A subset of cells expressing SV40 large T antigen contain elevated p53 levels and have an altered cell cycle phenotype

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Abstract. Cells transformed by the simian virus 40 (SV40) large T antigen (Tag) contain elevated levels of cellular p53 protein. To quantify this relationship, levels of p53 were measured in NIH 3T3 cells that expressed different concentrations of Tag. Using immunoblotting, average p53 levels were shown to increase linearly with Tag concentrations in these cell lines. Single-cell measurements were also performed using flow cytometry to measure p53 immunofluorescence. Surprisingly, the flow cytometry experiments showed that two distinct cell populations, based on p53 content, were present in cells expressing high levels of Tag. One cell population contained elevated p53 levels. A second population did not contain elevated p53, even though high concentrations of Tag were present in the cells. This latter cell population did not appear to arise because of mutations in either Tag or p53. The two cell populations also had phenotypic differences. In exponentially growing cells, Tag alters the cell cycle distribution (decreases the percentage of G₁ phase cells and increases the percentages of S and G₂ + M phase cells). This phenotype was maximum in the cell population containing elevated p53. A lesser phenotype was found in the cell population that did not contain elevated p53. These data show, firstly, that cells can express significant levels of Tag and not contain elevated levels of p53 and, secondly, that elevated p53 correlates with the altered cell cycle distribution produced by Tag in growing cells.

INTRODUCTION

The large T antigen (Tag) gene of simian virus 40 (SV40) encodes a protein that transforms mammalian cells in culture. Cells transformed by Tag contain elevated levels of the cellular p53 protein (Oren, Maltzman & Levine 1981, Reich, Oren & Levine 1983) mainly as a result of Tag binding directly to the short-lived p53 protein and increasing its half-life (Reihnsaus *et al.* 1990). There are other mechanisms whereby p53 levels can be increased in cells, independent of Tag. Some mutations in the cellular p53 gene, for example, can increase the half-life of the protein (Hinds *et al.* 1990). Cells transformed by Tag, however, do not

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routinely contain mutant p53 (Lin & Simmons 1990, Moore *et al.* 1992) and therefore transformation by Tag does not involve induction, or selection for, mutant forms of p53.

In addition to transformation, Tag affects the cell cycle distribution of exponentially growing cells. Tag expression reduces the percentage of cells in the G₁ cell cycle phase and increases the percentages of cells in the S and G₂ + M cell cycle phases (Sladek & Jacobberger 1992b). Using a set of recombinant retroviruses that express Tag at different concentrations in cells (Sladek & Jacobberger 1992a), a dose-response relationship between Tag concentration and this cell cycle phenotype was previously demonstrated (Sladek & Jacobberger 1992a).

This study sought to quantify the relationship between Tag and p53 by measuring p53 levels in NIH 3T3 cells expressing various concentrations of Tag. Using immunoblotting to measure the levels of both Tag and p53 in cells infected with retroviruses expressing Tag, cellular p53 levels were found to increase linearly with increasing Tag concentration. When p53 content in the same cells was examined on a single-cell basis using flow cytometry, however, two distinct cell populations were found. One cell population contained elevated p53 and a second cell population did not contain levels of p53 above background, even though the cells expressed high levels of Tag. Analysis of clones from an original Tag-expressing, NIH 3T3 cell line was inconsistent with either the presence of mutant p53 molecules that could not bind Tag or mutant Tag molecules that could not bind to p53 to explain the cell populations. Finally, Tag perturbed the cell cycle distribution of both the p53-elevated and p53-unelevated cell populations, but this phenotype was clearly greater in the population containing elevated p53.

MATERIALS AND METHODS

Cells, culture and viruses

NIH 3T3 cells infected with various Tag-encoding retroviruses (described below) were used in these studies (Sladek & Jacobberger 1992b). The cells were grown as previously described in Dulbecco modified Eagle medium supplemented with 5% (v/v) fetal bovine serum and 5% calf serum (Sladek & Jacobberger 1990, Yang & Sladek 1995).

Use of the Tag-encoding retroviral vectors pZIPTEX (Brown *et al.* 1986), Linker CMV T, Linker 101tk T and Linker tk T has been described previously (Sladek & Jacobberger 1992a). In addition to Tag, all of these viruses encoded a neomycin-resistance gene that conferred resistance to the drug G418 upon the infected cells. Infected cells used in these experiments were obtained after selection in 400 µg G418 per ml of medium. Unless otherwise stated, infected cell lines were uncloned and therefore contained cells representative of many independent infection events.

Immunoblotting

Growing cells were washed with PBS and lysed by addition of lysis buffer (50 mM Tris (pH 8.0), 5 mM EDTA, 150 mM NaCl, 0.5% Nonidet P-40, 1 mM phenylmethylsulphonyl fluoride and 0.01 mg of aprotinin per ml; Kierstead & Tevethia 1993) as described previously (Sladek, Fisher & Rubenstein 1994, Yang & Sladek 1995b). A volume of cell extract corresponding to a specific number of cells (indicated in the figure legend of each experiment) was boiled in loading buffer (48 mM Tris (pH 6.8), 18.5% sodium dodecyl sulphate, 3.94 mM β-mercaptoethanol, 31% glycerol and 3% bromophenol blue), analysed by SDS-polyacrylamide electrophoresis and transferred to nitrocellulose membranes as previously described (Sladek *et al.* 1994).

Immunodetection of Tag was performed by incubating membranes with anti-Tag mouse monoclonal antibody PAb108 (Pharmingen, San Diego, CA, USA). Immunodetection of p53 was performed using either anti-p53 mouse monoclonal antibody PAb122 (Pharmingen), PAb421 (Oncogene Research Products, Cambridge, MA, USA) or a sheep polyclonal antibody (Ab-7, Oncogene Research Products; Sladek *et al.* 1994, Sladek 1996). The membranes were then incubated with alkaline phosphatase-conjugated anti-mouse IgG and colour was developed with 5-bromo-4-chloro-3-indolyl phosphate and nitroblue tetrazolium (Friedrich, Laffin & Lehman 1993). Alternatively, membranes were incubated sequentially with rabbit anti-sheep IgG and donkey anti-rabbit antibody-HRP and processed for autoradiography using enhanced chemiluminescence reagents (Sladek 1996). In some cases, the immunoblots were quantified by densitometry.

Cell fixation, staining and flow cytometry

The procedures for cell fixation, antibody and propidium iodide staining, and flow cytometry have been described in detail (Jacobberger, Fogelman & Lehman 1986, Sladek & Jacobberger 1990). Briefly, cells were fixed with methanol, incubated with 1 μ g mouse anti-Tag antibody PAb416 or PAb419 (Oncogene Research Products) or anti-p53 antibody PAb421 and then stained with 2.1 μ g goat anti-mouse IgG F(ab')₂ fragments conjugated to fluorescein. Cells were treated with RNase A and then stained with propidium iodide. These amounts of primary and secondary antibodies were determined to be at saturating levels. Fluorescence measurements were made on a Cytofluorograph II flow cytometer (Ortho Instruments, Westwood, MA, USA) using the 488 nm line of an argon laser. Green fluorescence was collected through a 530/20 nm bandpass filter set and red fluorescence was collected above 640 nm. Data acquisition was triggered on red fluorescence. A doublet discriminator (peak vs. integrated red fluorescence) was used as the primary gate to eliminate cell aggregates.

Flow cytometric data analysis

Single parameter immunofluorescence histograms of cells containing a G₁ phase DNA content were obtained from bivariate immunofluorescence histograms as shown in Figure 1 and as described elsewhere (Sladek & Jacobberger 1993). Single parameter DNA histograms were obtained from bivariate immunofluorescence histograms also as described (Sladek & Jacobberger 1992b). The fraction of the total cell population present in each of the G₁, S and G₂ + M cell cycle phases was obtained from DNA histograms by mathematical modelling using ModFit software (Verity Software House, Topsham, MA, USA).

RESULTS

Immunoblot measurement of p53 in Tag-expressing cells

Cells transformed by Tag contain elevated levels of the cellular p53 protein (Oren *et al.* 1981, Reich *et al.* 1983). It was of interest to quantify the relationship between Tag and p53 using cell lines that had been previously constructed by infection with recombinant retroviruses expressing different levels of Tag (Sladek & Jacobberger 1992a). Both Tag and p53 levels were measured in these cell lines by immunoblotting. Results for a typical experiment are shown in Figure 1. Figure 1a shows an immunoblot of extracts made from cells infected with four different retroviral vectors that encode Tag (Sladek & Jacobberger 1992a) and for uninfected cells. Visually, it can be seen that as Tag levels increase (moving right to left in Figure 1a, p53 levels also increase. Figure 1b shows a plot of the relative levels of Tag and p53 obtained from the immunoblot in Figure 1a by densitometry. The data show a positive,

Figure 1. Quantification of Tag and p53 levels in cells by immunoblotting. In (a) cell extracts from NIH 3T3 cell lines infected with Tag-encoding recombinant retroviruses pZIPTEx (lane 1), Linker CMV T (lane 2), Linker 101tk T (lane 3), Linker tkT (lane 4) and uninfected cells (lane 5), were used in an immunoblot experiment to detect both Tag and p53, as described in *Materials and methods*. Each lane contained an amount of extract corresponding to 1.5×10^5 cells. The Tag and p53 bands are indicated by arrows on the left side of the immunoblot. The relative amounts of Tag and p53 on the immunoblot were quantified by densitometry and the values obtained were plotted in the graph shown in (b). The graph contains four data points through which a linear regression line was drawn ($r^2 = 1.0$).

linear relationship between the levels of Tag and p53. Therefore, as the levels of Tag increase, the levels of p53 in cells linearly increase.

Single-cell measurements of p53 in Tag-expressing cells

Because immunoblotting measures the average amount of antigen in a cell population, any cell subpopulations possessing antigen levels different from the average would not be detected. However, single cell measurements by flow cytometry of cells stained with antibodies specific for an antigen could reveal cell subpopulations that were present. Therefore, flow cytometry was used to examine p53 levels in Tag-expressing NIH 3T3 cells.

In the flow cytometry experiments, NIH 3T3 cells infected with the pZIPTEx retrovirus were examined (Brown *et al.* 1986). As shown previously in polyclonal, pZIPTEx-infected 3T3 cells, the levels of Tag are higher than any other vector tried (Sladek & Jacobberger 1992, Sladek & Jacobberger 1993). These cells were examined for p53 content by flow cytometry. The cells were also stained with propidium iodide to quantify DNA content. Therefore, it was possible to determine the levels of p53 in any phase of the cell cycle (G_1 , S, $G_2 + M$). Figure 2 shows the results of these experiments.

Figures 2a–c show bivariate immunofluorescence vs. DNA content plots for the cells. Figures 2d–f show single parameter immunofluorescence histograms derived from cells in the G_1 cell cycle phase of the respective bivariate plots (i.e. the cells within the boxes in Figure 2a–c).

Figures 2a,d show background immunofluorescence. This pattern was obtained when uninfected, control NIH 3T3 cells were stained with antibodies against either Tag or p53. Cellular p53 levels in these cells were below the threshold necessary for detection in the

Figure 2. Flow cytometric detection of Tag and p53 in Tag-expressing cells. Growing NIH 3T3 cells, uninfected or infected with the pZIPTEX retrovirus and selected in G418, were stained for either Tag or p53 as described in *Materials and methods* and analysed by flow cytometry. Panels (a–c) are bivariate contour plots of immunofluorescence vs. DNA content. Panels (d–f) are single parameter immunofluorescence histograms derived from cells with a G₁ phase DNA content (cells within the boxed regions) in (a–c), respectively. Cells rightward of the cursor in (d–f) are positive for either Tag or p53. Cells leftward of the cursor represent background (negative) staining. Panels (a,d) are from staining of uninfected cells with either Tag or p53 and represents background immunofluorescence. Panels (b,e) are from Tag staining, and (c,f) are from p53 staining of pZIPTEX-infected cells. To demonstrate the specificity of the PAb421 antibody used in the flow cytometry staining experiments, in (g) the same antibody was used to stain an immunoblot containing extracts from 3×10^5 pZIPTEX-infected cells.

experiments and these cells therefore gave the same staining pattern as uninfected NIH 3T3 cells stained for Tag.

In Figures 2b,e, pZIPTEX-infected cells were stained for Tag. Ninety-three per cent (from Figure 2b) of these cells were positive for Tag staining. As previously noted, a small percentage of cells (7% in this experiment) were found that did not stain positive for Tag, but nonetheless grew in the presence of G418, indicating that they were infected (Sladek & Jacobberger 1992b). The reason for this population is not known but it could be speculated that it results from the presence of defective virus or instability of the integrated provirus (Sladek & Jacobberger 1992b).

In Figures 2c,f, the pZIPTEX-infected cells were stained for p53. Fifty-three per cent (from Figure 2c) of these cells contained p53 levels above background staining. The remaining 47% of cells contained levels of p53 that were not detectable over background immunofluorescence. The specificity of the PAb421 antibody used to stain p53 in these experiments was examined by immunoblotting as shown in Figure 2g. A single p53 band was seen in blots of infected 3T3 cells, indicating that this antibody was specific for p53 and was not staining other antigens.

These data showed that a significant percentage of cells which expressed Tag (93 – 53 = 40%) did not contain elevated levels of p53. It is important to note that the p53 staining result was not a result of a single broad distribution of p53 fluorescence, the lower 47% of which was obscured by background. Rather, the results show that two distinct

fluorescence distributions were present that identified two cell populations clearly different in their p53 content (Figure 2c,f). Therefore, since 93% of all the cells expressed Tag but only 53% of all the cells contained elevated p53, almost one-half of the Tag-expressing cells failed to display levels of p53 above those present in cells containing no Tag. Although this result was obtained with NIH 3T3 cells, similar results were found in another murine fibroblast cell line, BALB/c-3T3 (clone A31; Aaronson & Todaro 1968), when it expresses Tag. This indicates that the result is more general and not specific to a single line of cells.

The cell cycle effects of Tag expression are maximum in cells containing elevated p53

From the results of staining the pZIPTEX-infected NIH 3T3 cells shown in Figures 2b,c, three cell populations can be defined in terms of Tag and p53 expression levels. The first population contains neither Tag nor elevated p53. These are the pZIPTEX-infected cells in Figure 2b that did not express Tag. It is known that these cells also do not contain elevated p53 for two reasons. First, by simultaneous staining with both Tag and p53 antibodies, it was directly shown that the pZIPTEX-infected cells which did not stain positive for Tag also did not contain elevated levels of p53 (data not shown). Second, elevated p53 levels have not been found in cells that do not express Tag (Figure 2a). Therefore, this cell population did not contain Tag and also did not contain elevated levels of p53.

The second cell population contains elevated p53. These are the cells that stained positive for elevated p53 levels in Figure 2c. In the present experiments, elevated p53 levels have been found only in cells that expressed Tag. Therefore, this cell population contained both Tag and elevated p53.

The third cell population does not contain elevated p53 but the majority of these cells do express Tag. From Figure 2c, it can be seen that 47% of all cells did not contain elevated p53. The 7% of cells that did not express Tag (from Figure 2b) are part of this population. If these Tag non-expressing cells are subtracted from all cells that do not contain elevated p53 ($47\% - 7\%$), the remaining cells (40%) are those which do express Tag but do not contain elevated p53 levels. Therefore, if the entire cell population that did not contain elevated p53 was considered (47% of all cells), it can be determined that 85% of this population ($40\% / 47\%$) does express Tag. Therefore, the third cell population contained Tag (85%) but did not contain elevated p53.

All three of these cell populations were present on the same culture dish. Therefore, differences in growth or cell cycle characteristics between the populations were not a result of the environment, but rather to properties of the cell populations themselves. Because the cells were stained for DNA content, in addition to being stained with antibodies, DNA histograms could be derived from each of the three cell populations. Analysis of these histograms (described in *Materials and methods*) yielded cell cycle phase fractions that were used to infer the growth phenotype of the cells. Previously, it was showed that Tag caused reduced percentages of G₁ and increased percentages of S and G₂ + M phase cells compared to cell populations not expressing Tag (Sladek & Jacobberger 1992).

The data in Table 1 are representative of a number of experiments analysed. The data show that the cell cycle phenotype was maximum in cells containing Tag and elevated p53. These cells exhibited a much lower percentage of G₁ and higher percentage of S and G₂ + M cells than did the control (Tag-negative, p53-unelevated) population. The cell cycle distribution of Tag-positive, p53-unelevated cells was also significantly affected compared to control cells, but to a lesser degree compared to Tag-positive, p53-elevated cells.

Previously, it was showed (Sladek & Jacobberger 1992) that the error in our measurement of cell cycle phase fractions was small (coefficient of variation < 5% for all phases). It was

Table 1 Cell cycle phase fractions of cell populations distinguished by Tag and p53 immunofluorescence

Cells	% of total cells		
	G ₁	S	G ₂ + M
Tag-positive, elevated p53	49.0	33.3	17.7
Tag-positive, unelevated p53	56.2	29.2	14.6
Tag-negative, unelevated p53	63.8	21.9	14.3

also shown that changes in cell cycle phase fractions of the order shown in Table 1 indicated significant changes in cell cycle phase durations (Sladek & Jacobberger 1992b). In those experiments, the G₁ cell cycle phase duration was decreased 18% and the G₂ + M phase duration was increased 29%. It was concluded therefore that the measured differences in cell cycle phase fractions shown in Table 1 are significant and that a major component of the cell cycle phenotype previously ascribed to Tag (Sladek & Jacobberger 1992b) requires elevation of p53 by Tag. However, Tag imparts a significant cell cycle phenotype to cells in absence of elevated p53.

Tag-containing cells without elevated p53 cannot be explained by mutations in either Tag or p53

Tag directly binds wild-type p53 protein, increases its half-life (Reihnsaus *et al.* 1990) and results in elevated p53 levels within the cell. Cellular p53 levels would not likely be elevated if Tag was mutated such that it could not bind p53. Likewise, mutant p53 molecules that could not bind to Tag might also not result in elevated levels of p53. It was a concern that the present uncloned line of pZIPTEx-infected NIH 3T3 cells, comprised of many independently infected clones, was a mixture of two genetically distinct cell populations. One population could have contained both wild-type Tag and p53 such that Tag bound to p53 and caused elevated p53 levels. A second population could have contained either mutated Tag or p53 such that Tag did not bind to p53 and no elevated p53 resulted. If two genetically distinct cell populations did exist, it was reasoned that it should be possible to isolate Tag-expressing single cell clones representative of the two populations; i.e. pure clones that had elevated p53 and separate pure clones that did not.

To determine this, the original pZIPTEx-infected NIH 3T3 line was plated at clonal density. Single colonies were cloned and expanded. Of 10 clones isolated, six were greater than 98% Tag positive. Of the other four clones, three were negative for Tag and one was 70% Tag positive and may have been a mixed clone. When the six Tag-positive clones were stained for p53 content, three of the clones contained only elevated p53 (Figure 3b). The other three clones contained both elevated and unelevated p53 populations, similar to what was seen in Figure 1 (immunofluorescence distributions for two of these three clones are shown in Figures 3c,d). Levels of Tag in clones of the two types (clones that contained only unelevated p53 and clones that contained both elevated and unelevated p53) were comparable.

Colonies from one of the original three colonies containing both elevated and unelevated p53 populations were recloned to obtain three new clones. All three of these clones expressed Tag and all three contained both elevated and unelevated p53 populations (as in Figures 3c,d and data not shown). Therefore, the existence of clones containing both elevated and unelevated p53, as shown in Figures 3c,d, was not a result of analysis of impure clones.

Figure 3. p53 immunofluorescence distributions of cloned cells. Exponentially growing cell clones were fixed, stained for p53 content and analysed by flow cytometry. Single parameter immunofluorescence histograms obtained from G₁ phase cells are shown. The clone in (a) did not contain Tag and the histogram shows that it also did not contain increased levels of p53. The staining in (a) therefore is representative of background staining. The clones in (b–d) were known to be greater than 98% Tag positive. The histogram in (b) shows that this clone had only increased levels of p53. The histograms in (c) and (d) show that both of these two clones had distinct populations with increased 53 and without increased p53.

These data show that clones with both elevated and unelevated p53 levels continue to segregate single cells that give rise to clones with both elevated and unelevated levels of p53. It is not believed that mutation of either Tag or p53 between cloning steps can account for this since the mutation rate would have to be 50%. This result is not easily understood in terms of the original notion of the existence of NIH 3T3 cells containing mutated p53 molecules that could not be bound by Tag, or mutated Tag molecules that could not bind p53, since clones that expressed Tag and did not contain any elevated p53 could not be isolated. However, there clearly appear to be two clonable populations of cells with distinct patterns of p53 expression levels (i.e. one clone type displays elevated p53 and one clone type displays both elevated and unelevated p53).

DISCUSSION

The goal of the experiments described in this paper was to examine the cellular distribution of p53 levels in proliferating cells expressing the Tag protein from SV40. It has been known for some time that p53 concentrations are elevated in cells transformed by Tag (Oren *et al.* 1981, Reich *et al.* 1983), but it was of interest to quantify the relationship between the two molecules more precisely.

To carry out these experiments, NIH 3T3 cells lines that had previously been infected with retroviral vectors expressing different levels of Tag were used (Sladek & Jacobberger 1992a). Levels of both Tag and p53 were measured in these cell lines, first using immunoblotting, a technique that measures the average level of an antigen in a cell population. The results showed that p53 levels increased as cells expressed increasing concentrations of Tag (Figure 1a). Quantification of the immunoblots showed that the relationship between levels of Tag and p53 was linear (Figure 1b).

In addition to immunoblotting, p53 levels were quantified independently using flow cytometric detection of cells stained with fluorescently labelled antibodies specific for p53. Since this technique measured p53 on a single-cell basis, it was possible to detect cell subpopulations containing p53 levels different from the average. Surprisingly, these experiments detected two populations of Tag-expressing cells that expressed different levels of p53. One cell population contained elevated p53 levels. A second population did not contain elevated p53, even though high concentrations of Tag were present in the cells.

The presence of a cell population expressing Tag but not containing elevated p53 levels is unusual. Since Tag binds to wild-type p53, increases its half-life and results in increased cellular levels of p53 (Reihnsaus *et al.* 1990), whether this cell population contained either mutated Tag or p53 such that the two molecules could not interact was a point of curiosity. If this were true, then the cells analysed in Figure 2 were likely a mixture of cells with mutant Tag or p53 as well as cells with wild-type Tag and p53. Given this, it should have been possible to isolate Tag-expressing single-cell clones where all of the p53 was elevated (cells with wild-type Tag and p53) as well as single-cell clones where no p53 was elevated (cells with either mutant Tag or p53).

In these cloning experiments, Tag-expressing clones were isolated in which all cells contained elevated p53 (Figure 3b). However, it was not possible to identify Tag-expressing clones that did not contain elevated p53. Instead, Tag-expressing clones that continued to display both cells with elevated p53 and cells without elevated p53 were identified (Figures 3c,d). The p53 staining pattern in these clones was similar to that found when the original uncloned populations were analysed (Figure 2c). Different clones did display different percentages of cells with elevated p53 (compare Figures 3c,d). No significant differences in Tag concentration were found in the cells that could be correlated with clone type.

In addition to mutation of Tag and p53, one other possibility was considered to explain the existence of cells that expressed Tag but did not contain elevated p53 levels. Since cells expressed a range of Tag concentrations (see distribution in Figure 2e), it could be that only cells with very high concentrations of Tag contained elevated p53. Although such a threshold level of Tag required for p53 elevation could exist, it was determined that, for the pZIPTEx-infected cells, the 25% of cells expressing the highest Tag levels had five-times as much Tag as the 25% of cells expressing the lowest Tag levels (data not shown). The range of Tag expression in these cells therefore was small compared to the 20 to 30-fold range of Tag concentration that could be produced in cells by the retroviral vectors described (Sladek &

Jacobberger 1992a). This does not exclude the possibility of existence of a threshold, but if one did exist, the threshold would have to be very tight.

Tag expression has previously been shown to alter the cell cycle of proliferating cells by decreasing the percentages of cells in the G₁ phase and increasing the percentages of cells in the S and G₂ + M phases (Sladek & Jacobberger 1992a). It was previously showed that this cell cycle alteration was caused by a decrease in the G₁ phase duration and an increase in the G₂ + M phase duration (Sladek & Jacobberger 1992b). In the experiments described in this paper, perturbation of the cell cycle was found to be most significant in Tag-expressing cells containing elevated levels of p53 (Table 1). Tag-expressing cells that did not contain elevated p53 still displayed an altered cell cycle phenotype, as compared to control cells not expressing Tag. This phenotype, however, was less significant than in the cells that contained elevated p53. Therefore, elevated p53 clearly is associated with the most significant cell cycle effects produced by Tag. However, Tag can perturb the cell cycle of proliferating cells in the absence of p53 elevation.

Finally, these data suggest that the presence of high levels of Tag in cells is not sufficient to result in elevated p53. It has been shown, in certain non-permissive, untransformed cells, abortively infected with SV40 virus, that p53 was not stabilized even though directly bound by Tag (Deppert, Haug & Steinmayer 1987, Reihsaus *et al.* 1990). Other data have suggested an additional mechanism for stabilization of p53 that is independent of Tag binding. In Tag-transformed cells, p53 unbound by Tag has a longer half-life than p53 in untransformed cells containing no Tag (Deppert & Haug 1986, Deppert, Steinmayer & Richter 1989). It is suggested that, in addition to genetic components, there may also be physiological components that determine whether p53 levels will be increased in cells expressing Tag.

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