p53 Inhibits JC Virus DNA Replication in Vivo and Interacts with JC Virus Large T-Antigen

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The onset of DNA replication is an important step within the life cycle of the human neurotropic polyomavirus JC. In this study, evidence that both the human and the murine tumor suppressor protein p53 strongly inhibit JCV DNA replication in vivo is presented. This inhibition is dose-dependent and not a secondary effect of a decreased expression of JCV large T-antigen in response to p53. Using deletion mutants of murine p53 and tumor-derived point mutations of human p53, the basis of the suppression of JCV DNA replication by p53 was dissected. Deletion of either the amino- or the carboxy-terminal domain of murine p53 did not interfere with the repression of JCV DNA replication. However, deletion of the highly conserved central region of p53 abolished the inhibitory effect on replication. The tumor-derived human mutant p53(His273) inhibited JCV DNA replication significantly, whereas another tumorigenic mutant, p53(His175), had no inhibitory effect. Concomitantly, a direct protein–protein interaction between p53 and JCV large T-antigen was lost in mutants which did not affect JCV DNA replication. These results strongly suggest that p53 inhibits JCV DNA replication by interacting with JCV large T-antigen.

INTRODUCTION

JC virus (JCV), a member of the papovavirus family, is the etiologic agent of progressive multifocal leukoencephalopathy in human (for review see Berger and Concha, 1995). Exposure to JC virus usually occurs early in life, and results in the persistence of an archetypic virus in a large percentage of the human population. Reactivation of the latent virus in individuals suffering from immunosuppression can lead to selective infection and cytolysis of the myelin-producing oligodendrocytes of the central nervous system and results in severe demyelination. Viral isolates from infected brain tissue, such as prototypic JC viral strain MAD-1 (Frisque et al., 1984), exhibit a marked specificity for glial cells (Kenney et al., 1984). The tissue tropism of JCV is defined on the level of transcription and is conferred by the hypervariable noncoding region, which not only serves as origin of DNA replication (Lynch and Frisque, 1990; Sock et al., 1991, 1993) but also as promoter for the early regulatory and the late capsid genes (Kenney et al., 1984). Unlike the tissue specificity of the early gene activity, viral DNA replication occurs in any primate cell type when the early protein, T-antigen, is provided (Feigensbaum et al., 1987). In contrast to simian virus 40 (SV40), JCV is highly restricted in its ability to transform cells in culture (Walker and Frisque, 1986). Binding of p53 by SV40 large T-antigen appears to be important for the immortalization of rodent and human cells (Lin and Simmons, 1991; Zhu et al., 1991). The basis for JCVs restricted transforming behavior might be the difference in complex formation between JCV large T-antigen and p53 (Trowbridge and Frisque, 1993).

The p53 gene is the most frequently mutated gene found in human cancers and has been established as a tumor suppressor gene (for reviews see Levine et al., 1991; Donehower and Bradley, 1993; Prives and Manfredi, 1993). In vitro transformation assays have revealed that wild-type p53 inhibits cell transformation induced by activated oncogenes (Eliyahu et al., 1989). Inactivation of the p53 gene plays a critical role in malignant transformation, probably by allowing cells to escape from normal growth control. Previous studies have demonstrated that wild-type p53 can mediate direct growth arrest of proliferating cells at the G1–S boundary (Diller et al., 1990; Martinez et al., 1991). Accumulating evidence suggests that p53 may be directly involved in the regulation of DNA replication. For example, murine and human wild-type p53 inhibits the SV40 DNA replication in vivo (Braithwaite et al., 1987) and in vitro (Friedmann et al., 1990; Wang et al., 1989). Murine and human wild-type p53 binds to the SV40 large T-antigen, which acts as a helicase and also binds to the cellular DNA polymerase α (Gannon and Lane, 1987). The central highly conserved domain of p53 is responsible for this interaction (Tan et al., 1986; Jenkins et al., 1988). Wild-type p53 inhibits the initiation and propagation of SV40 DNA replication, most likely by preventing DNA unwinding by the large T-antigen helicase (Wang et al., 1989). p53 was also found to relocate to
the sites of DNA replication in herpes virus-infected cells (Wilcock and Lane, 1991), indicating that p53 may interact directly with components of the DNA replication machinery. In support of this observation, purified p53 has recently been shown to bind the cellular replication factor A in vitro (Dutta et al., 1993; He et al., 1993; Li and Botchan, 1993).

Unlike the well-studied interaction of p53 with SV40 large T-antigen (reviewed in Ludlow, 1993) and its influence on SV40 DNA replication (Friedman et al., 1990; Wang et al., 1989; Braithwaite et al., 1987), little is known about the interaction between p53 and JCV large T-antigen. Early studies found either no evidence for a protein complex between JCV large T-antigen and p53 in JCV virus-infected human fetal brain or kidney cells (Major and Traub, 1986) or the interaction was limited to the large T-antigen of JCV-S86, a strain which was isolated from owl monkey astrocytoma cells and differed from both the Mad-1 and Mad-4 strains (Major et al., 1987). Bollag et al. (1989) reported that the subpopulation of large T-antigen that forms a complex with cellular p53 in Rat2 cells is smaller in JCV-transformed cells than in SV40- or BK virus-transformed cells. It was further reported that the ratio of JCV large T-antigen to p53 in the corresponding complex in Rat2 cells differs from the stoichiometry found for the complex between SV40 large T-antigen and p53 (Trowbridge and Frisque, 1993).

In this study we show that wild-type human and the highly homologous wild-type murine p53 inhibit the replication of JCV in human glial cells. The central domain of murine p53 is essential for this suppression of JCV DNA replication. A tumor-derived mutant human p53, (His273), did also inhibit JCV DNA replication, whereas another mutant, p53 (His175), had no such effect. Furthermore we present evidence that binding of p53 to JCV large T-antigen is responsible for the repression of JCV origin-dependent DNA replication in vivo.

MATERIALS AND METHODS

Cells and baculovirus

U138 and U87MG human glioblastoma cells were propagated in RPMI 1640 and EMEM medium, respectively, supplemented with 10% fetal calf serum at 37° and 5% CO₂. Spodoptera frugiperda insect cells (Sf9) were grown in TC100 medium supplemented with 10% fetal calf serum at 27° and infected at an m.o.i. of 10 with recombinant baculovirus vJCTHxaII which expresses the large T-antigen of JC virus.

Antibodies

The monoclonal anti-p53 antibodies PAb421 and RA3-2C2 as well as the anti-SV40 T-antigen antibody PAb416, which cross-reacts with the JCV large T-antigen, were purified from supernatants of hybridoma cells by chromatography on protein A–Sepharose and dialyzed against a buffer containing 1% Nonidet P-40, 150 mM NaCl, and 50 mM Tris - HCl, pH 8.0.

Plasmids and construction of p53 deletion mutants

pJC433 is a pUC18-based plasmid containing the sequence of JC virus (Mad-1 strain) from map positions 4981 to 279 (Sock et al., 1993). The plasmid pRSV–JCT which expresses JCV large T-antigen has been described before (Sock et al., 1993).

Plasmids with cDNA sequences for mouse p53 were a generous gift of Dr. V. Rotter. In order to construct a series of p53 deletion mutants, the coding sequence for wild-type mouse p53 was inserted into the pBKS-II+ (Stratagene) as an EcoRI/HindIII fragment. For in vitro mutagenesis two synthetic oligonucleotides were used to introduce an AflII restriction site and a stop codon into the p53 cDNA (oligonucleotide 1: 5’ TGGGAACCTT CTGTA- CGCT TAAGCTTTTA GGTTCTGTGT TTG 3’; oligonucleotide 2: 5’ CAAAGAGAGC GCTGCCC * TGGGAACCTT CTG * 3’, oligonucleotide 2: 5’ CAAAGAGAGC GCTGCCC * TGGGAACCT TCT TAAG * GGTTCGTGTT TG 3’, oligonucleotide 2: 5’ CAAAGAGAGC GCTGCCC * TGGGAACCT TCT TAAG * GGTTCGTGTT TG 3’).

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Replication assay

U138 and U87MG human glioblastoma cells were plated at a density of 5 × 10⁵ per 6-cm dish in DMEM medium supplemented with 10% FCS 24 hr before transfection. Cells were transfected with 1 μg pJC433 and 1 μg of p53 expression plasmid applying the calcium phosphate technique followed by a glycerol boost 16 hr posttransfection. The JCV T-antigen was supplied by cotransfection of 1 μg of pRSV–JCT. At 72 hr posttransfection cells were harvested and low-molecular-weight DNA was extracted according to Hirt (1967). Repli-
cation was assayed by testing the DpnI sensitivity of the retrieved plasmid DNA as described (Sock et al., 1991). Quantification of Southern blots was performed using the ImageQuant software on a Molecular Dynamics PhosphorImager. Experiments (each performed in triplicates) were repeated at least three times for each plasmid.

**Extraction of JCV T-antigen and Western blotting**

Sf9 cells infected with recombinant baculovirus were lysed 48 hr after infection in a buffer containing 1% NP-40, 150 mM NaCl, 50 mM Tris- HCl, pH 8.0, 0.5 mM PMSF, and 1 mg/ml aprotinin at 0°C for 30 min. After centrifugation at 15,000 rpm and 4°C for 15 min, the supernatant was collected. For Western blotting protein samples were transferred from SDS-PAGE (10% polyacrylamide) onto nitrocellulose filters for 2 hr at 150 mA. Nitrocellulose was blocked with 10% dry milk in PBS for 2 hr. JCV T-antigen was detected by the anti-T-antigen antibody 279 (pJC433; Sock et al., 1991). Deletion mutants of murine p53 were described by the chimera Mad-1 from map positions 4981 to 5262/390 and 306/390 could be detected only with the antibody RA3-2C2 but not with PAb421. Figure 1C shows the expression of the human wild-type and mutant p53 proteins, detected with PAb421. Endogenous p53 of the U87MG cells was not detected with either antibody, indicating that expression and/or stability might be extremely low.

JCV DNA replication is inhibited by expression of human and mouse p53

A plasmid containing the complete regulatory region of JCV virus (Mad-1) from map positions 4981 to 279 (pJC433; Sock et al., 1991) which includes the viral origin of replication was tested for its ability to replicate in the presence of JCV virus large T-antigen and human or murine p53. The human glioblastoma cell lines U138 and U87MG were chosen for these studies. The JCV virus T-antigen and the human or murine p53 were expressed transiently in the human glioblastoma cells from the expression vectors pRSV-JCT and the various pSG5-p53 constructs, respectively, which were cotransfected with the replication test plasmid pJC433. Cotransfection of the expression vectors for both the human and the murine p53 results in a dose-dependent inhibition of replication of pJC433 in U87MG cells (Fig. 2, Table 1), as well as in U138 cells (Table 1). In the case of the human p53, 0.1 μg of transfected p53 expression plasmid led to a decrease in the replication rate of pJC433 in U87MG cells by 14.5%. Transfection of 0.3 and 0.5 μg resulted in a decrease by 21.3% and 40.7%, respectively, whereas at 1 μg the replication of pJC433 is reduced by 75 to 80% (Fig. 2B, Table 1). Similar results were obtained for murine p53. Transfection of 0.1 μg p53 expression plasmid led to a decrease in the replication rate in both cell lines by 8.6%. Increasing the amount of transfected expression plasmid to 0.3 and 0.5 μg resulted in a further decrease by 13.4 and 37.8%, respectively. After transfection of 1 μg expression plasmid the replication of pJC433 is reduced by 80 to 85% (Fig. 2A, Table 1).

The central domain of murine p53 is responsible for inhibition of JCV DNA replication

To dissect the structural basis of the inhibitory effect of murine p53 on JCV DNA replication we used deletion mutagenesis to remove distinct domains of murine p53. The deletion mutagens were named by the amino acids that were preserved (Fig. 1). All mutants were tested in standard in vivo replication assays

**Coimmunoprecipitation of JCV large T-antigen and murine p53**

p53 proteins were transcribed and translated in vitro using the TNT-coupled reticulocyte lysate (Promega) according to the manufacturer's instructions. Monoclonal antibody PAb416 preadsorbed to protein A-Sepharose was incubated with [35S]methionine-labeled p53 and baculovirus-produced JCV virus T-antigen for 2 hr at 4°C in binding buffer (150 mM NaCl, 50 mM Tris-HCl, pH 8.0, and 1% NP-40). Beads were washed four times with binding buffer, boiled in Laemmli sample buffer, and the proteins were size separated by SDS-PAGE (15% polyacrylamide). Coimmunoprecipitated 35S-labeled p53 was detected by autoradiography.

**RESULTS**

Expression of wild-type and mutant p53 in human glioblastoma cells

To analyze whether human and murine p53 inhibit JCV DNA replication, p53 cDNAs were cloned in the expression vector pSG5. Besides wild-type human p53 we assayed two tumor-derived human p53 mutants. One of the mutants— (His 273)— displays the wild-type conformation of human p53, whereas mutant (His175) was shown to have an altered structural conformation (Lane and Ben-Naim, 1990). Deletion mutants of murine p53 were specifically designed to target the conserved regions of murine p53, including the domains responsible for p53-T-antigen interaction (Fig. 1A). Expression of each of the p53 proteins in U87MG glioblastoma cells was analyzed by Western blotting using the p53-specific monoclonal antibodies PAb421 and RA3-2C2. Cells were transfected with 3 μg of the various p53 expression plasmids. Figure 1B demonstrates that all mutants are expressed although mutants Δ262/390 and Δ306/390 could be detected only with the antibody RA3-2C2 but not with PAb421. Figure 1C shows the expression of the human wild-type and mutant p53 proteins, detected with PAb421. Endogenous p53 of the U87MG cells was not detected with either antibody, indicating that expression and/or stability might be extremely low.
FIG. 1. Construction and expression of p53 deletion mutants. (A) Construction of deletion mutants of murine p53 and correlation to structural domains. A series of deletion mutants of murine p53 was designed and constructed as described under Materials and Methods. The extent of the deletions generated is indicated. The conserved boxes I to V, the nuclear localization signals, the oligomerisation domain, the N-terminal transactivation domain, and the binding domains for several proteins are shown below. (B) Expression of wild-type p53 and deletion mutants in U87MG cells. Cells were transfected with 3 μg of the various p53 expression plasmids. Protein samples were analyzed by Western blotting applying the p53-specific antibodies PAb421 (a) and RA3-2C2 (b). (C) Expression of wild-type and mutant human p53 in U87MG cells. Cells were transfected with 3 μg of the p53 expression plasmids. Protein samples were analyzed by Western blotting applying the p53-specific antibody PAb421.

upon cotransfection of U138 and U87MG cells with the replication test plasmid pJC433 and the expression plasmid for JCV T-antigen pRSV-JCT (Fig. 3, Table 2). Whereas no replication could be detected in the absence of JCV T-antigen, pJC433 replicated in the presence of JCV T-antigen (Fig. 3). The JCV DNA repli-
cation in both cell lines was inhibited by p53 to the same extent. The degree of inhibition of JCV DNA replication by p53 was virtually unchanged for constructs that were deleted either in the amino-terminal (Δ10/34 and Δ10/71) or in the carboxy-terminal domains (Δ306/390). Removal of the central domain of p53 comprising the conserved boxes II to V, however, abolished the inhibitory effect on the replication activity. From the series of deletion mutants tested the border of the inhibitory core domain of p53 can be localized between amino acids 71 on the amino-terminal side and 262 near the C-terminus.

Effects of human tumor-derived mutant p53 on JCV DNA replication

To study whether tumor-derived mutants of human p53 affect JCV DNA replication in vivo we have analyzed two different point mutations. One of them (His 273) was previously shown to retain the wild-type conformation of p53, whereas the other one (His 175) disturbs the structural conformation of the p53 protein. These mutants were tested in standard in vivo replication assays upon cotransfection of U87MG cells. Figure 4 and Table 3 demonstrate that JCV DNA replication was inhibited by wild-type and mutant (His 273) p53. The degree of inhibition was virtually the same for both p53 proteins. However, the mutant (His175) did not suppress JCV DNA replication in vivo at all (Fig. 4, Table 3). In conclusion p53 affects JCV DNA replication only in wild-type conformation.

p53 does not repress JCV T-antigen expression

p53 functions as a transcriptional regulator and can activate transcription when bound to a promoter through a p53 response element (Farmer et al., 1992; Funk et al., 1992), but it also can negatively regulate a variety of genes lacking such a response element (Ginsberg et al., 1991; Subler et al., 1994).

We asked whether coexpression of p53 in U87MG cells affects the level of JCV T-antigen necessary for replication and exerts the observed replicational repression this way. Therefore, pRSV-JCT was cotransfected with increasing amounts of pSG5–mp53cD and levels of JCV T-antigen were analyzed by Western blotting using the anti-T-antigen antibody PAb416 and the anti-p53 antibody PAb421. pRSV–JCT-transfected cells showed a clearly visible signal at 94 kDa, corresponding to JCV T-antigen even in the presence of high levels of coexpressed murine p53 (Fig. 5). Cotransfection of 5 μg of p53 expression plasmid (lane 4), compared to 1 μg (lane 2) used for replication studies, had no effect on the level of JCV T-antigen expressed in U87MG cells.

In the in vivo replication system the expression of JCV large T-antigen is driven by the LTR promoter of the Rous sarcoma virus. To find out whether murine p53 modulates the transcriptional activity of this promoter we studied the activity of a luciferase reporter gene which was under the control of the LTR promoter. Cotransfection of the pRSVluc reporter plasmid and the p53 expression plasmid into U87MG cells revealed that increased levels of the p53 expression vector do not down-modulate the expression of luciferase (data not shown). Thus the LTR promoter driving the JCV T-antigen expression is not impaired by overexpression of murine p53.

p53 interacts physically with JCV T-antigen in vitro

To investigate whether the inhibition of JCV DNA replication by p53 directly depends on the interaction of p53 with JCV T-antigen, coimmunoprecipitation experiments were carried out. For these experiments p53 proteins were translated in vitro in the presence of [35S]-methionine. Efficient expression and labeling of p53 was analyzed by SDS–PAGE (Fig. 6). Immuno precipitations were carried out with the anti-T-antigen antibody PAB416 coupled to protein A-Sepharose. We first dissected the structural basis of the interaction of murine wild-type and mutant p53 with JCV T-antigen. JCV T-
antigen and equal amounts of each of the \([^{35}S]\)-methionine-labeled p53 proteins were incubated and subsequently precipitated with the anti-T-antigen resin. Figure 6A clearly shows that \([^{35}S]\)-methionine-labeled murine wild-type p53 coprecipitates with JCV large T-antigen. Comparable results were obtained for some of the p53 deletion mutants. Deletion of the N-terminal region of p53 (p53Δ10/34, p53Δ10/71) or of the C-terminal domain containing the nuclear localization and oligomerization signals (p53Δ306/390) did not interfere with the ability of p53 to form stable complexes with JCV T-antigen (Fig. 6A). However, mutations affecting the central region of the p53 protein (p53Δ71/213, p53Δ71/266, p53Δ71/310, p53Δ262/390) lost their competence for the interaction with JCV T-antigen.

In further experiments we analyzed the interaction of JCV T-antigen with human wild-type and mutant p53 (Fig. 6B). Mutant p53 (His 273) bound to JCV T-antigen with similar relative affinity as wild-type human p53. Mutant p53 (His 175), however, failed to interact with JCV T-antigen. These results argue that a structurally intact central region of p53 is necessary and sufficient for the interaction between p53 and the JCV T-antigen and for the repression of T-antigen-dependent JCV DNA replication.

**DISCUSSION**

DNA replication is an important step in the life cycle of papovaviruses. Not only are viral progeny genomes produced during this process, but also important changes take place which cause the virus to switch from the early to the late phase of viral gene expression. DNA replication has been well studied in SV40; however, much less is known about the mechanism and the regulation of DNA replication of the related papovavirus JC. The aim of this work was to increase our understanding of the replication properties of this virus which is endemic in humans and can cause PML in immunocompromised individuals.

The studies presented here show that p53 represses JCV DNA replication by interacting with JC virus T-antigen which is necessary for initiation and elongation of DNA replication (Feigenbaum et al., 1987). It has been postulated that wild-type p53 plays a negative regulatory role in SV40 DNA replication in vivo, possibly by downregulating SV40 DNA replication late in the lytic cell cycle (Gannon and Lane, 1987; Kienzle et al., 1989; Stürzbecher et al., 1988; Wang et al., 1989). This negative regulatory effect of p53 in SV40 DNA replication often is considered a

### TABLE 1

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<tr>
<th>p53 expression plasmid</th>
<th>% Replication in U87MG cells</th>
<th>0 μg</th>
<th>0.1 μg</th>
<th>0.3 μg</th>
<th>0.5 μg</th>
<th>1.0 μg</th>
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<tr>
<td>Murine</td>
<td>8.9 ± 1.5</td>
<td>91.4 ± 12.4</td>
<td>86.6 ± 9.3</td>
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<td>% Replication in U138 cells</td>
<td>100 ± 13.8</td>
<td>90.6 ± 7.2</td>
<td>88.4 ± 5.7</td>
<td>67.3 ± 6.3</td>
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<tr>
<td>Human</td>
<td>100 ± 6.8</td>
<td>85.5 ± 8.9</td>
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### TABLE 2

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<th>% Replication in U87MG cells</th>
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<td>100 ± 13.8</td>
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<td>wt</td>
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<td>Δ10/266</td>
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<tr>
<td>Δ306/390</td>
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<td>13.7 ± 14.3</td>
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**FIG. 4.** Repression of JCV DNA replication by human p53. U87MG cells were transfected with 1 μg of each of the expression plasmids pJC433, pRSV JCT and pSG5-hp53, respectively. Low-molecular-weight DNA was extracted and analyzed by Southern blotting. Arrow, replicated, DpnI-resistant pJC433 DNA.

model for the antiproliferative effect of wild-type p53, as it is consistent with the observation that mutant p53 has lost its inhibitory effect on SV40 DNA replication both in vitro and in vivo (Friedman et al., 1990; Miyamoto et al., 1990). This is in analogy to the loss of a growth-inhibitory function of mutant p53 in cellular proliferation (Mercer et al., 1990; Michalowitz et al., 1990).

The SV40 large T-antigen binding domain of p53 overlaps the sequence-specific DNA binding domain within the central region of the protein (Shaunlian et al., 1992; Ruppert and Stillman, 1993). This region of the p53 protein contains mutational hotspots in human tumors. From our deletion analysis, the acidic transactivation domain which is indispensable for stimulating transcription apparently to be important but not solely responsible for repressing JCV DNA replication. Moreover, deletion of the C-terminal region hardly impairs the replication-repressing activity. Structural motifs within the C-terminal region determine the ability of p53 to form homologous complexes (Stürzbecher et al., 1992). Thus, it appears that strong p53–p53 interaction is not required for the observed inhibitory activity of p53. This might be due to a weak nontetrameric oligomerization domain recently identified within amino acid 1–320 (Stenger et al., 1994). Interaction between large T-antigen and p53, on the other hand, is indispensable for the inhibitory activity of p53 on JCV DNA replication. Mutants that fail to bind to JCV T-antigen are not able to repress JCV DNA replication in vivo. In SV40 DNA replication, the initiation step reportedly involves the binding of large T-antigen to DNA sequence elements comprising the origin of replication as two lobes, each containing a hexamer of large T-antigen, followed by induction of structural distortions and gradual unwinding of the origin DNA (Borowiec et al., 1990). This unwinding of the SV40 origin of replication by large T-antigen is the target of inhibition by p53 at least in vitro (Stürzbecher et al., 1988; Wang et al., 1989). It is possible that any disturbance of this delicately balanced initiation complex by the binding of JCV large T-antigen to p53 even without the formation of p53 self-aggregates would result in a disruption of the initiation process.

Growth suppression by p53 may be mediated through transcriptional control of specific target genes or by interaction with other cellular components or both. Binding of large T-antigen to wild-type p53 may mimic the effect of tumor mutations and interfere with p53 function either by directly inhibiting DNA binding (Bargonetti et al., 1992) or by preventing interaction with a cellular protein that facilitates DNA binding. Alternatively, large T-antigen binding could prevent p53 association with other cellular proteins that mediate the effect of p53 on growth control. Analogy with

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<th>Replicational Repression by Wild-Type p53 and Tumor-Derived Mutants</th>
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<tr>
<td>p53</td>
<td>% Replication in U87MG cells</td>
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<tr>
<td>Mock</td>
<td>100 ± 6.8</td>
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<tr>
<td>wt</td>
<td>22.5 ± 7.3</td>
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<td>His 175</td>
<td>94.3 ± 7.8</td>
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<td>His 273</td>
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**FIG. 5.** p53 does not repress JCV T-antigen expression. U87MG cells were transfected with 3 μg of JCV T-antigen expression plasmid pRSV JCT and increasing amounts of pSG5–mp53cD. pSG5 plasmid was added to bring the total amount of transfected DNA to 8 μg. Protein samples were analyzed by Western blotting applying the anti-p53 PAb421 and anti-T-antigen PAb416 antibodies. Lane 1, without pSG5–mp53cD; lanes 2, 3, and 4, 0.5, 1, and 5 μg of pSG5–mp53cD.
FIG. 6. (A) Interaction of murine p53 deletion mutants with the JCV large T-antigen. (a) In vitro translation of p53 deletion mutants. [35S]Methionine-labeled p53 proteins were separated after translation by SDS-PAGE (15% polyacrylamide) and visualized by autoradiography. (b) Interaction of p53 with JC virus T-antigen in vitro. [35S]Methionine-labeled wtp53 and deletion mutants were incubated with JC virus T-antigen and coimmunoprecipitated by anti-T-antigen antibody PAb416 coupled to protein A-Sepharose. Precipitates were separated by SDS-PAGE (15% polyacrylamide) and analyzed by autoradiography. (B) Interaction of human p53 with the JCV large T-antigen. (a) In vitro translation of p53. [35S]Methionine-labeled p53 proteins were separated after translation by SDS-PAGE (15% polyacrylamide) and visualized by autoradiography. (b) Interaction of p53 with JC virus T-antigen in vitro. [35S]Methionine-labeled murine (mu wt) and human (hu wt) wild-type and human mutant p53 (M175 and M273) was incubated with JC virus T-antigen and coimmunoprecipitated by anti-T-antigen antibody PAb416 coupled to protein A-Sepharose. Precipitates were separated by SDS-PAGE (15% polyacrylamide) and analyzed by autoradiography.

the retinoblastoma protein Rb suggests that, for each of these tumor suppressor proteins, large T-antigen could recognize a protein binding domain that normally interacts with cellular factors to regulate growth. Additionally, the inhibition of JCV DNA replication as well as the SV40 DNA replication by p53 suggests a common mechanism for at least the JCV and the SV40 papovavirus to overcome the stringent cell cycle control by p53. The initiation of viral replication depends on the functional inactivation of endogenous p53, since endogenously expressed wild-type p53 does not negatively modulate SV40 DNA replication in vivo (von der Weth and Deppert, 1993). The ratio of p53 to large T-antigen in vivo replication is low, leaving the majority of large T-antigen in a free, replication-competent state. In contrast, exogenous supplementation of p53 results in a titration of free T-antigen and subsequent inhibition of viral DNA replication. Therefore, we assume that, in analogy to the functional elimination of free large T-antigen by excess p53 in vitro replication assays, overexpression of wild-type p53 might eliminate replicational activities of large T-antigen in vivo. More detailed analysis of p53 domains involved in the regulation of viral and cellular DNA replication should lead to new insights into fundamental mechanisms of cell cycle and growth control by p53.

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Rat2 cells containing prototype, variant, mutant, and chimeric JC virus and SV40 virus genomes. Virology 196, 458–474.


