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Energy Requirement for Degradation of Tumor-Associated Protein p53

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A 53,000-dalton protein (p53) present in large amounts in several types of tumorigenic cells was rapidly degraded in nontumorigenic BALB/c 3T3 fibroblasts ($t_{1/2}$, ~0.5 h) but not in tumorigenic methylcholanthrene-induced mouse sarcoma cells ($t_{1/2}$, >2 h). In 3T3 cells, dinitrophenol and 2-deoxyglucose, agents which reduce ATP production, inhibited the rapid degradation of p53 and the slower breakdown of total cell protein. After removal of these agents, the degradation of both p53 and total cell proteins resumed at their normal rates. Inhibitors of intralysosomal proteolysis (Ep475 and chloroquine) did not reduce the rate of degradation of p53. Thus, in 3T3 cells, p53 appears to be degraded by a nonlysosomal, ATP-dependent proteolytic system similar to that previously shown to degrade short- and long-lived proteins in growing fibroblasts. The immunoreactive p53 which remained in ATP-depleted cells had the same molecular weight as the p53 in the control cells. No intermediate products of p53 degradation were detected by immunoprecipitation in either ATP-depleted or control cells. Hence, ATP seems to be required for an initial step in the degradation of p53. Although the amount of labeled p53 was increased in simian virus 40-transformed and methylcholanthrene-induced mouse sarcoma cells, the amount of p53 labeled during a 3-h pulse in Moloney virus- and Rous sarcoma virus-transformed cells and untransformed 3T3 cells was similar. Thus, an increased net rate of p53 accumulation is not a common feature of transformed tumorigenic cells.

Several types of tumorigenic, transformed mammalian cells contain high levels of a 53,000-dalton phosphoprotein, called p53 (31, 32). By contrast, levels of p53 are 10- to 100-fold lower in untransformed fibroblasts (19, 40, 49, 50). The p53 polypeptide was first discovered as a protein which coprecipitated with the large tumor antigen (T-ag) of simian virus 40 (SV40) during immunoprecipitation of extracts of SV40-transformed cells with tumor-specific antisera (28). Linzer et al. (31) used temperature-sensitive mutants of SV40 to show that a functional viral T-ag is required to maintain high p53 levels in SV40-transformed mouse cells. The level of p53 also increases during lytic infection of cells with SV40 (21).

The amount of p53 is also markedly elevated in some transformed cells which lack the SV40 T-ag. Immunochemical studies have shown an association between a p53 protein and the tumor antigens of Abelson leukemia virus- and adenovirus-transformed cells (50, 52). Methylcholanthrene-induced mouse sarcoma (Meth A) cells and mouse F9 embryonal carcinoma cells also contain high levels of p53 (14, 30).

The level of p53 is high in 12-day-old mouse embryos but low at 16 days (7, 38). Thus, the increased level of p53 in some transformed cells (e.g., F9 embryonal carcinoma cells) may be due to reversion of the cells to a specific stage of embryonic development. However, it has also been reported that p53 may be a normal cell surface component on a subclass of B-lymphocytes in adult mice (10, 50). Hence, some transformed cells (i.e., Abelson virus-transformed T-cells) may contain high levels of p53 as a consequence of their cell type and not because of their transformed phenotype.

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The half-life of p53 is less than 1 h in untransformed mouse 3T3 fibroblasts but greater than 22 h in SV40-transformed 3T3 cells (40). This greater stability presumably is responsible for the 25- to 100-fold increase in the level of p53 seen in SV40-transformed cells. In fact, the levels of translatable mRNA for p53 are similar in untransformed and SV40 transformed cells (40). In mouse F9 embryonal carcinoma cells, the half-life of p53 is approximately 3.5 h (41). Since the levels of translatable mRNA for p53 are similar in F9 and 3T3 cells, the high level of p53 found in F9 cells is also probably due to its greater stability.

A characteristic feature of tumorigenic, transformed animal cells is the loss of control of cell proliferation (9, 43). One model for the control of proliferation proposes that a short-lived protein (R-protein) accumulates during the G_1 phase of the cell cycle (45); cells become committed to DNA synthesis when sufficient R-protein accumulates (48, 54). This protein may be more stable in some transformed cells, and the resulting accumulation of the protein could cause uncontrolled cell proliferation (6, 34). Since p53 is short-lived in untransformed cells and stable in SV40-transformed cells, it shows some of the properties proposed for the putative R-protein. Mercer et al. (35) demonstrated that microinjection of antibodies to p53 into serum-stimulated mouse 3T3 cells inhibits entry of the cells into S phase. To examine the possible role of p53 in cell transformation, we measured the level and stability of p53 in untransformed control BALB/c 3T3 cells and various transformed 3T3 cell lines. Additional studies were designed to gain further information about the process responsible for the rapid degradation of p53 in untransformed cells.

MATERIALS AND METHODS

Cell lines and culture conditions. Mouse 3T3 cell lines were obtained from the following sources. The MA-3T3, RS-3T3,

BP-3T3, and SV-3T3 cell lines were derived from in vitro transformation of BALB/c 3T3 mouse embryo cells with Moloney sarcoma virus (1), Rous sarcoma virus B77 (57), benzo[a]pyrene (42), and SV40 (SV-29 and SV-34 are different clones) (53), respectively. Clone 122, a hybrid cell line producing anti-p53 immunoglobulin, prepared by Gurney et al. (19), was obtained from the Salk Institute. Meth A cells, derived from a methylcholanthrene-induced tumor in a BALB/c mouse (15), were provided by Lloyd J. Old (14).

The 3T3-related cells were cultured in Dulbecco modified Eagle medium (DME; Flow Laboratories, Inc.) supplemented with 4 mM glutamine, 100 U of penicillin per ml, 100 μg of streptomycin per ml, and 10% bovine serum. Cultures were incubated at 37°C in a water-saturated 10% CO_2–90% air atmosphere. Cell lines were passaged twice weekly, and fresh cells were obtained from frozen stocks every 6 to 9 weeks. All cell lines were determined to be free of mycoplasmas from the ratio of [^{3}H]uridine to [^{3}H]uracil incorporation (54).

Production of anti-p53 antibodies. Clone 122 cells, which produce anti-p53 antibodies (19), were grown in DME containing 10% fetal bovine serum. The cultures were inoculated at 2×10^5 cells per ml. When the cell number reached 10^6 cells per ml, the cultures were harvested, the cells were removed by centrifugation at $500 \times g$ for 10 min, and the culture medium was sterilized by filtration through a 0.2-μm membrane filter (Nalgene Labware Div., Nalge/Sybron Corp.). Robert Croy generously provided the culture medium from clone 122 cells that contained monoclonal antibodies against p53. DME containing 10% fetal bovine serum was used as the nonimmune culture medium.

Preparation of cell extracts for immunoprecipitation. Cells (2×10^6 to 3×10^6 per dish) were placed in 3 ml of methionine-free DME. [^{35}S]methionine was added (150 μCi per plate, 900 Ci/mmol), and the cells were incubated at 37°C for 3 h. After being labeled, the cells were washed 3 times with serum-free DME, and 0.5 ml of extraction buffer (50 mM Tris chloride, pH 8.0, 5 mM EDTA, 0.6 M NaCl, 0.5% Nonidet P-40, 5 mg of bovine serum albumin per ml, 2 mM phenylmethylsulfonyl fluoride) was added to each plate (31). The phenylmethylsulfonyl fluoride was added to the extraction buffer from a 200 mM stock solution just before cell extraction. The whole-cell extracts were frozen immediately at −20°C.

Immunoprecipitation of p53. Immunoprecipitation was performed as described by Linzer et al. (31). All work was done at 4°C. The cell extracts were thawed on ice and sonicated (twice for 10 s each in a Branson sonicator setting no. 2). Portions (200 to 400 μl) of extract, always containing 4×10^7 cpm of [^{35}S]methionine-labeled proteins, were placed into 1.5-ml conical plastic tubes and brought to a volume of 500 μl with extraction buffer. A 200-μl amount of a 10% suspension of Formalin-fixed *Staphylococcus aureus* cells (Bethesda Research Laboratories) was added, and after 15 min the cells were removed by centrifugation at $12,000 \times g$ for 2 min. Samples (300 μl) of the supernatant fluid were placed into each of two tubes; 200 μl of either anti-p53 culture medium or nonimmune culture medium was added, and after 1 to 2 h 200 μl of *S. aureus* cells was added. After 15 to 30 min, the cells were collected by centrifugation, washed 3 to 4 times with wash buffer (50 mM Tris chloride, pH 7.4, 0.5 M NaCl, 5 mM EDTA, 5% sucrose, 1% Nonidet P-40, 0.25% gelatin [type 1; Sigma Chemical Co.]) and once with distilled water. The bound proteins were eluted by boiling for 5 min in 50 μl of electrophoresis sample buffer (62.5 mM Tris chloride, pH 6.8, 5% 2-mercaptoethanol, 10% glycerol, 2.3% sodium dodecyl sulfate, 0.001% bromphenol blue).

Gel electrophoresis. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was performed as described by Laemmli (27). Protein samples in 10 to 40 μl of electrophoresis sample buffer were applied to a gel slab composed of a 10% acrylamide separating gel and a 5% acrylamide stacking gel, and electrophoresis was performed at a constant current of 15 to 25 mA per gel for 5 to 10 h. For fluorography, the gel was soaked for 1 h in En^{3}Hance (New England Nuclear Corp.) and then for 1 h in distilled water; it was then dried and exposed to Kodak X-AR5 film at −70°C for 2 to 7 days. Calibration strips containing known amounts of radioactivity were prepared by the method of Garrels (17) and exposed to the film along with the dried gel. The film was developed with a Kodak X-Omat processor, and the optical density of areas of the autofluorogram was determined by scanning with a recording densitometer (Helena Laboratories). Care was taken to ensure that the measurements were made within the linear portion of the optical density range of the film.

Measurement of overall protein degradation. Cells (2×10^6 to 3×10^6 per dish) were labeled with [^{35}S]methionine as described above and then washed 3 times in DME containing 2 mM unlabeled methionine.

The cells were incubated in DME containing 2 mM unlabeled methionine, and 500-μl portions of the medium were taken at time zero and every 30 min. A 50-μl amount of 100% trichloroacetic acid was added to each portion (10% final trichloroacetic acid concentration), the portions were centrifuged at $12,000 \times g$ for 2 min at 4°C, and the acid-soluble radioactivity in the medium was measured by liquid scintillation counting. After 90 min the cells were harvested as described above for immunoprecipitation. To determine the amount of radioactivity in cell proteins, a sample of the cell extract was made 10% in trichloroacetic acid and centrifuged at $12,000 \times g$ for 2 min at 4°C. The resulting pellet was dissolved in 0.1 N NaOH, and radioactivity was measured by liquid scintillation counting. The amount of acid-precipitable radioactivity present in cell proteins at the start of the chase (time zero) was calculated by taking the amount of acid-precipitable radioactivity present in the cell extract at the end of the chase and adding the amount of the label released into the medium as acid-soluble material during the 90-min chase period. The percentage of protein degradation at each time point was calculated by dividing the amount of acid-soluble radioactivity in the medium at that time by the amount of acid-precipitable radioactivity present in the cells at time zero (39). This technique accurately measures overall protein degradation (39).

Chemicals. Chloroquine was purchased from Sigma. [^{35}S]methionine was purchased from New England Nuclear Corp. Ep475 is a derivative of the fungal product E-64 and was a gift from K. Hanada (Taisho Pharmaceutical Co., Ltd., Yoshincho, Japan).

RESULTS

To measure the accumulation of p53, cells were incubated with [^{35}S]methionine, and proteins were precipitated from cell extracts with anti-p53 monoclonal antibody (19). Control precipitates made with nonimmune culture medium, and the specific immunoprecipitates obtained with anti-p53 antibodies were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Extracts of SV40-transformed cells contained proteins with molecular weights of 53,000 and 82,000 which were specifically precipitated by anti-p53 antibodies

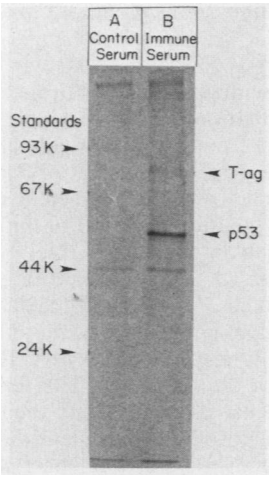

FIG. 1. Immunoprecipitation of p53 from an extract of SV40-transformed 3T3 cells (clone SV-29). The molecular weight standards were phosphorylase a (93,000 [93K]), bovine serum albumin (67K), ovalbumin (44K), and trypsinogen (24K). The positions of SV40 T-ag and p53 are shown.

(Fig. 1). Contaminating proteins of ca. 40,000 and 150,000 daltons were seen in both control and immune precipitates.

Turnover of p53 in 3T3 cells. To measure the degradation of p53, 3T3 cells were labeled with [^{35}S]methionine for 3 h and then placed in medium containing unlabeled methionine. Extracts of cells were prepared immediately after the labeling period and at various times during the chase period. The amount of labeled p53 decreased (Fig. 2) with a half-time between 25 and 40 min in several different experiments.

Energy requirement of p53 loss from 3T3 cells. Previous studies have shown that the intracellular degradation of short-lived proteins requires ATP (18, 24, 46; Gronostajski and Goldberg, manuscript in preparation). We therefore tested the effect of inhibitors of ATP production on the rate of p53 degradation. Initial experiments showed that treating cells with 0.5 mM dinitrophenol and 12.5 mM 2-deoxyglucose reduced ATP levels to less than 5% of normal values within 5 min and that this effect was reversible (24; R. M. Gronostajski, Ph.D. thesis, Harvard University, Cambridge, Mass., 1982). At these concentrations, the inhibitors of ATP production reduced the rate of degradation of total ^{35}S-labeled protein by about 75% (Fig. 3). These agents also

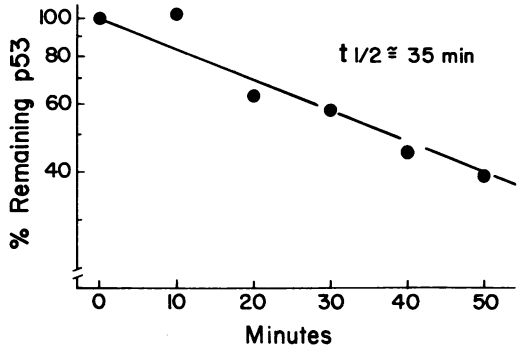

FIG. 2. Half-life of p53 in 3T3 cells. The amount of radioactive p53 (●), determined by densitometric scanning of the autofluorogram of the dried gel, was normalized to the 100% value obtained at zero time.

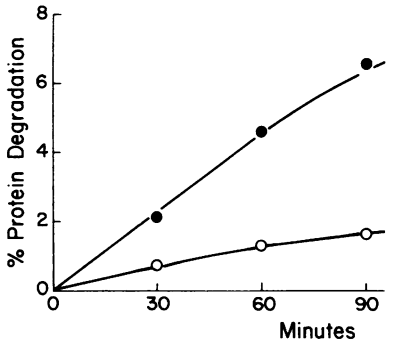

FIG. 3. Inhibition of degradation of total ^{35}S-labeled cell proteins by ATP production inhibitors. 3T3 cells were labeled and then chased (○) in glucose-free medium with 0.5 mM dinitrophenol and 12.5 mM 2-deoxyglucose or (●) in medium containing 25 mM glucose (control). Protein degradation was measured by the appearance of acid-soluble ^{35}S-labeled material in the culture medium (see the text).

prevented the rapid loss of p53 (Fig. 4A). Quantitation of the autofluorogram (Fig. 4B) confirmed that p53 had a half-life of about 33 min in control cells, whereas in inhibitor-treated cells it was completely stable for 60 min. The molecular weight of the p53 antigen was indistinguishable between control cells and cells treated with proteolysis inhibitors (Fig. 4A, cf. lane A with D and E). In addition, no intermedi-

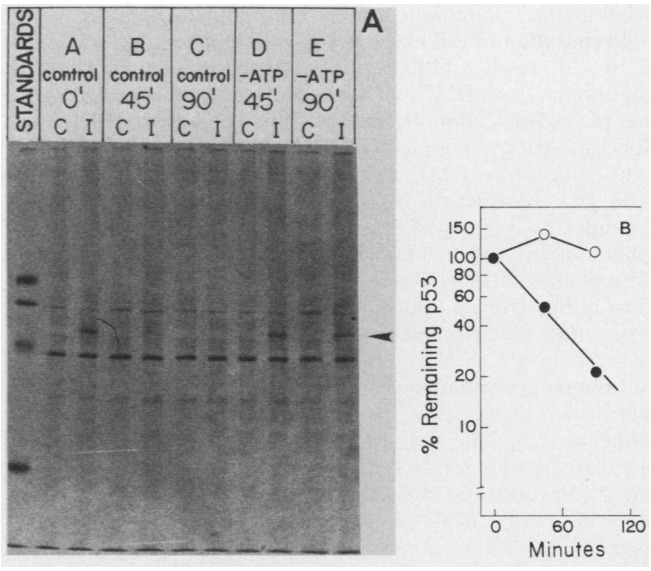

FIG. 4. p53 degradation is blocked by ATP production inhibitors. (A) 3T3 cells were labeled and then chased in nonimmune control culture medium (lanes C) or with medium containing monoclonal anti-p53 immunoglobulin (lanes I). Control reactions contained 25 mM glucose; −ATP reactions contained 0.5 mM dinitrophenol and 12.5 mM 2-deoxyglucose. Lanes A, B, and C, extracts of control cells taken at 0 (A), 45 (B), and 90 (C) min; lanes D and E, extracts of treated cells taken at 45 (D) or 90 (E) min. The arrow marks the position of p53. (B) The autofluorogram shown in A was scanned with a recording densitometer, and the radioactivity in the p53 remaining in control (●) and treated (○) cells was measured during the chase period. The background optical density measured from the lanes containing the nonspecific precipitates was subtracted from the values obtained for the p53-specific precipitates. The amount of p53 at each time point was normalized to the 100% value obtained at zero time.

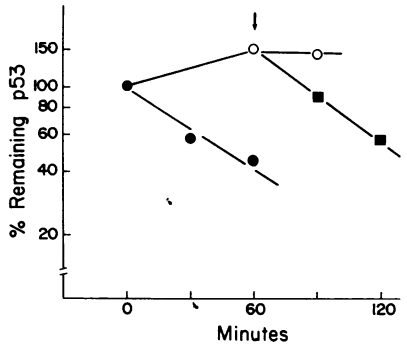

FIG. 5. Reversal of inhibition of p53 degradation. 3T3 cells were labeled and then washed and incubated in either glucose-free medium with dinitrophenol and 2-deoxyglucose (○) or medium containing 25 mM glucose (●). After 60 min (arrow), the inhibitor-treated cultures were washed and placed into medium containing 25 mM glucose (■). Cells were harvested at the times indicated, and the amount of p53 in extracts from each time point was normalized to the 100% value obtained at zero time.

ate of p53 degradation (i.e., smaller antigenic material) was detected under either chase condition. The inhibition of p53 degradation was completely reversed after removal of the inhibitors and readdition of glucose (Fig. 5), as was the inhibition of overall protein degradation and the reduction of ATP levels (data not shown).

Since lysosomes can degrade certain cellular proteins (18), we attempted to prevent the degradation of p53 with inhibitors of lysosomal function. Previous studies showed that inhibitors of lysosomal acidification (NH_4Cl, chloroquine, etc.) and inhibitors of lysosomal proteases (leupeptin, Ep475, etc.) did not prevent the degradation of intracellular long- or short-lived proteins in growing fibroblasts (Gronostajski, Ph.D. thesis; Gronostajski and Goldberg, manuscript in preparation). In accord with these reports, we found that neither Ep475 (Fig. 6) nor chloroquine (data not shown) decreased the rate of disappearance of p53. Thus, the loss of p53 appears to involve a nonlysosomal, ATP-dependent proteolytic system.

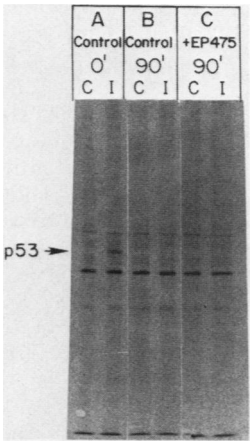

FIG. 6. Degradation of p53 in the presence and absence of Ep475. Proteins were precipitated with nonimmune control culture medium (lanes C) or medium containing monoclonal anti-p53 immunoglobulin (lanes I). The autofluorogram shows proteins precipitated from control cultures at 0 (A) and 90 (B) min and from an Ep475-treated culture at 90 min (C).

Levels of p53 in untransformed and transformed cells. Since it has been reported that p53 levels are high in several types of transformed cells (12, 14, 50), we measured the amount of p53 that accumulated during a 3-h labeling period in nontumorigenic 3T3 cells and in 3T3 cells transformed to tumorigenicity with different agents. Surprisingly, the amounts of p53 which accumulated in RS-3T3 and MA-3T3 cells were similar to the amount of labeled p53 in untransformed 3T3 cells (Fig. 7). RS-3T3 cells contained 150% of the amount of labeled p53 found in 3T3 cells. However, MA-3T3 cells accumulated 50% less p53 than did 3T3 cells. As expected, the amount of labeled p53 in two SV40-transformed 3T3 cell lines (SV-29 and SV-34) was 7- to 10-fold higher than the amount in 3T3 cells (Fig. 7). In the SV40-transformed cells, SV40 large T-ag coprecipitated with p53, whereas no proteins coprecipitated with p53 in 3T3, RS-3T3, or MA-3T3 cells.

Stability of p53 in Meth A cells. DeLeo et al. (15) reported that p53 levels were also high in a cell line derived from a chemically induced mouse sarcoma (Meth A). We therefore examined the rate of degradation of p53 in the Meth A cell line (Fig. 8). In accord with the earlier studies, we observed a substantially higher level of p53 in Meth A cells than in 3T3 cells. In contrast to the rapid disappearance of p53 from 3T3 fibroblasts, p53 was stable in Meth A cells. In 1 h, little or no p53 was lost from the Meth A cells (Fig. 8), whereas more than 70% of the p53 was degraded in 3T3 fibroblasts (Fig. 4). A protein of molecular weight 68,000 was also present in immunoprecipitates of p53 from extracts of Meth A cells (Fig. 8). It is uncertain whether this protein has any association with p53 or was merely a contaminant in the immunoprecipitate.

DISCUSSION

These studies suggest that (i) the rapid loss of immunoreactive p53 from nontransformed 3T3 cells occurs via a nonlysosomal, ATP-dependent process; (ii) the elevated amount of p53 in Meth A cells may result from inhibition of p53 degradation; and (iii) tumorigenic RS-3T3 and MA-3T3 cells accumulate amounts of p53 similar to that in nontumorigenic 3T3 cells, and a high rate of p53 accumulation is not required for tumorigenicity.

The last conclusion holds only if the p53 antigen measured in each type of transformed cell is identical. The proteins recognized by this antibody in F9 embryonal carcinoma,

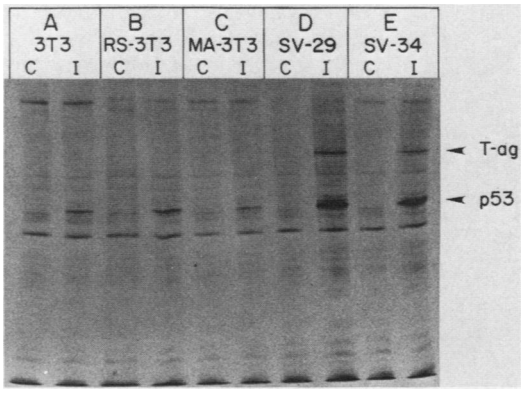

FIG. 7. Comparison of p53 labeling in normal and transformed 3T3 cells. Proteins were precipitated by nonimmune control culture medium (lanes C) or medium containing monoclonal anti-p53 immunoglobulin (lanes I).

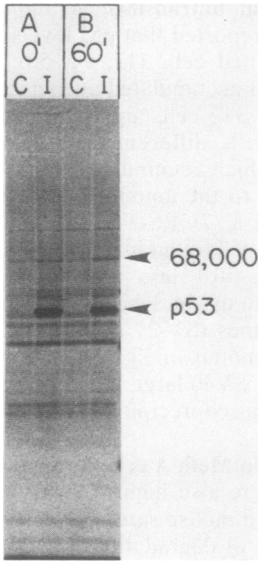

FIG. 8. Lack of degradation of p53 in Meth A cells. Meth A cells were labeled and then washed and incubated in chase medium containing unlabeled methionine. Cells were harvested and extracts were prepared at 0 (A) and 60 (B) min of the chase period. Proteins were precipitated by nonimmune control culture medium (lanes C) and medium containing monoclonal anti-p53 immunoglobulin (lanes I). The arrows mark the positions of p53 and a 68,000-dalton protein which coprecipitated with p53.

SV40-transformed, adenovirus-transformed, and embryonic mouse cells all have identical peptide maps (7, 52). Thus, the p53 protein we examined is most likely identical in all of our mouse cell lines.

Since the half-life of p53 in untransformed 3T3 cells is about 30 min (Fig. 2), the 3-h labeling period used (Fig. 7) will label the p53 pool in these cells essentially to equilibrium. We also have preliminary evidence that the half-life of p53 in MA-3T3 and RS-3T3 cells is between 0.5 and 1 h; thus, p53 is also labeled almost to equilibrium in these cell lines by a 3-h pulse of methionine (not shown). However, since p53 has a half-life of more than 12 h in SV40-transformed 3T3 cells (40; unpublished data), the amount of p53 present in this cell line is greatly underestimated by a 3-h labeling period (3). Thus, the steady-state level of p53 in SV40-transformed 3T3 cells is actually about 50 to 100 times the level in untransformed 3T3 cells, not 7 to 10 times as we found (Fig. 7).

The 3-h labeling period used here is similar to that used previously to determine the amount of p53 that accumulates in various normal and tumorigenic cells (7, 37, 49, 50). This technique gives a good estimate of the steady-state level of p53 in cells only if the half-life of the protein is less than ca. 1 h (3). The similar intensities of the p53 bands shown in Fig. 7 indicate that the half-life of p53 is similar in all of these lines and, therefore, must be short (unless slower degradation is closely compensated for by slower synthesis). Therefore, p53 at 3 h must in all these cases be near equilibrium level. Although the steady-state level of p53 was not measured in these studies, these data (Fig. 7) confirm and extend previous findings which showed that many tumorigenic cells differ from SV40-transformed cells and fail to accumulate high levels of p53.

Fate of immunoreactive p53 in 3T3 cells. The rapid loss of p53 closely resembles general intracellular protein degradation (24; Gronostajski, Ph.D. thesis; Gronostajski and Goldberg, manuscript in preparation) in the following ways. (i) Degradation of p53 and total short- and long-lived proteins was rapidly prevented by inhibitors of ATP production (Fig. 3 and 4). (ii) These inhibitions were rapidly reversed after removal of the inhibitors and addition of glucose to the culture medium (Fig. 5). (iii) Neither type of degradation was prevented by Ep475, an inhibitor of lysosomal proteases (2, 20, 22). Thus, the loss of p53 appears to involve a nonlysosomal, ATP-dependent system similar to that which degrades short- and long-lived proteins in growing hamster fibroblasts (Gronostajski, Ph.D. thesis; Gronostajski and Goldberg, manuscript in preparation).

Our studies further indicate that metabolic energy, probably in the form of ATP, is required for an initial rate-limiting step in the degradation of p53. ATP depletion prevents the loss of both the enzyme activity (24, 36, 47) and the immunoreactive material that accompany the degradation of short-lived enzymes (24, 36) and "abnormal" proteins (4, 5, 16, 23, 25, 26; F. S. Boches, Y. Klemes, and A. L. Goldberg, Fed. Proc. **39**:1682, 1980). When the degradation of a normal cell protein (p53) was inhibited by ATP depletion, the undegraded protein had the same subunit molecular weight as the native protein (Fig. 3A). ATP must therefore function in an initial step in the degradation of p53. If ATP were required only for some step after the initial rate-limiting step, then the p53 band would have disappeared during ATP depletion and products of the degradation of p53 with molecular weights below 53,000 would have accumulated.

An alternative explanation, that newly synthesized p53 was rapidly converted into a non-immunoreactive form in an energy-dependent manner, is unlikely. Oren et al. (40), using a polyclonal anti-p53 antiserum, observed rapid loss of immunoreactive p53 in 3T3 cells. In addition, Rotter et al. (50) used a different monoclonal anti-p53 antibody and reported that p53 disappears rapidly in mouse cells.

Another explanation for the rapid loss of immunoreactive p53 from 3T3 cells would be that p53 is secreted into the culture medium. The half-life of p53 in 3T3 cells (~35 min) was similar to the time required to secrete proteins from lymphoma cells (55) and rat liver cells (8). However, it has not been possible to detect p53 in the medium of 3T3 cells after cell labeling and medium sampling (J. Campisi, personal communication). Therefore, intracellular proteolysis appears to be the most likely explanation for the rapid loss of p53 from 3T3 cells.

The inhibitors used in these studies, dinitrophenol and 2-deoxyglucose, have been used previously to deplete intracellular ATP pools and inhibit intracellular proteolysis (18, 24, 46, 47). These agents can also inhibit protein transport and glycosylation, functions which may influence the rate of protein turnover (18, 55). Also, the general disruption of cellular metabolism by these drugs (ion pumping, etc.) might affect intracellular protein degradation. However, the rapid reversal of proteolysis inhibition (Fig. 5) and reaccumulation of ATP (not shown) after removal of the inhibitors and readdition of glucose suggests that no permanent damage to the cells occurs during energy depletion.

Stabilization of p53 in Meth A cells. It has been suggested that p53 is stabilized in SV40-transformed cells through interaction with SV40 T-ag (40); these proteins form a complex in solution and cosediment through sucrose density gradients (21, 33). The mechanism for the stabilization of p53 in transformed Meth A cells is unknown. A 68,000-dalton protein was present at low levels in immunoprecipitates of p53 from extracts of Meth A cells (Fig. 8). Further studies

are required to determine whether this protein has any specific association with p53. Ruscetti and Scolnick (51) recently reported that monoclonal antibodies directed against p53 precipitate a protein of about 70,000 daltons along with p53 in extracts of mouse erythroleukemia cells. Also, a 68,000-dalton nuclear protein in normal mouse cells shares an antigenic determinant with SV40 T-ag (11, 29). It will be of interest to determine whether this T-ag-related protein encoded by mouse cells is related to the 68,000-dalton protein seen in immunoprecipitates from Meth A (Fig. 8) and erythroleukemia cells or to a 68,000-dalton protein involved in cell cycle control (13).

Relation between p53 and cell transformation. MA-3T3 and RS-3T3 tumor cells did not accumulate high levels of p53 (Fig. 7), nor did BP-3T3 cells (44). In fact, MA-3T3 cells accumulated only 50% of the amount of labeled p53 found in untransformed 3T3 cells. Thus, a high rate of accumulation of p53 is not required for tumorigenicity. These data extend the findings of Mora et al. (38), who showed that p53 levels did not correlate with the degree of tumorigenicity of spontaneously transformed mouse cell lines. Also, p53 is not detectable in HeLa cells, a highly tumorigenic human tumor cell line (12). Although some tumor cells do not accumulate high levels of p53, this protein may still have an important role in tumorigenicity. Recently, Sompayrac et al. (56) showed that F8dl, a deletion mutant of SV40, can transform mouse cells and that the level of p53 is not elevated in these transformants. Interestingly, this mutant virus transforms cells at only 2% of the wild-type SV40 frequency. Also, p53 is a marker for primary tumors in mice (49). These data suggest that stabilization of p53 may be important for the initiation, but not the maintenance, of transformation. It is possible that the functional activity of p53 may be regulated independently of the amount of the protein; for example, the effect of phosphorylation on p53 activity is unknown.

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