcriptional activity were found to increase severalfold during cellular aging. These observations suggest a mechanism of p53 activation during cellular senescence, in the absence of increased expression, that may be a significant factor in blocking the growth of senescent cells through inducing the expression of p21.

MATERIALS AND METHODS

Cell Culture and Metabolic Labeling. Hs68 [ATCC CRL 1635, from newborn foreskin, reaches 85 mean population doublings (MPDs) in culture], WI38 (CCL 75, from embryonic lung, reaches 58 MPDs), HF [a gift from S. Cohen (Stanford University), from newborn foreskin, reaches 70 MPDs], and A2 [a gift from S. Goldstein (University of Arkansas for Medical Sciences), forearm fibroblast from a 20-year-old donor, reaches 75 MPDs] HDFs were cultured and labeled with [^{35}S]methionine as described (32). In all experiments, low-passage (young) cells were between 32 and 40 MPDs and high-passage (old) cells were used at 78–81 MPDs (Hs68), 54–56 MPDs (WI38), 65–69 MPDs (HF), or 70–73 MPDs (A2). Data shown were generated by using Hs68 cells unless otherwise noted; 85 ± 8% of young cells and 7 ± 4% of old cells used in these experiments could initiate DNA synthesis, as judged by incorporation of bromodeoxyuridine in a 36-h period (data not shown).

Immunoprecipitation and Immunoblot Analysis. Equal amounts of precipitable [^{35}S]methionine or [^{32}P]phosphate radioactivity of whole cell lysates from young (Y) or old (O) HDFs were immunoprecipitated under nondenaturing conditions by using the p53 monoclonal antibodies PAb240, PAb122, PAb421, or DO-1 or a control hybridoma supernatant. PAb240 recognizes only mutant forms of p53 (33), Pab122 (34) and Pab421 (35) recognize C-terminal regions of wild-type p53, and DO-1 recognizes an N-terminal region of p53 that is also bound by MDM-2 (36). Immunoblot analysis was done by using PAb421 hybridoma supernatant as described (37).

Microinjected Constructs and Immunofluorescence. Cells were fixed 12, 18, 24, or 36 h after microinjection in the nuclei with constructs RSV-CAT, wtp53-CAT, or mup53-CAT (each at 0.2 mg/ml) and were stained for the presence of bacterial chloramphenicol acetyltransferase (CAT) as described (38) by using polyclonal anti-CAT antibodies (1:150 dilution) obtained

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Abbreviations: MDM-2, mouse double minute 2; CDK, cyclin-dependent kinase; PCNA, proliferating cell nuclear antigen; HDF, human diploid fibroblast; MPD, mean population doubling; CAT, chloramphenicol acetyltransferase; GAPDH, glyceraldehyde-phosphate dehydrogenase; EMSA, electrophoretic mobility shift assay; CRE, cAMP-response element.

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from 5 prime → 3 prime Incorporated. Concatamerized sequences in the wtp53-CAT construct that respond specifically to wild-type p53 protein (39) were (5′-CCTGCCTG-GACTTGCCTGG-3′)$_{13}$. Sequences in the mup53-CAT construct were (5′-CCTTAATGGACTTTAATGG-3′)$_{15}$ (7).

PCR Analysis of mRNA. PCRs were done as described (40) and aliquots of the products were equalized to give equivalent signals from the internal control mRNA (glyceraldehyde-phosphate dehydrogenase, GAPDH), electrophoresed through 2% agarose gels containing ethidium bromide (0.2 mg/ml), UV-illuminated, and photographed by using Polaroid film, and multiple (three) exposures were analyzed by computerized densitometric scanning and averaged. Primers were designed to contain a G+C content of 40–60% and spanned introns to prevent signals from genomic DNA.

Mobility Shift Assays. Equal amounts of protein extracted from the nuclei of young or old HDFs in the presence of four protease inhibitors (41) were mixed with 10^4 cpm of ^{32}P-radiolabeled probes of the sequences: CRE wild-type, 5′-TCGACGGTATCGATAAGCTATGACGTCATC-CGGGGGATC-3′; CRE mutant, 5′-TCGACGGTATCGAT-AAGCTATAAGATTATCCGGGGGATC-3′; p53 wild-type, 5′-TAGACTTGCCTGGACTTGCCTGGCTA-3′; p53 mutant, 5′-TAGACTTTAATGGACTTTAATGGCTA-3′. Electrophoretic mobility shift assays (EMSAs) contained 10 μg of protein and were performed as described (41). Dried gels were exposed to x-ray film for 12 h at −80°C prior to development of films for cAMP-response element (CRE) probes or for 80 h at −80°C prior to development for p53 probes. Reactions preincubated with unlabeled oligodeoxynucleotides used a calculated 10-fold molar excess (20 ng) and reactions preincubated with antibodies used 1 μl of hybridoma supernatants concentrated 10-fold.

Glycerol Gradients. Young and old Hs68 cells labeled with [^{35}S]methionine as described above were lysed under nondenaturing conditions at 0°C, layered over 10–30% (vol/vol) glycerol gradients, and centrifuged immediately at 4°C for 18 h at 46,000 rpm in a Beckman SW 50.1 rotor. Gradients were fractionated from the bottom of the tubes into aliquots that were further divided and immunoprecipitated with the p53 monoclonal antibodies Pab421 and DO-1 or with control hybridoma supernatant.

RESULTS

p53 Expression Is Similar in Young and Old Fibroblasts. Previous reports have indicated that the level of p53 does not increase as HDFs age in culture (27, 31). Our results, using several independent methods, confirm this observation. Indirect immunofluorescence showed that p53 staining was predominantly nuclear and of similar intensity in fields of low (Fig. 1*A*)- and high (Fig. 1*B*)-passage HDFs chosen at random. By using a multiplex "primer-dropping" variation of the reverse transcriptase-coupled PCR, which is very precise (40), p53 mRNA, p21 mRNA, and GAPDH mRNA were analyzed in low-passage (young) and high-passage (old) cells. The levels of mRNA for p53 were similar in young and old cells (Fig. 2*A*), whereas p21 mRNA levels increased 9.6 ± 2.08 times (mean ± SD, n = 3) as described (13). The amounts of newly synthesized (Fig. 2*B*) and total (Fig. 2 *C* and *D*) p53 protein were also similar in young and old cells, in agreement with previous reports (27, 31) and with our results using immunofluorescence and reverse transcriptase-coupled PCR.

***In Vitro* DNA Binding Activity of p53 Increases During Cellular Aging.** Since p53 levels remained constant but p21 expression increased, we next examined p53 activity. Proteins were extracted from the nuclei of asynchronous young and old cells and were tested for DNA-binding activity by using a consensus binding site for the CRE binding protein in an EMSA. Similar amounts of shifted complex were recovered

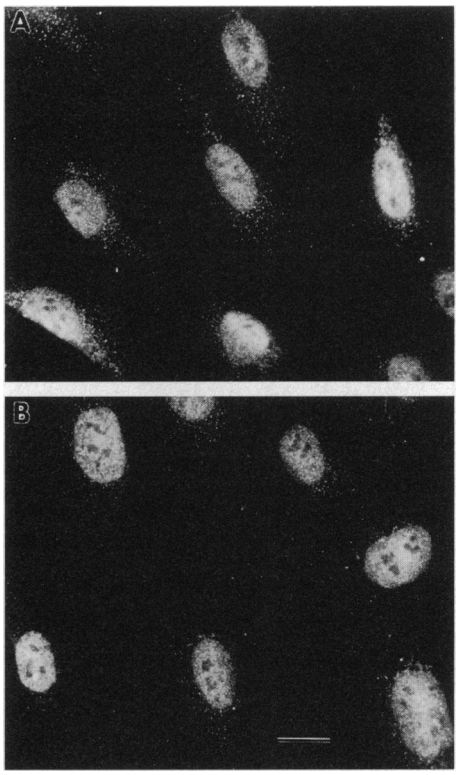

FIG. 1. p53 immunofluorescence in low- and high-passage HDFs. Young (*A*) and old (*B*) Hs68 fibroblasts were fixed, stained with a mixture of three p53 monoclonal antibodies in antibody excess, and photographed (38) under the same conditions to allow comparisons. (Bar = 10 μm.)

when equal amounts of proteins extracted from young or old cells were incubated with a wild-type CRE probe (Fig. 3*A*, lanes 2 and 3) that were not seen with a mutant CRE probe (Fig. 3*A*, lanes 4 and 5), corroborating previous observations (41). When similar reactions were performed by using an oligodeoxynucleotide containing a consensus p53 binding site, specific and nonspecific shifted complexes were seen (Fig. 3*B*). When extracts were incubated with PAb421 [which activates sequence-specific binding of p53 to DNA (8, 42)] prior to incubation with nuclear extracts, two specific complexes of similar low mobility were seen that were severalfold more abundant in extracts from old cells (Fig. 3*B*, lanes 7 and 8). These complexes did not form when incubating with nonspecific antibodies (lanes 5 and 6) or with PAb421 in the absence of nuclear extract (lane 2). Formation of these complexes on labeled probes was blocked by incubation of extracts with a 10-fold excess of unlabeled wild-type p53 oligonucleotide (lanes 9 and 10) but was not affected by incubation with mutant p53 oligonucleotide (lanes 11 and 12), demonstrating that they were specific for the consensus p53 binding site. PAb421-induced binding to p53 sites also increased with increased passage levels in all other strains of primary HDFs tested. Lanes 13 and 14 show results obtained with extracts from the HF strain and confirm that nuclear extracts from high-passage cells (lane 14) show greater p53 binding activity than extracts from low-passage cells (lane 13). Fig. 3*C* shows the results of densitometer scans of products from nine EMSAs comparing binding avidity of nuclear extracts from young and old cells under the conditions used for lanes 7 and 8. On average, binding increased 4.6 ± 1.9 times (mean ± SD, n = 9). The fact that very low levels of p53 binding activity were detected in extracts from growing (Fig. 2) or quiescent (data not shown) young cells is consistent with other reports indicating that p53 binding activity is very low compared to other DNA binding activities and is not detected in extracts from untransfected

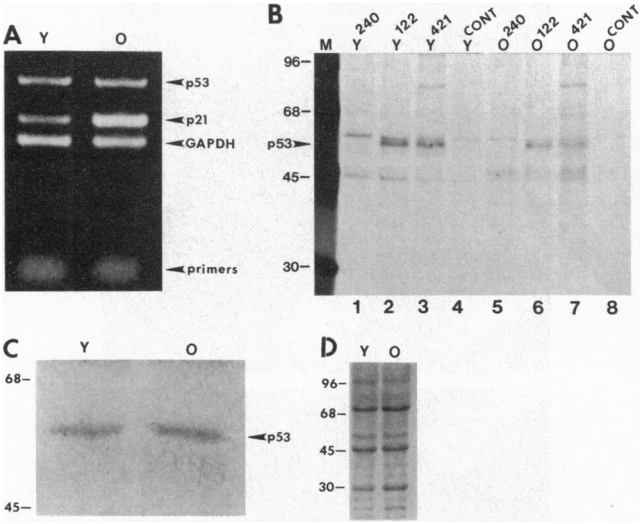

FIG. 2. Expression of p53 in young and old HDFs. (*A*) Arrowheads indicate the relative amounts of p53, p21, and GAPDH mRNA in young (Y) and old (O) cells. Gene products encoding p53, p21, and GAPDH were PCR-amplified in the same tube for 26, 22, and 19 cycles, respectively, by using a "primer-dropping" method (40). GAPDH served as an internal control. (*B*) Equal amounts of radioactivity from [^{35}S]methionine-labeled whole cell lysates from young (Y) or old (O) asynchronously growing Hs68 cells were immunoprecipitated under nondenaturing conditions by using the p53 monoclonal antibodies indicated. (*C*) The arrowhead indicates the signal obtained when cell lysates containing equal amounts of protein from low-passage (Y) or high-passage (O) cells were examined by immunoblot analysis with PAb421. (*D*) Coomassie brilliant blue staining pattern of aliquots of protein samples used for immunoblot analysis.

established cell lines (8, 42). Therefore, despite similar levels of p53 expression in young and old HDFs, the p53 extracted from near-senescent cells was ≈4.6-fold more active as a DNA binding protein *in vitro*.

Expression of Constructs Containing p53 Binding Sites Increases in Senescent Cells. We next examined the activity *in vivo* of endogenous p53 in young and old cells by microinjecting reporter constructs containing multiple copies of wild-type (wtp53-CAT) or of mutant (mup53-CAT) p53 binding sites located 5′ of a minimal polyoma promoter and the bacterial CAT gene (7, 39). Constructs encoding the CAT gene driven by the Rous sarcoma virus long terminal repeat (RSV-CAT) were injected as a positive control to determine whether near-senescent cells were capable of gene expression from a constitutive promoter. Both young (Fig. 4*A*) and old (Fig. 4*B*) fibroblasts expressed high levels of CAT protein when microinjected with the RSV-CAT expression construct. In contrast, clearly higher levels of immunodetectable CAT were observed in high-passage (Fig. 4*D*) vs. low-passage (Fig. 4*C*) fibroblasts after injection with the wtp53-CAT construct in four of four experimental trials. Low-passage cells showed a wide range in wtp53-CAT expression levels (see arrows in Fig. 4*E*) that were severalfold lower than levels seen in high-passage cells (Fig. 4*F*). Neither low- nor high-passage HDFs expressed detectable amounts of CAT after injection of the mup53-CAT construct (data not shown). These results corroborate the observation of increased p53 DNA binding activity seen *in vitro* in the EMSA and strongly suggest that the activity of p53 as a sequence-specific transcriptional activator increases as cells approach the end of their replicative lifespan in culture.

Levels of MDM-2 Expression and Binding to p53 Do Not Decrease in High-Passage Cells. Posttranslational modifications reported to affect p53 binding activity and p53 transcriptional activity include oxidation state (43), phosphorylation (42, 44), and binding to other proteins such as MDM-2 (5, 21).

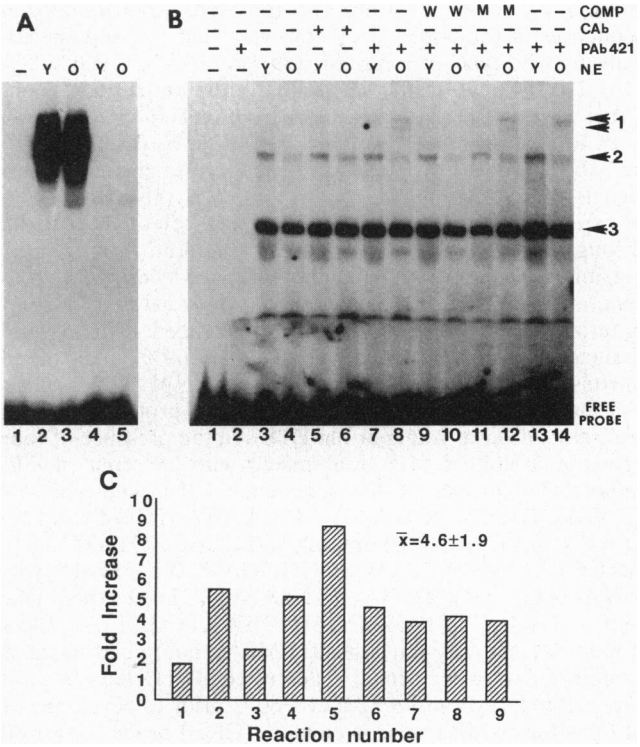

FIG. 3. DNA binding activity *in vitro*. (*A*) Total nuclear proteins from young (Y) or old (O) HDFs were mixed with 10^4 cpm of ^{32}P-radiolabeled wild-type (lanes 1–3) or mutant (lanes 4 and 5) synthetic CRE oligonucleotide probe. After electrophoresis, the dried gel was exposed to x-ray film for 12 h at −80°C. (*B*) Binding reactions with extracts from young (Y) and old (O) Hs68 cells (lanes 3–12) or HF cells (lanes 13 and 14) were performed as in *A* by using an oligonucleotide containing a p53 binding site. The specific bands identified by arrow 1 show a PAb421-inducible DNA–protein complex described in cells transfected with p53 expression constructs (8, 42). Bands identified by arrows 2 and 3 were nonspecific since they were unaffected by preincubation with p53 antibodies (compare lanes 5 and 6 and lanes 7 and 8) or by preincubation with wild-type (lanes 9 and 10) or mutant (lanes 11 and 12) unlabeled p53 oligonucleotide competitor. Similar results were obtained by using extracts from the A2 and WI38 primary fibroblast strains (data not shown). Binding reactions were preincubated with buffer (lanes 3 and 4), control hybridoma supernatant (CAb, lanes 5 and 6), PAb421 (lanes 7 and 8 and lanes 13 and 14), PAb421 plus excess unlabeled wild-type p53 oligonucleotide (lanes 9 and 10), or PAb421 plus excess unlabeled mutant p53 oligonucleotide (lanes 11 and 12). After electrophoresis, the gel was dried and exposed to x-ray film for 80 h at −80°C. (*C*) Bands corresponding to p53-specific shifted complexes from nine pairs of binding reactions using lysates from young and old cells were quantitated by scanning densitometry and the ratio of binding seen in old cell vs. young cell extracts was plotted.

Preparation of nuclear extracts using varied levels of reducing agents did not affect the ability of p53 to bind DNA by EMSA (data not shown), suggesting that oxidation of amino acid residues was not inhibiting binding in young cells. Since the MDM-2 protein has been shown to inhibit the transcriptional activity of p53 (5, 21), we examined MDM-2 mRNA levels by using reverse transcriptase-coupled PCR. Fig. 5*A* shows that three strains of young and old cells express very similar levels of MDM-2 mRNA when reactions are normalized to p53 levels (or to GAPDH; data not shown), indicating that the MDM-2/p53 mRNA ratio does not decrease with increased *in vitro* age. We next determined the relative amounts of p53 bound by MDM-2 protein as an estimate of MDM-2 activity. Both MDM-2 and certain p53 monoclonal antibodies including DO-1 bind the same region (aa 18–23) of the human p53 protein (36). If MDM-2 differentially bound and inactivated

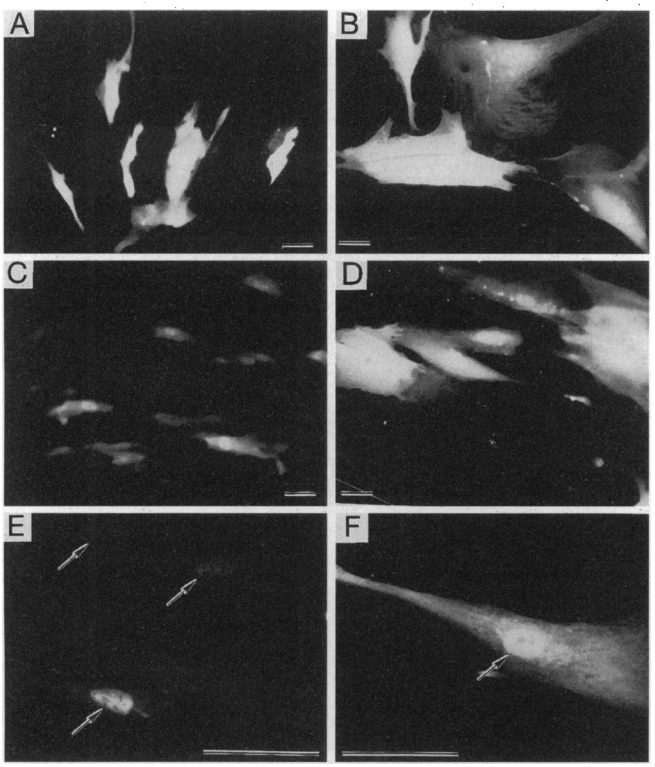

FIG. 4. Transcriptional activation *in vivo.* Young (*A, C,* and *E*) and old (*B, D,* and *F*) HDFs microinjected with a constitutively expressed RSV-CAT construct (*A* and *B*) or with the p53-responsive wtp53-CAT construct (*C–F*) were fixed 24 h later. Signal intensity was much greater for cells injected with RSV-CAT, and so to allow direct comparison between young and old cells, conditions of exposure and printing were identical between *A* and *B, C* and *D,* and *E* and *F.* HDFs increase in size as they approach senescence (41). (Bars = 50 μm.)

p53 in young and old cells, different amounts of p53 should be available for binding by the DO-1 antibody, but not by C-terminal-specific antibodies such as PAB421, under nondenaturing immunoprecipitation conditions. The PAB421 and DO-1 antibodies appear to bind similar amounts of p53, suggesting that the different p53 activities in young and old fibroblasts are not due to differential binding of MDM-2 (Fig. 5*B*). To more clearly visualize the relative amounts of p53 and MDM-2, nondenatured lysates prepared from young and old cells were sedimented through glycerol gradients, fractionated, and immunoprecipitated with p53 (Fig. 6 *A* and *B*) or control (Fig. 6*C*) antibodies. The relative amounts of a p53 antibody-specific protein of 92 kDa (probably MDM-2) were very similar in young (Fig. 6*A*) vs. old (Fig. 6*B*) cells, corroborating data from PCR analysis that showed that the levels of MDM-2 mRNA did not decrease in old cells and from immunoprecipitation studies with the p53 N-terminal-specific antibody (DO-1).

Net Levels of p53 Phosphorylation Do Not Change with Increased Passage. We have reported (41) that a protein that regulates transcription (serum response factor) is inactivated by hyperphosphorylation during cellular aging, and reports from another group (44) indicated that p53 transcriptional activity can be inhibited by hyperphosphorylation. We therefore analyzed the phosphorylation state of p53 in young and old Hs68 fibroblasts by immunoprecipitating [^{32}P]orthophosphate-labeled lysates containing the same amounts of total incorporated label. Immunoprecipitation with p53 (PAB421 and DO-1) but not control antibodies resulted in comparable signals being obtained from young and old cell lysates (Fig. 5*C*), suggesting that p53 is phosphorylated to similar degrees in young and old fibroblasts.

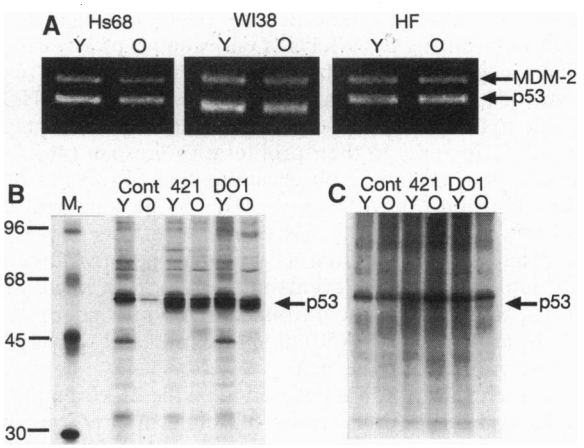

FIG. 5. Posttranslational modification of p53. (*A*) Steady-state levels of MDM-2 mRNA were assayed in three strains of young (Y) and old (O) HDFs. MDM-2 levels were standardized against p53 to give more precise estimates of the ratio of MDM-2 to p53 (40). (*B*) Lysates from young (Y) and old (O) Hs68 cells containing equal amounts of precipitable ^{35}S radioactivity were prepared and immunoprecipitated under nondenaturing conditions with control antibody (Cont) or the p53 antibodies PAB421 or DO-1. The sample from old cells precipitated with control antibodies was lost during preparation. (*C*) Lysates containing equal amounts of precipitable ^{32}P radioactivity were prepared and immunoprecipitated as described in *B.*

DISCUSSION

Based on the increased expression of p53 seen with DNA damage (22, 23), p53 has been proposed to be a component of cell cycle checkpoints that inhibit the progression of cells through the cell cycle until damaged DNA is repaired (11, 14, 20, 23). This proposal is consistent with the observation that overexpression of wild-type p53 inhibits cell proliferation (3–6). Conversely, inhibition of p53 function has been reported to extend the proliferative lifespan of human fibroblasts, suggesting a significant role in cellular senescence and a

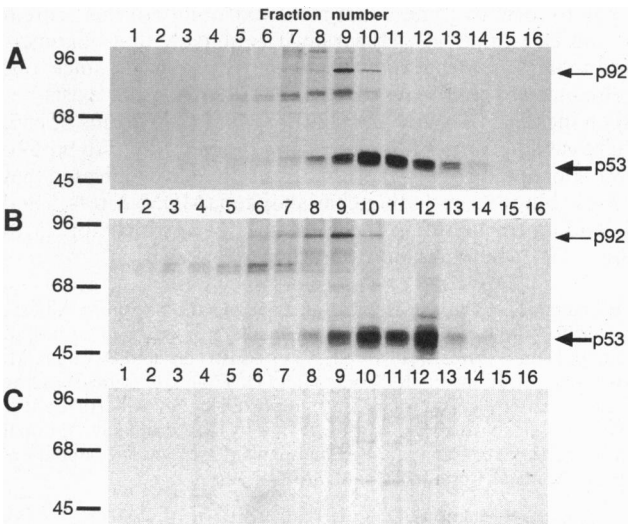

FIG. 6. Glycerol gradient fractionation of p53-containing complexes. Lysates from young and old Hs68 fibroblasts were prepared, centrifuged, fractionated, and immunoprecipitated. In all cases, 5×10^8 cpm of [^{35}S]methionine-labeled lysate was immunoprecipitated. (*A*) Lysate from young cells was immunoprecipitated with a mixture of excess PAb421 and DO-1 antibodies. (*B*) Lysate from old cells was immunoprecipitated as in *A*. (*C*) Lysate from young cells was immunoprecipitated with a control hybridoma supernatant. Bands common to *A–C* are nonspecific. Gels were exposed for 48 h at −80°C after impregnation with fluor.

possible link between senescence and DNA damage. By selectively neutralizing the effects of endogenous p53 by using a dominant negative approach in which an amphotropic retroviral vector directed expression of human mutant p53, HDFs were able to temporarily "escape" from senescence, as judged by an ≈30% increase in their proliferative lifespan (46). This result is supported by the observations that fibroblasts from p53-knockout mice have been reported to be immortal (47, 48). However, these results may be due to effects of the p53 mutant that are distinct from its dominant negative function (2), or additional cellular events in the knockout mice, since blocking p53 activity by the introduction of viral oncoproteins (49) or by antisense RNA (50) did not significantly extend the fibroblast proliferative lifespan.

A major effector of p53 is believed to be the 21-kDa inhibitor of CDKs and PCNA that arrests cell cycle progression when overexpressed and that is transcriptionally induced by p53 (11, 12). The observation that p21 expression increases severalfold as cells approach the end of their *in vitro* replicative lifespan (13) has led to our observations that p53 binding and transcriptional activities are increased in high-passage cells, but in the absence of increased p53 expression. It therefore appears that signals that link DNA damage to increased p53 expression are not activated as HDFs approach senescence. Instead, increased binding and transcriptional activity of p53 and increased levels of p21 expression appear to be due to alternate mechanism(s) that increase the activity of p53 in the absence of increased p53 protein and perhaps also induce p21 expression by additional p53-independent pathways. Such an additional mechanism has been implicated by experiments in which fibroblasts with extended lifespans due to the presence of a temperature-sensitive simian virus 40 tumor antigen show increased expression of p21 with increased passage levels (51). However, the presence of tumor antigen increases the endogenous levels of p53 in these cells severalfold and only a small and variable proportion of the cellular p53 appears to be bound by tumor antigen, raising the possibility that altered amounts and/or activities of p53 contribute to increased p21 expression in this model system.

Increased transcriptional activity of p53 may be due to altered protein–protein interactions of p53 such as the ability of p53 to form oligomers that affect the ability of this protein to bind DNA (52, 45). However, it is unlikely that oligomerization increases the activity of p53 in old cells since our preliminary experiments suggest that a correlation exists between increased activity and slower sedimenting forms of p53, whereas higher-order oligomers have been reported to bind to DNA more avidly (52, 45). Previous studies have indicated that p53 also binds several other characterized (MDM-2; refs. 5 and 21) and newly identified (26) proteins, all of which appear to inhibit DNA binding and transcriptional activation.

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1. Hollstein, M., Sidransky, D., Vogelstein, B. & Harris, C. (1991) *Science* **253,** 49–53.
2. Levine, A. J. (1993) *Annu. Rev. Biochem.* **62,** 623–651.
3. Chen, P. L., Chen, Y., Bookstein, R. & Lee, W. H. (1990) *Science* **250,** 1576–1580.
4. Diller, L., Kassel, J., Nelson, C. E., Gryko, M. A., Litwak, G., Gebhardt, M., Bressac, B., Ozturk, M., Baker, S. J., Vogelstein, B. & Friend, S. H. (1990) *Mol. Cell Biol.* **10,** 5772–5781.
5. Michalovitz, D., Halevy, D. & Oren, M. (1990) *Cell* **62,** 671–680.
6. Ullrich, S. J., Anderson, C. W., Mercer, W. E. & Appella, E. (1992) *J. Biol. Chem.* **267,** 15259–15262.
7. Kern, S. E., Kinzler, K. W., Bruskin, A., Jarosz, D., Friedman, P., Prives, C. & Vogelstein, B. (1991) *Science* **252,** 1708–1711.
8. Funk, W. D., Pak, D. T., Karas, R. H., Wright, W. E. & Shay, J. W. (1992) *Mol. Cell Biol.* **12,** 2866–2871.
9. Zambetti, G. P., Bargonetti, J., Walker, K., Prives, C. & Levine, A. (1992) *Genes Dev.* **6,** 1143–1152.
10. Farmer, G., Bargonetti, J., Zhu, H., Friedman, P., Prywes, R. & Prives, C. (1992) *Nature (London)* **358,** 83–86.
11. el-Deiry, W. S., Tokino, T., Velculescu, V. E., Levy, D. B., Parsons, R., Trent, J. M., Lin, D., Mercer, W. E., Kinzler, K. W. & Vogelstein, B. (1993) *Cell* **75,** 817–825.
12. Xiong, Y., Hannon, G. J., Zhang, H., Casso, D., Kabayashi, R. & Beach, D. (1993) *Nature (London)* **366,** 701–704.
13. Noda, A., Ning, Y., Venable, S. F., Pereira-Smith, O. M. & Smith, J. R. (1994) *Exp. Cell Res.* **211,** 90–98.
14. Waga, S., Hannon, G. J., Beach, D. & Stillman, B. (1994) *Nature (London)* **369,** 574–578.
15. Ginsberg, D., Mechta, F., Yaniv, M. & Oren, M. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 9979–9983.
16. Santhanum, U., Ray, A. & Sehgal, P. B. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 7605–7609.
17. Chin, K. V., Ueda, K., Pastan, I. & Gottesman, M. M. (1992) *Science* **255,** 459–462.
18. Seto, E., Usheva, A., Zambetti, G. P., Momand, J., Horikoshi, N., Weinmann, R., Levine, A. J. & Shenk, T. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 12028–12032.
19. Truant, R., Xiao, H., Ingles, C. J. & Greenblatt, J. (1993) *J. Biol. Chem.* **268,** 2284–2287.
20. Kastan, M. B., Zhan, Q., el-Deiry, W. S., Carrier, F., Jacks, T., Walsh, W. V., Plunkett, B. S., Vogelstein, B. & Fornace, A. J., Jr. (1992) *Cell* **71,** 587–597.
21. Momand, J., Zambetti, G. P., Olson, D. C., George, D. & Levine, A. J. (1992) *Cell* **69,** 1237–1245.
22. Kastan, M. B., Onyekwere, O., Sidransky, D., Vogelstein, B. & Craig, R. W. (1991) *Cancer Res.* **51,** 6304–6311.
23. Lu, X. & Lane, D. P. (1993) *Cell* **75,** 765–778.
24. Hayflick, L. & Moorhead, P. S. (1961) *Exp. Cell Res.* **25,** 585–621.
25. Goldstein, S. (1990) *Science* **249,** 1129–1133.
26. Iwabuchi, K., Bartel, P. L., Li, B., Marraccino, R. & Fields, S. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 6098–6102.
27. Rittling, S. R., Brooks, K. M., Cristofalo, V. J. & Baserga, R. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 3316–3320.
28. Seshadri, T. & Campisi, J. (1990) *Science* **247,** 205–209.
29. Richter, K. H., Afshari, C. A., Annab, L. A., Burkhart, B. A., Owen, R. D., Boyd, J. C. & Barrett, J. C. (1993) *Cancer Res.* **51,** 6010–6013.
30. Stein, G. H., Drullinger, L. F., Robetorye, R. S., Pereira-Smith, O. M. & Smith, J. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 11012–11016.
31. Afshari, C. A., Vojta, P. J., Annab, L. A., Futreal, P. A., Willard, T. B. & Barrett, J. C. (1993) *Exp. Cell Res.* **209,** 231–237.
32. Riabowol, K., Schiff, J. & Gilman, M. Z. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 157–161.
33. Gannon, J. V., Greaves, R., Iggo, R. & Lane, D. P. (1990) *EMBO J.* **9,** 1595–1602.
34. Harlow, E., Crawford, L. V., Pim, D. C. & Williamson, N. M. (1981) *J. Virol.* **39,** 861–869.
35. Wade-Evans, A. & Jenkins, J. R. (1985) *EMBO J.* **4,** 699–706.
36. Picksley, S. M., Vojtesek, B., Sparks, A. & Lane, D. P. (1994) *Oncogene* **9,** 2523–2529.
37. Tsai, L.-H., Lees, E., Faha, B., Harlow, E. & Riabowol, K. (1993) *Oncogene* **8,** 1593–1602.
38. Riabowol, K. T., Draetta, G., Brizuela, L., Vandre, D. & Beach, D. (1989) *Cell* **57,** 393–401.
39. Pietenpol, J. A., Tokino, T., Thiagalingam, S., el-Deiry, W. S., Kinzler, K. W. & Vogelstein, B. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 1998–2002.
40. Wong, H., Anderson, W. D. & Riabowol, K. (1994) *Anal. Biochem.* **223,** 251–258.
41. Atadja, P. W., Stringer, K. S. & Riabowol, K. (1994) *Mol. Cell Biol.* **14,** 4991–4999.
42. Hupp, T. R., Meek, C. A., Midgley, C. A. & Lane, D. P. (1992) *Cell* **71,** 875–886.
43. Delphin, C., Cahen, P., Lawrence, J. J. & Baudier, J. (1994) *Eur. J. Biochem.* **223,** 683–692.
44. Zhang, W., McClain, C., Gau, J. P., Guo, X. Y. & Deisseroth, A. B. (1994) *Cancer Res.* **54,** 4448–4453.
45. Hainaut, P., Hall, A. & Milner, J. (1994) *Oncogene* **9,** 299–303.
46. Bond, J. A., Wyllie, F. S. & Wynford-Thomas, D. (1994) *Oncogene* **9,** 1885–1889.
47. Harvey, M., Sands, A. T., Weiss, R. S., Hegi, M. E., Wiseman, R. W., Pantazis, P., Giovanella, B. C., Tainsky, M. A., Bradley, A. & Donehower, L. A. (1993) *Oncogene* **8,** 2457–2467.
48. Tsukada, T., Tomooka, Y., Takai, S., Ueda, Y., Nishikawa, S., Yagi, T., Tokunaga, T., Takeda, N., Suda, Y., Abe, S., Matsuo, I., Ikawa, Y. & Aizawa, S. (1993) *Oncogene* **8,** 3313–3322.
49. Shay, J. W., Wright, W. E., Brasiskyte, D. & Van der Hagen, B. A. (1993) *Oncogene* **8,** 1407–1413.
50. Hara, E., Tsurui, H., Shinozaki, A., Nakada, S. & Oda, K. (1991) *Biochim. Biophys. Res. Commun.* **179,** 528–534.
51. Tahara, H., Sato, E., Noda, A. & Ide, T. (1995) *Oncogene* **10,** 835–840.
52. Friedman, P. N., Chen, X., Bargonetti, J. & Prives, C. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 3319–3323.