ABSTRACT

In response to DNA damage, cells transduce a signal that leads to accumulation and activation of p53 protein, transcriptional induction of several genes, including p21, gadd45, and gadd153, and cell cycle arrest. One hypothesis is that the signal is mediated by DNA-dependent protein kinase (DNA-PK), which consists of a catalytic subunit (DNA-PKcs) and a regulatory subunit (Ku). DNA-PK has several characteristics that support this hypothesis: Ku binds to DNA damaged by nicks or double-strand breaks, DNA-PKcs is activated when Ku binds to DNA, DNA-PK will phosphorylate p53 and other cell cycle regulatory proteins in vitro, and DNA-PKcs shares homology with ATM, which is mutated in ataxia telangiectasia and involved in signaling the p53 response to ionizing radiation. The hypothesis was tested by analyzing early passage fibroblasts from severe combined immunodeficient mice, which are deficient in DNA-PK. After exposure to ionizing radiation, UV radiation, or methyl methane-sulfonate, severe combined immunodeficient and wild-type cells were arrested normally. Therefore, DNA-PK is not required for the p53 response or cell cycle arrest after DNA damage.

INTRODUCTION

When cells are exposed to DNA-damaging agents, they initiate a complex response that includes the arrest of cell cycle progression until the damage is repaired (1, 2). Agents that induce this response include IR, UV radiation, and chemical agents such as MMS. IR induces DNA nicks, double-strand breaks, and base damage; UV radiation induces cyclobutane pyrimidine dimers, 6–4 photoproducts, and other lesions, including DNA nicks acquired during excision repair of photoproducts; and MMS alkylates DNA, leading to spontaneous and enzymatic depurinations and DNA nicks from excision repair of abasic sites.

Several components of the pathways that arrest the cell cycle in response to DNA damage have now been identified in mammalian cells. One component of the G1 checkpoint is p53, a Mr 53,000 protein that is capable of binding to specific DNA sequences as a transcriptional activator and is normally present in the cell at very low levels. When cells are exposed to DNA damage, p53 undergoes posttranslational modification that stabilizes the protein and contributes to its accumulation (3–5). Cells lacking p53 fail to undergo G1 arrest following exposure to DNA-damaging agents (6, 7). The p53 gene is mutated in 50% of all human cancers, which suggests that the p53-dependent G1 checkpoint pathway is critical for suppressing carcinogenesis.

The p21 gene (also named Cip1, Waf1, Cap20, or Sdi1) includes a p53 DNA-binding site in its promoter region and is transcriptionally activated by p53 (8). Thus, p21 is induced by DNA damage and p21 protein interacts directly with cyclin-dependent kinases, inhibiting their activity and arresting the cell cycle (6, 9, 10).

Several genes are induced by growth arrest and DNA damage (gadd) and include the gadd45 and gadd153 genes (11). Like p21, the gadd45 promoter contains a p53-binding site, and gadd45 transcription is induced by IR via a p53-dependent pathway (12). The gadd45 gene is also induced by UV radiation and MMS by a p53-independent pathway. The gadd153 gene is induced by UV radiation and MMS by a p53-independent pathway and is not induced by IR (13).

Despite progress in identifying many of the damage-response genes, the molecule that detects DNA damage and initiates the signal for the subsequent cellular response remains a mystery. One candidate molecule is DNA-PK, a serine/threonine protein kinase composed of a catalytic subunit, DNA-PKcs, and a regulatory subunit, Ku (14). The catalytic subunit DNA-PKcs is a Mr 465,000 polypeptide that is normally inactive, but is activated by the binding of Ku to one of its DNA substrates. Ku was first identified as an autoantigen in several autoimmune diseases; it is a heterodimer of Mr 70,000 and 86,000 (Ku70 and Ku86) that binds to DNA ends, nicks, gaps, and stem-loop structures (15, 16). These Ku substrates are noteworthy since DNA ends are induced by IR, and DNA nicks are produced as an intermediate during excision repair of UV- and MMS-induced damage. Such strand breaks appear to trigger the p53-dependent damage response (17).

DNA-PK can phosphorylate a number of proteins in vitro, including Ku, p53, and other proteins with roles in replication or regulation of the cell cycle: RPA, c-myc, c-fos, c-jun, and topoisomerases I and II (18). DNA-PK appears to be required for the phosphorylation of Ku and RPA in vivo (19). Furthermore, the phosphorylation of p53 by DNA-PK might be physiologically significant: DNA-PK phosphorylates p53 in vitro at serines 15 and 37 in the amino terminal transactivation domain of p53, and site-directed mutagenesis of serine 15 leads to stabilization of p53, suggesting that phosphorylation of that site might affect the lifetime of p53 (20).

The kinase domain of DNA-PKcs shares homology with the kinase domain from the gene (ATM) that is mutated in ataxia telangiectasia (21). Mutations in the ATM gene lead to IR hypersensitivity, reduced ability to arrest DNA synthesis and mitosis, and a delayed p53 response after IR. Thus, the ATM gene acts upstream of p53 in the G1 checkpoint pathway (4, 6, 22), raising the possibility that DNA-PK might also act in a checkpoint pathway.

DNA-PK has an established role in detecting and repairing DNA double-strand breaks. Mutant rodent cells in three X-ray-sensitive complementation groups (groups 4, 5, and 7) are defective in repairing the double-strand breaks induced either by IR or by V(D)J recombination (23, 24). Group 5 cells lack Ku DNA end-binding activity (25, 26, 27), are rescued by transfection of Ku86 cDNA (50, 28), and have mutations in the Ku86 gene (29). Group 7 cells contain Ku DNA end-binding activity, but contain severely decreased levels of immunoreactive and enzymatically active DNA-PKcs protein (30–32). Furthermore, group 7 cells are rescued by centromeric fragments of
human chromosomal DNA containing the \( \text{DNA-PK}_{\text{cat}} \) gene (30–32). Group 7 includes cells from the scid mouse, which is hypersensitive to IR and lacks mature B and T cells due to a deficiency in V(D)J recombination (33).

DNA-PK has several hallmarks that would be expected for a molecule that couples DNA damage to arrest of the cell cycle. First, DNA-PK binds to DNA damaged by nicks or double-strand breaks and already has an established role in recognizing and repairing DNA damage in intact cells. Second, its kinase activity is quiescent under normal conditions but is activated by DNA damage. Third, potential targets for DNA-PK include p53 and other proteins that play roles in regulating the cell cycle. Fourth, the homology of DNA-PK to ATM suggests that DNA-PK might play a similar role in regulating the cell cycle after DNA damage.

In this article, we test the hypothesis that DNA-PK is essential for transducing DNA damage into the subsequent cellular responses. Since established cell lines often have abnormal regulation of their cell cycles, studies were performed on early passage fibroblasts derived from newborn scid mice, which are defective in DNA-PK. The scid and wild-type cells were exposed to DNA damage and compared for accumulation of p53 protein, induction of the p21, \( \text{gadd45} \), and \( \text{gadd153} \) genes, and G1 and G2 checkpoint responses.

**MATERIALS AND METHODS**

**Cell Culture.** Early passage embryo fibroblast cultures derived from p53 deficient (−/−) mice were generously provided by L. Donehower (34). The p53 −/− cells were grown in DMEM supplemented with 10% FCS. Early passage skin fibroblast cultures were generated from newborn scid (33) and Balb/c mice generously provided by L. Weissman. The scid mice were originally derived from the C.B-17 mouse lineage which differs from Balb/c exclusively at the immunoglobulin heavy chain locus IgH (33). Agouti wild type mice were generously provided by G. Barsh. Within 3–8 h after birth, newborn mice were sacrificed and the dorsal and ventral skins were surgically removed. Individual skins were washed repeatedly with PBS, and all traces of blood and connective tissue were removed. The skins were then placed in 10 ml of trypsin-EDTA (0.05% trypsin and 0.53 mM EDTA), which had been prewarmed to 37°C where they were dispersed by extensive cutting and vigorous pipetting. The culture was incubated at 37°C for 30 min, 5 ml of fresh warm trypsin-EDTA were added, and the culture was incubated further at 37°C for 15 min. The suspension was transferred to a 50-ml conical tube, and the remaining large fragments were allowed to settle to the bottom. The supernatant was carefully removed, and the cells were pelleted by spinning for 5 min at 1000 rpm. The cells were then resuspended in DMEM supplemented with 10% FCS and plated onto 10-cm tissue culture dishes. All cultures were maintained at 37°C in 5% CO2. Newborn human fibroblasts (NHFs) were prepared and maintained as described previously (6, 35). For treatments with DNA-damaging agents, 2 × 106 cells were plated onto 10-cm tissue culture dishes 24 h before treatment. For UV treatment, cells were rinsed with PBS and exposed, (uncovered) to UV radiation from a germicidal lamp at a flux of 1 J/m²s. Following treatment, fresh medium was added to the plates for the remaining incubation. For MMS treatment, cells were placed in fresh medium containing 100 μM MMS and incubated at 37°C for the duration of the experiment. For IR treatment, cells were exposed to a 137Cs source with a flux of 11.5 Gy/min. To test IR sensitivity of the primary fibroblast cultures, 3 × 105 scid or Balb/c cells were plated in duplicate and treated with 0, 2.5, 5, or 10 Gy. Five days after treatment, the remaining cells were trypsinized and counted.

**Western Blot Analysis.** Cells were lysed in a SDS-containing buffer [0.125 mM Tris-HCl (pH 6.8), 4% SDS, 20% glycerol, 0.008% bromphenol blue, and 50 mM DTT] at a concentration of 106 cells/ml, immediately boiled for 10 min, and loaded directly onto the gel or stored at −80°C. Samples (2 × 105 cell equivalents/lane) were resolved by a 7.5% SDS-polyacrylamide gel, transferred to nitrocellulose GSWP membrane (Millipore, Bedford, MA), and probed with monoclonal p53 antibody 240 or M19 (Santa Cruz Biotechnology, Santa Cruz, CA) followed by horseradish peroxidase-conjugated goat anti-mouse IgG or swine anti-goat IgG, respectively (Bio-Rad, Richmond, CA). Antibody binding was detected using enhanced chemiluminescence (Amer sham Corp., Arlington Heights, IL).

**Northern Blot Analysis.** Total RNA was prepared using RNA STAT-60 reagent (Tel-Test “B,” Friendswood, TX) according to the manufacturer’s instructions. Probes used for Northern blot analysis included Cip1/cDNA (from S. Elledge), \( \text{gadd45} \) and \( \text{gadd153} \) cDNA (from A. Fornace), and human \( \beta \)-actin cDNA (from P. Berg). They were labeled with [32P]dCTP by random hexamer labeling. Total RNA (10 μg) was denatured and run on a 0.8% formaldehyde agarose gel. The RNA was transferred to a Hybond nylon filter (Amersham Corp.) and cross-linked with the Stratalinker UV source (Stratagene, La Jolla, CA). The filter was hybridized to 32P-labeled probes at 65°C for 24 hr in 1× Nondenaturing wash (14% SDS, 130 mM NaOHPO4, 14 mM EDTA, and 0.2% Triton X-100). The filter was washed sequentially in 0.5×, 0.25×, and 0.1× Nondenaturing wash for 30 min each at 65°C. The filter was then autoradiographed on XAR-5 X-ray film at −80°C. To reprobe, the filter was stripped by washing in 0.03× Nondenaturing wash for 2 or more hr at 65°C and then hybridized to the new 32P-labeled probe.

**Cell Cycle Checkpoint Response.** Fibroblast cultures were assayed for G1 arrest by incubating asynchronously proliferating cells with 10 μM BrdUrd (Sigma Chemical Co., St. Louis, MO) for 2 h, beginning 6–8 h after mock treatment or exposure to IR. BrdUrd-labeled nuclei were quantified by flow cytometry after incubation with FITC-labeled anti-BrdUrd antibody (Becton Dickinson, Bedford, MA) as described previously (35). G2 delay was assayed as the fraction of cells in mitosis at various times after exposure of asynchronously proliferating cells to IR as described previously (35, 36). To determine the mitotic fraction, mitotic figures were counted in 50–200 consecutive high-power fields. Enough fields were viewed to ensure that at least 20 mitotic cells were counted in the sham control.

**RESULTS**

**Preliminary Experiments on Immortalized Cell Lines.** Our initial strategy in testing the possibility that DNA-PK might be involved in signaling DNA damage-mediated cell cycle regulation was to utilize immortalized human mouse and hamster cell lines containing defects in the individual components of DNA-PK. Western blot analysis of p53 protein levels in wild-type and mutant immortalized hamster cells revealed high levels of constitutive p53 expression, which was not induced significantly upon treatment with IR or UV (data not shown). In immortalized wild-type (C.B-17) and scid (SF-7) mouse cells, the constitutive expression level of p53 was also abnormally high (data not shown). In nonimmortalized primary cells, the basal expression of p53 is extremely low due to the short half-life of p53 protein (3, 37). Therefore, we chose to pursue these studies in early passage fibroblasts.

**Phenotype of Early Passage Fibroblast Cells.** Early passage fibroblast cultures from newborn scid and wild-type (agouti and Balb/c) mice were tested for two relevant phenotypes. The early passage scid cells contained normal levels of Ku DNA end-binding activity (data not shown) as was found for immortalized scid cells using methods described previously (26). In addition, the cells were tested for sensitivity to IR. The scid cells were approximately 10-fold more sensitive than wild-type cells at a dose of 10 Gy (Fig. 1), which was the same level of sensitivity as previously measured for immortalized scid cell lines (38, 39).

**DNA Damage-induced Accumulation of p53.** The accumulation of p53 in response to UV radiation was determined by measuring p53 protein levels over time after exposing cells to UV radiation (Fig. 2). The p53 accumulation in wild-type cells followed a prolonged time course from 4 h to at least 12 h, as reported previously (22). The p53 accumulation in scid cells was not significantly different from that in wild-type cells in four independent experiments with two different antibodies (M19 and 240).

The accumulation of p53 after IR was determined in early passage scid cells (Fig. 3). IR at doses of 2–20 Gy has been reported to
ACCUMULATION OF p53 AND CELL CYCLE ARREST IN scid CELLS

induction pattern: they induced gadd45 very strongly with MMS treatment and only weakly with UV (40 J/m²) or IR treatment (10 Gy; Fig. 4A). We observed the same induction for a range of UV doses (10–50 J/m²) and a slightly increased induction when the IR dose was

Fig. 3. scid cells accumulate p53 with a normal time course after IR damage. Early passage fibroblasts from wild-type Balb/c and scid mice were treated with 10 Gy IR, and cell lysates were harvested after 0, 1, 2, or 4 h. The accumulation of p53 was detected using Western blot with p53 antibody 240 and quantitated by densitometry.

increase p53 levels, with a brief peak (approximately 2–5-fold) 1–2 h after irradiation (5, 7). Therefore, wild-type, scid, and p53−/− cells were analyzed with Western blot for p53 protein expression 1, 2, and 4 h after treatment with 10 Gy IR. Wild-type and scid cell lines showed no significant difference in the p53 induction profile over a 4-h time course after treatment with 10 Gy in this and two other independent experiments.

DNA Damage-induced Transcription of gadd45, p21, and gadd153. Wild-type and scid cells were treated with MMS, UV, or IR, and total RNA was harvested 4 h after treatment (Fig. 4). Northern blot analysis of total RNA was used to monitor the expression of several genes known to be induced by DNA damage: gadd45, p21, and gadd153. The blot was first probed for β-actin to confirm equal loading of mRNA (Fig. 4D). The membrane was then stripped and rehybridized to a probe for gadd45, which is induced after IR by a p53-dependent pathway and after UV by a p53-independent pathway (4, 40). Wild-type and scid early passage fibroblasts showed the same

Fig. 4. DNA-PK activity is not required for induction of p21, gadd45, or gadd153. Early passage fibroblasts from wild-type Balb/c and scid mice were treated with DNA-damaging agents, and total RNA was harvested 4 h later. Northern blots were probed for expression of gadd45 (A), p21 (B), gadd153 (C), and β-actin (D). Cells were treated as follows: 0, no treatment; MMS, 100 µg/ml; UV, 40 J/m² UV radiation; and IR, 10 Gy IR. The blot was first probed with β-actin as a control for equal loading of mRNA, then stripped, and probed sequentially for gadd45, p21, and gadd153. Bands corresponding to each of these genes are indicated on the left, and the residual bands from previous probed are indicated in parentheses.
Table 1: G₁ checkpoint response after IR in scid and wild-type cells

<table>
<thead>
<tr>
<th>Cells</th>
<th>2-3 N (%)</th>
<th>3-4 N (%)</th>
<th>G₁ arrest (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Balb/c (n = 2)</td>
<td>33</td>
<td>112</td>
<td>67</td>
</tr>
<tr>
<td>agouti (n = 2)</td>
<td>43</td>
<td>106</td>
<td>57</td>
</tr>
<tr>
<td>scid (n = 3)</td>
<td>28</td>
<td>103</td>
<td>72</td>
</tr>
<tr>
<td>human (n = 1)</td>
<td>4</td>
<td>152</td>
<td>96</td>
</tr>
</tbody>
</table>

ACCUMULATION OF p53 AND CELL CYCLE ARREST IN scid CELLS

Fig. 5. G₁ checkpoint is intact in scid cells. Asynchronously dividing early passage fibroblasts were mock treated (A–C) or exposed to 8 Gy IR (D–F). Data are shown for wild-type Balb/c (A and D); scid (B and E); and NHFs (C and F). Cells were analyzed by flow cytometry, which reveals the distribution of nuclei in G₀, G₁, S-phase, and G₂-M for diploid and tetraploid cells. The first and second halves of the S-phase for diploid cells are marked by the boxes (first half, left box; second half, right box). The G₁ checkpoint response was quantitated for Table 1 by measuring selective depletion of the first half of the S-phase compartment (35). Data for NHFs were obtained by staining the cells using the same methods used for the mouse cells, but appear with different axes because a different flow cytometer was used for that particular experiment.

raised to 20 Gy (data not shown). Again, no significant difference was observed in scid and wild-type cells.

The p21 gene is induced after several forms of DNA damage by a p53-dependent pathway (6, 8, 41). Although the expression of p21 can be regulated in the absence of p53 (43), its induction after exposure to IR appears to be dependent on the presence of wild-type p53 (6, 42). Wild-type and scid cells induced p21 strongly in response to MMS, UV, and IR (Fig. 4B). Dose-response experiments demonstrated increasing p21 induction with increasing doses of UV (10–50 J/m²) and the same induction of p21 for a range of IR doses (10–20 Gy) (data not shown). Again, no significant difference in p21 induction was observed in scid and wild-type cells.

The gadd153 gene is induced by UV and MMS but not by IR in Chinese hamster ovary cells (11). Additional studies have linked gadd153 induction to a p53-independent UV response (44). In our experiments with primary mouse fibroblasts, gadd153 was strongly induced with MMS, only weakly induced in response to IR, and (in contrast to Chinese hamster ovary cells) weakly suppressed by UV treatment (Fig. 4C). Again, no significant difference was observed in scid and wild-type cells.

G₁ Checkpoint Response. Although scid cells had a normal damage response in the specific factors that we examined (p53, p21, gadd45, and gadd153), it remained possible that the physiological cell cycle response might nevertheless be abnormal. Therefore, the G₁ checkpoint response was quantified in early passage cultures of newborn mouse skin fibroblasts using flow cytometry (35). Cells were irradiated with 8 Gy IR to trigger G₁ arrest and then incubated with BrdUrd 6–8 h after irradiation for quantification of the S-phase cells. As demonstrated with the wild-type Balb/c fibroblasts (Fig. 5, A and D), the G₁ checkpoint arrested progression of G₁ cells into the S-phase. Cells that were in the S-phase at the time of irradiation continue progression albeit at a reduced rate. As a result of the inhibition of entry into the S-phase, the S-phase compartment is depleted after irradiation. By 6–8 h after irradiation of the wild-type Balb/c fibroblasts, the fraction of BrdUrd-labeled cells in the first half of the S-phase (2–3N DNA content) was reduced by 67% for Balb/c and by 57% for agouti wild-type cells (Fig. 5 and Table 1). Irradiation of the scid fibroblasts reduced the fraction of diploid cells in the first half of the S-phase by 72% (Fig. 5, B and E), a value similar to that seen for the wild-type fibroblasts and consistent with an intact G₁ checkpoint.

Notably, NHFs were more sensitive than the three mouse skin fibroblast strains to radiation-induced G₁ arrest. In parallel experiments with human fibroblasts from three different neonates, 8 Gy produced 96% G₁ arrest (Fig. 5, C and F, and Table 1) and 1.5 Gy produced 75–93% G₁ arrest. With the mouse fibroblasts, G₁ arrest could not be detected after 1.5 Gy, necessitating use of the higher 8-Gy dose.

Table 1: G₁ checkpoint response after IR in scid and wild-type cells

<table>
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<td>57</td>
</tr>
<tr>
<td>scid (n = 3)</td>
<td>28</td>
<td>103</td>
<td>72</td>
</tr>
<tr>
<td>human (n = 1)</td>
<td>4</td>
<td>152</td>
<td>96</td>
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G2 Checkpoint Response. Mammalian cells also have a G2 checkpoint that is activated after DNA damage. The G2 checkpoint was assessed by measuring inhibition of mitosis after IR. Low doses of IR will cause G2 cells to delay entry into mitosis without affecting completion of mitosis (35, 36). Consequently, the fraction of cells in mitosis drops following IR (Fig. 6). After a transient delay, the cells restored progression from G2 into M and mitosis recovered. The recovery in scid cells was delayed compared to Balb/c cells. This, however, is consistent with the scid repair defect, which leads to persistent DNA double-strand breaks. For both Balb/c and scid cells, inhibition of mitosis was maximal 2 h after treatment with 2 Gy IR. Therefore, the G2 checkpoint was quantified 2 h after irradiation for additional cell lines: agouti wild-type mouse cells and NHFs as well as Balb/c and scid cells. The scid and wild-type mouse cells displayed the same degree of inhibition of mitosis (Table 2): 78–86% after 2 Gy and 99–100% after 4 Gy. As with the G1 checkpoint, the G2 checkpoint response of mouse fibroblasts appeared to be attenuated in comparison to human skin fibroblasts, in which mitosis was inhibited by 99.7% 2 h after treatment with 1.5 Gy IR.

DISCUSSION

DNA-PK has a number of properties that have made it a candidate for transducing DNA damage into a signal for p53 accumulation and cell cycle arrest. Its Ku subunit binds to DNA double-strand breaks, which are induced by IR, and to DNA nicks, which appear as intermediates during the nucleotide excision repair of bulky adducts. It is already known to be involved in the repair of DNA double-strand breaks and therefore has a role in the cellular response to DNA damage. The kinase is activated by Ku binding to the damaged DNA and will phosphorylate p53 in vitro as well as other proteins involved in regulating the cell cycle. Phosphorylation of p53 induces a conformation change that activates its transcriptional activity (45). The kinase domain of DNA-PK is also homologous to the ATM gene, which is known to signal p53-mediated cell cycle arrest (4, 6). Therefore, it was important to test carefully the possible role of DNA-PK in cellular responses to DNA damage.

Cells from scid mice were used to test the role of DNA-PK, since these cells express no detectable kinase activity from DNA-PK (30), contain reduced levels of the catalytic subunit DNA-PKα protein (31), and are rescued by yeast artificial chromosomes carrying the DNA-PKα gene (30, 31). The available immortalized scid and wild-type mouse cell lines expressed constitutively high levels of p53, suggesting that such cell lines might have abnormal p53 and cell cycle responses to DNA damage. Therefore, our experiments were performed with early passage skin fibroblasts derived from newborn mice.

Accumulation of p53 protein was normal in scid and wild-type cells after treatment with IR or UV. No differences were seen even when p53 was measured at different times after different doses of the DNA-damaging agents. Similarly, the induction of the p21, gadd45, and gadd153 genes occurred normally in scid and wild-type cells after treatment with IR, UV, or MMS. Finally, scid and wild-type cells underwent normal G1 arrest and G2 delay in response to IR.

These observations demonstrated that the p53-dependent pathway for signaling DNA damage is intact in scid cells and does not require the kinase activity of DNA-PK. First, there was normal accumulation of p53 in scid cells. Second, there was normal induction of two genes, p21 and gadd45, which are transcriptionally activated by p53. It was important to measure p21 and gadd45 induction, because transcriptional activation by p53 depends on both the accumulation and posttranslational conformational changes in p53 (46). The possibility that DNA-PK might affect the function of p53 without affecting its accumulation was ruled out by the normal induction of p21 and gadd45.

The observations also demonstrated that several p53-independent damage signaling pathways do not require DNA-PK. The gadd45 and gadd153 genes are induced by UV and MMS independently of p53, and the p21 gene could potentially be induced by p53-independent pathways. Similarly, G2 delay following DNA damage does not require p53 (36, 42). Again, no abnormalities were seen in scid cells for each of these p53-independent responses.

There are several possible interpretations of our results. The interpretation that we favor is that DNA-PK does not act on p53 in vivo and is not involved in activating the G1 and G2 checkpoints after DNA damage. A second possible interpretation is that the scid mutation does not lead to complete loss of function of the DNA-PKα gene. There is evidence that the DNA-PKα polypeptide is present in scid cells albeit at reduced levels, although the enzymatic activity is undetectable. Thus, the residual levels of DNA-PK might somehow induce the accumulation and activation of p53 despite the absence of kinase activity in the scid cells. Elucidation of the scid mutation will help address this possibility, which we consider unlikely.

A third possible interpretation is that DNA-PK is involved in cell cycle regulation, but the loss of DNA-PK activity is concealed by the existence of overlapping pathways that activate p53 and induce cell cycle arrest independently of DNA-PK. For example, p53 is activated for sequence specific binding by short single strands of DNA by a mechanism that does not require any other proteins (47). Furthermore, p53 will bind directly to insertion-deletion mismatches, suggesting that it may be capable of acting directly as a sensor for DNA damage (48). The p53 response may also be regulated by the gene that is mutated in ataxia telangiectasia (ATM). Ataxia telangiectasia cells are

### Table 2: G2 checkpoint response after IR in scid and wild-type cells

<table>
<thead>
<tr>
<th>Cells</th>
<th>2 Gy (%)</th>
<th>4 Gy (%)</th>
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<tbody>
<tr>
<td>Balb/c</td>
<td>22 (n = 2)</td>
<td>0 (n = 1)</td>
</tr>
<tr>
<td>agouti</td>
<td>14 (n = 1)</td>
<td>0 (n = 1)</td>
</tr>
<tr>
<td>scid</td>
<td>14 (n = 5)</td>
<td>1 (n = 4)</td>
</tr>
</tbody>
</table>
abnormally sensitive to IR and show delayed accumulation of p53 and impaired G$_1$ arrest (4, 6, 22). Thus, the ATM gene acts upstream of p53 in the damage response pathway. The ATM gene shares homology with the putative kinase domain in DNA-PK$_{cs}$ (21, 49), suggesting that the ATM protein might be functionally similar to DNA-PK in being capable of phosphorylating p53 directly.

Given the possibility that redundant pathways may exist for transducing DNA damage into the p53-dependent response and cell cycle arrest, the data do not completely rule out a role for DNA-PK. However, the experiments do rule out an essential role for DNA-PK in both G$_1$ and G$_2$ checkpoint responses and strongly suggest that if there is a role, it must be a minor one. We were unable to find any evidence for an abnormal damage response in scid cells, apart from the known defect in double-strand break repair. The scid cells responded normally after several different forms of DNA damage. Both the magnitude and the time course of the response were indistinguishable from the response in wild-type cells when we measured accumulation of p53, induction of p21, gadd45, and gadd153, and arrest of the cell cycle in G$_1$. Although G$_2$ delay was prolonged in scid cells, this result was consistent with the prolonged presence of DNA double-strand breaks in the repair-deficient scid cells and clearly showed that the G$_2$ checkpoint was intact.

Given the normal response of scid cells when analyzed with respect to so many parameters, we believe that the best interpretation of the data is that DNA-PK is not involved in the p53-dependent response nor in the arrest of the cell cycle after cells are exposed to DNA-damaging agents.

ACKNOWLEDGMENTS

We thank Yan-Ru Chen and Gregory Barsh for guidance in establishing the early passage fibroblast cultures and for the agouti mice; H. Leo Aguila and Irving Weissman for helpful discussions and for the gadd45 and gadd153 probes; Stephen Elledge for the Cip1 probe; and Lawrence Donehower for the gadd45 and gaddl53 genes. We thank Y. H. Lee and Al Fornace for helpful discussions and for the gadd45 and gadd153 probes; Stephen Elledge for the Cip1 probe; and Lawrence Donehower for the p53$^-/$ cells.

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DNA-dependent Protein Kinase Is Not Required for Accumulation of p53 or Cell Cycle Arrest after DNA Damage


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