Cell-Specific Modulation of Papovavirus Replication by Tumor Suppressor Protein p53

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Small DNA tumor viruses like human papillomaviruses, simian virus 40, and adenoviruses modulate the activity of cellular tumor suppressor proteins p53 and/or pRb. These viruses replicate as nuclear multiplicity extrachromosomal elements during the S phase of the cell cycle, and it has been suggested that inactivation of p53 and pRb is necessary for directing the cells to the S phase. Mouse polyomavirus (Py), however, modulates only the pRb protein activity without any obvious interference with the action of p53. We show here that Py replication was not suppressed by the p53 protein indeed in all tested different mouse cell lines. In addition, E1- and E2-dependent papillomavirus origin replication was insensitive to the action of p53 in mouse cells. We show that in hamster (Chinese hamster ovary) or human (osteosarcoma 143) cell lines the replication of both Py and papillomavirus origins was efficiently blocked by p53. The block of Py replication in human and hamster cells is not caused by the downregulation of large T-antigen expression. The deletion analysis of the p53 protein shows that the RPA binding, proline-rich regulatory, DNA-binding, and oligomerization domains are necessary for p53 action in both replication systems. These results indicate that in mouse cells the p53 protein could be inactive for the suppression of papovavirus replication.

p53 is one of the key proteins which ensures the genomic integrity of higher eukaryotic cells (18, 19, 21). This function is believed to be expressed through the transcriptional activation and repression activities of the protein, which result in cell cycle block or in the induction of apoptosis of cells after the activation of p53 (21). This protein also participates in a mitotic spindle checkpoint (6) as well as in the prevention of reduplication of DNA before the completion of mitosis. The latter function does not require the transactivation activity of p53 (27).

In addition, it has been shown that the p53 protein is directly involved in the control of DNA repair and replication (18). p53 interacts with several cellular proteins involved in DNA repair and replication, like the DNA helicases, replication protein A (RPA), and RAD51 (8, 30, 36, 46). It has been demonstrated that the p53 protein truncated in its C terminus blocks nuclear DNA replication in Xenopus egg extracts (5).

Small DNA tumor viruses in general encode proteins which interact with and modulate the activity of cellular tumor suppressor proteins. The interaction of the p53 protein with simian virus 40 (SV40) large T antigen (LT Ag) impairs the helicase activity of this protein (35, 45) as well as the ability of p53 to activate transcription (40). Specific p53 binding sites have been identified in the SV40 replication origin (2), LT Ag of another member of the papovavirus family, human JC virus, also binds p53, resulting in the inhibition of viral replication (34). The adenovirus E1B 55-kDa protein interacts with p53, thus blocking p53-dependent transcription (49). The binding of hepatitis B virus HBX protein to p53 abolishes the p53 sequence-specific DNA binding and hence the ability to activate transcription, which leads to the impairment of p53-dependent apoptosis (14, 47). In the case of herpes simplex virus infection, p53 has been colocalized with proliferating-cell nuclear antigen, DNA polymerase α, DNA ligase, and RPA in the DNA replication sites (48). The E6 proteins of both the high-risk and low-risk human papillomaviruses (HPVs) interact with p53 (22, 32). The HPV-16 and HPV-18 E6 proteins interact with p53 and direct its degradation through the ubiquitin degradation pathway (16). Several viral proteins modulate the pRB protein activity. Adenovirus E1A protein (10), HPV E7 protein (11), SV40 LT Ag, and mouse polyomavirus (Py) LT Ag (9) all interact with and modulate the activity of pRB. It is believed that modulation or inactivation of the tumor suppressor proteins by viral factors is necessary to direct the cells into the S phase of the cell cycle, thus providing necessary conditions for replication.

In our previous work we demonstrated that p53 could efficiently block the amplificational replication of the papillomavirus origin. This activity of p53 is determined by the RPA binding, proline-rich regulatory, DNA binding, and oligomerization domains of the p53 protein (20). The inhibition of replication seemed to be direct, not making use of the abilities of p53 to block the cell cycle or direct cells to apoptosis. We also showed that the N-terminal transcriptional activation and C-terminal regulatory domains are not needed for the suppression of replication. Our data suggested that papillomaviruses could use the p53 protein to control the amplificational replication of viral genome in basal cells, where the initial amplification of viral DNA takes place. Py LT Ag binds pRb, but the virus has not been shown to have a mechanism for neutralizing p53, raising the question whether papillomavirus replication is not susceptible to the action of p53. It has been shown that under normal circumstances p53 is unable to suppress the replication of Py DNA in vivo and in vitro (17, 23). Moreover, Py transformation apparently does not interfere with the transactivation activities of the p53 protein in REF52 cells after irradiation (24).

We studied the effect of p53 on the replication of papillomavirus and Py origins in mouse cells. Surprisingly we found that p53 was equally inactive for the suppression of Py and papillomavirus replication. At the same time, both papilloma-
virus and Py replication was efficiently blocked by p53 in Chinese hamster ovary (CHO) cells and in human cells. Our data indicate that in mouse cells p53 does not interfere with the viral functions and must be using different activities from those used in other cell lines.

MATERIALS AND METHODS

Plasmids. Bovine papillomavirus type 1 (BPV-1) E1 expression vector pCGEag, E2 expression vector pCGE2, and minimal replication origin plasmid pUCAT153 have been described previously (4, 44). Human p53 deletion mutants were created by PCR and expressed from the pCG vector (20). The mutant protein SReΔN39 expression vector was generated by removing the cytomegalovirus (CMV) promoter from the pCG vector with EcoRI and EcoRII and replacing it with the SRe promoter from the vector pBBS5G (29) by blunt-end cloning. Py origin-containing plasmid pmtu1046/CAT (Py wt ori) contains a replication-stimulating transcriptional enhancer segment adjacent to the origin; the mutant pmtu1047/CAT has the enhancer segment deleted (26). In the construct pmtu1047mE2RE/CAT (Py enh−/E2BS), the normal enhancer has been replaced by BPV-1 E2 binding sites (25). Py LT Ag expression vector pCGLT was generated from plasmid pLTBB, kindly provided by G. Magnusson (Py DNA with the LT Ag coding region from pLTBB). Escherichia coli the LT Ag coding region from pLTBB. The XbaI-XhoI fragment of pCGbeta was derived from the vector pCGE2. The XhoI site of pCGbeta was derived from the derivative of the vector pNP175, containing BPV-1 upstream regulatory region (URR) and pUC19 polylinker, by replacing the lacZ gene with the LT Ag coding region from plTB. Escherichia coli β-galactosidase expression vector pCGbeta was derived from the vector pCGE2. The XhoI site in pCGE2 was converted into the HindIII site by linker insertion, and the E2 coding sequence was replaced with the lacZ gene and β-globin intron from pP1Np175 using XbaI and HindIII cleavage.

Cells and transfections. The cell line CHO and its derivative CHO4.15 (expressing the BPV-1 E1 and E2 proteins) (29) was maintained in Ham's F12 medium supplemented with 10% fetal calf serum. Mouse NIH 3T3 fibroblasts, mouse COP5 cells constitutively expressing Py T Ag (41), and BALB/c murine lymphoma cells producing the BPV-1 E1 and E2 proteins) (29) was maintained in Ham's F12 medium supplemented with 10% fetal calf serum. Mouse embryonic stem cells (ES cells) were cultured in conditioned ES culture medium (Bethesda Research Laboratories) containing leukemia inhibitory factor (10 U/ml). The electroporation experiments were carried out as described previously (43), using an Innotro ElectroPorator at a capacitance setting 975 μF. The voltage settings were 230 V for CHO and CHO4.15 cells, 170 V for human osteosarcoma 143 cells, 220 V for COP5 cells, 200 V for NIH 3T3 cells, 210 V for 101 cells, and 150 to 190 μg/ml for ES cells. Transient-transfection assays were performed as described previously (43). The transfection efficiencies were determined by in situ staining of the cells transfected in parallel with a β-galactosidase-expressing plasmid, pCGbeta.

For β-galactosidase assays (31) CHO and NIH 3T3 cells were transfected with 500 to 1,000 ng of the lacZ expression vector and 250 ng of p53 expression constructs. β-Galactosidase expression was measured 24 h posttransfection, and the relative optical density was measured using the samples with no added p53 as controls.

Immunoblotting. The expression level of LT Ag and p53 constructs was estimated by Western blot analysis of CHO4.15, CHO and COP5 cells transfected with 500 to 1,000 ng of LT Ag expression plasmid and 100 to 3,000 ng of p53 expression plasmid and processed 24 h after transfection. The cells were lysed in sodium dodecyl sulfate (SDS) loading buffer and analyzed by SDS-polyacrylamide gel electrophoresis (PAGE) (10% acrylamide) by standard methods (31). The LT Ag was detected using mouse F4 monoclonal antibody (28) as the primary antibody and peroxidase-conjugated goat anti-mouse immunoglobulin G as the secondary antibody; for p53, a mixture of pAb240 and pAb421 antibodies was used as the primary antibody. Enhanced chemiluminescence Western blotting detection reagents (Amersham) were used to detect the signals. Equal numbers of transfected cells were loaded onto the gel for that purpose, the cells were counted, and the transfection efficiencies of the cell lines were estimated within each experiment.

RESULTS

Replication of the Py and papillomavirus origins is not suppressed by the p53 protein in mouse fibroblasts. p53 protein suppresses the papillomavirus origin-dependent replication in hamster and human cells (20). We decided to study the presumed differential effect of the p53 protein on the replication of Py and papillomavirus origins in mouse cells, because previous studies suggested that Py replication is not influenced by this protein (17, 23). We first studied the effect of wild-type p53 and different p53 mutants on the replication of Py origin in the mouse COP5 cell line. These cells are derivatives of mouse C127 cells and express constitutively all Py T Ags. We electroporated 50 ng of Py origin plasmid together with 250 ng of wild-type p53 and different mutant p53 expression plasmids into the cells and found that Py replication is not blocked by the p53 protein in these cells, as expected (Fig. 1A). In the following experiment we cotransfected 150 ng of the BPV-1 minimal-origin plasmid pUCAlu, 50 ng of Py origin plasmid 1046, 1,000 ng of the BPV-1 E1 protein expression vector pCGEag, and 500 ng of E2 protein expression vector pCGE2 into the COP5 cells by electroporation. The plasmids containing the Py and papillomavirus origins for replication were replicated in these cells (Fig. 1B, lanes 1). Surprisingly, wild-type p53 and the double-deletion mutant ΔN39ΔC362, which both suppress papillomavirus amplificational replication in CHO cells, were completely inactive in suppression of both viral origins in COP5 cells (Fig. 1B, lanes 2 and 3, and Fig. 1C).

Since we were using human p53, which possibly could be inactive in mouse cells, we tested the effects of human and mouse p53 on Py replication. We used two different Py origin configurations, and in both cases neither human nor mouse p53 was able to suppress LT Ag-dependent replication (Fig. 1D). This demonstrates clearly that the suppression of papovavirus amplificational replication does not occur in mouse COP5 cell line. There could be several explanations for the absence of the p53-induced replication block. (i) The absence of the replication block could be specific to COP5 cells, suggesting that inactivation of certain function had occurred in the process of selection when creating the Py T Ag-expressing cell line on the basis of C127 fibroblasts. (ii) The inability of p53 activity to suppress replication could be specific to mouse fibroblasts and may not occur in other types of cells or in undifferentiated cells of mouse origin. (iii) The absence of the replication block could be a general species-specific feature of mouse cells, pointing to an inactive state of the p53 protein with respect to the control of replication in those cells. (iv) The expression level of p53 could be so low that it could not induce the block of Py or papillomavirus replication in mouse cells.

We tested a set of mutant p53 proteins (schematically depicted in Fig. 2A) for their activity to suppress the amplificational replication of the BPV-1 origin in CHO4.15 cell line (20). In those cells p53 was able to block the replication of the papillomavirus origin. We have shown that oligomerization, DNA binding, RPA binding, and proline-rich domains of the p53 protein were essential for efficient inhibition, while the N-terminal transcriptional activation and C-terminal regulatory domains were dispensable for this activity. We tested the effect of the same p53 proteins on the replication of Py origin in two fibroblast cell lines derived from mouse primary fibroblasts. 101(1) is a p53-null line, and NIH 3T3 contains wild-type p53. The stability and expression level of the mutant p53 proteins was tested in 101(1) by Western blot analysis. The pattern of expression was the same as in CHO4.15 (20).

Both cell lines were transfected with Py wt ori-containing plasmid (50 ng) and either pCGLT or pCGE2 (100 ng). In NIH 3T3, papillomavirus replication could also be detected when 250 ng of the reporter plasmid pUCAlu was used at pCGEag and pCGE2 concentrations of 1,000 and 500 ng, respectively. The amount of cotransfected p53 was 250 ng. We found that in 101(1) cells as well as in NIH 3T3 cells, the expression of p53 or any of the p53 mutants did not suppress Py replication (Fig. 2B and C; Fig. 3). These data also show that the inability of p53 to suppress replication was not a
peculiar feature of COP5 cells but occurred in at least two other cell lines based on mouse fibroblasts.

p53 does not inhibit Py replication in totipotent nondifferentiated ES cells. We had to test the possibility that the replication block could be specific to mouse fibroblasts and might not occur in other types of mouse cells. For this purpose, we used undifferentiated cells of mouse origin, ES cells. We succeeded in detecting Py replication when using 50 ng of the Py wt ori reporter plasmid at 100 ng of transfected pUELt. We found that 250 ng of cotransfected p53 expression constructs did not suppress the replication (Fig. 4). Moreover, the constructs with the point mutation Arg248Trp seemed to activate replication. We have observed the same kind of activation by the same constructs in CHO cells. From this experiment, it became clear that fibroblasts were not the only mouse cells where papovavirus replication was not blocked. This was characteristic of all the mouse cells studied.

p53 represses the replication of Py origin plasmids in CHO cells. We performed simultaneous transient-replication assays of different Py origin plasmids and BPV-1 origin-containing plasmid pUCAU in CHO-K1 cells. For the simultaneous replication of the papillomavirus and Py origin-containing reporter plasmids, CHO cells were transfected with pCGL (25 to 250 ng), pCGEag (250 ng), and pCGE2 (250 ng), encoding the Py LT, BPV-1 E1, and BPV-1 E2 proteins, respectively. To have comparable signals in the replication assay, we used 100 ng of pUCAU and either 50 ng of Py origin plasmids *pmu1046/CAT*, wt, which carries a transcriptional enhancer segment adjacent to the origin, or *pmu1047/CAT*, where the enhancer segment was deleted and which supports replication on a very low level, or *pmu1047mE2RE/CAT*, where the normal enhancer has been replaced with BPV-1 E2 binding sites. In the case of the latter construct, the E2 protein provides the replicational enhancer function to the Py origin and this hybrid origin replicates at a comparable level to the wild-type homologue (Fig. 5A, lanes 1 and 3). Cotransfection of 250 ng of human wild-type p53 protein expression plasmid almost completely suppressed the replication of both BPV1 and Py origins (lane 2); so did ΔN39ΔC362, the minimal p53 deletion mutant active in the suppression of papillomavirus replication (20) (lane 4). All the Py origins studied were suppressed efficiently by the p53 protein in CHO cells (Fig. 5). The extent of the Py replication block was not rescued by the increase in the concentration of LT Ag. We increased the amount of the cotransfected LT Ag expression plasmid from 25 to 100 ng at the constant level of transfected p53. This resulted in an increase of replication without p53, but in the presence of the tumor suppressor the suppression still had the same fold effect (data not shown). Therefore, the possibility that the replication was suppressed due to the low level of expressed LT Ag was excluded. Mouse wild-type p53 also blocked the replication of the Py origin (Fig. 5B, lane 4). Coexpression of middle T and small T
Ags separately and in combination with each other did not abolish the effect of p53 on the replication (data not shown).

To confirm that p53 is expressed at comparable levels in mouse and hamster cell lines, we performed a Western blot analysis of several p53 mutants that, due to the lack of the Mdm2 binding site in the N terminus, appeared to be more stable and more easily detectable. We studied comparatively the expression in CHO and COP5. Equal numbers of transfected cells were loaded onto the gel after counting of the cells and determination of the transfection efficiencies in all cases. Figure 6 shows that all the studied mutants were expressed on the same level in both cell lines, undermining the possibility that the replication in CHO cells is suppressed due to the better expression of p53.

Although p53 is able to repress the CMV promoter in CHO cells, its effect on papovavirus replication must be direct. It is well known that p53 is a potent repressor of transcription and that it may repress several cellular and viral promoters (37). The transactivation functions of p53 are probably regulated by cellular factors, and the regulation of promoters can be cell specific (7). We studied the effect of the p53 protein on the activity of different promoters. Using β-galactosidase assays (31), we showed that in CHO cells the expression of p53 considerably reduces the expression of β-galactosidase directed by the CMV promoter in the expression vector pCG (39). At the same time, the expression of p53 had little effect on the β-galactosidase expression from the constructs where the expression was directed by the Rous sarcoma virus (RSV) long terminal repeat (LTR) (Fig. 7A). We also studied the effect of human p53 on the CMV promoter in the mouse cell line NIH 3T3. In this case, p53 did not have much influence on β-galactosidase expression (Fig. 7B). There was a slight decrease only in the case of wild-type p53, but since wild-type p53 is a very multifunctional protein, the apparent reduction in reporter expression could be caused by the apoptosis. These data show that the effect of p53 on the replication of the Py origin in CHO cells could have two components: (i) the expression of LT Ag from the CMV promoter of pCG has been reduced, and (ii) there is a direct effect on the replication of the origin. To evaluate the direct effect of p53 on the Py origin-dependent replication, we expressed LT Ag from the RSV LTR (pUELT). The expression level of LT Ag in response to different p53 constructs was studied by enhanced

FIG. 2. Effect of different p53 proteins on the transient replication of Py wt ori plasmids in 10(1) cells. (A) Schematic representation of designed p53 mutants. Numbers indicate positions on the amino acid sequence. (B) Southern blot analysis. Episomal DNA was extracted at 72 and 96 h posttransfection, digested with BamHI and DpnI, and probed with radiolabelled pUCAlu. Py ori indicates 200 pg of the marker plasmid linearized with BamHI, pmu1046/CAT (50 ng) and 100 ng of pUELT were transfected into the cells, and 250 ng of wild-type (wt) or mutant p53 expression plasmids was added. (C) Relative inhibition of replication. The replication signals of three independent experiments were quantified with a PhosphorImager, and signals from the cells transfected with origin plasmids only were used as a control to normalize the results.
plasmids only were used as a control to normalize the results. The replication signals of three independent experiments were quantified with a PhosphorImager, and signals from the cells transfected with origin plasmids were introduced into the cells. (B) Relative inhibition of replication by different p53 mutants together with 250 ng of wild-type (wt) or mutant p53 expression plasmids were transfected with 1,000 ng of LT Ag expression plasmid and 250 to 500 ng of Py wt ori-containing plasmid in CHO4.15 cells; with COP5 cells only the origin plasmid and ΔN39 expression plasmid were introduced exogenously. In CHO4.15 cells the extent of suppression of replication (Fig. 9A) was proportional to the amount of introduced p53 (Fig. 9). The expression level of LT Ag remained constant at the same time (Fig. 9B).

We also studied comparatively the expression levels of LT Ag and ΔN39 and the resulting effect on replication in the CHO and COP5 cell lines. In this case, equal numbers of transfected cells were used in the Western blot experiment after counting of the cells and determination of the transfection efficiencies of both cell lines. CHO cells were transfected at approximately the same efficiency as COP5 cells (15 to 30%), while the transfection efficiency of CHO4.15 cells was greater (about 70%). At the same time, the expression level of proteins (β-galactosidase, p53, and LT Ag) was much higher in CHO4.15 cells than in the other two cell lines. Therefore, the replication was not as intensive in CHO cells as it was in CHO4.15 cells, especially when the expression of LT Ag was directed by RSV LTR. COP5 cells that express LT Ag endo-

The same structural determinants of the p53 protein necessary for the suppression of papillomavirus replication are needed to inhibit the replication of Py origin. We tested the effect of the p53 mutants (Fig. 2A) in transient-coreplication assays of the BPV-1 minimal origin-containing plasmid and Py wt ori-containing plasmid in the CHO4.15 cell line. BPV-1 E1 and E2 replication proteins were constitutively expressed from integrated expression vectors in this cell line (29). Py LT Ag was expressed from the expression plasmid pUELlt from the RSV LTR promoter that was shown not to be modulated by p53 in this cell line. RSV LTR yielded a much lower level of LT Ag than the constructs with the CMV promoter. However, it was sufficient to support the replication of the Py origins to very high levels.

We used 100 ng of BPV-1 replication plasmid pUCAlu, 100 ng of pUELlt, and 25 to 50 ng of Py wt ori plasmid. Coreplication of Py and papillomavirus replication origins resulted in robust replication of the Py origin; papillomavirus origin replication was less intense (Fig. 8A). The results were consistent with those of papillomavirus replication (20). Wild-type p53, the C-terminal regulatory domain-defective mutant ΔC362, the N-terminal deletion mutant ΔN39 lacking the transactivation domain, and the double-deletion mutant ΔN39ΔC362 all suppressed the replication of both origins, confirming that in CHO the amplificational replication of papovaviruses is successfully inhibited by p53 (Fig. 8).

Different effect on replication in response to introduced p53 in CHO and COP5 cell lines is not caused by cell-specific differences in p53 expression level or changes in the concentration of LT Ag. To be convinced that the different effect of p53 on Py and papillomavirus replication in mouse and hamster cell lines does not depend on an insufficient level of the p53 protein in mouse cells and is not mediated by fluctuations in the concentration of LT Ag, we studied the expression level of both p53 and LT Ag under the conditions of Py virus replication. Of all the mutants capable of suppressing replication, we choose ΔN39 because it is easily detectable on Western blots, it lacks the transcriptional activation domain, and it does not possess the ability of wild-type p53 to induce apoptosis (21). We transfected increasing amounts of ΔN39 into the cells together with 500 ng of the Py LT Ag expression plasmid pUELlt and 50 ng of Py wt ori-containing plasmid in CHO4.15 and CHO cells; with COP5 cells only the origin plasmid and ΔN39 expression plasmid were introduced exogenously. In CHO4.15 cells the extent of suppression of replication (Fig. 9A) was proportional to the amount of introduced p53 (Fig. 9). The expression level of LT Ag remained constant at the same time (Fig. 9B).

FIG. 3. Replication of Py and papillomavirus ori plasmids in NIH 3T3 cells in the presence of different p53 proteins. (A) Southern blot analysis. Episomal DNA was extracted at 22 and 40 h posttransfection, digested with BamHI and DpnI, and probed with radiolabeled pUCAlu. Py ori and pUCAlu indicate 200 pg of the marker plasmid linearized with BamHI, pmu1046/CAT (50 ng) and 100 ng of pCGLT or 250 ng of pUCAlu, 1,000 ng of pCGEag, and 500 ng of pCGE2 together with 250 ng of wild-type (wt) or mutant p53 expression plasmids were introduced into the cells. (B) Relative inhibition of replication by different p53 mutants. The replication signals of three independent experiments were quantified with a PhosphorImager, and signals from the cells transfected with origin plasmids only were used as a control to normalize the results.

FIG. 4. Relative inhibition of replication of Py wt ori by different p53 mutants in COP5 cells. pmu1046/CAT (50 ng), 100 ng of pUELlt, and 250 ng of p53 expression plasmids were used in transfection experiments. The replication signals of two independent experiments (40 h posttransfection) were quantified with a PhosphorImager, and signals from the cells transfected with origin plasmids only were used as a control to normalize the results.

FIG. 9A) was proportional to the amount of introduced p53 (Fig. 9). The expression level of LT Ag remained constant at the same time (Fig. 9B).

We also studied comparatively the expression levels of LT Ag and ΔN39 and the resulting effect on replication in the CHO and COP5 cell lines. In this case, equal numbers of transfected cells were used in the Western blot experiment after counting of the cells and determination of the transfection efficiencies of both cell lines. CHO cells were transfected at approximately the same efficiency as COP5 cells (15 to 30%), while the transfection efficiency of CHO4.15 cells was greater (about 70%). At the same time, the expression level of proteins (β-galactosidase, p53, and LT Ag) was much higher in CHO4.15 cells than in the other two cell lines. Therefore, the replication was not as intensive in CHO cells as it was in CHO4.15 cells, especially when the expression of LT Ag was directed by RSV LTR. COP5 cells that express LT Ag endo-
genously yield very high replication levels (Fig. 10A). The expression levels of ΔN39 in CHO and COP5 cells are comparable (Fig. 10B) but remain lower than in CHO4.15 cells. Therefore, in CHO cells we could get the same extent of suppression as in CHO4.15 cells at higher concentrations of the p53 plasmid. To raise the levels of p53 in the cells, we expressed it from the construct SRαΔN39, where the expression was directed by the strong SRα promoter. Under these conditions, we could observe obvious suppression of replication in CHO cells at p53 concentrations of 500 and 1,000 ng, while in COP5 cells the replication signals remained constant (Fig. 10A) and were not influenced even when the amount of introduced p53 was raised to 3,000 ng (data not shown). The level of LT Ag in the cells remained constant in both cases (Fig. 10B). Since the F4 antibody used for the detection of LT Ag recognizes all three Py antigens present in COP5 cells, the expression of the middle T Ag can also be seen on the figure. These data convincingly show that although the p53 expression level is similar in CHO (hamster) and COP5 (mouse) cell lines and the level of LT Ag remains constant, in hamster cell lines the replication is inhibited but in mouse cells it is not.

**Py replication is supported in 143 cell line, and p53 suppresses Py replication in 143 cells.** It was interesting to investigate how p53 modulated the replication of Py in the cells of other organisms, more or less permissive for Py replication. We

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**FIG. 5.** p53 represses the replication of papovavirus origins in CHO cells. The results of Southern blot analyses are shown. Episomal DNA was extracted from cells at 72 and 96 h after transfection and digested with BamHI and DpnI. Filters were probed with radiolabeled pUCAlu plasmid. Py ori and pUCAlu indicate 200 pg of the marker plasmids linearized with BamHI. (A) Coreplication of the Py origin and pUCAlu. The cells were transfected with 50 ng of either pmu1046/CAT or pmu1047/E2RE/CAT, 100 ng of pUCAlu, 250 ng of pCGLT, 250 ng of E1 and E2 expression vectors pCGEag and pCGE2, and 250 ng of p53 expression plasmid. (B) Replication of the enhancerless Py origin. The cells were transfected with 50 ng of pmu1047/CAT, 25 ng of pCGLT, and 250 ng of p53 expression plasmid.

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**FIG. 6.** Comparative expression of different p53 mutants in CHO and COP5 cells. The results of enhanced chemiluminescence Western blot analysis are shown. The cells were transfected with 500 ng of different p53 expression constructs. Equal numbers of transfected cells were loaded onto the SDS-10% polyacrylamide gel after counting of the cells and determination of the transfection efficiencies of the cell lines. p53 proteins were detected using a mixture of pAb240 and pAb421 antibodies.

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**FIG. 7.** β-Galactosidase assays in the presence of p53 proteins in CHO cells (A) and NIH 3T3 cells (B). β-Galactosidase expression directed by either the CMV or RSV LTR promoter was measured 24 h posttransfection. The signals of three independent experiments were quantified, and the relative optical density (on the y axis) was calculated using the samples with no added p53 as controls. The cells were transfected either with 500 ng of the construct pCGβeta (CMVβ-gal) or with 1,000 ng of the construct pNP175 (LTRβ-gal) and with 250 ng of p53 expression constructs.
assumed that in primate cells Py replication, like papillomavirus replication, could be blocked. Although it has long been considered that Py neither transforms nor grows in human cells (12), Py LT Ag-dependent replication was recently reported in human 293 and C-33A cells (38). Supporting the results of Sverdrup et al. (38), we replicated Py wt ori-containing reporter plasmid (500 ng) in human osteosarcoma 143 cells when using 1,000 ng of pCGLT per transfection. Cotransfection of 250 ng of p53 expression constructs resulted in the suppression of Py replication in wild-type p53 and the mutants active in the suppression of replication in CHO cells (Fig. 11). These results suggest that the absence of the p53-mediated replication block is characteristic of mouse cells, since papovavirus amplificational replication was suppressed in other cell lines tested.

DISCUSSION

p53 suppresses neither Py nor papillomavirus replication in mouse cells but the replication is blocked in hamster cells. We studied the effect of the p53 protein on the amplificational replication of Py and papillomavirus origins in the hamster cell line CHO-K1, in different mouse cell lines, and in human 143 cells. The modulation of replication of both origins by p53 had a similar pattern. Py and papillomavirus origin replication was not suppressed in mouse COP5 cells by any of the p53 mutant proteins. Moreover, the replication was not inhibited in any mouse fibroblast cell line or in the ES cells. Our results from mouse cells are consistent with earlier published data, which showed that mouse Py replication was not inhibited by the p53 protein unless 4 to 16 additional p53-specific RGC sites were included in the plasmid (17, 23). These data suggest that Py replication is not susceptible to the action of p53 in these cells, although the p53 protein is expressed and is active in other functions. To our surprise, the replication of the papillomavirus origin was also not blocked in mouse cells. We also showed that mouse wild-type p53 protein had no effect on Py and papillomavirus replication in mouse cells. Our data show convincingly that the p53 protein is incapable of suppressing Py and papillomavirus DNA replication in all the mouse cell lines studied.

In contrast to its action in mouse cells, the p53 protein was fully competent in suppressing papovavirus replication in hamster cells. We studied the LT Ag-dependent replication of the Py origin in hamster (CHO) cells, tested many p53 deletion mutants in the papovavirus replication assay, and found that the replication of both Py and papillomavirus origins was blocked by p53 in CHO cells. The same determinants of p53, i.e., the intact DNA binding, oligomerization, RPA binding, and proline-rich domains, were essential for efficient inhibition of both replication origins. The different effects of p53 on papovavirus replication in hamster and mouse cell lines could not be the consequence of low expression level of p53 in mouse cells, because all the p53 mutant proteins studied were expressed at comparable levels in mouse (COP5) and hamster (CHO) cell lines. Moreover, we studied comparatively the expression of p53 and its possible effect on the level of LT Ag in CHO and COP5 cells under the conditions of the replication assay. These experiments demonstrated clearly that although the expression level of p53 is quite similar in these cell lines and this does not cause any changes in the level of LT Ag, the replication block occurs only in CHO cells.

Mouse Py replication has been studied mostly in mouse cells, although the host range specificity of Py can be extended to all rodent cells (13). The belief that the mouse Py origin does not replicate in human cells was partially based on early-infection studies and on in vitro experiments showing that mouse DNA polymerase α primase interacts more efficiently with LT Ag than its human analogue does (3, 13, 33). Although for a long time Py was believed not to replicate in human cells, we were able to detect the replication of the Py wt ori-containing plasmid in human osteosarcoma 143 cells. This was consistent with the results of a previous study (38), where Py replication was successfully detected in human 293 and C-33A cells. The efficiency of LT Ag-dependent replication of mouse Py origin in human cells is much lower than in mouse cells. This is probably a reflection of less efficient interaction of LT Ag with polymerase α; however, the replication of the Py origin could be clearly
detected in 143 human osteosarcoma cells and was efficiently blocked by the p53 protein, as had been the case with the papillomavirus origin (20). In 143 cells, the same domains of the p53 protein as in hamster cells were needed for the block of Py origin replication. This led us to the conclusion that in mouse cells p53 is unable to suppress the amplificational replication of certain papovaviruses whereas in hamster and human cells this protein is fully functional.

High-risk and low-risk papillomaviruses encode the E6 protein, which is capable of interacting with the p53 protein, while the E6 protein from the high-risk viruses directs the degradation of p53 by the ubiquitin degradation pathway. The ability of the E6 protein to bind p53 suggests that the virus has developed a tool to modulate cellular functions through the p53 protein or that it protects itself from the action of p53 through that interaction. Many human and primate DNA viruses, like adenoviruses, human and monkey Py, herpesviruses, and papillomaviruses, encode proteins which modulate p53 activity. However, mouse Py does not encode proteins that interact with the p53 protein. This suggests that in mouse cells p53 does not interfere with viral functions; therefore, there is no need for the virus to develop a defense mechanism.

The putative mechanism of action of p53. We have shown previously that the p53 protein carries an activity for the suppression of papillomavirus DNA replication in hamster and human cells (20). Here we show that mouse Py replication is blocked by the same DNA binding, oligomerization, proline-rich regulatory, and RPA binding domains of the p53 protein which were needed for the suppression of papillomavirus replication in hamster and human cells. At the same time, the replication of both viral origins was insensitive to the wt p53 protein in mouse cells while the mutant Trp248 even activated replication.

There could be several reasons for the lack of the p53-induced replication block of certain papovavirus origins in mouse cells. First, the p53 protein could be preferentially in an inactive conformation in mouse cells, not allowing the expression of suppression of DNA replication. Second, mouse cells might lack the signal transduction pathway that could sense DNA amplificational replication in hamster and human cells. Third, the pathway necessary for blocking replication could be...
missing in mouse cells. Fourth, the replication mode of papovaviruses could be different in mouse cells and might not expose the determinants recognized by the p53-dependent pathway.

In vitro replication studies have shown that papillomaviruses and Py resemble each other in their utilization of a number of cellular replication factors, including DNA polymerase α/primase, DNA polymerase δ, RPA, proliferating-cell nuclear antigen and topoisomerases (for a review, see reference 42). The viral E1 and E2 proteins in papillomavirus and LTAg in Py cooperate in a number of these replication factors on the origin. We showed here that the action of p53 on the replication of papillomavirus and Py was identical in hamster, human, and mouse cells, which might suggest that p53 uses the same mechanisms for the block of replication of all papovaviruses. We suggest that the target for the p53 action is the single-stranded DNA generated by viral helicases in hamster and human cells, which is recognized by the p53 protein in cooperation with the single-stranded-DNA binding protein RPA. Previous studies have shown that interactions between RPA and p53 via a 20-amino-acid RPA binding region in the N-terminal part of p53 were necessary for coordinating the RPA-dependent DNA repair after UV damage and perhaps regulating some p53-dependent pathways (1). On the basis of our data, the interaction of p53 with RPA through the RPA binding domain could facilitate the recognition of single-stranded DNA by p53 and stabilize the complex. The unknown signal transduction pathway, which could be mediated through the proline-rich regulatory domain of p53, could be capable of blocking the action of replicative helicases on the viral DNA. Further work is needed to elucidate this signal transduction pathway leading to the suppression of replication in human and hamster cells and to understand why in mouse cells this pathway is not functional.

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